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PHYSICAL ACTIVITY FOR THE PREVENTION OF CARDIOVASCULAR DISEASES

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FIZIČKA AKTIVNOST U PREVENCIJI KARDIOVASKULARNIH OBOLJENJA

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ABSTRACT

Over the last decade, the quantity and quality of scientific literature examining the relationship between physical activity (PA) and cardiovascular diseases (CVD) have significantly increased. Data from the literature now unequivocally show that physical inactivity is one of the major risk factors for CVD. It is believed that obesity, the prevalence of which has tripled over the last three decades, and physical inactivity among children are the main factors that will increase the prevalence of CVD in this century. The cardiovascular benefits of exercise are multifactorial and include important systemic effects on skeletal muscle, the peripheral vasculature, metabolism, and neurohumoral systems, as well as beneficial alterations within the myocardium itself. Thus, exercise does much more than change traditional risk factors, such as blood pressure, blood lipids, glucose tolerance and insulin resistance, metabolic syndrome, and overweight and obesity. Evidence from epidemiologic studies suggests that the preventive effects of PA may be achieved by 150 minutes of moderate PA a week, while increases in the intensity and volume of exercise lead to further health benefits. This dose–response gradient is curvilinear, with the largest gains from the first hour of weekly exercise. However, although much progress has been made in this field, existing studies performed on human subjects do not clearly show what type, intensity, and duration of exercise is most beneficial to cardiovascular fitness and metabolic optimization. Animal-based exercise studies may provide more information and help to elucidate the abilities of different training regimens to reduce the risk of CVD.

Keywords: *cardiovascular diseases, physical activity, physical fitness, prevention*

SAŽETAK

Kvantitet i kvalitet naučne literature na temu veze između fizičke aktivnosti i kardiovaskularnih oboljenja (KVO) značajno je porastao u toku poslednje decenije. Naučni podaci sada nedvosmisleno pokazuju da je nedovoljna fizička aktivnost jedan od glavnih faktora KVO rizika. Smatra se da su gojaznost, čija se prevalenca u poslednje tri decenije utrostručila, i fizička neaktivnost dece glavni faktori rizika koji će u 21. veku povećati prevalencu ovih oboljenja. Kardiovaskularni benefiti vežbanja su multifaktorijalni, i uključuju važne sistemske efekte na skeletne mišiće, perifernu vaskulaturu, metabolizam, neurohumoralni sistem, kao i promene u samom srčanom mišiću. Dakle, efekti vežbanja se ne ogledaju samo u promeni tradicionalnih KVO risk faktora kao što su krvni pritisak, lipidni profil, tolerancija glukoze, rezistencija na insulin, metabolički sindrom, prekomerna uhranjenost i gojaznost. Dokazi dobijeni iz jakih epidemioloških studija ukazuju na to da se preventivni efekti fizičke aktivnosti mogu postići umerenom fizičkom aktivnošću od oko 150 minuta nedeljno, dok se sa povećanjem obima ili intenziteta fizičke aktivnosti dobijaju dodatni zdravstveni benefiti. Ovaj dozno-zavisni gradijent je krivolinijski, a najveći zdravstveni benefiti dobijaju se implementacijom prvog sata vežbanja nedeljno. Ipak, iako je napravljen veliki napredak na ovom polju, postojeće studije sprovedene na humanoj populaciji ne pokazuju jasno koji tip, intenzitet i obim vežbanja je najefikasniji u povećanju kardiovaskulanog fitnesa i metaboličkoj optimizaciji. Animalni modeli vežbanja bi mogli pružiti više informacija i pomoći u rasvetljavanju benefita različitih trenažnih režima u smanjenju rizika od KVO.

Ključne reči: *kardiovaskularna oboljenja, fizička aktivnost, fizički fitnes, prevencija*

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ABBREVIATIONS

ACSM - American College of Sports Medicine
AHA - American Heart Association
BP - blood pressure
CRP - C-reactive protein
CVD - cardiovascular diseases
CHD - coronary heart disease
eNOS - endothelial nitric oxide synthase
ET-1 - endothelin-1
HbA1c - glycated haemoglobin
HDL-C - high-density lipoprotein cholesterol
IL-6 - interleukin-6
LDL-C - low-density lipoprotein cholesterol
NO - nitric oxide
PA - physical activity
PF - physical fitness
ROS - reactive oxygen species
TNF - tumour necrosis factor
T2D - type 2 diabetes
WHO - World Health Organization

PHYSICAL ACTIVITY/FITNESS AND HEALTH

Physical activity (PA) and physical fitness (PF) have been linked with health and longevity since ancient times. A positive correlation between PA and health was recognized more than 4000 years ago in China, but Greek physicians of the 5th and 4th centuries BC were the ones who established a tradition of maintaining positive health through the combination of diet and exercise. Currently, physical inactivity, poor diet and smoking are the root causes of approximately one-third of deaths in developed countries (1). These risk factors often underlie today's leading chronic disease killers of heart disease, stroke, diabetes and cancer.

The strength of the association between PF and all-cause mortality risk, which has been reported in numerous epidemiological studies, suggests that PF is of equal or greater importance as a mortality predictor than other established disease risk factors. Nevertheless, the Norwegian epidemiologist Gunnar Erikssen asserts that "modern day humans are dying because of a lack of physical exercise" (2). The World Health Organization (WHO) reported that physical inactivity is the 4th leading risk factor for global mortality, accounting for 6% of deaths globally (3). Recently, a published analysis of the worldwide burden of disease showed that physical inactivity is responsible for 6% of the incidence of coronary heart disease (CHD), 7% of type 2 diabetes (T2D), 10% of breast cancer, and 10% of colon cancer (4). It is posited that physical inactivity among children and obesity, the prevalence of which has tripled in the last 30 years, are the main risk factors that will increase the prevalence of these diseases in the 21st century (1). However, although PA and PF are associated with the prevention of premature mortality, they do not appear to extend the natural lifespan (1).

PHYSICAL ACTIVITY/FITNESS AND CARDIOVASCULAR DISEASES

Advances in understanding the pathophysiological basis of cardiovascular diseases (CVD) have led to significant reductions in the prevalence and incidence of these diseases since the middle of the last century (5), but CVD still represent the main cause of death worldwide (6), especially in Eastern Europe (7). In Europe, approximately 42% of deaths in women and 38% in men are due to CVD (8), mostly CHD and stroke. Although genetic factors and age play major roles in the occurrence and development of these diseases, other factors, such as elevated blood pressure (BP), high levels of cholesterol, insulin resistance, diabetes, obesity and behavioural factors such as smoking, poor diet and physical inactivity, also have a great influence on CVD development (9).

The observation that PA can protect against heart attack was first made in the 1950s, when the first cross-sectional studies compared incidence rates of CHD attacks in men in a variety of occupations. One of the first studies reported that the conductors of English double-decker buses experienced roughly half the number of heart attacks as the drivers (10). A similar difference was noticed between postmen and their sedentary colleagues who sorted the mail (11). Since the early work of Morris and colleagues (10,11), from the works of Blair, Paffenberger and colleagues in the 1980s and 1990s (12-15) to today, numerous longitudinal studies have explored the correlation between PA/PF and the relative risks of morbidity and mortality (16-20). The US Physical Activity Guidelines Advisory Committee reported that, based on analysis of 60 studies with a total of more than 300 000 participants, moderate PA decreases the risk of CHD by 19% in males and 22% in females, while vigorous exercise has even better effects, with a 32% reduction among males and a 38% reduction among females (21). Similar results were reported on the relationship between PA and stroke (22-24). There was no evidence on different effects of PA in different populations, including men vs women, premenopausal vs postmenopausal women, or middle-aged vs older (>65 years) populations. Those results are consistent with the systematic review and meta-analysis on the association of PA with all-cause and cardiovascular mortality, which included 24 studies with a total of more than 650 000 participants (25). This analysis showed that cardiovascular mortality was 35% lower in the most active compared with the least active subjects. Adjusting only for age, the risk reduction increased to 47%. In stud-

ies that examined CVD risk based on PF (results achieved on a test of cardiovascular endurance) as opposed to data on the amount and intensity of PA reported by questionnaire, PF was related to a 57% lower CVD risk (26). The key message obtained from those studies is that, in sedentary subjects, even small increases in PA, and consequently PF, significantly reduce CVD risk (27). One of the first studies to demonstrate an association between PF and all-cause mortality risk was the Aerobics Center Longitudinal Study (12). In this study, based on time to exhaustion in a maximal treadmill exercise test, subjects were classified into 5 groups of fitness. The results showed that the risk of all-cause mortality during follow-up was 3.44 times higher in men and 4.65 times higher in woman with lowest physical fitness level when compared to subjects with the highest fitness level, but more importantly, even small improvements in fitness among totally unfit subjects reduced the risk by half. These trends remained after statistical adjustment for age, smoking, cholesterol level, systolic BP, fasting blood glucose level, parental history of CHD and follow-up interval. A 2012 analysis of data from 6 cohorts with a total of 655 000 adults followed for a median of 10 years supports the idea that even minimal levels of physical activity can extend lifespans (28). Compared with their inactive peers, women who reported a low amount of PA (~11 minutes/day) had a 2.1-year longer life expectancy after age 40, while women who exercised 30 minutes/day had additional gains in life expectancy (3.6 years). Women with an activity level of 60 to 90 minutes/day experienced further gains (4.0 years), proving that the dose–response gradient is curvilinear, with the largest gains from the first hour of weekly exercise. The weight of the evidence from numerous studies strongly points towards a favourable relation between increases in habitual aerobic exercise and cardiovascular health outcomes, including CHD morbidity and mortality, stroke, control of BP, atherogenic dyslipidaemia, vascular function measures and cardiorespiratory fitness (21).

PHYSICAL ACTIVITY AND REDUCED CARDIOVASCULAR RISK: BIOLOGICAL MECHANISMS

The cardiovascular benefits of exercise are multifactorial and include important systemic effects on skeletal muscle, the peripheral vasculature, metabolism, and neurohumoral systems, as well as beneficial alterations within the myocardium itself (29, Table 1). Molecular mechanisms through which exercise exerts its favourable effects on CVD are clearly presented in a recent paper by Gielen and colleagues (30). Thus, exercise does much more than change traditional risk factors such as BP, blood lipids, glucose tolerance and T2D, metabolic syndrome, and overweight and obesity (31). For example, in the women's health study (32), less than half of the improvement in the risk for CHD could be attributed to improvements in traditional risk factors. Additionally, only ~59% of the risk reduction for all forms of CVD could be attributed to the effects of exercise on traditional factors. This means

Table 1. Effects of physical activity on the cardiovascular system (30)

PHYSICAL ACTIVITY			
Cardiac effects	**Vascular effects**	**Neurohumoral and autonomic effects**	**Non-cardiovascular effects**
Normal LV function: Ischaemia/reperfusion protection Prevention of age-related diastolic dysfunction Physiologic hypertrophy *Systolic heart failure:* Reverse left ventricular remodelling Left ventricular ejection fraction ↑ Improved neurohumoral activation Arrhythmia prevention *Diastolic heart failure:* Prevention of diastolic function Improvement of left ventricular relaxation and compliance *Cardiac valves:* Prevention of valve degeneration Prevention of calcification	*Aorta:* Aorta stiffness ↓ Aortic compliance ↑ *Conduit vessel:* Endothelial vasodilatation ↑ Production of nitric oxide ↑ Oxidative stress ↓ *Resistance vessel and microcirculation:* Vasculogenesis Sensitivity to adenosine ↑ *Capillary bed:* Capillary vessel formation ↑ *Venous circulation:* Venular capillaries ↑ *Pulmonary artery:* Endothelial function ↑ Pulmonary artery pressure in CHF ↓	Sympathetic tone ↓ Parasympathetic tone ↑ *In chronic heart failure:* Norepinephrine Angiotensin II Atrial natriuretic peptide Brain natriuretic peptide *Antiarrhythmic effects:* Normalization of heart rate variability Hyperpolarization Attenuated automaticity	*Skeletal muscle:* Oxidative phosphorylation ↑ Muscle hypertrophy Calcium handling ↑ *Ventilation:* Vital capacity ↑ Tidal volume ↑ Max inspiratory and expiratory force ↑ *Haemorheology:* Blood viscosity ↓ Coagulability ↓ O_2 transport capacity ↑

that only ~40–60% of the relative risk of CHD and CVD in general can be explained by how exercise and PA modify traditional risk factors.

PA and BP. Despite being healthy, physically inactive people and people with poor fitness levels have a 30% higher risk of developing high BP compared with healthy physically active/fit people (33). Furthermore, for every 20/10-mmHg increase in BP, there is a doubling of mortality from both ischaemic heart disease and stroke (34). A recently published meta-analysis (35) shows that in healthy people, aerobic types of PA decrease resting BP by an average of 2.4/1.6 mmHg (systolic/diastolic BP). In prehypertensive subjects, the corresponding decrease is 3.1/1.7 mmHg, and in hypertensive subjects it is 6.9/4.9 mmHg. Resistance training can also induce lowering of BP (36). It has been long thought that static resistance exercises should be avoided due to an acute hypertensive response, but recent research shows that this type of exercise has the greatest potential for systolic BP reduction (37). A meta-analysis of randomized controlled trials shows that resistance training reduces systolic BP by an average of 3.2 mmHg and diastolic BP by an average of 3.5 mmHg (38). Although these reductions seem modest, a systolic BP reduction of 3 mmHg in average populations has been estimated to reduce cardiac morbidity by 5-9%, stroke by 8-14%, and all-cause mortality by 4% (36). BP lowering is the result of the reduction in total peripheral resistance due to changes in the diameter of blood vessels, which are attributed to the smaller influence of the sympathetic system on the peripheral blood vessels and the effects of local vasodilators such as nitric oxide (39).

PA and blood lipids. The role of blood lipids in the pathology of atherosclerosis is well established, and dyslipidaemia is understood to be an important contributing factor for CHD. Cross-sectional studies have consistently shown a positive association between the volume and intensity of aerobic activities and high-density lipoprotein cholesterol (HDL-C) levels and a negative association with triglyceride levels (40), especially in subjects with initially high levels of these blood lipids (26). A meta-analysis of 52 exercise training trials demonstrated an average increase in HDL-C levels of 4.6% and average reductions in triglyceride and low-density lipoprotein cholesterol (LDL-C) concentrations of 3.7% and 5.0%, respectively (15,16). In contrast, the effects of resistance training on blood lipids are not as consistent in the available literature (41).

PA and T2D. A great number of studies have shown that PA is significantly related to improved glucose tolerance and decreased risk of T2D (21, 26), which is a consequence of increased sensitivity of muscle and other tissues to insulin. A meta-analysis of 10 cohort studies with a total of more than 300 000 participants found that compared with inactivity, and after adjustment for body mass index, moderate-intensity PA predicted a 17% reduction in diabetes risk (42). In one study, brisk walking for 30 minutes/day 5 days/week was associated with a 25% reduction in diabetes risk (43), while another stated that participants who walked 2 to 3 hours/week were 34% less likely to develop diabetes (44). Cardiorespiratory fitness, as assessed by a bicycle ergometer or treadmill test, also correlates inversely with the incidence of T2D (45). The effects of resistance exercise on T2D risk remain unknown due to few published studies on this relationship. One study found that resistance training was associated with a decline in glycated haemoglobin (HbA1c) levels, which are measured primarily to identify the average plasma glucose concentration over prolonged periods of time (46). Although the effects of resistance training on T2D risk are not clearly proven scientifically, leading organizations for diabetes prevention and treatment recommend that diabetics include this type of exercise in their lives (47).

Exercise also has a role in secondary prevention in subjects with T2D. Heart disease and stroke account for approximately 65% of deaths among people with diabetes, and individuals classified as prediabetic are also at increased risk for CVD (34). Recent meta-analyses of diabetic cohorts report risk reductions of 29% for CVD incidence (48) and 37% for CVD mortality (49) for those in the highest versus lowest PA category. Walking 2 to 4.5 hours/week is associated with a 46% reduction in CVD mortality compared with walking less than 2 hours/week (49). Structured exercise training that consists of aerobic exercise, resistance training, or both, is associated with HbA1c reduction in patients with type 2 diabetes (36). A review of 9 trials examining the effect of exercise training in patients with T2D reported an average reduction of HbA1c of 0.5% to 1% (50). One study showed that each percentage point reduction in HbA1c was associated with a 35% reduction in microvascular complications (51), whereas an increase of 1 percentage point in HbA1c was associated with a 28% increase in mortality risk, independent of other CV risk factors (52).

Finally, taking into consideration the effects of PA on BP, blood lipids and glucose tolerance, it is clear that there is a strong relationship between the amount of PA and metabolic syndrome. Several cross-sectional and prospective studies strongly suggest dose-response relationships between the amount of PA and metabolic syndrome in men and women (21).

PA and obesity. Exercise serves to counteract excess caloric consumption, thereby reducing the risks of obesity. Numerous longitudinal epidemiological studies have found that PA levels are negatively correlated with weight gain (53-55). Prevention of weight gain is an effective way to prevent the development of undesirable changes in the metabolic CVD risk factors, and even small (less than 3%) or no decrease in body weight as a result of PA leads to significant beneficial changes in those risk factors (56). Furthermore, PA also counterbalances the risk caused by overweight (BMI of 25.0 to 29.9) on CVD mortality or events (57). Increasing PA levels from low to moderate ensures a maintenance of body weight, while increasing PA levels from low to high helps individuals lose weight (58). In the Women's Health Study, 60 minutes/day of moderate PA was associated with maintenance of weight or an increase in minor weight loss (less than 2.3 kg) during 13 years of follow up (59). Anoth-

er study showed that PA for 30 or more minutes per day is strongly associated with a significantly lower likelihood of weight gain of more than 5%, with the strongest effect in overweight subjects (60). In this study, even a small increase in activity (11-20 minutes/day) in sedentary subjects appeared to be beneficial. Resistance exercise also positively influences the maintenance of weight, although evidence from the literature is less consistent than in the case of aerobic activity. Muscle mass decreases with age, but resistance training increases muscle mass and consequently increases resting energy consumption (61). Weight loss induced by resistance training is not as obvious due to the simultaneous decrease in fat mass and increase of muscle mass (36), meaning that although weight does not significantly change, body composition improves.

Aerobic PA also decreases total abdominal adiposity and intra-abdominal adiposity (26). This is important because excessive central adiposity, especially visceral fat, is related to the development of hyperlipidaemia, hypertension, insulin resistance, T2D, and cardiac diseases, while fat in the extremities is a small risk factor (62, 63). Although there is genetic predisposition to having visceral fat tissue, ageing, together with a high-fat diet and sedentary lifestyle, are also important determinants of this fat tissue. According to limited scientific evidence, the effects of resistance training on abdominal obesity seem to be small and inconsistent (21, 36).

PA and inflammation. An inactive lifestyle leads to the accumulation of visceral fat, and this is accompanied by adipose tissue infiltration by pro-inflammatory immune cells, increased release of adipokines and the development of a low-grade systemic inflammatory state (64). A chronic low-grade inflammatory state, as indicated by elevated levels of circulating inflammatory markers such as interleukin-6 (IL-6), tumour necrosis factor (TNF) and C-reactive protein (CRP), has been established as a predictor of risk for numerous diseases, including CVD. Regular exercise reduces the risk of chronic metabolic and cardiorespiratory diseases, partially due to the anti-inflammatory effects of PA. PA has been shown to decrease chronic low-grade inflammation, which is an important factor in the pathogenesis of atherosclerosis and insulin resistance (65, 66). The anti-inflammatory effects of regular exercise may be mediated via both a reduction in visceral fat mass (with a subsequent decreased release of adipokines) and the induction of an anti-inflammatory environment (67-69). Following acute exercise, there is a transient increase in circulating levels of anti-inflammatory cytokines, whereas chronic exercise reduces basal levels of pro-inflammatory cytokines (65). Cross-sectional studies indicate that increasing levels of PA/PF are associated with reductions in circulating levels of TNF-α and IL-6 and increased levels of anti-inflammatory substances such as IL-4 and IL-10 (70-72). Cross-sectional studies also consistently demonstrate an inverse relationship between serum CRP and both PA level and cardiorespiratory fitness (73). For example, one study found that 10 months of aerobic exercise, but not flexibility and resistance exercise, significantly reduced serum CRP by 10–15% (74). Exercise training also induces the expression of antioxidant and anti-inflammatory mediators in the vascular wall that may directly inhibit the development of atherosclerosis (65).

PA and oxidative stress. Atherosclerosis has been described as an inflammatory response to oxidized LDL in the artery wall (75). Although acute exercise increases production of reactive oxygen species (ROS), exercise-induced plasma oxidative stress may stimulate an arterial antioxidant response, which should inhibit LDL oxidation, inflammation and ultimately atherosclerosis (76). Chronic exercise appears to enhance antioxidant defences in skeletal muscle, the circulation and the vasculature by a variety of mechanisms. Regular exercise training increases the activity of the antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase (77). Chronic exercise also reduces markers of oxidative stress in the plasma, including F2-isoprostanes (78) and myeloperoxidase (79), whose circulating levels are also associated with CVD risk (80,81). Furthermore, exercise increases the activity of endothelial nitric oxide synthase (eNOS), whose activity has many direct and indirect effects on oxidative stress and inflammation (65). Exercise-induced upregulation of vascular eNOS expression is closely related to the frequency and intensity of physical forces within the vasculature, especially shear stress. Laminar flow, which is augmented during moderate and intense physical activities, upregulates eNOS expression, while oscillatory forces, which are associated with hypertension, lead to increased NADPH-oxidase activity and augment oxidative stress (82).

PA and arterial stiffness and compliance. PA also exerts several direct effects on the vascular wall. Adults who regularly perform aerobic PA demonstrate smaller or no age-associated increases in large elastic artery stiffness, reductions in vascular wall endothelial function, and increases in carotid artery intimal medial thickness (26, 83, 84). Aerobic exercise training improves carotid artery compliance and improves vascular endothelial function through several mechanisms. The effects of exercise on the vascular wall may be induced via the impact of repetitive increases in shear stress on the endothelium, which transduce structural and functional adaptations that decrease arteriosclerotic risk. The age-related losses in endothelial function are also affected by restoration of nitric oxide (NO) availability consequent to prevention of ROS production due to regular PA (85). An exercise-induced increase in arterial compliance is also mediated by a reduction in plasma endothelin-1 (ET-1) concentration, as well as the elimination of ET-1-mediated vascular tone (82). The beneficial effects of PA on the vascular wall may also be enhanced by decreased sympathetic and increased parasympathetic outflow caused by PA (31, 86). When aerobic exercise is combined with resistance training, there is no evidence of increased arterial stiffness (87), though less is known about the independent effects of resistance training on arterial stiffness. The results of the studies on this relationship are contradictory (88, 89).

RECOMMENDATIONS ON THE TYPE AND DOSE OF PA FOR CVD PREVENTION

Being physically active does not necessarily mean playing sports. PA is defined as any bodily movement produced by skeletal muscles that results in an energy expenditure significantly beyond resting level (such as cleaning or digging), while systemic execution of PA for a specific purpose, i.e., maintenance or development of one or more fitness components, is termed exercise or exercise training (90). PF is a set of attributes that enables an individual to perform PA, and it encompasses cardio respiratory fitness, muscular strength, muscular endurance, flexibility, and body composition as the most important health-related components (91). The effects of exercise training may vary with different exercise modalities (like endurance training or resistance exercise) and dose parameters, specifically programme length, session duration, frequency, and workload or intensity. Dynamic aerobic endurance exercise involves large muscle groups in dynamic repetitive activities that result in substantial increases in heart rate and energy expenditure (37). Resistance training is an activity in which each effort is performed against a specific opposing force generated by resistance and is designed specifically to increase muscular strength, power, and/or endurance (37).

According to the WHO, a significant decrease in CVD risk may be achieved by moderate-intensity aerobic PA (3-6 times higher oxygen consumption than at rest) performed at least 5 days per week for 30 minutes per day (150 minutes per week), and increases in intensity and/or volume of PA provide additional health benefits (92). However, the shapes of any dose-response relations have not been well defined, potentially because of the inaccuracy involved in assessing physical activity (data regarding the amount of PA is usually self-reported by subjects; pedometers, heart rate monitors and other modern technologies are rarely used for monitoring PA in those studies). Additionally, the obtained data are hard to analyse due to the variability of human subjects and numerous confounding factors. Thus, it is not clear what type, intensity, and duration of exercise is most beneficial to cardiovascular fitness and metabolic optimization. The existing data in the literature mainly concern the relationship between CVD prevention and volume of exercise, with less information about intensity and none for frequency and duration of sessions. Many studies suggest that moderate exercise intensity is sufficient to reduce the risk of CVD, but a recent review article suggests that high-intensity aerobic interval training results in a greater beneficial adaptation of the heart compared with that observed after low-to-moderate exercise intensity (93). In a study of health professionals, high exercise intensity was associated with reduced all-cause mortality independent of the duration of activity (17). Another study showed that a single weekly bout of high-intensity exercise reduced the risk of cardiovascular death by approximately half, and no additional benefit from increasing the duration or the number of exercise sessions per week was observed (94). However, it is important to understand that among individuals with very low fitness or with physical limitations, vigorous physical activity may be difficult to achieve and may be contraindicated in some cases. Furthermore, there is very limited data related to the effects of short bouts of PA (~10 min or less) or the accumulation of those shorts bouts during the day, though they seem to be effective in increasing cardio-respiratory fitness (21). One prospective study in men examined the relation between brief bouts of exercise and clinical CVD. After controlling for total energy expenditure, exercise sessions lasting 15, 30, or 45 minutes offered equal protection against incident CVD in subjects followed for 5 years (95). Incorporating such findings into public health messages may help convince busy individuals to treat exercise as a manageable part of their daily routine rather than as a time-consuming activity reserved for rare occasions (96).

Aerobic exercise, such as walking, cycling and stair climbing, was the type of PA that the majority of studies explored in relation to CVD prevention. A meta-analysis that explored active commuting and cardiovascular risk showed that people who walk or cycle to work have an 11% lower risk from CVD mortality, CHD, stroke, hypertension and diabetes (97). Walking, which is the most common and feasible type of PA, has been the most often investigated, and the results of those studies are unambiguous (27, 97-99). The effects of walking on CVD mortality are dose-dependent (98), with the pace of walking being a more important factor than total distance (26). Walking for just 1 hour per week was associated with a reduced risk of CVD outcome (27), while 30 minutes of normal walking each day for 5 days per week (150 minutes per week) was associated with a 19% lower risk of CVD (99). Cycling, as a popular leisure time activity and in some countries a mode of commuting, has been investigated to a lesser extent than walking, and due to some methodological problems, the results of those studies are not as strong. However, evidence from randomized controlled trials indicates that the intensity of the spontaneously chosen speed of cycling is more important than the intensity of walking, and it is sufficient to lead to significant increases in aerobic capacity and to induce favourable changes in selected cardiovascular risk factors. In one prospective study, the risk of fatal and non-fatal CVD incidence among cyclists compared with non-cyclists was found to be 18% (100), while in other studies it was 25-37% (101). Stair climbing, which is a high-intensity aerobic activity, was also found to be effective for improving cardiorespiratory fitness and preventing CVD (102). Stair climbing for 8–12 minutes a day may improve aerobic capacity in previously untrained individuals by more than 10%, which may be associated with a 15% reduction in CVD mortality (103).

