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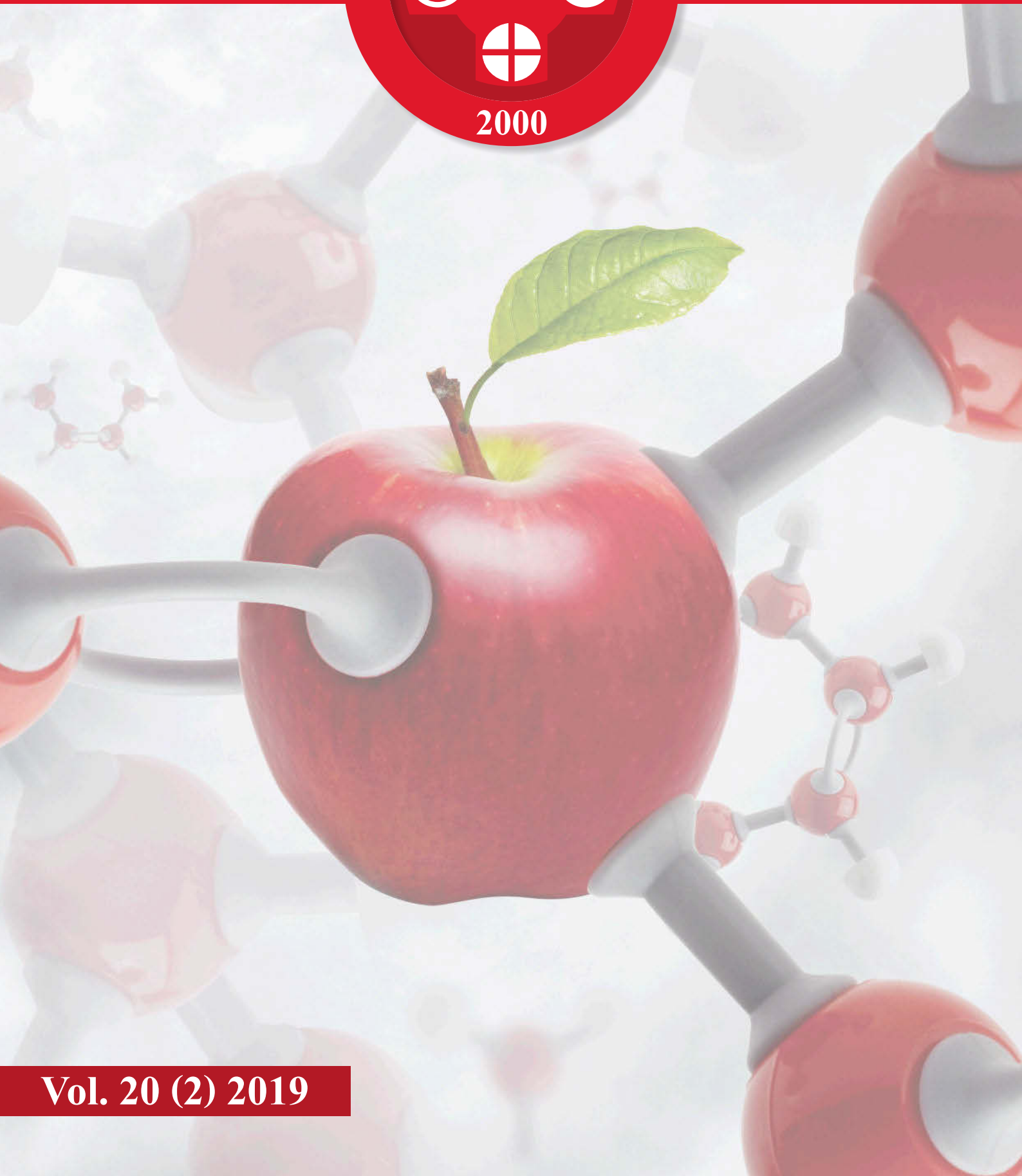

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OLEIC ACID – HEALTH BENEFITS AND STATUS IN PLASMA PHOSPHOLIPIDS IN THE SERBIAN POPULATION

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OLEINSKA KISELINA-UTICAJ NA ZDRAVLJE I STATUS U FOSFOLIPIDIMA PLAZME U SRPSKOJ POPULACIJI

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ABSTRACT

Oleic acid has many beneficial effects on human health. One of the main dietary sources of oleic acid is olive oil. Non-Mediterranean European countries, including Serbia, have low habitual olive oil consumption, but other vegetable oils also contain different amounts of oleic acid. In infants and young children milk is the most important source of fatty acids, including oleic acid. Furthermore, fatty acid composition of plasma phospholipids reflects dietary intake and fatty acid metabolism. In patients with serious chronic diseases fatty acid status is altered independently on the intake. Here we reviewed status of oleic acid in healthy persons as well as in patients with different chronic diseases in Serbia.

Keywords: *plasma phospholipids, oleic acid, olive oil*

SAŽETAK

Oleinska kiselina ima ima višestruke pozitivne efekte na život i zdravlje ljudi. Jedan od glavnih izvora oleinske kiseline je maslinovo ulje. Evropske zemlje, koje ne pripadaju mediteranskoj regiji, među kojima je i Srbija, retko koriste maslinovo ulje u ishrani, ali i druga biljna ulja sadrže oleinsku kiselinu u različitom procentu. Kod odojčadi i dece mlađeg uzrasta, mleko je najvažniji izvor masnih kiselina, uključujući i oleinsku. Dijetetski unos masnih kiselina i njihov metabolizam u telu, odražava masnokiselinski sastav u fosfolipidima plazme. Kod pacijenata sa teškim hroničnim oboljenjima, masnokiselinski sastav je promenjen nezavisno od unosa. U ovom radu je prikazan status oleinske kiseline kod zdravih osoba, kao i kod pacijenata sa različitim hroničnim oboljenjima u Srbiji.

Ključne reči*: fosfolipidi plazme, oleinska kiselina, maslinovo ulje*

ABBREVIATIONS

FA- fatty acid
IDL- intermediate-density lipoprotein
HDL- high-density lipoproteines
MUFA- monounsaturated fatty acid
OA- oleic acid
PUFA- polyunsaturated fatty acid
SFA- saturated fatty acid
VLDL- very-low-density lipoprotein

OLIVE OIL AS A KEY COMPONENT OF MEDITERRANEAN DIET

Numerous epidemiological, clinical and experimental research have shown that consumption of Mediterranean diet rich in olive oil has a profound influence on a number of health outcomes, including obesity, metabolic syndrome, cardiovascular disease and diabetes mellitus (1). Intensive olive oil intake is inversely associated with both systolic and diastolic blood pressure (2), and with stroke incidence in elderly populations (3). A major component that is responsible for beneficial properties of olive oil is oleic acid (OA). Olive oil contains up to 80% monounsaturated fatty acids (MUFA), mostly in the form of OA, relatively low content of saturated (SFA) and polyunsaturated fats (PUFA), but also several antioxidant components, including phenolic compounds that could partially account

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for the observed healthful effects of the Mediterranean diet (4). Non-Mediterranean European countries, including Serbia, have low habitual olive oil consumption. However, olive oil is not the only source of OA, it is also a component of other vegetable oils, nuts and animal products (5, 6), although its concentration depends on the type of food, as well as on the region.

Besides the favorable effects of oleic acid intake in adults, it is also essential in infants and children nutrition. OA is a component of tissues and membranes, and a major fatty acid (FA) component of brain myelin phospholipid, which is mainly formed during the two years after birth (7). Since OA is rapidly deposited during myelination, its proportion in brain total lipids increases with progressive central nervous system myelination (8). Thus intake of OA is of a great importance in post-natal life. In infants and young children milk is the most important source of fatty acids, including OA. Several epidemiological studies reported different content of OA and other FA in human colostrum (9), transitional and mature milk (10-12), suggesting that FA composition of breast milk is markedly influenced by geographic differences in maternal dietary composition. The similar situation has been shown in cow's milk samples, as various interactions were found among diet type, cow type and altitude, indicating that a combination of these factors contributes to the characteristic FA profile of the respective milk (13).

Fatty acids have been traditionally considered as precursors for the biosynthesis of macromolecules and as a source of energy, as well as constituents of cellular membranes. However, emerging evidences from several lines suggest that dietary FA serve multiple function in the body, and are linked not only to health promotion, but also to disease pathogenesis. Therefore, the choice of dietary FA could markedly influence overall health in humans. Depending on the dietary intake of FA, blood phospholipids' FA composition will change (14). In healthy people, the FA profile of the serum/plasma phospholipid is an indirect biomarker of the average dietary FA intake during the last 3 to 6 weeks, while the composition of erythrocyte phospholipids reflects the intake during the preceding months (15, 16). Nevertheless, a number of physiological and pathologic conditions can influence the FA status in blood. Here we reviewed status of OA in plasma phospholipids of healthy people of different ages, in professional athletes and in patients with chronic diseases in Serbia.

OLEIC ACID STATUS IN PLASMA PHOSPHOLIPIDS

Apparently healthy subjects

Several papers reported FA status in apparently healthy subjects in Serbia (14, 17-22). Their content of OA in plasma phospholipids is presented in Table 1. As it can be seen in the Table, healthy people have similar level of OA in plasma from birth to older ages. Slightly lower level of OA was found in young adults, but it can be attributed to their specific dietary intake which mostly includes fast food with low level of OA.

Elite athletes

The status of OA in plasma phospholipids of elite athletes is displayed in Table 2. In comparison to sedentary healthy subjects, athletes mostly have lower levels of OA.

Table 1. The content oleic acid in plasma phospholipids in apparently healthy subjects in Serbia

Author, year (ref No)	Sample size	Gender (M/F)	Mean age	mol% of OA
Arsić et al., 2012 (17)	12	n.a.	At birth (34-36 weeks of gestation)	11.91 ± 0.73
Arsić et al., 2012 (20)	14	0/14	23.67±1.56	9.03 ± 1.03
Tepšić et al, 2009 (14)	16	16/0	24.0 ±3.0	11.03 ± 1.50
Tepšić et al, 2013 (19)	19	19/0	24.44 ± 3.40	10.46 ± 1.66
Cvetković et al., 2010 (18)	29	15/14	53(23-71)	11.70±1.79
Ristić et al., 2006 (22)	29	17/12	55 ± 9	11.71 ± 1.24
Popović et al., 2009 (21)	15	12/3	60 (54-68)	11.85 ± 1.69

Table 2. The content oleic acid in plasma phospholipids of elite athletes in Serbia

Author, year (ref No)	Type of sport	Sample size	Gender	Mean age	mol% of OA
Petrovic et al., 2016 (23)	Handball	17	F	17.2 ±0.93	7.81±0.49
Petrovic et al., 2016 (23)	Handball	15	M	18.5±1.06	8.49±0.97
Tepšić et al, 2009 (14)	Basketball	23	M	21 ±4	10.69 ± 1.00
Arsić et al., 2012 (20)	Football	19	F	21.19±2.45	10.42±1.21
Arsić et al., 2012 (20)	Waterpolo	15	F	21.71±4.5	8.85±1.56
Tepšić et al, 2013 (19)	Box	16	M	22.41±3.28	14.06 ± 1.50
Tepšić et al, 2009 (14)	Football	24	M	24 ± 5	11.01 ± 0.95

Table 3. The content oleic acid in plasma phospholipids in patients with different diagnoses in Serbia

Author, year (ref No)	Diagnosis	Sample size	Gender (M/F)	Mean age	mol% of OA
Ristić et al., 2006 (22)	hemodyalized	37	21/16	52 ± 10	13.05 ± 2.21
Ristić-Medić et al., 2009 (24)	hyperlipidemia	78	25/53	56 (45-65)	11.20± 1.0
Ristić-Medić et al., 2006 (25)	Type 2 diabetes	28	n.a.	57 ±7	10.46± 2.31
Cvetković et al., 2010 (18)	non-Hodgkin lymphoma	47	26/21	57 (19–74)	13.8±1.4
Popović et al., 2009 (21)	obstructive jaundice	13	10/3	59 (54-68)	14.31± 2.19
Ristić-Medić et al., 2013 (26)	Alcoholic liver cirrhosis	20	18/2	62 (46-72)	18.83± 4.54
Veselinovic et al., 2017 (27)	Rheumatoid arthritis	60	0/60	63.1± 9.6	8.64 ± 1.03

In particular handball players, both male and female, and female water polo players (14, 20, 23). On the contrary, boxers had significantly higher levels of OA, but their general fatty acid status is altered (19).

Chronic non-communicable diseases

Since FA profiles in plasma phospholipids also depend on the metabolism, they are often altered in chronic diseases. Table 3 presents the OA proportions in patients with different diagnoses. It seems that metabolic disorders, such as hyperlipidemia (24) and diabetes mellitus type 2 (25) do not affects metabolism of OA and its level remain similar as in the control group. Patients with non-Hodgkin lymphoma (18) and patients on hemodialysis (22) had higher levels of OA (13-14 mol%). Serious liver diseases, such as obstructive jaundice (21), and in particular alcoholic liver cirrhosis (26), markedly increased level of OA in plasma phospholipid, while in contrast rheumatoid arthritis (27) decreased OA in comparison to control subjects. The observed changes are unlikely to be a consequence of increased (or in patients with rheumatoid arthritis decreased) intake, but rather of altered activity of delta-9 desaturase. The reasons for these alterations remain to be clarified.

HEALTH BENEFITS OF OLEIC ACID

Oleic acid is the major component that is responsible for health benefits of the Mediterranean diet, rich in vegetables, fruits and particularly olive oil (28). It has been shown that diets with OA are associated with a decreased risk of coronary heart disease, cardio-metabolic risk, obesity, type 2 diabetes and hypertension (29, 30). Observational studies from Mediterranean cohorts have suggested that dietary MUFA, in particular OA, may be protective against stroke (3), age-related cognitive decline and Alzheimer's disease (30). In addition, insulin sensitivity is relatively impaired by diets that are low in oleic acid, or rich in trans MUFA or saturated palmitic acid (31). Rising evidence exists that saturated palmitic acid causes insulin resistance via stimulation of inflammatory signaling and production of cytosolic lipid compounds (diacylglycerol and ceramide). OA acts in an opposite direction, suggesting that dietary or pharmacologic intervention that facilitate transport of FA into the mitochondria would be beneficial (32). Moreover, substitution of dietary saturated fat by OA and/or PUFA has been described to reduce the cardiovascular risk by reducing blood lipids, mainly cholesterol, LDL-cholesterol and triglycerides (33, 34). Nevertheless, Pedersen et al. reported that in healthy men the olive oil diet resulted in higher concentrations of VLDL, IDL, and LDL particles (number and lipid content) than the rapeseed oil and sunflower oil diets (35).

Furthermore, the authors did not find any differences between proportion of OA in serum phospholipids (as a biomarker of dietary intake) of patients with hyperlipidemia or T2D and healthy controls (25). The percentage of OA in adults in this study varied from 10.3% in young females, to 11.9% in older male participants. The results from other studies showed a strong ecological correlation between olive oil intake and oleic acid in plasma phospholipids (36). For instance, a multicenter European cross-sectional study found that participants from northern countries had lower intake of olive oil and lower OA in plasma phospholipids (9.4-10.5%) than people from Spain, Italy and Greece (11.2-12.7%) (36), while another study reported 9.5-9.8% of OA in plasma phospholipids of female and male subjects in Australia, respectively (37).

Recent findings suggest potential protective effects of OA on the promotion and progression of several human cancers. Several case-control and cohort studies have shown that OA and olive oil are associated with a reduction in the risk of cancer, mainly breast, colorectal and prostate cancer (38). Although underlying mechanism requires further investigation, protective action on cancer may be mediated through several mechanisms, including alterations in the composition and structure of tumor cell membranes, effects on eicosanoid biosynthesis or intracellular signaling pathways, beneficial influence on cellular oxidative stress and DNA damage, and modulation of the immune system or gene expression (38-41). Menendez et al. have recently revealed that OA suppressed the overexpression of HER2 (erbB-2), a well-characterized oncogene playing a key role in the etiology, invasive progression and metastasis in several human cancers (41, 42). They have found that OA directly affected the expression of a cluster of interrelated human cancer genes (i.e., HER2, FASN and PEA3). However, studies reported higher level of OA in plasma phospholipids of patients with non-Hodgkin lymphoma and obstructive jaundice (including patients with

hepatocellular carcinoma) than in healthy subjects (18, 21), although we believe that these results arised from an altered delta-9 desaturase activity, rather than an increased intake of oleic acid. Nevertheless, a recent study showed significantly lower intake of olive oil in patients with lung cancer than in the corresponding healthy control subjects (43). Thus an appropriate dietary intervention must be carried out in animal models and human pilot studies to confirm the possible anti-cancer effects of OA.

DIETARY INTAKE OF OLEIC ACID

In addition to olive oil, another rich source of OA is rapeseed oil, with 64% of OA (44), thus we suppose that all health benefits related to olive oil consumption that could be attributed to OA, can also be applied to rapeseed oil consumption. With an average concentration of about 8 g/l in whole cow's milk, milk and dairy products substantially contribute to the dietary intake of oleic acid in many countries (45). However, high consumption of milk and/or dairy products is thought to contribute to cardiovascular disease, primarily by increasing saturated fat intake, and for that reason many advisory bodies recommend avoiding high-fat dairy foods (46). Several studies have shown that the percentage of undesirable SFA in cow's milk can be replaced with OA by inclusion of different feed components in cow's nutrition. Komprda et al. have recently shown that feed mixture containing rapeseed, rapeseed oil and rapeseed cakes significantly decreased the content of palmitic acid and increased the content of stearic, oleic, linoleic and α-linoleic acid in milk of these cows (47). Another study has demonstrated that supplementation of a basic diet with oilseed, linseed and sunflowerseed improved the milk quality from a nutritional point of view by a large reduction in the content of saturated FA and an increase in the levels of MUFA and PUFA (48). Thus dietary intervention in cows can contribute to healthier milk composition, particularly in terms of its FA content.

Regarding its role in myelinisation, OA is very important in infants' nutrition. Both human milk and infant formulas had significantly higher content of OA than cow's milk (49). Moreover, it is the most abundant FA in breast milk and formulas, and an important source of energy for the infants (7). To provide an optimal content of OA regardless on the diet, only 25% of OA in breast milk originates from the diet during the past 48h, while the remaining 75% originates from the body stores, mostly adipose tissue, or it is produced by interconversion from other FA (50). Nevertheless, Yuhas et al. found different proportions of OA in breast milk from nine countries, which varied from 21.8% in Philippines to 36.5% in China, suggesting a significant impact of the diet (12). Although OA is not an essential fatty acid, its content in human milk is of a great importance, because it reduces the melting point of triacylglycerides, thus providing the liquidity required for the formation, transport and metabolism of milk fat globules (51). Higher demands for OA in infancy for brain development suggest that an increased dietary intake of OA in lactating mothers would enhance nutritional quality of breast milk.

In conclusion, content of oleic acid in serum phospholipids in Serbian population is lower than in Mediterranean countries, but higher than in Northern Europe and it increases during life. Considering beneficial effects of OA, its increased dietary intake would lead to improvement of overall health in the Serbian population.

Acknowledgments

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GLYCOREGULATION DURING PREGNANCY

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GLIKOREGULACIJA TOKOM TRUDNOĆE

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ABSTRACT

Pregnancy is a period marked by profound changes in a woman's hormonal status and metabolism, including the development of a carbohydrate-intolerant state. Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy.

The aim of this study was to estimate and analyse the parameters of glycaemic control during pregnancy. We stratified patients into the following three groups according to OGTT results: normal glucose tolerance (NTG), gestational impaired glucose tolerance (GIGT) and GDM.

We investigated 92 pregnant women, diagnosed with vital and desired pregnancy up to 12 weeks of gestation, who had signed informed consent forms. Among them, 7 pregnant women had a spontaneous abortion, while 8 pregnant women dropped out, so a total of 77 pregnant women completed the trial. Most of the women examined had no risk factors (48%), while 35% of the women had one risk factor. The current study demonstrates that normal glucose tolerance was shown in 59 (76.6%) participants, while some form of glucose intolerance (GIGT or GDM) was shown in 18 (23.4%) patients. Our findings revealed an increase in glucose intolerance with advancing pregnancy (in the second and third trimester). In conclusion, we demonstrate that the difference in the quality of glycaemic control during pregnancy is manifested in the second and third trimester, until it manifests in the first trimester. These findings underpin the clinical significance of discovering GDM.

Keywords: *Pregnancy, glycoregulation*

SAŽETAK

Trudnoća je period koji karakterišu znatne promene u hormonskom statusu i metabolizmu žene, ali i stanje intolerancije ugljenih hidrata. Gestacijski dijabetes (GDM) se definiše kao bilo stepen netolerancije glukoze sa početkom ili prvim pojavljivanjem tokom trudnoće.

Cilj ovog istraživanja je bio da se procene i analiziraju parametri metaboličke kontrole tokom trudnoće. Prema rezultatima OGTT-a, podelili smo pacijente u tri grupe: normalna tolerancija glukoze (NTG), smanjena tolerancija na glukozu (GIGT) i GDM. Ispitivali smo 92 trudnice kod kojih je postavljena dijagnoza vitalne i željene trudnoće do 12 nedelje, koje su potpisale informisani pristanak. Među njima, 7 trudnica je imalo spontani pobačaj, dok je 8 trudnica odustalo, tako da je 77 trudnica završilo ispitivanje. Većina ispitanih žena nije imalo faktore rizika (48%), dok je 35% imalo jedan faktor rizika.

Ova studija pokazuje da je NTG zastupljena kod 59 (76,6%) ispitanica, dok neki oblik intolerancije glukoze (GIGT ili GDM) ima 18 (23,4%) ispitanica. Naši rezultati pokazuju porast intolerancije glukoze sa napredovanjem trudnoće (u drugom i trećem trimestru). Pokazano je i da se razlika u kvalitetu glikoregulacije tokom trudnoće manifestuje u drugom i trećem trimestru, dok se ne manifestuje u prvom trimestru. Ovi nalazi podupiru klinički značaj otkrivanja GDM.

Ključne reči: *trudnoća, glikoregulacija*

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INTRODUCTION

Pregnancy is a period marked by profound changes in a woman's hormonal status and metabolism (1). During pregnancy, β cells normally increase their insulin secretion to compensate for a decrease in insulin sensitivity (2). Pregnancy is a carbohydrate-intolerant state, but only a small proportion of pregnant women will develop diabetes (1). In all pregnancies, insulin resistance increases as the pregnancy develops due to rising circulating concentrations of insulin antagonists such as cortisol, prolactin, human placental lactogen, and leptin. This resistance results in increasing need for insulin, especially in the second half of pregnancy. If insulin secretion is impaired, the enhanced insulin resistance will give rise to hyperglycaemia (3).

During the first two-thirds of gestation, the mother is in an anabolic condition, increasing her fat depots (4). During the last third of gestation, the mother switches to a catabolic condition (5). Insulin resistance tends to increase in physiological pregnancy, following gradually rising levels of foeto-placental hormones (1). In response to this imbalance, the pancreas normally compensates with a higher insulin secretion (4). When this compensatory mechanism fails, glucose intolerance develops (6).

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy (4,7). It does not exclude the possibility that unidentified glucose intolerance may have preceded the pregnant state (8,9). The prevalence varies for different populations, and ranges from 7 to 18% (7). Screening for this disease is recommended for the high-risk and intermediate groups any time during pregnancy, or between 24 and 28 weeks in the latter group (4). The diagnosis of GDM is based on an oral glucose tolerance test, OGTT (9). GDM usually appears in the third trimester as the placenta matures and placental hormones contribute to insulin resistance, which usually recedes after the placenta is delivered (10). Glucose crosses the placenta and stimulates foetal insulin production, while maternal or exogenous insulin does not (4). Since many patients with GDM develop type 2 diabetes, and both diseases are characterized by insulin resistance, some authors postulate that they represent points on a continuum of glucose intolerance (11).

The traditional risk factors for GDM are high maternal age, weight and parity, previous delivery of a macrosomic infant and family history of diabetes (3). Although controversy exists regarding the specific protocols to apply, screening for GDM by OGTT in pregnancy has become a standard element of obstetrical care (12). With this testing, GDM is diagnosed based on blood glucose levels that exceed specific glycaemic thresholds (2). These criteria were chosen to identify women at high risk for developing diabetes after pregnancy, or were derived from criteria used for nonpregnant individuals and not necessarily to identify pregnancies with increased risk for adverse perinatal outcome (8). Clinical detection of GDM is carried out to identify pregnancies at increased risk for perinatal morbidity and mortality (10,13). Most women with GDM appear to have β-cell dysfunction that occurs against a background of chronic insulin resistance (14).

GDM is associated with increased foetal and maternal complications, including congenital malformation, macrosomia, birth injuries, hypoglycaemia and pre-eclampsia, increased incidence of caesarean section and postpartum diabetes in mother (9). Clinical evidence has confirmed that the main goal of glycaemic management in GDM is to keep the mother's blood glucose levels within the normal range (6).

Women with GDM are at high risk for having or developing diabetes after pregnancy (7,13). After pregnancy, the main focus of clinical care should be on reducing the risk of diabetes and detecting and treating diabetes that does develop. Measurement of fasting glucose in the postpartum period will identify women with increased fasting glycaemia who should have an OGTT sometime during the first 2-6 months postpartum and, if not diabetic, annual testing for diabetes (14).

The aim of this study was to estimate and analyse the parameters of glycaemic control during pregnancy.

MATERIALS AND METHODS

Subjects.

This study included 92 women with a diagnosis of vital and desired pregnancy in the first trimester, and 77 of them finished the study. Exclusion criteria for all subjects included age <18 years, diabetes, diseases of the pancreas, liver diseases, kidney diseases, hypertension, corticosteroid treatment, cancer, physical disabilities or psychiatric disorders. The study was performed according to the principles of good clinical practice and was approved by the Ethical Committee of Clinical Center of Kragujevac. Written informed consent was obtained from all participants.

The study protocol included the following four visits for each pregnant woman: in the first (10-14 weeks of gestation), second (20-24), and third trimesters (30-34) and 8 weeks after delivery.

Pregnant women underwent a diagnostic 3 h, 100-g oral glucose tolerance test (OGTT) after a 12 h overnight fast. Venous blood samples for measurement of plasma glucose levels and insulin concentrations were drawn in the fasting state and at 0 min, 1 h, 2 h and 3 h after ingestion of the 100-g glucose drink. None of the respondents had disturbance of glycaemic control at baseline.

HbA1c was determined with immunoinhibition commercial reagents and latex agglutination inhibition process (measured absorbance caused agglutination at 700 nm), expressed as a percentage (%) (reference value 4-7).

Based on the criteria using the OGTT proposed by the *American Diabetes Association* (ADA), GDM was diagnosed if two or more values reached or exceeded the following thresholds: fasting glucose >5.3 mmol/L, 1 h

Table 1. Anthropometric values, personal history and risk factorsrisk factors

Maternal age (min-max)	30,8±4,7 (19-41)
≤25	4/77 (5,2%)
25-34	56/77 (72,7%)
≥35	17/77 (22,1%)
Body weight in first trimester (kg) (min-max)	63±8,49 (48-90)
Body height (m) (min-max)	169±5,7 (155-186)
Body mass index (BMI-kg/m2)	22±2,6 (17-30,88)
BMI 18,5-24,8 kg/m2	70/77 (90,9%)
BMI 25-31,0 kg/m2	9/77 (9,1%)
Weight gain until delivery (kg) (min-max)	12,7±1,49 (9-16)
Obesity (%)	3/77 (3,9%)
Hypertension (%)	4/77 (5,2%)
Previously treated sterility (%)	7/77 (9,1%)
Previous use of metformin (%)	3/77 (3,9%)
Previous gestational diabetes mellitus (%)	8/77 (23,5%)
Smoking Yes	18/77 (23,4%)
No	47/77 (61%)
Previous (until pregnancy)	12/77 (15,6%)
Concomitant disease	
Autoimmune thyroid disease	20/77 (25,9%)
Hypertension	6/77 (7,8%)

glucose >10.0 mmol/L, 2 h glucose >8.6 mmol/L, and 3 h glucose >7.8 mmol/L (15,16).

The OGTT result was considered normal (normal glucose tolerance, NGT) if plasma glucose levels in the fasting state and at 1 h, 2 h and 3 h during the OGTT were less than the thresholds of the ADA criteria. Based on the four blood glucose values obtained during the three-hour 100 g OGTT, participants were classified as having either GDM (defined by two or more values above criterion thresholds), gestational impaired glucose tolerance (GIGT) (defined by only one value above criterion thresholds) or normal glucose tolerance (NGT) (no elevated values).

Statistical analysis

The median value, standard deviation (SD), minimum and maximum value, as well as the normality of distribution of all continuous variables were examined. Continuous variables with normal distribution were presented as the mean ± SD in the text and tables, and those with a distribution that was not normal as the median and range of the minimum-maximum value. Continuous variables were tested for normality of distribution and were compared among four subgroups using the one-way analysis of variance or the Kruskal-Wallis test. Categorical variables are presented as proportions and were assessed with the $\chi 2$ test or Fisher's exact test. A p-value less than 0.05 was considered statistically significant. All statistical calculations were carried out using commercial, standard software package SPSS, version 20.0.

RESULTS

We investigated 92 pregnant women with a diagnosis of vital and desired pregnancy up to 12 weeks of gestation who had signed informed consent forms. Among them, 7 pregnant women had a spontaneous abortion, while 8 pregnant women dropped out, so a total of 77 pregnant women completed the trial.

Demographic characteristics of the subjects are summarized in Table 1.

The largest number of the study population was aged 25-34 years (72.7%), with an average age of 30.8 ± 4.7 (19-41) years, usually with medium (51.9%) and high (40.3%) education levels. Body mass index in the first trimester was most often within the reference range for the general population, while 9% of the women were overweight.

Of the study population, 23.4% of the pregnant women smoked during pregnancy. The most commonly associated disease during pregnancy was an autoimmune thyroid disease (25.9%), followed by antiphospholipid syndrome (9%) and hypertension (7.8%). Of the 34 respondents who were multipara, 23.5% of pregnant women had had previous GDM, as shown in Table 1.

The following risk factors for abnormal glucose regulation (GDM or GIGT) were taken into consideration: obesity, age over 35 years, previous GDM, birth weight in previous pregnancies > 4 *kg*, family history of diabetes, previous use of metformin, previously treated polycystic ovary syndrome, parity ≥3. Most examined women had no risk factors (48%), while 35% had one risk factor (Table 2).

The examination of the existence of abnormal glucose regulation (GDM or GIGT) during pregnancy by trimester demonstrated an increase in disorders of glucose tolerance with advancing pregnancy (in the second and third trimester). After childbirth, all subjects showed normal glucose tolerance (Table 3).

ANOVA-repeated measurements are compared fasting glucose (FBG) at 4 times: OGTT1, OGTT2, OGTT3 and OGTT4. A significant effect of time, Vilks lambda = 0.527, F = 22.175, p < 0.001, multivariate partial eta squared=0.473.

There are intergroup differences in the values of FBG (during the test period), namely: FBG1 compared to FBG2 (p = 0.001), FBG1 compared to FBG3 (p < 0.001), FBG2 compared to FBG 4 (p < 0.001), FBG3 in relation to FBG4 (p < 0.001), but there is no significancet when comparing

Table 2. Frequency of risk factors for pathological glucose tolerance

No. of risk factors	**0**	**1**	**2**	**≥3**	**total**
Normal glucose tolerance, NGT	31/59	19/59	6/59	3/59	59 (100%)
Gestational impaired glucose tolerance, GIGT	2/9	6/9	1/9	0	9 (100%)
Gestational diabetes mellitus, GDM	4/9	2/9	2/9	1/9	9 (100%)

Table 3. Frequency of abnormal glucose regulation (subject-stratified according to glucose tolerance status) during examined period

OGTT	1. trimester	2. trimester	3. trimester	After delivery
Normal glucose tolerance, NGT	71 (92,2%)	67 (87%)	69 (89,6%)	77 (100%)
Gestational impaired glucose tolerance, GIGT	5 (6,5%)	6 (7,8%)	4 (5,2%)	0
Gestational diabetes mellitus, GDM	1 (1,3%)	4 (5,2%)	4 (5,2%)	0

FBG1 in relation to FBG4 (p = 0.209) and FBG2 compared to FBG 3 (p = 0.504) (Table 4).

One-way analysis of variance indicated that there was no statistically significant difference in mean HbA1c during the test period (p> 0.05), as shown in Figure 1.

According to OGTT results, we stratified patients into three groups as follows: normal glucose tolerance (NTG), gestational impaired glucose tolerance (GIGT) and gestational diabetes mellitus (GDM).

It has been shown that the average HbA1c in the first trimester is highest in the GDM group, compared to in the second and third trimester; after birth, it is highest in GIGT group. Combined analysis of variance estimated the impact of time and degree of glucose tolerance on HbA1c. There was a statistically significant effect of time on HbA1c (Vilks lambda=0.849, multivariate partial eta square=0.151, p=0.008). There is no interaction between the factors of time and the factor of glucose tolerance (Vilks lambda=0.879, multivariate partial eta squared=0.062, p=0.152). Separate influence on glucose tolerance was significant (F=4.134, multivariate partial eta squared=0.059, p=0.046). The intergroup differences compared HbA1c between NTG and GIGT (Vilks lambda=0.842, multivariate partial eta squared=0.158, p=0.011), between NTG and GDM (Vilksov lambda= 0.856, multivariate partial eta squared=0.476, p=0.018) and compared GIGT and GDM (Vilks lambda=0.524, multivariate partial eta squared=0,476, p=0.025).