In contrast, no large epidemiological studies have explored the relationship between CVD and anaerobic

exercise, such as strength (resistance) training or short-distance running. However, it is believed that resistance exercise may lower the risk for CHD (104). For example, among 44 000 men in the Health Professionals Follow-up Study, those who trained with weights for more than 30 minutes per week were 23% less likely to develop CHD during an 8-year follow-up period than those who did not train with weights (105). However, although additional research is needed to confirm this protective effect, the American College of Sports Medicine (ACSM) and the American Heart Association (AHA) have published a report in which, in addition to the previously recommended 30 minutes of daily moderate aerobic activity, more intensive aerobic activity and 2 sessions of resistance training per week are added to the recommendations (106). Their inclusion of resistance training for CVD prevention and reduction was based on the known effects of resistance training on certain biological CVD risk factors (36,104). Using scientific evidence and expert opinion, the AHA and the ACSM published guidelines on resistance training for individuals with and without CVD (107, 108). The guidelines for individuals without CVD is to include 2-3 sessions of resistance training per week, each consisting of 8-10 exercises covering the major muscle groups, with moderate training load (30-40% of one repetition maximum for upper body exercises and 50-60% of one repetition maximum for lower body exercises). Exercisers should aim for 8 to 10 repetitions in a set, and when 12 to 15 repetitions can be accomplished with little difficulty, the weight should be increased (36).

PA and BP. For the prevention and management of hypertension, leading health organizations recommend regular aerobic physical activity, such as brisk walking, at least 30 minutes per day, most days of the week (109). Vigorous exercise was not found to be different from moderate-intensity exercise in its ability to reduce blood pressure, with some studies showing that exercise at 40% of oxygen consumption reserve produces a greater reduction in systolic blood pressure than exercise at 65-75% of oxygen consumption reserve (110,111).

PA and blood lipids. There is no general consensus on the optimal exercise prescription for improving the blood lipid profile, as very few studies attempted to evaluate the relationship between training load and blood lipids. However, in the majority of available studies on this theme, the exercise intervention was performed at moderate to high intensity, 3 to 5 times per week, for at least 30 minutes per session.

PA and T2D. To improve glycaemic control, assist with weight maintenance, and reduce risk of CVD, 150 minutes of moderate-intensity, or 90 minutes of vigorous-intensity aerobic PA per week is recommended (34). The physical activity should be distributed over at least 3 days per week, with no more than 2 consecutive days without physical activity. Any amount of PA is better than no activity, but higher intensity and more frequent exercise sessions increase this preventive effect. Unless contraindicated, people with T2D should be encouraged to perform resistance exercise 3 times per week, targeting all major muscle groups (34).

PA and obesity. Although there is large inter-individual variability, the literature suggests that the majority of people need more than 150 minutes per week of moderate PA to maintain a stable weight (less than 3% change in body weight), while clinically significant weight loss (at least 5% of body weight) may be achieved by a combination of adequate diet and moderate-intensity aerobic PA of 150–250 minutes per week (21,56,112-114). The ACSM recommends an initial goal of a reduction in body weight of 5% to 10% through a combination of moderate-intensity exercise and calorie restriction sufficient to produce a negative caloric balance of 500 to 1000 kcal per day (115). To prevent the regaining of lost weight, approximately 250 to 300 minutes of PA per week are needed (26).

PA and inflammation. Based on available research, the mode, intensity and duration of exercise required to optimize the anti-inflammatory effects cannot be established, and the mechanisms of these effects need to be elucidated. High training loads may be needed to maximize the anti-inflammatory effects of PA, but this may cause a small increase in infection risk (70). An independent contribution of an exercise-induced reduction in visceral fat, apart from other exercise-induced anti-inflammatory mechanisms, to reducing inflammation in adipose tissue, insulin resistance and risk of chronic disease also remains to be determined (70).

PA risks. PA confers risks as well as benefits, but it is important to recognize that regular activity of moderate or vigorous intensity is associated with an overall decrease in CVD events that far outweighs the transient heart risks associated with sporadic exertion (21). The absolute risk associated with a bout of moderate or vigorous physical activity is low and is on the order of 2 to 3 additional myocardial infarctions and 1 additional sudden cardiac death per 10 000 person-years of exercise (116). The risk is even lower among people who are habitual rather than sporadic exercisers.

It is very important to understand that the physiological and biochemical improvements that occur in the muscles, heart, and vascular function that occur with regular PA do not depend on an enormous physical effort, but rather on a physical effort that is greater than that to which an individual is accustomed. Brisk walking is generally recommended as the type of exercise that meets the criteria for moderate physical activity, but brisk walking may not be of sufficient intensity to meet the minimum criteria for moderate PA in a normal, healthy individual of college age, while it may be considered vigorous activity for someone older than 65 years. Thus, the dose of PA must be defined relative to an individual's age, physical condition and limitations, and gradual increases of training load should be performed in order to allow for bodily adaptation. Although athletes may require work at a high intensity to improve their fitness and performance, sedentary or relatively inactive individuals require little.

CONCLUSION

Over the last decade, the quantity and quality of scientific literature on the relationship between PA and CVD have significantly increased. It is now known that exercise does much more than change traditional risk factors such as blood pressure, blood lipids, glucose tolerance and insulin resistance, metabolic syndrome, and overweight and obesity. PA also influences novel cardio-metabolic risk factors, endothelial function, haemostasis, inflammatory defence systems, the myocardium, and others. This means that even in the absence of positive changes in traditional risk factors, increasing the PA level can decrease the risk of CVD. Evidence from epidemiologic studies suggests that preventive effects of PA may be achieved by 150 minutes of moderate PA per week, while increases in the intensity and volume of exercise lead to further health benefits. However, although much progress has been made in this field, existing studies performed on human subjects do not clearly identify the type, intensity, and duration of exercise that are most beneficial to cardiovascular fitness and metabolic optimization. Information from athletes or patients is difficult to analyse due to the variability of human subjects and confounding comorbidities and medications. Animal (rat) models, in contrast, often allow for more invasive, extensive, and homogenous experimental designs than human models. These rat-based exercise studies may provide more information and help to elucidate the benefits of different training regimens to reduce the risk of CVD.

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THE PLATINUM(II) COMPLEXES INDUCED OXIDATIVE STRESS OF ISOLATED RAT HEART

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PLATINA (II) KOMPLEKSI INDUKUJU OKSIDACIONI STRES IZOLOVANOG SRCA PACOVA

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ABSTRACT

Interest for the clinical application of transition metal complexes as chemotherapeutic agents initially started with discovery of cisplatin. Despite the remarkable clinical success, cisplatin treatment is limited due to its resistance and side effects. Over the last 40 years, numerous transition metal complexes were synthesized and investigated in vitro and in vivo in order to establish a metallopharmaceutical that will exert less toxicity and equal or higher potency. We have compared the cardiotoxicity of 2 platinum complexes, one ligand, and a starting salt for complex synthesis using an experimental model of an isolated, perfused rat heart according to the Langendorff technique. The cardiotoxicity was assessed by comparison of oxidative stress induced following the perfusion of the following compounds: Dichloro(1,2-diaminocyclohexane)platinum(II), cisplatin, potassium-tetra-chloroplatinum(II) and 1,2-diaminocyclohexane, which were perfused at increasing concentrations from 10^{-8} to 10^{-4} M for 30 minutes. The oxidative stress was assessed by determination of superoxide anion radical, hydrogen peroxide, thiobarbituric acid reactive substances, and nitric oxide from the coronary venous effluent. Our results showed that the levels of oxidative stress parameters were not significantly affected by perfusion with all the tested compounds and were not dose-dependent. These results could be of importance to further investigations concerning the effects of platinum-based potential anticancer drugs on the heart.

Key words: *cisplatin, 1,2-diaminocyclohexane, isolated rat heart, oxidative stress, perfusion, platinum(II) complexes*

SAŽETAK

Interesovanje za kliničku primenu kompleksa prelaznih metala kao hemioterapijskih lekova započeto je otkrićem cisplatine. Uprkos izvanrednom kliničkom uspehu, lečenje cisplatinom je ograničeno zbog njene rezistencije i neželjenih efekata. Tokom poslednjih 40 godina sintetisan je veliki broj kompleksa prelaznih metala i ispitan in vitro i in vivo sa ciljem uvođenja metalofarmaceutika koji bi imao manju toksičnost i istu ili veću potentnost. Mi smo poredili kardiotoksičnost dva kompleksa platine, jedan ligand i so potrebnu za početak sinteze kompleksa, koristeći eksperimentalni model izolovanog, perfundovanog srca pacova metodom po Langendorfu. Kardiotoksičnost se procenjivala upoređivanjem oksidacionog stresa indukovanog perfuzijom tih supstanci. Dihloro(1,2-diaminocikloheksan) platina (II), cisplatina, kalijum-tetra-hloroplatinat i 1,2-diaminocikloheksan su primenjeni u rastućim dozama od 10^{-8} do 10^{-4} M tokom 30 minuta. Oksidacioni stres je određivan merenjem superoksid anjon radikala, vodonik peroksida, indeksa lipidne peroksidacije i azot-monoksida u koronarnom venskom efluentu. Naši rezultati su pokazali da nivoi parametara oksidacionog stresa nisu bili značajno povišeni niti dozno-zavisni nakon perfuzije svih ispitivanih supstanci. Ovi rezultati bi mogli biti od značaja za buduća istraživanja potencijalnih antitumorskih lekova zasnovanih na platini u pogledu efekata na srce.

Ključne reči: *cisplatina, 1,2-diaminocikloheksan, izolovano srce pacova, oksidacioni stres, perfuzija, kompleksi platine(II)*

ABBREVIATIONS

CDDP - cisplatin
GSH - glutathione
DACH - 1,2-diaminocyclohexane
DNA - deoxyribonucleic acid
H_2O_2 - hydrogen peroxide
$K_2[PtCl_4]$ - potassium-tetra-chloroplatinum(II)
mtDNA- mitochondrial deoxyribonucleic acid
NO - nitric oxide
$O_2^{\bullet -}$ - superoxide anion radical
$Pt^{(II)}DACHCl_2$ - dichloro (1,2-diaminocyclohexane) platinum(II)
ROS - reactive oxygen species
TBARS - Thiobarbituric Acid Reactive Substances

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INTRODUCTION

Clinical use of metal-based anticancer drugs began in 1970s, several years after the accidental discovery of the antitumour effects of cisplatin (CDDP) by Rosenberg in 1965 (1, 2). Cisplatin is one of the most effective chemotherapeutic agents for the treatment of various cancers, such as lung, bladder, neck, ovarian, and testicular (3). It has a potent cytotoxic effect due to its ability to cross-link DNA through a covalent coordinate bond to the N7 atoms of guanine and adenine. This molecular mechanism prevents replication and transcription of DNA and finally leads to apoptosis and cell death (4). Other targets of cisplatin are glutathione and metallothioneins, to which binding has been associated with the development of resistance and toxicity (5). Accordingly, the clinical use of cisplatin is severely limited, especially by dose-dependent side effects, such as nephro-, oto-, neuro-, hepato-, and cardiotoxicity (6, 7). Over the past decades, medicinal chemists have been devoted to the development of a large number of novel platinum complexes with less toxicity and more antitumour success (8, 9).

Many preclinical and clinical researchers have suggested that chronic cisplatin therapy is associated with severe side effect such as cardiotoxicity (10, 11). Cardiotoxicity may be an early or late complication after treatment with cisplatin. Acute manifestations are accompanied with electrocardiographic changes and arrhythmias, while late complications are primarily associated with the development of cardiomiopathy and congestive heart failure (12-14). Experimental evidence supports the hypothesis that cisplatin and its analogues promote cardiotoxicity through the formation of reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), Thiobarbituric Acid Reactive Substances (TBARS), and nitric oxide (NO) (15, 16). Numerous studies reported that cisplatin has a potential to induce oxidative damage in heart tissue by causing peroxidation of the cell membrane and dysfunction of mitochondria (17, 18).

The present study was performed in order to assess the cardiotoxicity of cisplatin and its analogue, $Pt^{(II)}DACHCl_2$ (dichloro(1,2-diaminocyclohexane)platinum (II)), in isolated rat heart by means of oxidative stress markers in the coronary venous effluent.

MATERIALS AND METHODS

Preparation of isolated rat hearts

Male Wistar albino rats (n=60, 12 per group, age 8 weeks, body mass 180-200 g) were killed by cervical dislocation (Schedule 1 of the Animals/Scientific procedures, Act 1986, UK) after short ether narcosis. After emergency thoracotomies and sudden arrest by superfusion with ice-cold physiological solution, the hearts were rapidly excised and perfused via the aorta according to Langendorff's technique at a constant coronary perfusion pressure of 70 cm H_2O. The composition of Krebs-Henseleit solution was as follows (mmol/l): NaCl (118); KCl (4,7); $CaCl_2$ x $2H_2O$ (2,5); $MgSO_4$ x $7H_2O$ (1,7); $NaHCO_3$ (25); KH_2PO_4 (1,2); glucose (5,5). The solution was balanced with 95% O_2 and 5% CO_2 at 37°C, with a pH value of 7,4. Immediately after the establishment of automatic operation by opening the left atrium of the heart and dissecting the mitral valve, the sensor was inserted (*transducer BS4 73-0184, Experimetria Ltd, Budapest, Hungary*) into the left ventricle for continuous registration of myocardial function.

Physiological assay and experimental protocol

After the stabilization period of 30 minutes, the hearts were perfused with different concentrations (from 10^{-4} to 10^{-8} M) of following substances: CDDP, $Pt^{(II)}DACHCl_2$, $K_2[PtCl_4]$, DACH and Krebs-Henseleit solution (control group).

Biochemical assays

Samples of coronary venous effluent were collected at the end of the period of perfusion with each of the tested compounds (30, 60, 90, 120 minutes).

Superoxide determination

Superoxide anion radical ($O_2^{\cdot-}$) levels were measured in the coronary venous effluent by nitro blue tetrazolium (NBT) in TRIS buffer at a wavelength of 530 nm. Krebs-Henseleit solution was used as a blank probe (19).

Hydrogen peroxide determination

Hydrogen peroxide (H_2O_2) levels were determined by measuring the oxidation of phenol red in a reaction catalysed by horseradish peroxidase (HRPO). The level of H_2O_2 was measured at 610 nm (20).

Nitrite determination

The nitrite level (NO_2^-) was measured and used as an index of nitric oxide (NO) production using Griess's reagent. A total of 0,5 ml of perfusate was precipitated with 200 µl of 30% sulphosalicylic acid, vortexed for 30 min and centrifuged at 3000 g. Equal volumes of the supernatant and Griess's reagent, containing 1% sulphanilamide in 5% phosphoric acid/0,1% naphtalene ethylenediamine dihydrochloride, were added and incubated for 10 min in the dark and measured at 543 nm. The nitrite levels were calculated using sodium nitrite as a standard (21).

Determination of TBARS (Index of lipid peroxidation)

The degree of lipid peroxidation in the coronary venous effluent was estimated by TBARS (Thiobarbituric Acid Reactive Substances) using 1% thiobarbituric acid (TBA) in 0,05 sodium hydroxide (NaOH), incubated with coronary effluent at 100°C for 15 minutes and measured at 530 nm. Krebs-Henseleit solution was used as a blank probe (22).

Substances

$Pt^{(II)}DACHCl_2$ was synthesized according to *Galanski* and *Keppler* (23). Cisplatin, $K_2[PtCl_4]$, DACH and substances necessary for the preparation of Krebs-Henseleit buffer were purchased from the company Sigma-Aldrich GmbH, Germany.

Statistical Analysis

Experimental data were expressed as the arithmetic mean value (X) ± standard deviation (SD). Linear regression on logarithmically transformed data was used to determine the concentration-response relationship. This was calculated according to the method of least squares. The effect of different concentrations of experimental substances was expressed as a percentage of the maximal response. Analysis of variance was used to test significance of the linear regression with p values lower than 0.05 considered statistically significant. For all experimental substances, we calculated the EC_{50}, the concentration eliciting 50% of the maximum response.

RESULTS

The results are summarized in Tables 1, 2, 3, and 4.

Under CDDP, $Pt^{(II)}DACHCl_2$, $K_2[PtCl_4]$, and DACH perfusion of isolated rat heart (from 10^{-8} to 10^{-4} M), the levels of oxidative stress parameters ($O_2^{\cdot-}$, H_2O_2, NO, and TBARS) were not affected significantly.

$O_2^{\cdot-}$. CDDP: F=1,02, df_1=4, df_2=25, p>0,05; $Pt^{(II)}DACHCl_2$: F =0,09, df_1=4, df_2=25, p>0,05; $K_2[PtCl_4]$: F=0,73, df_1=4, df_2=25, p>0,05; DACH: F=0,87, df_1=4, df_2=25, p>0,05.

H_2O_2. CDDP: F=0,81, df_1=4, df_2=25, p>0,05; $Pt^{(II)}DACHCl_2$: F=0,01, df_1=4, df_2=25, p>0,05; $K_2[PtCl_4]$: F =0,82, df_1=4, df_2=25, p>0,05; DACH: F=0,67; df_1=4, df_2=25, p>0,05;

NO. CDDP: F=0,75, df_1=4, df_2=25, p>0,05; $Pt^{(II)}DACHCl_2$: F=0,06, df_1=4, $df2$=25, p>0,05; $K_2[PtCl_4]$: F=1,19, df_1=4, df_2=25, p>0,05. DACH: F=1,22, df_1=4, df_2=25, p>0,05.

TBARS. CDDP: F=1,06, df_1=4, df_2=25, p>0,05; $Pt^{(II)}DACHCl_2$: F=0,1, df_1=4, df_2=25, p>0,05; $K_2[PtCl_4]$: F=0,15, df_1=4, df_2=25, p>0,05; DACH: F=1,08; df_1=4, df_2=25, p>0,05.

DISCUSSION

The mechanisms of the anticancer effects of cisplatin are relatively well-known, but the cellular and molecular mechanisms involved in its cardiotoxicity are still not clear (24). Cardiotoxicity is a severe side effect that can occur during cisplatin treatment and is a limiting factor of cisplatin use in chemotherapy (25, 26). The best studied antitumour drugs with cardiotoxic effects are anthracyclines; however, in recent years, much attention has been paid to the mechanisms of cisplatin-induced cardiotoxicity. Many preclinical and clinical studies indicate that the use of anthracyclines is associated with myocardial damage, which is initiated by the formation of oxidative free radicals. Sawyer et al. (27) showed that apoptotic cells could be found in rat cardiomyocytes upon exposure to doxorubicin. Additionally, several investigations have shown the role of mitochondrial damage in a few models of nephro- and ototoxicity induced after cisplatin administration (28, 29). Mitochondria have a central role in myocardial tissue homeostasis, since cardiomyocytes are cells with high energy metabolism. Cardiac myocytes can accumulate a large amount of cisplatin and establish enhanced mtDNA damage. Thus, impairment in mitochondrial function leads to apoptosis of myocytes and endothelial cells and consequent cardiac dysfunction. Mitochondrial dysfunction can develop via various mechanisms, such as the loss of mitochondrial membrane potential, depletion of mitochondrial antioxidant enzymes, and increase in oxidative and nitrosative stress, which can induce cell death (30). The increase of oxidative stress and apoptosis in rat liver after cisplatin treatment (31) and overproduction of ROS in rat heart tissue (32) may lead to cardiovascular complications.

Hence, excessive production of ROS (superoxide anion radical, hydrogen peroxide, thiobarbituric reactive substances, and nitric oxide), in addition to the impairment of the defence system of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase), contributes to oxidative stress damage of the heart tissue (33). The levels of antioxidants, such as glutathione (GSH), are decreased significantly during cisplatin therapy with a high cumulative dose (34).

Cisplatin causes severe cardiotoxic effects that can impair the quality of life thus a Pt(II)DACHCl2 complex was synthesized and tested for cardiotoxicity in the isolated rat heart. Platinum (II) complexes with 1,2-diaminocyclohexane (DACH) as a ligand display high cytotoxic activity in tumours with a primary resistance to cisplatin, as well as lower nephro- and myelotoxicity (35). Success of oxaliplatin, which incorporates the 1R,2R-DACH ligand as platinum (II) complex, raised research interest over the past few decades for platinum-DACH complexes. Platinum (II) analogues have a strong potential for binding to sulphur donor groups, such as glutathione and metallothioneins. Consequently, before this complex reaches the DNA of the cancer cells, it can interact with many compounds, resulting in its inactivation and side effects (26).

In our study, isolated rat hearts were perfused with two platinum complexes in divergent concentrations as follows: 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M. The obtained results revealed that administration of cisplatin and $Pt^{(II)}DACHCl_2$ induced production of $O_2^{\cdot-}$ (Table 1) similar to when applied in a lower concentration range (10^{-8} - 10^{-6} M), whereas at high-

Table 1. The effects of CDDP, $Pt^{(II)}DACHCl_2$, DACH, K_2PtCl_4 perfusion on $O_2^{\cdot-}$

n= 12	$X \pm SD$ (nmol min^{-1} g^{-1})				
	Control	Cisplatin	$Pt^{(II)}DACHCl_2$	DACH	K_2PtCl_4
10^{-8}	55,72 ± 18,09	23,80 ± 14,61	30,46 ± 26,89	38,54 ±25,64	36,40 ± 22,90
10^{-7}	73,52 ± 40,52	43,96 ± 22,35	37,05 ± 43,37	19,65 ± 12,54	29,16 ± 18,95
10^{-6}	45,04 ± 26,20	27,93 ± 26,95	29,73 ± 15,86	18,20 ± 13,27	52,90 ± 47,37
10^{-5}	70,17 ± 42,31	14,36 ± 8,84	65,30 ± 94,53	14,82 ± 5,14	21,47 ± 15,83
10^{-4}	49,87 ± 15,36	13,14 ± 12,36	46,80 ± 40,59	8,50 ± 4,28	8,95 ± 5,16

Table 2. The effects of CDDP, $Pt^{(II)}DACHCl_2$, DACH, K_2PtCl_4 perfusion on H_2O_2

n = 12	$X \pm SD$ (nmol min^{-1} g^{-1})				
	Control	Cisplatin	$Pt^{(II)}DACHCl_2$	DACH	K_2PtCl_4
10^{-8}	23,54 ± 13,26	13,61 ± 4,33	17,54 ± 5,89	24,47 ± 10,02	2,97 ± 2,74
10^{-7}	18,58 ± 5,43	11,90 ± 6,50	18,49 ± 7,85	18,71 ± 4,48	1,40 ± 1,25
10^{-6}	19,67 ± 7,48	9,28 ± 5,03	18,11 ± 6,51	17,35 ± 2,82	1,08 ± 0,53
10^{-5}	17,30 ± 10,94	8,95 ± 5,30	20,94 ± 7,42	11,74 ± 3,00	0,55 ± 0,51
10^{-4}	20,01 ± 14,97	4,47 ± 2,96	13,93 ± 4,83	3,98 ± 1,13	0,26 ± 0,20

Table 3. The effects of CDDP, $Pt^{(II)}DACHCl_2$, DACH, K_2PtCl_4 perfusion on NO

n = 12	$X \pm SD$ (nmol min^{-1} g^{-1})				
	Control	Cisplatin	$Pt^{(II)}DACHCl_2$	DACH	K_2PtCl_4
10^{-8}	5,20 ± 4,39	10,24 ± 4,79	9,55 ± 6,21	6,69 ± 2,53	12,19 ± 3,96
10^{-7}	3,72 ± 2,40	10,60 ± 4,20	10,44 ± 6,70	4,09 ± 2,30	8,90 ± 3,90
10^{-6}	2,35 ± 1,94	8,99 ± 2,22	5,76 ± 3,12	3,94 ± 1,34	7,58 ± 1,12
10^{-5}	2,80 ± 1,33	6,29 ± 4,16	7,57 ± 6,86	3,18 ± 2,13	3,81 ± 0,62
10^{-4}	2,05 ± 1,46	3,12 ± 0,85	5,00 ± 2,80	1,97 ± 1,92	1,60 ± 0,82

Table 4. The effects of CDDP, $Pt^{(II)}DACHCl_2$, DACH, K_2PtCl_4 perfusion on TBARS

n = 12	$X \pm SD$ (μmol min^{-1} g^{-1})				
	Control	Cisplatin	$Pt^{(II)}DACHCl_2$	DACH	K_2PtCl_4
10^{-8}	14,72 ± 9,91	28,68 ± 23,76	23,96 ± 15,19	29,01 ± 12,59	40,04 ± 18,38
10^{-7}	11,73 ± 10,26	20,65 ± 16,24	22,42 ± 18,58	35,69 ± 17,36	48,84 ± 18,59
10^{-6}	14,95 ± 10,50	24,56 ± 11,41	17,45 ± 12,55	31,37 ± 25,78	37,10 ± 22,36
10^{-5}	12,58 ± 9,23	15,97 ± 10,15	14,57 ± 14,41	23,63 ± 12,71	18,35 ± 6,52
10^{-4}	13,56 ± 8,10	8,49 ± 4,60	17,92 ± 28,18	5,50 ± 2,58	7,03 ± 4,39

er concentrations (10^{-5}- 10^{-4} M), the $Pt^{(II)}DACHCl_2$ complex induced significant elevation of $O_2^{\cdot-}$ in comparison with CDDP.

Table 1. The effects of CDDP, $Pt^{(II)}DACHCl_2$, DACH, K_2PtCl_4 perfusion on $O_2^{\cdot-}$

Cisplatin applied in a lower concentration range can induce apoptosis via the overproduction of free radicals in renal tubular cells (36), whereas higher concentrations induce necrosis via $O_2^{\cdot-}$ and H_2O_2. This result indicates that cardiotoxicity may occur in a dose-dependent manner.

The data obtained in our study showed that the $Pt^{(II)}DACHCl_2$ complex induced higher levels of hydrogen peroxide production (Table 2) through the whole range of applied doses (10^{-8} - 10^{-4} M), which is in contrast to the CDDP results.

Table 2. The effects of CDDP, $Pt^{(II)}DACHCl_2$, DACH, K_2PtCl_4 perfusion on H_2O_2

Numerous effects of oxidative stress are observed in many diseases and are also implicated in the toxicity induced by anticancer agents such as cisplatin. There is *in vivo* and *in vitro* evidence that cisplatin induces oxidative stress, which is involved in renal damage and severe nephrotoxicity. This is confirmed by the increasing levels of $O_2^{\cdot-}$, H_2O_2 and hydroxyl radicals, as well as by the depletion of the antioxidants GSH-peroxidase and GSH-reductase (17).

The present study determined the nitrite level (NO_2^-) as an index of nitric oxide (NO) production (Table 3). It was shown that formation of NO is higher at lower doses of each complex (10^{-8}- 10^{-7} M) and is lower at higher doses (10^{-6} - 10^{-4} M).

Table 3. The effects of CDDP, $Pt^{(II)}DACHCl_2$, DACH, K_2PtCl_4 perfusion on NO

Demkow and coauthors (37) observed a significant increase of NO production in lung cancer patients after cisplatin administration. In the presence of a superoxide anion radical, nitric oxide forms peroxynitrite, which is more reactive and toxic, and has a potential for apoptosis of cells such as cardiomyocytes (38). In accordance with these results, Zhou et al. (39) reported significant elevation of serum NO after chemotherapy in patients with lung cancer. Chirino et al. (17) also showed that high production of peroxynitrite plays a role in the pathogenesis of nephrotoxicity after cisplatin application. Peroxynitrite can induce cell damage by developing lipid peroxidation, causing DNA damage and mitochondrial dysfunction. In contrast, Colakogullari and coworkers (40) did not observe high concentrations of NO as an early effect of cisplatin therapy. This variation can be attributed to different study protocols.

In our research, TBARS was measured as an index of lipid peroxidation, and it is observed that levels of lipid peroxidation were higher at lower doses of cisplatin (10^{-8}-10^{-6} M) in comparison with $Pt^{(II)}DACHCl_2$ (Table 4).

Table 4. The effects of CDDP, $Pt^{(II)}DACHCl_2$, DACH, K_2PtCl_4 perfusion on TBARS

However, the $Pt^{(II)}DACHCl_2$ complex caused an increase in the levels of lipid peroxidation when applied at the highest dose (10^{-4} M), which is in contrast to cisplatin. Cardiotoxicity associated with cisplatin therapy could also be the consequence of increased lipid peroxidation in myocardial cells that resulted in irreversible modification of cell functions. Free radicals can cause serious damage to tissue, reacting with membrane lipids, proteins and nucleic acids (41). After cisplatin is distributed to a cell, it is aquated into a highly reactive form, which can react with GSH and metallothioneins. Depletion of GSH levels and antioxidants results in the accumulation of ROS (42, 43).

The current study compared the $Pt^{(II)}DACHCl_2$ complex to CDDP for their ability to develop cardiotoxicity by the production of free radicals. Over the complete dosage range tested, neither complex produced a statistically significant elevation of ROS or induced evidently cardiotoxic effects. Data presented in this research could be useful for future investigations of platinum-based chemotherapeutic agents. These results suggest that further elucidation of platinum (II) analogue-induced cardiotoxicity should add significantly to our understanding of this phenomenon and the role of platinum complexes with DACH ligands in anticancer treatment.

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THE EVALUATION OF ANGIOTENSIN-CONVERTING ENZYME INHIBITORS IN RENAL ELIMINATION WITH SELECTED MOLECULAR DESCRIPTORS

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PROCENA RENALNE ELIMINACIJE INHIBITORA ENZIMA KOJI KONVERTUJE ANGIOTENSIN SA ODABRANIM MOLEKULSKIM DESKRIPTORIMA

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ABSTRACT

Angiotensin-converting enzyme (ACE) inhibitors modulate the function of the renin-angiotensin-aldosterone system, and they are commonly prescribed antihypertensive drugs especially in patients with renal failure. In this study, the relationships between several molecular properties of eight ACE inhibitors (enalapril, quinapril, fosinopril, ramipril, benazepril, perindopril, moexipril, trandolapril) and their renal elimination data, from relevant literature, were investigated. The 'molecular descriptors of the ACE inhibitors, which included aqueous solubility data (logS); an electronic descriptor, polar surface area (PSA);, a constitutional parameter, molecular mass (Mr); and a geometric descriptor, volume value (Vol), as well as lipophilicity descriptors (logP values), were calculated using different software packages. Simple linear regression analysis showed the best correlation between renal elimination data and lipophilicity descriptor AClogP values (R^2 = 0.5742). In the next stage of the study, multiple linear regression was applied to assess a higher correlation between the ACE inhibitors' renal elimination data and lipophilicity, AClogP, with one additional descriptor as an independent variable. Good correlations were established between renal elimination data from the literature and the AClogP lipophilicity descriptor using the constitutional parameter (molecular mass (R^2 = 0.7425)) or the geometric descriptor (volume value (R^2 = 0.7224)) as an independent variable. The application of computed molecular descriptors in evaluating drug elimination is of great importance in drug research.

Keywords: *Angiotensin-converting enzyme inhibitors; lipophilicity; molecular mass; elimination.*

SAŽETAK

Inhibitori enzima koji konvertuje angiotenzin (ACE) modifikuju funkciju renin-angiotenzin-aldosteron sistema i predstavljaju često propisane lekova za sniženje pritiska, posebno kod pacijenata sa insuficijencijom bubrega. U ovom radu, za osam odabranih ACE inhibitora (enalapril, kvinapril, fosinopril, ramipril, benazepril, perindopril, moeksipril, trandolapril) ispitan je odnos između osobina njihovih molekula i njihove eliminacije putem bubrega. Za ispitivane inhibitore ACE korišćenjem različitih softverskih paketa izračunate su vrednosti nekoliko molekulskih deskriptora: rastvorljivost u vodi (logS), elektronski deskriptor – polarna površina molekula (PSA), molekulska masa (Mw), geometrijski deskriptor – volumen molekula (Vol) kao i deskriptor lipofilnosti (logP vrednosti). Primenom proste linearne regresione analize najbolja zavisnost dobijena je između podataka o eliminaciji inhibitora ACE putem bubrega i deskriptora lipofilnosti, AClogP vrednosti (R^2 = 0.5742). U sledećoj fazi istraživanja primenjena je metoda višestruke regresione analize (MLR) kako bi se dobila bolja zavisnost između podataka o eliminaciji ACE inhibitora putem bubrega i njihove lipofilnosti (AClogP vrednosti) uz primenu dodatnog molekulskog deskriptora kao nezavisno promenljive. Dobre korelacije su dobijene između podataka o eliminaciji putem bubrega i deskriptora lipofilnosti AClogP, uz primenu molekulske mase (R^2 = 0.7425) ili zapremine molekula (R^2 = 0.7224) kao nezavisno promenljive. Mogućnost primene izračunatih molekulskih deskriptora u proceni eliminacije lekova je od velikog značaja u njihovom istraživanju.

Ključne reči: *Inhibitori enzima koji konvertuje angiotenzin; lopofilnost; molekulska masa; eliminacija.*

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INTRODUCTION

Angiotensin-converting enzyme (ACE) inhibitors are the most commonly prescribed antihypertensive drugs today. They are a significant group of drugs widely used in the treatment of hypertension, congestive heart failure and renal failure, especially in patients with diabetes mellitus or proteinuria (1).

According their chemical structures, ACE inhibitors can be classified into three groups: sulfhydryl-containing inhibitors (exemplified by captopril), dicarboxylate-containing (exemplified by enalapril) and phosphonate-containing inhibitors (exemplified by fosinopril). The ACE inhibitors are pro-drugs, and, following administration, they undergo ester hydrolysis into their active di-acid metabolites, with the exception of lisinopril, which is already in the di-acid form (1).

Even though they have the same usage indications, they demonstrate differences in their pharmacokinetic and pharmacodynamic properties, which may affect their clinical efficacy. The ACE inhibitors demonstrate their antihypertensive effect through their active metabolites by modulation of the renin-angiotensin-aldosterone enzymatic system and selective dilation of efferent renal arterioles. In hypertensive patients with renal failure, particularly of diabetic aetiology, ACE inhibitors are used as the drug of choice because, in addition to their antihypertensive effects, they slow the progression of microalbuminuria and proteinuria (2-5).

Some ACE inhibitors have dual routes of elimination, renal and faecal, which may be important for patients with renal failure. They can be applied in patients with end-stage renal failure who are treated with renal replacement therapy, haemo or peritoneal dialysis (1).

ACE inhibitor's pharmacological properties (absorption, protein binding, distribution, activity, duration of action and elimination) and their relationship with lipophilicity were investigated in numerous studies by chromatographic methods, spectrophotometry, capillary electrophoresis or spectrofluorimetry. ACE inhibitors were determined in pharmaceutical formulations and biological material. There are few data on the elimination of ACE inhibitors by peritoneal dialysate (6-17).

In our previous studies, we investigated the lipophilicity of several ACE inhibitors under different chromatographic conditions (18-20) and the correlation between ACE inhibitors' chromatographic or *in silico* lipophilicity data and with their protein binding (PPB) data (21) or absorption (22). In continuation of these studies, our aim was to correlate ACE inhibitors' molecular descriptors (electronic descriptor - polar surface area (PSA); constitutional parameter - molecular weight (Mw); geometric descriptor - volume value (Vol); aqueous solubility data (log*S*)) with their renal elimination data to determine a reliable relationship appropriate for evaluating renal elimination of the investigated group of drugs. The selection of appropriate molecular descriptors was established.

MATERIALS AND METHODS

The eight most often prescribed ACE inhibitors were investigated.

1. **enalapril maleate**, (*S*)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-*L*-alanyl]-*L*-proline maleate;
2. **quinapril hydrochloride,** [3*S*-[2[*R**(*R**)],3*R**]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl] amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid hydrochloride;
3. **fosinopril sodium,** (4*S*)-4-cyclohexyl–1-[[(*R*)-[(1*S*)-2-methyl-1-(1-oxopropoxy)- propoxy](4-phenylbutyl) phosphinyl]acetyl]-*L*-proline, sodium salt;
4. **ramipril,** (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3–phenylpropyl]amino]-1–oxopropyl] octahydrocyclopenta[b]pyrrole–2–carboxylic acid;
5. **benazepril hydrochloride**, (3*S*)-3-[[1-(ethoxycarbonyl)-3–phenylpropyl]amino]-2,3,4,5–tetrahydro–2-oxo–1*H*-1–benzazepine–1–acetic acid hydrochloride;
6. **perindopril erbumin,** 2-methylpropan-2-amine (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxopentan-2-yl]amino]propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid;
7. **moexipril,** (3*S*)-2-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-6,7-dimethoxy-3,4-dihydro-1*H*-isoquinoline-3-carboxylic acid**;**
8. **trandolapril,** [2*S*-[1[*R**(*R**)],2*α*,3a*α*,7a*β*]]-1-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic acid.

The software package Molinspiration Depiction Software (Molinspiration Cheminfirmatics) (23) was used for the calculation of the electronic descriptor polar surface area (PSA), the constitutional parameter molecular weight (Mw) and the geometric descriptor volume value (Vol) (Table 1). The ACE inhibitors' lipophilicity descriptors, different log*P* values (Alog*Ps,* AClog*P,* AB/log*P,* milog*P,* Alog*P,* Mlog*P,* KOWWINlog*P,* XLOG*P*2*,* XLOG*P*3), and aqueous solubility data (log*S*), were calculated using the Virtual Computational Chemistry Laboratory software package (24).

The elimination data of the investigated compounds (Table 1) were obtained from the relevant literature (1) and using software package DrugBank (25).

Microsoft Excel 2003 and Origin 7.0 PRO (Origin Lab Corporation, USA) were used to perform the statistical analysis of the regression.

RESULTS

In this paper, the correlations between renal elimination data of selected ACE inhibitors and their calculated molecular properties were examined.

Table 1. The ACEi calculated molecular descriptors and renal elimination data collected from relevant literature (*); predicted from (A) AClogP and Vol; (B) AClogP and Mw values.

ACEi	Ren. el.*	AC logP	Vol	Mw	logS	PSA	Ren. el.A	Ren. el.B
1	100	1.52	357	376	-2.92	96	101	101
2	96	2.08	411	439	-3.81	96	77	78
3	50	3.05	539	564	-4.70	110	45	44
4	60	2.07	396	417	-3.58	96	73	72
5	88	2.09	395	424	-4.24	96	71	73
6	100	1.58	358	368	-2.63	96	97	94
7	100	1.87	462	499	-4.21	114	109	111
8	33	2.39	413	430	-3.98	96	54	53

*Ren. El. values obtained from literature (Lemke and Williams, 2013)
The numbers denote ACEi.

The five molecular descriptors (PSA, Mw, Vol, log*P*, log*S*) of the ACE inhibitors were calculated using different software packages as well as elimination data of investigated compounds from the relevant literature are shown in Table 1.

In the first stage of the investigation, correlations between the renal elimination data and calculated molecular descriptors of the ACE inhibitors were investigated using simple linear regression analysis. The renal elimination data and the 'molecular descriptors, (Vol, Mw and log*S*) of the ACE inhibitors showed correlations with correlation coefficients (R^2) lower than 0.2. Next, the relationship between different lipophilicity descriptors, log*P* values and renal elimination data were examined. The strongest correlation was found between AClog*P* and the renal elimination data (R^2 = 0.5742).

Following these results, in the next stage of the study, the relationship between renal elimination data and two different molecular descriptors of the ACE inhibitors were investigated using multiple linear regression (MLR) analysis. The AClog*P* was chosen as the first independent variable since it showed the best correlations with the ACE inhibitors' renal elimination data. The application of five calculated molecular descriptors (PSA, Mw, Vol, log*P* and log*S*) of ACE inhibitors was investigated by MLR analysis.

Good correlations were established between renal elimination data obtained from the literature and the AClog*P* lipophilicity descriptor using the constitutional parameter molecular mass (R^2 = 0.7425) or the geometric descriptor volume value (R^2 = 0.7224) as an independent variable. The values of predicted renal elimination were calculated according to the following equations:

$$\text{Renal el.}_{\text{pred 1}}\,(\%) = 101.1189(\pm 48.0996) - 74.9464(\pm 24.0853)\text{AClog}P + 0.3199(\pm 0.1957)\text{Vol}$$

with n = 8; R^2 = 0.7224; S.D. = 16.6729; F = 6.5073 Eq. 1.

$$\text{Renal el.}_{\text{pred 2}}\,(\%) = 102.1234(\pm 44.0093) - 73.0586(\pm 21.3470)\text{AClog}P + 0.2918(\pm 0.1614)\text{Mw}$$

with n = 8; R^2 = 0.7425; S.D. = 16.0606; F = 7.2073 Eq. 2.

The results obtained using MLR analysis by applying two different descriptors as independent variables are presented in Table 1 and in Fig. 1.

DISCUSSION

The clinical success of drugs depends mostly on their absorption, distribution, metabolism or route of elimination (ADME) (26). Lipophilicity is one of the most important molecular properties that influence these values, but a number of other molecular properties (such as molecular

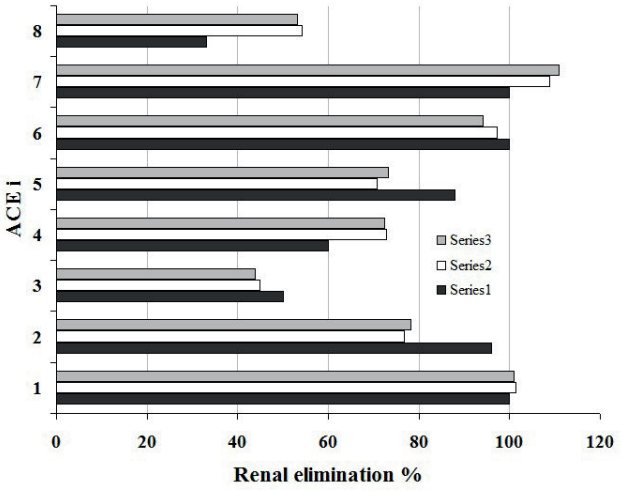

Fig. 1. The relationship between ACEi renal elimination collected from relevant literature and (Lemke and Williams, 2013) (Series 1) and predicted in MLR using AClogP and Vol (Series 2); AClogP and Mw values (Series 3). The numbers denote ACEi.

weight (Mw), molecular volume (Vol), polar surface area (PSA) and solubility data (logS)) also play important roles in drug absorption, tissue penetration, degree of distribution, degree of plasma protein binding and route of elimination (27-29).

According to the available literature, several authors investigated drugs belonging to the ACE inhibitor group, their pharmacological properties and their similarities or differences (6-10). Their acidity, lipophilicity, solubility and absorption were evaluated based on their molecular structures with the application of computer programs (27-29).

Various authors have also suggested several assays that could be employed in investigations of different drug eliminations (30-32). Most of these methods still have certain limitations, and a new approach for fast, reliable and cost-effective evaluation of the route of elimination of ACE inhibitors should be developed. The decrease in complexity and size of the average drug molecule, as well as its low log*P* values and high water solubility, can lead to higher probability of drugs being rapidly cleared via renal elimination (33). Since a drug's route and degree of elimination may affect a drug's duration of action and activity, the application of computed molecular descriptors in the prediction of a drug's elimination are of great importance, especially for the newly synthesized drugs.

In this study, eight ACE inhibitors—,enalapril maleate, quinapril hydrochloride, fosinopril sodium, ramipril, benazepril hydrochloride, perindopril erbumin, moexipril and trandolapril—were studied to evaluate correlations between their renal elimination data obtained from the relevant literature and calculated molecular descriptors. According to the data from the literature, the degree of renal elimination of the ACE inhibitors can vary from 33% to 100% (Table 1). The lowest values of renal elimination were found for trandolapril (approximately 33%), while enalapril, perindopril and moexipril dominantly exhibit renal elimination (approximately 100%).

The correlations between the ACE inhibitors' calculated molecular descriptors and their renal elimination data obtained from relevant literature were examined. The applicability of calculated molecular descriptors in ACE inhibitor elimination evaluation was investigated. The main topic of this study was to establish an approach using simple or multiple linear regression analysis capable of predicting the renal elimination of selected ACE inhibitors based on their molecular properties.

In the first stage of the study, the relationship between all calculated log*P* values (Clog*P*, Alog*Ps*, AClog*P*, AB/log*P*, milog*P*, Alog*P*, Mlog*P*, KOWWINlog*P*, XLOG*P*2, XLOG*P*3) and renal elimination data for ACE inhibitors was investigated. Amongst all log*P* values, only AClog*P* provided a relatively good correlation (R^2 = 0.5742) with the 'renal elimination data of the ACE inhibitors.

Second, the relationship between the renal elimination data and calculated molecular descriptors of the ACE inhibitors was investigated using MLR analysis with application of two independent variables, AClog*P* and one of the following: polar surface area (PSA), molecular weight (Mw), volume value (Vol) or solubility (logS) to assess their higher correlation. The best correlations were established between the ACE inhibitors' renal elimination data and the AClog*P* lipophilicity descriptor using the molecular weight (R^2 = 0.7425) or volume (R^2 = 0.7224) as an independent variable, indicating that these molecular properties are critical for ACE inhibitors' route of elimination. The established correlations are presented by Eq. 1 and Eq. 2. They indicate that the molecule's lipophilicity has a dominant influence on the ACE inhibitor's renal elimination, and the increase in lipophilicity led to a decrease in their renal elimination.

The correlation observed between the ACE inhibitors' renal elimination data and their *in silico* molecular descriptors (lipophilicity parameter (AClog*P)* and constitutional parameter (molecular mass) or geometric descriptor (volume value)) can be considered good, as proposed by Asuero et al. (34), due to the limited number of compounds. These correlations confirmed the calculation of descriptors as the technique suitable for evaluation of renal elimination of the selected compounds.

CONCLUSION

A relatively good correlation was obtained between the renal elimination data and the calculated molecular lipophilicity descriptor (AClogP) (R^2 = 0.5742). Furthermore, using MLR analysis with two different descriptors as independent variables and the lipophilicity descriptor (AClog*P)* and molecular mass or volume value as independent variables, better correlations were established (with R^2 = 0.7425 and R^2 = 0.7224, respectively). The possible application of computed molecular descriptors in evaluating drug routes of elimination can be highly useful in drug research.

The present study may be considered an effective assay and could be used as a fast, easy and cost-effective screening technique for route of elimination evaluation. The proposed methodology confirmed that lipophilicity, together with other molecular properties, is essential in a drug's route of elimination.

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THE AUTONOMIC REPERCUSSIONS OF FETAL AND MATERNAL INTERACTION IN PRE-ECLAMPSIA

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AUTONOMNE REPERKUSIJE FETALNE I MATERNALNE INTERAKCIJE U PREEKLAMPSIJI

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ABSTRACT

Pre-eclampsia (PE) is one of the severe complications of pregnancy that leads to fetal deterioration. The aim of the investigation was to determine the role of maternal respiratory sinus arrhythmia (RSA) in regulation of fetal circulatory system in case of healthy pregnancy and in PE.

The investigation of maternal and fetal HRV and umbilical venous blood flow velocity spectral analysis in 106 patients at 34-40 weeks of gestation was performed. 30 of them had healthy pregnancy and were involved in the Group I. In Group II 44 pregnant women with mild-moderate PE were observed. 32 patients with severe PE were monitored in Group III. The maternal sympathetic overactivity modulated HRV in PE. The suppression of RSA was explored in preeclamptic patients. The Doppler spectrograms of the umbilical veinous blod flow had the oscillatory peak with a frequency about 0.5 Hz. The above peak characterized the participation of the maternal RSA in fetal hemodynamics. Strong relationship between maternal RMSSD and amplitude of RSA associated peak, maternal and fetal RMSSDs was found in healthy pregnancy. No considerable relationship was revealed between the maternal RMSSD and the amplitude of 0.5 Hz frequency peak, the maternal and fetal RMSSDs in the patients with severe PE. The maternal RSA propagated its influence on the fetal umbilical venous blood flow and the fetal autonomic nervous regulation in normal gestation. The control of fetal hemodynamics diminished in the mild-moderate PE and even disappeared in severe PE.

Keywords: *autonomic nervous regulation, maternal respiratory sinus arrhythmia, fetal umbilical venous hemodynamics, pre-eclampsia*

SAŽETAK

Preeklamspija (PE) je jedna od teških komplikacija trudnoće koje dovodi do propadanja ploda. Cilj ovog istraživanja bio je da utvrdi ulogu respiratorne sinusne aritmije majke (RSA) u regulaciji sistema fetalne cirkulacije u slučaju zdrave trudnoće i preeklampsije.

Istraživanje maternalne i fetalne HRV i spektralna analiza brzine protoka venske krvi pupčanika je sprovedeno kod ukupno 106 pacijentkinja gestacijske starosti 36-40 nedelja. Prvu grupu je činilo 30 pacijentkinja sa normalnom trudnoćom. Drugu grupu je činilo 44 pacijentkinje sa blagom do umerenom PE. Pri PE, preterana aktivnost simpatikusa majke modifikovala je HRV. Supresija RSA je otkrivena kod pacijentkinja sa preeklapmsijom. Na dopler-spektrogramu praćenjem venskog protoka pupčanika, primećen je oscilatorni pik sa frekvencijom od 0.5 Hz. Navedeni pik karakteriše učešće maternalne RSA u fetalnoj hemodinamici. Jaka povezanost između maternalne RMSSD i amplitude oscilatornog pika RSA, maternalne i fetalne RMSSD je primećena u zdravoj trudnoći. Nije utvrđena povezanost između maternalne RMSSD i amplitude pika frekvence 0.5 Hz i između maternalne i fetalne RMSSD kod pacijentkinja sa teškim formama PE. Maternalne RSA utiču na venski protok pupčanika kao i na fetalnu autonomnu nervnu regulaciju pri normalnoj trudnoći. Pri blagoj do umerenoj PE, ova kontrola fetalne hemodinamike se smanjuje, dok pri teškim formama PE, kontrola čak potpuno izostaje.

Ključne reči: *autonomna nervna regulacija, respiratorna sinusna aritmija majke, fetalna venska hemodinamika pupčanika, preeklapmsija*

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INTRODUCTION

Autonomic nervous regulation plays an important role in the successful scenario of healthy pregnancy. The emphasis on parasympathetic stimulation occurs in the first half of pregnancy. Gestational autonomic resetting provides increased blood volume and systemic vasodilation (1, 2). The circulatory response to trophoblastic invasion into spiral arteries contributes to the optimal utero-placental hemodynamics. This morphological transformation in the placental vascular bed makes the vessels absolutely tolerable to vasoactive substances in physiological condition (2, 3, 4). In case of shallow trophoblastic invasion placental ischemia provokes well known synthesis and release of vasoconstrictors (1, 2, 3, 4). Further both endothelial dysfunction and thrombophilia enhance vasoconstriction. The augmented vascular tone is also associated with abnormally increased sympathetic activity (5). Pre-eclampsia (PE) is a gestational disease caused by failed placentation that leads to fetal compromise (1, 2, 3, 4, 5, 6).

Since maternal and fetal circulatory systems are anatomically distinct from each other the question of their interaction becomes very relevant. The periods of maternal and fetal cardiac synchrony were explored (7, 8). It was found that maternal relaxation, physical and mental activities could induce fetal autonomic response (9, 10, 11, 12). In several studies maternal respiratory sinus arrhythmia (RSA) was determined as an evident factor of maternal and fetal heart rate synchronization (7, 13).

RSA captures parasympathetic impact on the heart rate variability (HRV). This physiological phenomenon provides nonlinearity of the cardiac function and cardiorespiratory synchronization (6). RSA is known to have a modulating impact on heart rate, cardiac output, blood pressure and peripheral vascular tone of end-organs (2, 11, 12, 13). The decreased RSA is a sign of the cardiac failure (2, 4, 5). Fetal RSA is one of the main factors of cardiac rhythm complexity in physiological condition. The lack of fetal parasympathetic regulation till the last weeks of healthy pregnancy was found (12). But fetal respiratory activity is strongly associated with an increased vagal domain region of HRV (6, 14).

It is possible to speculate that maternal RSA-associated hemodynamic fluctuations could penetrate through placental barrier. Therefore, these hypothesized fluctuations could be considered a possible coupling mechanism of the maternal and fetal circulatory systems. The placental vascular bed acts as an intermediary in the oscillatory processes between the mother and the fetus. The umbilical vein could be approached as a "mirror" of the oscillatory processes in the mother-placenta-fetus system since the cord is not an innervated tissue. Fetal RSA is involved in its adaptive response to chronic placental insufficiency (14). The investigation of the relationship between maternal and fetal HRV parameters could contribute to a better understanding of their role in PE. The root mean square of successive heartbeat interval differences (RMSSD) was considered as a RSA-related parameter (14).

The investigation's **aim** was to determine the role of maternal RSA in regulating the fetal circulatory system in case of healthy pregnancy and in pre-eclamptic patients.

MATERIALS AND METHODS

The study protocol was approved by the Bioethics Committee of the Kharkiv Medical Academy of Postgraduate Education. The eligible participants were informed about the study's methodology, its aims, objectives, indications and eventual complications before enrollment. Patients from the department of maternal-fetal medicine were selected randomly. All the patients who met the inclusion criteria gave written informed consent to participate (15). The inclusion criteria: diagnosed PE based on the blood pressure higher than 140/90 mm Hg in two separate occasions 6 hours apart, a positive proteinuria test in two mild-stream urine samples collected 4 hours apart. The exclusion criteria: multiple pregnancy, eclampsia, pre-existing medical disorders like diabetes mellitus, metabolic syndrome, cardiac diseases, renal disease, thyrotoxycosis and chronic hypertension. If blood pressure was 140 to 159 mmHg systolic and 90 to 109 mmHg the patient was included in mild-moderate PE Group. Severe PE was diagnosed in case of blood pressure was higher 160 mmHg systolic and 110 mmHg diastolic or (and) thrombocytopenia, serum creatinine more than 1.1 mg/L, elevated blood concentration of liver transaminases to twice normal concentration, pulmonary oedema, cerebral or visual disturbances. The patients who had no gestational complications and medical disorders including chronic infections and tobacco smoking were enrolled in the control Group. All patients included in the study were inhabitants of Eastern Ukraine. The study was conducted from January 2013 to October 2014.

106 patients at 34-40 weeks of gestation were enrolled. 30 of them had healthy pregnancy and were included into the Group I (control). In Group II, 44 pregnant women with mild-moderate PE were observed. 32 patients with severe PE were monitored in Group III.

All examined pre-eclamptic patients received antihypertensive drugs. The choice of antihypertensive agent was made according to the type of central maternal hemodynamics (CMH) determined by bio-impedance cardiography. It was estimated the values of cardiac index (CI) and total peripheral vascular resistance (TPVR). The hyperkinetic type of CMH was associated with high CI and low TPVR. The pre-eclamptic women with eukinetic type of CMH had high or normal CI and increased TPVR. And the pre-eclamptic patients with low CI and high TPVR had the hypokinetic type of CMH (3). The pregnant women with hyperkinetic type of CMH took carvedilol 6.25-12.5 mg 2 times daily, in case of the eukinetic type – methyldopa 250-500 mg 4 times a day and in cases of the hypokinetic one – methyldopa 500 mg 4 times daily combined with nifedipine 20 mg 2 times daily.

Doppler ultrasonography was performed with the ultrasound system "Voluson 730" (GE Healthcare, USA). The Doppler spectrogram of the venous umbilical blood flow was subjected to further processing. The curves of maximum blood flow velocity were isolated and their spectral components determined. The spectra were calculated with a sampling step of Δt=0.01 seconds for the sample of 256 points. The resulting spectrum was obtained by averaging over all the samples of this contingent (16).