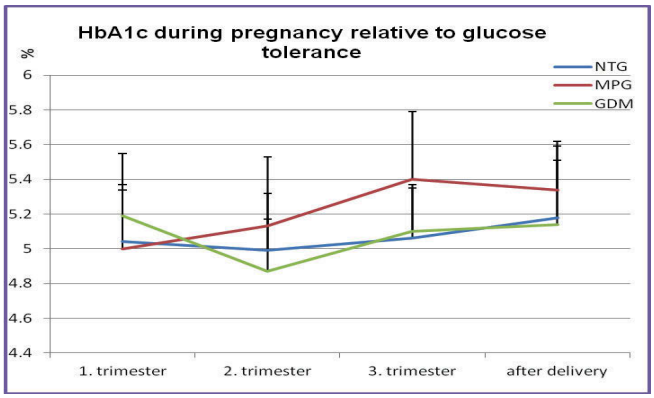

Figure 1. Average values of HbA1c during OGTT (n=77)

Table 4. Average values of glycaemia and insulinaemia during OGTT (n=77)

Glycaemia (*mmol/L*) $X\pm SD$	1st trimester	2nd trimester	3rd trimester	After delivery
0 min	4.42±0.48	4.13±0.49	4.04±0.51	4.55±0.39
60 min	6.34±1.37	6.97±1.83	7.44±1.45	
120 min	5.47±1.37	5.97±1.47	6.71±1.45	5.2±1.09
180 min	4.28±1.32	4.5±1.47	5.18±1.6	

Table 5. Average values of glicemiaglycaemia and HbA1c during OGTT. Normal glucose tolerance, NTG (n=59)

Glycaemia (*mmol/L*) $X\pm SD$	1st trimester	2nd trimester	3rd trimester	After delivery
0 min	4.38±0.38	4.07±0.48	3.92±0.43	4.51±0.39
60 min	5.99±1.45	6.42±1.4	6.96±1.24	
120 min	5.24±1.08	5.47±0.98	6.34±0.99	5.16±0.97
180 min	4.21±1.29	4.3±1.28	4.85±1.37	
***HbA1c* (%)**	5.04±0.3	4.99±0.33	5.06±0.29	5.18±0.41

Table 6. Average values of glicemiaglycaemia and HbA1c during OGTT. Gestational impaired glucose tolerance, GIGT (n=9)

Glycaemia (*mmol/L*) $X\pm SD$	1st trimester	2nd trimester	3rd trimester	After delivery
0 min	4.7±0.47	4.41±0.59	4.49±0.66	4.55±0.43
60 min	7.31±2.73	8.06±1.81	8.91±1.62	
120 min	6.23±1.83	7.01±1.75	7.14±1.4	4.88±1.61
180 min	4.73±1.13	4.97±1.61	5.37±1.52	
***HbA1c* (%)**	5.0±0.37	5.13±0.4	5.4±0.39	5.34±0.28

Table 7. Average values of glicemiaglycaemia and HbA1c during OGTT. Gestational diabetes mellitus, GDM (n=9)

Glycaemia (*mmol/L*) $X\pm SD$	1st trimester	2nd trimester	3rd trimester	After delivery
0 min	4.35±0.32	4.23±0.37	4.39±0.46	4.47±0.25
60 min	7.63±2.2	9.49±1.91	9.1±1.19	
120 min	6.18±2.06	8.19±2.54	8.74±2.18	5.78±1.17
180 min	4.21±1.74	5.34±2.17	7.17±1.82	
***HbA1c* (%)**	5.19±0.36	4.87±0.3	5.1±0.27	5.14±0.37

DISCUSSION

GDM is known to be associated with adverse outcomes (AOs) to the mother and the foetus, and the risk for AOs increases with the degree of hyperglycaemia in women with GDM (17). GDM is increasingly recognized

not only as a serious and frequent complication of pregnancy but also as an opportunity for early prevention of diabetes and other diseases across the lifespan (18). It is expected that the global prevalence of GDM, which is currently from <1 to 28%, will continue to grow. It has become clear that there is a large gap between the enormous global number of pregnant women with GDM and the small proportion of women who were diagnosed and left on their own (19). Although the risk of AO associated with GDM is well known, the impact on the health of mothers and neonates is less well known regarding borderline GDM (GIGT), which is characterized by values between NTG and GDM. In the last ten years, the influence of different degrees of tolerance to glucose complications revealed significantly increased risk of preeclampsia, caesarean section, hypoglycaemia and hyperbilirubinemia in GIGT compared to NTG (20,21). Older age, weight gain and BMI are associated with pregnancy outcome at the GIGT. There are data linking elevated BMI with numerous maternal and foetal complications, such as foetal death, preeclampsia, GDM, macrosomia and complicated labour (22,23). A small weight gain is associated with small size for gestational age, while increased body weight is associated with an increased risk of macrosomia, caesarean section and excessive retention of weight after childbirth (24).

Glucose of the mother crosses the placenta, and maternal hyperglycaemia leads to intrauterine hyperglycaemia, foetal hyperinsulinaemia and possible modifications to the growth and development of the foetus (25). Mild hyperglycaemia in women with GDM is associated with increased foetal growth and perinatal morbidity. Less serious forms of glucose intolerance are also associated with increased foeto-maternal morbidity (26).

Women with GDM have a 17–63% risk of developing type 2 diabetes within 5-16 years. There is also some evidence that further pregnancies accelerate the rate of decline of β cell function in women with GDM (3). The severity of glucose intolerance has been reported to influence the rate of preeclampsia. In some studies, less severe forms of glucose intolerance, even in women who were not diagnosed with GDM, have been associated with preeclampsia. Hyperinsulinaemia, which is often associated with glucose intolerance, may also contribute to the pathogenesis of essential hypertension or development of preeclampsia. Other investigators have found that demographic variables that include race, age, and weight also confer an increased risk of preeclampsia (25,26).

The new proposed diagnostic criteria will result in a prevalence of 17.8% for GDM, doubling the number of pregnant women diagnosed with GDM. These criteria are based on the correlation of glucose and 1.75-fold increased risk of large size for gestational age in the HAPO study. Supporting the Pendersen hypothesis, the results showed a clearly increased risk of these outcomes, and the risk gradually increases with the level of glucose (17,27). While the concentration of FBG shows a trend towards lower as pregnancy progresses, the rise in blood glucose after eating is greater and longer lasting as a result of impaired, insulin-mediated glucose usability, suppression of endogenous glucose production and inadequate insulin increase in the first phase of insulin secretion (28).

In our study, we included 92 pregnant women who met the inclusion criteria, and none of the participants had disturbance of glycaemic control at baseline. The following risk factors for glucose intolerance (GIGT or GDM) were taken into consideration: obesity, older age (over 35 years), previous GDM, birthweight neonates in previous pregnancies> 4 kg, positive family history of DM, previous use of metformin (previously treated polycystic ovary syndrome), and parity ≥3. Most respondents had no risk factors (48%), while 35% had one risk factor. The current study demonstrates that normal glucose tolerance was shown in 59 (76.6%) participants, while some form of glucose intolerance (GIGT or GDM) was shown in 18 (23.4%) patients. are nNumerous risk factors thathave been shown to influence the degree of disorder of glucose tolerance (r = 0.034).

There are no clear guidelines for the determination of HbA1c during pregnancy, but it is not routinely recommended for women with GDM. The lifetime of erythrocytes during pregnancy is shortened to 90 days, so its determination is only possible within shorter intervals than in the state outside of pregnancy. The degree of change in HbA1c in pregnancy reflects glycaemic control for the past few weeks. Therefore, the determination of HbA1c during pregnancy is more likely to be used for therapeutic decisions in pregnancy complicated with diabetes, including GDM. Strict glycaemic control is essential to minimize the morbidity and mortality of the mother and foetus (21, 25). Women with GDM have several basic defects in glucose metabolism that are unmasked physiologically by IR late in pregnancy, including the production of increased basal insulin and β cell dysfunction. In these women, FBG increases, indicating increased basal and the production of β-cell dysfunction (29).

The treatment of GDM is intended to decrease adverse pregnancy outcomes (28). The HAPO Study (27) reported associations of maternal 'moderate' hyperglycaemia with risks of adverse pregnancy outcome in a very heterogeneous cohort of 25,505 pregnant women from 15 centres in nine countries. This helped to publish the most recent diagnostic criteria by the IADPSG (8), where the same test is used for both screening and diagnosis.

Testing HbA1c in NTG patients has shown a mild insignificant increase as the pregnancy progresses. The HbA1c in patients with GDM at the beginning of pregnancy is elevatedis but is corrected in more heavily pregnant women. This is likely due to therapeutic intervention for the GDM. Pregnant women with GIGT have a good initial state, but as the pregnancy advances, HbA1c increases significantly. There were statistically significant differences in HbA1c between NTG and GIGT, as well as between GIGT and GDM. Pregnant women with GIGT

remain unrecognized but are at higher risk of adverse outcomes than those with GDM, so it is necessary to detect GIGT to establish good glycaemic control. There is therapeutic intervention only in patients with GDM. An increase of insulin levels during OGTT is expected as pregnancy progresses. There is no one respondent with adverse pregnancy outcomes who had at least one risk factor. Pregnant women with adverse outcomes have significantly higher frequency of glucose intolerance, with adverse outcomes equal to that of GIGT and GDM, so GIGT is not harmless GDM.

Our findings revealed an increase in glucose intolerance with advancing pregnancy (in the second and third trimester). After delivery, all participants exhibited normal glucose tolerance. The weakness of our study is the small cluster/cohort of women. In conclusion, we demonstrate that the difference in the quality of glycaemic control during pregnancy is manifested in the second and third trimester, until it manifests in the first trimester. These findings underpin the clinical significance of discovering GDM.

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Conflicts of interest

The authors declare no financial or commercial conflicts of interest.

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EVALUATION OF SAFETY AND SUCCESSFULNESS OF THE COIL EMBOLIZATION OF INTRACRANIAL ANEURYSMS

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EVALUACIJA BEZBEDNOSTI I USPEŠNOSTI KOIL EMBOLIZACIJE INTRAKRANIJALNIH ANEURIZMI

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ABSTRACT

Intracranial aneurisms are ongoing problem for neurosurgeons and especially for interventional neuroradiologists due to its morbidity and mortality. The method of choice for treatment of the unruptured and ruptured intracranial aneurysms is endovascular coiling on account of its minimal invasiveness and high effectiveness. The aim of our study was to evaluate the safety and successfulness of endovascular coiling procedure in unruptured and ruptured intracranial aneurysms.

Our study was designed as case series and consisted of patients older than 18 years, who underwent endovascular coiling of unruptured and ruptured intracranial aneurysms and follow-up examination 6 months after the interventional procedure. The procedures were performed from December 2010 to December 2016, by experienced interventional neuroradilogists (more than 400 performed embolizations each) at the Department for Interventional Neuroradiology, Clinical Center Kragujevac, Serbia.

There were 681 patients (average age 47.5 ± 11.2 years) treated with endovascular coiling, out of them 324 (234 females, 90 males) had unruptured intracranial aneurysm and 357 (138 females, 219 males) had ruptured intracranial aneurysm. In our series, total complication rate was 11.71 %. Analysis of the results after first endovascular procedure has shown that complete aneurysm occlusion was accomplished in 546 patients (80.3%), near-complete in 81 patients (11.8%), and incomplete in 54 patients (7.9 %).

Our results were satisfying regarding the procedure's success, safety, outcomes and study material. However, further technical development of the materials and constant training of the interventional radiologists, are a necessity in order to improve treatment outcomes and patients' benefit.

Key words: *endovascular coil, embolization, intracranial aneurysm, safety, complication, evaluation*

SAŽETAK

Intrakranijalne aneurizme su problem kako za neurohirurge, tako i za interventne neuroradiologe zbog svog morbiditeta i mortaliteta. Metoda izbora za lečenje nerupturiranih i rupturiranih intrakranijalnih aneurizmi je endovaskularni koiling zbog minimalne invazivnosti i visoke efikasnosti. Cilj naše studije je bio da se proceni bezbednost i uspešnost procedure endovaskularnog koilovanja kod nerupturiranih i ruptiranih intrakranijalnih aneurizmi.

Naša studija je dizajnirana kao serija slučajeva i sastojala se od pacijenata starijih od 18 godina, koji su podvrgnuti endovaskularnom koilovanju nerupturiranih i rupturiranih intrakranijalnih aneurizmi i naknadnom pregledu 6 meseci nakon interventne procedure. Procedure su sprovedene od decembra 2010. do decembra 2016. godine od strane iskusnih interventnih neuroradiologa (više od 400 izvedenih embolizacija) na Odeljenju za interventnu neuroradiologiju Kliničkog centra Kragujevac, Srbija.

Bilo je 681 pacijenta lečenih endovaskularnim koilovanjem (prosečna starost 47.5 ± 11.2 godina), od kojih je 324 (234 žena, 90 muškaraca) imalo nerupturiranu intrakranijalnu aneurizmu, a 357 (138 žena, 219 muškaraca) je imalo rupturiranu intrakranijalnu aneurizmu. U našoj seriji, ukupna stopa komplikacija bila je 11.71%. Analiza rezultata nakon prve endovaskularne procedure pokazala je da je kompletna okluzija aneurizme izvršena kod 546 pacijenata (80.3%), skoro-kompletna okluzija kod 81 pacijenta (11.8%) i nepotpuna okluzija kod 54 pacijenta (7.9%).

Naši rezultati su zadovoljavajući u pogledu uspeha, bezbednosti, ishoda i studijskog materijala. Međutim, dodatni tehnički razvoj materijala i stalna obuka interventnih radiologa su neophodni u cilju poboljšanja ishoda lečenja i koristi pacijenata.

Ključne reči: *endovaskularni koil, embolizacija, intrakranijalna aneurizma, bezbednost, komplikacija, evaluacija*

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ABBREVIATIONS

IA- intracranial aneurysm,
SAH - subarachnoid hemorrhaghe,
RIA – ruptured intracranial aneurysm,
MR – magnetic resonance,
UIA – unruptured intracranial aneurysm,
PAE-perianeurysmal edema

INTRODUCTION

Intracranial aneurisms (IA) are ongoing problem for neurosurgeons and especially for interventional neuroradiologists due to its morbidity and mortality. Its prevalence was estimated to be between 0.5% and 6 %, in adults (1). They are multiple in 10-30% of cases (2). In majority of patients, aneurysm ruptures which results in subarachnoid hemorrhage (SAH) (3). In developed countries, such as United States, around 27000 new patients with SAH after ruptured intracranial aneurysm (RIA) occur, while the incidence of SAH in the western countries is around 6-10 per 100 000 inhabitants per year (4,5). In Serbia the incidence of rupture and SAH goes between 8 to 10 per 100 000 inhabitants per year (5). These patients ussually come with „sudden severe headache of atypical quality", coma or severe neurologic disfunction, but they can also have mild clinical presentation (6,7). Cerebrospinal fluid examination and imaging with magnetic resonance (MR) imaging and MR angiography are used as diagnostic modalities (5). Still, mortality rates after subarachnoid hemorrhage are around 40% and due to this fact, diagnosis and treatment of intracranial aneurysm before the rupture are cruical (8).

Diagnose of the unruptured intracranial aneurisms (UIA) is growing due to the increasing use of modern imaging modalities (9,10). The prevalence of UIA is around 1-7%, and some authors suggest that 30% of general population has an UIA (8,11). These patients present with palsies of the cranial nerves or brain stem compression (2). Most of the UIA are small (65-85%), with diameter less than 5mm or up to 7 mm in diameter and they have the risk of rupture less or qual than 1% per year (12). The rupture of the UIA is rare, but high in mortality (13). It was noticed that there is a raise of cost associated with the diagnosis and treatment of the UIA in the United States (14). This was due to the expanded use of MR angiography and computed tomography angiography (12,14).

The method of choice for treatment of the unruptured and ruptured intracranial aneurysms is endovascular coiling on account of its minimal invasiveness and high effectiveness (3,4,14,15). This technique is presented by Gugliemi's discovery of detachable coils and the essence of the procedure is to exclude the aneurysm from the circulation by use of detachable coils after suitable placement of a microcatheter in the aneurysm (11,16). It is proven that endovascular embolization shortens hospitalization length and improves recuperation on account of its ability to prevent aneurismal rupture and intracranial hemorrhage (17). Because of that more advanced way of treatment was designed in the form of bioactive coils (18). However, despite its effectiveness, the treatment is occasionally accompanied with perianeurysmal edema (PAE) (15). The relationship between the occurrence of the perianeurysmal edema and use of bioactive coils in the treatment is still under research (15,17). Other complications of the coil embolization are: cerebral vasospasm, artery occlusion, aneurysm perforation, coil migration, thromboembolism etc. (2).

The aim of our study was to evaluate the safety and successfulness of endovascular coiling procedure in unruptured and ruptured intracranial aneurysms.

MATERIALS AND METHODS

Our study was designed as case series and consisted of patients older than 18 years, who underwent endovascular coiling of unruptured and ruptured intracranial aneurysms and follow-up examination 6 months after the interventional procedure. The procedures were performed by experienced interventional neuroradilogists (more than 400 performed embolizations each) at the Department for Interventional Neuroradiology, Clinical Center Kragujevac, Serbia from December 2010 to December 2016.

The study participants had given written consent before the study onset, and the Ethics Committee of the Clinical Center had approved the study. The Helsinki Declaration as well as applicable guidelines were followed.

Every patient was subjected to digital subtraction angiography of brain, MR imaging of brain and multidetector computed tomography of brain, prior to the interventional endovascular procedure. Treatment strategy was chosen based on the diagnostic images and clinical manifestation. The embolizations were made by „etachable platinum coils: pure platinum, hydrophilic and combination of platinum and hydrophilic coils". Self-expanding stents were used for prevention of the coil extrusion in aneurysms with broad neck.

The aneurysms were categorized according to their size and location. Technical failure was defined as „an attempted embolization procedure during which coils could not be successfully deployed". Any procedural or other subsequent complication was recorded and analyzed in order to collect information about procedure's safety. The successfulness of the endovascular coils was estimated by initial post-embolization angiography and then by digital subtraction angiography after 6 months, using the following categories: complete occlusion of an

Figure 1. Ruptured aneurysm on anterior communicating artery, 10 x 8 mm

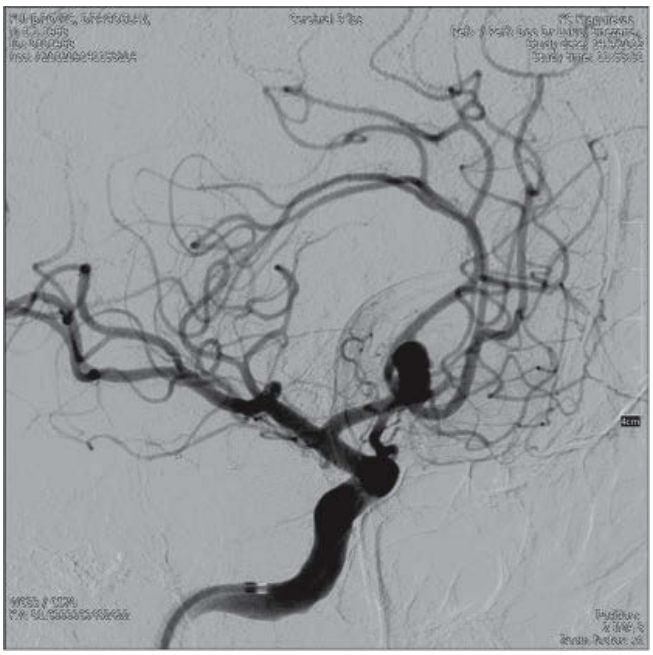


Figure 2. Postembolization angiography on same patient as Fig.1

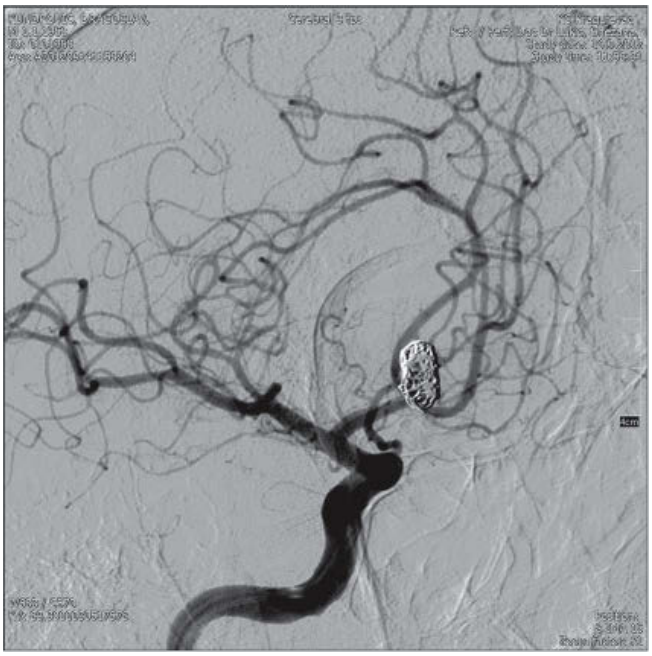


Figure 3. Unrptured aneurysm on left internal carotid artery, 12 x 10 mm

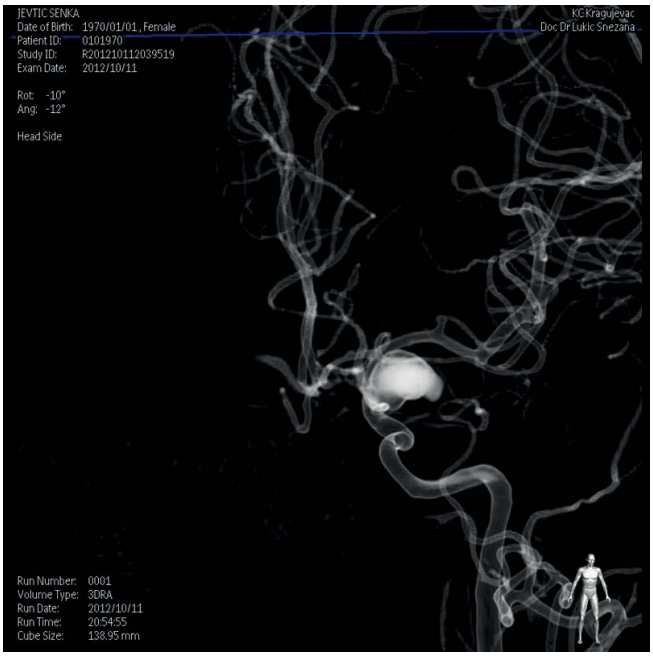
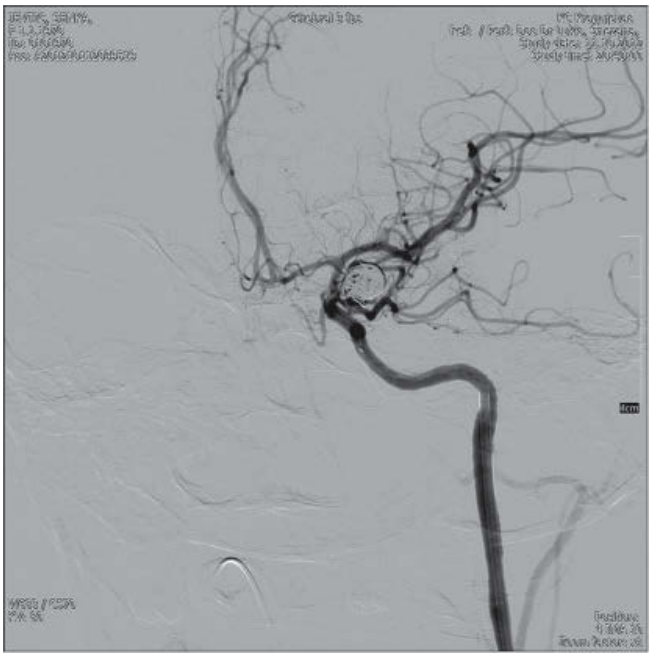


Figure 4. Postembolization angiography on same patient as Fig.3



aneurysm (98%–100%), near-complete occlusion (90%–98%), and incomplete occlusion (<90%). Patients were followed after the procedure and Modified Rankin Score was used to determine clinical outcome (scores 0-2: good outcome; scores 3-5: dependency, cannot attend own bodily needs and carry out daily activities without assistance; score 6: death).

Statistical analysis of the data was accomplished with the descriptive statistics, primarily using medians, means and standard deviations for continuous variables, and percentages and odds for categorical variables. The differences among study groups were considered significant if the probability of null hypothesis was <0.05.

RESULTS

There were 681 patients (average age 47.5 ± 11.2 years) treated with endovascular coiling, out of them 324 (234 females, 90 males) had unruptured intracranial aneurysm and 357 (138 females, 219 males) had ruptured intracranial aneurysm. Figures 1-4 present patients with ruptured and

Table 1. Aneurysm characteristics and patients' Modified Rankin Score results

	Number of patients	Percentage, %
Location of ruptured aneurysm		
Carotid artery	242	35.5
Middle cerebral artery	93	13.6
Anterior communicating or cerebral artery	159	23.4
Posterior circulation	187	27.5
Size of aneurysm		
≤10 mm	306	45
≥25 mm	48	7
<15 mm	231	34
>15 mm	177	26
Modified Rankin Score		
Good (score:1-2)	531	78
Dependency (score: 3-5)	112	16.5
Death (score 6)	38	5.5

Table 2. Occurrence of the endovascular coiling procedure complications

	Number of patients	Percentage, %
Perianeurysmal edema	248	36.4
Symptomatic perianeurysmal edema	44	6.5
Cerebral vasospasm	195	28.6
Thromboembolism	20	3
Artery occlusion	3	0.4
Aneurysm perforation	2	0.3
Coil migration	0	0

unruptured aneurysms before and after endovascular coiling procedure. Also, mean aneurysm size was calculated to be 18.85 mm in diameter. Location of the aneurysm, its size and Modified Rankin Sore are presented in Table 1.

Technical success of the procedure was assessed by the evaluation of the procedure and after 6 months examination. Analysis of the results after first endovascular procedure has shown that complete IA occlusion was accomplished in 546 patients (80.3%), near-complete in 81 patients (11.8%), and incomplete in 54 patients (7.9 %). These rates were also investigated after six months and it was determined that complete occlusion persisted in 528 patients, near-complete in 75 patients, and incomplete in 38 patients. In thirty eight patients, outcome was death. Occlusion rate was investigated depending on the aneurysm size. Our results revealed that the complete occlusion was successful in all patients with aneurysms <10 mm in diameter, after first endovascular treatment and after 6 months of follow-up. Nevertheless, angiograms of aneurysms >10 mm in diameter have shown that after 6 months, 35 patients had recanalization of the aneurysm, even though their aneurysms were completely occluded after the first procedure. The endovascular coiling procedure in this study led to a significant decrease in aphasia, confusion and frequency of motor deficit in the upper and lower extremities.

Procedure safety was estimated based on the occurrence of the coiling complications. In our series, total complication rate was 11.71 %. Death rate was 5.5%. The occurrence of individual complications, due to the endovascular coiling procedure are presented in Table 2.

DISCUSSION

It has been published by several authors that endovascular embolization of intracranial aneurysms with detachable coils is an effective method for patients' treatment (19,20). Previously, the golden standard for the treatment of IA was surgical clipping (21). Thanks to the technical advancement, this conception has changed and interventional procedures have found their way in this field (20,22). Gugliemi's discovery of detachable coils brought the opportunity to treat the patients with IA more efficiently and more effective (16). This technique as previously described has its complications.

Different studies have shown that their mortality rates varied from 0.6% to 29% (4,23,24). One of the published studies had presented that death rate of patients with IA who underwent coil embolization was 6.21 % (25). This study was performed on both ruptured and unruptered intracranial aneyrisms and it stated that 1.74% of deaths was due to the technical complications and in 4.47% of patients the main cause of death was severity of the hemorrhage (25). In our series, mortality rate was 5.5% which is a good result compared to the published literature and is in good accordance comparing to the other studies (23-25).

The coils embolization outcomes were compared with the surgical treatment and it was found, by the other authors, that the risk of significant disability was 22.6% lower, as well as risk of death in favour of coiling procedure (2,13). Our case series showed good clinical outcome, as 78% of patients had grade 1 or 2 on Modified Rankin Score, which is in accordance with previous studies (Jahromi: 63% and Sluzewski: 84%) (19,26). Also, all of our patients with small aneurysms (less than 10 mm) had successful treatment, while larger aneurysms demonstrated predisposition for new coiling. This was justified with the aneurysm size and their closeness to the large blood vessels which is delicate factor that may lead to extensive ischemia after potential compression.

Our study has also evaluated the complications of the procedure. In our series complication rate was 11.71 %, which is similar with previous studies (12.4%) (27). We have shown that none of the patients with incomplete occlusion had repeated rupture after six month follow-up examination, which may be due to the smaller sample size than previous study which reported that 1% out of 1005 patients had rebleeding (28). It was also shown that the risk of rebleeding is around 1.4% by another study (11). Our rate of the perianeurysmal edema is in agreement with previously published papers in which PAE rate was 33.3% of all cases (29). Also, our symptomatic PAE rate was

6.5%, and other studies have shown that this rate can be very small or it goes up to 14.3% (15,30).

In conclusion, our results were satisfying regarding the procedure's success, safety, outcomes and study material. The material that is used for the endovascular procedures has relevant role in the treatment of patients with intracranial aneurysms. However, further technical development of the materials and constant training of the interventional radiologists, are a necessity in order to improve treatment outcomes and patients' benefit.

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THE FACTORS THAT DETERMINE REPRODUCTIVE HEALTH CHARACTERISTICS OF ADOLESCENTS IN SERBIA

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ČINIOCI KOJI ODREĐUJU ODLIKE REPRODUKTIVNOG ZDRAVLJA ADOLESCENATA U SRBIJI

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ABSTRACT

The aim of the work is to assess vulnerability degree of reproductive health in adolescent population in Serbia by analyzing their sexual behavior. The paper is based on data from a National health survey of the population in Serbia in 2013 (without Kosovo and Metohija region), conducted by the Ministry of Health of the Republic of Serbia. For the purposes of this study, the data used are referred to households and population of age 15 and over, so that the final number for analysis is a sample of 1722 respondents aged 15 -24. The study included demographic characteristics of respondents (age, gender, settlement type, region) and the characteristics of reproductive health: sexual behavior, use of contraceptive protection, knowledge and attitudes towards HIV, protection of reproductive health. χ^2 test was applied for testing differences in frequency of categorical variables. All results with a probability that equals to or is less than 5% ($p \leq 0.05$) are considered statistically significant. Among the respondents, there were more than half (53.5%) of sexually active adolescents. The highest percentage of them- 16.6% responded that they had first sexual intercourse at the age of 17. 53.4% of adolescents had sexual intercourse with one partner, 26.4% of respondents had sexual intercourse with two or more partners. The most commonly used contraceptive method was interrupted intercourse (coitus interruptus) with 34.7% of respondents.

Key words: *reproductive health, adolescents, Serbia, National Health Research*

SAŽETAK

Cilj rada je da se proceni stepen ugroženosti reproduktivnog zdravlja adolescentske populacije u Srbiji analizom njihovog seksualnog ponašanja. Rad se zasniva na podacima iz nacionalnog istraživanja zdravlja stanovništva Srbije u 2013. (bez podataka za Kosovo i Metohiju), koje je sprovelo Ministarstvo zdravlja Republike Srbije. Za potrebe ovog istraživanja, korišćeni su podaci o domaćinstvima i stanovništvu starosti 15 i više godina, tako da je u konačni uzorak za analizu ušlo 1722 ispitanika starosti 15 do 24 godine. U istraživanju su korišćene demografske karakteristike ispitanika (starost, pol, tip naselja, region) i obeležja reproduktivnog zdravlja: seksualno ponašanje, korišćenje kontraceptivne zaštite, znanje i stavovi o HIV-u, zaštita reproduktivnog zdravlja. χ2 test je primenjen za testiranje razlika u učestalosti kategorijskih varijabli. Svi rezultati sa verovatnoćom koja je jednaka ili manja od 5% ($p \leq 0,05$) su smatrane statistički značajnim. Među ispitanicama je bilo više od polovine (53,5%) seksualno aktivnih adolescenata. Najveći procenat njih 16,6% se izjasnio da je prvi put stupio u seksualne odnose sa 17 godina. Seksualni odnos sa jednim partnerom upražnjavalo je 53,4% adolescenata, a sa dva ili više partnera 26,4% ispitanika našeg istraživanja. Najčešće korišćeno kontraceptivno sredstvo je bio prekinut snošaj 34,7%.

Ključne reči: *reproduktivno zdravlje, adolescenti, Srbija, Nacionalno istraživanje zdravlja*

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INTRODUCTION

One of the modern societies features is an increase in sexual activity among young people. This is manifested by the growing proportion of adolescents sexually active, by a growing number of those who had the first sexual experience in the period of early adolescence and lowering the average age when the first sexual intercourse is realized. Sexual activity is followed by new health problems. This can result in unwanted pregnancy and the occurrence of diseases that are transmitted through sexual contact. Risk for the damage of reproductive health depends on the adopted model of sexual behavior, as well as on many other determinants of physiological characteristics and health habits of an individual, and it also depends on number of factors related to socio-cultural environment. Reproductive health of young people in the age of adolescence is particularly affected which is explained by the unfinished processes of their physical and psychosocial maturation. Young people are susceptible to various influences of immediate and wider social environment and the environment affects -to a greater or lesser extent their knowledge, attitudes and behavior in the areas of sexuality and reproduction (1).

THE AIM OF STUDY

The aim of the paper is to assess the vulnerability degree of adolescent reproductive health in Serbia by analyzing their sexual behavior.

METHOD

Data source and type of study

The paper is based on data from a national health survey of the population of Serbia in 2013 (no data for Kosovo and Metohia region). This is the third national survey of health of the population, conducted by the Ministry of Health of the Republic of Serbia. The survey was conducted in accordance with the methodology and instruments of the European Health Survey - Second Wave (EHIS-wave 2). We used a nationally representative random sample, stratified two-stage sample with a known probability of selection of sample units at every stage of sampling. The sample was selected so, as to provide a statistically reliable estimate of the large number of indicators that indicate the health of the population at the national level. The mechanisms that have been used to obtain a random sample of households and respondents, represent a combination of the two sampling techniques: stratification and multi-stage sampling. For the purposes of this study, the data on households and population age 15 and over were used, so that the final sample for analysis entered 1722 respondents aged 15 to 24.

Variables

Demographic characteristics (age, gender, type of settlement, region) were used as independent variables. Reproductive health is described and analyzed through the following characteristics: sexual behavior (sexual relations with a steady partner and someone who is not, the use of contraceptive protection, (opinion on condom use, used contraceptive type), reproductive health protection (antenatal age at the first visit to a gynecologist, gynecologist visits in the last 12 months, the reason for the visit, abortions).

Statistical data processing

All data of interest are presented and analyzed by adequate mathematical-statistical methods appropriate for the data type. χ^2 test was applied to test the difference in the frequency of categorical variables. All results with the

Table 1. Sexual behavior adolescents in Serbia in relation to sociodemographic characteristics

Variables	Sexual intercourse with one partner (%)	p	Sexual contact with two or more partners (%)	p	Condom used during the last sexual contact (%)	p	Used condom with occasional partners (%)	p
Sex								
Male	52.9	p<0.001	77.2	p<0.001	57.1	p<0.05	69.9	p<0.001
Female	47.1		22.8		42.9		30.1	
Settlement type								
Urban setting	58.1	p<0.05	57	p<0.05	63	p<0.05	58.6	p>0.05
Ruralna setting	41.9		43		37		41.4	
Region								
Belgrade	25.4	p<0.001	21.5	p<0.001	25.8	p<0.001	22.6	p<0.001
Vojvodina	22.9		31.1		26.4		26.3	
Shumadia and Western Serbia	25.9		24.4		21.5		22.9	
South and Eastern Serbia	25.8		23		26.4		28.2	

probability that is equal to, or less than 5% ($p \leq 0.05$) were considered statistically significant. Statistical analysis was performed using a commercial, standard software package SPSS, version 19.0. (The Statistical Package for Social Sciences software (SPSS Inc., version 19.0, Chicago, IL).

RESULTS

From a total of 1722 respondents aged 15-24 (average age 19 ± 2.1 years) 49% of boys and 51% girls were interviewed. 55.5% of the surveyed adolescents were from urban areas. 30% of adolescent respondents were from Sumadia and West Serbia regions.

The survey results show that there are more than half (53.5%) sexually active adolescents, boys (52.9%) more often than girls (47.1%), ($p < 0.001$). The highest percentage 16.6% of them confirmed that they had their first sexual experience at the age of 17 years old , girls (53.4%) more often than boys (46.6%), the difference was statistically significant ($p<0.05$) . In the last 12 months in relation to the survey time 73.2% of adolescents had sexual intercourse. Sexual intercourse with one partner was with 53.4% of adolescents, girls more often ($p<0.05$) and adolescents from the South and East Serbia (27.8%) ($p<0.05$). 26.4% respondents from our survey had sexual contact with two or more partners, slightly more boys (67,4%) ($p<0.05$) and adolescents from Sumadia and West Serbia (35.4%) ($p< 0.05$). 2.9% of respondents (male and female alike) declared sexual relations with a person of the same sex. Young men more often than girls (77.2% vs. 22.8%) had sexual intercourses with a periodic partner in the last 12 months in in relation to the survey time ($p<0.05$). During the last sexual contact, a condom was used by 48.5% of adolescents, more frequently young men 57.1% compared to 42.9% of women ($p<0.05$). Also, the practice was more often with adolescents in urban areas 63% and those originated from the South and East Serbia regions (26.4%) and Belgrade (26.4%) ($p<0.05$). Only 26.4% of respondents used condom with occasional partners- more frequently boys 77.2% than girls 22.8% ($p<0.05$). The most commonly used contraceptive was interrupted intercourse (coitus interruptus) 34.7%. Next contraceptive according to frequency was male condom in 31.9%, infertile days 16.7% and a day after pill 13.9%. There is a statistically significant difference in contraceptive types used among adolescents from different regions of Serbia, as well as in respect of whether the respondents originate from an urban or rural environment. Interrupted intercourse (coitus interruptus) was the most common form of contraception among adolescents from Sumadia and West Serbia 48.3%. Male condom as the most common form of contraception was used by adolescents from the South and East Serbia 28.4%, while a contraceptive pill was used by adolescents from Vojvodina Region 31.3% and 31.3% of Belgrade. Among adolescents from rural areas the most frequently used contraceptive was infertile days method of 55.6% respondents, while the other methods of birth control were more common among adolescents originating from urban areas ($p<0.05$).