The fetal and maternal HRV parameters were obtained with the fetal noninvasive computer electrocardiographic system "Cardiolab Baby Card" (Scientific Research Center "KhAI-Medica", Ukraine). The Ukrainian ECG recordings were included in the Physio Net database (17). The recording lasted for 10 minutes in the normal maternal sitting position. The values of total power (TP) and its spectral compounds, i.e. the very low frequency (VLF), the low frequency (LF), the high frequency (HF) and LF/HF ratio or sympatho-vagal balance, were determined. The temporal characteristics of the fetal HRV: the standard deviation of normal to normal intervals (SDNN), RMSSD, the proportion of the number of pairs of NNs differing by more than 50 ms divided by the total number of NNs (pNN50), the amplitude of mode (the most frequent value of NN interval or the highest column in the histogramm) – the number of NN intervals included in the pocket corresponding to the mode measured in percentages (%) (AMo) and the stress index – SI = AMo(%) / (2×Mo×Var); Var = NNmax – NNmin; (SI) were calculated (18). The fetal frequency bands of HRV were explored by David M. et al. (19).

The results thus obtained were analyzed with an ANOVA test to compare data between groups. The significance was set at p-value <0.05. For the statistical analysis of relationship between X and Y, the correlations coefficients were estimated with Spearman's test. Microsoft Office 2010 Excel software was used for statistical analysis (Washington , USA).

RESULTS

The mean age values were 26.5±4.1; 25.8±7.2 and 25.4±6.3 years in Group I, Group II and Group III respectively. The mean values of the gestational age were 37.1±3.6; 36.9±2.5 and 36.7±1.8 weeks in Group I, Group II and Group III respectively. The body mass index values in the same groups were 24.9±5.1; 28.5±7.8 and 29.6±8.3. So the mean values of body mass index in PE were significantly higher than in healthy pregnancy Group (p<0.05).

The study of CMH types revealed an increased both CI and TPVR mean values in mild-moderate PE Group (table 1). The values of CI and TPVR changed in opposite directions in severe PE, therefore, demonstrated an increase in pre- and afterload on maternal heart. CI was decreased and TPVR increased in Group III. The hyperkinetic type of CMH was found in 86.4 % and the eukinetic one in 13.6 % of the patients in Group II. 59.4 % of women in Group III had the hypokinetic type of CMH and 40.6 % of severe pre-eclamptic patients had a eukinetic pattern of CMH. Hyperdynamic circulation was typical for mild-moderate PE and hypodynamic one was found in severe PE. Therefore, the patients of Group III had centralization of blood flow.

Table 1. The parameters of bioimpedance cardiography in the study population

Index, units of measure	Group I	Group II	Group III
CI, L/min/m^2	3.6±0.8	3.9±1.2*	2.2±1.1*†
TPVR, dyn·s/cm^5	1214.5±128.2	1371.0±203.4*	2460.2±318.6*†

* –the differences were statistically significant compared to the control group (p<0,05);
† – the differences were statistically significant compared to the group II (p<0,05).

Abbreviations:
CMH – central maternal hemodynamics;
CI –cardiac index;
TPVR – total peripheral vascular resistance.

The obtained data showed a suppressed autonomic tone in pre-eclamptic patients (table 2). The most considerable power was determined in the maternal VLF domain region in all study groups. It was connected with a relative

Table 2. Maternal HRV parameters in the study population

Index	Group I	Group II	Group III
SDNN, ms	119. 8±14.1	102.5±9.0*	82.6±10.4*†
RMSSD, ms	41.6±8.5	22.7±6.2*	16.3±4.8*†
pNN5O, %	12.8±3.2	6.5±1.9*	1.8 ±0.6*†
AMo, %	34.6±5.1	50.4±11.3*	65.4±12.1*/†
SI, c.u.	115.2±16.8	403.9±34.5*	1362.6 ± 243.4*/†
TP, ms^2	3084.6±565.7	1568.2±347.2*	825.6±117.9*†
VLF, ms^2	2361.2±485.3	1130.8±181.4*	541.6±85.2*†
LF, ms^2	349.5±42.6	310.3±51.6*	231.9±52.4*†
HF, ms^2	375.4±56.1	128.6±31.4*	53.1±13.6*†
LF/HF	0.9±0.3	2.2±0.6*	4.5±1.1*†

* – the differences were statistically significant compared to Group I (p<0.05);
†– the differences were statistically significant compared to Group II (p<0.05).

Abbreviations:
SDNN – the standard deviation of normal to normal intervals;
RMSSD – the root mean square of successive heartbeat interval differences;
pNN50 – the proportion of NN pairs differing by more than 50 ms divided by total number of NNs;
AMo - the mode amplitude (the most frequent value of NN interval or the highest column in the histogramm) – the number of NN intervals included into the pocket corresponding to the mode measured in percentages (%) (AMo);
SI – the stress index SI = AMo(%) / (2×Mo×Var);
Var = NNmax – NNmin;
TP – the total power;
VLF – the very low frequency;
LF – the low frequency;
HF – the high frequency.

Table 3. Fetal HRV parameters in the study population

Index	Group I	Group II	Group III
SDNN, ms	45.8±13.1	29.4±8.3*	10.2±4.5*†
RMSSD, ms	22.4±3.4	14.2±2.6*	8.1±0.8*†
pNN5O, %	4.2±1.1	2.0±0.4*	1.1±0.3*†
AMo, %	39.6±14.1	50.2±11.6*	65.9±13.4*/†
SI, c.u.	169.3±42.7	496.1±65.8*	1467.3 ± 405.8*/†
TP, ms²	1513.6±329.1	896.2±163.5*	424.9±93.7*†
VLF, ms²	1252.8±248.3	692.8±91.3*	251.8±44.2*†
LF, ms²	184.3±26.5	151.9±34.1*	135.0±19.6*†
HF, ms²	77.6±9.4	53.6±8.2*	38.9±10.4*†

* – the differences were statistically significant compared to Group I ($p<0.05$);
† – the differences were statistically significant compared to Group II ($p<0.05$).

Abbreviations:
SDNN – the standard deviation of normal to normal intervals;
RMSSD – the root mean square of successive heartbeat interval differences;
pNN50 – the proportion of NN pairs differing by more than 50 ms divided by the total number of NNs;
AMo – the mode amplitude (the most frequent value of NN interval or the highest column in the histogramm) – the number of NN intervals included in the pocket corresponding to mode measured in percentages (%);
SI – the stress index SI = AMo(%) / (2×Mo×Var);
Var = NNmax – NNmin;
TP – the total power;
VLF – the very low frequency;
LF – the low frequency;
HF – the high frequency.

predominance of the hypothalamic-pituitary-adrenal axis domain region among all spectral components of HRV in sitting position in a state of rest. The maternal HRV in PE demonstrated an augmented activity of the central sympathetic circuit. This peculiarity was associated with the relative increase of AMo, SI and LF and indicated an abnormal pattern of gestational autonomic resetting. The mean sympatho-vagal balance (LF-to-HF ratio) values were 0.9±0.3, 2.2±0.6 and 4.5±1.1 respectively in Group I, Group II and Group III. This gradual growth of sympatho-vagal balance was associated with the progedient severity of PE. The mean values of short-term parameters: the RMSSD, the pNN5O and the HF were lower in Group II and Group III. The lack of parasympathetic regulation was revealed in PE. The decreased impact of RSA on maternal hemodynamics was found.

The fetal HRV parameters demonstrated a suppressed autonomic nervous regulation with an abnormal relative elevation of the sympathetic domain region values in PE (table 3). The values of fetal SDNN and TP were lower in Group II and Group III. However, the shares of AMo, SI and LF grew relatively in the total spectra of fetal HRV in the pre-eclamptic patients. The revealed tendency was associated with an almost complete loss of the cardiac rhythm nonlinearity in Group III. The decrease of RMSSD, pNN5O and HF in the patients with PE was determined in

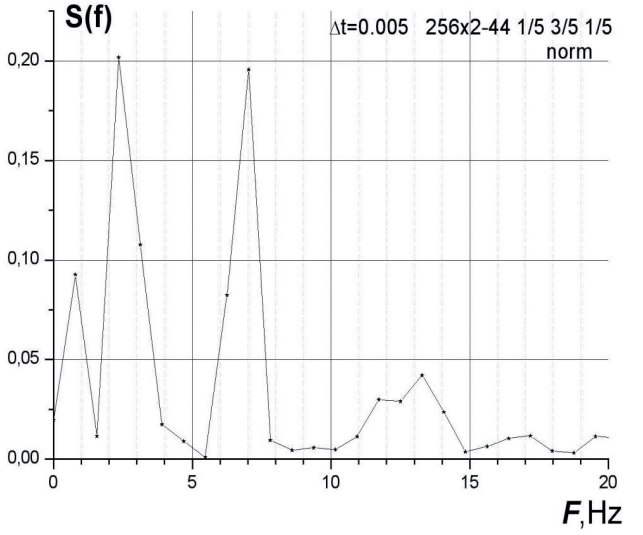

Figure 1. The spectral characteristics of the umbilical venous blood flow velocity of a patient from healthy pregnancy Group.

Table 4. The amplitudes of the spectral peaks of umbilical blood flow velocity in the study population

Frequency of peak	Group I	Group II	Group III
0,5 Hz, c.u.	0.19±0.04	0.15±0.03*	0.03±0.01*†
2 Hz, c.u.	0.16±0.03	0.19± 0.05*	0.18±0.04*†
7 Hz, c.u.	0.18±0.03	0.16±0.04*	0.05±0.01*†

* – the differences were statistically significant compared to Group I ($p<0.05$);
† – the differences were statistically significant compared to Group II ($p<0.05$).

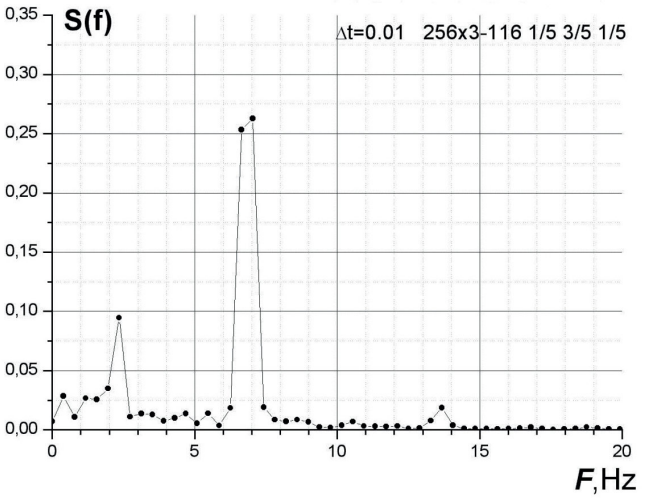

Figure 2. The spectral characteristics of the umbilical venous blood flow velocity of a moderate pre-eclamptic patient in Group II.

Group II and Group III. So, the reduced fetal vagal activity was found in PE. Fetal RSA according to RMSSD value was decreased too.

The investigation of the spectral characteristics of the venous blood flow velocity in the study population revealed the origin of the controlling signals. In Group I, the hemodynamics in the umbilical vein was characterized with three mostly pronounced spectral peaks: 0.5 Hz, 2 Hz and 7 Hz (figure 1). The above peaks had corresponding amplitudes (table 4). In Group II, there was a decrease in the amplitude of the 7 Hz peak. The apparent peak was recorded at a frequency of 2 Hz. The 0.5 Hz peak was considerably lower (figure 2). In Group III, the two peaks in the regions of 0.5 Hz and 7 Hz were very low. The highest peak in this Group was revealed at 2 Hz frequency. The pulsatile pattern of blood flow in Group III was found.

It was found a weak relationship between sympatho-vagal balance and CI (R=-0.30; p<0.05), sympatho-vagal balance and TPVR (R=0.32; p<0.05) in healthy pregnancy Group (table 5). In mild-moderate PE Group the strength of correlation in the pairs: sympatho-vagal balance versus CI (R=-0.34; p<0.05) and sympatho-vagal balance versus TPVR (R=0.38; p<0.05) was found almost on the previous level. The strength of correlation was strong in the same pairs: sympatho-vagal balance versus CI (R=-0.63; p<0.05) and sympatho-vagal balance versus TPVR (R=0.70; p<0.05) in severe PE Group.

The investigation of statistically significant correlations between the maternal and fetal RMSSD's and the amplitudes of 0.5 Hz peak in the study population revealed certain regularities (table 6). The most considerable positive correlation was determined in the healthy pregnancy Group between the maternal RMSSD and 0.5 Hz peak amplitude (R=0.64; p<0.05), the maternal RMSSD and the fetal RMSSD (R=0.51; p<0.05). The positive weak correlation between the maternal RMSSD and the amplitude of 0.5 Hz frequency peak (R=0.34; p<0.05) was determined in Group II. The positive weak correlation between the maternal and fetal RMSSD's in women with mild-moderate PE (R=0.36; p<0.05) was also found. No considerable relationship was revealed between the maternal RMSSD and the amplitude of 0.5 Hz peak (R=0.20; p<0.05), the maternal and fetal RMSSD (R=0.18; p<0.05) in the patients with severe PE.

Table 5. Statistically significant (p<0.05) Spearman's correlations between the maternal sympatho-vagal balance and CI, maternal sympatho-vagal balance and TPVR in the study population.

Pairs of parameters (X versus Y)	Group I	Group II	Group III
Sympatho-vagal balance versus CI	R=-0,30	R=-0,34	R=-0,63
Sympatho-vagal balance versus TPVR	R=0,32	R=0,38	R=0,70

Table 6. Statistically significant (p<0.05) Spearman's correlations between the maternal RMSSD and the amplitude of RSA associated peak, the maternal and fetal RMSSDs in the study population.

Pairs of parameters (X versus Y)	Group I	Group II	Group III
Amplitude of the RSA-associated peak (0.5 Hz) vs the maternal RMSSD	R=0.64	R=0.36	R=0.20
Maternal RMSSD vs fetal RMSSD	R=0..51	R=0.32	R=0.18

DISCUSSION

The obtained data supported the well-known hyperdynamic model of the mild-moderate PE. The persistent gestational hypervolemia with an augmented periferal vascular tone provided an increased cardiac output. Further hemodynamic crossover to hypovolemia and low cardiac output manifested hypodynamic circulation in severe PE (3). The increased sympathetic activity in the mild-moderate PE could be considered a compensatory reaction. Such circulatory response was directed to the support of the maternal organs perfusion. Severe PE was associated with maximal sympathetic tone augmentation. High peripheral vascular resistance and hypovolemia caused hypoperfusion of the end-organs in severe pre-eclamptic patients. Therefore, severe PE destroyed gestational adaptive autonomic response and contributed to the development of cardiac failure.

A parasympathetic regulation was proved more dominant than the sympathetic one in reproductive-aged women. The parasympathetic division of autonomic nervous system was found to be involved in the gestation regulatory resetting and provided an increased level of ergo-, trophotropic reactions (20). Therefore, the vagal regulation demonstrated a considerable ability to protect the gravidity in the first half of healthy pregnancy (2, 5, 20). The current study revealed an increased power of the sympathetic domain region in the total HRV spectra of pre-eclamptic women. It was previously determined that the sympathetic tone was more than 3 times higher in PE than in normotensive pregnant women (5). In addition to the sympathetic hyperactivity, a decreased parasympathetic tone was also found in PE. The RSA is known to have a strong relationship with the parasympathetic division of autonomic nervous regulation (14, 20). Therefore, the suppression of parasympathetic regulation reduced the role of RSA in the total level of maternal HRV in PE. So, the RSA had a diminished influence on the maternal hemodynamics in PE.

Maternal sympathetic overactivity is associated with gestational age and heart rate in PE. The relationship with such parameters as: maternal age, hemoglobin and body mass index have not been confirmed (21). The increased sympathetic tone modulates maternal HRV by suppression of vagal domain region.

The parasympathetic regulation typically has its physiological decline because of the elevated intraabdominal pressure and the decreased diaphragmatic motility in the last trimester of pregnancy (20). PE additionally increased intraabdominal pressure because of the abdominal compartmentalization (4). Breathing disorders could play a certain role in the total scenario of PE. The autonomic imbalance was found to be strongly associated with the respiration abnormalities in PE. Thus, the suppression of autonomic circuit in PE has a relationship with the endothelial dysfunction, oxidative stress, maternal inflammation and thrombophilia (2). The increased abnormally sympatho-vagal balance was a sign of the vasoconstriction and hypoperfusion. Previously the placental bed and renal vessels were determined as the highest vascular resistance areas in PE (3, 5). Even reasonable usage of antihypertensive drugs failed to influence the autonomic balance restoration. Therefore, severe PE destroyed the basis for the pregnancy vagal-mediated mechanism of fluid retention and vasodilation. That is why the only efficient treatment of the severe pre-eclamptic patients is a pregnancy termination. Possibly the postpartum decrease of the intraabdominal pressure could contribute to the sympatho-vagal balance rehabilitation.

The fetal HRV demonstrated an abnormal pattern of the autonomic nervous regulation in PE. A gradual reduction of fetal sympatho-vagal balance in the last trimester of healthy pregnancy was previously showed (21, 22, 23). The increased parasympathetic regulation was a sign of the fetal maturation in the late gestation. The revealed fetal hypersympatheticotonia and the lack of vagal activity were characteristic of PE (18). Thus, PE caused fetal distress and changed its autonomic response. It was found in the previous study that fetal RSA in the growth restricted fetuses was on the same level with fetal RSA in healthy pregnancy (14). This peculiarity was regarded as an adaptive response in case of normal utero-placental hemodynamics. The decreased fetal RSA in pre-eclamptic patients was explored in this study. PE had a negative impact on fetal RSA with a destructive action on the cardiorespiratory synchronization phenomenon.

Several investigations described the fetal and maternal cardiac rhythm synchronization (7, 8, 13). The data obtained in the study explained the participation of maternal RSA in the fetal hemodynamics regulation. The explored strong correlation between 0.5 Hz peak amplitude and RMSSD in healthy pregnancy Group confirmed the role of the maternal RSA as a possible driver of the umbilical venous circulation. The hypothesis that the maternal RSA played a trigger role and the respiration induced hemodynamic fluctuations penetrated through placental vascular bed was supported. The determined amplitudes of three spectral peaks (0.5 Hz, 2 Hz and 7 Hz) in the umbilical vein blood flow velocity were almost equal. The 2 Hz peak was associated with fetal cardiac activity (about 2 beats per minute). The origin of the 7 Hz peak is unclear (16). Fetal umbilical vein captured this frequency from unknown pace-maker. It was possible to speculate the approximately identical contribution of the above-mentioned controlling signals to the umbilical venous hemodynamics. The peak with the frequency of about 0,5 Hz is found also in the total power spectrum of blood flow velocity variability in the umbilical artery (24). It was suggested that the maternal RSA played a significant role in the umbilical hemodynamics and supported a continuous non-pulsatile pattern of the venous blood flow. The umbilical vein served the fetal peripheral heart and provided it with oxygen and nutrients (16).

The limitations of the study were associated with cross-sectional design and small size of samples. The future investigations should also include parameters of hemodynamic fluctuations in gestational hypertension and chronic hypertension. Since fluctuations have nonlinear nature the nonlinear correlation between maternal and fetal HRV will be investigated onward.

The maternal parasympathetic regulation and RSA had a protective impact on the circulatory response of the mother and fetus in PE. The increased maternal autonomic balance supported perfusion of the end organs and coupling with fetal hemodynamics in mild-moderate PE while vagal-mediated reactions were safe. Hypokinetic type of CMH in severe PE was associated with the reduction of RSA. The deteriorated placental perfusion and the decreased motion of the RSA-associated hemodynamic fluctuations were due to hemodynamic failure. Therefore, the fetal cardiovascular system was absolutely separated from the maternal organism and lost the control in severe PE (confirmed with the considerable reduction of an RSA-associated peak and the pulsatile pattern of umbilical hemodynamics). For this reason, the loss of fetal and maternal hemodynamic coupling could be considered a presumable pathogenetic mechanism of the fetal distress in PE; this hypothesis, though, requires further investigations. It is possible to speculate that the testing for maternal sympatho-vagal balance, fetal and maternal RMSSD could be used in future as biophysical markers of the severe PE and fetal distress.

CONCLUSION

The maternal RSA propagated its influence on the fetal umbilical venous blood flow and the fetal autonomic nervous regulation in healthy pregnancy. The hypokinetic type of CMH in severe PE decreased the RSA-mediated hemodynamic fluctuations. It was associated with umbilical vein pulsatile pattern and fetal deterioration.

Conflict of Interest

No conflict of interest was declared by the author.

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HENOCH-SCHÖNLEIN PURPURA NEPHRITIS IN CHILDREN: PROGNOSIS AND TREATMENT EXPERIENCES

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NEFRITIS U HENOH-ŠENLAJNOVOJ PURPURI KOD DECE: ISKUSTVA U PROGNOZI I TERAPIJI

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ABSTRACT

Henoch-Schönlein purpura is a type of systemic small vessel vasculitis. The dominant manifestation is the cutaneous component, the illness is self-limiting in nature, and the prognosis and outcome depend mostly on renal manifestations. We analysed the associations among clinical and laboratory parameters with the prognosis and outcome of HSP in children hospitalised at the Paediatrics clinic, Clinical Centre, Kragujevac between January 2011 and January 2012. Children who developed nephritis were older on average and all manifested with arthritis, abdominal complaints, microhaematuria, elevated D-dimer levels in the serum, and significant proteinuria and microalbuminuria (≥ 300 mg/L), and two children had pre-existing allergic conditions. All three children with repeatedly positive proteins in the morning sample urine test had significant proteinuria (≥0,5 g/24 h) and microalbuminuria (≥ 300 mg/L). These children had more bursts of rash and more severe and lasting abdominal pain and arthritis compared to children with normal urine tests. They were therefore treated with glucocorticoids and an angiotensin-converting enzyme inhibitor. The glomerular filtration rate measured by determining creatinine clearance was normal in all patients. These patients were diagnosed with Henoch-Schönlein purpura nephritis, and their condition was regularly monitored. Analysis of this group of patients demonstrated that the average age of 8 years and abdominal complaints were indicative of nephritis development.. Because both of these parameters are easily noted at disease onset, we suggest careful monitoring of disease course in these children.

Keywords: *nephritis, Henoch-Schönlein purpura, children*

SAŽETAK

Henoh-Šenlajnova purpura je sistemski vaskultis malih krvnih sudova. Dominantna manifestacija je kutana komponenta, bolest je najčeće samoograničavajuća, a ishod i prognoza najviše zavise od renalnih manifestacija. Bavili smo se povezanošću kliničkih i laboratorijskih pokazatelja u prognozi i ishodu Henoh-Šenlajnova purpure kod dece hospitalizovane na Klinici za pedijatriju Kliničkog centra Kragujevac, u periodu od januara 2011. do januara 2012. godine. Deca u grupi sa nefritisom su bila starije srednje životne dobi, sva su ispoljila artritis, abdominalne tegobe, mikrohematuriju, povišene vrednosti D-dimera u serumu, značajnu proteinuriju i mikroalbuminuriju (≥ 300 mg/L), a kod dvoje je utvrđeno ranije postojanje alergijskih stanja. Kod svo troje dece sa ponovljenim pozitivnim nalazom proteina u urinu nađena je značajna proteinurija (≥0,5 g/24 h) i mikroalbuminurija (≥ 300 mg/L). Ova deca su imala i više naleta ospe, izraženije i dugotrajnije abdominalne tegobe i artritis od dece sa urednim nalazom u urinu. Njima je ordinirana kortikosteroidna terapija uz inhibitor angiotenzin-konvertujućeg enzima. Jačina glomerularne filtracije merena klirensom kreatinina bila je uredna kod svih pacijenata. Kod svih je postavljena dijagnoza Henoh-Šenlajn purpura nefritis i njihovo stanje se redovno prati. U našoj grupi ispitanika se ispostavilo da je prosečna starost dece od 8 godina i postojanje abdominalnih tegoba, predznak nastanka nefritisa. Budući da su oba parametra lako uočljiva na samom početku bolesti, smatramo da je potrebno brižljivo praćenje toka bolesti kod ove dece.

Ključne reči: *nefritis, Henoh-Šenlajn purpura, deca*

ABBREVIATIONS

ACE-I - angiotensin converting enzyme inhibitor;
C3 - complement component 3;
EULAR - European league against rheumatism;
Gd-IgA1 - Galactose deficient IgA1 antibodies;
HSP - Henoch-Schönlein purpura;
HSPN - Henoch-Schönlein purpura nephritis;
IgA - immunoglobulin A;
ISKDC - International Study of Kidney Disease in Children;
KDIGO - Kidney Disease Improving Global Outcome;
PRES - paediatric rheumatology european society;
PRINTO - The paediatric rheumatology international trials organisation

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INTRODUCTION

Henoch-Schönlein purpura (HSP) is a systemic small vessel vasculitis. It occurs in some people as a consequence of the immune response to exogenous and endogenous antigens, during which aberrant IgA1 antibodies are produced. Because of this response, HSP is also called IgA vasculitis even though aberrant IgA antibodies cannot be confirmed in all patients. The dominant clinical manifestation is the cutaneous eruption, a purpuric rash that usually develops in several bursts. Skin changes begin to pale after a couple of days, changing their colour from dark red to reddish-yellow, and finally fading away after a few days without leaving a scar. The diagnosis is usually based on this clinical finding. The commonly used 2010 EULAR/PRINTO/PRES criteria (1) include palpable purpura and one of the following: abdominal pain, IgA deposits in biopsy findings, arthritis/arthralgia, or nephropathy. The disease is most often self-limiting, and the outcome and prognosis depend mostly on renal manifestations.

Nephropathy occurs in about a third of patients with HSP, of which 30-50% develop haematuria and/or proteinuria, 7% develop nephritic or nephrotic syndrome, and 1-2% develop chronic kidney disease. Nephropathy clinically manifests within the first 4 weeks in 75% of patients, rarely 9 months after HSP onset, and is an extremely rare first sign of HSP. Galactose deficient IgA1 antibodies (Gd-IgA1) are probably most important for the pathogenesis of HSP nephritis (HSPN). The presence of these antibodies in HSP patients' serum predisposes them to nephritis (2,3). The immune system recognizes aberrantly glycosylated IgA antibodies, making anti-glycan antibodies, which together form immune complexes. These immune complexes bind to mesangium and initiate the process of renal damage by activating the complement cascade that eventually leads to leukocytoclastic vasculitis. This pathologic process matches that in IgA nephropathy (4). The relationship between the two diseases is demonstrated by the case of a girl that developed symptoms characteristic of HSPN 5 years after the initial onset of IgA nephropathy (5). A retrospective study conducted in Turkey involving 430 patients showed that girls, children with atypical presentations, and early introduction of glucocorticoids have an increased risk of producing renal damage, and that relapses are more frequent in children treated with glucocorticoids (6).

The variety of clinical presentations, possible complications, and treatment approaches for HSP led us to retrospectively explore the relationships of clinical and laboratory parameters with the prognosis and outcomes of HSP and especially with the treatment and outcomes of HSP nephritis in children hospitalised at the Paediatrics Clinic, Clinical Centre, Kragujevac in the period between January 2011 and January 2012.

PATIENTS AND METHODS

The data on the patients treated at the Paediatrics Clinic, Clinical Centre, Kragujevac from January 2011 to January 2012 were collected retrospectively. Each patients' data were obtained from written and electronic medical files, and demographic, clinical, and laboratory information were extracted.

The patients were diagnosed with HSP if they manifested the characteristic rash, abdominal symptoms, arthritis, or other symptoms, according to the 2010 EULAR/PRINTO/PRES criteria (1). Microhaematuria was defined as the presence of 5 or more erythrocytes per field at 500x amplification; macrohaematuria was defined as visible red urine with correlating microscopic findings of erythrocytes in the urine sample test; significant proteinuria was defined as urine protein concentration above 0,5 g in the 24-hour urine collection test; hypertension was defined as multiple measurements of blood pressure above the 95th percentile for age, height and gender. The creatinine clearance reference range from 1,47 to 2,28 mL/sec/1.73 m^2 was used. Because there was a small number of patients included in the study, only descriptive statistical methods were used.

RESULTS

Patient clinical and laboratory data are shown in table 1. We included a total of six patients, 3 boys and 3 girls, aged 3 to 14 years. All children were diagnosed with HSP based on clinical presentation of purpuric, non-thrombocytopenic rash and other non-cutaneous manifestations. Five children presented with arthritis, and abdominal discomfort without gastrointestinal bleeding was noted in three. None of the included children manifested with macrohematuria, hypertension or elevated IgA serum level. We identified some sig-

Table 1. Patient clinical and laboratory data.

Patients[1]	No nephritis (3)	Nephritis (3)
Gender	1:2 (m:f)	2:1 (m:f)
Age (mean)	5,33	8,00
Purpura	3 (100%)	3 (100%)
Arthritis	2 (66,7%)	3 (100%)
Abdominal discomfort	0	3 (100%)
Microhaematuria	2 (66,7%)	3 (100%)
Macrohaematuria	0	0
D-Dimer (>230 ng/mL)	2 (66,7%)	3 (100%)
Proteinuria (>0.5 g/24[h])	1 (33,3%)	3 (100%)
Creatinine clearance[2]	0	2 (66,7%)
Microalbumin (>300 mg/L)	0	3 (100)
Cholesterol	0	2 (66,7%)
Infectious agent confirmed	1 (33,3%)	1 (33,3%)
Total serum IgA (>4 g/L)	0	0
Coplement level[3]	0	1 (33,3%)
Allergies	0	2 (66,7%)

[1] The total number and the percentage of patients are shown for each group.
[2] The number of cases with values below reference is shown. Reference values are 1.47 to 2.28 mL/sec/1.73 m2.
[3] The number of cases with values below reference is shown. Reference values are C3 0,9 to 1,8 g/L and C4 0,1 to 0,4 g/L.

nificant differences among children who developed nephritis and those who did not. As presented in table 1, children in the nephritis group were older on average (8,00 years old), and all manifested with arthritis, abdominal discomfort, microhematuria, elevated D-dimer levels, significant proteinuria (>0,5 g/24 h) and microalbuminuria (>300 mg/L). Two of three children in the nephritis group had prior confirmation of multiple allergies, whereas the children in the group without nephritis had no allergic conditions.

All patients were initially put on a hypoallergenic diet and an antihistamine. Antimicrobials were introduced in those patients with signs of infection. The general condition of the patients as well as clinical and laboratory parameters were monitored regularly.

Patients in both groups had repeated bursts of the typical rash. Finding proteins in the morning urine sample tests was particularly important because it signified the development of renal damage and the occurrence of nephritis. In three children with repeated positive proteins in the morning urine sample test, a 24-hour urine collection test was performed. Significant proteinuria (>0.5 g/24 h) and microalbuminuria (>300 mg/L) were found in all three. These children had more bursts of rash and more pronounced and lasting abdominal pain and arthritis than children with normal urine findings. Two out of the three children who developed signs of renal damage were known to have allergies to nutritive allergens and medications. Two of these three children had an increased cholesterol level during the course of illness. These test results were understood as signs of disease progression towards renal involvement and the development of HSP nephritis, so treatment with prednisone and angiotensin converting enzyme inhibitor (ACE-I) was initiated. Prednisone treatment succeeded in stopping further bursts of rash in two patients, whereas in the third patient, the rash persisted until an infectious agent was isolated from the stool sample and treated adequately. Positive proteins in the morning urine sample test with significant proteinuria and hypercholesterolemia continued under prednisone treatment for several weeks while the glomerular filtration rate remained normal in all three children. Renal biopsy was performed in one of the patients because of the nephrotic range proteinuria, and the histology results indicated a mesangioproliferative glomerulonephritis with crescents and positive staining for IgA and C3 in the mesangium. Treatment of this patient was then continued with azathioprine and an ACE-I. Urinary remission was achieved in all patients within 5 weeks of initiating nephritis treatment. There were no relapses of purpura or nephritis. All patients are presently without any specific therapy and maintaining normal renal function; their condition is monitored through regular follow-up.

DISCUSSION

We looked into the relationships among clinical and laboratory parameters and the prognosis and outcome of HSP, and the results indicate the significance of nephritis in HSP morbidity. Older age, abdominal complaints, previous allergic conditions, and duration and intensity of symptoms indicate that the development of nephritis is more likely in patients with such a presentation. Although our study involved a small number of patients, the results are in line with the results of most other studies that have been performed on a much larger case series. Older age, abdominal complaints and persistence of rash have been confirmed as risk factors for developing nephropathy (7,8). Positive signs of type I hypersensitivity reactions are common in HSP patients, and earlier studies have shown that they are predictors of nephropathy (9,10). Identification of new and careful monitoring of known patients with type I hypersensitivity reactions would have been easy and useful, but no such recommendations exist because the new results dispute the rationale for such actions (11). Female gender has been shown to predict a poorer long-term outcome (12), but we could not confirm this in our group.

Because HSP is the most common vasculitis in children, HSPN is a respectively common form of glomerulonephritis, which was illustrated by the results of the study conducted in Dalmatia, where during a 10-year period, of all the renal biopsies performed, 10,8% of glomerulonephritis cases were due to the renal involvement of HSP (13). HSPN is usually manifested by acute phases of glomerular inflammation, during which histology examination can reveal endocapillary and mesangial proliferation. Older lesions contain fibrin deposits and epithelial crescents that could resolve completely or evolve to chronic lesions. The histology results of a mesangioproliferative glomerulonephritis with crescents and positive IgA and C3 staining in one of the patients matches the findings observed in HSPN patients (14). Complete and lasting urinary remission in this patient was achieved only after introducing azathioprine. Patients in the nephritis group had persistent rash and pronounced abdominal complaints for which they were treated with glucocorticoids early in the course of illness before signs of renal damage were evident. However, that treatment did not prevent the development of nephritis. To date, there have not been any treatment methods that significantly shortened the duration of HSP, and there is conflicting evidence in the literature that, on the one hand, suggest that early glucocorticoid treatment reduces the chance of persistent renal disease, relapses and the need for surgical interventions (15), and on the other, that a short course of glucocorticoids is not justified in preventing persistent renal disease (7). There are only a few quality clinical trials on treatment options, especially on treating nephropathy with immunosuppressants (16).

Many investigators aimed their research at determining risk factors for poor outcomes and finding adequate therapies. The blood pressure level at the disease onset is not a good indicator of the outcome (17). Isolated haematuria and/or mild proteinuria early in the course of illness usually has a good prognosis, but 18% of patients with mild proteinuria have poor outcomes (12). Clinical

and biochemical prognostic parameters that could point to the occurrence of severe nephropathy or end-stage renal disease are haematohesia, persistence of rash, signs of nephritic or nephrotic syndrome and finding numerous glomerular crescents (18). Other researchers have published the results of multivariate risk factor analysis that show a glomerular filtration rate below 70 mL/min/1,73 m^2 and growing proteinuria after 3 years of follow-up to better correlate with progression to chronic renal disease than reduced renal function, severe proteinuria, hypertension or presence of crescents at disease onset (17). The prognostic value of histology change grades according to the International Study of Kidney Disease in Children (ISKDC) classification has been investigated several times, and it appears to be significant, especially for short-term outcomes (12). There are results that show the possibility of using only the presence of crescents for predicting outcomes in a way that if over 50% of glomeruli contain crescents or demonstrate signs of sclerosis, there is a greater likelihood for the occurrence of progressive renal disease, renal insufficiency or end stage renal disease (7).

Our patients who developed nephritis had all been treated with ACE-Is and glucocorticoids. Our patients who developed nephritis had all been treated with ACE-Is and glucocorticoids. The glucocorticoid doses did not exceed 2 mg/kg, nor did we use pulse therapy in any of the cases. The nephritis, however, was not controlled efficiently in the one patient whose proteinuria reached the nephrotic range, where treatment was continued successfully with azathioprine after the biopsy results were reviewed. KDIGO guidelines for HSPN based the choice of treatment on the degree of proteinuria, and because there was no high quality evidence for the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers or immunosuppressants in HSPN treatment, the recommendations are founded on the research of IgA nephropathy treatment (19). This guideline does not consider the histology findings to be significant due to the results that were published by Ronkainen and colleagues stating that the first kidney biopsy findings for each patient do not typically correlate with the long-term outcome (20). Additionally, many papers have been published on using various immunosuppressants and other therapies with good outcomes in case series or isolated cases of patients with high grade proteinuria or histology changes. Use of glucocorticoids, ACE inhibitors and azathioprine in our group had good long-term effects in all patients with nephritis. The decisions on which type of treatment to use were made were based on experience or on the results of the case series, which showed that intensifying treatment reduces the chance for developing chronic renal disease and that delaying treatment can produce an unfavourable outcome. Previous statements related mainly to the treatment of patients with severe renal disorders at disease onset, which can manifest itself as renal failure, nephrotic, nephritic or nephritic-nephrotic syndrome and numerous glomerular crescents. These presentations are shown to be predictors of a poor outcome (21). Similar biopsy results at the onset of HSPN have a variable evolution (22), but the significance of the degree of proteinuria for nephritis outcomes has been validated several times (12,17). Still, the unknowns about HSPN pathophysiology make it difficult to find clues from basic research as to which type of treatment is best. New research should indicate the best choice and the role of immunosuppressants as well as other therapies in HSPN.

CONCLUSION

HSPN is a rare disease with the potential for development of long-term renal damage. The occurrence of nephritis merits long-term follow-up of these patients for possible end-stage renal disease. Renal damage in HSP has a good overall prognosis in childhood, but in some cases, nephritis progresses to renal failure. Even low grade histology changes carry a risk for chronic renal disease. On the other hand, high grade lesions can resolve completely. The treatment of HSPN relies on many immunosuppressants with confirmed efficacy in case series. However, the evidence from well-designed, randomised controlled trials are needed, especially for the treatment of severe cases of HSPN. The choice of therapy should be based on clinical and laboratory parameters of HSPN severity. The patient age and abdominal complaints were indicators of nephritis in our group. Because both of these parameters are easy to monitor at any time during the disease course, we suggest careful follow-up of these HSP patients for early and adequate detection of nephritis.

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IMPACT OF REHABILITATION ON HEALTH RELATED QUALITY OF LIFE IN PATIENTS WITH HIP OSTEOARTHRITIS

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UTICAJ REHABILITACIJE NA KVALITET ŽIVOTA U VEZI SA ZDRAVLJEM KOD PACIJENATA SA OSTEOARTRITISOM KUKA

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ABSTRACT

Hip osteoarthritis (OA) is a degenerative, progressive musculoskeletal system disease in adult individuals. Both genders demonstrate a similar prevalence at 11.5% for men and 11.6% for women. During the initial stage of hip OA, conservative treatments may significantly decrease pain, provide functional improvement and enhance health related quality of life (HRQoL).

The aims of the study were to evaluate the quality of life of patients with hip osteoarthritis and to estimate the impact of a comprehensive rehabilitation intervention on their HRQoL.

This was a prospective, observational study of 50 consecutive patients with hip osteoarthritis who were referred to an outpatient rehabilitation intervention. To assess their HRQoL before and after rehabilitation, we used the SF-36 and the Lequesne index for hip OA.

The mean age was 61.7±8.3 years, and 56% of the patients were women. After rehabilitation, the SF-36 RE and RP subscales and the Lequesne pain subscale showed the most significant improvement, although all of the SF-36 and Lequesne domains showed significant improvement. Before rehabilitation, the Lequesne ADL subscale was most correlated with the SF-36 PF subscale (rho=–0.908). After rehabilitation, the total Lequesne score was highly correlated with the SF-36 PF subscale (rho=–0.895). Age, education and the duration of disease were significantly correlated with all of the Lequesne subscales before and after rehabilitation.

This study showed that patients with hip osteoarthritis had a substantially low HRQoL, but all health dimensions showed statistically significant improvements after outpatient rehabilitation intervention.

Keywords: Hip osteoarthritis, Rehabilitation, Health related quality of life

SAŽETAK

Osteoartritis (OA) kuka je degenerativna, progresivna bolest mišićnokoštanog sistema kod odraslih. Oba pola pokazuju sličnu učestalost od 11,5% kod muškaraca i 11.6% kod žena. U početnoj fazi OA kuka, konzervativni tretmani mogu značajno da smanje bol, obezbede funkcionalni napredak i poboljšaju kvalitet života u vezi sa zdravljem (HRQoL).

Ciljevi: Ciljevi ove studije su bili evaluacija kvaliteta života pacijenata sa osteoartritisom kuka i procena uticaja sveobuhvatne rehabilitacione intervencije na njihov HRQoL.

Ovo je bila prospektivna, opservaciona studija kojom je obuhvaćeno 50 uzastopnih pacijenata sa osteoartritisom kuka koji su upućeni na ambulantnu rehabilitacionu intervenciju. Za procenu njihovog HRQoL pre i posle rehabilitacije koristili smo SF-36 i Lekejn indeks za osteoartritis kuka.

Prosečna starost je bila 61.7±8.3 godina, i 56% pacijenata su bile žene. Posle rehabilitacije, najznačajnije poboljšanje su pokazale SF-36 RE i RP subskale i Lekejn Pain subskala, ali su i svi SF-36 i Lekejn domeni pokazali značajno poboljšanje. Pre rehabilitacije, Lekejn ADL subskala je najviše korelirala sa SF-36 PF subskalom (rho=–0.908). Posle rehabilitacije, Totalan Lekejn skor je jako korelirao sa SF-36 PF subskalom (rho=–0.895). Starost, obrazovanje i dužina trajanja bolesti su značajno korelirali sa svim Lekejn subskalama pre i posle rehabilitacije.

Ova studija je pokazala da pacijenti sa osteoartritisom kuka imaju žnačajno lošiji HRQoL, ali su sve dimenzije zdravlja pokazale statistički značajno poboljšanje posle ambulantne sveobuhvatne rehabilitacione intervencije.

Ključne reči: *Osteoartritis kuka, Rehabilitacija, Kvalitet života u vezi sa zdravljem*

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ABBREVIATIONS

ADL - Activity of Daily Living
BP - Bodily Pain
GH – General Health perceptions
HRQoL - Health related quality of life
IFS - Interferential current stimulation
MH – Mental Health
OA - Osteoarthritis
PF - Physical Functioning
PEMF - Pulsed electromagnetic field therapy
RE - Emotional Role
RP - Physical Role
SF – Social Functioning
SF-36 - 36-Item Short Form Health Survey
VT – Vitality

INTRODUCTION

Hip osteoarthritis (OA) is a degenerative, progressive musculoskeletal system disease in adult individuals (1). It is firstly characterized by both progressive articular cartilage loss (2, 3) and appositional new bone formation in the subchondral trabeculae at the joint margins (osteophytes) (3). Hip OA is a disorder ubiquitous all over the world, specifically in the elderly. Because of differences in the definition of OA, such as those based on radiographs, symptoms, or self-report as well as differences in the characteristics of study samples, the estimated prevalence of hip OA greatly differs among studies. The prevalence rates are higher among studies that used the radiographic definition of OA than among studies that used the self-reported or symptomatic definition, varying from 0.9% to 45%. Both genders demonstrate similar prevalences at 11.6% for women and 11.5% for men.

Hip OA is a strongly disabling condition. A major symptom is pain, and as the disease progresses, the pain becomes permanent and more restricting (4). Patients also complain about functional impairment in everyday activities, such as walking, stair climbing, car driving, and household and garden working, and they report symptoms of anxiety and depression. These limitations in physical activity and pain are further accompanied with a deterioration in quality of life (4-7). Hip OA has an unfavourable natural course as the intensity of symptoms and the degree of disability have a tendency to increase over time (8).

During the initial stage of the disease, conservative treatments may significantly decrease pain, provide functional improvement, and enhance health related quality of life (HRQoL). In this context, the appropriate options for therapeutic management are physical therapy and physical exercise programmes. However, joint replacement is needed to manage the disease during the advanced stage (5).

Although quality of life cannot be described with bodily symptoms or the absence of an illness, social and emotional perceptions have an impact on this concept (9). HRQoL uses measurements of health outcomes that have been demonstrated to affect patients (7). This concept is frequently applied to describe the impact of a disease or intervention on an individual, but studies have used more than one definition. The World Health Organization (WHO) defines quality of life as an individual's self-perception of life within the context of their living conditions, culture and values. HRQoL tools measure the influence of health status on life and encompass the physical, emotional and social dimensions of health (10).

The aims of the present study were to evaluate the quality of life of patients with hip osteoarthritis and to estimate the impact of a comprehensive rehabilitation intervention on their HRQoL.

MATERIALS AND METHODS

This was a prospective, observational, descriptive study of 50 patients with hip OA. The patients were recruited from the services for physical medicine and rehabilitation of the Clinical Center Kragujevac. From September 2015 to December 2015, all patients with hip osteoarthritis were consecutively asked to participate in the study and referred to an outpatient rehabilitation intervention. The study was approved by the ethics committee of the Clinical Center Kragujevac. We explained the purpose, risks and benefits of the study to all patients, and they gave their verbal consent to participate.

The inclusion criteria were as follows: patients of either gender, patients aged greater than 40 years old and patients with primary hip osteoarthritis. The exclusion criteria were as follows: patients with concomitant systemic inflammatory rheumatic diseases; patients in the terminal stage of malignancy; patients with severe respiratory, heart or renal failure; patients with peripheral vascular diseases of the lower extremities; patients with psychiatric disorders and patients who refused to participate in the study.

The patients received the same comprehensive rehabilitation intervention. It consisted of physical therapy, which comprised pulsed electromagnetic field therapy (PEMF) and interferential current stimulation (IFS), and therapeutic exercises. The PEMF therapy was performed using a Magomil 2 (Electronic Design, Belgrade), which emits a low-frequency field. The intensity of the magnetic field was 20 mT, and its frequency was 99 Hz. All patients underwent a 30-minute treatment of PEMF therapy five times per week for a period of two weeks. IFS was delivered using an alternating current generator (IF53, PROXIMA, Niš) with a medium frequency. All patients received IFS therapy at a frequency from 1 to 100 Hz and at a tolerable intensity. It was applied once a day for 20 minutes, five times per week, over a two-week period.

The therapeutic exercises involved flexibility (range of motion) exercises in conjunction with strength exercises that were intended to maintain or improve hip joint mobility and to increase the muscle strength of the hip joint stabilizer. The exercise therapy programme comprised an individual, physiotherapist-supervised session that lasted for 30 minutes five times per week over the two-week period.

The included patients were interviewed by the same physician before (baseline) and after rehabilitation (follow up). Socio-demographic and clinical data, such as gender, age, the level of education and the duration of disease, were collected for every patient through a personal interview. We used the 36-Item Short Form Health Survey (SF-36) to assess patient HRQoL. It is a generic instrument composed of 36 items that measures eight domains of health status by self-report. Physical functioning, physical role, bodily pain and general health perceptions are the four domains that estimate physical health. The other four domains, vitality, social functioning, emotional role, and mental health, evaluate mental health. As there is no total score, each domain is analysed individually and is scored from 0 to 100, where a lower score correlates with a lower health status (7, 11, 12).

We also used the Lequesne algofunctional index to assess HRQoL among those with hip osteoarthritis. It is a disease-specific instrument that gathers information about symptoms and physical function. It is an 11-item questionnaire composed of three sections; five items address pain or discomfort, two items address the maximum distance walked and whether a walking aid is required and four items address activities of daily living. Each domain is scored from 0 to 8, and the sum of all items is the Lequesne index score, which ranges from 0 to 24. A higher score correlates with a poorer status of health. A total index score from 1 to 4 indicates mild disability, from 5 to 7 indicates moderate disability, from 8 to 10 indicates severe disability, from 11 to 13 indicates very severe disability and equal or greater than 14 indicates extremely severe disability (11, 13).

The statistical analyses were performed by using the statistical software package SPSS version 20.0 for Windows (SPSS Inc.). All domains of the SF-36 and Lequesne index were not normally distributed, and Wilcoxon's rank sum test was used to analyse the change in scores of both instruments before and after the rehabilitation intervention. Spearman's correlation coefficient was calculated as measure of the association intensity to assess the correlations among all domains of the SF-36 and Lequesne index. A non-parametric Mann-Whitney U test was used to compare differences between genders. The results are expressed as the means ± standard deviations. The alpha level for significance was set to $p \leq 0.05$.

RESULTS

The sample consisted of 50 patients whose mean age was 61.7±8.3 years (range from 47 to 75 years), and 56% of the patients were women. A total of 60% of the patients had received a secondary school education, 22% had a high school/university education and 18% had a primary school education. The mean duration of disease was 4.42±2.5 years (range from 1 to 10 years).

Table 1 summarizes the mean, median, and interquartile range for each domain of the SF-36 and Lequesne index. After the comprehensive rehabilitation intervention, the SF-36 RP and RE subscale scores were significantly improved compared with the scores for these domains at

Table 1: Descriptive statistics and features of score distributions for health status measures in hip osteoarthritis patients (n=50)

SF-36	Mean ± SD[1]	Median[1]	Interquartile (25th-75th)[1]	Mean ± SD[2]	Median[2]	Interquartile (25th-75th)[2]	Wilcoxon´s rank test	
							Z	P
PF	38.7±25.7	40.0	10.0-60.0	73.5±23.7	80.0	48.7-95.0	-6.168	**
RP	13.0±23.8	0.0	0.0-25.0	78.0±23.5	75.0	68.8-100.0	-6.115	**
RE	14.0±23.4	0.0	0.0-33.3	82.0±26.3	100.0	66.7-100.0	-6.151	**
VT	32.2±21.4	35.0	10.0-51.2	81.8±21.1	55.0	30.0-70.0	-6.432	**
MH	47.9±16.3	48.0	36.0-64.0	66.1±15.2	68.0	56.0-80.0	-6.482	**
SF	31.2±22.3	31.2	12.5-50.0	58.5±23.1	62.5	37.5-75.0	-6.543	**
BP	27.6±19.3	32.5	10.0-45.0	55.2±21.5	55.0	32.5-77.5	-6.306	**
GH	35.1±20.5	37.5	15.0-55.0	59.3±20.4	60.0	40.0-80.0	-6.252	**
Lequesne								
Pain	5.1±1.8	4.5	4.0-7.0	1.6±1.8	1.0	0.0-3.0	-6.202	**
Distance	3.8±2.2	3.0	2.0-6.0	2.5±2.0	2.0	1.0-4.0	-6.029	**
ADL	7.7±4.1	7.5	4.0-12.0	4.7±3.7	4.5	2.0-8.0	-5.997	**
Total	16.5±7.7	16.0	10.0-24.0	8.7±6.4	7.0	3-0-15.0	-6.175	**

SF-36- 36-Item Short Form Health Survey; PF-Physical Functioning; RP-Physical Role; RE- Emotional Role; VT – Vitality; MH – Mental Health; SF – Social Functioning; BP- Bodily Pain; GH – General Health perceptions; ADL-Activity of Daily Living.

[1] Before rehabilitation

[2] After rehabilitation

*p≤0.05 **p≤0.01

Table 2: Spearman´s correlation coefficient of SF-36 and Lequesne subscale scores for patients with hip ossteoarthritis

	SF-36							
Lequesne	PF[1]	RP[1]	RE[1]	VT[1]	MH[1]	SF[1]	BP[1]	GH[1]
Pain[1]	-0.712**	-0.427*	-0.569**	-0.750**	-0.637**	-0.668**	-0.626**	-0.702**
Distance[1]	-0.871**	-0.513**	-0.611**	-0.789**	-0.724**	-0.751**	-0.745**	-0.721**
ADL[1]	-0.908**	-0.436*	-0.582**	-0.796**	-0.652**	-0.744**	-0.777**	-0.714**
Total[1]	-0.907**	-0.486**	-0.627**	-0.841**	-0.726**	-0.784**	-0.780**	-0.765**
Lequesne	PF[2]	RP[2]	RE[2]	VT[2]	MH[2]	SF[2]	BP[2]	GH[2]
Pain[2]	-0.669**	-0.388*	-0.441**	-0.620**	-0.630**	-0.579**	-0.528**	-0.583**
Distance[2]	-0.832**	-0.641**	-0.479**	-0.808**	-0.711**	-0.779**	-0.782**	-0.720**
ADL[2]	-0.866**	-0.550**	-0.584**	-0.730**	-0.606**	-0.707**	-0.706**	-0.684**
Total[2]	-0.895**	-0.602**	-0.683**	-0.811**	-0.733**	-0.789**	-0.767**	-0.741**

SF-36- 36-Item Short Form Health Survey; PF-Physical Functioning; RP-Physical Role; RE- Emotional Role; VT – Vitality; MH – Mental Health; SF – Social Functioning; BP- Bodily Pain; GH – General Health perceptions; ADL-Activity of Daily Living.
[1] Before rehabilitation
[2] After rehabilitation
*p≤0.05 **p≤0.01

baseline. For the SF-36, the RE and RP domains achieved the largest improvement, while the GH domain showed the smallest improvement. For the Lequesne index, the pain domain showed the most significant improvement. All health dimensions of the SF-36 and Lequesne index showed significant improvement after intervention as observed in table 1.

We also investigated the associations of all of the SF-36 subscales with all of the Lequesne subscales before and after comprehensive rehabilitation intervention. As shown in table 2, before rehabilitation, we found that the Lequesne ADL subscale was better correlated with the SF-36 PF subscale (rho=–0.908) than the rest of the SF-36 subscales. The total Lequesne score was highly correlated with the SF-36 PF subscale (rho=–0.895) after rehabilitation. The Pearson correlation coefficients were negative between the Lequesne subscales and the SF-36 subscales, indicating that the scores decreased with improvements in HRQoL. All associations were highly correlated, but only the correlations between the Lequesne ADL subscale and the SF-36 RP before rehabilitation and between the Lequesne pain subscale and the SF-36 RP after rehabilitation were statistically significant.

Table 3 shows significantly positive correlations of age and the duration of disease with all of the Lequesne domains. There were also significant negative correlations between education and all of the Lequesne subscales. There were no statistically significant differences in all of the Lequesne domains before and after rehabilitation between women and men.