DISCUSSION

In adolescence period young people assume new roles and responsibilities and proper sexual behavior is of vital importance. Risky sexual behavior in adolescence could have serious consequences for health in later life. Risk sexual behavior practice adopted during adolescence can often continue throughout adulthood (2). The national research examining sexual behavior among students showed that more than a quarter of adolescents had sexual intercourse, more often young men. About 25% of them had not used a condom during their last sexual intercourse. Unprotected sexual contacts were more common in adolescents who used psychoactive substances. The adolescents given guidelines on pregnancy prevention and sexually transmitted infections rarely manifested risky sexual behavior (3). In our study, more than half of the adolescents were sexually active, during which they did not use a condom, while 26.4% of adolescents had sexual contact with two or more partners. Other studies also noted risky sexual behavior of young people, such as a rare use of condoms and multiple partners (2). Also, many studies have shown that the level of risky sexual behavior among young people is different in comparison to the sex, race and ethnicity. A survey of African American students showed that 46.7% of them ever had sexual intercourse, that 37% of sexually active students did not use a condom during last sexual intercourse. It was found that the black race students tend to have sexual intercourse more often, had greater number of sexual partners, as well as they more often used condoms during last sexual contact, as compared to the white students. It was also found that there was no difference related to gender (4). Many teens believe that risky sexual behavior increases their chances of getting infected by HIV and sexually transmitted diseases, but nevertheless the results show that even 39% of sexually active adolescents do not use a condom during the last sexual intercourse, that more than 10% of girls aged 15-19 replied that they had multiple partners and the short time intervals between having a sexual contact with two different partners (5). Such risky sexual behavior increases the risk of HIV, sexually transmitted diseases and unwanted pregnancies (6,7). Among teens, nearly 90% of heterosexually acquired HIV cases are in girls (8), and among the estimated 19 million cases of sexually transmitted infections that occur each year in the United States, girls aged 15-19 have the highest rates of gonorrhea and chlamydia compared to young men of the same age (9). It was also observed that adolescents originating from poorer urban areas start sexual activity at an earlier age (10). Sexually transmitted infections are serious public health problem worldwide, particularly af-

fecting young people, this is confirmed by the data - 70% of patients with sexually transmitted infections are aged between 15 and 24 years (3). Reducing risky sexual behavior and sexual education that provides correct information about reproductive health protection can reduce the incidence of sexually transmitted infections as well as to reduce the number of unplanned pregnancies among adolescents (12).

CONCLUSION

It can be concluded, based on the survey results, that the reproductive health of adolescents in Serbia is threatened to a significant degree. The surveyed respondents are observed with high degree of irresponsibility in sexual behavior. It is essential to inform and educate young people about reproductive health in order to prevent numerous unwanted consequences of ignorance and risk behavior. It is also important to choose the right method to pass important information to young people, in particular to inform the marginalized and socially disadvantaged young people. Cooperation between governmental and non-governmental organizations is necessary in order to improve reproductive health as well as multiple efforts of the community and society in whole.

Conflict of Interest

Authors declare no conflict of interest.

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OXIDATIVE STRESS PARAMETERS AFTER ABDOMINAL HYSTERECTOMY AND THEIR RELATIONSHIPS WITH QUALITY OF RECOVERY

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PARAMETRI OKSIDATIVNOG STRESA POSLE ABDOMINALNE HISTEREKTOMIJE I NJIHOVA POVEZANOST SA KVALITETOM OPORAVKA

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ABSTRACT

Study aimed to investigate relationship between oxidative stress markers and postoperative recovery in woman after abdominal hysterectomy, as well as to test the hypothesis that different analgesics differently influence redox status.

The quality of recovery was evaluated with a QoR-40 questionnaire in fifty-one patients who underwent abdominal hysterectomy, preoperatively and on the 1st, 2nd, 3rd postoperative days (POD1,2,3). Blood samples were collected at baseline (T0), 3 (T1), 24 (T2), 48 (T3) and 72 (T4) hours after surgery. Oxidative stress markers concentrations (TBARS, NO_2^-, H_2O_2, O_2^-) as well as antioxidative enzymes (SOD, CAT, and GSH) were analyzed.

QoR-40 total score significantly declined on POD1 and POD2 and returned to baseline levels on POD3 ($p<0.001$). H_2O_2 levels significantly decreased from T0 to T3 and then, increased at T4 ($p=0,011$). Changes of TBARS and H_2O_2 from T0 to T3 showed significant and negative correlation ($r=-0.303$, $p=0.046$). There was no significant correlation between QoR-40 total score and any parameter of oxidative stress response ($p>0.05$). Changes in TBARS levels from T0 to T3 were statistically significant between the study subgroups primarily due to increase of the concentrations in patients receiving paracetamol ($p=0.031$). Patients age, duration of surgery and cigarette smoking status showed significant influcences on and association with some oxidative stress response markers (TBARS, O_2^-, CAT) ($p<0.05$).

Women who underwent hysterectomy had significant changes of H_2O_2 and TBARS activity however, those changes were not associated with changes of QoR-40 total scores during recovery.

Keywords: *hysterectomy; postoperative period; oxidative stress.*

SAŽETAK

Studija je imala za cilj da ispita povezanost između markera oksidativnog stresa i postoperativnog oporavka kod pacijentkinja podvrgnutih abdominalnoj histrektomiji kao i da testira hipotezu da različiti analgetici različito utiču na redoks status.

Kod 51 pacijentkinje podvrgnute abdominalnoj histrektomiji zbog benigne bolesti uterusa kvalitet postoperativnog oporavka je evaluiran sa upitnikom QoR-40 , preoperativno, kao i prvog, drugog i trećeg postoperativnog dana (POD 1, 2 i 3). Uzorci krvi su uzeti preoperativno (T0), 3 (T1), 24 (T2), 48 (T3) i 72 (T4) sata posle operacije. Analizirane su koncentracije markera oksidativnog stresa (TBARS, NO_2^-, H_2O_2, O_2^-) kao i enzima antioksidacione odbrane (SOD, CAT i GSH).

Vrednosti ukupnog QoR-40 skora su značajno opale u POD1 i POD2 i vratile se na preoperativne vrednosti u POD3 ($p<0.001$). Koncentracije H_2O_2 su značajno opale od T0 do T3 i porasle u T4 ($p=0,011$). Promene od T0 do T3 u vrednostima TBARS-a i H_2O_2 su u značajnoj međusobnoj negativnoj korelaciji ($r=-0.303$, $p=0.046$). Nije bilo značajne korelacije između ukupnog QoR-40 skora i bilo kog parametra oksidativnog stresa ($p>0.05$). Promene vrednosti TBARS-a od T0 do T3 su bile značajno različite između studijskih podgrupa pre svega zbog porasta koncentracija kod pacijentkinja koji su primale paracetamol ($p=0.031$). Starost pacijenata, trajanje operacije kao i pušenje pokazali su povezanost sa pojedinim markerima stres odgovora (TBARS, O_2^-, CAT) ($p<0.05$).

Kod pacijentkinja podvrgnutih histerektomiji dolazi do značajnih promena u H_2O_2 i TBARS-u, ali one nisu značajno udružene sa kvalitetom oporavka.

Ključne reči: *histrektomija; postoperativni oporavak; oksidacioni stres.*

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ABBREVIATIONS

ASA –American Society of Anesthesiologist physical status classification
CAT- catalase
ECG - electrocardiogram
$EtCO_2$ – end tidal carbon dioxide
GSH - reduced glutathione
H_2O_2 - hydrogen peroxide
MAC - minimum alveolar concentration
MDA - malonyldialdehyde
NIBP – non-invasive blood pressure
NO - nitric oxide
NO_2^- - nitrite
NPRS - numeric pain rating scale
NSAID – non-steroidal anti-inflammatory drugs
O_2^- - superoxide anion radical
PACU - post-anesthesia care unit
POD- postoperative day
QoR-40 - quality of recovery 40-item questionnaire
SOD - superoxide dismutase
SpO_2 – peripheral capillary oxygen saturation
TBARS - thiobarbituric acid reactive substances
GPx - glutathione peroxidase

INTRODUCTION

The abdominal hysterectomy, the most common major gynecological procedure, disturb patients quality of life during postoperative period despite of modern advances in both surgery and anesthesia (1, 2). Re-establishing physical and psychological balance as well as disappearing of unpleasant symptoms, take certain amount of time during period known as postoperative recovery (3). Since postoperative recovery is nowadays recognized as a valid clinical outcome, many efforts have been done in developing instruments for its measurement as well as for exploring its pathophysiological basis (4, 5).

Surgical trauma activates several biological pathways and among them, there is increased production of reactive species causing the state known as the oxidative stress (6, 5). On the other side, the researchers have found positive association between increased oxidative stress markers and some symptoms, which participated in health-related quality of life perception like fatigue, depression, anxiety, nausea and headache (7-10). Although patients are combating with many of these symptoms during postoperative recovery the studies, which measured quality of life after surgery in details, and using validated rating questionnaires were not common.

Some additional issues increase the complexity of the matter and, consequently, the need for further investigations. For example, the synthesis of reactive species depends on the type and the extent of the intervention, anesthesia techniques and anesthetics (11-14). In addition, analgesics could modulate oxidative stress response during postoperative period either decreasing (e.g. morphine) or increasing it (e.g. ibuprofen) (15, 16). In some cases, researchers elucidated the mechanisms of stress response alterations in fine details. Paracetamol, a phenolic compound, has a large antioxidative capacity due to inhibition of myeloperoxidase, the enzyme that generates the high amounts of the pro-oxidants (17). Therefore, the studies investigating the oxidative stress response after surgery in the variety of settings and with the diversity of putative modulators are still necessary.

The current knowledge about oxidative stress markers after abdominal hysterectomy is much less than those concerning other major surgeries with more profound ischemia-reperfusion injury (e.g. vascular, cardiologic surgery, tourniquet-used interventions). Abdominal hysterectomy triggers detectable changes of oxidative stress status and studies identified several factors that contributed to production of free radicals during the intervention (e.g. peritoneal closure, retention of ovaries, hormonal changes) (18-20). However, we are not aware of any study that simultaneously investigated the relationships of oxidative stress parameters and postoperative recovery as well as the effects of therapeutic interventions.

Therefore, in this study, we hypothesized that oxidative stress injury after abdominal hysterectomy deteriorated quality of life during the postoperative recovery period and that multimodal analgesia with analgesics, that have proven or proposed antioxidant activity, will give faster recovery measured with a validated rating questionnaire.

PATIENTS AND METHODS

The study was designed as interventional, time-series, non-therapeutic trial within which there was four groups of patients according to the primary analgesic drug given during the early postoperative recovery. The cohort was formed from women who were underwent abdominal hysterectomy in Clinical Center "Kragujevac", Kragujevac, Serbia, successively, from October 2011 to February 2013, up to the number of pre-calculated total sample size. Eligible subjects met the following inclusion criteria: females, total abdominal hysterectomy due to benign disease (leiomyoma), ASA physical status I or II and the receiving one or two of the study analgesics (morphine, ketoprofen, ketorolac, paracetamol). Exclusion criteria were: previous chronic use of anti-inflammatory drugs (i.e. steroids, NSAIDs), antipsychotics and opioids, known previous hypersensitivity to study drugs and history of medi-

cally important kidney or liver disease. The study design was comparable with similar previous published studies (1, 4). Study approval was obtained from Clinical Center Kragujevac Institutional Ethics Review Board and all study participants gave the written informed consent.

Anesthesia and analgesia

All patients were premedicated with 0.07 mg/kg i.m. midazolam and during anesthesia, standard monitoring were applied (ECG, NIBP, SpO_2, $EtCO_2$ and capnography). Induction of anesthesia was achieved with fentanyl 2-3 mcg/kg i.v., propofol 1.5-2.0 mg/kg i.v. rocuronium 0.6 mg/kg i.v. Patients were ventilated with an oxygen-air mixture (FiO_2=0.4) and $EtCO_2$ stabilized at 35-40 mm Hg. Anesthesia was maintained the with sevoflurane at 1-1,5 minimum alveolar concentration (MAC), fentanyl 1 mcg/kg titrated to the dose which avoided the increase arterial blood pressure values above 20% of baseline, and additional doses of rocuronium to keep satisfactory surgical relaxation. All patients received ondansetron 4 mg prior to the end of surgery. Neuromuscular blockade was antagonized with 0.05 mg/kg i.v. neostigmine and 0.01 mg/kg i.v. atropine. Patients were awakened and extubated in the operating room and transferred to the post-anesthesia care unit (PACU) upon following simple commands.

There were four analgesic drug protocols and according to that four groups: At the beginning of peritoneal closure all subjects received 0.15 mcg/kg morphine (M group). Some subjects additionally received 100 mg ketoprofen (MK group) or 30 mg ketorolac (MZ group) or 1000 mg paracetamol (MP group) respectively, in i.v. infusion over 30 minutes at the beginning of surgery plus 0.075 mcg/kg at the same time point as in M group.

In the PACU, pain was assessed on regular 10-minute intervals and patients received additional 2 mg morphine boluses in order to maintain the Numeric Pain Rating Scale (NPRS) score £3. Discharge readiness from the PACU was assessed by using the modified Aldrete's score every 15 minutes until patients met discharge criteria (score ≤9) (21). Administrations of study analgesics were continued on the ward according to the following scheme during the first 48 hours after surgery: subjects in the M subgroup received i.v. boluses of morphine 5 mg every 4 hours , in MK subgroup received ketoprofen 100 mg/ 8 h i.v. infusion, in MZ - ketorolac 30 mg / 8 hours i.v. infusion and in MP received paracetamol 1000 mg every 6 hours i.v. infusion. Additional analgesia for patients in all subgroups was performed on demand with the administration of 2 mg of i.v. boluses of morphine until achieving NPRS score of £3.

Patients' variables and quality of postoperative recovery assessments

Perioperative data collected included subject's age, ASA physical class, smoking habits, duration of surgery. One of the investigators not involved with patient care performed perioperative data collection. Pain intensity was measured using a 10-point Numeric Pain Rating Scale (NPRS) on 10-minute intervals in PACU and on every four hours on the ward. The postoperative recovery was the primary study outcome and it was assessed using the researcher-assisted, 40-item questionnaire which was specifically designed and validated to measure a patient's health status after surgery and anesthesia (22). This questionnaire measures 5 dimensions of recovery: emotional state (9 items), physical comfort (12 items), psychological support (7 items), physical independence (5 items) and pain (7 items). The sum of the individual components generates an aggregate score, which we considered to be the primary study variable, ranging from 40 (the worst) to 200 (the best) points. We validated the Serbian language version internally, based on previous recommendation for clinical researchers (1).

Oxidative stress markers measurements

Venous blood samples were collected before the surgery in the preoperative room before saline infusion (T0), 3 hours (T1), 24 hours (T2) , 48 hours (T3) and 72 hours (T4) after the surgery. Samples (5–10 mL) were taken in tubes. Blood samples were taken from antecubital veins into test tubes containing sodium citrate anticoagulant. Blood samples were processed and stored immediately. Blood was centrifuged to separate plasma and red blood cells (RBCs). Biochemical parameters were measured spectrophotometrically.

Index of lipid peroxidation (Thiobarbituric Acid Reactive Substances, TBARS)

The degree of lipid peroxidation in plasma was estimated by measuring thiobarbituric acid reactive substances (TBARs) using 1% thiobarbituric acid (TBA) in 0.05 M NaOH, incubated with plasma at 100°C for 15 min and read at 530 nm. Distilled water was used as a blank probe. TBA extract was obtained by combining 0.8 mL of plasma and 0.4 mL of trichloroacetic acid (TCA), and then the samples were put on ice for 10 minutes and centrifuged for 15 min at 6000 rpm (23).

Nitrite (NO_2^-)

Nitric oxide (NO) decomposes rapidly to form stable metabolite nitrite/nitrate products. Nitrite (NO_2^-) was determined as an index of nitric oxide production with Griess's reagent: 0.1 mL 3 N perchloric acid (PCA), 0.4 mL 20 mM ethylenediaminetetraacetic acid (EDTA), and 0.2 mL plasma were put on ice for 15 min, then centrifuged for 15 min at 6000 rpm. After pouring of the supernatant, 220 μL K2CO3 was added. Nitrites were measured at 550 nm of wavelength. Distilled water was used as a blank probe (24).

Superoxide anion radical (O_2^-)

The level of superoxide anion radical (O_2^-) was measured using nitro blue tetrazolium (NBT) reaction in TRIS-buffer combined with plasma samples and read at 530 nm. Distilled water was used as a blank probe (25).

Hydrogen peroxide (H_2O_2)

The protocol for measuring hydrogen peroxide (H_2O_2) was based on oxidation of phenol red in the presence of horseradish peroxidase. Two hundred µL sample with 800 µL phenol red solution (PRS) and 10 µL horseradish peroxidase (PD) were combined (1:20). The level of H2O2 was measured at 610 nm (26).

Determination of activities of antioxidant enzymes

Hemoglobin determination, necessary for the calculation of activity of endogenous antioxidants, was performed according to the Drabkin method. *Superoxide dismutase (SOD) activity* was determined by the epinephrine method of Misra and Fridovich (27). A hundred µL lysate and 1 mL carbonate buffer were mixed, and then 100 µL of epinephrine were added. Detection was performed at 470 nm. Plasma levels of *reduced glutathione (GSH)* are determined spectrophotometrically by Buetler's method, based on the oxidation of glutathione (GSH) by 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (28) . *Catalase (CAT) activity* was determined according to Beutler. Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6 : 1 v/v) to remove hemoglobin. Then 50 µL catalase buffer, 100 µL sample, and 1 mL 10 mM H2O2 were mixed. Detection was performed at 360 nm. Distilled water was used as a blank probe (28).

Statistical analysis

Sample size calculation assumed differences between baseline and final QoR-40 total score of at least 10 points (~5%), for pairwise comparisons (matched pairs), with a=0.05 and b=0.2, based on data from similar studies (1, 29). The preliminary calculated size was increased 1.5 times (assumption of non-parametric distribution and higher variability of study data than expected), establishing the final study population of at least 45 subjects . Statistical analysis included description methods, analysis of variance (one-way and repeated measures pairwise comparisons), Friedman test, Kruskal Wallis test, Wilcoxon Signed Rank test, and correlations (Pearson, Spearman's rho). For the purpose of secondary analysis the patients have been divided into four subgroups according to the prescribed analgesic protocol: morphine only (M), morphine plus ketoprofen (MK), morphine plus ketorolac (MK) and morphine and paracetamol (MK). All statistical tests were performed two-sided and the differences were considered statistically significant at the level of p£0.05.

RESULTS

Patients' characteristics

Study population included 51 females, average age 51.6±78 years (the mean ± standard deviation-SD), the youngest was 39 and the oldest was 69 years old. Among them 76.5% were ASA I (n=39), 23.5% ASA II (n=12) and 43.1% were smokers (n=22). Average duration of surgery was 74.8±26.4 minutes (minimum 35 minutes and maximum 120 minutes). There were four study subgroups according to the principal analgesic drug protocol: 19 women (37.3%) received morphine (M), 14 women (27.5%) received morphine plus ketoprofen (MK), 6 women (13.7%) received morphine plus ketorolac (MZ) and 7 women (13.7%) received morphine plus paracetamol (MP). Five patients received analgesic combinations which did not fit into abovementioned, prespecified classification and their data had been not included for analgesic subgroup analysis. There were no statistically significant differences in patients characteristics between subjects from different groups (p>0.05). In addition, exclusion criteria in our study eliminated differences in distribution of variety of other factors that could affect our outcomes.

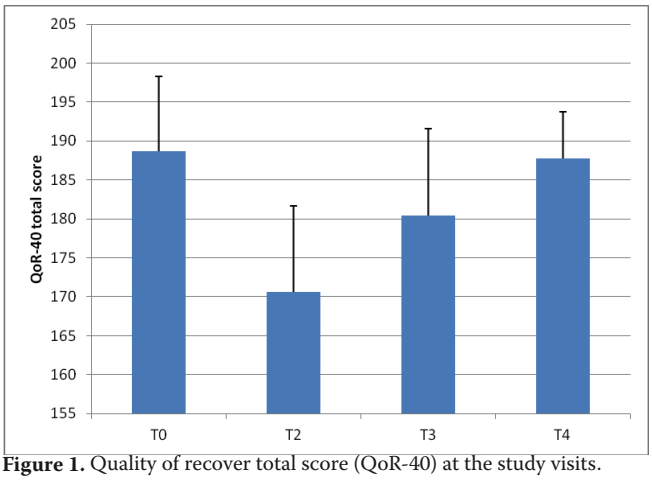

Figure 1. Quality of recover total score (QoR-40) at the study visits.

Quality of recovery

Total QoR-40 score decreased during early postoperative period and returned at preoperative values within three days (figure 1). In general, the scores during the study changed significantly (p<0.001; ANOVA-repeated measures) with significantly lower values on the POD1 and POD2 (p<0.05; ANOVA-repeated measures, pairwise comparisons).

In general, observed changes of QoR-40 total scores were similar between subgroup of patients receiving different analgesics drugs. Only on the first postoperative day the differences were statistically significant between groups (p=0.039; one-way ANOVA). The patients in MK group had a smaller drop of the scores (n=8, the mean decrease = 10.6 points, SD=6.5, min = -1, max = 18) than patients in MZ group (6, 23.2, 17.2, 7, 54) or M group (11, 25.6, 12.0, 4, 46).

Oxidative stress markers

The values of oxidative stress partameters in the whole study population were presented in the table 1. The de-

Table 1. The values of oxidative stress markers before (T0), 3 (T1), 24 (T2), 48 (T3) and 72 (T4) hours after the surgery in whole study population (n=51)

Parameter	T0	T1	T2	T3	T4
TBARS (μmol/ml)	3.23±2.26	3.63±2.62	3.74±2.43	3.64±2.30	3.49±2.17
NO_2 (nmol/ml)	10.37±7.62	9.58±7.88	10.15±7.93	9.38±7.56	10.12±7.05
H_2O_2 (nmol/ml)*	2.47±2.32	1.94±1.41	1.85±1.43	2.36±1.83	2.96±2.33
O_2 (nmol/ml)	6.91±4.25	6.54±3.83	8.53±5.07	7.48±5.27	6.19±4.66
SOD (U/gHgbx10⁴)	584.09±1065.07	695.88±1298.00	507.21±675.36	570.68±692.91	605.77±672.69
GSH (nmol/ml)	5826.29±4093.37	6586.89±8246.42	6869.54±6654.74	8059.70±12197.24	7556.66±8731.99
CAT (U/gHgbx10⁴)	133.45±144.76	103.06±87.99	97.27±117.51	115.43±123.14	102.15±143.04

Table 2. The values of oxidative stress markers before (T0), 3 (T1), 24 (T2), 48 (T3) and 72 (T4) hours after the surgery in whole study population (repeated measures analysis)

Parameter	N	T0	T1	T2	T3	T4
TBARS (μmol/ml)	29	0.13±11.21	0.04±10.97	0.71±9.70	9.70±9.70	0.26±10.24
NO_2 (nmol/ml)	29	1.42±23.47	1.46±31.44	1.38±26.60	0.04±35.20	1.75±23.01
H_2O_2 (nmol/ml)*	27	2.65±2.30	2.11±1.31	2.08±1.25	2.59±1.54	2.96±2.34
O_2 (nmol/ml)	29	6.24±3.04	6.52±3.77	7.91±4.67	6.56±4.67	6.29±4.72
SOD (U/gHgbx10⁴)	31	642.80±1237.00	889.62±1522.65	359.47±354.45	626.52±791.88	605.77±672.69
GSH (nmol/ml)	31	5454.66±4464.90	6531.01±9169.65	6974.39±7429.45	9515.30±14367.34	7556.66±8731.99
CAT (U/gHgbx10⁴)	30	134.95±160.69	84.27±76.76	109.14±134.52	117.00±141.61	102.15±143.04

*p<0.05 between study visits

scriptive statistics included all study subjects (n=51), i.e. those with missing data at some visits.

TBARS levels values across five measurements were not statistically significant (p=0,633; Fiedman test). Although the parameter, in general, increased during the study the individual variability was very high and the differences did not reach the threshold of statistical significance (table 2).

Nitrite (NO_2^-) values across five study visits were not statistically significant (p=0,633; Fiedman test). The individual variability was even higher than in other parameters (table 2).

Hydrogen peroxide (H_2O_2) values significantly decreased from baseline to T3 and then, increased at the end of the study period (table 2). Overall, the differences across the study visits were statistically significant (p=0,011; Fiedman test). Statistically significant differences of hydrogen peroxide (H_2O_2) values in patients' serum samples have been observed between the following study visits T0 and T2 (p=0.044; Wilcoxon Signed Ranks test), T1 and T3 (p=0.040; Wilcoxon Signed Ranks test), T1 and T4 (p=0.036; Wilcoxon Signed Ranks test), T2 and T3 (p=0.003; Wilcoxon Signed Ranks test and, T2 and T4 (p=0.007; Wilcoxon Signed Ranks test).

Superoxide anion radical (O_2^-) values across five study visits were not statistically significant (p=0,293; Fiedman test). The individual variability was slight and it seems further suggesting of the stability of the production of this molecule during the study period (table 5).

There were not statistically significant differences of superoxide dismutase (SOD) values during the study (p=0,769; Fiedman test). The individual variability was rather high and notable difference particularly between T1 and T2 visit did not significantly affected overall trend of the parameter (table 2).

There were not statistically significant differences of reduced glutathione (GSH) values during the study (p=0,114; Fiedman test). The individual variability was high, too and notable difference particularly between T0 and T3 (almost doubling the mean values) visits did reached statistical significance (table 2).

Catalase (CAT) values across five measurements were not statistically significant (p=0,317; Fiedman test). Although the parameter, in general, decreased during the study the differences did not reach the threshold of statistical significance (table 2).

Correlation analysis of oxidative stress markers and QoR-40

There were no significant correlation between the values of parameters of oxidative stress response, except in the case of TBARS and H_2O_2. The values of changes from T0 to T3 of TBARS and the values of changes in the same period (T0 to T3) of H_2O_2 significantly and negatively correlated with each other (r=-0.303, p=0.046; Spearman's rho). Finally, there were no significant correlation between total QoR-40 and any parameter of oxidative stress response, neither for their absolute values nor their changes between study visits.

Oxidative stress markers, analgesics and other factors

The differences of the means of TBARS changes from the baseline to T3 were statistically significant between

the analgesic study subgroups (p=0,031; Kruskal Wallis test). These alterations originated from statistically significant differences between the values of ketorolac and paracetamol subgroups (p=0.004; Mann-Whitney-Wilcoxon test) as well as between morphine and paracetamol subgroups (p=0.015; Mann-Whitney-Wilcoxon test). Overall, the largest changes was noted in paracetamol study subgroups in which there was substantial increase of TBARS activity from T0 to T3 visits and then, the sudden drop to the end of the study period (figure 2).

There were no statistically significant differences between valuse of other oxidative stress response markers (NO_2^-, H_2O_2, O_2^-, SOD, GSH, CAT) during the study period regarding different analgesics subgroups (p>0.05; Kruskal Wallis test).

Patients age, duration of surgery and cigarette smoking status showed some significant influcences on and association with the oxidative stress response markers. Patients age negativelly correlated with TBARS changes from T0 to T2 (r=-0.359, p=0.032; Spearman's rho) and positively with CAT changes (r=0.363, p=0.025; Spearman's rho). Duration of surgery correlated negatively with O_2^- changes from T0 to T1(r=-0.388, p=0.016; Spearman's rho) and positively with CAT changes from T0 to T2 positively (r=0.327, p=0.045; Spearman's rho). There were statistically significant differences of O_2^- changes from T0 to T1 between the cigarette smokers and non-skomers (p=0.005; Mann-Whitney-Wilcoxon test) as well as from T0 to T2 (p=0.008; Mann-Whitney-Wilcoxon test) (figure 3).

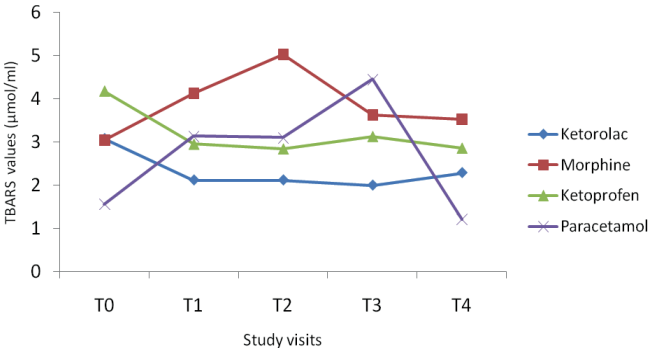

Figure 2. Median values of TBRAS (µmol/ml) between the analgesic study subgroups during the study period; ketorolac (n=6), morphine (n=19), ketoprofen (n=14), paracetamol (n=7), all non-opioid subgroup subjects received baseline analgesia with morphine (see methods).

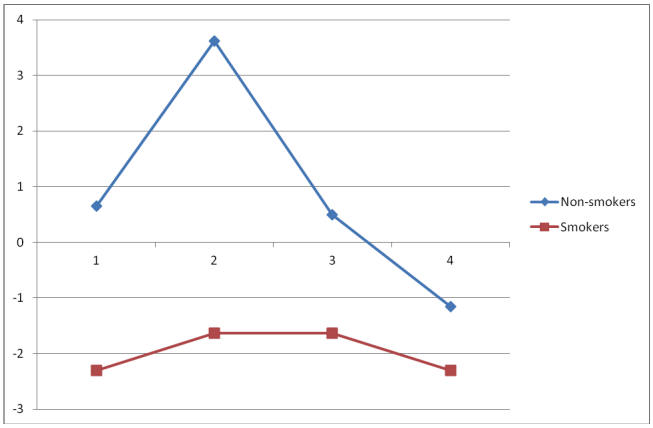

Figure 3. The median of changes from baseline (TO) of O_2^- values in subgroup of cigarette smokers and non-smokers.

In addition, there were statistically significant changes of SOD from T0 and T1 between cigarette smokers and non-smokers (p=0.045; Mann-Whitney-Wilcoxon test). In the smokers the median of change was -268.62 (-1554.74 and -81.40, percentile 25 and 75, respectively) and in the non-smokers the median of change was 252.34 (-32.56, 529.10)

DISCUSSION

The results of our study revealed that there was significant decrease of hydrogen peroxide (H_2O_2) in women after abdominal hysterectomy in all patients 24 hours after surgery. In addition, there was significant increase of index of lipid peroxidation (measured as thiobarbituric acid reactive substances - TBARS activity) during 48 hours postoperatively in the MP subgroup. Correlation analysis confirmed this type of negative relationships between these two parameters of oxidative stress response. Only a few studies published so far investigated parameters of oxidative stress response in women after hysterectomy (18-20, 30). In addition, data about H_2O_2 activity lack and, therefore, our results represent the novelty in the field.

The several enzyme cascades are involved in the synthesis and the degradation of H_2O_2 including SOD, CAT, GPx, GSH and myeloperoxidase (31). However, in our study activities of SOD and CAT were insignificantly changed which suggest that other, unmeasured, alternative pathways were involved in observed decrease of H_2O_2. It is known for example that H_2O_2 could be removed with reactions mediated with free metal ions (e.g. Fe^{2+}, Cu^{2+}) during which very toxic, hydroxyl radical ($OH^{\bullet}$) is formed (Fenton's reaction). Therefore, it seems that, in fact, the decrease of H_2O_2 was, in essence, rather a marker of the very pro-oxidation state then an indicator of higher activity of oxidative stress defense mechanism during early postoperative period after abdominal hysterectomy (32). Indeed, there were a plenty of proteins such as ferritin, transferrin, hemoglobin, myoglobin and other metalloproteins which could release free metal ions from the tissues during the surgical trauma, then sequestering H_2O_2, liberating ($OH^{\bullet}$) and finally making pro-oxidant state in the immediate postoperative period (32-34). Our finding about significant, negative correlation between H_2O_2 (decrease) and TBARS (increase), which is a marker of lipid peroxidation, further support that conclusion.

The effect of analgesic drugs could be additional mechanism of the decrease of H_2O_2 in our study subjects. All women received baseline morphine analgesia and the others paracetamol or a non-steroidal anti-inflammatory drug (NSAID), as well. Previous researches showed that morphine could dose-dependently decrease free radical

synthesis in some surgical patients and increase H_2O_2 degradation exploiting remarkable antioxidative activity (16, 35). It is known that NSAIDs and paracetamol could also increase synthesis of OH• during Fenton's reaction which is also a mechanism of H_2O_2 sequestration, as described above (36).

The time-limited and paracetamol-dependent increase of TBARS (marker of lipid peroxidation) detected in our study could be consequences of at least two circumstances: methodological specificity of the TBARS assay and effects of analgesic drugs. The assay used for determination of TBARS is based on measurement of malonyldialdehyde (MDA) concentration. The synthesis and degradation of this metabolite is variable depending on many factors such as the types and content of polyunsaturated free fatty acid in lipid membrane and the contribution of both non-enzymatic and non-lipid pathways (37).

Morphine, during experimental oxidative stress conditions, which included the presence of adenosine diphosphate (ADP) and ferrous ion, prevented the lipid peroxidation due to inhibition of oxygen consumption and H_2O_2 generation (38). However, in other methodological settings this effect was either absent or achieved with high doses of morphine (39 ,40). The use of paracetamol, on the other side, is associated with the consumption of a powerful antioxidant molecule, GSH, due to conjugation of its metabolites during the phase 2 of the drug's biotransformation (41, 42). This fact support our results that the subgroup of women, which received paracetamol, was more susceptible for development of pro-oxidant state than the others.

In our study, the concentration of other parameters of oxidative stress (SOD, CAT, NO, O_2^-), as well as GSH did not differ in successive plasma samples, either in whole patients population or in individual ones subgroups. Although the results of other, previously published studies in the field are not fully consistent and some of them support our findings this part of our study could be interpreted bearing the mind several its limitation (43). Firstly, our study sample was not very large, being underpowered for detecting significant differences but of smaller magnitudes. Secondly, the influence of other confounders, not included in our analysis could not be excluded. Finally, it is difficult to extrapolate our results to the patients treated in other clinical setting, such as receiving other type of anesthetic and analgesics drugs. Obviously, future research with more focused designs is needed in order to provide additional valid evidences.

The effects of age, duration of surgery and cigarette smoking in our study are further evidences about the disturbances of pathways of oxidative stress response at the time of at abdominal hysterectomy and during postoperative period. We noted disturbances of concentrations/activities of TBARS, O_2^-, CAT and SOD pointing to activation of some pro-oxidant mechanisms and compensatory synthesis of some enzymes of antioxidative defense. All these factors are known triggers of oxidative stress response cascades but small numbers of cases in our research as well as the parameter- and time-dependent changes precluded more detailed analysis of their influence on the main study outcomes (44, 45).

The quality of recovery in women included in our study did not correlate with any of measured oxidative stress response parameter. The use of quality of recovery with QR-40 instrument had been validated in many studies with surgical patients in general anesthesia including women after abdominal hysterectomy (1). However, according to our knowledge, there were no studies, that particularly explored association between postoperative quality of recovery (e.g. QR-40 scores) and pro-oxidant reactive species or biomarkers of oxidative stress defense. In one recent research both MDA and quality of recovery score were assessed during 24 hours after Gynecologic Laparoscopic Surgery but no direct relationships between the two variables were established and reported (46). In general, studies dealing with postoperative recovery more often investigated other important biological pathways like cytokine response than oxidative stress parameters (4).

In conclusion, our findings suggest that during the hysterectomy women experienced a detectable degree of damage of cell membranes by lipid peroxidation type and that the subgroup that received paracetamol is under the highest risk. Of all the analgesics used, paracetamol has the highest potential for exhaustion of antioxidant defense mechanisms particularly in the presence of other stressors, which liberate reactive, pro-oxidant species as if it is a surgical intervention. Our study is, as we are aware, the first one, which in a comparative design, by the same method, examined the differences between different analgesics on parameters of lipid peroxidation and other indicators of oxidation stress during abdominal hysterectomy.

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ATHEROGENIC IMPACT OF HOMOCYSTEINE: CAN *HMG-CoA* REDUCTASE INHIBITORS ADDITIONALLY INFLUENCE HYPERHOMOCYSTEINAEMIA?

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ATEROGENI POTENCIJAL HOMOCISTEINA: DA LI INHIBITORI *HMG-CoA* REDUKTAZE MOGU UTICATI I NA HIPERHOMOCISTEINEMIJU?

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ABSTRACT

The strong association among the risk of coronary artery diseases (CAD), high levels of LDL-C and low levels of HDL-C is well established. Hyperhomocysteinaemia (HHcy) is an independent risk factor for cardiovascular disease (CVD) and causes endothelial dysfunction, a hallmark of atherosclerosis. In this study, we ascertained the influence of statins on the atherogenic index, as an indicator and a significant adjunct for predicting atherosclerosis in hyperhomocysteinaemic male Wistar albino rats. For 4 weeks, the animals were fed with one of the following diets (Mucedola SRL., Milan, Italy): standard rodent chow; a diet enriched in methionine with no deficiency in B vitamins or a diet enriched in methionine and deficient in B vitamins. The animals were simultaneously exposed to a pharmacology treatment with atorvastatin at dose of 3 mg/kg/day i.p. or simvastatin, at dose of 5 mg/kg/day i.p. We measured weight gain, food intake, and FER and determined the concentrations of biochemical parameters of dyslipidaemia (TC, TGs, LDL-C, VLDL-C, and HDL-C), AI, and CRR. A histopathological examination was conducted on portions of the right and left liver lobes from each animal. A connection between Hhcy and dyslipidaemia was indicated by the findings of biochemical and histological analyses, suggesting that Hhcy was a pro-atherogenic state. An improvement in the lipid profile along with a decrease in the atherogenic index by statins suggests that atorvastatin and simvastatin could be useful antiatherogenic agents, with protective activities during hyperhomocysteinaemia.

Keywords: *atherogenic index, hyperhomocysteinaemia, liver, HMG-CoA reductase inhibitors*

SAŽETAK

Između rizika za nastanak koronarnih arterijskih bolesti (CAD), visokih nivoa LDL-C i niskih nivoa HDL-C je utvrđena jaka povezanost. Hiperhomocisteinemija (HHcy) je nezavistan faktor rizika za kardiovaskularne bolesti (CVD), uzrokuje endotelnu disfunkciju kao obelezje ateroskleroze. U ovoj studiji, utvrdićemo uticaj statina na aterogeni indeks mužjaka pacova Vistar albino soja sa hiperhomocisteinemojom, kao važnog indikatora i značajnog činioca u prognozi ateroskleroze. Životinje su hranjene sa jednom od sledećih dijeta tokom četiri nedelje (Mucedola SRL., Milan, Italy): standardnom hranom za pacove, hipermetioninskom dijetom bez deficita B vitamina ili hipermetioninskom dijetom sa deficitom B vitamina. U isto vreme, životinje su bile izložene farmakološkom tretmanu atorvastatinom u dozi od 3 mg/kg/dan i.p. ili simvastatinom u dozi od 5 mg/kg/dan i.p. Merili smo stopu prirasta telesne mase, unos hrane, FER i određivali koncentracije biohemijskih parametara dislipidemije (TC, TGs, LDL-C, VLDL-C, HDL-C), AI, CRR. Histolopatološka istraživanja sprovedena su na uzorcima desnog i levog lobusa jetre svake životinje. Povezanost Hhcy i dislipidemije je potvrđena biohemijskim i histološkim analizama u našem istraživanju, navodeći na činjenicu da je Hhcy pro-aterogeno stanje. Poboljšanje lipidnog profila zajedno sa smanjenjem aterogenog indeksa usled upotrebe statina, navodi na zaključak da atorvastatin i simvastatin mogu biti upotrebljeni kao antiaterogeni agensi sa protektivnim delovanjem u toku hipehomocisteinemije.

Ključne reči: *aterogeni indeks, hiperhomocisteinemija, jetra, inhibitori HMG-CoA reduktaze*

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INTRODUCTION

A lipid profile consists of a group of biochemical tests often used in predicting, diagnosing and treating lipid-related disorders including atherosclerosis. Generally, hyperlipidaemias are of interest to the physician in the context of risk factors for ischaemic heart disease (IHD) and peripheral vascular disease. The first step in the diagnosis of hyper- and hypolipoproteinaemias is to define the lipoprotein pattern by a chemical analysis of plasma lipids and lipoproteins. Abundant evidence has accumulated relating the concentrations of lipids (total cholesterol and triglycerides) and their associated blood transporting lipoproteins (HDL-C, LDL-C, and VLDL-C) with the occurrence of atherosclerosis in general and coronary artery disease (CAD) in particular (1-3).

The strong association among the risk of coronary artery diseases (CAD), high levels of LDL-C and low levels of HDL-C has been well established. However, the enormous contribution of triglycerides (TGs) to cardiovascular risk has been underestimated, especially in our environment (4). Indeed, high levels have been associated with an increased incidence of CAD and an increased population of small dense LDL-C particles (5, 6). Many studies have evaluated the relationship between TGs and HDL-C, and it has been shown that the ratio of TGs to HDL-C is a strong predictor of myocardial infarction. Universally, the atherogenic index of plasma (AIP), calculated as the log (TG/HDL-C), is used by some practitioners as a significant predictor of atherosclerosis (7-9).

On the other hand, homocysteine (Hcy) is a sulfur-containing amino acid, and the metabolism of Hcy is influenced by folic acid and vitamin B_{12} deficiencies, which lead to high Hcy levels in the blood (10). In 1969, McCully made initial observations linking plasma Hcy concentrations and arteriosclerotic vascular disease (11). Hyperhomocysteinaemia (HHcy) has been associated with an increased risk of venous thrombosis (11), myocardial infarction (12), peripheral vascular disease (12), and coronary artery disease (13). Many subsequent studies have shown that high Hcy levels may present a risk factor for atherosclerosis. Hyperhomocysteinaemia (HHcy) is an independent risk factor for cardiovascular disease (CVD) and causes endothelial dysfunction, a hallmark of atherosclerosis (14). The mechanisms of hyperhomocysteinaemia (HHcy) that promote atherosclerosis are seldom explored and always indefinite. Possible mechanisms for the association between homocysteine and atherosclerosis include stimulation of smooth muscle cell growth, reduction in endothelial cell growth and endothelial cell relaxation, and decreased synthesis of high-density lipoproteins (12-15).

One possible therapeutic agent could be an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is known as a statin. Several large trials published recently years have clearly shown that treatment with statins reduces coronary heart disease morbidity and mortality rates, and the beneficial effects manifest sooner than expected. Statin treatment affects variables such as endothelial function, cellular immunity, lipoprotein oxidation, rheological factors, and stabilization of atherosclerotic plaque (16).

In this study, we ascertained the clinical effects of statins on the atherogenic index, as an indicator and a significant adjunct for predicting atherosclerosis in hyperhomocysteinaemic male *Wistar albino* rats.

MATERIAL AND METHODS

Chemicals

All the reagents and substances were purchased from Sigma-Aldrich (Sigma–Aldrich Chemie GmbH, Germany), including Atorvastatin calcium salt trihydrate ≥98% ($C_{33}H_{34}FN_2O_5$x0.5 Ca x1.5 H_2O) MW: 604.69, (product number: PZ0001-25MG) and Simvastatin ≥97% (C25H38O5), MW: 418.35, (product number: S6196-25MG). All the food for the animals was purchased from Mucedola Corporation (Milan, Italy).

Ethical Approval

All the research procedures were carried out in accordance with European Directive for the welfare of laboratory animals No: 2010/63/EU and the principles of Good Laboratory Practice (GLP). The protocol for the current study was approved by the Ethics committee for experimental animal wellbeing of the Faculty of Medical Sciences of the University of Kragujevac, Serbia (No: 01-11794).

Experimental design

The animals were acclimatized for two weeks in the animal house at the Faculty of Medical Sciences in Kragujevac before dietary manipulation. Two rats were housed per wire floored cage in an air-conditioned room (22 ± 2°C), with 12 h light/dark cycles and had free access to standard or special laboratory chow diet and water ad libitum. The study was conducted on adult male *Wistar albino* rats (n=72; 4 weeks old), in which HHcy was achieved by dietary manipulation. For 4 weeks, the animals were fed with one of the following diets (*Mucedola SRL., Milan, Italy*): standard rodent chow; a diet enriched in methionine with no deficiency in B vitamins (folic acid, B_6 and B_{12}) or a diet enriched in methionine and deficient in B vitamins (folic acid, B_6 and B_{12}) (Table 1). At the same time, the animals were exposed to a pharmacology treatment with atorvastatin at a dose of 3 mg/kg/day i.p. or simvastatin at a dose of 5 mg/kg/day i.. at the same time every day, according to the equivalent therapeutic doses of these statins. The animals were divided into nine (9) different groups as follows:

1. Animals fed with standard rodent chow without the administration of statins (vehicle);
2. Animals fed with a diet enriched in methionine with no deficiency in B vitamins (folic acid, B_6 and B_{12}) without the administration of statins (mild Hhcy);
3. Animals fed with a diet enriched in methionine and deficient in B vitamins (folic acid, B_6 and B_{12}) without the administration of statins (severe Hhcy);
4. Animals fed with standard rodent chow with the administration of atorvastatin, at a dose of 3 mg/kg/day i.p. (atorvastatin);
5. Animals fed with standard rodent chow with the administration of simvastatin, at a dose of 5 mg/kg/day i.p. (simvastatin);
6. Animals fed with a diet enriched in methionine with no deficiency in B vitamins (folic acid, B_6 and B_{12}) with the administration of atorvastatin, at a dose of 3 mg/kg/day i.p. (ato+mild Hhcy);
7. Animals fed with a diet enriched in methionine with no deficiency in B vitamins (folic acid, B_6 and B_{12}) with the administration of simvastatin, at a dose of 5 mg/kg/day i.p. (sim+mild Hhcy);
8. Animals fed with a diet enriched in methionine and deficient in B vitamins (folic acid, B_6 and B_{12}) with the administration of atorvastatin, at a dose of 3 mg/kg/day i.p. (ato+severe Hhcy);
9. Animals fed with a diet enriched in methionine and deficient in B vitamins (folic acid, B_6 and B_{12}) with the administration of simvastatin, at a dose of 5 mg/kg/day i.p. (sim+ severe Hhcy).

Determination of weight gain, food intake, and FER

The starting date of the experiment was set as day "0," and the animals' body weights were measured every 24 hr. Body weight was measured until the final date of the experiment. The FER was obtained using the amount of food intake and the body weight increase during the entire experimental period; FER = increase of body weight (g)/ amount of food intake (g) during the experiment.

Determination of the biochemical parameters of hyperlipidaemia

After a 4 week-dietary manipulation, the animals were sacrificed, and blood samples were collected by exsanguination. Prior to the blood collection, to measure the plasma parameters, the animals were fasted for 12 h in order to minimize the interference of food intake in the results of the lipid profile. In the serum samples, we determined the concentration of homocysteine and lipids, such as total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), and very low-density lipoprotein-cholesterol (VLDL-C). TC was measured by the method previously reported (17), and TG was measured by the colorimetric method previously reported (18). LDL-c was measured by the Friedewald formula (19). HDL-C and VLDL-C were determined using the method described by Zlatkis and Zak (20).

Determination of the Atherogenic Index (AI) and the Cardiac Risk Ratio (CRR)

The AI was calculated as LDL-C/HDL-C, and the CRR was calculated as (TC/HDL-C) (21).

Liver histology

For the histological examination, portions of the right and left liver lobes from each animal were fixed in 10% formalin and embedded in paraffin, a 7-micron thick section was stained with H&E, and histological and morphometric analyses were performed. The liver histology was scored using a modification of the system developed by Brunt et al. (22). Briefly, the degree of steatosis, hepatocyte ballooning, lobular inflammation, and portal inflammation was scored separately in a blinded way. Each variable was graded from zero to three. The sum of the scores (degree of steatosis, hepatocyte ballooning, lobular inflammation, and portal inflammation) was considered the total pathology grade. The extent of steatosis was commonly evaluated and reported semi-quantitatively. The most reproducible method followed the acinar architecture dividing the liver parenchyma in thirds and assessing the percentage involvement by the steatotic hepatocytes as follows: 0%-33% (or 0%-5%, 5%-33%) was considered mild, 33%-66% was considered moderate, and > 66% was considered severe steatosis. The grading system for hepatic steatosis was as follows: grade 0, no fat; grade 1, steatosis affecting less than 33% of the hepatic parenchyma; grade 2, steatosis affecting 34–66% of the hepatic parenchyma; and grade 3, steatosis affecting more than 66% of the hepatic parenchyma (23) (Table 1).

Table 1. Scoring system and total pathology grade of rat livers (degree of steatosis, hepatocyte ballooning, and lobular inflammation)

Item	Definition	Score
Steatosis	<5% (no fat)	0
	5-33% (Grade 1)	1
	>33-66% (Grade 2)	2
	>66% (Grade 3)	3
Lobular Inflammation	No foci	0
	< 2 foci per 200 x field (Grade 1)	1
	2-4 foci per 200 x field (Grade 2)	2
	> 4 foci per 200 x field (Grade 3)	3
Ballooning	None	0
	Few balloon cells	1
	Many cells/prominent ballooning	2

Table 2. Effects of statins on the body weight gain, food intake, and FER values of rats with hyperhomocysteinemia (from a high-methionine diet after 4 wk)

Groups	Initial weight (g)	Final weight (g)	Weight gain[a] (g)	Food intake (g)	FER[b]
Vehicle group	96.00±9.06	413.00±13.15*	317.00±11.01*	934.12±7.01*	0.34±0.01*
Mild HHcy	104.63±6.96	356.75±24.89*	252.12±9.02*	659.25±8.07*	0.38±0.02*
Severe HHcy	105.88±6.66	258.75±32.48	152.87±8.24	562.68±5.06	0.27±0.01
Atorvastatin per se	104.02±5.55	356.90±23.22*	255.88±10.11*	822.92±6.07*	0.31±0.01*
Atorvastatin + mild HHcy	95.98±7.23	277.33±24.22	181.35±12.09	687.41±4.05*	0.26±0.02
Atorvastatin +severe HHcy	83.86±4.24	259.30±31.23	175.44±11.17	494.85±4.08	0.35±0.01*
Simvastatin per se	100.14±3.58	314.40±24.25*	214.26±14.12*	781.64±9.04*	0.27±0.02
Simvastatin + mild HHcy	93.13±5.55	326.15±25.63*	233.02±13.13*	675.50±4.01*	0.34±0.03*
Simvastatin + severe HHcy	91.38±4.98	232.12±32.54	140.74±11.12	524.00±4.03	0.27±0.01

[a]Body weight gain: final weight (g) – initial weight (g).
[b]Feed efficiency ratio (FER) = body weight gain (g)/food intake (g).

STATISTICAL ANALYSES

The statistical analyses were performed using SPSS for windows version 22. The results are presented as the mean ± standard deviation (mean ± SD). Testing for significance was done using a Student's T-test and an analysis of variance (ANOVA), where applicable. Multiple comparisons of the mean differences among the variables were done using the Tukey post hoc test. P values less than or equal to 0.05 were considered significant.

RESULTS

Homocysteine concentrations among the groups

As we previously published (24), the rats fed with standard rodent chow had normal levels of Hcy (<15 μM/L), the rats fed with a methionine-enriched diet with B vitamins had mild elevated Hcy levels (15-31 μM/L), and the rats fed with a methionine-enriched diet that was deficient in B vitamins had extremely elevated Hcy levels (>31 μM/L).

Weight gain, food intake and FER

The initial weights of all the animals were similar and not statistically significantly different, but after four weeks of the dietary treatment, the final weights were significantly different among the groups. The weight gain and final weight were significantly higher in the groups fed with standard rodent chow (vehicle group, atorvastatin and simvastatin groups) and the groups fed methionine-enriched food (mild Hhcy and simvastatin + mild Hhcy groups) than in the other groups (Table 2). The food intake was significantly higher in the groups fed the standard and methionine-enriched diets than in the groups fed the methionine-enriched and B-deficient diets. The FER was significantly higher in the mild-Hhcy group without statin administration and in the groups with statin treatment (atorvastatin, atorvastatin +severe Hhcy, simvastatin + mild Hhcy groups) than in all the other groups (Table 2).

Lipid profile, Cardiac Risk Ratio (CRR) and Atherogenic Index (AI)

The levels of all the lipoproteins were significantly different between the control groups. Tthe TC, TGs, LDL-C and VLDL-C levels were higher in the severe Hhcy and especially in the mild Hhcy groups than in the vehicle group (Table 3).

During the mild and severe Hhcy, atorvastatin at a dose of 3 mg/kg/day significantly reduced the levels of TC and the levels of TG, LDL-C and VLDL-C. On the other hand, the levels of all these lipoproteins were significantly higher after the atorvastatin administration in the group fed the standard food than in the vehicle group (Table 3). The concentration of HDL-C was not significantly changed (Table 3). The CRR and AI were significantly increased in the group fed standard food and were decreased in the groups with mild and severe Hhcy after the atorvastatin treatment (Table 3).

In the simvastatin + mild Hhcy and simvastatin + severe Hhcy groups, the levels of TC, TGs, LDL-C and VLDL-C were significantly decreased compared to those in the mild Hhcy and severe Hhcy groups, while in the simvastatin group, the values of TC, TGs, LDL-C and VLDL-C were significantly increased compared to those in the vehicle group (Table 4). The concentration of HDL-C was significantly affected only in the simvastatin group (decreased) (Table 4). The CRR and AI were significantly increased in the group fed standard food and were decreased in the groups with severe Hhcy after the simvastatin treatment (Table 3).

Table 3- Effects of atorvastatin on serum TC (mg/dl), HDL-C (mg/dl), TGs (mg/dl), LDL-C (mg/dl) levels and atherogenic index of albino rats (Values are mean± SE from 8 animals in eacg group), P values: <0.05 when compared with *vehicle control, ¶ mild Hhcy, §severe Hhcy.

Groups	TC	HDL-C	TGs	LDL-C	VLDL-C	Cardiac Risk Ratio TC/HDL-C	Atherogenic Index LDL-C/HDL-C
Vehicle group	101.94±3.77	62.93±2.73	59.29±1.12	27.15±1.00	26.95±0.09	1.62±0.09	0.43±0.06
Mild HHcy	221.62±4.85	86.10±3.22	108.85±4.37	113.75±3.81	49.48±1.25	2.57±0.26	1.32±0.13
Severe HHcy	148.26±2.85	52.51±1.98	99.12±3.85	75.93±1.98	45.05±1.34	2.82±0.24	1.45±0.15
Atorvastatin *per se*	128.18±3.21*	60.23±1.95	86.73±1.98*	50.60±0.98*	39.42±1.11*	2.13±0.13*	0.84±0.08*
Atorvastatin + mild HHcy	204.64±4.89¶	93.82±2.55	74.34±2.11¶	95.95±2.54¶	33.79±1.00¶	2.18±0.16¶	1.02±0.11¶
Atorvastatin + severe HHcy	103.48±2.11§	47.49±1.53	30.09±0.81§	49.97±1.08§	13.68±0.02§	2.18±0.16§	1.05±0.09§

Hepatic histology

The semi-quantitative histology results showed no visible lesions in the control group, whereas in the groups with mild and severe Hhcy, the liver sections showed areas of infiltration with lipid cells and degeneration of hepatocytes (Figure 1).

According to the different dietary treatments, fatty changes in the liver were distributed differently among the groups.

In the groups fed standard rodent chow and with normal levels of homocysteine, grade 1 fatty changes (5-33% fat) were present in 70% of the animals, and grade 2 fatty changes (33-66% fat) were present in 10% of the animals.

In the groups fed the methionine-enriched (mild Hhcy) diet, grade 3 (>66% fat) steatosis was present in 100% of the animals.

In groups fed the methionine-enriched and B-deficient (severe Hhcy) diet, grade 1 steatosis (5-33% fat) were present in 75% of the animals, grade 2 steatosis (33-66% fat) was present in 12.5% of the animals, and grade 3 steatosis (>66% fat) was present in 12.5% of the animals.

By the semi-quantitative measurements, the percent of lipid area was present as follows: the lipid field in the vehicle group was 0.14%, in the atorvastatin group, it was 2.9%, and in the simvastatin group, it was 2.3%. In the mild Hhcy group, the lipid field was 20.96%, but in the ato+mild Hhcy group, it was lower, at 7.96%; the lipid field was similarly lower in the sim+mild Hhcy group, at 8.68%. In the severe Hhcy group, the lipid field was 36.32%, but in the ato+severe Hhcy group, it was lower, at 31.11%; the lipid field was similarly lower in the sim+severe Hhcy group, at 27.85% (Figure 2). The percent of lipid area in the ato and sim groups were significantly lower than those in the groups without drug treatment. Additionally, the percentage of fatty areas was significantly lower in the normohomocysteinaemic groups than in the hyperhomocysteinaemic groups (Figure 2).

DISCUSSION

Thirty years ago, the atherogenic properties of homocysteine were discovered by observation of arteriosclerosis in children with homocystinuria caused by aan inherited deficiency of three different enzymes (9-14). Hyperhomocysteinaemia is generally recognized as an independent risk factor for coronary, cerebral, and peripheral athero-

Table 4- Effects of simvastatin on serum TC (mg/dl), HDL-C (mg/dl), TGs (mg/dl), LDL-C (mg/dl) levels and atherogenic index of albino rats (Values are mean± SE from 8 animals in eacg group), P values: <0.05 when compared with *vehicle control, ¶ mild Hhcy, §severe Hhcy.

Groups	TC	HDL-C	TGs	LDL-C	VLDL-C	Cardiac Risk Ratio TC/HDL-C	Atherogenic Index LDL-C/HDL-C
Vehicle group	**101.94±3.77**	**62.93±2.73**	**59.29±1.12**	**27.15±1.00**	**26.95±0.09**	**1.62±0.09**	**0.43±0.06**
Mild HHcy	**221.62±4.85**	**86.10±3.22**	**108.85±4.37**	**113.75±3.81**	**49.48±1.25**	**2.57±0.26**	**1.32±0.13**
Severe HHcy	**148.26±2.85**	**52.51±1.98**	**99.12±3.85**	**75.93±1.98**	**45.05±1.34**	**2.82±0.24**	**1.45±0.15**
Simvastatin *per se*	**122.00±1.98***	**46.72±2.11***	**76.99±2.32***	**59.88±2.05***	**35.00±1.32***	**2.61±0.21***	**1.28±0.11***
Simvastatin + mild HHcy	**172.20±2.14¶**	**70.66±2.84**	**38.94±0.09¶**	**93.75±3.55¶**	**17.70±0.07¶**	**2.44±0.27**	**1.33±0.15**
Simvastatin + severe HHcy	**142.86±1.57**	**62.93±2.16**	**79.65±2.15§**	**64.00±1.78§**	**36.20±1.21§**	**2.27±0.32§**	**1.02±0.09§**

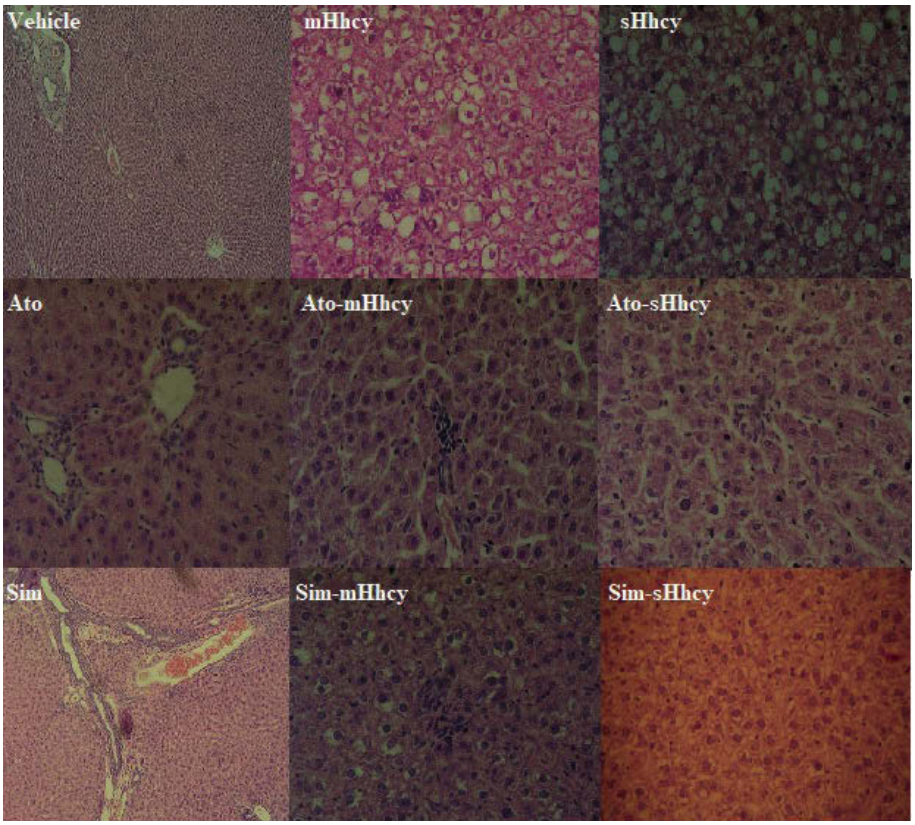

Figure 1. Histology results showing the effect of mild (mHhcy) or severe hyperhomocysteinaemia (sHhcy) and atorvastatin/simvastatin (ato/sim) on liver tissue. Representative haematoxylin–eosin (H&E)-stained liver sections (×400 and ×200 objectives). After 4 weeks, feeding a methionine-purified diet induced mainly micr ovesicular steatosis, with a macrovesicular component. After 4 weeks of feeding a methionine-purified and B-deficient diet, the pattern of steatosis was macrovesicular and a scattered foci of inflammatory cells. In severe Hhcy, diffuse and extensive macrovesicular steatosis with foci of mononuclear inflammatory cells occurred. Compared to the groups without the statin treatment, extensive macrovesicular steatosis was reduced after the ato/sim treatment in states of mild and severe Hhcy.

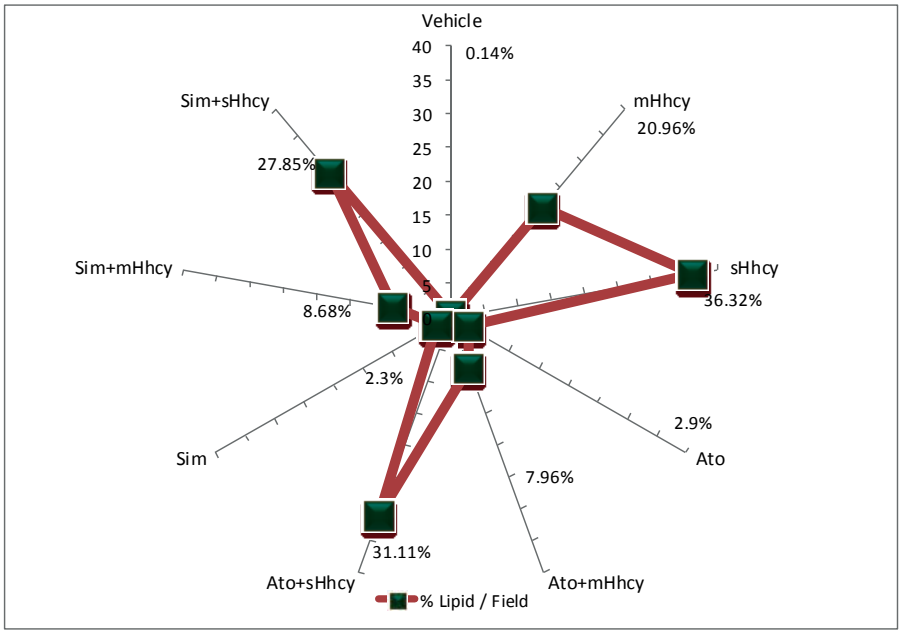

Figure 2. Distribution of fatty changes in the liver among all the groups.

sclerosis (9) and numerous investigators report that elevated levels of tHcy, an amino acid formed during methionine metabolism, are related to CVDs. Elevated levels of tHcy are associated with an increased risk of atherothrombotic events, and the acute phase of myocardial infarction when there is an elevation of reactant proteins. Although the direction of the causality between elevated tHcy and CVD is not definitive, increasing clinical and epidemiological research supports the hypothesis that tHcy is an independent risk factor for CVD (9-15).

We hypothesized that Hcy has high atherogenic potential to induce dysregulation in the metabolism of lipoproteins in the liver, with the protective role of statins in this connection. In this study, we used two the most frequently used HMG-CoA reductase inhibitors, atorvastatin and simvastatin, at therapeutically equivalent doses. Additionally, we ascertained the utility of statins on the atherogenic index as an indicator and a significant adjunct for predicting atherosclerosis in hyperhomocysteinaemic male *Wistar albino* rats.

In the first part of the study, we evaluated the morphometric characteristics of the animals and the concentrations of the lipoproteins in the serum of the animals after four weeks of dietary and pharmacological treatment. Our results confirm elevated lipids (TC, Tgs, VLDL-C, LDL-C) in the hyperhomocysteinaemic rats compared to the rats with normal Hcy levels. The literature strongly suggests an association between Hcy and dyslipidaemia, and increased plasma Hcy leads to the formation of atheromatous changes, which ultimately cause myocardial infarction (MI) (11, 12). Potential mechanisms by which elevated Hcy induces increases in lipids may be in the structure of Hcy, which contains reactive a sulfhydryl group that undergoes oxidation to the disulfide at a physiological pH. Therefore, upon oxidation, Hcy forms Hcy thiolactone. It is complexed with LDL-particles. This LDL-Hcy thiolactone complex aggregates and deposits in the form of thioco and then thioretinamide. These intermediates promote the proliferation and fibrosis of smooth muscles. During the conversion of thioco to thioretinamide, highly reactive oxygen species are generated, which cause several changes in the intima of the blood vessels and endothelial dysfunction and ultimately result in atherosclerotic plaques (9-15).

Furthermore, treatment with atorvastatin and simvastatin significantly reduces the elevated levels of lipids induced by hyperhomocysteinaemia. In comparison of these statins, simvastatin strongly reduced levels of TC, Tgs, VLDL-C and LDL-C compared to atorvastatin (Table 3 and 4). It is known that Hhcy increases the activity of HMG-CoA reductase, with an increased biosynthesis of cholesterol as a consequence. By blocking the conversion of HMG-CoA to L-mevalonate, statins decrease the levels of cholesterol (13, 14).

Additionally, protein targets for the modification by Hcy-thiolactone in human blood include fibrinogen, low-density lipoprotein, and high-density lipoprotein. At the same time, the primary target of the therapy is LDL-C. The latest LDL Guidelines (NCEP 2004 Update) suggest that levels higher than 70 mg/dl may be very high risk for CAD or as atherosclerotic levels of LDL-C (25). In the hyperhomocysteinaemic rats without statin treatment, we found high levels of LDL-C in mild and severe Hhcy, which were higher than 70 mg/dl. According to the latest guidelines, this elevation of LDL-C indicates a very high risk for CAD. Treatment with statins, in our study, significantly decreased the levels of LDL-C in all the groups (Table 3 and 4).

A previous study suggested that decreased levels of HDL-C is one of the potential mechanisms of Hhcy-induced CVD. Additionally, statins might increase the levels of HDL-C and, by these alterations, achieve a protective role. Interestingly, in our study, the levels of HDL-C were not significantly altered. One of the possible explanations for this result is the very short time of Hhcy in vivo. According to King *et al*, only more than an 8-week duration of Hhcy is capable of decreasing the cardioprotective HDL-C fraction of lipoproteins, and different doses of statins differently affect the HDL-C concentration in various experimental models (26).

The atherogenic index indicates the deposition of foam cells or plaque or fatty infiltration or lipids in the heart, coronaries, aorta, liver and kidneys (5-11). The higher the AI is, the higher the risk of damage to the abovementioned organs. According to high levels of most of the measured lipoproteins in our study, the atherogenic index and cardiac risk ratio were similarly affected (Tables 3 and 4). In addition, the AI, calculated from the standard lipid profile, acts as an adjunct that significantly adds predictive value beyond that of the individual lipids and/or the TC/HDL-C and LDL/HDL-C ratios; in the present study, an increase in the AI and CRR were found, indicating atherosclerotic vascular damage (27).

In one of the few similar studies, Bhandari *et al* examined the ameliorative role of atorvastatin in methionine-induced Hhcy and haematological changes in albino rats. They used atorvastatin at a dose of 0.02 mg/kg bw per os for 30 days during a co-treatment with a high-methionine diet. They concluded that an oral treatment of atorvastatin significantly reduced the levels of Hcy, TC, TGs, LDL-C and VLDL-C, and increased the levels of HDL-C compared to the control group. These results are similar to ours, with differences in the dynamics of the levels of HDL-C, probably, because of the very different doses of atorvastatin (0.02 mg/kg/day and 3 mg/kg/day) that were used (28).

Matte and colleagues evaluated the activities of aminotransferases in the liver and plasma of hyperhomocysteinaemic rats. Wistar rats received a daily subcutaneous injection of Hcy from their 6th to their 28th day of life. Twelve hours after the last injection, the rats were sacrificed, and the liver and plasma were collected. Hyperhomocysteinaemia decreased the antioxidant defences and total thiol content and increased the lipid peroxidation in the liver of the rats, characterising a reliable oxidative stress. The histological analysis indicated the presence of inflammatory infiltrate, fibrosis and a reduced content of glycogen/glycoprotein in the liver tissue sections from the

hyperhomocysteinaemic rats. The aminotransferase activity was not altered by homocysteine. Their data showed a consistent profile of liver injury elicited by homocysteine, which contributed to explaining, at least in part, the mechanisms involved in human liver diseases associated with hyperhomocysteinaemia (29).

Zhang et al examined the influence of mild hyperhomocysteinaemia on the atherosclerotic inflammatory processes in rats. They concluded that elevated plasma homocysteine stimulates the activation of nuclear factor kappa B and, consequently, increases the expression of the inflammatory factors in vivo, which contribute to atherogenesis by enhancing the inflammatory response of the vascular endothelium (30).

Additionally, many other experimental studies also confirmed our results of the high atherogenic potential of Hcy and the protective role of atorvastatin and simvastatin in this connection (31, 32).

In a number of prospective clinical studies, in order to study the connection between homocysteine and lipid metabolism in atherosclerosis, homocysteine was determined in lipoprotein fractions in men with hypercholesterolaemia. All the lipoprotein fractions contained a considerably higher level of homocysteine in hypercholesterolaemia compared to normolipaemic men, varying from a 2.2 to 7.2 times higher estimated per unit volume of serum. The atherogenic index for cholesterol, the LDLChol/HDLChol, was 2.2 times higher in the hypercholesterolaemic group than in the normolipidaemic group. The results suggested that the analysis of the homocysteine content of the serum and lipoprotein fractions might prove to be useful for assessing the risk, prognosis and response to therapy in persons with atherosclerosis (33-35).