DISCUSSION

In our study, the mean age of patients with hip OA was 61.7±8.3 years, which was similar to the mean age reported in other studies. The majority were women, corresponding to the sex distribution of cohorts from other studies (7, 14-19). The education level of our patients was, on average, higher than that of patients in other studies; the majority of the populations from those studies had completed up to primary school (14, 15, 17) or were illiterate (17, 18). The average duration of hip OA in our group of patients was 4.42 ± 2.5 years, which was similar to that reported by Boutron et al. (5.4 ± 4.8 years; n=1581) and other studies (15, 17, 18).Before rehabilitation, our patients showed low

Table 3: Spearman´s correlation coefficients between socio-demographic and clinical characteristics and Lequesne subscale scores

Lequesne[a]	Age[a]	Education[a]	Duration of disease[a]	Gender[b]
Pain[1]	0.547**	-0.521**	0.684**	Z= -1.005
Distance[1]	0.775**	-0.542**	0.794**	Z= -0.406
ADL[1]	0.833**	-0.653**	0.841**	Z= -0.461
Total[1]	0.798**	-0.615**	0.842**	Z= -0.577
Pain[2]	0.433*	-0.471**	0.558**	Z= -0.524
Distance[2]	0.788**	-0.538**	0.777**	Z= -0.020
ADL[2]	0.793**	-0.601**	0.730**	Z= -0.390
Total[2]	0.774**	-0.627**	0.780**	Z= -0.118

[a] Spearman´s correlation
[b] non-parametric Mann-Whitney U test

average scores for the RP, RE and BP domains of the SF-36. In fact, the average SF-36 RP domain score reported in this study was lower than that recorded in other studies (12, 14, 20-23), while Krauss et al. reported the highest score for this domain (5). After rehabilitation, the SF-36 RP domain showed the greatest improvement, followed by the RE domain. These results were consistent with the results of other studies (19, 20), although one study observed lower scores in the SF-36 RP domain after rehabilitation (23). The SF-36 RS domain showed the largest improvement after rehabilitation; this finding contradicted the results observed in other studies (14, 19, 23), with some studies reporting high scores for that domain before rehabilitation (1, 12). Generally, hip OA patients receive low scores for the SF-36 BP domain (1, 12, 14, 15, 19-24), which was in accordance with our results; thus, after rehabilitation, this was one of the domains that showed moderate improvement in our patients, although other studies reported that this domain showed the greatest improvement (14-16, 20, 23, 25). A Cochrane review from 2014 (26) included nine studies (549 patients) and showed that exercise therapy reduces pain and improves physical function immediately after treatment. After rehabilitation, there was a significant improvement in the SF-36 VT domain; this finding was in agreement with the results from other studies (6, 23). This domain was the second highest in score, immediately after the RE domain. After rehabilitation, statistically significant improvements were shown in all SF-36 domains (20, 24).

Before rehabilitation, our patients scored an average total Lequesne index score of 16.5 ± 7.7 (extremely severe). Similar studies demonstrated serious disability in their study populations with total Lequesne scores that ranged from 10.51 to 13.67 (11, 14, 17, 18). In our study, the worst score for the Lequesne index was in the ADL domain; this was also reported in the study by Konstantinidis et al. (11). However, in the studies by Basaran et al. (18) and Nadrian et al. (17), the pain domain showed the worst score. After rehabilitation, statistically significant improvements were observed in all domains of the Lequesne index.

Before rehabilitation, our results showed that the Lequesne ADL domain was most significantly correlated with the SF-36 PF domain; however, Konstantinidis et al. (11) found that the strongest significant correlation existed between the Lequesne ADL domain and the SF-36 SF domain. The correlations among all other subscales in the present study were significant and negative, whereas in the aforementioned study, the correlations among the other subscales were also negative but not statistically significant. In a study by Basaran et al., all of the Lequesne subscales showed a moderate correlation with the SF-36 PF and BP subscales, but the relationships lacked statistical significance (18).

Our study also noted that there were significant positive correlations between all of the Lequesne subscales (except for pain after rehabilitation) and the age of the patient, which was confirmed in the study by Nadrian et al. (17). According to the results from Konstantinidis et al. (11), a significant positive correlation only existed between age and the Lequesne distance subscale, indicating that the correlations of age with the other subscales were not significant. Education showed significant negative correlations with all of the Lequesne subscales in our study and in the study by Nadrian et al. (17). Moreover, we found significant positive correlations between the duration of disease and all of the Lequesne subscales before and after rehabilitation, while Nadrian et al. (17) observed a significant positive correlation between the pain domain and the total Lequesne score and also between the Lequesne distance and ADL subscale scores. In a study by Basaran et al. (18), age, education and the duration of the disease did not show significant correlations with all of the Lequesne subscales.

CONCLUSION

According to the results of this study, patients with hip OA had a substantially low HRQoL. This study also showed significant improvements in all health dimensions of patients who underwent an outpatient, comprehensive rehabilitation intervention, as evaluated by both the generic SF-36 and disease-specific Lequesne index for hip OA.

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EMERGENCY OR DELAYED SURGICAL TREATMENT OF UNSTABLE SUPRACONDYLAR HUMERAL FRACTURES IN CHILDREN?

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HITANA ILI ODLOŽENA HIRURŠKA INTERVENCIJA NESTABILNOG SUPRAKONDILARNOG PRELOMA HUMERUSA KOD DECE?

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ABSTRACT

Supracondylar humeral fractures (SCHF) are the most common elbow fractures in children, representing 3% of all paediatric fractures. Treatment options for SCHF in children are based on the Gartland classification. Treatment of non-displaced fractures (type I) is non-operative. Plaster immobilization for 3 to 4 weeks is recommended, depending on the age of the child and fracture healing. Treatments of displaced supracondylar fractures (type II and III) of the humerus in children are still undefined in clinical practice. Because of divided opinions, the aim of this study was to evaluate whether delayed or immediate surgical treatment has an advantage in the treatment of supracondylar fractures in children. This is a prospective – retrospective clinical study. This study included 64 patients from 5 to 15 years old; 47 (73.4%) were boys and 17 (26.6%) were girls. The most common age range (59.4%) in this study was 5-8 years old. All patients were diagnosed with supracondylar fractures at the Institute for Orthopaedic Surgery "Banjica". We analysed 17 parameters, which were obtained either from direct patient interviews or from their medical history. All patients were divided into two groups with matched characteristics. Group I consisted of 26 patients who had immediate operations. Group II consisted of 38 patients who had delayed operations.

Based on the results of the analysed parameters, consisting primarily of functional results, the absence of subjective symptoms and myositis ossificans one year after surgery suggests that emergency surgical treatment of displaced supracondylar humeral fractures is optimal.

Key words: *Supracondylar humeral fractures, Functional result, Myositis ossificans, Cubitus varus, Cubitus valgus.*

SAŽETAK

Suprakondilarni prelomi humerusa su najčešći prelomi lakta kod dece i predstavljaju 3% od svih pedijatrijskih preloma. Opcije za tretman ovog preloma kod dece, zasnovane su na Gartland-ovoj klasifikaciji. Tretman nedislociranog preloma (tip I) je neoperativan. Većina autora preporučuje imobilizaciju gipsom 3 do 4 nedelje u zavisnosti od zarastanja i uzrasta deteta. Tretman dislociranog preloma (tip II i tip III) još uvek nije univerzalno definisan. Upravo zbog podeljenih mišljenja, cilj ove studije bio je da ispita da li hitno ili odloženo hirurško lečenje ima prednsti u tretmanu suprakondilarnog preloma kod dece. Ovo je bila prospektivno-retrospektivna klinička studija. U studiji je učestvovalo 64 pacijenata od 5 do 15 godina, 47 (73,4%) dečaka i 17 (26,6%) devojčica. Većina (59,4%) pacijenata u studiji imala je od 5 do 8 godina. Svim pacijentima dijagnostikovana je suprakondilarna fraktura na Institutu za ortopediju "Banjica". Analizirano je 17 parametara, a same informacije su dobijene direktno od pacijenata ili iz medicinske istorije. Svi pacijenti su bili podeljeni u dve grupe sa sličnim karakteristikama. Grupu I sačinjavalo je 26 pacijenata koji su bili hitno operisani, a grupu II 38 pacijenata koji su imali odloženu operaciju.

Analizirani parametari, prvenstveno funkcionalni rezultati kao i prisustvo subjektivnih simptoma i myositis ossificans-a, godinu dana nakon operacije, favorizuju hitno hirurško lečenje dislociranih suprakondilarnih preloma humerusa.

Ključne reči: *Suprakondilarni prelom humerusa, Funkcionalni rezultati, Myositis ossificans, Cubitis varus, Cubitis valgus.*

ABBREVIATIONS

SCHF – Supracondylar humeral fractures

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INTRODUCTION

Supracondylar humeral fractures (SCHF) are the most common elbow fractures in children, representing 3% of all paediatric fractures. The annual incidence of these fractures is estimated to be 177.3 per 100,000 children. They have a seasonal distribution, being more frequent in the summer months, and occur more often in the left elbow (1). Boys are more affected by this fracture than girls (2, 3) and the prevalence decreases after 12 years of age (4).

The elbow is a hinge joint formed by the distal humerus, radial head and proximal ulna. The distal humerus has two surfaces that articulate with both forearm bones. Specifically, the capitellum articulates with the radial head and the trochlea interacts with the articular surface of the olecranon. The elbow is a very complex anatomical area with many related structures. All these structures must be well understood by the paediatric orthopaedic surgeon for proper supracondylar fracture management (5). Supracondylar fractures of the humerus in children are fractures located at the distal end of the humerus, from the proximal end of the distal metaphysis to the distal transversal humeral shaft (6).

SCHF are classified into two types, according to the mechanism of injury. The flexion type is present in only 2%, whereas the extension type is present in 98% of all injuries. In extension fractures, the Gartland classification is used to describe the severity of the injury as well as to focus therapeutic management. Such fractures are divided into three types, according to the degree of fracture displacement measured in the lateral view on a plain radiograph (7). Type I is very stable and includes non-displaced or minimally displaced fractures. Type II includes slight displacement fractures (>2 mm) with an intact posterior cortex. Type III includes completely displaced fractures. This type of fracture is unstable and requires operative treatment (6, 8).In extension type fractures, the mechanism of fracture involves a fall on the extended arm. In this type of fracture, the radial and median nerve and brachial artery are more prone to injury, while in flexion type fractures ulnar nerve injury is more likely (8). Radiographic diagnosis includes an anteroposterior view of the distal part of the humerus, an anteroposterior radiograph of the elbow and a lateral radiograph of the elbow. Initial radiographs may show no evidence of a non-displaced fracture, except signs of a posterior fat-pad. It is sometimes necessary to perform a radiographic diagnosis of the uninjured elbow to confirm the fracture in the injured elbow (7).

Treatment options for SCHF in children are based on the Gartland classification. Treatment of non-displaced fractures (type I) is non-operative. Plaster immobilization for 3 to 4 weeks is recommended, depending on the age of the child and fracture healing (9, 10). Treatments of displaced supracondylar fractures (type II and III) of the humerus in children are still undefined in clinical practice. Some authors defend a conservative approach to stable type II fractures without malrotation or displacement (11, 12) while the American Academy of Orthopaedic Surgeons (AAOS) has recommended surgical treatment for all type II fractures (13). All described treatments can be classified into 4 categories: orthopaedic repositioning, repeated orthopaedic repositioning, delayed surgical treatment in the case of unsatisfactory repositioning and emergency surgical treatment.

Because of divided opinions, the aim of this study was to evaluate whether delayed or immediate surgical treatment has an advantage in the treatment of supracondylar fractures in children.

PATIENTS AND METHODS

This is a prospective – retrospective clinical study. The study included 64 patients, whose ages ranged from 5 to 15 years old. All patients were diagnosed with supracondylar fractures at the Institute for Orthopaedic Surgery "Banjica". In the period between 1990 and 2007, data were obtained either from direct patient interviews or from their medical history.

The patients were characterized using the Gartland classification method. All patients in this study had surgery using either the posterior or lateral approach. The lateral approach was used more frequently. For fixation, Kirchner pins (two, three or four) were used. These were swiftly removed after 3 to 6 weeks. Exclusion criteria from the study included the following: Gartland type I fractures (non-displaced), open fractures and cases with serious neurovascular complications demanding other specific operative management.

All patients were divided into two groups. Group I consisted of 26 patients who had immediate operations (within two days after the fracture). Group II consisted of 38 patients who had delayed operations (at least two days after fracture). Patients were also divided into three groups by age: from 5 to 8, from 9 to 12 and from 13 to 15 years old.

We analysed 17 parameters (7 numeric and 10 descriptive). Numeric parameters included the following: ages, tracking time (in months), days from fracture until operation, functional movement (in degrees), the Baumann's angle (before and after operation), humerocapitellar angle (before and after operation) and time to bone healing. Descriptive parameters included: sex, functional results after one year, continuous anterior humeral line (before and after operation), continuous coronoid line (before and after operation), nerve lesions (before and after operation), vascular complications (before and after operation), myositis ossificans, cubitus varus, cubitus valgus and subjective symptoms at the end of the monitoring period.

The most important parameter was the functional result of the elbow after one year. It was estimated by the Hardacre functional score and included the range of movement, presence of subjective complaints, deformity and radiographic changes (14). The results were described as excellent, good, satisfactory or poor. An excellent result

Table 1. Patient characteristics

			Frequency	Percentage
Groups	I		26	40.6
	II		38	59.4
Sex	Male	I	19	29.7
		II	28	43.7
	Female	I	7	10.9
		II	10	15.7
Age	5-8 years	I	16	25
		II	22	34.4
	9-12 years	I	7	10.9
		II	9	14.1
	13-15 years	I	3	4.7
		II	7	10.9

included a full range of motion in the elbow and no subjective problems and deformities, with the possibility of mild radiographic changes. A good result included less than the full range of movement, with a loss of less than 10 degrees for each movement or changes in the humeral corner of the elbow with some radiographic changes but without subjective problems and deformities. A satisfactory result included a loss of 10 to 20 degrees for each movement, changes in the humeral corner of the elbow of more than 5 degrees without deformities and with or without subjective problems. A poor result included loss of more than 20 degrees from each movement and changes in the humeral corner of the elbow outside the normal range with subjective problems and deformities.

Statistical analyses were performed using the statistical package SPSS 20.0 for Windows. The results are expressed as the means ± standard deviations from the mean (SD). Data distribution was checked with the T-test, x^2-test, analysis of variance, the Fisher and Kruskal-Wallis test and descriptive statistical methods. The alpha level for significance was set to $p < 0.05$.

Table 2. Fracture characteristics before and after operation for both groups

			Frequency	Percentage	Cumulative percentage
Functional result#	Poor		6	9.4	9.4
	Satisfactory		12	18.8	28.1
	Good		17	26.6	54.7
	Excellent		24	45.3	100.0
Continuous anterior humeral line	Before operation	No	63	98.4	98.4
		Yes	63	1.6	100.0
	After operation	No	14	21.9	21.9
		Yes	50	78.1	100.0
Continuous coronoid line	Before operation	No	58	90.6	90.6
		Yes	6	9.4	100.0
	After operation	No	4	6.3	6.3
		Yes	60	93.8	100.0
Nerve lesions	Before operation	No	60	93.8	93.8
		Yes	4	6.3	100.0
	After operation	No	55	85.9	85.9
		Yes	9	14.1	100.0
Vascular complications	Before operation	No	64	100.0	100.0
		Yes	/	/	/
	After operation	No	63	98.4	98.4
		Yes	1	1.6	100.0
Myositis ossificans	No		51	79.7	79.7
	Yes		13	20.3	100.0
Cubitus varus#	No		56	87.5	87.5
	Yes		8	12.5	100.0
Cubitus valgus#	No		63	98.4	98.4
	Yes		1	1.6	100.0
Subjective symptoms	No		42	65.6	65.6
	Yes		22	34.4	100.0

#1 year after operation

Table 3. Differences between groups I and II before operation

		I	II	Difference frequency
Continuous anterior humeral line	No	25	38	p>0.05
	Yes	1	0	
Continuous coronoid line	No	1	5	p>0.05
	Yes	25	33	
Nerve lesions	No	24	36	p>0.05
	Yes	2	2	
Vascular complications	No	26	38	/
	Yes	/	/	
Baumann's angle		4.50	1.29	p>0.05
Humerocapitellar angle		-30.00	-24.39	p>0.05

*p<0.05; **p<0.01.

RESULTS

The study included 64 patients, all of whom were followed for a minimum of 12 months. Forty-seven (73.4%) of the patients were boys and 17 (26.6%) were girls. Most patients (59.4%) in this study were in the age range of 5-8 years old. All patient characteristics were matched among the groups (Table 1).

Fracture characteristics both before and after the operation in both groups are presented in Table 2. The maximum follow-up was 66 months and the minimum was 12 months (average 14.9 months). Patients in group I waited approximately 5.33 days for surgery, while patients in group II waited approximately 59.58 days. Patients from group I waited an average of 1.46 days, while patients from group II waited an average of 7.97 days. Minimal functional movement was achieved at the 12th postoperative day. However, one patient did not manage to achieve functional movement. The average elbow flexion one year after the operation was 129.30 degrees. The preoperative minimal Baumann's angle was 2.59 degrees, on average. After the operation, the average angle was 15.77 degrees. The mean humerocapitellar angle was -26.26 degrees preoperatively and 32.55 degrees postoperatively. The average time to bone healing was 40 days for both groups.

After processing the data, there was no statistically significant difference between the groups before surgery (Table 3).

The differences between group I (emergency surgical) and group II (delayed surgical) after surgery are shown in Table 4. The emergency surgical group (group I) successfully accomplished continuous anterior humeral lines and had less frequent myositis ossificans. Functional results and subjective symptoms indicate that there is a highly significant difference between the groups. Patients from group I had significantly better results one year after operation than patients from group II.

DISCUSSION

Supracondylar fracture of the humerus is the most common elbow fracture in children. It is also one of the

Table 4. Postoperative differences between groups I and II

		I	II	Difference frequency
Functional result#	Poor	0	6	**
	Satisfactory	1	11	
	Good	1	16	
	Excellent	24	5	
Continuous anterior humeral line	No	2	1 2	*
	Yes	24	26	
Continuous coronoid line	No	0	4	p>0.05
	Yes	26	34	
Nerve lesions	No	23	32	p>0.05
	Yes	3	6	
Vascular complications	No	26	37	p>0.05
	Yes	/	1	
Myositis ossificans	No	24	27	*
	Yes	2	11	
Cubitus varus#	No	25	31	p>0.05
	Yes	1	7	
Cubitus valgus#	No	25	38	p>0.05
	Yes	1	/	
Subjective symptoms	No	25	17	**
	Yes	1	21	
Baumann's angle		17.77	14.39	*
Humerocapitellar angle		37.15	29.58	**
Extension movement#		-1.15	-10.66	**
Flexion movement#		135.4	125.1	**

#1 year after operation; *p<0.05; **p<0.01.

most difficult fractures to treat (15). Fracture is more common in boys (Table 1); this is explained by the fact that boys are more restless than girls (16). Despite the high incidence, we still do not have a generally accepted treatment for this fracture. Because of this, the aim of this study was to evaluate whether delayed or immediate surgical treatment has an advantage in the treatment of supracondylar fractures in children.

Patients in both groups showed excellent functional results, but this rate did not exceed 50% (Table 2). This result was expected, given the complexity of treatment. Postoperative establishment of continuous anterior humeral and coronoid lines shows the success of surgical treatment. The percent and number of vascular complications was similar to the literature data (17, 18). Myositis ossificans is an indicator of damaged soft tissue around the elbow and is associated with poorer functional outcomes. The presence of myositis ossificans was higher than that in the available literature (19). Cubitus varus and cubitus valgus describe elbow deformities one year after operation. Based on the results, we can see that approximately 13% of all patients exhibited observed deformities (Table 2). At the end of follow-up 34.4% of patients reported subjective symptoms. Symptoms are not categorized by their character. However, patients mainly complained of pain in the elbow during meteorological changes.

Before operation we examined the continuity of the humeral and coronoid lines, nerve lesions and vascular complications as well as the Baumann's and humerocapitellar angle because these parameters are indicators of operative success. The results showed that there were no preoperative differences between the groups (Table 3).

Differences between the emergency surgical (group I) and delayed surgical cohorts (group II) are presented in table 4. Functional results of elbows one year after operation were better in group I. These results are in agreement with previously presented literature data (20-22, 8). Twenty-four patients from group I had excellent functional results, while only 5 patients from group II showed the same functional results. Unfortunately, there are few similar studies which investigated this type of fracture in children. Research performed by Bojovic and co-workers found that good results were achieved by treating patients (with open and closed reposition) within the first twelve hours after fracture (22). Additionally, in a prospective study of 93 children, Ducic and co-authors recommended a selective approach to the initial treatment of displaced supracondylar fractures in children based on fracture subtype. They also recommended that closed reduction should always be attempted first (23).

Postoperatively, 2 patients from group I and 12 patients from group II did not have established continuity of the anterior humeral line. Statistics indicate successful postoperative reposition in the sagittal plane among the emergency surgical group (group I). However, the available literature does not have enough data regarding this parameter. Postoperatively, the rates of continuous coronoid lines, nerve lesions and vascular complications were similar between the groups. Frequency data were correlated with data from the available literature (24, 25). Based on these results, we can say that the operative technique in both groups was good, with anatomical repositioning of the fragments. Myositis ossificans, an indicator of soft tissue damage, occurred more frequently in group II than in group I. This parameter is very important, as it can lead to the formation of large ossifications that block movement of the elbow. Cubitus varus and cubitus valgus are described as cosmetic deformities with little functional significance, including chronic pain, snapping elbow and increased risk of lateral condyle and other secondary fractures (5). Differences between groups in these parameters were not recorded. There were 8 patients with cubitus varus, 1 patient from group I and 7 patients from group II. We also found a highly significant difference in the presence of subjective complaints one year after surgery between groups. Twenty-one patients from group II complained of subjective symptoms while only 1 patient from group I had similar complaints, but the available literature has not considered this parameter. The value of Baumann's angle after surgery was within the physiological range in all patients. However, there was a statistically significant difference between the groups. Better results were achieved with the humerocapitellar angle. Furthermore, extension and flexion movements in group I suggest that emergency surgical treatment of SCHF results in is better and faster rehabilitation. However, several limitations to this study need to be acknowledged. The initial choice of treatment was based on the expert opinion of a senior orthopaedic surgeon. Attitudes for or against orthopaedic methods in an injured child were based on personal experiences in interpreting clinical findings or available radiographic data. Considering that these fractures are associated with numerous complications, further investigation should examine different procedures for the treatment of SCHF in larger study samples.

CONCLUSION

In summary, a statistically significant difference between the functional results and the absence of subjective symptoms after one year of operation in group I suggests that emergency surgical treatment of displaced supracondylar fractures of the humerus is optimal. Furthermore, according to many authors, the difference in the occurrence of myositis ossificans also favours emergency surgical treatment, which is most responsible for poor final results.

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THE EFFICACY OF A POSTERIOR SUB-TENON'S CAPSULE TRIAMCINOLONE INJECTION IN PATIENTS WITH NON-INFECTIOUS INTERMEDIATE UVEITIS AND POSTERIOR UVEITIS

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EFIKASNOST ZADNJE SUBTENONSKE INJEKCIJE TRIAMCINOLONA KOD PACIJENATA SA INTERMEDIJALNIM I ZADNJIM UVEITISOM NEINFEKTIVNE ETIOLOGIJE

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ABSTRACT

To investigate the efficacy of a posterior sub-Tenon's capsule triamcinolone injection for treating eyes with non-infectious posterior and intermediate uveitis.

A total of 31 eyes from 20 patients with non-infectious posterior uveitis and 18 eyes from 10 patients with non-infectious intermediate uveitis that inadequately responded to treatment with systemic corticosteroids and second-line immunosuppressive agents were enrolled in the study. All patients received a posterior sub-Tenon`s injection of 20 mg/ml triamcinolone. The parameters we examined included the following: best corrected visual acuity, central foveal thickness, fluorescein angiography score and intraocular pressure.

The mean best corrected visual acuity was significantly improved from the control visit, 0.15±0.30 logMAR (Snellen equivalent 0.7), compared to the baseline measurements, 0.60±0.30 logMAR (Snellen equivalent 0.25; P<0.05). The mean central foveal thickness (CFT) and the mean score for fluorescein angiography (FA) were significantly decreased from the baseline (CFT: 320±34 μm; FA mean score: 5.9±1.9) compared to the twelve-week control visit (CFT: 235±30 μm; FA mean score: 1.2±1.1; P<0.001). Five eyes had intraocular pressure spikes that required a topical anti-glaucomatous treatment.

A posterior sub-Tenon`s injection of triamcinolone can significantly improve visual acuity and decrease macular oedema in patients with non-infectious posterior and intermediate uveitis. Complications were minimal, and there were no eyes that required surgical treatment for elevated intraocular pressure. The results suggest that the posterior sub-Tenon`s injection of triamcinolone is an important form of therapy for non-infectious posterior and intermediate uveitis.

Keywords: *Intermediate uveitis, posterior uveitis, triamcinolone, fluorescein angiography*

SAŽETAK

Ispitivanje efikasnosti zadnje subtenonske injekcije triamcinolona (PSTI) u terapiji očiju sa intermedijalnim i zadnjim uveitisom neinfektivne etiologije.

u studiju je uključeno ukupno 31 oko od 20 pacijenata sa zadnjim uveitisom i 18 očiju od 10 pacijenata sa intermedijalnim uveitisom neinfektivne etiologije koji neadekvatno reaguju na kombinaciju terapije sistemskih kortikosteroida i druge linije imunosupresivnih medikamenata. Svi pacijenti su primili zadnju subtenonsku injekciju 20 mg/ml triamcinolona. Parametri koje smo pratili su: najbolje korigovana vidna oštrina, centralna fovealna debljina, skor fluoresceinske angiofrafije i intraokularni pritisak.

Sredna vrednost najbolje korigovane vidne oštrine je statistički značajno poboljšana na kontrolnoj poseti 0.15±0.30 logMAR (Snellen ekvivalent 0.7) u odnosu na bazalnu 0.60±0.30 logMAR (Snellen ekvivalent 0.25), P<0.05. Srednja centralna fovealna debljina (CFT) i srednji skor fluoresceinske angiografije (FA) su bili statistički značajno smanjeni na kontroli u 12 nedelji (CFT: 235±30 μm; FA srednji skor: 1.2±1.1) u odnosu na bazalni (CFT: 320±34 μm; FA srednji skor: 5.9±1.9), P<0.001. Pet očiju sa skokom intraokularnog pritiska je zahtevalo lokanu antiglaukomatoznu terapiju.

Zadnja subtenonska injekcija triamcinolona može statistički značajno poboljšati vidnu oštrinu i smanjiti edem makule kod pacijenata sa zadnjim i intermedijalnim uveitisom neinfektivne etiologije. Komplikacije su bile minimalne i nije bilo očiju koje su zahtevale hirušku terapiju povišenog intraokularnog pritiska. Rezultati sugerišu da zadnja subtenonska injekcija triamcinolona je važan oblik u terapiji zadnjih i intermedijalnih uveitisa neinfektivne etiologije.

Ključne reči: *intermedijalni uveitis, zadnji uveitis, triamcinolon, fluoresceinska angiografija*

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INTRODUCTION

Non-infectious posterior and intermediate uveitis comprises a group of diseases associated with inflammation of the eye that can lead to vision loss. A number of patients with uveitis have macular oedema. Macular oedema is treated with systemic medications, but the treatment does not always prevent vision loss. In some eyes, macular oedema persists even after the inflammation is controlled and requires an additional treatment to improve vision. This approach is currently completed with and additional systemic corticosteroid and/or ocular corticosteroid injections (1).

Triamcinolone is a long-acting synthetic corticosteroid that is effective against a certain level of inflammation and uveitic macular oedema when it is administered by an intravitreal or sub-Tenon`s capsule injection as a derivative acetonide (2). Intravitreal injections carry the risk of glaucoma (3,4,5), cataracts (6) and severe vision-threatening ocular complications in patients with posterior and intermediate uveitis, such as intraocular haemorrhage, retinal detachment and endophthalmitis (7,8).

A periocular posterior sub-Tenon`s capsule injection of corticosteroids is an alternative route through which a certain amount of a drug can be delivered to the posterior segment of the eye via transscleral absorption. An image from a previous study showed that the fluid injected in the sub-Tenon space diffuses into the surrounding orbital tissues (9). The rationale behind the usage of this technique lies in its ability to inhibit the arachidonic acid pathway, regulate the production of cytokines, and reduce the breakdown of the blood-retinal barrier (10). Therefore, we used the posterior route to administer steroids. The best-fit line, according to the literature data, suggests that the effect of a posterior sub-Tenon`s triamcinolone injection in a 20-mg dose lasts approximately 1 year (11).