CONCLUSION

The connection between Hhcy and hyperlipidaemia, is indicated by our biochemical and histological analyses, makes Hhcy a pro-atherogenic state. The semi-quantitative histological measurements confirmed the negative effects of Hhcy in hepatic tissue and the protective effects of statins in Hhcy. The improvements in the lipid profile, along with the decrease in the atherogenic index by statins, might suggests that the antiatherogenic action of atorvastatin and simvastatin are in part dependent on their significant influence on homocysteine metabolism.

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CONFLICT OF INTEREST

No conflicts of interest, financial, or otherwise, are declared by the authors.

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QUALITY OF LIFE AND ANXIETY IN MILITARY PERSONNEL

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KVALITET ŽIVOTA I ANKSIOZNOST KOD PROFESIONALNIH VOJNIH LICA

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ABSTRACT

The quality of life of military personnel represents their self-evaluation of the quality of their own mental and physical health, social relations, and the environment in which they live and work. The aim of our study was to evaluate the relationship between quality of life and anxiety levels in military personnel of the Serbian Armed Forces.

The cross-sectional study included a total of 311 randomly selected professional military personnel (officers, non-commissioned officers and professional soldiers) between 23 and 53 years of age (mean, 35.3±7 years) without current mental health problems. For the purpose of this study, we used the World Health Organization Abbreviated Instrument for Quality of Life Assessment (WHOQOL-BREF) and the Beck Anxiety Inventory (BAI). The statistical analysis included parametric and non-parametric descriptive statistics.

Professional military personnel showed high satisfaction with their quality of life in the categories of social relations (82.52), psychological health (82.10) and physical health (81.68), while the satisfaction scores in the category environment category were the lowest (62.77). The average value for the total BAI score was 4.83±5.66. Quality of life decreased, while anxiety increased, with increasing age of the military personnel. Higher BREF score values were associated with lower scores on the BAI questionnaires in all subscales (p<0.001).

Military personnel of the Serbian Armed Forces showed a high degree of satisfaction with their own quality of life. Learning techniques to easily overcome everyday stress would reducedreduce anxiety and improve the quality of life in military personnel of the Serbian Armed Forces.

Key words: *quality of life, anxiety, military personnel, Serbian Armed Forces*

SAŽETAK

Kvalitet života profesionalnih vojnih lica predstavlja njihovu samoprocenu kvaliteta sopstvenog zdravlja kako psihickog tako i fizickog, kao i socijalnih odnosa i sredine u kojoj zive i rade. Cilj našeg istraživanja bio je da proceni odnos između kvaliteta života i nivoa anksioznosti profesionalnih vojnih lica Vojske Srbije.

Studija preseka obuhvatila je ukupno 311 profesionalnih vojnih lica (oficira, podoficira i profesionalnih vojnika) odabranih metodom slucajnog izbora, starosti između 23 i 53 godine (35,3±7 godina u proseku) bez dijagnostikovanog mentalnog problema. Za potrebe ove studije koristili smo Upitnik za procenu kvaliteta zivota Svetske Zdravstvene organizacije (skracena verzija) (WHOQOL-BREF) i Bekov upitnik za procenu anksioznosti (BAI). Statistička analiza uključivala je parametarsku i neparametarsku deskriptivnu statistiku.

Profesionalna vojna lica pokazala su veliko zadovoljstvo kvalitetom života u segmentima socijalnih odnosa (82,52), psihičkog zdravlja (82,10) i fizičkog zdravlja (81,68), dok je segment životne sredine bio najniži (62,77). Prosečna vrednost ukupnog BAI skora bila je 4,83±5,66. Kvalitet života se smanjivao, dok se anksioznost povećavala sa godinama života vojnih lica. Visoke ocene BREF-a u svim domenima pratile su niže ocene BAI upitnika (p<0,001).

Profesionalna vojna lica Vojske Srbije su pokazala visok stepen zadovoljstva svojim kvalitetom života. Tehnike učenja lakšeg prevladavanja svakodnevnog stresa dovele bi do smanjenja anksioznosti i poboljšanja kvaliteta života profesionalnih vojnih lica Vojske Srbije.

Ključne reči: *kvalitet života, anksioznost, profesionalna vojna lica, Vojska Srbije*

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ABBREVIATIONS

QOL - Quality of life
WHO - World Health Organization
BREF or WHOQOL-BREF - World Health Organization Abbreviated Instrument for Quality of Life Assessment
BREF 1 - How would you rate your quality of life?
BREF 2 - How satisfied are you with your health?
DOM 1 - Physical Health
DOM 2 – Psychological Health
DOM 3 – Social Relations
DOM 4 – Environment
BAI - Beck Anxiety Inventory
SD – Standard deviation

INTRODUCTION

The military organization is a unique system characterized by a specific environment with an established system of work, in which the activity of an individual is regulated by rules and norms and the basic relationship is commanding. The structure of military units is hierarchical, and their operation is guided by rules of order and discipline (1,2). Discipline, in the military context, is more than a requirement to obey the orders of a superior; it also involves following a set of general rules of behaviour, even in the face of distractions that include lethal threats. The emphasis on discipline serves two purposes: it facilitates the accomplishment of difficult missions, and it supports the creation and maintenance of spirit and morale. It is also characterized by specific symbolism of the military organization and commands, uniformity (uniformity of norms, behaviour, life and work clothes), accentuated ritualism (greetings, addressing, holding of the body), carrying a weapon, and other factors. (2,3).

Military personnel face numerous stressors on a daily basis in their professional lives that affect their quality of life in the military environment. They are subject to the system of command, must respect the principle of subordination, undergo extreme training, etc., which may cause them to experience stress more frequently than the civilian population. At the same time, stressors in their family environment cannot be neglected (4,5).

Quality of life (QOL) includes subjective well-being, life satisfaction, perceptions of social relationships, physical health, economic status, and functioning in daily activities and work (6). Quality of life is defined as an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. The concept is very broad and includes variables related to one's physical health, psychological health, level of independence, social relationships and relationships to salient features of their environment (7).

In accordance with the above concepts, the quality of life of military personnel refers to their ability to engage in professional activities in the military environment and to participate in social activities, along with intrapsychic abilities that imply good health and psychological and intellectual functioning, both in the military environment and with their family (3,4). Quality of life refers to the subjective experience of the quality of life of military personnel during professional military service, as opposed to the objective characteristics of the military environment (1). Social support is very important and may be a protective factor against the development of some psychological problems, including anxiety and depression, in poor working conditions (8).

Many studies have assessed the quality of life in different civilian environments or different aspects of illnesses (hypertension, migraine, diabetes) (9,10); however, few of these studies were carried out in the military environment (3,11-14). In particular, no studies have investigated the relationship between job stress, anxiety and quality of life in the military environment.

The aim of our study was to evaluate the relationship between quality of life and anxiety levels in military personnel of the Serbian Armed Forces.

METHODS

A cross-sectional study was conducted in the three barracks of infantry units of the Serbian Armed Forces, whose total military personnel number met the required sample size of the respondents, and in which all military personnel were exposed to approximately the same professional burden. The study was conducted in September 2016.

This study was conducted with the approval of the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac. The study was approved by the General Staff of the Serbian Armed Forces. In addition, a special permit for the research in the units of the Serbian Armed Forces was obtained from the Ministry of Defense

Subjects

The study included a total of 311 randomly selected professional military personnel (officers, non-commissioned officers and professional soldiers), aged 23 to 53 years, who were on military duty for at least three years at a relatively high professional load, including duty service, guard duty, overtime work, or an inability to take days off.

The sample was consecutive, i.e., participation in the study was offered to all members of the professional military unit who, during the study period, met the criteria for inclusion and exclusion. The inclusion criteria were as follows: subjects had to be professional military personnel of the Serbian Armed Forces (officers, non-commissioned officers and professional soldiers), work under a significant workload (guard, on-call, overtime, inability to use free days), be age 23 to 53 years, and have at least three years of active professional military service. The exclusion criterion was a diagnosed psychiatric illness.

Written informed consent was obtained from all participants prior to participation in the study, and the investigation included only those who volunteered to take part in it. Thus, each respondent could drop out of the research if he or she felt that the questions in any way disturbed his or her mental well-being. All participants were assured anonymity and that only group-level findings would be reported.

The size of the sample was determined based on the formula for determining sample size. Ten percent was added to this number because of the possibility that questionnaires would not be fully completed. Using this method, we obtained a sample size of 311 respondents, with a previous decision to set the alpha error level at 0.05 and the beta level at the limit of 0.01, which yields a study strength of 90% (15).

Psychological instruments

The demographic **questionnaire** included questions regarding age, gender, education, marital, professional and health status.

Psychometric assessments of quality of life and anxiety were made using the World Health Organization Abbreviated Instrument for Quality of Life Assessment (WHOQOL-BREF) (16) and the *Beck Anxiety Inventory* (BAI) (17).

The **World Health Organization Abbreviated Instrument for Quality of Life Assessment (WHOQOL-BREF)** is a self-report measure consisting of 26 items on a five-point Likert scale. This instrument was developed to measure physical, psychological, social and environmental aspects of subjective well-being. Domain scores were scaled in a positive direction (higher scores denote better QOL), with a score range of 4-20 that was transformed to a 0-100 scale following the standard procedure defined in the World Health Organization Abbreviated Instrument for Quality of Life Assessment user manual. The World Health Organization Abbreviated Instrument for Quality of Life Assessment could be applied cross-culturally. The World Health Organization Abbreviated Instrument for Quality of Life Assessment scores correlated strongly (r = 0.89) with the original WHOQOL - 100 domain scores. Permission to use the Serbian version was obtained from WHO (World Health Organization) (16).

*The **Beck Anxiety Inventory** **(BAI)*** is a non-specific, self-report inventory that is used for measuring the severity of anxiety in children and adults. The questions used in this inventory detect common symptoms of anxiety that the subject has experienced during the past week (including the day of the assessment). It served as the primary outcome for measuring the severity of anxiety in participants suffering from different primary anxiety disorders. The BAI assesses emotional, physiological and cognitive aspects of state anxiety. It consists of 22 items, rated on a four-point Likert scale ranging from 0 = *not at all* to 3 = *severely*. Categorical anxiety levels consist of minimal (0–7 points), mild (8–15), moderate (16–25) and severe (26–63) anxiety (17).

Statistical analyses

Statistical analysis included parametric and non-parametric descriptive statistics, depending on the nature of data. Data analysis was carried out using SPSS software version 20.0.

To test whether all numerical parameters and scores were normally distributed, we used the Kolmogorov-Smirnov test. The results demonstrated that in all monitored and calculated parameters and scores there was a normal distribution (z was less than 1.96, and $p<0.05$), so that it was possible to apply parametric methods in further analyses.

RESULTS

The demographic variables of the military personnel are shown in Table 1. The average age of the professional military personnel was 35.3±7 years. There were statistically significant differences in education and gender. There were more subjects with completed military education (military academy) (60.8%) than with other categories of completed education ($\chi 2 = 8.167$; $p<0.01$), and the number of male subjects was much higher than the number of females ($\chi 2 = 11.478$; $p<0.01$).

There was also a statistically significant difference in marital status ($\chi 2 = 8.167$; $p<0.01$); more military personnel were more married (62.7%) than were in other marital status categories.

The presence of chronic somatic diseases (diabetes, hypertension, hypo/hyperthyrosis, migraine, etc.) was observed in a small number of subjects (8,0%), who demonstrated statistically significant differences in the observed variable ($\chi 2 = 11.558$; $p<0.01$) compared to healthy subjects.

Table 1. Demographic characteristics of military personnel

Variable	%	χ2	P
Gender (male)	91,3	11,478	< 0.01**
Age (>30 years)	79,5	5,598	< 0.01**
Education (>12 years)	60,8	8,167	< 0.01**
Marital status (married)	62,7	8,167	< 0.01**
Health status (somatic diseases presence)	8,0	11.558	< 0.01**

** $p < 0.01$

Table 2. Values of the total scores of the BREF and BAI questionnaires

Questionnaire		Min	Max	X	SD
BREF	**Domain**				
	DOM 1 (Physical health)	28.57	100.00	81.68	13.88
	DOM 2 (Psychic health)	29.16	100.00	82.10	13.23
	DOM 3 (Environment)	9.37	100.00	62.77	17.81
	DOM 4 (Socail relathionship)	41.67	100.00	82.52	12.29
	BREF 1 - How would you rate your quality of life?	1.00	5.00	3.61	0.84
	BREF 2 - How satisfied are you with your health?	2.00	5.00	4.06	0.77
BAI		0.00	39.00	4.83	5.66

WHOQOL-BREF (World Health Organization Abbreviated Instrument for Quality of Life Assessment)
BAI (*Beck Anxiety Inventory*)

The total scores on the BREF domains and BAI questionnaire are shown in Table 2. The results of the BREF questionnaire showed approximately the same values for the domains of Psychological Health, Physical Health and Social Relations, while the scores were the lowest for the Environment domain. The average value for total BAI score was 4.83±5.66.

The average values for the total BAI score increased with the age of the military personnel, and a strongly statistically significant difference was detected between respondents of differing ages (p<0.001) (Table 3).

The average total BREF scores decreased with the age of the respondents. Strongly statistically significant differences were found for Psychiatric Health and the Social Environment (p<0.001), as well as for Physical Health (p<0.05) (Table 3).

In the total BREF scores of all domains, the highest average values were observed in subjects with minimal anxiety levels, followed by individuals with a mild level of anxiety. The lowest average total BREF scores were reported by respondents with moderate and high levels of anxiety. In all situations, the BREF questionnaire values were negatively linked to the mentioned scores, indicating that higher BREF scores in the respondents were associated with lower scores on the BAI questionnaires. There were strongly statistically significant differences among all subjects (p<0.001) (Table 4).

There were statistically significant differences between the total score on the BREF question 1 (How would you rate your quality of life?) and the individual BREF questionnaires for Social and Physical Health (p<0.001), as well as for the Environment and Psychological Health domains (p<0.05).

There were statistically significant differences between the total score on the BREF question 2 (How satisfied are you with your health?) and the individual BREF question-

Table 3. Correlation of BREF and BAI total scores according to the age of military personnel

Questionnaire		Age (years)	N	X	SD	F	p
BAI		<30	98	3.99	3.69	6.820*	0.001***
		31-40	140	4.34	5.63		
		>40	73	6.92	7.26		
BREF	**DOM 1 (Physical health)**	<30	98	83.34	12.81	4.204	0.016*
		31-40	140	82.63	13.11		
		>40	73	77.64	15.96		
	DOM 2 (Psychic health)	<30	98	84.27	12.87	6.939	0.001***
		31-40	140	83.12	12.04		
		>40	73	77.23	14.80		
	DOM 3 (Environment)	<30	98	84.78	10.64	9.388	0.001***
		31-40	140	83.69	11.82		
		>40	73	77.28	13.84		
	DOM 4 (Social relathionship)	<30	98	63.65	16.74	0.819	0.442
		31-40	140	63.37	17.51		
		>40	73	60.45	19.72		

BAI (*Beck Anxiety Inventory*)
WHOQOL-BREF (World Health Organization Abbreviated Instrument for Quality of Life Assessment)
***p < 0.001
*p < 0.05

Table 4. Correlation of the total scores of BAI and BREF questionnaires

BREF Domain	BAI Level	N	X	SD	F	P
DOM 1 (Physical health)	Minimal	275	84.04	13.41	49.619	0.001**
	Mild	19	69.55	15.04		
	Moderate/ severe	17	57.14	14.35		
	Total	311	81.68	15.54		
DOM 2 (Psychic health)	Minimal	275	84.17	11.04	36.349	0.001**
	Mild	19	70.17	13.21		
	Moderate/ severe	17	62.50	15.68		
	Total	311	82.10	12.70		
DOM 3 (Social relathionship)	Minimal	275	83.78	11.21	13.588	0.001**
	Mild	19	74.93	17.85		
	Moderate/ severe	17	71.02	13.46		
	Total	311	82.01	12.90		
DOM 4 (Environment)	Minimal	275	64.79	11.21	17.627	0.001**
	Mild	19	50.32	16.19		
	Moderate/ severe	17	43.94	15.51		
	Total	311	62.77	17.81		

BAI (*Beck Anxiety Inventory*)
WHOQOL-BREF (World Health Organization Abbreviated Instrument for Quality of Life Assessment)
*** $p < 0.001$

naires for Social Relations ($p<0.001$), as well as for Psychic Health and Physical Health domains ($p<0.005$) and Environment ($p<0.05$).

DISCUSSION

In this study, we wanted to determine how job strain in the military environment affects anxiety and quality of life. Individuals in some professions, such as firefighters, rescue professionals and military professionals, deserve special attention regarding health care because they are exposed to extreme physical, mental and social demands in their daily work and, therefore, are susceptible to different health risks (3,18). Studies have reported that in the military environment, stress arises from risky assignments or missions, problems in interactions with peers and those of higher rank, sleep deprivation from shift work, the demand for physical fitness, deployment and separation from family, and additional noncombat work assignments such as disaster relief, peacekeeping and other humanitarian actions (2,9,11).

Pearlini and Schooler described four primary social roles: marital, parental, professional and domestic (19). Problems with maladaptation to the military environment contribute to the simultaneous presence of several problems related to these roles, as well as their mutual conflict (2,11).

According to some studies, marital situation is an important factor that may be associated with individuals' quality of life (2,6,20,21). In our study, approximately 2/3 of the military personnel were married, and many families included children. Domestic problems with children and spouses have an important impact on the professional lives of military personnel. The downsizing of military manpower and budget cuts increase the workload and stress levels of the few remaining military personnel. Family support is a top priority. The US Army has established exclusive programs addressing every aspect of family life to help service members and their loved ones. Some examples of such programs include affordable family housing, military spouse education, child care, affordable shopping, youth education and development, family health care, family advocacy, services for families with special needs, family citizenship, family recreation, financial stability, family relocation and family counselling. The aim of these programs is to maximize service member families' stability and quality of life (22).

In accordance with other studies, we found that the respondents reporting a significantly high average quality of life were younger and healthier. Their perception of quality of life was significantly better that of thefor younger than for older respondents; this finding was expected because the younger respondents did not have chronic diseases, were psychologically and physically healthy and experienced good social relations (3). In our study, physical health, that

is, the absence or the presence of chronic disease, proved to be the most important factor affecting all segments of quality of life as well as anxiety. This finding was expected and is in line with the results of other investigations (23-25).

The anxiety levels measured using the BAI questionnaire were significantly higher in older respondents, as well as in respondents with chronic somatic illnesses. These findings were expected, given that those respondents have experienced stress and overwork for many years and have become worn out over time. This observation has been demonstrated in other studies (3,26,27).

Respondents with higher anxiety reported significantly lower perceptions of quality of life in all categories; this finding emphasizes how strongly stress and consequent anxiety broadly affect the life and functioning of individuals (7,11,14).

In our study, among the four domains of the WHO-QOL-BREF, the highest mean satisfaction rating was found for DOM 3 (Social Relationships), implying good interpersonal relationships. This observation is of exceptional significance for the military environment in peacetime, and is even more important during wartime conditions. Interpersonal relations in the military reflect the nature of the military activity, the character of the society to which the military belongs, the complexity of the work with combat techniques, the special conditions of the work, and the military subordination. Each member of the military collective was in an interactive relationship, i.e., interacting with other members of that collective. The cohesiveness and efficiency of the military unit depends on the quality of interpersonal relations. Cohesiveness is expressed through feelings of belonging and loyalty to the collective, an atmosphere of security, the ability to establish friendly relationships, emotional relationships, and well-structured time. The social domain evaluates issues related to personal relationships, sexual activity, practical social support, and feelings of being respected and accepted. Adverse social relationships and job characteristics have been associated with poorer health, and some studies have found that social support may act as a buffer and protect against the development of depression or anxiety in environments with poor working conditions (4, 5, 9,11,14)

Because this was a primarily healthy study population, it was expected that scores would be high for DOM 1 (Physical Health), which measures self-assessment of satisfaction with one's own physical health. High scores were also reported for DOM 2 (Psychological Health), which was expected in a military environment where people are prepared to deal with stressful situations on a daily basis and are ningtrained under extreme conditions (28).

Our study showed that mental health, including anxiety and social relationships, but not physical health, was impaired most strongly in older military personnel. Therefore, the relationship between quality of life and health perception is crucial for the sustained prosperity of military personnel and the strength of troops (29). Emotional distress significantly affects the combat readiness and opportunities of the individual and thus the entire army (4).

The environmental domain was an important issue in the workplace (6, 4). Moreover, the lowest mean score was shown for DOM 4 (Environment). This finding was expected due to poor working conditions, unsolved housing issues and low salaries. Social support is very important and may be protective against the development of anxiety and depression under adverse working conditions (3, 6). Mental and physical health undoubtedly have a strong impact on overall quality of life and are significantly influenced by stress at work. Individuals with a higher rank and positions with greater accountability are more vulnerable to the effects of stressors (2, 9, 30).

In DOM 4, there were different variations in responses (SD very high) among groups. This variation may be associated with different social groups, where those who had a real-life housing or income issue or were out of work were likely to be more satisfied with the environment (2,6).

There was no statistically significant difference in responses based on gender and marital status. Women in the military environment have a similar perception as their male colleagues in all domains of quality of life. They bear the same professional burdens as men, but also have obligations in other social roles, primarily marital (5,8,11).

CONCLUSION

In spite of the many stressors and high demands at work, military personnel of the Serbian Armed Forces reported a high degree of satisfaction with their own quality of life, especially in the fields of psychological and physical health and social relations. Learning techniques to easily overcome everyday stress would lead to a reduction in anxiety and improve the quality of life of military personnel of the Serbian Armed Forces.

LIMITATIONS

The published data were approved by the competent authorities. We did not have approval to obtain other information that might be relevant to the study, such as the military rank of the members and the formation position. Also, the sample was composed primarily of men, which is a limitation that could not be overcome.

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PECULIARITIES OF FREE-RADICAL PROCESSES AND NEUROPSYCHOLOGICAL STATUS IN PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS AND POSSIBILITY OF CORRECTING THEIR IMPAIRMENTS WITH ANTIOXIDANT THERAPY

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KARAKTERISTIKE SLOBODNO-RADIKALSKIH PROCESA I NEUROPSIHOLOŠKOG STATUSA KOD PACIJENATA SA HRONIČNIM GENERALIZOVANIM PARODONTITISOM I MOGUĆNOST KOREKCIJE NJIHOVIH OŠTEĆENJA ANTIOKSIDATIVNOM TERAPIJOM

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ABSTRACT

This clinical study included a total of 71 patients with chronic generalized periodontitis (CGP). The chemiluminescence method was used to determine the level of generation of reactive oxygen species by leukocytes (basal and stimulated), as well as the content of malonic dialdehyde in blood plasma and antiperoxide activity of plasma. The anxiety level was measured using the Spielberger's test. Patients with chronic generalized periodontitis in the exacerbation phase were found to have a natural significant increase in both basal and stimulated PICL, as well as an increase in blood plasma malonic dialdehyde 1.6-, 3.9- and 1.4-fold, respectively, compared with apparently healthy donors (all $p<0.05$), and a significant 2.1-fold decrease in plasma APA compared with healthy donors ($p<0.001$). In the remission phase, all parameters were normalized, however, they did not reach the norm. We revealed significant dependence of reactive oxygen species generation by leukocytes, lipid peroxidation and antiperoxide activity of plasma on the severity of the course of chronic generalized periodontitis, whereas plasma antiperoxide activity decreased. In patients with the severe course, after the treatment with AO, situational and personal anxiety increased, depression deteriorated, unlike patients with mild-to-moderate course in whom this symptomatology virtually disappeared. In patients with the severe course of periodontitis after surgical manipulations, the parameter in the Hounsfield units increased gradually, on days 14, 30 and 90 on average, 1.3 , 2.4 and 3.2-fold, respectively, thus suggesting that bone tissue density increased faster after surgical methods of treatment in patients with severe chronic generalized periodontitis.

Keywords: *free radical processes, neuropsychological status, generalized periodontitis, antioxidant therapy*.

SAŽETAK

Ova klinička studija obuhvatila je ukupno 71 bolesnika sa hroničnim generalizovanim parodontitisom (HGP). Metod hemiluminescencije je korišćen za određivanje nivoa produkcije reaktivnih vrsta kiseonika posmatranjem leukocita (bazalnih i stimulisanih), kao i sadržajem malonil-dialdehida i aktivnosti antiperoksidaze u plazmi. Nivo anksioznosti je meren korišćenjem Spilbergerovog testa. Pacijenti sa hroničnim generalizovanim parodontitisom u fazi egzacerbacije su naišli na prirodno značajno povećanje i bazalnog i stimulisanog parametra intenziteta hemiluminescencije, kao i povećanja malonil-dialdehida u plazmi 1,6-, 3,9 i 1,4 puta, u poređenju sa očigledno zdravim donatorima ($r<0,05$), i značajno 2,1-struko smanjenje aktivnosti antiperoksida u plazmi (APA) u poređenju sa zdravim donorima ($r<0,001$). U fazi remisije, svi parametri su normalizovani, ali nisu dostigli normu. Otkrivena je značajna zavisnost reaktivnih vrsta kiseonika od leukocita, lipidne peroksidacije i antiperoksidne aktivnosti plazme na težinu hroničnog generalizovanog parodontitisa, dok je aktivnost plazma antiperoksida smanjena. Kod bolesnika sa teškim tokom bolesti, nakon tretmana sa AO, situaciona i lična anksioznost su se povećale, depresija se pogoršala, za razliku od pacijenata sa blagim do umerenim tokom, kod kojih je ova simptomatologija praktično nestala. Kod pacijenata sa teškim tokom parodontitisa nakon hirurških intervencija, parametar u Hounsfield jedinicama povećavao se postepeno, u srednjim danima 14, 30 i 90, odnosno 1,3-, 2,4- i 3,2-puta, što ukazuje na to da je gustina koštanog tkiva povećana brže posle hirurških metoda lečenja kod pacijenata sa teškim hroničnim generalizovanim parodontitisom.

Ključne reči: *slobodni radikali, neuropsihološki status, generalizovani parodontitis, antioksidativna terapija.*

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INRODUCTION

The treatment of inflammatory periodontal diseases is currently one of the important problems in dentistry. According to the WHO, the incidence of periodontal diseases in people over 40 years old exceeds 95%, with the diseases being aggressive and almost untreatable [7].

Currently, a large body of studies exists on periodontitis. However, despite improved surgical, therapeutic and prosthetic techniques, periodontal diseases have a steadily progressing course. Besides, the problem of priority of etiopathogenesis is still controversial.

The last several decades have seen extensive studies of free-radical processes whose role was proven in a wide variety of nosological entities. However, the number of studies addressing the state of oxidative stress in patients with periodontitis appears to be sparse.

Free-radical processes (FRPs) are known to be general biological mechanisms of defence and damage of tissues [11, 12]. In health, they participate in energetic processes, transport of electrons in the chain of respiratory mitochondria, proliferation and differentiation of cells, in regulation of enzyme activity, etc. Besides, FRP is an indispensable link of any inflammation, associated with production of reactive oxygen species (ROS) by phagocytes. This evolutionally developed secretory function of phagocytes is required for killing of bacteria; however, an abrupt increase of oxygen consumption during phagocytosis leads to the fact that instead of O_2 reduction to H_2O leukocytes mainly generate ROS. Overproduction of ROS initiates free-radical lipid peroxidation (LPO), which damages tissues, first of all, the biological membranes [2, 14]. The appearance of a large number of free radicals is associated with impaired transport of electrons in the mitochondrial chain; dissociation of oxidative phosphorylation under the effect of LPO leads to profound deficit of energy, accompanied and followed by changed functions of enzymes, carbohydrates and proteins, including proteins of DNA and RNA, with the resulting loss by the cell of its regulatory functions, accompanied by the emergence of abnormal proteins and stimulation, apart from a direct damaging effect, of secondary destructive processes. Deep disorders of the membranous and, consequently, total architectonics lead to cell death. This process is referred to as oxidative stress.

Given that a significant role in the etiopathogenesis of periodontitis is assigned to bacterial flora closely related to activity of free radicals and immune responses, FRPs are one of the links of the development and course of periodontitis.

In this connection, it appears appropriate and currently important to study the state of systemic free-radical homeostasis and assess prompt pathogenetically substantiated correction of oxidative stress reactions in patients with periodontal diseases. It served as the basis for carrying out the present study.

PATIENTS AND METHODS

This clinical study included a total of 71 patients [35 (49.3%) men and 36 (50.7%) women] suffering from CGP, aged from 17 to 77 (mean age 47.22±6.67 years).

Once enrolled into the study, the patients were randomly divided into two groups. Group One (comparison group) comprised 32 (45.1%) patients aged from 30 to 73 (mean age 44.79±6.35 years) receiving conventional therapy including the standard set of surgical, therapeutic and prosthetic manipulations. Group Two (study group) was composed of 39 (54.9%) patients aged from 28 to 76 (mean 51.22±7.92 years). All patients from this group additionally received AO therapy [cytoflavin 1-2 tablets twice daily for 25 days, mexidole 1 tablet (125 mg) three times daily and ascorbic acid 1 dragee (100 mg) three timed daily] in the composition of conventional treatment. Eighteen (16.7%) patients additionally received a calcium preparation (calcium D_3), 1 chewing tablet twice daily for 30 days. The groups of patients were homogeneous and statistically did not differ.

The anxiety level was determined by means of the Spielberger's test [Spielberger C. D., 1966]. It was used to obtain tentative standards: low anxiety level – 20-24 points, moderate level – 35-44 points and high level – 46 points and more. The total score was obtained by calculating the results with the help of the key taking into consideration inverted statements. The obtained data were processed in accordance with the software on an IBM PC. The test is an informative method of measuring the level of state anxiety at a given moment (reactive anxiety as a state anxiety about an event) and personal anxiety (trait anxiety as a personal characteristic). The self-assessment scale consists of two parts, separately evaluating reactive anxiety (items № 1-20) and personal anxiety (items № 21-40).

The dynamics of the anxiety state before and after treatment was assessed using the first half of the scale (statements № 1-20), whereas the dynamics of the therapeutic course was assessed by means of the second half of the scale (statements № 21-40).

Personal anxiety characterizes permanent proneness of people to perceive a wide range of situations as dangerous or threatening and to respond to such situations with elevations in the intensity of their state anxiety. Very high reactive anxiety induced impaired attention, sometimesimpaired fine coordination. Very high personal anxiety directly correlated with the presence of neurotic conflict, with emotional and neurotic breakdowns and with psychosomatic diseases.

In order to determine the normal value of CL of the parameters of GROSL we examined a total of 30 apparently healthy donors (12 men and 18 women). The donors' age varied from 19 to 57 years (mean 39.66±1.67 years).

Therapeutic policy in patients corresponded to the standards and included a set of surgical, therapeutic and prosthetic measures. All patients were subjected to removal of dental deposits, smoothing and polishing of the exposed root portion, immobilization of movable teeth by splinting. Local therapy consisted of mouth rinsing with antiseptics, applications on the mucous membrane of the gingival margin, and administration of anti-inflammatory agents to the periodontal pocket.

The patient's condition was assessed in dynamics (at admission, on days 14 and 30 of the therapy) based on a set of clinical, laboratory and instrumental methods: studying case history and complaints, examining of the oral cavity, gingival margin of the mucous membrane with the assessment of the degree of gingival edge recession and teeth motility, studying salivary total protein, alkaline phosphatase (ALP), MDA and catalase, blood plasma FRP by the GROSL values – a basal parameter of intensity of chemiluminescence (PICLb) and zymozan-stimulated chemiluminescence intensity (PICLs), by indices of plasma APA, MDA, X-ray picture based on orthopantomogram with analysis of reduction of the interalveolar septum height and the level of teeth root exposure, measuring periodontal pockets depth with a periodontal probe, periodontal index (PI), Fedorov-Volodkin hygiene index (HI), index of the degree of inflammatory events, gingivitis, papillary marginal alveolar index (PMA).

Chemiluminescent (CL) parameters of GROSL were examined using chemiluminescence-adapted luminometer LKB "Wallac" (Sweden) at the standard temperature of 36.9° C. We determined the level of the basal (spontaneous) CL in the standard volume of leukocyte suspension with the standard leukocytic concentration (2500 in 1 μl). After adding a non-specific activator (0.1 ml of 1% zymosan solution) we determined PICLs. MDA, a secondary by-product of LPO, was determined according to the technique described by Douest J.C. (1983). The methodology of studying plasma APA is based on measuring and comparing the indices of plasma CL induced by hydrogen peroxide and spontaneous plasma CL (Ind/Sp CL). The calculated parameter of the ratio is a value inversely proportional to plasma APA. The lower this ratio is, the higher APA, and vice versa.

The obtained data were statistically processed using Microsoft Excel software and statistical data analysis package Statistica 8.0 for Windows (StaSoft Inc., USA) and SPSS 15.0. Differences were regarded as statistically significant if $p<0.05$. Quantitative variables were tested for normality of distribution using the Shapiro-Wilk criterion. The obtained findings were evaluated using the following methods of statistical analysis: Pearson's chi-square test (contingency table analysis), Student's t-test and Newman-Keuls criterion for multiple comparisons. Independent non-parametric samples were analysed using Mann-Witney test, whereas for multiple comparisons we used the Kraskell-Wallis criterion. Dependent non-parametric samples were analysed using the Wilcoxon criterion, with multiple comparison performed by means of Friedman's criterion.

RESULTS

The values of the GROSL (PICLb and PICLs), LPO (blood plasma MDA) and plasma APA (plasma induced-to-spontaneous CL ratio) are shown in Table 1.

Table 1. *PICLb (mV/s × 10^6 leukocytes), PICLs (mV/s × 10^6 leukocytes), blood plasma MDA (μmol/L) and plasma induced-to-spontaneous CL ratio in healthy donors*

	PICLb	PICLs	Plasma MDA	Plasma induced-to-spontaneous CL ratio
Healthy donors	67.56±5.23	448.61±33.56	3.21±0.15	2.76±0.14

The results of studying PICLb in healthy donors and patients with CGP in the phase of exacerbation and remission are shown in Fig. 1.

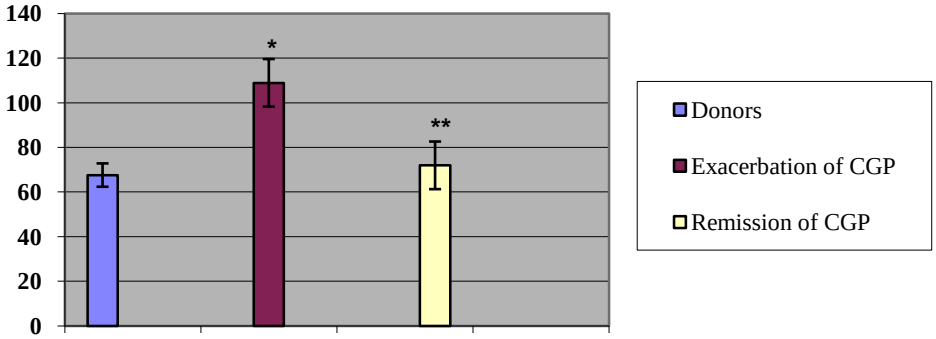

Figure 1. *PICLb (mV/s × 10^6 leukocytes) in healthy donors and patients with CGP in the phase of exacerbation and remission;*
* p<0.05 compared with healthy donors; ** - p<0.05 compared with exacerbation phase.

The average value of PICLb in patients with CGP in the exacerbation phase amounted to 108.90±10.67 mV/s × 106 leukocytes, which was 1.6-fold higher than that in donors, p<0.05. The average value of PICLb in patients with CGP in the remission phase amounted to 71.92±10.67 (mV/s × 106 leukocytes), which was 1.5-fold less than that in patients in the exacerbation phase (p<0.05) and non-significantly above the norm.

The results of studying PICLs in healthy donors and patients with CGP in the stage of exacerbation and remission are shown in Fig. 2.

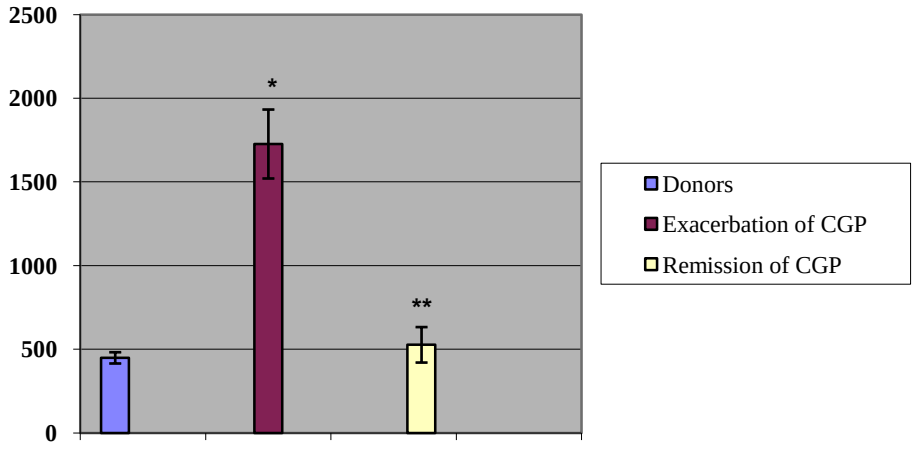

Figure 2. *PICLs (mV/s × 10^6 leukocytes) in healthy donors and patients with CGP in the phase of exacerbation and remission;*
* p<0.05 compared with healthy donors; ** - p<0.05 compared with exacerbation phase.

The average value of PICLs in patients with CGP in the exacerbation phase amounted to 1726.53±206.00 mV/s × 106 leukocytes, which was 3.9-fold higher than that in donors (p<0.001). The average value of PICLs in patients with CGP in the remission phase amounted to 526.53±106.00 (mV/s × 106 leukocytes), which was 3.3 times less than in patients in exacerbation phase (p<0.05) and non-significantly above higher than the norm.

The results of studying blood plasma MDA in healthy donors and patients with CGP in the phase of exacerbation and remission are shown in Fig. 3.

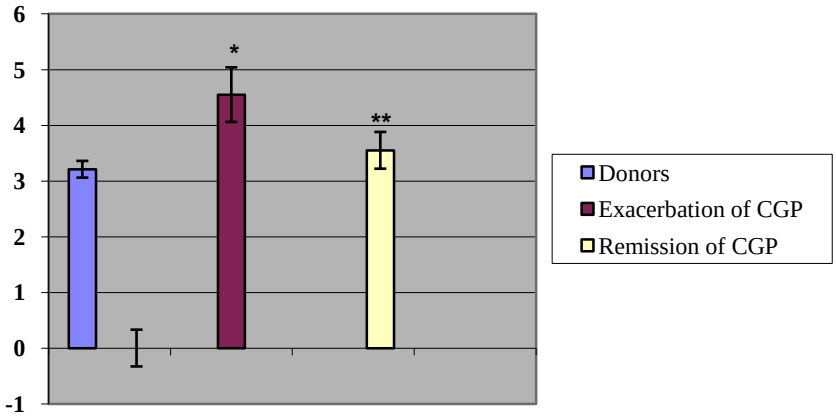

Figure 3. *Blood plasma MDA (μmol/L) in healthy donors and patients with CGP in the phase of exacerbation and remission;*
* p<0.05 compared with healthy donors; ** - p<0.05 compared with exacerbation phase.

Averagely, blood plasma MDA in with CGP in the exacerbation phase amounted to 4.55±0.43 μmol/L, which was 1.4 times higher than blood plasma MDA in donors (p<0.05). Blood plasma MDA in remission-phase CGP patients averaged 3.55±0.33 μmol/L which was 1.3 times less than in exacerbation-phase CGP patients (p<0.05) and non-significantly above the norm.

The results of studying the value of the plasma induced-to-spontaneous CL ratio in donors and patients with CGP in the exacerbation and remission phase are shown in Figure 4.

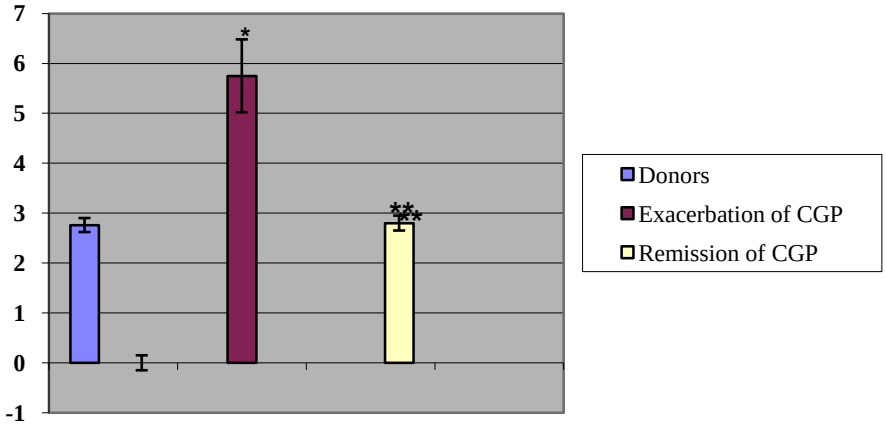

Figure 4. *Blood plasma induced-to-spontaneous CL ratio in healthy donors and patients with CGP in the phase of exacerbation and remission;*
* p<0.05 compared with healthy donors; ** - p<0.05 compared with exacerbation phase.

The average value of the blood plasma induced-to-spontaneous CL ratio in exacerbation-phase CGP patients amounted to 5.75±0.7, which was 2.1 times higher than that in donors (p<0.05). The value of the plasma induced-to-spontaneous CL ratio in patients with remission-phase CGP averaged 2.80±1.5, which was 2.1 times less than in patients in the exacerbation phase (p<0.05) and non-significantly above the norm.

Analyzing and generalizing the above described data made it possible to draw a conclusion that patients with exacerbation-phase CGP were found to have: a) a natural significant increase in both basal and stimulated PICL compared with healthy donors; b) increased blood plasma MDA as compared with that in healthy donors (p<0.05); c) a significant decrease in plasma APA compared with healthy donors (p<0.05).

Patients with remission-phase CGP as compared with the exacerbation phase were found to have the following:

a) a regular, significant decrease in both basal and stimulated GROSL;

b) a significant decrease in blood plasma MDA;

c) a significant enhancement of plasma APA;

d) all parameters remained above the norm; however, p>0.05.

Taking into consideration a presumptive pathogenetic role of ROS and LPO in the development of CGP, we found it feasible to examine the parameters of GROSL, plasma LPO and APA in patients with a mild, moderate and severe course of the disease (Figures 5, 6 and 7).

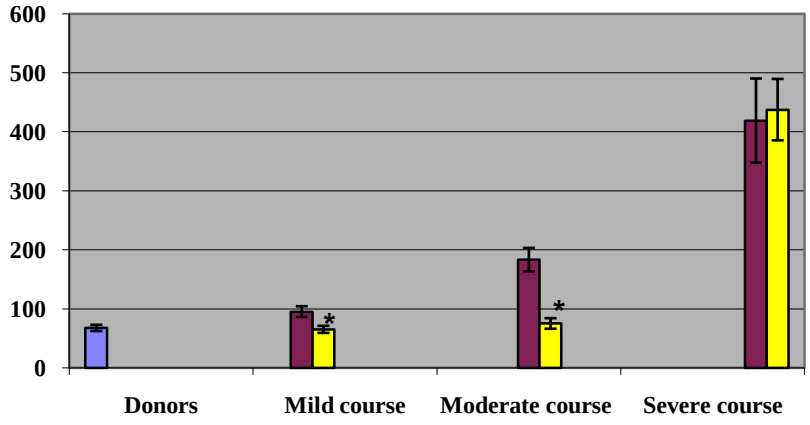

Figure 5. *PICLb (mV/s × 10^6 leukocytes) in patients presenting with mild (n=9), moderate (n=15) and severe (n=4) course and treated by AO;*
- prior to the treatment, - after the treatment;
* p<0.05 compared with the exacerbation phase.

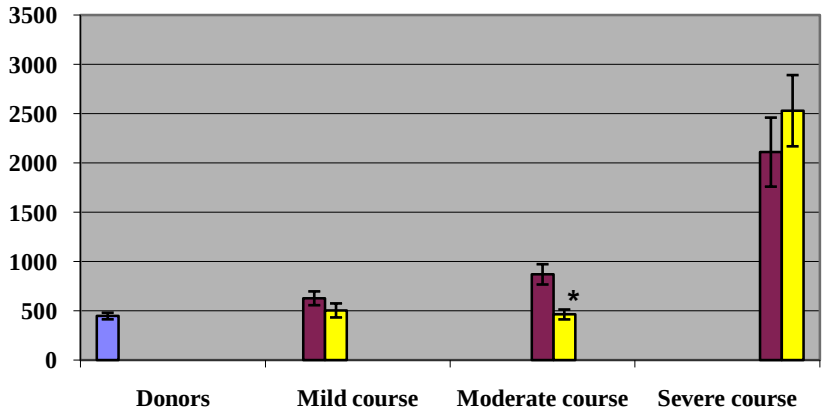

Figure 6. *PICLs (mV/s × 10^6 leukocytes) in patients presenting with mild (n=6), moderate (n=10) and severe (n=4) course and treated by AO;*
- prior to the treatment, - after the treatment;
* p<0.05 compared with the exacerbation phase.

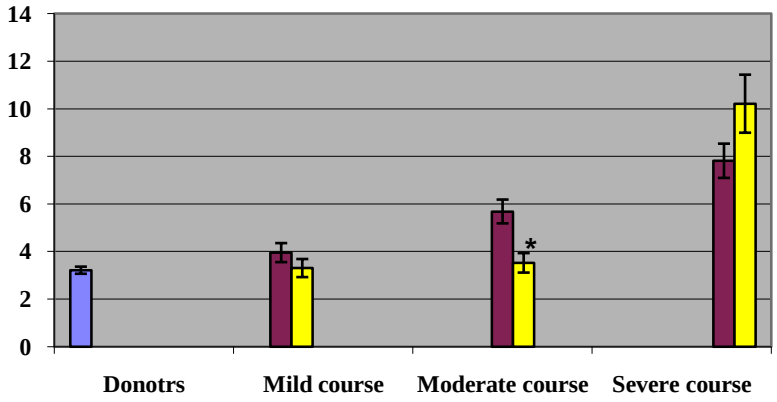

Figure 7. *Blood plasma MDA (μmol/L) in patients presenting with a mild (n=9), moderate (n=15) and severe (n=4) course and receiving treatment with AO; - prior to the treatment, - after the treatment;*
* p<0.05 compared with the exacerbation phase.

The PICLb in patients with a mild course of the disease (n=24) averagely amounted to 94.58±18.77 mV/s × 106 leukocytes, which was 1.4-fold higher than that in donors (however p>0.05); the PICLs averaged 455.87±57.98 mV/s × 106 leukocytes, which practically did not differ from that in donors (p>0.05).

Blood plasma MDA in patients with mild CGP (n=24) averagely amounted to 3.85±0.26 μmol/L which was 1.2 times higher than that in donors (p<0.05).

The blood plasma induced-to-spontaneous CL ratio in patients with mild CGP (n=24) averagely amounted to 2.82±0.17, not virtually differing from that in donors, p>0.05.

Hence, patients with mild CGP were found to have a significant elevation of only blood plasma MDA.

The value of PICLb in patients with moderate CGP (n=35) averagely amounted to 175.66±22.76 mV/s × 106 leukocytes, thus exceeding 2.6-fold the average PICLb in donors (p<0.001); the PICLs averaged 852.36±98.22 mV/s × 106 leukocytes, thus exceeding 1.9-fold the PICLs in donors (p<0.001).

Blood plasma MDA in patients with moderate CGP (n=35) averagely amounted to 4.50±0.32 μmol/L, thus exceeding 1.4-fold the average blood plasma MDA in donors (p<0.001).

The value of the plasma induced-to-spontaneous CL ratio in patients with moderate CGP (n=35) averagely amounted to 3.86±0.28, exceeding 1.4-fold that in donors (p<0.05).

Hence, patients with a moderate course of the disease demonstrated a significant increase in both basal and stimulated GROSL, as well as blood plasma MDA and a decrease in plasma APA.

The value of PICLb in patients with severe CGP (n=13) averagely amounted to 445.90±54.00 mV/s × 106 leukocytes, which was 6.6-fold higher than that in donors (p<0.001). the value of PICLb averagely amounted to 2108.47±317.78 mV/s × 106 leukocytes, which was 4.7 times higher than that in donors (p<0.001).

Blood plasma MDA in patients with severe CGP (n=13) averagely amounted to 7.38±0.82 μmol/L, which was 2.3 times higher than that in donors (p<0.001).

The value of the plasma induced-to-spontaneous CL ratio in patients with severe CGP (n=13) averagely amounted to 5.24±0.68, thus being 1.9-fold higher than that in donors (p<0.05).

Hence, patients with a severe course in the exacerbation phase demonstrated a significant increase in both basal and stimulated GROSL, blood plasma MDA and a decrease in plasma APA.

Comparing the CL parameters of GROSL in various severity of CGP, the following differenced were detected: a) between PICLb and PICLs in patients with mild CGP on the one hand, and patients with moderate and severe CGP on the other, in favour of an increase in the latter (p<0.05 for all parameters) and between PICLb and PICLs in patients with moderate and severe course in favour of the latter (p<0.05 for both parameters); b) patients with moderate and severe CGP had increased blood plasma MDA as compared with patients with mild CGP (p<0.05 and p<0.05) and in patients with severe CGP it was also higher than in patients with moderate CGP (p<0.05); c) plasma APA was significantly lower in patients with severe CGP as compared with patients with mild-to-moderate CGP (p<0.05 and p<0.05, respectively).

We also studied peculiarities of situational and personal anxiety, as well as depression, once present in patients with CGP, depending on the disease severity (Table 2).

Table 2. *Parameters of anxiety and depression in mild, moderate and severe periodontitis according to the findings of the Spielberger's test*

Severity of periodontitis	Situational anxiety	Personal anxiety	Depression
Control group	18.1 ± 3.9	21.4 ± 5.4	4.2±1.3
Mild			
before treatment	33.8 ± 3.6*	46.1 ± 3.6 *	13.1 ± 2.4*
after treatment	20.5 ± 2.8 ##	29.1 ± 3.2##	6.2 ± 0.8##
Moderate			
before treatment	39.8 ± 2.7*	53.4 ± 4.0 *	16.6 ± 4.8*
after treatment	27.4 ± 2.9##	34.4 ± 4.2 * ##	10.4 ± 3.0*
Severe			
before treatment	49.9 ± 5.0 * **	68.7 ± 5.1 * ** #	19.2 ± 4.4*
after treatment	55.9 ± 5.1* ** #	78.5 ± 7.2* ** #	24.6 ± 5.1* ** #

Designations: * - p<0.05 as compared with the control group;
** - p<0.05 as compared with mild course;
# - p <0.05 as compared with moderate course;
- p< 0.05 as compared with patients before treatment of the respective groups.

It was revealed that the increased severity of CGP was associated with elevations in both situational anxiety and personal anxiety, as well as depression. In patients with severe CGP, situational and personality anxiety were 1.5-fold higher than in patients with mild CGP (p<0.05), and depression was also 1.5-fold higher; however, p>0.05.

Having detected in previous studies that after the carried out treatment there remained some patients showing neither clinical effect nor dynamics of alterations in the parameters of free-radical processes, we found it feasible to explore the effect of AO therapy on patients with CGP depending on severity.

Patients with mild and moderate CGP under the influence of AO therapy demonstrated a decrease in PICLb 1.5- and 2.4-fold, respectively (p<0.05 and p<0.05, respectively, Fig. 5). However, quite opposite results were obtained in patients with severe CGP. PICLb in these patients after the carried out AO therapy increased 1.1-fold (p>0.05). Thus, AO therapy in patients with severe CGP was accompanied and followed by increased PICLb.

Similar results were obtained in studying PICLs and blood plasma MDA under the effect of AO therapy (Figures 6 and 7). In all patients, AO therapy according to these indices also turned out to be ineffective.

Besides, patients with severe CGP had no clinical improvement. The signs of inflammation failed to regress; the depth of the periodontal pocket did not change, with no dynamics of dental scales either.

Hence, antioxidant therapy in patients with severe CGP resulted in neither clinical improvement nor a decrease in free-radical processes.

Simultaneously, patients with severe CGP after AO treatment were found to have elevated situational and personality anxiety, deteriorated depression, unlike patients with mild-to-moderate CGP in whom this symptomatology virtually disappeared.

Therefore, we came to a conclusion that these patients should be subjected to surgical treatment. The latter included

curettage of periodontal pockets, periodontal flap operations, and tooth extraction.

In patients with a severe course of periodontitis after surgical manipulations, the parameter in Hounsfield units increased gradually, on days 14, 30 and 90 averagely 1.3-, 2.4- and 3.2-fold, respectively.

The findings of the analysis of dental cone beam computed tomography with the help of determining bone tissue density in Hounsfield units suggested that bone mineral density increased faster after surgical methods of treatment in patients with severe chronic generalized periodontitis. This is an indirect sign of success of carried out manipulations.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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DEVELOPING CHARACTERISTICS AND COMPETENCES OF A HEALTH CARE MANAGER: LITERATURE REVIEW

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RAZVOJ KARAKTERISTIKA I KOMPETENCIJA MENADŽERA U ZDRAVSTVU: PREGLED LITERATURE

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ABSTRACT

Broadly educated professionals are a rely valuable resource for the Russian public health system in the current socio-economic conditions. Their capability to flexibly switch the focus and content of their activities in the changing and challenging environment is essential for successful performance.

The purpose of our study is to analyze the international evidence on managing the process of competencies' development in healthcare managers.

Competence-oriented education and training of future healthcare managers is of special interest. In light of this, the study of international data on the organization of the processes of key competence development is important. Here with, it is necessary to take into account the content of the studies, resources and technologies, factors that influence on this process, the development and evaluation of models aimed at improvement of the education of healthcare management professionals at medical schools.

We believe that the results of this review will help to develop and offer a number of practical steps aimed at optimizing the education and training of healthcare managers. In addition to a review of international data, this article presents an innovative project for the development of professional competencies of health managers – the Academy of Talent Development in Healthcare (ATDH). This project involves students of the Sechenov University who want to learn how to lead healthcare teams efficiently.

Keywords: *Healthcare managers, public health, leadership, management competences, medical education.*

SAŽETAK

Široko obrazovani profesionalci su dragocen resurs za ruski javni zdravstveni sistem u trenutnim socio-ekonomskim uslovima. Njihova sposobnost da fleksibilno promene fokus i sadržaj svojih aktivnosti u promenjivom i izazovnom okruženju je neophodna za uspešan rad. Svrha naše studije je da analizira međunarodne dokaze o upravljanju procesom razvoja kompetencija kod menadžera u zdravstvu.

Posebno je interesantno obrazovanje i obuka budućih zdravstvenih menadžera orijentisanih na kompetencije. U tom smislu, važno je proučavanje međunarodnih podataka o organizaciji procesa razvoja ključnih kompetencija. Pri tome je neophodno uzeti u obzir sadržaj studija, resursa i tehnologija, faktore koji utiču na ovaj proces, razvoj i evaluaciju modela koji imaju za cilj poboljšanje obrazovanja stručnjaka zdravstvenog menadžmenta u medicinskim školama.

Verujemo da će rezultati ovog pregleda pomoći da se razvije i ponudi niz praktičnih koraka usmerenih ka optimizaciji obrazovanja i obuke menadžera zdravstva. Pored pregleda međunarodnih podataka, ovaj članak predstavlja inovativni projekat za razvoj profesionalnih kompetencija zdravstvenih menadžera - Akademije za razvoj talenata u zdravstvu (ART). Ovaj projekat uključuje studente Univerziteta Sečenov koji žele da nauče kako da efikasno vode zdravstvene timove u svojim ustanovama.

Ključne reči: *Menadžeri u zdravstvu, javno zdravlje, liderstvo, kompetencije menadžmenta, medicinsko obrazovanje.*

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INRODUCTION

Broadly educated professionals are a rely valuable resource for the Russian public health system in the current socio-economic conditions. Their capability to flexibly switch the focus and content of their activities in the changing and challenging environment is essential for successful performance (1−3). In this regard, the selection and placement of personnel should be carried out with particular carefulness. Both their special training in public health and healthcare management and their general level of skills must be at high level. These professionals must possess the necessary skills and competencies in their chosen professional field (2, 4).

As it often happens, the inexperienced specialists, having rather a vague idea about the organization of health care, are appointed as the heads of facilities, including positions of chief doctors of hospitals and out-patient clinics. Here with a lack of clear confidence in the properly chosen professional pathway of healthcare managers leads to the situation that even the healthcare managers with significant administrative experience prefer to see themselves rather as clinical specialists (physicians, surgeons, etc.), but not healthcare managers. At the same time, it was found that the higher the administrative position is, the larger is the number of administrative functions to perform, compared to the number of clinical functions. The results from a study on the job satisfaction in healthcare managers demonstrate that the reasons for low satisfaction often lie in their own inability to organize their work and activities of subordinates, as well as in the lack of systematic approach to performance arrangement (3−5).

Certainly, a combination of specific training in management and working experience is needed to improve the quality of performance of the healthcare managers Therefore, an increase in the quality of education of the future healthcare managers is required for performance of the management functions in their future professional activity (2, 4).

In light of the above, the analysis of the international data on the development of the skills required for the effective implementation of administrative functions in health care organization is of interest.

The analysis of international data led us to the need of a complete modification of training for health managers that meets the long-term health system requirements. That is why we set up an innovation training program for healthcare managers at Sechenov University, which includes extensive use of active learning methods such as educational games, trainings, case studies, round-table discussions, master classes, mass open online courses, project development, training videos and many others modern educational approaches.

Objective of the study

The purpose of our study is to analyze the international literature on managing the process of competencies development in health care managers.

Current health care system

From the 90s, along with increasing requirements for higher professional education, the paradigm of achieving the outcome of the educational process has changed. The transition to student-centered education enhances the interaction between education and culture, and the culture plays a role of essential prerequisite for the realization of the creative potential of a person, which determines the inclusion in the educational process of the so-called culture-based technologies (2, 5). Today, most experts consider that besides purely professional knowledge and skills the university graduate should be able to apply practically, the graduate should also have the appropriate personality traits, developed cognitive, emotional and axiological spheres (1, 3, 6).

Some authors point out the conflicts in the modern Russian educational system, between:

- the university educational outcomes and the needs of the labor market;
- the declared humanistic nature of education and the real image of a graduate;
- the integrative nature of the activities of the future graduate and ineffective teaching methods;
- a desire to create a joint educational space with other (mostly European) countries and the reluctance of the Russian higher education to take into account emerging global trends in education (3, 8).

The most important survey on this problem was conducted by prof. Starodubov V. in 2014 (45) in Russia. The survey covered 33000 healthcare managers in the Russian Healthcare (HC) sector. The survey results demonstrate that there is an equal chance to meet a male or female in this professional field. They are rather young with the average age of 49. Most of them work for more than 10 years as administrators. If we take a look at their educational background, we can see that they all are medical doctors and 1/3 of them have not been trained in healthcare management. 22% of HC managers have got an additional degree either in management, economics, or law and psychology. Russian HC managers have rich working experience, but they are not aware of modern management skills and technologies. They rely mainly on their personal qualities and try to avoid risky decisions.

Also, we can see that almost 40% of HC managers in Russia came to work at their position by chance. About 28% were promoted by the personal pool and 33% managers had in their career plan. Our recent retrospective study (50) has discovered that only 7% of healthcare managers had decided upon their future specialization at the University, 20% –3 years following the graduation and 73 % – had chosen this career even later. It is an interesting fact that generally 95% of students make the decision on the specialization either in therapy or in surgery in early years at medical schools. Currently, most medical public organizations are managed by doctors, who started their career

as clinicians and were promoted to the top administrative positions.

But, in most cases, these people have no special knowledge and competences in management. The most common solutions are suggested by introduction of master's programs in healthcare management available to students with non-medical background.

The programs should be competency-based and provide knowledge, skills and characteristics.

The competency-based approach to training healthcare professionals consists of 5 main blocks:

The 1st block is professional skills, combined with professional and social responsibility and personal engagement in continuing professional development.

The 2nd block is the knowledge of a health system, patients' needs and human resources, understanding the environment of the organization and individual contribution of a specialist.

The 3rd block is business administration which includes strategic, financial and general management, risk management, HR management and quality assurance.

The 4th block covers communication skills, including partnerships and negotiations.

The 5th block - Leadership is formed by the ability to translate the vision, influence and involve people and build attitudes.

The strategy of modernization of modern higher education

Because of the changed requirements of vocational training and social maturity of university graduates, many experts consider the need to improve the educational process for the purpose of its orientation towards the maximum realization of the creative potential of the student and the development of the student motivation towards further self-development. Therefore, the strategy of modernization of modern higher education is based on the introduction of competency-based approach, which is now recognized as one of the most promising approaches to the modernization of the educational process (5–8). This approach aims at the developing student knowledge and skills, as well as a number of key competencies in intellectual, communication, information and other areas (3, 8). In accordance with this set, the structuring of the learning process must be carried out, which will allow students to be able to effectively master these competencies during education.

Currently, most researchers consider the competency-based approach as the most important way of solving the challenges facing the entire sector of education, believing that its implementation should provide influx of competitive specialists and thus contribute to the economic development of the country, promote the social and labor adaptation of youth, maintain Russian scientific and educational potential and the enhance international cooperation prospects (2, 5, 7, 8).

Analysis of international data shows that the following factors should be taken into account in the process of justification of the training model of specialist-organizer of health care:

positions that are assumed in the implementation of health management programs. It is shown that the most in-demand are experts in epidemiology, economics and health management;

educational background. Compulsory basic education, as a rule, is a bachelor's degree of the University;

principles of healthcare managers training, which are based on so-called dual education (medical and non-medical), which is implemented at two levels (basic education and specialization in health management);

educational programs that promote careers of the healthcare managers.

The main attention is paid to the training of practical skills in solving the tasks of the effective management of resources (labor, material, financial) in medical organization.

According to common ideas of international experts on the model of training managers, this model primarily includes innovation, creativity and competence in the field of management. In addition, the manager must demonstrate entrepreneurship, high motivation and responsibility. An ideal manager is a person who looks to the future, manages people by involvement and personal example and strives to the ultimate goal "no matter what" (9). Similar properties are emphasized in the new management model, under which an ideal manager is a responsible person who has the real interest in management and devotes himself/herself to the interests of the organization (10). These characteristics have been proposed by the representatives of the national health service of Finland during the study of the involvement of clinical experts in health system management process (9).

Considering the views of international researchers on various aspects of the competence-based approach in medical education, we note that Griewatz J. et al. (2016) studied the opinion of four professors from German universities on the importance of development of different competences and implementation in the "domestic roles" in the training as the healthcare managers (11). It has been shown that most teachers appreciate the importance of the role of "medical expert", "communicator" and "employee". Other internal roles, such as "health advocate" and "teacher" are less perceived by study participants (11).

Andreasson J. et al. (2016) evaluated the significance of leaders qualities in various sectors of the healthcare system and the importance of the role of different qualities with re-

spect to improving the process of health care delivery. The surveys of managers in Swedish hospitals were carried out. The central topic of discussion in this case was the so-called "coaching of participation", which is implemented both "vertically" and "horizontally". The latter approach refers to the management strategy for motivating and engaging their employees to the activity for the improvement of the quality of health care. It has been shown that the improvement of health care processes requires a "coaching" management, built on close interactions between a manager and specialists of the individual units, here with managers must share the responsibility for the results with their teams of specialists (12).

Schoenmaker S. et al. (2013) studied the integration of the development of a number of management skills in the frameworks of medical education in Australian medical colleges. In particular, it was shown that the role of a leader is one of the most important competencies of the future doctor, but it attracts too little attention during the training. The aim of the study was to study the perception of managerial skills by young doctors. The survey of 1376 young professionals was carried out in respect to their evaluation of their own needs in the development of manager skills. Most of the respondents assessed the level of their knowledge and management skills as average and pointed to the need for the further development of these knowledge and skills (13).

The aim of the Liang Z. et al. (2013) study was to evaluate the key competencies required for middle and senior managers in public hospitals of both urban and rural areas of Australia. The authors also highlighted 6 key competencies required for training of future managers of health services: management, leadership, organizational behavior, human resource management, strategic planning and financial management (14).

Foreign experts also agree that for the purposes of effective management of diagnostic and treatment processes, in terms of the authors of “Clinical practice guidelines", it is necessary to carry out the justification and development of a model, which would consider the "central role of clinicians in planning, healthcare delivery and the improvment of healthcare quality" (15, 16). In recent years, there have been many studies about the characteristics of training and the activity of clinical specialists who hold the management positions in the health care system (17−19).

Literature review

A number of authors developed an idea of creating an environment in which clinicians could develop their skills and gain the necessary knowledge that would later enable them to become managers. A number of programs are devoted to the study of factors influencing the decision of clinical professionals about moving to leadership positions, including the projects of the European program of cooperation in the field of science and technology "Enhancing the role of clinicians in the management of the European health care system" (20).

In medical institutions of Ireland, the involvement of clinical specialists into the process of health care management is considered a "key factor determining the effectiveness of the medical institution" (21). A series of studies carried out not only in Europe but also in several other countries, including Australia, were dedicated to study characteristics of the governance of medical institutions (17, 22).

The concept of involving clinicians in the health management process was met with interest in Norway after the introduction of the unitary management system in 2001. According to the recommendations of the Office of the Auditor General of Norway, clinicians should be more actively involved in the process of making strategic and budgetary decisions to improve the economic efficiency of the health system (23).

The experience of Japanese companies with their high efficiency is also of interest and it shows that it is expensive to conduct management training on the basis of including to the natural traditional method of leaders’ development through the official promotion of certain elements inherent in an artificial method that involves extensive target training.

A considerable amount of research is devoted to various aspects of leadership; herewith the leadership is understood as the ability to motivate others to achieve change, while the management is defined as the achievement of results through planning, organization and problem-solving (24-26). Edmonstone J. (2011) designates as the clinical leaders those professionals who are involved in patient care and simultaneously involved in the management process (27). It has been shown that professional dominance and autonomy are the most important motivating factors for the appointment of professionals to managerial positions (28, 29).

According to most experts in the field of management training, one of the fundamental functions of a leader is a vision of the future. Also, a creative approach is needed to develop new solutions that can lead the industry or organization to success. Ackoff R., Purdehnad J., (2009) believe that, along with the skills necessary to all administrators and managers, leaders must have the qualities such as credibility, imagination, honesty, cooperation and adaptability (30).

Vision of the future and creative foundation of leadership are closely linked. Individuals who have the creativity potential form the creative class, which includes all of those who are involved in the creation of new forms in any sphere of activity. The system of values of such sort of individuals includes individuality, self-expression, meritocracy and transparency (31).

The foregoing is relevant to the issue of leadership training in medical institutions. As it is known, the health care system in the developed countries is considered a strategically important sector of the economy, which requires adequate regulation and control. In recent years, the process of health care becomes more technologically advanced, being supported by the standardization, implementation of information and communi-

cation technologies, the use of high-tech methods of diagnosis and treatment. All of these processes contribute to the change of management principles of both a medical organization and the health care system as a whole. A major role in this process must be played by the principles of effective leadership, which, according to some researchers, use all known arsenal of theoretical approaches to leadership, including tvarious theories of leadership (*Leader Member Exchange Theory, LMX*; *Transformational Leadership Theory*; *Authentic leadership Theory*; *Servant Leadership Theory*; *Resonant Leadership Theory*) (32).

From the standpoint of the Harvard Business School, the management is an arbitrary (focused) influence on people and their community, which leads to their conscious and active behavior and activity.

The main differences between leadership and management are as follows:

the management provides the organization of the whole group, while the leadership characterizes psychological relations arising in the group "vertically"; that is, from the viewpoint of domination and subordination relations;

the management is a natural and necessary attribute of the emergence of a formal organization, while leadership arises spontaneously as a consequence of the people interactions.

The leader is the subject of group norms and expectations that are formed spontaneously in interpersonal relationships. The successful implementation of the model of "distributed" leadership is possible when two conditions are met: the group is composed of people who are on the same intellectual, cultural and social level, and they have effective communication skills and the bases of behavior of both leader and the subordinate. As a result of this interaction a sense of deeper satisfaction with the achieved results arises. The most successful healthcare organizations consider all of its employees, including all doctors, as potential leaders in the field of their activity (33).

Various countries pay considerable attention to issues of leadership skills development of medical staff. Harvard Business School carries out the educational program "Management of the provision of health services" since 2009. This program is designed for senior-level health care specialists. The program has sparked interest in many countries: only in the first year, 67 people from 16 countries took part in this program. Its main goal is to help professionals in the medical field to form leadership, strategic and financial competences for the improvement of the quality of healthcare (34, 35).

According to sociologists, desire of clinicians to be nominated for positions, which involve broad powers, is largely conditional upon the fact that the work of the clinical specialist is constantly associated with struggle against various hindering factors and the overcoming of obstacles. The studies of the activities of clinicians in the healthcare management support this view. For example, Doolin B. (2001) showed that New Zealand doctors make decision about the managerial position with purposes of some kind of "protection of the clinical practice against intervention on the part of hospital administration" (22).

Forbes T. et al. (2004) carried out a survey of experts in Scotland and found that many doctors have decided to perform the duties of a manager to "protect their profession from influence by unauthorized persons or those they consider as inappropriate persons for the role of the healthcare leader" (36). Similar results were obtained in a survey of experts in Norway (37, 38). According to Edmonstone J. (2011), this is largely due to the fact that public health and, in particular, clinical medicine is characterized by the so-called representative, rather than traditional hierarchical management system. The gist of it is that doctors have a responsibility not only in front of governments and higher authorities, but also in front of colleagues who perceive them as representatives of their views and interests (27). Other researchers point out that, though the majority of authors focused on doctors, the same situation can be observed when analyzing the views of other health professionals, such as nurses (9). In particular, Norway is one of the countries where this category of health care workers directly compete with doctors for management positions. According to Johansen and Gjerberg (2009), Norwegian nurses occupy management positions to improve their professional status (38). This fact confirms the sociological theory, according to which the post of the manager is seen as a tool to strengthen the position in the main profession.

Developing characteristics and competences of health care manager

Despite the all facts regarding the motivation, both cases involve voluntary participation in the process. If the first approach describes the administrative process from the strategic point of view, then, according to the second approach, people hold administrative positions because of the interest in the work and motivation.

In general, the authors agree that the process of development of a doctor as a leader can be divided into three stages: awareness of the leading position, the adoption of the role of the manager and the gaining of experience in this activity. Most of the surveyed executives thought that they were rather persuaded to become managers, while they themselves did not show an active desire for this (39, 40).

Certain resemblance to the Russian practice can be traced, in which an entry into the leadership position without the proper level of training to do the job is observed quite often. As reported by foreign participants of the study, they are often forced to learn the principles of management "on the fly", being left to themselves (41).

Some authors describe a situation where doctors became managers by accident (36, 42), in terms of foreign researchers, such cases are treated as "the concept of path dependency" and include a scenario in which the doctor accidentally takes a

control position, and in some cases is "stuck" in this position (42). The individual experts give assent to the higher post purely because of financial reasons and later regret their choice, but the return to the former position is not possible (43). In connection to this, a number of clinicians think that the cessation of clinical activity is certain "point of no return" after the passage of which the doctor does not have other option but to fully devote himself/herself to administrative work (44).