The purpose of this study was to evaluate the results of a posterior sub-Tenon`s triamcinolone injection (PSTI) in the treatment of eyes with non-infectious posterior and intermediate uveitis.

SUBJECTS AND METHODS

This was a prospective interventional study that lasted from January 2013 to June 2014 and included 31 eyes from 20 patients with non-infectious posterior uveitis and 18 eyes of 10 patients with non-infectious intermediate uveitis that inadequately responded to treatment with a systemic corticosteroid and second-line immunosuppressive agents. Laboratory tests were performed to rule out infectious uveitis if the subject did not already have an infectious type of uveitis at the time of the uveitis diagnosis.

Examination was conducted at baseline, 1 week after treatment, 4 weeks and 12 weeks after treatment.

The following examinations were required for all patients who were included in the study: (1) best corrected visual acuity (BCVA), determined with illuminated logMAR charts (logarithm of minimum angle of resolution), (2) IOP of both eyes measured with Goldmann applanation tonometry, (3) ophthalmic examination including dilated ophthalmoscopy and a slit-lamp examination (for lens assessment), (4) fluorescein angiography (FA) and (5) optical coherence tomography (OCT).

Table 1. Modified Fluorescein Angiographic Grading System for Macular Oedema

Grade	Characteristics
0	No perifoveal hyperfluorescence
1	Faint perifoveal hyperfluorescence; specific localization of hyperfluorescence was too difficult because of very minimal leakage
2	Evident perifoveal hyperfluorescence in an area centred on the fovea of less than 1 optic disc diameter
3	Evident perifoveal hyperfluorescence in an area centred on the fovea between 1 and 1.5 optic disc diameter(s) in size

The clinical evaluation of macular oedema was performed during a stereoscopic slit-lamp fundus examination using both non-contact (+90 diopters) and Goldmann contact lens and colour photographs of fundus. The tests for evaluating macular oedema were grouped into three categories, according to whether they analysed the underlying pathogenesis, the effect of macular oedema on the retina, or its impact on visual function: a) the test for assessing macular function was the control of visual acuity, b) the test for detecting disturbances in the blood-retinal barrier was the fluorescein angiogram of the fundus, and c) the test for detecting retinal tissue thickness was optical coherence tomography.

BCVA was measured using logarithmic visual acuity charts (logMAR) and recorded as the number of standard letters that were read correctly. These measurements were taken in all subjects at baseline (score upon entering the study), 1 week after treatment and 12 weeks after treatment.

Optical coherence tomography (OCT) detected and measured small changes in the macular thickness and quantitatively estimated them. At OCT, we measured central foveal thickness in micrometres. The central foveal thickness represents the thickness at the point of intersection of 6 radial scans. OCT was performed with a Stratus OCT device (Stratus 3000, software version 4.0.1 Carl Zeiss Meditec, Inc., Dublin, California, USA).

For fluorescein angiography, we recorded the following information: Retinal oedema was measured in three concentric areas of the centre foveae, with a width of 500 microns each. We graded these variables as none (0), mild (1), moderate (2), and severe (3). Fluorescein angiograms were graded according to a modified grading system (Table 1), which included a combination of the criteria reported by Yannuzzi and the proposed grading system of the Angiography Scoring for Uveitis Working Group (12,13). Macular oedema was assessed through images obtained at least 5 minutes after intravenous injection of 5 mL 20% sodium fluorescein. In this study, only the subjects in which there was obvious leakage in the centre (grades 2, 3) were

classified and analysed as having macular oedema. We did not have any patients with evident perifoveal hyperfluorescence in an area centred on the fovea of more than 1.5 optic disc diameters (grade 4). We defined clinical improvement as an improvement in the total score of these criteria after 12 weeks compared to our baseline clinical findings.

In the evaluation of macular oedema with optical coherence tomography, we recorded information on retinal thickening in the macula at baseline and 12 weeks after the application of a posterior Sub-Tenon`s injection of triamcinolone.

The IOP was measured by Goldmann applanation tonometer immediately before and one day after a posterior Sub-Tenon`s injection of triamcinolone. Repeat measurements of IOP were collected with Goldmann applanation tonometry 1 week after PSTI and then at intervals of 1 to 4 weeks for at least 3 months, according to the patients' clinical needs of the patients.

At follow-up appointments, if the IOP was > 25 mm Hg but < 30 mmHg, one topical anti-glaucoma medication was started. If the IOP was > 30 mm Hg but < 35 mmHg or > 35 mmHg but < 40 mmHg, 2 or 3 topical anti-glaucoma medications were started, respectively. If the IOP was > 40 mmHg, 3 topical anti-glaucoma medications and oral acetazolamide were administered. Lens status was noted in a slit-lamp examination, and lens opacities were graded (as present or absent and whether they were visually significant) by LOCS III (Lens Opacities Classification System III) (14). We applied this scale in short-term clinical trials for the drug triamcinolone, which has cataractogenic potential.

Data Analysis

Visual acuity was converted to the Logarithm of Minimum Angle Visual Acuity a (LogMAR) for the purpose of statistical analysis. Changes in BCVA and CFT over time were analysed with a paired sample *t*-test. The statistical analysis was performed using SPSS (version 15.0, SPSS Inc., Chicago, IL, USA).

Ethical approval

Ethical approval was obtained from: Ministry of Health, Republic of Serbia No: 500-01-00035, Commission for Health Technology Assessment.

TREATMENT PROTOCOL

Sub/Tenon`s Injection

The standardized procedure for the preparation of the ocular surface prior to the injection of triamcinolone is as follows: (1) administration of topical ofloxacin (Uniflox, Unimed pharma) on the day of injection (use of 3 drops over a period of at least 15 minutes); (2) administration of topical 0.5% tetracaine anaesthetic drops on the conjunctiva inferonasally to the limbus for 3 minutes before injection; (3) application of two to three drops of 5% povidone-iodine in the lower fornix that were allowed to dry for 30-60 seconds; and (4) usage of a sterile eyelid speculum to separate the eyelids.

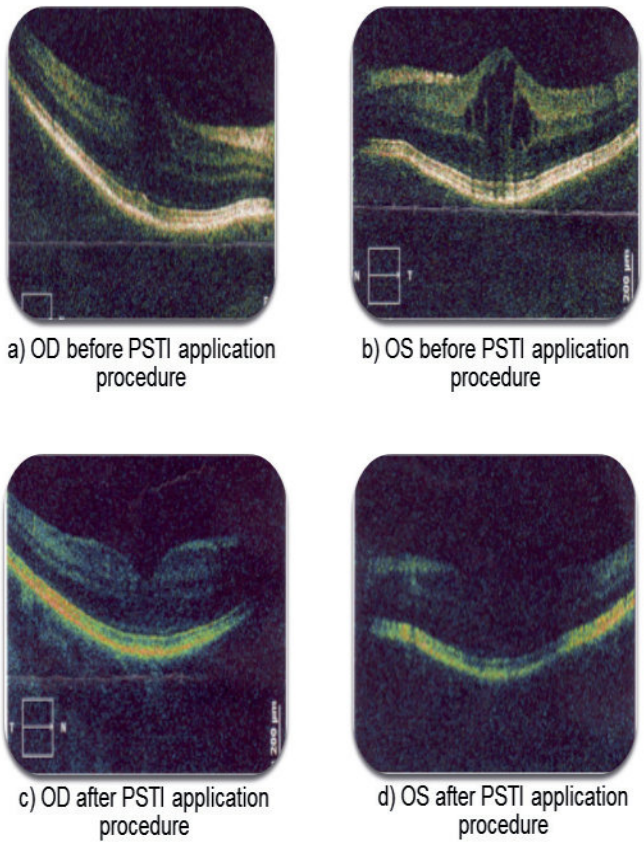

Figure 1. Macular oedema of the right and left eye of the same patient before (a, b) and after the PSTI procedure (c, d)

The study drug was formulated and manufactured as a sterile, triamcinolone acetonide injectable suspension (20 mg per 1 ml, brand name Kenalog 40, Krka, Novo mesto, Slovenia). The patient was instructed to look in the contralateral direction of the eye that was to receive the injection. The upper eyelid was lifted, and the inferonasal conjunctival fornix was penetrated with the needle bevel down. After the tip of the needle entered the fornix, the needle was rotated by 180 degrees with the bevel up. The needle advanced along the sclera posteriorly and slowly with a side-to-side sweeping motion until the hub of the needle was reached. Then, an injection of 1 ml 20 mg/ml triamcinolone was given (Figure 1). The eyelid speculum was removed. The subjects were instructed to use topical ofloxacin 4 times a day for 3 days following the injection.

RESULTS

Forty-nine eyes from 30 patients were included in the study, including 20 patients with posterior uveitis and 10 patients with intermediate uveitis.

Table 2. Details and follow-up for the uveitis group of patients treated with a posterior sub-Tenon`s injection triamcinolone

Uveitis group, N = 30, eyes = 49	
mean follow-up time	14.7±8.6 months
mean ages	39.5±24.5 years
duration of uveitis	21±7 months
Unilateral / bilateral	18(40%) / 12(60%)
posterior uveitis / intermediate uveitis	n = 20/10 eyes = 31/18

Table 2 summarizes the ophthalmic data and other clinical data collected from the participating subjects. The patients had a mean follow-up time of 14.7±8.6 months (range, 3-36) after PSTI. The mean age of the patients was 39.5±24.5 years (range, 28 - 77) in the PSTI group. The average duration of uveitis was 21±7 months (range, 5 - 34). Eighteen (40%) patients had a bilateral version of the disease.

Table 3. Evaluation of visual function before and after a posterior sub-Tenon`s injection triamcinolone

BCVA N=49 eyes	Before treatment (baseline score)	1 week after the treatment	12 weeks after treatment
Mean BCVA scores logMAR	0.60±0.30	0.17±0.30	0.15 ± 0.30
Mean BCVA Snellen equivalent)	0.25(0.35-0.22).	0.67(0.63-0.32)	0.7(0.78 - 0.32)
paired t - test		P< 0.05	P < 0.05

Table 4. Fluorescein angiography analysis of CME

	baseline	12 weeks
Mean FA score ± SD	5.2± 1.9	1.2 ± 1.1
paired t-test		P < 0.001

Table 5. Optical coherence tomography analysis of CME

n =49 eyes	baseline	12 weeks	average improvement
Mean central foveal thickness (±SD)	320 ± 34 μm	235±30 μm	85 μm
P			< 0.001

Table 6. Intraocular pressure before treatment, 12 weeks and 18 weeks after PSTI

n =49 eyes	baseline	12 weeks	18 weeks
mean IOP±SD	20 ± 1.4 mmHg	22±2.1 mmHg	21 ± 2.1 mmHg
P			> 0.05

Table 7. Intraocular pressure for the study subjects

IOP	n(%)	Anti-glaucoma medication
mild	4(8%)	/
rise of > 25 mmHg	3(6%)	1 topical anti-glaucoma medication
rise of > 30 mmHg	1(2%)	2 topical anti-glaucoma medications
rise of > 35 mmHg	/	/
rise of > 40 mmHg	1(2%)	3 topical anti-glaucoma medications and oral acetazolamide
Σ	9 (18%)	

Cystoid macular oedema was defined throughusing three categories of tests: visual function, fluorescein angiography and OCT.

Tests for evaluating visual function: Best Corrected Visual Acuity was measured using the Logarithm of Minimum Angle Visual Acuity. Before treatment, the mean BCVA score was 0.60±0.30 logMAR (Snellen equivalent 0.25 mean, range, 0.35 - 0.22). After the first week of treatment, the corresponding value was 0.17±0.30 logMAR (Snellen equivalent 0.67 mean, range 0.63 - 0.32). The twelve-week score was 0.15±0.30 logMAR, (Snellen equivalent 0.7 mean, range 0.78 - 0.32) (Table 3). When the baseline scores were compared to thosethese scores with an unpaired t-test, there was a statistically significant difference between the BCVA before treatment, 1 week after treatment and 12 weeks after treatment (P<0.05).

Fluorescein angiography analysis: The mean baseline score before treatment was 5.2±1.9 (mean, SD). Twelve weeks after treatment, the corresponding value was 1.2±1.1 (mean, SD) (Table 4.)

Optical coherence tomography (OCT): OCT was analysed in 49 eyes of 30 patients. The mean central foveal thickness (±SD) decreased sharply by 85 μm from 320±34 μm at baseline to 235±30 μm at 12 weeks. In the treatment group, a significant reduction in the mean macular thickness (P<0.001) was detected 12 weeks after the injection (Table 5).

Table 8. Lens status of the study subjects

lens status	cataracta complicata uveitica	cataracta complicata medicamentosa	cataracta senilis	suma	phacoemulsification with IOL implantation
N(%)	4(8%)	1(2%)	2(4%)	N=7(14%)	4(8%)

Intraocular pressure: The mean IOP increased from 20 mmHg at baseline (range 18 - 22 mmHg) to a mean maximal value of 32 (range 22 - 40 mmHg) and then decreased to 21 mmHg (range 17 - 23 mmHg) at the end of the study (Table 6).

Four eyes (8%) out of 49 eyes in the PSTI group developed mild elevation of intraocular pressure (up to 25 mmHg). We managed to control them without topical anti-glaucoma agents, which corresponded to final IOP levels of 15 and 16 mmHg.

During the study period, 3 eyes (6%) had an IOP rise of > 25 mmHg, whereas 1 eye (2%) had an IOP rise of >30 mmHg. One eye (2%) had a maximal IOP > 40 mmHg. After the injection, the mean time for detecting an IOP rise of > 5 mmHg was 4 weeks. Five eyes (10%) required some form of anti-glaucoma medication during the follow-up. The mean duration of the anti-glaucoma treatment was 17 weeks (min 4, max 20) (Table 7).

L**ens Changes:** Seven eyes (14%) had cataracts (mean age, 44 years (range 28-65), and the remaining patients had a clear lens (mean age, 42 years (range 27-64). These patients had a mean follow-up time of 15 months (range 3-26). Four eyes (8%) had complicated cataracts due to the effects of chronic uveitis, 1 eye (2%) had a cataract as an effect of corticosteroids and 2 eyes (4%) had senile cataracts. Four eyes (8%) were treated with phacoemulsification and lens implantation (Table 8).

DISCUSSION

This study investigated the use of a posterior sub-Tenon's capsule triamcinolone injection for the treatment of macular oedema of non-infectious posterior and intermediate uveitis that could not be controlled with systemic medications (15). Macular oedema is the major cause of the loss of visual acuity in uveitis patients. In oedema, the blood-retinal barrier was damaged by an alteration in tight junctions between the retinal capillary endothelial cells and pigmented epithelial cells with consequent leakage in the retinal tissue.

This study demonstrated that 12 weeks after a sub-Tenon`s triamcinolone injection, there was a statistical improvement in visual acuity and a significant reduction in retinal thickness. After 12 weeks, we also observed no statistically significant variations in IOP.

The main outcome measure of our study was the visual acuity score. Our results showed that early improvement after treatment (at 12 weeks) was significant. In the study, after a single posterior sub-Tenon`s capsule triamcinolone injection, we observed a statistically significant difference between the BCVA before treatment, 1 week after treatment and 12 weeks after treatment (P<0.01). Moreover, control of macular oedema was achieved for a mean duration of 12 weeks. Triamcinolone performed well as a long-acting corticosteroid and was effective against inflammation in uveitic macular oedema.

This study showed through measurement of the mean CFT by OCT and FA scores that a posterior sub-Tenon's capsule triamcinolone injection was significantly effective in the treatment of posterior uveitis and intermediate uveitis macular oedema throughout the follow-up period (16,17).

In this study, although the mean IOP increased between the baseline measurements and the 1-month visit, it returned to the baseline level before the end of the study. A single sub-Tenon`s capsule injection of triamcinolone did not cause a statistically significant increase in IOP (P>0.05) in this study. Only 9 (18%) out of 49 eyes had an IOP elevation that required medical treatment during the follow-up period. The rise in IOP reflectsed the pharmacokinetic properties of PSTI. However, a careful observation of IOP was necessary after administration of the posterior sub-Tenon`s capsule injection of triamcinolone.

A Sub-Tenon`s injection may be safer because there was no incidence of endophthalmitis.

During the 15-month follow-up period, 1 (2%) out of 49 eyes developed posterior subcapsular opacities due to the corticosteroids. This effect of a posterior sub-Tenon`s capsule triamcinolone injection might be caused by the solubility of triamcinolone in the sub-Tenon`s space, which promotes long-term steroid release (18). We think that 20 mg/ml PSTI had a shorter action period, and as a result, it led to lower rates of cataract progression; ,this concentration also led to lower rates of secondary glaucoma.

A previous report (19,20) found orbital fat prolapse and ptosis occurred in some patients after a superotemporal sub-Tenon's corticosteroid injection, but we did not have any similar complications with the inferonasal approach. After injection, nearly 50% of the solution resided in the orbital tissues anterior to the globe equator (11). This anterior diffusion of the solution in our patients did not contribute to the development of ptosis or fat prolapse after the posterior triamcinolone injection. This different approach for injection in our patients resulted in the absence of proptosis, and there was no need for multiple injections.

CONCLUSION

We have shown that a posterior sub-Tenon`s capsular triamcinolone injection is effective and safe for the treatment of intermediate and posterior uveitis. The assessment of the efficiency of macular oedema reduction was statistically significant at all levels of our evaluation by visual acuity, FA, and OCT. Additionally, the level of complications in the form of increased IOP and cataracts was not significant.

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ENCOPRESIS IN CHILDREN: AN OVERVIEW OF RECENT FINDINGS

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ENKOPREZA KOD DECE: NAJNOVIJA SAZNANJA

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ABSTRACT

The term 'encopresis,' derived from ancient Greek ἐγκόπρησις / egkóprēsis, which means stool, was first introduced in 1926 by Weissenberg to describe the loss of stool in underwear as the faecal equivalent of enuresis. The soiling of underwear is defined as the accidental passage of very small amounts of faeces into underpants. Quantitatively, the content of stool between encopresis and soiling is difficult to determine, and it is especially difficult for parents assess it. Therefore, a new term was adopted – faecal incontinence – that encompasses both encopresis and soiling.

Faecal incontinence is defined as the discharge of faeces in socially awkward situations at least once per month in children ≥ 4 years old. In approximately 95% of cases, faecal incontinence in children is not organic in origin, but instead appears as a functional gastrointestinal disorder. In 80% of children with functional faecal incontinence, the symptoms are associated with functional constipation. The remaining 20% of the cases involve no signs of faecal retention and are defined as non-retentive functional faecal incontinence.

This paper aims to present the latest findings within this area of paediatric gastroenterology.

Keywords: *encopresis, children, incontinence*

SAŽETAK

Termin Encopresis je prvi uveo 1926 god. Weissenberg (Staro grčki naziv ἐγκόπρησις / egkóprēsis, stolica) da okarakteriše pojavu stolice u donjem vešu kao ekvivalent noćnog mokrenja. Prljanje je definisano posebno, kao nenamerna pasaža vrlo malih količina stolice u ličnom rublju. Kvantitativno, sadržaj stolice između enkopreze i prljanja je teško odrediti, pogotovu roditelji teško mogu učiniti tu procenu, zato je usvojen nov naziv fekalna inkontinencija koji podrazumeva enkoprezu i prljanje zajedno.

Fekalna inkontinencija se definiše kao ispuštanje stolice u socijalno neadekvatnim uslovima najmanje jednom u toku meseca u razvojnom periodu deteta ≥ 4 godine. U oko 95% slučajeva, fekalna inkontinecija kod dece nije organskog porekla već se ispoljava kao funkcionalni poremećaj gastrointestinalnog trakta. U 80% dece sa funkcionalnom fekalnom inkontinencijom simptomi asociraju sa funkcionalnom konstipacijom. U preostalih 20% slučajeva ona je bez znakova fekalne retencije, definisana kao ne- retentivna funkcionalna fekalna inkontinencija.

Ovaj rad ima za cilj da prikaže najsavremenija saznanja iz ove oblasti dečje gastroenterologije.

Ključne reči: *enkopreza, deca, inkontinencija*

INTRODUCTION

The ability to retain faeces has been associated with the study of privacy and order in our culture for approximately 200 years. The ancient Romans spent time together each morning and defecated around each other. This was recorded by the Minister of Finance at the time – Hadrian – who also introduced a tax on "villains" and defended his position with strong arguments. It was only with the invention of the water closet (WC) that privacy-related defecation was developed and occurred with the sense of shame. The link between urges, privacy, order, cleanliness and bowel emptiness has been used for more than 150 years in pedagogy; in other words, even sitting on the toilet is considered part of one's upbringing; and, finally, to exist on one's own has become an integral part of an intelligible picture of a man (1).

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Table 1. Rome III criteria for functional defecation disorders in children with a developmental age of at least 4 years

Suggested terminology	Definition
Faecal incontinence	*Passage of stools in an inappropriate place*
Organic faecal incontinence	*Faecal incontinence resulting from organic disease (e.g., neurological damage or sphincter abnormalities)*
Functional faecal incontinence	*Non-organic disease that can be subdivided into:*
– Constipation-associated faecal incontinence (80%)	
– Non-retentive (non-constipation associated) faecal incontinence (20%)	

Definition

The term 'encopresis', derived from ancient Greek ἐγκόπρησις / egkóprēsis, which means stool, was first introduced in 1926 by Weissenberg as the faecal equivalent of enuresis to describe the loss of stool in underwear (2, 3). Later, Bellman defined encopresis as the repeated voluntary or involuntary passage of normal stools into inappropriate places, such as into clothes or onto the floor after the age of 4 years without any organic cause (4). Soiling is specifically defined as an unintentional passage of very small amounts of stool into underwear. Quantitatively, the content of stool between encopresis and soiling is not easy to determine, and it can be especially difficult for parents to assess it. Therefore, a new term was adopted – faecal incontinence – that encompasses both encopresis and soiling (5, 6). Both conditions are commonly associated with functional constipation. At first, it was thought that all children with faecal incontinence had constipation, but subsequent findings revealed that faecal incontinence can occur without signs of constipation, which created confusion in the interpretation of the problem (7). In some parts of the world, doctors consider encopresis to be a mental disorder, and others use the term 'encopresis' in relation to soiling or faecal incontinence. Whatever it is called, the situation is very unpleasant for the child, and it is difficult for parents to accept the same. Due to the absence of a consensus on the interpretations of encopresis and other functional disorders of the gastrointestinal tract, a group of experts in paediatric gastroenterology established the criteria for childhood functional gastrointestinal disorders, known as Roma II, in the year 2000. Disorders of defecation included: functional constipation, functional faecal retention and functional non-retentive faecal soiling. Later, numerous studies evaluated the acceptability of these types of classifications in clinical practice, and it became clear that the first paediatric Roma criteria were too restrictive and were insufficient for many patients with specific functional gastrointestinal diseases such as constipation and abdominal pain (8). Therefore, the terms were redefined in 2006 as part of a set of criteria known as Roma III; the term 'faecal incontinence' was adopted as a substitute for encopresis and soiling to indicate organic faecal incontinence or functional faecal incontinence (9-11). Table 1.

The Roma III criteria are currently used to define functional faecal incontinence. Table 2.

Epidemiology

The prevalence of functional faecal incontinence has been found to vary between 1 and 4% in children > 4 years old and between 1 and 2% in 7-year-old children. The rate in children aged 10 and 11 years was found to be 1.6%. This condition was observed three to six times more often in boys than in girls (3:1 to 6:1) (12-14).

Children with functional non-retentive faecal incontinence (FNRFI) experience faecal incontinence as their only symptom. In contrast to children with functional constipation, they have normal stool consistencies. Symptoms such as abdominal pain, rectal bleeding, difficulty defecating, poor appetite, palpable abdominal masses and palpable rectal masses are significantly less common in these children compared to those with constipation (15). Nocturnal faecal incontinence is less common in children with FNRFI (12%) compared to children with constipation (30%) (9), while the frequency of diurnal and nocturnal enuresis in them is higher

Table 2. Rome III criteria for functional faecal incontinence in children with a developmental age of at least 4 years

Suggested terminology	Definition
Functional constipation	Most fulfil ≥ 2 criteria at least once per week for ≥ 2 months prior to diagnosis, with insufficient criteria for the diagnosis of irritable bowel syndrome: 1. ≤ 3 defecations in the toilet per week 2. ≥ 1 episode of faecal incontinence per week 3. History of retentive posturing or excessive volitional stool retention 4. History of painful or hard bowel movements 5. Presence of a large faecal mass in the rectum 6. History of large-diameter stools, which may obstruct the toilet
Functional non-retentive faecal incontinence (FNRFI)	Must fulfil all of the following for ≥ 2 months prior to diagnosis: 1. Defecation into places inappropriate to the social context at least once per month 2. No evidence of an inflammatory, anatomic, metabolic, or neoplastic process that explains the subject's symptoms 3. No evidence of faecal retention

(40-45%) than in children with constipation (25-29%), which suggests that children with FNRFI lack the normal physiological stimuli needed to go to the toilet (16-18). These children attended paediatric clinics for the first time at an older age than those who had constipation (on average 9.2:6.5 years). It is surprising that only 29% of these children had ever visited a doctor to address the problem (19, 20). Very often, FNRFI is not recognized as a distinct clinical entity by general practitioners and paediatricians, which frequently results in inadequate treatment with a negative response in the follow-up and deepens the problem. Approximately 30 - 40% of children with FNRFI have never been toilet trained successfully, while the majority have been completely toilet trained before and regressed to incontinence. Children may blame the occurrence of faecal incontinence on "not having time to go to the toilet", or they may state that "I could not leave my computer game" or "I felt the urge to go, but I was too late".

Pathophysiology

The exact mechanism of FNRFI is generally unknown. In the literature, there are controversial ideas regarding its aetiology that focus on anorectal motility and sensation, genetics, and mental and psychiatric disorders. In any case, it is complex and multifactorial. Defecation is a complex action that takes place between the pelvic floor muscles, autonomic and somatic nervous systems and anal sphincter muscles. It consists of involuntary and voluntary actions that are both reflexive in nature. In infants and young children, myelination of the corticospinal tract is not yet complete, so they lack the ability to volitionally defecate. In most cases, this myelination is complete at the age of approximately 18 months, although the exact age can vary. At the age of 3 years, 98% of children are 'clean'. Girls tend to gain control sooner due to their accelerated maturation, which is also reflected in their earlier bladder control. The process of control over defecation and urination is an issue of development and cannot be accelerated by intensive toilet training. A child's initiative is the only proven indicator that they have developed the pathways needed to desire to be "clean" and "dry". Abnormal dynamics of defecation are one of the factors involved in the pathophysiology of faecal incontinence. The use of so-called radiopaque markers (*colonic transit time*) and anorectal manometry enable the evaluation of sphincter function, while a *rectal barostat* is a tool used to investigate rectal compliance and sensation. Pathological findings on *colonic transit time (CTT)* were found in approximately 50% of constipated children, while findings within the normal range were found in all of the children with FNRFI. This points to the presence of normal intestinal motility in these children (21, 22). In assessing anorectal function, anorectal manometry has indicated that there is no significant damage to anorectal sensorimotor function in these children compared to healthy volunteers (23, 24). The *rectal barostat* method has indicated that an increase in rectal compliance, rather than a reduction in rectal sensitivity, is the pathophysiological mechanism in functional constipation in children. In children with high rectal compliance, a large-volume stool is necessary to "trigger" an immediate sensation. It is not known whether genetic predisposition plays a crucial role in this, but in approximately 20% of children with FNRFI, a positive family history was reported. It is questionable whether it is a matter of genetic tendency or if it is the result of psychosocial and/or environmental effects, as psychiatrists have considered incontinence to be a result of emotional instability (conduct disorders, reduced alertness, lack of will, hyperactivity, poor social adaptability, learning difficulties), which is reflected in impulsive and unconscious defecation. Paediatricians believe that psychological problems are secondary and are a result of social incapacitation in children with faecal incontinence. Frustration and shame due to the inability to control defecation and occasional incontinence lead to comorbid psychological disorders in these children, which can be improved after successful treatment (25).