Mo T.D. (2008) compiled a record about Norwegian doctors occupying positions of public health executives and their motivation of administrative activities. Some specialists have reported that they took up a manager position "out of curiosity" (37). Forbes T. et al. (2004) described some of the interviewed experts by the term "investors", because they are actively engaged in administrative work as an alternative to clinical activity (36). However, most of the senior leaders of health care, according to Ham's S. et al. (2011), relate to their work positively (45). The same authors reported problems such as lack of training of doctors for administrative positions: a considerable part of specialists is initially unable to cope with the increased workload. Polls showed that in most of the cases there is a lack of proper organizational support of these specialists, and during the transition to an administrative position, the doctors often do not receive practical advice and assistance (45). According to Ham C. et al. (2011), transition to the administrative position after clinical work is accompanied by a significant change in the conditions of the activity, because a clinical practice in those countries is often routine and standardized, while administrative activities are usually very dynamic. The specialists at the beginning of their leadership activity often feel overwhelmed by that; according to the author, it is a consequence of their inability to delegate some of their work to other specialists (45). Specialists with a longer experience of administrative work reported, as a rule, their ability to effectively organize the work. Herewith, the senior executives, who participated in the study, noted that they learned how to use the experience of other colleagues: "It should be understood that the existing gaps in expertise and experience can be filled by the help of other specialists. Thus, more experienced colleagues can provide the proper level of support" (44).

Kane-Urrabazo C. (2006) noted that the delegation of tasks is one of the main goals of a medical manager (46). Specialists of the UK national health system, after reviewing their own leadership activities, reported a need in increasing the degree of transfer of the functions to other personnel and reduction of the independently performed work (47). These data suggested the need to develop specialists in the field of health care management with the skills of effective delegation of authority.

International experience of training of the management specialists for health care shows that trends in the development of this education include the transition from the programs of medical additional vocational education in public health and health care to the programs based on the management, an increase in the proportion of students with a basic non-medical education, as well as an increase in the number of training programs in the field of public health management in non-medical educational institutions (primarily in economic ones), an increase in the proportion of non-state educational and consulting centers offering the services in the field of advanced professional training for the specialists of healthcare management.

The above trends condition the emergence of new problems, directions and principles of the organization and content of education of the healthcare management professionals, especially, the training of middle and senior executives in accordance with their management level, prior education and personal preferences.

It is well-known that the level of training of medical specialists depends on the university learning technologies. These technologies should be based on the principles of the scientific organization of educational process, structuring the learning objectives, the optimum combination of fundamental and special disciplines, as well as theoretical and practical training. The control of the quality of the learning management, compliance of training programs and characteristics of future specialists are required. In turn, the effective application of the above principles is impossible without the continuous improvement of teaching staff training and provision of an adequate level of university infrastructure.

In the field of educational activities, it is necessary to implement the target orders for training of specialists in accordance with their official functions; the establishment and intensification of interaction between universities and medical organizations, including governance bodies of public health; the development and licensing of the respective programs of additional vocational education; the provision of the opportunities for the high education; the use of modern educational technologies, including those based on IT resources and distance technologies. Training of healthcare management should be based on the principles of continuity of education throughout working life, succession and consistency between the levels and stages of education.

Other authors concluded that involvement of motivated professionals in the leadership positions must be as early as possible (46, 47), that will increase the number of prospective candidates for these posts and thus reduce the proportion of individuals who have become managers "by chance" (22). Researchers believe that it is necessary to create a "direct career paths" for healthcare managers with the provision of training opportunities with the aim of eliminating the need observed in practice called "learning on the go".

It is also reported about the need to take into account the experience of organizations, which develop successfully functioning systems for the recruitment and training of potential, such as Mayo Clinic, which creates a support system for novice managers (48). Besides, some authors consider that mentoring is an effective method of training, because early involvement of mentors in the process of training and education

allows to educate the candidates for the posts of healthcare managers more effectively. As one of such measures, the creation of social networks for the exchange of experience between clinical managers is considered.

It should be noted that some foreign authors (27, 49) criticize the set of measures, used in the frameworks of the programs of the national health system for leadership training, and thus believe that the use of competency approach oversimplifies the concept of training of future health care leaders. Edmonstone J. andWestern J. (2002, 2011) believe that this approach can only have limited application in the framework of the modern health care system, which is becoming increasingly complex. According to them, an approach based on practical experience is more effective, because it helps in solving a number of problems, which unexpectedly arise in front of health authorities. The authors note that both approaches have their positive and negative characteristics that must be considered (27).

The Academy of Talent Development in Healthcare (ATDH)

In 2016, in Sechenov University, on the base of the decision of Faculty of Preventive Medicine on Public Health chair the Academy of Talent Development in Healthcare (ATDH) was founded. This project is intended for students of the Sechenov University who have shown an initiative to deepen their knowledge of the discipline "Public Health and Healthcare" andwho want to become leaders in the team and chief physicians of medical organizations.

A special feature of the training is the development of the Academy's corporate culture, which includes the following rules: the main participants in the class are students, teachers act as consultants; attendance is voluntary; mastering of each planned subject and passing the test is an obligatory condition of education; any sensible initiative in learning is welcomed; to get the result you need to work hard and stand on the shoulders of giants, respect the environment and the experience of your predecessors. This approach allows us to create high professionalism, innovative thinking, creative mentality, modern worldview culture and high social responsibility among specialists.

During the out-of-class time, which is part of student self-management, the teaching staff of the department conducts individual consultations on the successful accomplishment of tasks in the student council, the unions of students, the volunteer center, the student sports camp "Sechenovets", among schoolchildren, scientific societies, etc.

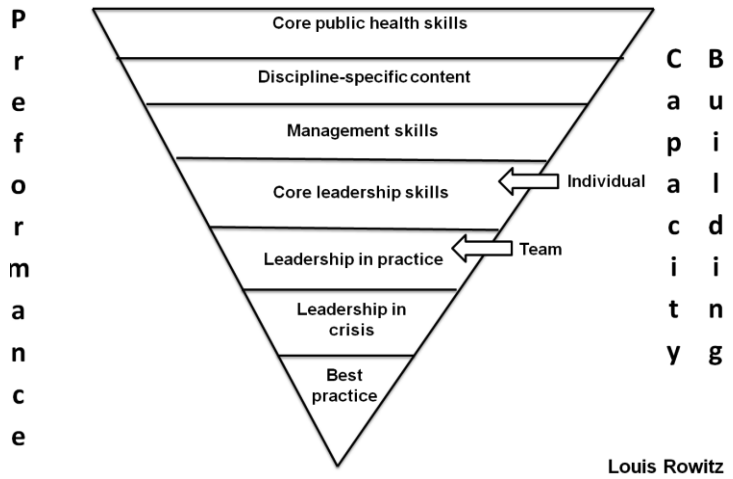

Figure 1: The leadership pyramid is the core idea of teaching program in The Academy of Talent Development in Healthcare

Each level of the pyramid requires a determination of the specific competencies necessary to master. Building capacity is divided into individual Competency and Team competency. Public Health professionals improve their skills by training in Core public health disciplines, Discipline – specific content, in Management module, PH workforce and Leadership.

Public Health professionals increased their personal skills, which can then be translated into team-based and other collaborative processes. The bottom of the pyramid emphasizes the importance of best practices.

We use modern didactic concept, which includes developmental teaching and problem-based learning, self-education, creative activity of students, communication training with professional activity, interactive learning and flexibility in solving the problems. Professors and teachers use active learning methods: work in small groups, business games, role play, case study and others.

Case studies are very important in training healthcare managers. They give an opportunity to simulate real situations from the routine of healthcare management. We use case studies for undergraduate and master students as well as for those

who take part in the Academy of Talent Development in Healthcare. The case studies are combined in a practical module "innovation in HC management"«, which covers 10 topics, including: effective HC manager, sharing of powers and responsibilities, complex decision making, development of the image and culture of HC organization.

One year ago we have initiated a research project aimed at early detection of students with a strong potential in management and promotion of the competences necessary for successful career in healthcare management. We enrolled 200 volunteers and randomized them into study and control groups. Enrollment criteria included:

1. Study for 4-6 years
2. Extracurricular activities
3. Strong motivation for success
4. Good communication skills
5. Adaptability

Both groups are currently being assessed on psychophysiological characteristics and leadership abilities.

Currently, there is ongoing research in individual approaches to training healthcare managers. Its main objective is to develop a pattern for individual curriculum and early career development for students with strong leadership capacities.

The primary outcome is an educational model for Health care manager training aiming at leadership competences development based on individual educational path.

The secondary outcomes include:

1. Detection of correlation between personal and psychophysiology characteristics that will give an opportunity to create a system of criteria aiming at potential Health care manager identification.

2. Development of a program of vocational route for higher education system for a competitive Public health specialist training.

3. Development of "The Academy of Talent Development in Healthcare"» with the aim of vector training of public health managers, for residency pre-training.

4. Implementation of database containing social, psychophysiological, demographical and personal characteristics of potential HealthCare leaders.

We also used physiological study to measure the adaptive capacity by EEG and ECG results. To assess the switching and distribution of attention, we used Red-black Schulte-Gorbov tables under EEG. We also evaluated "Intellectual lability" by assessing the ability of a subject to switch attention, multiple problem solving with no mistakes, etc.

We reviewed literature to identify physiological parameters present in people with strong managerial skills.

To assess the personal characteristics we used survey data via the use of personality tests: "leadership style test" (P. Hersey and K. Blanchard) and "Self-limitation analysis" (M. Woodcock and D. Francis). We assume that these questionnaires will help assess the readiness of young specialist to work in the field of health management.

We also used sociological methods to assess the impact of social factors and health status on leadership characteristics development.

Future perspectives

Future perspectives in development of public health care system are the following:

To identify and compare most significant social, psychophysiological and personal characteristics of students, residents and professionals in the field of Public Health and Medical Care Organization, which will help to collect data on social, psychophysiological, social-psychological characteristics and health status of students at different years of medical training at university.

To justify the system of socio-psychological and psychophysiological criteria of professionals in the field of Public Health and Medical Care Organization, with the aim of creation of Student's career guidance program defining the vector of training in a particular specialty.

To develop a flexible specialist training model in the field of Public Health and Medical Care Organization, including the design and implementation of individual educational routes, using modern educational methods. We believe that detection of correlation between psycho-physiological characteristics and leadership competences will give an opportunity to create a system of criteria aiming at potential HealthCare leaders' identification. This methods can be used in early stages of students training. The introduction of early identification of students with leadership characteristics will enable to create individual educational paths for such students, increase the effectiveness of career guidance and selection to continue the training in residency and create conditions for more efficient use of human resources.

CONCLUSION

Human resources development is the most important problem of the public health in Russia. At the same time, Russian public health is dynamic; it is characterized by rapid structural and functional changes. This increases the demands for economic methods in management. The demand for professionals with skills to manage healthcare organizations at various levels of the public health system is significantly increasing. The role of professionals competent in the strategic management methods increases. Given the urgency of the various aspects of the implementation of competency-based approach,

we believe that the education and training of future healthcare organizers, based on the development of key competences, is of special interest. In light of this, the review of international data on organization of key competence development is important. Herewith it is necessary to take into account the educational content, resources and technologies, the determination of the factors that influence this process, the development and evaluation of models aimed at improvement of the education of healthcare management professionals at medical schools.

We believe that the results of such analysis will help to develop and offer a number of practical measures aimed at improving education and training of healthcare management experts in the Russian higher education and ultimately enhance the effectiveness of the Russian health care organizations.

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FORENSIC GENETICS AND GENOTYPING

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FORENZIČKA GENETIKA I GENOTIPIZACIJA

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ABSTRACT

Forensic genetics represents a combination of molecular and population genetics. Personal identification and kinship analysis (e.g. paternity testing) are the two main subjects of forensic DNA analysis. Biological specimens from which DNA is isolated are blood, semen, saliva, tissues, bones, teeth, hairs. Genotyping has become a basis in the characterization of forensic biological evidence. It is performed using a variety of genetic markers, which are divided into two large groups: bi-allelic (single-nucleotide polymorphisms, SNP) and multi-allelic polymorphisms (variable number of tandem repeats, VNTR and short tandem repeats, STR). This review describes the purpose of genetic markers in forensic investigation and their limitations. The STR loci are currently the most informative genetic markers for identity testing, but in cases without a suspect SNP can predict offender's ancestry and phenotype traits such as skin, eyes and hair color. Nowadays, many countries worldwide have established forensic DNA databases based on autosomal short tandem repeats and other markers. In order for DNA profile database to be useful at a national or international level, it is essential to standardize genetic markers used in laboratories.

Keywords: *Forensic genetics; Genotyping; Genetic markers; Databases*

SAŽETAK

Forenzička genetika predstavlja spoj molekularne i populacione genetike. Identifikacija i utvrdjivanje rodbinskih veza (npr. očinstva) su osnovni zadaci forenzičke DNK analize. Biološki materijali iz koga se izoluje DNK su krv, semena tečnost, pljuvačka, tkiva, kosti, zubi, dlake. Genotipizacija je postala osnov u karakterizaciji forenzičkih bioloških dokaza. Ona se izvodi pomoću različitih genetičkih markera koji su podeljeni u dve velike grupe: bialelski (pojedinačni nukleotidni poliorfizam, SNP) i multialelski polimorfizmi (varijabilni broj uzastopnih ponovaka, VNTR i kratki uzastopni ponovci, STR). Ovaj revijski rad ukazuje na značaj genetičkih markera u forenzičkoj istrazi i ograničenja u njihovoj primeni. STR lokusi su trenutno najinformativniji genetički markeri u identifikacije, ali u slučajevima gde nema osumnjičenih SNP može da ukaže na poreklo prestupnika i fenotipske karakteristike, kao što su boja kože, očiju i kose. U mnogim zemljama širom sveta uspostavljene su fonezičke DNK baze podataka koje se baziraju na autozomnim STR i drugim markerima. Da bi baze podataka o DNK profilu bile korisne na nacionalnom i inernacionalnom nivou neophodnoje da se standardizuju genetički markeri koji se koriste u laboratorijama.

Ključne reči: *forenzička genetika, genotipizacija, genetički markeri, DNK baze podataka*

ABBREVIATIONS

bp – base pairs
cfDNA – cell-free fetal DNA
CODIS – Combined DNA Index System
DNA – deoxyribonucleic acid
EDNAP – European DNA Profiling Group
ENFSI – European Network of Forensic Science Institute
ESS – European Standard Set
EU – European Union
FBI – Federal Burean of Investigation
HV1 - hyper variable region 1
HV2 - hyper variable region 2
MPS – Massively Parallel Sequencing
mRNA – messenger ribonucleic acid
mtDNA – mitochondrial DNA
NA – nucleic acid
PCR – polymorphism chain reaction
RNA – ribonucleic acid
RFLP- restriction fragment length polymorphism
SNP – single nucleotide polymorphism
SSR – simple sequence repeat
STADNAP – Standardization of DNA Profiling
STR – short tandem repeats
UV light - ultraviolet light
USA - United States of America
VNTR – variable number of tandem repeats

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INTRODUCTION

Forensic genetics is an interdisciplinary science that uses techniques from statistics and computer science and connects them biology and law (1, 2). Typical contexts for forensic DNA analysis are disputes on kinship (e.g. paternity testing), corpse identification, forensic casework (criminal matters), missing person investigation and mass-fatality identification (e.g. terrorist attacks, airplane crash, tsunami disaster, war crimes) (2-4). Personal identification and relatedness to other individuals are the two main subjects of forensic DNA analysis (2).

Early molecular biology procedures were based on the analysis of protein polymorphisms (e.g. blood group) and phenotype polymorphisms (e.g. eyes and hair color). The discrimination potential of these systems is low compared to DNA polymorphisms (3, 5). Any two humans are 99,9% identical in their nucleotide sequences (1, 2, 4). The remaining 0,1% indicates that, on average, for every 1,000 nucleotides there is one nucleotide that varies among people and defines different alleles of the corresponding gene, and for two randomly chosen individuals there are about 3 million bases differentiating haploid genomes (6). Polymorphisms in the DNA molecule are not uniformly distributed; in some regions of the genome they are densely packed. These hypervariable genetic loci are the source of forensic DNA typing and can be used to both differentiate and correlate individuals (1, 4). The forensic application of DNA typing methods over the past thirty years constitutes a major progression in the examination of biological evidence. With its remarkable sensitivity and power of discrimination, DNA analysis has become the most important in the fields of forensic science and forensic medicine (7).

SOURCES OF DNA

Biological specimens from whom DNA is isolated and typed are: blood and bloodstains, semen and seminal stains, saliva, tissues, organs, bones, teeth, hairs, fingernails, urine, and other body fluids (e.g. vaginal) (3, 7-10).

Blood samples provide large amounts of cells which contain DNA (11). It is possible to reproduce genetic profiles even from four-year-old dried blood stains (5). Many studies showed that blood was not a suitable substrate for personal identification in the case of blood transfusion, peripheral blood stem cells transplantation or bone marrow transplantation due to the presence of donor cells in the recipient blood cells (12, 13). Chaudhary et al. have shown that the saliva also contain donor cells (12). Other common biological sources for forensic DNA profiling in these cases are fingernails and hair follicles (12). However, some studies have shown donor chimerism (presence of donor cells in the recipient) in these sources, but very rarely (12).

Saliva is a potentially useful source of genomic DNA for genetic studies since it can be collected in a painless and non-invasive manner. The inhibitory substances are much lower and less complex in saliva (e.g. proteases from food particles, bacterial cells) (14) than in blood (e.g. heme, hemoglobin, lactoferin, immunoglobin G) (15, 16, 17). Saliva transports exfoliated epithelial cells from buccal mucosa which contains DNA and the mean number of epithelial cells per 1 mL of saliva is about 4.3×10^5, which makes it a suitable source of genomic DNA (8, 11, 18). Some studies have shown that DNA of perpetrator may be detected in the victim's oral cavity up to 1 hour after intense or constrained kissing (8, 10). Saliva can also be placed on human skin through kissing, biting, sucking and licking. Kenna et al. have reported that salivary DNA persists on the skin a minimum of 96 h (19). For example, it is possible to extract DNA from bite marks within this period, but amount of saliva deposited on the skin is usually very small in bite marks (19, 20). In addition, the concentration of salivary DNA varied from donor to donor and from day to day (19). Also many factors can cause DNA damage in saliva, such as: diet, brushing habits, eating habits, smoking, cancer in the oral cavity, fungal infection, etc. Problem with buccal cells is DNA contamination. Food particles often remain in the oral cavity which contaminates the exfoliated epithelial cells from buccal mucosa and leads to less yields of DNA and incorrect results (6).

The fingernail hyponychium can provide a valuable source of evidential material for investigation (8, 21). Traces of skin (especially if the victim scratched the perpetrator), body fluids and hairs may collect under the nails. The persistence of foreign DNA under the nails generally lasts up to 6 h (8). Cook and Dixon in their study detected foreign DNA in 13% of samples after 24 h (21).

Hairs from the victim or from the putative offender are frequently found at crime scenes. Isolation and analysis of DNA molecule from the hair root could provide the identification of the perpetrator. Hairs with intact root in the mitotically active anagen-growing phase mainly contain informative DNA profiles, while the inactive, naturally shed hairs in the telogen phase rarely yield informative DNA profiles. Unfortunately, 95% of the hairs found at a crime scene are inactive or in telogen phase (22).

Bones and teeth are available evidence for homicide committed several years ago or for homicides where the victim was not found promptly and they are often the only surviving material that can be tested especially in mass fatality incidents and mass graves (23). Bone and teeth samples protect DNA through their physical and/or chemical resistance to environmental degradation (23). Teeth with their anatomical location and morphological structure (enamel, dentine, pulp and cementum) provide unique protection to endogenous DNA from post-mortem degradation (24). The dental pulp also provides a rich source of DNA (25).

It should be noted that several factors affect the ability to obtain a DNA profile from biological samples: quantity of DNA, sample degradation and sample purity. The quantity and quality (purity and degradation) of DNA that can be extracted from biological sources are different and can vary

as they are strongly influenced by the time after death and environmental conditions. The DNA quality really influences on genotyping and polymorphism chain reaction (PCR) amplification. Another common problem is the presence of inhibitors of DNA analysis and PCR amplification (26).

Quantity

In 1993, Decorte and Cassiman published that the conventional DNA typing needs at least 50 to 500 ng of high molecular weight DNA (5). Nowadays, optimal amount of DNA is well defined and typically ranges from 100-200 pg to 2-3ng (1 ng is considered the optimum amount for most commercial kits) (27, 28). The quantity of DNA is different in different biological samples (Table 1). For comparison, total weight of DNA in one single cell is less than 7 pg (1). The amount of DNA in each hair depends on the anatomic place of collection (e.g. head, beard, pubis) and varies between individuals. Also, the hair melanin is significant inhibitor of PCR DNA amplification, and therefore roots are preferable. Hair chemical treatments may furthermore decrease amount of DNA (8).

Degradation

All biological specimens are susceptible to degradation. For example, prolonged exposure of even a large blood stain to the environment can degrade the DNA and make it unsuitable for further analysis (7). Environmental sources of degradation are chemical (acids, bases, strong oxidizing agents), physical (heat and sunlight) and biological (bacteria and fungi) (1, 3). The most common source of degradation is biological in the form of mold and mildew. This is most commonly because moist materials are packed in a tightly sealed nylon bags which contributes to the development of microorganisms (1). In order to prevent DNA degradation, a forensic examiner must correctly select the type of material used for collection and storage (e.g. paper versus plastic bags) and ensure complete drying of the sample prior to packaging (8).

In cadavers, DNA degrades very quickly, which is the consequence of rapid bacterial increase, especially in those that are exposed to hot temperatures. At the same time, DNA in dead cells degrades under the influence of endonucleases and exonucleases (5, 26). The level of degradation also depends on the type of analyzed tissue (29). In comparison to soft tissues, hard tissues are greatly resistant to autolysis and decomposition caused by environmental factors. Therefore, bones, teeth and nails are the only source of DNA in some forensic cases (3, 29). Miloš et al. have shown that femur provided the best success rates while clavicle, ulna, and radius provided the lowest success rates (23). Maciejewska et al. have shown that if human remains are exposed to high temperature, samples of soft tissues of the highest weight (thickness) should be collected for the genetic analysis, because they give the best chance of successful identification (29).

Table 1. Quantity of DNA in diffrent biological samples (1, 7)

Type of sample	Amount of DNA
Liquid blood	30 000 ng/ml
Stain of blood	200 ng/cm^2
Liquid semen	250 000 ng/ml
Postcoital vaginal swab	0-3000 ng/swab
Hair(with root) plucked	1-750 ng/root
Hair (with root) shed	1-12 ng/root
Liquid saliva	5 000 ng/ml
Oral swab	100-1500 ng/swab
Urine	1-20 ng/ml
Bone	3-10 ng/mg
Tissue	50-500 ng/mg

Purity

In biological specimens, besides the human traces, can also be found traces which originated from other organisms (plants and microorganisms), but the main sources of contamination originate from people. Contamination with microorganisms primarily depends on the way the samples are kept after collection (11). A distinction should be made between the so-called mixed sample and contaminated sample. Mixed sample contains DNA from two or more contributors, where the mixing occurred during the crime itself, usually with small amounts of DNA from potentially numerous contributors (10, 30). Contaminated sample contains DNA from the person who did not participate in the commission of the criminal offense and his or her DNA **is** deposited over the biological trace during the collection, storage and analysis. A sample may also be contaminated after it is collected from the crime scene and comes from the investigating officers, laboratory technicians and laboratory plastic ware. The issue of contamination is overcome by forming dedicated, specialized laboratories and by implementing protocols to reduce the risk of intra-laboratory contamination (28).

GENOTYPING

Genotyping has become a basis in the analysis of forensic biological evidence which allows analyses of an extensive choice of biological specimens (27). It is also known as DNA typing or DNA fingerprinting. It is called a "fingerprint" because it is very unlikely that any two people would have exactly the same DNA information, in the same way that it is very unlikely that any two people would have exactly the same physical fingerprint. Unlike clinical genetic research in which the result of DNA analysis itself is an information, in forensic genetics only by comparing DNA profiles obtained from one biological trace with the DNA profile from another biological trace we obtain the necessary information (1). The first method used for DNA fin-

gerprinting is RFLP (restriction fragment length polymorphism), which detects the repeated sequences by defining a specific pattern to the variable number of tandem repeats, which forms the DNA fingerprint of a person. Using a special enzyme, called restriction endonuclease, that acts as a molecular scissors, DNA is cut into fragments on specific restriction sites. The chopped fragments have varying lengths. Gel electrophoresis is done to separate the cut fragments based on their size. A Southern blot (transferring the fragments to a nitrocellulose membrane) is then performed, and a radioactive probe is used to analyze the DNA. This RFLP analysis requires large quantities of DNA and requires long waiting time to obtain results (31). Nowadays DNA fingerprinting is based on PCR (1, 32). The remarkable sensitivity of the DNA typing assays permits even very small quantities of DNA to be genotyped (27). The regions of DNA that have most often been used in forensic analysis are located in non-coding genome regions (they are not genes) (33). The gene that codes for an exacting protein contributes to only 2–5% of DNA, whereas the remaining 95% are non-coding DNA (25). Therefore we mark them as *markers* (19). However, some of these non-coding markers are linked with visible traits (3). In forensic DNA analysis we used a variety of genetic markers and divided them into two large groups: multi-allelic polymorphisms (variable number of tandem repeats - VNTR and short tandem repeats - STR) and bi-allelic (single-nucleotide polymorphisms - SNP) (1, 3).

Variable number of tandem repeats - VNTR

In 1985, Alec Jeffreys have shown that highly polymorphic DNA segments (minisatellites or variable number of tandem repeats) are able to generate individual specific DNA fingerprints (34). The VNTRs contain repeated motif of 6–100 bp (3, 10). The number of repeats varies from person to person. Because different alleles consist of different numbers of repeats, VNTR alleles can be identified by their lengths. The VNTR loci chosen for forensic use are on different chromosomes, or sometimes very distant on the same chromosome, so they are separately inherited (33). The VNTR loci are suitable for identification since they have a very large number of alleles, often a hundred or more (1, 3, 33). The great number of alleles means that the number of possible genotypes is huge. For example, one VNTR in humans is a 17 bp sequence of DNA repeated between 70 and 450 times in the genome, so that total number of base pairs at this locus could vary from 1190 to 7650 (2). Another advantage of VNTRs for genotyping is that none of the alleles is very common. Different alleles are much more comparable in frequency than multiple alleles of most genes. This is due to high mutation rate and the fact that most mutations increase or decrease the length of a VNTR by one or a few units (33). Due to high numbers of rare alleles, VNTR loci alone often lead to much higher exclusion (or inclusion) probabilities than single STR loci alone, which often have quite common and widespread alleles (1, 2). The major limitation of VNTR analysis is that DNA has to be better preserved (1). In the early 1990s, forensic DNA analysis moved from markers which contain large core repeat units and large amplicon size to short tandem repeats (STRs), and the first available commercial kits for typing multiple STRs in a single reaction became available in the early 2000s (10). Nowadays the use of VNTR has been replaced by STR (short tandem repeats).

Short tandem repeats - STR

Today the power of forensic DNA analysis is in polymorphisms at the short tandem repeat (STR) loci and the number of STR loci used (35). STRs, known as microsatellites or simple sequence repeats (SSRs), contain repeated motif from1-5 for some authors, 2-7 or 2-10 nucleotides in length for others, that are repeated in tandem from approximately a half dozen to several dozen times (25, 36-38). The small amplicon size of STRs (typically ranging from 100 to 500 bps in length) makes them suitable for the analysis of degraded DNA samples (2, 9, 27, 36, 38, 39). The STR loci are currently the most informative genetic markers for identity testing (3, 4, 28, 38). For example, the identity of the murdered Romanov family was confirmed using DNA extracted from bone fragments and amplified for 5 STR loci (HUMTH01, HUMVWA31, HUMF13A1, HUMFES/FPS, and HUMACTPB2), and amelogenin (40). Also forensic applications of STRs has allowed the reopening and solving old and forgotten cases and has also led to the exoneration of prisoners convicted through miscarriages of justice (32).

Limitation of using STRs is the high mutation rate of STR loci (10^{-3}–10^{-5}), which makes them very informative, but also less stable (3). High mutational rates also lead to extensive polymorphism and increase the probability of isolated populations diverging rapidly at these STR loci, which makes them particularly useful in the study of population genetics (38).

Use of multiple STR loci in a single test enables a high power of discrimination without the use of large amount of DNA (e.g. 1 ng or less). PCR amplification of multiple STR loci at the same time is possible with different colored fluorescent dyes and different sized PCR products (36). This is an ideal technique for genotyping due to the probability that identical alleles in two individuals decrease with the increase in the number of polymorphic loci examined and also can save time and money, but difficulties may happen when coamplifying several loci. Multiplex PCR involves using a number of sets of PCR primers and allows targeting multiple locations throughout the genome. The problem is that primers for one locus can complex with those of other loci and completely inhibit the amplification. This effect may be avoided by leaving a specific STR locus under certain conditions (2).

Severely degraded DNA samples could contain only very short DNA template molecules (under 100 bp) making conventional STR typing (100–500 bp) unsuccessful.

Damaged DNA templates (e.g. old bones, hair shafts) and minute amounts of cells occasionally lead to the elimination of single or, in the worst case, all alleles (2). In this case STR typing can be improved by moving the PCR primers closer to the STR repeat region, which reduces sizes of product while retaining the same information (3, 28, 36, 41). Thus, STR products reduced in lengths are marked as mini STR and if they are smaller than some of the fragmented DNA template molecules, genetic characterization of the sample may then be possible (28, 36). Butler have shown that the utility of these mini-STR assays has been confirmed in studies involving degraded bone samples and aged blood and saliva stains (36). Success rates in recovering information from severely degraded DNA samples are improved with mini-STR systems compared to conventional STR systems (36). Gill et al. described the acceptance of three new mini-STRs (D10S1248, D14S1434, D22S1045) into the European standard Interpol loci which now include 10 STR loci (42). Mulero et al. described the conversion of eight STRs (D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, and FGA) into mini-STRs (41). The amplicon range for mini-STRs is 71–250 bp in length. Consequently, mini-STRs could be accommodated into one multiplex analysis. It would be better if all STR kits are reconfigured into mini-STR kits for routine analysis of forensic evidence. Only one attempt would be needed to obtain a DNA profile for limited-quantity samples. More efforts will continue to convert all current forensically relevant STRs into mini-STRs and to incorporate in one multiplex amplification kit (28).

Single nucleotide polymorphisms - SNP

Single nucleotide polymorphisms (SNPs), i.e. single base difference among two different individuals, are another type of genetic markers and have been considered as additional informative markers (2, 4). SNPs are base substitutions or insertions/deletions that occur at single positions in the genome (9). SNPs in humans occur on average every 1/2000 bases (2).They have much smaller amplicons than those of mini-STRs, as short as 50-60 bp (3, 4, 28). This is very important when dealing with highly degraded DNA samples (3, 4, 39).

Budowle and van Daal claim that SNPs are not the same and they categorized SNPs into categories: 1. identity-testing SNPs for individualization, 2. lineage-informative SNPs, sets of tightly-linked SNPs for identifying missing persons through kinship analyses, 3. ancestry-informative SNPs for establishing biogeography ancestry with high probability, 4. phenotype-informative SNPs for establishing phenotypic characteristics such as skin color, hair color, or eye color with high probability and 5. SNPs for pharmocogenetic investigations for determining the cause of death (9, 28). The information contained in the human genome may provide insights into personal characteristics such as ethnicity, physical and physiological characteristics (43). When there is no suspect, SNPs that describe phenotypic traits (such as pigmentation of skin, facial features, height, hair color and eye color) would enable a genetic prediction of appearance for investigative leads to identify the perpetrator or at a minimum help confirm or refute the more refractory eyewitness description (28, 43-45). Walsh et al. with six eye color-SNPs adjusted prediction of colors of the iris with accuracies of over 90% (46). Lui et al. suggested the used 9 SNPs (rs183671, rs12203592, rs10756819, rs1393350, rs17128291, rs12913832, rs2924567, rs4268748, rs6059655) as suitable markers for DNA prediction of skin color in Europeans and neighboring populations (44). The same phenotype-informative SNPs could be used to facilitate facial reconstructions for identifying missing persons (28). For example, in the 2004 Madrid train bombings source population of the suspects was concluded by using 34 ancestry-informative SNPs (43). In contrast to the personal information contained in STR markers, the resources regarding SNP markers are mostly population-oriented and can provide robust information on biogeographic ancestry that STRs can't provide due to their high global heterozygosity and greater mutation rate (3, 45).

Although SNPs are bi-allelic and consequently less polymorphic than multi-allelic STRs, they have two known advantages over STRs. The first advantage is that SNPs are more stable genetic markers with low mutation rates on the order 10^{-8} and so changeless likely over generations which is crucial concerning inheritance cases, missing per-

Table 2. Advantages and disadvantages of VNTR, STR and SNP genetic markers for DNA profiling (1-4, 9, 27, 28, 33, 36, 38, 39)

	Advantages	Diadvantages
VNTR	-Large number of allels >100 -none of allels is very common -high number of rare allels -VNTR loci alone often lead to much higher exclusion (or inclusion) probabilities than single STR loci alone	-because the large amplicon size of VNTRs DNA has to be better preserved
STR	-small amplicon size makes them suitable for for the analysis of degraded DNA samples -high mutation rate lead to extensive polymorphisms which is useful in the study of population genetics -high power of discrimination without the use of large amount of DNA	-high mutation rate makes them less stable
SNP	-smaller amlicon size than sSTRs makes them suitable for highly degraded DNA samples -ancestry and phenotype SNPs can predict ethnicity and phenotypic traits -low mutation rates which is helpful in inheritance cases and missing person cases	-because they are bi-allelic and consequently less polymorphic -individually less informative than STRs -large panels of SNPs (50–100) are need for genotyping

son cases, and situations where no direct reference sample may be available (3, 4, 9, 28, 39). The second advantage is that amplicon's size is less than 60 bp in length (39).

However, SNP analysis also presents some limitations. It is generally accepted that SNPs are individually less informative than STRs (4, 9).The informativity may be increased by analyzing many unlinked SNPs, in order to achieve the level of discrimination typical for the 13 core STR loci, so large panels of SNPs (50–100) need to be genotyped (3, 4, 9, 39). **Sanchez et al. have shown** that the applied SNP typing multiplex with 52 SNPs in European populations provides a higher statistical power than 15 human identity STRs (39). Another limitation is that SNPs can provide only weak evidence of familial relationships or resolve mixtures of DNA from two or more individuals in a single sample, precisely because they are only bi-allelic (3, 9, 45).

SNP markers offer a useful and important extension to a routine STR-based DNA typing (3, 4). SNPs give an almost unlimited source of human genome diversity for analyzing (3). An advantage of SNPs is that research and development at present is underway to improve analytical capabilities, possibly making large multiplex assays and complete automation possible. Consequently, the large battery of required SNPs can be used and can provide the power of discrimination currently obtained with STR kits. So a battery of SNPs with a high power of discrimination is desired (28). Some scientists go even further, taking into consideration that SNPs will replace STRs in forensic investigation (4).

Table 2 shows comparison of VNTR, STR and SNP genetic markers.

MITOCHONDRIAL DNA

Mitochondrial DNA (mtDNA) exists within cytoplasmic mitochondria as a separate small genome and contains a region (control region or D loop) which is composed of two hyper variable segments (2, 5, 47). Two hyper variable segments of the control region, HV1 and HV2, are most variable between individuals and are therefore of special interest for forensic studies (3, 28). The principal advantage of mtDNA is that it is present in 500- 2000 copies per cell (3, 5, 43, 47). Nuclear DNA, in spite of its great power of identification, is present only in cells with nucleus and in only two copies per cell (47). Many copies of mtDNA increase the chances that some copies of mtDNA will survive in highly degraded forensic samples and permit typing a great range of samples that would otherwise be unthinkable (2, 3, 47). Such samples are highly degraded stains, bones, teeth, saliva, fingernails, and hair shafts (3, 28, 47). As mentioned before, most of hairs founded at crime scene, are shed hairs and do not contain a root. However, the hair shafts still contain many copies of mtDNA (5). Another characteristic of mtDNA is that it is maternally inherited, so even distant maternal relative can provide a comparative reference sample with an expectation of a match (43, 47, 48). In identifying missing persons it is very important to compare mtDNA of unidentified remains with that of a possible maternal relative (25).

Due to lack of recombination, mtDNA can be analyzed as a single, highly informative multi-allelic locus (haplotype). A big advantage of using mtDNA is high mutation rate in the mitochondrial genome which causes high sequence variation. MtDNA is almost ten times more prone to mutation than nuclear DNA. Limitation of mtDNA is heteroplasmy or the presence of more than one mtDNA type in an individual, which is an uncommon characteristic of the mitochondrial genome and may complicate the interpretation (43).

Y CHROMOSOME

As Y chromosome is passed to the son from his father, analysis of markers on this chromosome helps in the identification of male lineages and inferring paternal genetic ancestry for judicial and investigative purposes (25, 49-51). Whereas these markers are transmitted as haplotypes in the same way as single locus alleles, they have the lack of recombination.

Nowadays there are many commercial kits available for Y-STR haplotyping which are useful in reconstructing paternal relationship (52). Given that Y-STRs markers have relatively low mutation rate of about 10^{-3} per locus per generation, they have proven useful for testing short to medium timescale paternal relationships. Other markers used for Y chromosomes are Y-SNPs and, with an average mutation rate of about 3×10^{-8} per nucleotide per generation, they are suitable for studying distant relationships between male individuals and populations (50). Barra et al. have determined the male fetal Y-STR haplotype in maternal plasma during pregnancy and have estimated if the fetus and alleged father belong to the same paternal lineage (52). The main limitation is that it is only applied for mothers bearing a male fetus and its conclusion will be if alleged father and fetus belong to the same paternal lineage or not. Consequently, the test should not be performed in population with a high rate of endogamy (52).

Y-STRs are also used to resolve the male component of mixed DNA when a high female background is present (e.g. sexual assault cases) (43, 49, 52). The vasectomized or naturally azoospermic rapist leaves no sperm, in such cases,Y-specific profiling is effective, even from samples with 1:4000 male:female DNA ratio (32). In the last few years the number of Y-STR markers for routine use in forensic and population genetics has grown significantly (49).

X CHROMOSOME

The X chromosome is transmitted between both sexes in each generation, transferring different genetic information from uniparental genomes (53). Markers located on this chromosome have a particular inheritance pattern, be-

cause women are dizygous and men are hemizygous (54). The X chromosome markers (X-STRs and X-SNPs) show higher efficiency parameters than autosomes in special kinship investigations (as well as incest cases), involving mainly female offspring (48, 53, 54). The STRs on X chromosome can only be used in kinship analysis involving daughters, because there is no allele inherited by descent in a father-son relationship (54). Therefore, a growing number of scientists researching X markers uses them for studying the genetic structure of human populations, ancestry proportions in admixed populations and for forensic investigations (53).

EXTRACELLULAR DNA AND RNA

In 1978, Stroun et al. reported the presence of an RNA form in a nucleoprotein complex spontaneously released from human blood lymphocyte and frog auricle cultured cell systems (55). Extracellular nuclear acids (NAs) have been detected in a variety of biological specimens, including serum, plasma, saliva, urine, milk, bronchial lavage, cell culture supernatants. It is not defined how NAs are released from cells, whether by active secretion or as a result of apoptosis, or some combination of these mechanisms. Extracellular RNA may be protected from degradation by RNases, because they are being packaged into apoptotic bodies, integrated within nucleoprotein complexes with phospholipids and proteins. Analysis of extracellular mRNA profiling in forensic science has also been investigated (56). In 2003, using standard RT-PCR and gel electrophoresis, Juusola and Ballantyne reported that mRNAs specific for saliva and semen can be detected in stains as old as 10 weeks (15,57). The mRNAs have been used for identification of human body fluids or tissues, which is very useful for forensic investigations to identify the origin of biological samples (3, 58). In 2005, Juusola and Ballantyne demonstrated the distinction of stains originating from different body fluids such as blood, semen, saliva and vaginal secretions (59). But the main part of the RNA preserved in salivary stains is cellular (15).

In 1997, Lo *et al.* detected "fetal" DNA in the plasma of pregnant women (60). So, the non-invasive prenatal testing is possible by detection of cell-free fetal DNA (cfDNA) in maternal circulation. The cfDNA originates from the placenta cells and apoptosis is the main mechanism of releasing it to the mother's circulation. Length of fetal DNA sequences in maternal plasma is mostly 150 bp and is rarely longer than 250 bp, and their final disappearance from maternal circulation occurred after 1–2 days postpartum. Today, there are many situations where it would be desirable to do the non-invasive prenatal paternity testing by the analysis of the circulating cell-free fetal DNA (e.g. unclear paternity in case of women with more than one sexual partner who are unsure of the actual father) (52). Tumor-specific cell-free DNA has also been found in the circulation in many kinds of tumors. The presence of placental and tumor-specific cell-free RNA in plasma was also found (15).

DNA DATABASE

While the human genome contains thousands and thousands of STR markers, only a small core set of loci has been used in forensic DNA and human identity testing. Millions of STR profiles are formed worldwide every year by private laboratories, university and government performing different forms of human identity testing, including DNA data basing, forensic casework, kinship analysis, missing persons and mass disaster victim identification (36).

Currently, more than 60 countries worldwide have established forensic DNA databases based on STRs, and these databases continue to grow fast. Now, China has more than 27 million entries in its forensic database (43). Since 1997, The Federal Bureau of Investigation (FBI) uses a standard set of 13 specific STR loci, collectively referred to as Combined DNA Index System (CODIS) markers, and the sex-differentiating amelogenin locus AMEL-X/Y (1). The CODIS databank houses the largest number of DNA profiles compared to any other forensic DNA database (10, 28). In this database, the chances that more than one individual shares a 13-loci DNA profile are approximately one in one billion (10). The 13 routinely-used CODIS STR markers are: CSF1PO, FGA, THO1, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11; and 15 markers are13 CODIS loci plus D2S1338 and D19S433 (28, 43). Although CODIS is used strictly in the USA and Canada, sometimes it is used to match probability in mass disasters outside USA. Thus, after Madrid terrorist attack in 2004, the CODIS database was used to match probabilities of 220 body remains against 98 reference samples, including 67 samples from relatives, representing 40 family groups and 27 antemortem direct references (2).

The forensic DNA databases of the most countries contain two types of profiles: profiles from convicted offenders and/or arrestee profiles (these profiles are from known sources and forensic profiles obtained from crime scenes (from unknown sources) (3, 61). Then CODIS utilizes computer software to automatically search its two indexes for matching DNA profiles and this technology is authorized by USA law (3).

In order for DNA profile database to be useful at a national or international level, it is essential to standardize the genetic markers used among laboratories (28). So, a number of organizations are currently involved in developing and promoting DNA databases across the European Union (EU). The European DNA Profiling Group (EDNAP) was established in 1988 with the aim of forming systematic procedures for data-sharing across the European community. The Standardization of DNA profiling in the EU (STADNAP) enhances usage of DNA profiling across the EU in order to help better detection of 'mobile serial offenders'. The European Network of Forensic Science Institutes (ENFSI) aims to undertake the standardization of forensic practices in support of policing across the whole

of the EU (2). Recently, EU legislation has made all 28 members of the EU to participate in a network of national DNA databases (62).

To improve the discrimination power and the success in analyzing degraded DNA samples, the ENFSI and the EDNAP published in 2005 a list of three additional new mini-STR loci: D2S441, D10S1248 and D22S1045 and two additional polymorphic loci, D1S1656 and D12S391 (3). The FBI has suggested adding more autosomal STR loci to its present core set. The additional loci are mostly from those that the European system has chosen to supplement its core loci, assist with international data sharing and also increase discriminating power for missing person identifications. Also, one Y chromosome STR marker (DYS391) was added by the FBI into the new core loci, but the reason was to confirm amelogenin null values sometimes present in DNA typing. Many of Chinese crime laboratories have included Y-STRs in the standard protocol for sexual assault cases. However, because of the limitation in detection technology, only up to 25 to 30 autosomal STR and/or Y-STR loci can be multiplexed in a single kit and analyzed, which apparently indirectly limits the marker capacity of the DNA database to support forensic investigations. In the future, with Massively Parallel Sequencing (MPS) technologies it will be possible to type simultaneously all forensically-relevant autosomal STRs, Y STRs, X STRs, SNP and whole mitochondrial DNA genome sequences, comprising between 400-500 markers and much more (61). Addition of more loci increases discrimination power helps in missing persons' cases and makes a distinction between family members in closely related communities. In addition, with expanded locus overlap between numerous databases, international collaboration and data exchange would be easier. For that reason European and US forensic communities have taken steps toward these goals with proposal of the expanded CODIS core loci and acceptance of the European Standard Set (ESS) (63).

Since the early 1990s in the Western Balkan region, DNA analysis in forensic sciences has significantly increased. A large number of people that were buried in mass graves are identified by DNA typing. Forensic DNA testing in Serbia is performed in 6 laboratories. The use of DNA typing in criminal procedures in Serbia is regulated by the Criminal Procedure Act. This Act enables the district lawyer to order police to collect the buccal swab samples from the suspect, with or without his or her consent and requires the forensic pathologist to collect the DNA samples from unidentified bodies, and to collect and preserve samples of any biological traces obtained during autopsy (62).

DNA profiles are different from fingerprints (which are useful only for identification), because DNA can provide insights into many intimate aspects of a person and their families including predisposition to particular diseases and perhaps predispositions to certain behaviors (2). Expanded forensic DNA database will probably lead to the violation of a number of individual and civil rights (62). To defend individual and civil rights is to forbid the inclusion of genetic susceptibilities information in crime databases (2). For their SNP panel, Sanchez et al. pragmatically selected SNPs that were located at least 100 kb from any known gene, presumably to minimize the potential for perceived privacy risks (39). So the possible connection to a disease gene might be a criterion to consider about privacy. Prediction of physical traits limited to skin, eye, and hair color and possibly height and facial features is less likely to be considered a privacy matter (9).

CONCLUSION

Biological evidence for DNA studies is nowadays considered the most important evidence for legal proof in courts of law. DNA typing analysis can be performed on a large variety of materials, such as cigarette ends, tissues on a gun muzzle and on bullets, dismembered and decayed body parts, paraffin embedded tumor tissue, dirt and skin under fingernails, epithelia of an offender from the victim's neck after strangling, burned corpses, dried chewing gum, skeletal remains, body parts after mass disasters, skeletonized flood victims and human feces and urine. An inverse relation between the degradation and the length of DNA sequence that can be successfully and reproducibly analyzed influences the choice of DNA profiling technology and markers to be analyzed.

The STR analysis is still the most important and commonly-used genetic technique in forensic science. In complex cases of kinship analysis, severely degraded DNA or prediction of phenotype traits of offender's, SNP, Y-STRs, X-STRs and mtDNA could be used to complement autosomal STR typing. Multiplexing or the ability to simultaneously assessment several genetic markers in one analysis is the key to forensic testing. More simultaneously typed markers will reduce consumption of often limited precious forensic evidence.

The success of DNA typing in a police investigation can be greatly enhanced by storing DNA profiles in a central database. It is the quality of DNA database laws that makes DNA an effective investigative tool. Effective DNA databases are being constructed and numerous forensic cases solved today through generating STR profiles with a common set of genetic markers.

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DIFFICULTIES IN THE DIAGNOSIS OF INCLUSION BODY MYOSITIS-CASE REPORT

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POTEŠKOĆE U POSTAVLJANJU DIJAGNOZE MIOZITISA SA INKLUZIVNIM TELAŠCIMA-PRIKAZ SLUČAJA

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ABSTRACT

Inclusion body myositis belongs to the group of idiopathic inflammatory myopathies. Two processes, one autoimmune and the other degenerative, appear to occur in parallel. There are two forms of inclusion body myositis, hereditary and sporadic.

Case report: 47-year-old woman with muscle weakness and atrophy of the distal and proximal muscles, and involvement of quadriceps and deep finger flexors was admitted for neuromuscular evaluation. These changes have been started gradually and insidiously over three years. Electromyography findings were nonspecific and for this reason in the beginning of the disease it was misdiagnosed as demyelization peripheral neuropathy. Muscle biopsy, with the presence of characteristic structures such as rimmed vacuoles and amyloid deposits definitely confirmed the diagnosis of inclusion body myositis. Conclusion: There are several difficulties in the diagnosis of inclusion body myositis as nonspecific EMG findings and overreliance on electrophysiology and lack of the cardinal histological features in muscle biopsy. Although this disease is rare and incurable, making the correct diagnosis is crucial to directing the patient to physical therapy for weakness and occupational therapy to improve a patient's ability in activities of daily living.

Keywords: *inclusion body myositis, clinical signs, muscle biopsy*

SAŽETAK

Miozitis sa inkluzivnim telašcima pripada grupi idiopatskih inflamatornih miopatija. U osnovi ovog oboljenja se odvijajau dva paralelana patogenetska procesa, inflamatorni i degenerativni. Postoje dva oblika miozitisa sa inkluzivnim telašcima: sporadični i hereditarni.

Prikaz slučaja: 47 godišnja žena, koja je imala mišićnu slabost, atrofiju distalne i proksimalne muskulature je hospitalizovana zbog evaluacije neuromuskularnog stanja. Opisane mišićne promene su nastale postepeno u periodu od poslednje tri godine. Elektromiografski nalaz je u početku bolesti bio nespecifičan, zbog čega je postavljena pogrešna dijagnoza demijelinizirajuće periferne neuropatije. Kasnije urađena biopsija mišića je pokazala specifične promene za miozitis sa inkluzivnim telašcima, kao što su vakuole i depoziti amiloida. Zaključak: Postoji više poteškoća prilikom postavljanja dijagnoze miozitisa sa inkluzivnim telašcima, kao što su nespecifičan nalaz elektromioneurografije i nedostatak kardinalnih patohistoloških promena prilikom neadekvatnog izvođenja biopsije mišića. Iako je bolest retka i slabo reaguje na konvencionalnu terapiju, postavljanje odgovarajuće dijagnoze je značajno zbog upućivanja pacijenta na fizikalnu i okupacionu terapiju, sa ciljem osposobljavanja pacijenata za aktivnosti svakodnevnog života.

Ključne reči: *miozitis sa inkluzivnim telašcima, klinički znaci, biopsija mišića*

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INTRODUCTION

Inclusion body myositis (IBM) belongs to the group of idiopathic inflammatory myopathies. It is estimated that prevalence ranges from 4.3 per million in the Netherlands to 33 per million in South-East Norway (1,2). There are two forms of IBM, hereditary IBM and sporadic IBM. Hereditary form includes the entire spectrum of autosomal dominant and autosomal recessive muscle disease from which suffers younger population. Sporadic form (sIBM) usually appears as a separate disease, or it is associated with other systemic autoimmune diseases (such as systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis, etc.) (3,4). In pathogenesis of IBM two processes might occur in parallel: a primary immune process due to T-cell-mediated cytotoxicity, and a non-immune process characterized by vacuolization and intracellular accumulation of amyloid-related molecules, probably due to MHC class I-induced stress.

Muscle weakness, as the main characteristic of the disease; have been started gradually and insidiously over many months and years. Considering the fact that there are slow development of the disease and nonspecific EMG findings, s-IBM is still difficult to diagnose. It remains frequently misdiagnosed as other neuromuscular conditions by health care professionals. The degenerative processes with amyloid accumulation distinguish sporadic inclusion body myositis from other inflammatory myopathies (5). Muscle biopsy, with the presence of characteristic structures such as rimmed vacuoles and amyloid deposits, using Congo red technique, definitely confirm IBM (5,6). Currently, no effective therapy exists.

CASE REPORT

A 47-year-old woman was referred to Department of Rheumatology of Internal Clinic for a second-opinion neuromuscular evaluation. Three years before, she noticed leg weakness, particularly in his thighs, and difficulties to stand up from sitting position. After few months, weakness of upper extremity appeared too. Physical examination performed by neurologist, showed lower muscle strength of all extremities with decrease patellar and Achilles reflex bilaterally. Biochemical analysis were in referent range, except total creatine kinase (CK) was mildly elevated at 211ng/ml (referent range <200ng/ml). Electromyography (EMG) was performed and a result was consisted with demyelization peripheral neuropathy more dominant on the lower limbs. Physical therapy partially improved her muscle strength for a short time. Three years after initial symptoms started, patient hospitalized again in the Clinic of Neurology, with signs of severe muscle weakness. This time physical examination showed hypoesthesia of right face side, weakness of neck flexors revealed "drooped neck sign". Weakness and atrophy of the distal and proximal muscles, and involvement of quadriceps, deep finger flexors and ankle dorsiflexors were observed too. Muscle reflex on lower extremities were absent. Repeated EMG showed increased membrane instability and early recruitment with fractionation of the motor unit potentials, showing a brief, small, abundant, polyphasic motor unit potentials (MUPs). Total CK level was elevated at 437 ng/ml (referent range <200ng/ml). Biopsy of the right deltoid muscle was performed and showed myopathic features, variation in muscle fiber size, endomysial fibrosis, chronic inflammatory cells with macrophages, rimmed vacuoles with basophilic stippling and amyloid deposits, using Congo red technique, characteristic of sIBM (Figure 1,2,3). Patient received three cycles of immunoglobulin therapy, 0.5 g/kg of IVIG, three times at monthly intervals. After that muscle strength partially improves for a short time. Treatment with glicocorticoids and immunosupresives (azatioprine) as an attempt to suppress inflammation, resulted with normalizing the levels of CK, and was not accompanied by significant improvement in muscle strength. After that observation treatment with glicocorticoids and immunosupresives was interrupted. Patient was directed to the physical therapy for weakness, education to prevent falls and occupational therapy to improve activities of daily living.

DISCUSSION

There are two parallel processes in IBM pathogenesis: one autoimmune and the other degenerative. In contrast to dystrophies, in IBM the autoinvasive $CD8^+$ T cells are cytotoxic and antigen-driven, invading muscle fibers expressing major histocompatibility complex class I antigen and costimulatory molecules. The concurrent degenerative features include vacuolization, filamentous inclusions and intracellular accumulations of amyloid-β-related molecules (6,7).

sIBM causes weakness and atrophy of the distal and proximal muscles, and involvement of quadriceps and deep finger flexors are clues to early diagnosis (5,7). Patients often present with falls because their muscle weakness, or with difficulty performing certain tasks, such as turning keys, owing to weakness of finger flexors. The tendon reflexes, although preserved early in the disease, can diminish in the late stages as the atrophy of major muscle groups becomes evident (7). Creatine kinase levels can initially be elevated up to 10-fold, but they remain only slightly elevated as the disease progresses.

EMG findings in IBM have reported a high frequency of spontaneous activity i.e. fibrillations, positive sharp waves and polyphasic motor unit potentials (MUPs) that can occur in a variety of chronic myopathies (4). In some patients, the finding of enlarged MUPs with prominent spontaneous activity can lead to a mistaken diagnosis of a neurogenic disorder, unless a muscle biopsy is performed. EMG findings are nonspecific and for this reason EMG is not included in the diagnostic research criteria for IBM (6,8). However, in clinical practice EMG has an important

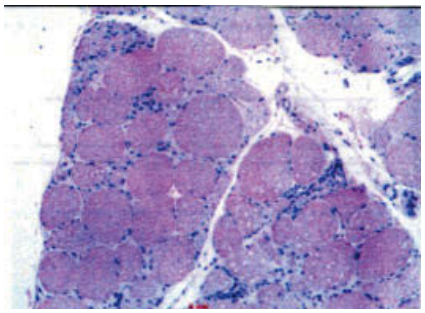

Figure 1. Histopathology finding: Lymphocite inflammatory infiltration of the mycle. There were aso generalized upregulation of sarcolemmal MHC I in zone of cellular infiltration and out of that zone. Expression of dyferlin was normal . (HEx19)

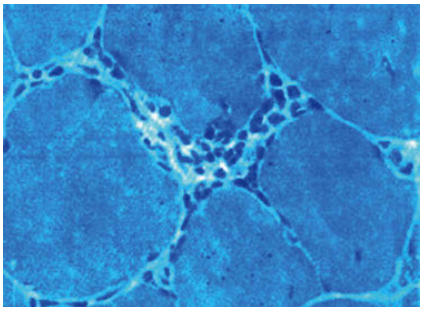

Figure 2. Histopathology finding: Signs of myophagocytosis (mod Gomori trichrome x 40)

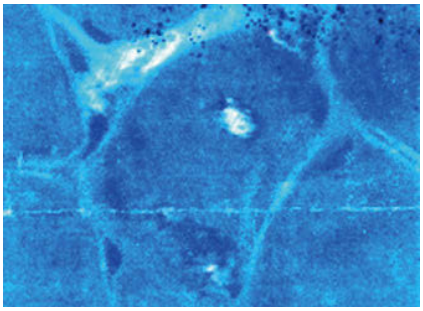

Figure 3. Histopathology finding: Presence of "rimmed vacuoles" in muscle fibers (Mod. Gomori trichrome x 40)

role in the evaluation of muscle weakness, and may alert the clinician that a muscle biopsy should be performed.

Muscle biopsy is essential for diagnosis. The main histological features are multifocal lymphocytic infiltrates invading non-necrotic muscle fibers, vacuoles in fibers not invaded by lymphocytes (these 'rimmed vacuoles' contain basophilic granular deposits, consisting of membranous whorls, around the edges), and Congo-red-positive amyloid deposits (6). Although individual pathological features are all non specific and can also be seen in other myopathies and neurogenic disorders, their co-occurrence in the same biopsy allows the diagnosis of sIBM (9). Clinicians may be misled by an incomplete biopsy appearance and lack of the cardinal histological features, with patchy inflammatory changes being more florid early and patchy degenerative changes more florid later in the disease course.

The cases described here confirm and highlight the fact that s-IBM is still difficult to diagnose and remains frequently misdiagnosed. Literature data shows that the time to diagnosis averaged 5.83 years. sIBM is often misdiagnosed as polymyositis, granulomatous myositis, myofibrillar myopathies, or motor neuron disease, amyotrophic lateral sclerosis, peripheral neuropathy and other diseases (10,11). It is frequently only suspected retrospectively when a patient does not respond to therapy.

Treatment of IBM is quite a challenge due to IBM resistance on corticosteroids and other immunosuppressive therapies and lack of controlled trials because of the rarity of the disease. Although the common immunotherapeutic agents available for inflammatory myopathies, such as corticosteroids, azathioprine, methotrexate, cyclosporine and cyclophosphamide are generally ineffective, some patients have responded to these therapies to a certain degree or for short periods (12). On the other hand, the mechanism by which IVIG affects muscle in inclusion body myositis has not been well-studied. However, it may work via suppression of T-cell activation (including cytotoxic T cells) and migration into muscle tissue and alterations in cytokine production (13). Some patients with IBM respond to intravenous immunoglobulin (IVIG) therapy (3, 13). Treatment with IVIG must be administered in the context of its known adverse effects. There is little evidence to advise the clinician on the proper dosing of IVIG and duration of therapy. Considering evidence-based guidelines there are insufficient evidences to support or refute use of IVIG in the treatment of inclusion body myositis (14, 15).

However, sIBM remains a disabling disease, with most patients requiring an assistive mobility device within 5 to 10 years of onset. In general, the older the age of onset, the more rapidly progressive is the course.

There are several difficulties in the diagnosis of s-IBM: nonspecific EMG findings and overreliance on electrophysiology, and lack of the cardinal histological features in muscle biopsy. For this reason, it is necessary to notice clinical signs that are crucial for diagnosis, such as long finger flexor and quadriceps weakness.

Declaration of interest

The authors declare no conflict of interest.

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SWOLLEN SCAPULA AS THE FIRST CLINICAL FEATURE OF DISSEMINATED MALIGNANT DISEASE

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OTOK SKAPULE KAO PRVA KLINIČKA MANIFESTACIJA DISEMINIRANE MALIGNE BOLESTI

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ABSTRACT

Metastases to the scapula are very rare as a primary clinical presentation of malignancies. In this Case Report have been described two cases of metastases to the scapula which gave the presenting clinical features of different disseminated carcinomas in patients in good performance status (ECOG 1) . In the first case, detailed examination revealed that swollen scapula was the result of cancer that had spreadfrom the rectum. In the second case, the scapulacancer metastasisoriginated from the cervix. Good condition of patients, despite widespread diseases, allowed the systemic and palliative treatment, which had resulted in the clinical improvement, slower progress of disease and improvement of patient's quality of life.

Keywords: *scapula, metastasis, rectal cancer, cervical cancer*

SAŽETAK

Metastaze na skapuli veoma retko daju prvu kliničku prezentaciju malignih bolesti. U ovom prikazu slučaja su opisana dva slučaja u kojima su metastaze na skapuli dale prezentujuće kliničke manifestacije različitih diseminiranih karcinoma kod bolesnika sa dobrim performans statusom (ECOG 1). U prvom slučaju detaljna ispitivanja su otkrila da je otok skapule posledica udaljenog širenja karcinoma rektuma. U drugom slučaju, metastaze na skapuli vodile su poreklo od karcinoma grlića materice. Dobro opšte stanje bolesnika, uprkos odmaklim stadijumima bolesti, omogućilo je sistemsko i paliativno lečenje, što je za rezultat imalo kliničko poboljšanje, sporije napredovanje bolesti i poboljšanje kvaliteta života bolesnika.

Ključne reči: *skapula, metastaze, karcinom rektuma, karcinom grlića materice*

ABBREVIATIONS

ECOG: Eastern Cooperative Oncology Group
FOLFOX4:FOL – Folinic acid (leucovorin), F – Fluorouracil (5-FU) and OX – Oxaliplatin
FOLFIRI:FOL – Folinic acid (leucovorin), F – fluorouracil (5-FU)and IRI – irinotecan

INTRODUCTION

Bone metastases are a common site of advanced malignancies and cause increased morbidity, including pain, hypercalcemia, pathologic fractures, and spinal cord compression, leading to surgery or bone radiation therapy for a symptomatic metastasis. They are usually a manifestation of distant relapse from many types of solid cancers, especially those arising in the lung, breast, and prostate (1) . Rarely, advanced cancers can be presented as initial bone pathology. This Case Report shows rare examples of different advanced solid tumors that have metastasized to the scapula. In each case, metastases to the scapula gave the initialclinical features of disseminated malignant diseases.

CASE REPORT

The first patient, 62 year-old-male, with no history of malignant disease, was admitted to the Department of Orthopedics with symptoms of pain and swelling of the right scapula and altered function of the right shoulder. As shown on the right-shoulder X-ray image (Figure 1.) , the bone structure of the acromion was not differentiated from the surrounding tissues. The only exception is the part that borders with the right acromioclavicular joint, where osteolytic changes were observed. The right-shoulder CT scan (Figure 2.) revealed an infiltrative tumor change (87x82mm) in the soft tissues of the right acromioclavicular joint. The composition of the infiltrate was non-homogeneous, with the

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Figure 1. The right shoulder X-ray: osteolytic changesof the rightacromioclavicular joint.

Figure 2. The right-shoulder CT scan: tumor infiltration in the soft tissues of the acromioclavicular joint.

presence of gas inclusions. The patient underwent shoulder joint arthroscopy with biopsy of revealed tumor changes. The chest CT scan (except for the already described tumor changes of the right scapula) showed enlarged axillary-, prepericardial- and supra-diaphragmatic lymph nodes. The abdominalCT scan revealed multiple liver metastases (Figure 3.) and enlarged celiac lymph nodes. Tumor markers CEA and CA 19-9 increased several times. Histopathological examination of the right shoulder tumor infiltrate showed the following finding: „tumor proliferation of well differentiated adenocarcinoma, most likely originating from the lower gastrointestinal tract (CDX2 +, CK20-/+, CK7-) " (Figure 4.) . Subsequent colonoscopy showed rectal tumor, 15cm from the *linea anocutanea.* Histopathological finding of the bioptate was as follows: „ Adenocarcinoma intestinicrassi (recti) , medium differentiated, histological grade G2" (Figure 5) . Subsequent NMR examination of the lumbosacral bones showed malignant infiltration of the L3 vertebral body with paraspinal propagation in L3-4 intervertebral foramen. Bone scintigraphy showed increased activity accumulation in lumbar and sacral vertebral bodies, in the left sacroiliac joint, left acetabulum, the proximal and distal right humerus, with infiltration of the scapula and clavicle. As the patient was in good performance status (ECOG 1) , treatment was continued with chemotherapy with FOLFOX4 protocol (6 cycles) , and palliative single shot radiotherapy (8Gy) of the right scapula and shoulder, with the good effect on pain management. After three months of stable disease (SD) , new CT scans revealed progression of lung-, liver- and bone metastases. Treatment continued with 6 cycles of chemotherapy with FOLFIRI protocol and bisphosphonates, with a therapeutic effect evaluated as SD. Furthermore, the patient did not respond to the scheduled evaluation of the disease.

The second patient, 74-year-old female without a history of cancer developed swelling of the left scapular bone. Initially, she felt just tactile discomfort in this area, without spontaneous pain. The patient contacted the general practitioner who referredher to the rheumatologist for suspectingrheumatological disease, and she was scheduled for rheumatological examination. In the meantime, amonth after firstscapular symptom occurred, she noticed discreet vaginal bleeding, so she underwent gynecological examination. The patient was in good performance status (ECOG 1) , and in addition to the above symptoms she started to feelfatigue and a lack of energy. Gynecological evaluation and cervical biopsy confirmed malignant neoplasm of the cervix with the following histopathological finding: „Screened fragments of tumor tissue are presented without preservation ofthe surrounding structure. Morphologically, tumor tissue corresponds to invasive squamous cell large cell carcinoma, G3". Further careful evaluation, radiographs, and subsequent imaging revealed extended malignancy with metastases to the lungs and infiltration of the posterior wall of the chest and the left scapula, metastases to the lymph nodes of the thorax, abdomen and pelvis, bladder infiltration with bilateral hydronephrosis grade II and III. After permanent catheterwas inserted, her urine output was measured at 1, 5 liters per day on average, with initial creatinine clearance 24ml/min. The treatment continued with systemic *cisplatin monochemotherapy (mCDDP) by*60% *reduced dose. After 3 cycles of the mCDDP therapeutic effect was evaluated as stable disease (SD) with clinical improvement in the form of minimal reduction of scapular swelling, complete reduction of tactile stimuliscapular pain and improvement in*creatinine

clearance*to 40 ml/min. After 6 cycles of the mCDDP CT scans revealed progression of disease.*

DISCUSSION

Bone is the third most common site of distant metastases, after the lung and liver (2, 3, 4) . Among solid cancers, breast, prostate, lung, thyroid, and kidney cancer account for 80 percent of all skeletal metastases, but also, many other primary malignant tumors can spread to the bones. Metastatic bone disease (particularly fracture) is a prominent contributor to the deterioration in quality of life in patients with cancer (5, 6) . Also, bone metastases represent a significant challenge to healthcare, in terms of thecurrent increase in morbidity from malignancies and aging population trend(7, 8) . However, ongoing improvements in systemic therapy extend the lives of patients with metastatic bone disease and enable a better quality of life (9, 10) . Scapula is not frequentlyaffected by metastases originated from the solid tumors. Metastases to the scapula are even more unlikely to occur as the first clinical presentation of disseminated malignant disease. So far, these cases have been described as part of advanced malignant lung disease as a result of expansion per continuitatem (11, 12, 13) , advanced hepatocellular carcinoma (14) , carcinoid tumors (15) , but also the cases of isolated metastasis to the scapula originated from colon cancer (16) , carcinoma of the lip (17) and bladder carcinoma (18) . In the case of our patients, metastases to the scapula occurred within advanced rectal and cervical cancer. At the time of detection of scapular swelling both patients were in good performance status, and based on it, the nature and severity of diseases could not be assumed. In both cases, after the completion of tumor evaluation, despite advanced stages of malignant diseases, good performance status of patients allowed continuation of systemic chemotherapy and (in the first case) palliative radiotherapy of the scapulaand shoulder.

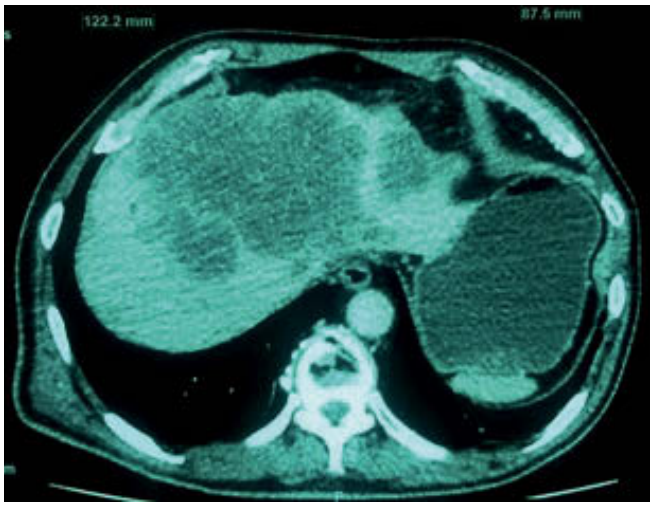

Figure 3. Abdominal CT scan shows multiple liver metastases.

CONCLUSION

Metastases to the scapula can show initial symptoms within extended malignant diseases of different primary tumors. During the initial diagnostic evaluation of the cause of the painful swelling of the scapula, healthcare providers have to develop simultaneous approach with different diagnostic procedures and make a broader evaluation of disease, as earlier diagnosis of possible advanced malignant processes can enable systemic treatment in case of patients with good performance status.

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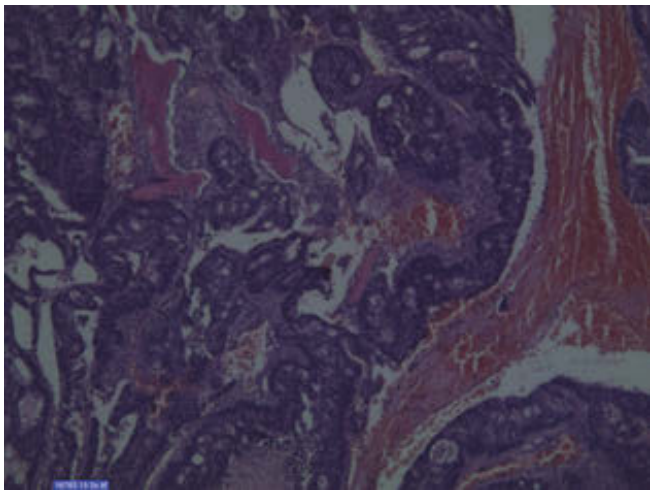

Figure 4. Tumor infiltrate of well differentiated adenocarcinoma originating from the lower gastrointestinal tractpervade the right shoulder joint synovialstructures.

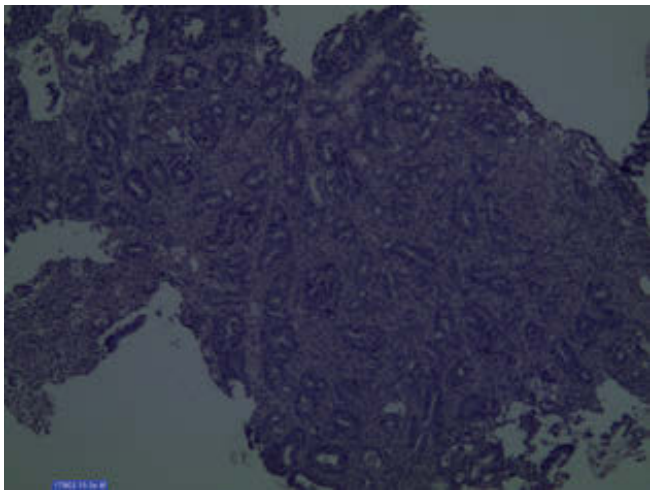

Figure 5. Pathohistological findings of rectal tumor biopsy showed medium -differentiated adenocarcinoma.

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