Evaluation

Normal *CTT* (90%) results with anamnesis and a normal stool appearance without "faecal masses" in the physical findings are sufficient for a differential diagnosis. Other tests such as anorectal manometry, *rectal barostat* testing and MRI of the spinal cord are rarely needed (26).

The medical history involves questions about the frequency and size of the child's stool, rectal bleeding, abdominal pain, painful defecation, etc. It is important to ask for the timing of defecation problems – daytime or nocturnal – and consider the situations associated with stool retention (playing outside, TV or computer use). FNRFI in most children usually occurs after school and before bedtime, while nocturnal faecal incontinence is associated with severe constipation. The child's nutritional history is also important, as is information concerning their bowel habits. Urinary tract abnormalities (*enuresis*), growth, drugs, neuromuscular development, any family history of defecation disorders, and information about psychological problems in the child and their family (birth of twins, parental divorce, illness in the family) must be considered. (27, 28)

Each child with a defecation disorder must undergo thorough physical and neurological examinations. A perianal inspection provides important information on the position of the anus, rectal faeces, redness, dermatitis, eczema, fissures, haemorrhoids, scars, etc. Digital anorectal examination is an invaluable tool in the assessment of perianal sensation, anal tone, rectal size, faecal volume and consistency, and voluntarily activated anal sphincter contraction and relaxation (29). No anorectal physiologic abnormalities were present in children with FNRFI (30).

Warning signs that should result in increased attention are the absence of meconium passage, early occurrences of constipation, an empty rectal ampulla, refractory constipation, etc. Disturbing neurological signs include motor and sensory dysfunction in the lower extremities, abnormal reflex activity and anorectal sensation. An MRI of the spinal cord is justified in these cases (31).

Treatment

In contrast with children with faecal incontinence caused by functional constipation, patients with FNRFI should not be treated with laxatives (32, 33). Education, toilet training, and positive motivation are the cornerstones of treatment for these patients (34). Children and their parents should be prepared for a long-term process with many ups and downs. The aim is to prevent accidents and achieve regular bowel emptying, emphasizing the importance of immediately going to the toilet (35, 36). In addition, the education of both children and their parents in colorectal physiology, defecation and faecal incontinence can help significantly. Finally, parents should know that children are not always aware of their faecal accidents; they should mitigate their child's guilt and explain the prevalence of the disorder and how cooperation is needed to treat it. Meticulously kept records and strict toilet training performed 3 times each day within 5 minutes after meals are the most effective methods (37-39). Small gifts can further increase motivation (40-42). No signs of improvement were noticed in these patients after the administration of laxatives, while the long-term administration of laxatives is required in children with constipation (43). The effects of loperamide, which increases the pressure in the internal anal sphincter and/or reduces rectal contraction, should be examined in paediatric patients (44). *Biofeedback training* has no additional effect in these groups of children (45). Successful treatment of children with FNRFI leads to improvements in most patients, which suggests that these children should be treated primarily in paediatric rather than psychiatric clinics and consulting rooms (46). The course of treatment is lengthy, symptoms often persist for a long time and relapses are possible.

CONCLUSION

Faecal incontinence rarely results from FNRFI, but it is crucial to make a differential diagnosis between FNRFI and functional constipation because each requires different approaches and treatments. A proper diagnosis is made through a case history and physical examination and is confirmed by the *colonic transit time*. Changes in behaviour designed to educate both children and their parents along with toilet training are the most effective therapies for FNRFI, while cases of functional constipation require long-term treatment with laxatives in addition to toilet training and diet modification.

The high percentage of relapses observed indicates the importance of intensive monitoring and follow-up for these patients.

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POSSIBLE USES OF DATA FROM HOSPITAL DISCHARGE REPORTS

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MOGUĆNOSTI KORIŠĆENJA PODATAKA IZ IZVEŠTAJA O HOSPITALIZACIJI

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ABSTRACT

Almost all countries in the world keep some form of hospital discharge report (HDR). Although there are many variations, every report contains such data as patient demographics, the main cause of hospitalization, comorbidities, the length of stay in hospital and outcome. The advantages of using data obtained from HDRs are numerous: The data from HDRs are already collected in a designated centre and thus easily available and relatively cheap; HDRs contain information for many previous years; they are sometimes more reliable than data obtained through any other method; and finally, they provide a large and representative database. HDRs databases can be connected with other databases using a unified patient identification number. The limitations of using data obtained through HDRs are as follows: inconsistencies in defining and coding diagnoses and applied procedures, common underestimations of comorbidity, limited possible applications in specific studies and partial coverage of inpatient institutions. The prediction that in the future, a growing number of diagnostic and treatment procedures will be performed on an outpatient basis will also limit the use of HDRs. When electronic recordkeeping becomes a practice, we may assume that these data will no longer be needed. There is no perfect model for collection and processing data regarding hospitalized patients. HDRs, with their advantages and disadvantages, currently represent the best way to perceive the size, type, quality and efficiency of the health care services provided to patients at the secondary and tertiary level.

Keywords: *Hospital discharge report, comorbidity, diagnostic related groups, health care quality, trend analysis*

SAŽETAK

Gotovo sve države sveta vode IOH u nekom obliku. Iako postoje mnoge varijacije, podatke kao što su: demografski podaci pacijenta, osnovni uzrok hospitalizacije, komorbiditeti, dužina boravka u bolnici (bolesnički dani), ishod, sadrži svaki izveštaj. Prednosti korišćenja podataka iz Izvestaja o hospitalizaciji: Podaci iz IOH-a su već prikupljeni u za to određenom centru, pa su lako dostupni i relativno jeftini, postoje za više godina unazad, ponekad su pouzdaniji od podataka dobijenih na drugi način (mogu da se vide pacijenti koji se ne vide ni u jednoj drugoj bazi podataka), veličina i reprezentativnost baze (smatra da se podaci iz IOH-a odnose na čitavo stanovništvo). IOH bazu je moguce vezati (preko jedinstvenog ID broja pacijenta) sa drugim bazama. Ograničenja korišćenja podataka dobijenih iz Izvestaja o hospitalizaciji: Nedoslednost u definisanju i kodiranju dijagnoza i primenjenih procedura, cesto potcenjivanje komorbiditeta, ograničena mogućnost upotrebe u specifičnim istraživanjima, delimični obuhvat stacionarnih ustanova. Predviđanja da će se u budućnosti sve veći broj dijagnostičkih i terapijskih procedura raditi ambulantno, dodatno će ograničiti upotrebu IOH-a. Kada elektronska evidencija postane praksa, može se pretpostaviti da ovi podaci više neće biti potrebni, s obzirom na količinu i kvalitet informacija koje će se dobijati iz elektronskih izveštaja. Ne postoji savršen model za prikupljanje i obradu podataka hospitalizovanih pacijenata. Izveštaj o hospitalizaciji sa svim svojim prednostima i manama za sada je najbolji način da se sagleda obim, vrsta, kvalitet i efikasnost usluga zdravstvene zaštite koje se pružaju pacijentima na sekundarnom i tercijarnom nivou.

Ključne reči: *Izveštaj o hospitalizaciji, komorbiditet, dijagnostički srodne grupe, kvalitet zdravstvene zaštite, analize trendova*

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INTRODUCTION

A hospital discharge report (HDR) is an individual report completed for each patient admitted to a hospital for "episodes of hospitalization" (diagnosis, treatment, rehabilitation and health care) over one night or for more than 24 hours. An episode of hospitalization is a time period calculated from the moment a patient is admitted until the moment he or she is discharged (1).

Almost all countries worldwide keep HDRs in some form. Although there are many variations, every report contains such data as patient demographics, the main cause of hospitalization, comorbidities, length of stay in hospital (hospital days) and outcome. In most countries, the costs of data collection and processing are covered by taxpayers (2).

"Hospital days" represents the number of days a patient spends in an inpatient medical institution for either treatment or medical testing. The number of hospital days represents the number of days spent in hospital, including the day of discharge (3).

"Average length of stay in hospital" refers to the average number of days that patients spent in hospital. It is calculated as a ratio of the total number of hospital days for all hospitalized patients per year to the total number of admissions or discharges (4).

The criteria for evaluating the quality of HDR data bases include the completeness and representativeness of data, consistency over time, accuracy, the existence of data from many previous years, the availability of data and the ability to connect with other databases (5, 6).

The most common problems when dealing with HDR databases are inconsistencies in the definitions and coding systems used for diagnoses and applied procedures (7), underestimations of comorbidity (8) and incomplete coverage of inpatient health institutions (9, 10).

Although the quality of available data varies, the analysis and the understanding of the variability of the data contained in HDRs is a skill that every doctor-researcher must possess (11).

The use of data from hospital discharge reports

Comorbidity analysis

Comorbidity is a term used to describe two or more disorders or illnesses affecting the same patient simultaneously or after one another. Comorbidity also includes possible interactions between a patient's illnesses that can negatively affect the course of each illness (12).

In addition to the basic causes of hospitalization, associated diseases have an impact on treatment outcomes (8). There are several different models for measuring comorbidity, which is important for individual risk assessment and for planning treatment costs and monitoring the quality of provided health services (13). The Charlson Comorbidity Index is the most widely accepted and used index. It includes 19 comorbidities, and each category has a specific comorbidity weight based on the relative risk of death within one year (a higher score indicates a worse prognosis) (14). Specific adapted versions of the Charlson Comorbidity Index can also be used in primary health care to make predictions about the costs of treating patients with chronic illnesses (15).

Diagnosis-related groups

Diagnosis-related groups (DRGs) represent a method for classifying hospitalized patients into groups that have similar clinical characteristics and require similar consumption of hospital resources. Since the 1990s, most developed countries have introduced a DRG-based payment system for hospitalized patients (11). The primary aim in introducing DRG systems is to increase the efficiency and transparency of hospital services (16). With DRG systems of classification, the costs of diagnostic procedures and treatments are pre-defined within a certain scale for a given DRG group (17, 18). The aforementioned cost predictability has been proven in numerous studies that have analysed the costs of treating certain health disorders (19).

The Republic of Serbia has adopted a classification system used in Australia (the Australian Refined Diagnosis Related Groups or AR DRG, version 6.0) that encompasses 698 diagnosis-related groups. It has been used since 2013 (20).

With the gradual transition from the old to the new method of funding hospitals, it is expected that the DRG system will significantly improve reporting and financing in our country by establishing a model for obtaining more accurate and higher-quality patient data that can also be connected with hospital costs data. The primary aim is to shorten the average length of hospitalization and to continuously compare the volume of work among hospitals to divide the available funds between health-care providers in a better and fairer way (20).

Routine monitoring of incidence

The reliability of HDR data for assessing the incidence of certain diseases that require hospitalization has been proven in several studies. The results obtained in Norway (21), MONIKA (22) and Rochster (23), which examined the sensitivity, positive predicative value and accuracy of HDR data using the stroke register as a gold standard revealed the strong sensitivity (86%) of HDR databases (21).

Trends analysis

HDR databases contain data for a number of previous years; consequently, they are suitable for analysing trends in hospitalization and re-hospitalization (24), hospital days (25, 26), morbidity, mortality (27) and health-care costs (28).

Mortality trends among hospitalized patients in the United States were analysed by the Center for Disease Control and Prevention using data from HDRs for the years 2000 to 2010. This analysis found that there was an 8% decrease in mortality, while the number of hospitalized patients increased by 11% (29).

Determination of hospital standards

A standard is the desired level of performance that can be achieved; the current level of performance can be compared against the standard. It represents a valuable means of ensuring and improving the quality of hospital performances. Standards should be measurable, flexible, accepted and adaptable to the needs of a given population (30).

Health system research

HDR data have been used in studies of national health care systems and in a study of health burden, the economic impact of diseases and the planning of future interventions (31). As a result, after a measles epidemic in Italy in 2002, the MMR vaccine was included in the obligatory vaccination schedule (32). HDR data can also be used in studies of and planning regarding medical interventions that can be performed only in a hospital environment. The efficiency of the system based on the services provided can be examined using information about whether a patient is directly admitted to a given institution or whether he/she is transferred from another (less specialized) institution (15). HDR data are used in analyses that focus on the effects of market competition on increases in hospital costs (33) and in studies analysing the differences in mortality with respect to the educational level of the employees at a given health institution (34). Research on health systems must take into account the racial and ethnic identity of a country's population because these variables can be limiting factors in the usage of certain health services (35).

Evaluation of the quality of health services

High-quality health care ensures the distribution of available resources in the most effective way to meet the health needs of users in terms of prevention and treatment in the safest possible way and without unnecessary losses. The work quality of inpatient health institutions is expressed through indicators of the quality of the inpatient health institution. These indicators include the mortality rate, the percentage of deaths during the first 48 hours of admission, the average length of hospital treatment, the average number of nurses per occupied hospital beds, the percentage of autopsied patients, the percentage of concordance in clinical and autopsy diagnoses, the percentage of patients readmitted to the intensive care unit during hospitalization and the percentage of patients monitored according to a health care process (36). Most of this information can be obtained from the HDR.

The need to evaluate work quality has arisen from the fact that there is a wide gap between health service expenditures and the relatively poor health status of the population. Patient safety is the main factor considered in the assessment of the work quality and services provided. There are over 200 clearly defined indicators of work quality, and 48 of them are related to safety (37). In some countries, there is a practice of public reporting (via the Internet) on work quality indicators for all levels of institutions. This type of public reporting not only provides necessary information to patients, but it can also be used to improve the work quality of health care institutions (38).

The Healthcare Cost and Utilization Project Quality Indicators (HCUP QIs) represents an approach for measuring the quality of health care using readily available data regarding hospitalized patients. The indicators that are monitored include the outcome measures (mortality and complications), usage and availability (39).

Advantages of using data obtained from hospital discharge reports

The data from HDRs are already collected in a designated central location and thus are easily available and relatively inexpensive to maintain and access; they contain information from many previous years (40); they are sometimes more reliable than data obtained through other methods (41) (e.g., information about certain patients that is invisible to any other database is available in the HDRs); and finally, the database is large and representative (data from HDRs is considered to refer to whole population). HDR databases can also be linked to other databases through unified patient identification numbers.

Limitations of using data obtained from hospital discharge reports

The inconsistencies in the defining and coding of diagnoses (7) and applied procedures, the common underestimation of comorbidity (8), the limited applicability to specific studies and the incomplete coverage of inpatient institutions (9,10) represent the limitations of data obtained through HDRs. The predictions that in the future, a growing number of diagnostic and treatment procedures will be performed on an outpatient basis will further limit the use of HDRs. When electronic records become a routine practice, we may assume that HDR data will no longer be needed given the quantity and quality of information that can be obtained from electronic reports. However, the question remains whether or when the routine use of electronic records will occur (42).

CONCLUSION

There is no perfect model for collecting and processing data regarding hospitalized patients. HDRs with their advantages and disadvantages currently represent the best way to perceive the size, type, quality and efficiency of the health care services provided to patients at the secondary and tertiary level.

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SPECIFIC POLYMORPHISM 4G/5G GENE FOR PAI-1 AS A POSSIBLE CAUSE OF CEREBRAL VENOUS THROMBOSIS: A CASE REPORT

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SPECIFIČNI POLIMORFIZAM 4G/5G GENA ZA PAI-1 KAO MOGUĆI UZROK CEREBRALNE VENSKE TROMBOZE: PRIKAZ SLUČAJA

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ABSTRACT

Thrombosis of veins and venous sinus (CVT) is the rare cerebral vascular disorder which makes less than 1% of all strokes. Thrombosis of veins and venous sinuses is picturesquely called "major neurological forger" since it is characterized by very varied clinical picture. Among the various causes of CVT, which can be of infective or non-infective nature, the congenital hyper coagulations especially stand out, diagnosis is based on highly sophisticated diagnostic tests.

We present the case of a female patient, 36 years old, who was hospitalized at the Clinic for Neurology in Clinical Center because of the diffuse headache she had for the last few days, with milder right-sided hemiparesis and one generalized tonic-clonic epileptic seizure. With nuclear magnetic resonance (MR/2D venography) the thrombosis of the upper and lower sagittal sinuses is confirmed. By appropriate laboratory tests, as well as by confirmatory immunological and genetic analyses, the impact of the most of the factors is excluded which can contribute to the occurrence of venous thrombosis. The only pathological findings which indicated the possible congenital thrombophilia as the cause of the sagittal sinus thrombosis was the determination of the specific polymorphism of the 4G/5G gene for plasminogen activator inhibitor 1.

According to our knowledge, this is the first decribed case of the possible impact of the specific polymorphism of the 4G/5G gene for plasminogen activator inhibitor of 1 on the development of cerebral venous thrombosis.

Keywords: *thrombosis of sagittal sinus; thrombophilia; polymorphism 4G/5G gene for PAI- 1*

SAŽETAK

Tromboza vena i venskih sinusa (CVT) predstavlja redak cerebrovaskularni poremećaj koji cini manje od 1% svih moždanih udara. Tromboza vena i venskih sinusa slikovito je nazvana „velikim neurološkim falsifikatorom" s obzirom da se odlikuje veoma raznolikom klinickom slikom. Medju brojnim uzrocima CVT, koji mogu biti infektivne i neinfektivne prirode, posebno se izdvajaju urodjene hiperkoagulooptije, cija dijagnoza se zasniva na visoko sofisticiranim dijagnostickim testovima.

Prikazujemo pacijentkinju, staru 36 godina, koja je hospitalizovana na Klinici za neurologiju KC zbog difuzne glavobolje unazad nekoliko dana, blaže desnostrane hemipareze i jednog generalizovanog tonicno-klonicnog epilepticnog napada. Nuklearnom magnetnom rezonancom (MR/2D venografija) dokazana je tromboza gornjeg i donjeg sagitalnog sinusa. Odgovarajucim orjentacionim laboratorijskim testovima, kao i potvrdnim imunoloskim i genetickim analizama, iskljucen je uticaj vecine faktora koji mogu doprineti nastanku venskih tromboza. Jedini patoloski nalaz koji je upucivao na mogucu urodjenu trombofiliju kao uzrok tromboze sagitalnih sinusa bio je utvrdjivanje specificnog polimorfizma 4G/5G gena za inhibitor aktivatora plazminogena 1.

Prema nasim saznanjima, ovo je prvi opisani slučaj mogućeg uticaja specificnog polimorfizma 4G/5G gena za inhibitor aktivatora plazminogena 1 na razvoj cerebralne venske tromboze.

Ključne reči: *tromboza sagitalnog sinusa; trombofilija; polimorfizma 4G/5G gena za PAI-1.*

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INTRODUCTION

Thrombosis of veins and venous sinuses (CVT) is a rare cerebrovascular disorder which makes less than 1% of all strokes (1). With the adult patients there is an incidence of 3-4 cases per million (1), while in pediatric population it is bigger and it numbers 7 cases per million children per year (2). This disorder is associated with the high risk of death outcome and significant rate of consequent mental disability. In addition, the distinctive variability concerning etiology and clinical manifestation, as well as disagreement in the approach of medical treatment, ranked CVT in the group of important public health problems, which is worldwide, even today, the real challenge from the diagnostic and therapy standpoint.

One of the most significant causes of CVT are congenital and acquired thrombophilia that occur at 29% of patients, 22% of which are hereditary hyper coagulate states (3). Congenital thrombophilia have particular importance from the diagnostic aspect, where genetic deficit of antithrombin III and protein C and S and also mutation of G169A factor of V coagulation (FV Leiden), gene for prothrombin (FII G20210A) and gene for methylenetetrahydrofolate reductase (MTHFR C677T), in earlier studies were identified as independent risk factors for the occurrence of CVT (3,4). On the other hand, for prothrombotic state due to genetic mutations in the plasminogen activator inhibitor 1 (PAI-1) there are no solid written evidence about the connection of such a disorder with risk of CVT, depending on polymorphism promoter region of PAI-1 gene.

In this case we present a young adult female patient with the thrombosis of sagittal venous sinus, who is confirmed to have the mutation of gene for PAI 1 5G/4G.

CASE REPORT

A female patient, 36 years old, was admitted in May 2015, at 6:50 PM at the Clinic of Neurology, Clinical Center Kragujevac, where she was sent from a regional hospital in Kosovska Mitrovica for the diffuse headache she had suffered for the past few days and one generalized tonic-clonic epileptic (GTC) seizure in the morning hours on the day of admission.

On admittance to our clinic, the patient was conscious, oriented, with stable vital parameters and with the finding of blood glucose in capillary blood (7,2 mmol/l) and the tongue bite as a confirmation of a previous epileptic seizure. In the neurological examination right-sided hemiparesis of the lower level was recorded with the score 5 on the National Institute of Health Stroke Scale (NIHSS).

In the personal anamnesis, the patient denied previous acute and other chronic diseases of importance. She had had three regular pregnancies with natural childbirths with no complications. She also denied diseases of hereditary significance.

Two more GTC seizures were observed right after the admittance of the patient. Computerized tomography of endo-cranium (CT) was initially performed where no pathological changes supratentorial and infratentorial were noticed. Anti-edematous therapy was immediately administered (Mannitol 20%, 125 ml/6 hours), anti-epileptic (Diazepam, intravenously, 10mg and tablets of Natrium-valproate in a fixed combination with valproic acid (333mg+145mg), daily dose 250mg/12 hours) and anti-aggregation therapy (100mg acetylsalicylic acid/24 hours). After that, the cardiologist was consulted who registered normal findings of the observed physiological systems. Basic laboratory analyses were made (complete blood test, C-reactive protein, glucose, urea, creatinine, jonogram) with the results within the limits of reference values.

The next morning, the patient was examined by magnetic resonance (MR cranial, MR venography and MR 2D venography) which confirmed the thrombosis of the upper and lower sagittal sinus, while high left in parietal lobe the signs of sub-acute ischemic lesions with hemorrhagic transformation were registered.

The second day of hospitalization, after the above mentioned additional radiological examinations, the therapy was changed in the sense that the suspended use of doze of acetylsalicylic acid and low molecular heparin, enoxaparin, in therapy doze 0,6 mg/12 hours, subcutaneously) was added. During the controlling neurological examination right-sided weakness persisted with the identical NIHSS as at the admittance to the hospital.

On the fifth day of hospitalization anti-edematous therapy was excluded and the appliance of anti-epileptics, natrium-valproate in the fixed combination with valproic acid was continued through the full therapeutic dose (500mg/12 hours).

During the further hospitalization ultrasound heart examination was done as well as color Doppler-sonography (CDS) of main neck arteries with positive results recorded.

Further laboratory tests were carried out with the aim to examine the cause of thrombosis, which meant to eliminate potential immunological and infective causes of a new neurological disorder: prothrombin and activated partial thromboplastin time were measured, concentration of D dimer and fibrin monomers, the activity of anti-thrombin and anticoagulant proteins C and S, the level of homocysteine, coagulation test with diluted Rusell's snake poison, lupus anticoagulant, activity and concentration of von–Wilebrand's factor and the activity of coagulation factors II, V and VIII; also the level of immunoglobulin types G, M and A, activity of C3 and C4 components of complement, titer of anti-nucleus, anti-mitochondrial and anti-smooth muscles antibodies, then concentration of the surface antigen of hepatitis B virus and the titer of specific antibodies against hepatitis C virus, HIV virus, Epstein-Bar virus and Borrelia Burgdorferi; the results of all listed analyses were normaland the tolerances without clinical importance.

In order to additionally examine etiology of thrombosis, genetic searches were carried out also (real time PCR)

to thrombophilia, which didn't confirm the mutation of gene for prothrombin (FII G20210A), factor V coagulation (FV Leiden) and methylenetetrahydrofolate reductase (MTHFR C677T), while simultaneously the existence of specific polymorphism 4G/5G(locus-675) in promote region PAI-1 gene was confirmed.

After two weeks of hospitalization, total regression of neurological deficit occurred and the patient didn't have epileptic seizures any more.

On discharge, instead of low molecular heparin, peroral anti-coagulant prophylaxis was added as therapy, tablets of dabigatran 150 mg/12 hours, with previously administered anti-epileptic therapy. During the routine three-month control tests after the discharge from the hospital, no neurological deficit was detected with the patient, she didn't have epileptic seizures, she was taking prescribed therapy regularly and she took it well.

DISCUSSION

Thrombosis of veins and venous sinus is picturesquely called "major neurological forger" considering that it is characterized by very diverse clinical picture. This disorder is noticed mainly with the persons younger than 50 (at about 80%), whereby 75% of cases are women (5). It is often manifested by headache (6), eyesight disturbances (7), epileptic seizures (8), consciousness disorder and focal neurological deficit as well as specific symptoms depending on the localization of pathological process. When the thrombosis of sagittal sinuses is suspected according to the clinical picture, properly set diagnosis requires complete evaluation by neuro-visualization methods as CT or MR venography depending on availability and characteristics of the patient, which present the "golden standard" for the confirmation of this disorder (1). Our patient according to all demographic and clinical standards presents the typical example of CVT, for whom due to the inability of adequate CT confirmation, diagnosis of thrombosis of the upper sagittal sinus was set by MR venography.

Among various causes of CVT, which can be of infective (sinusitis, otitis media, meningitis, system infections,etc) or non-infective nature (trauma, brain tumors, certain neurosurgical procedures, hematological malignancies, pregnancy and postpartum period, consuming of some medications, chronic inflammatory bowels diseases, system diseases of connective tissue, nephritic syndrome, cirrhosis of the liver,etc), congenital hyper coagulopathy stands out, it's diagnosis is based on highly sophisticated diagnostic tests (9). In the case of our patient, with appropriate laboratory tests, positive immunological and genetic analyses, disorders at the level and activity of the most factors which can influence the occurrence of vein thromboses, (prothrombine, anti-thrombin, anti-coagulation proteins C and S, factor V coagulation, methylenetetrahydrofolate reductase enzyme, anti-phospholipid antibodies,etc) were excluded while the determination of the specific polymorphism 4G/5G gene for PAI-1 was the only pathological result which indicated to the possible congenital thrombophilia.

Plasminogen activator inhibitor type 1 is an endogenous glycoprotein from the group of serine proteases, which is produced in endothelial cells, liver cells, fat, etc. (10) In the human body acts as the main inhibitor of a tissue plasminogen activator and urokinase, as a result of which a suppression of fibrinolytic activity occurs. (11) Its synthesis is regulated by the gene on chromosome 7 in whose promoter region at position -675 bp mutation is described which results in heterozygous variant of 4G/5G, with the 4G only transcriptionally active allelic variants responsible for increasing levels of PAI-1 in plasma, thus generally increases the risk of thrombosis (12). A recent study in a population of patients in Serbia showed that the frequency of this polymorphism is the most frequent, with an incidence of slightly more than 46%, while the recorded prevalence of allelic variants of homozygous 4G/4G and 5G/5G was quite a range of about 36%, or 19% (13).

According to previous studies, the effect of heterozygous variants of 4G/5G PAI-1 gene on the formation of arterial or venous thromboembolic complications is controversial. In fact, there is evidence that the presence of the 4G/5G polymorphism of PAI-1 can significantly increase the risk of coronary ischemic disease (14), including myocardial infarct (15), and venous thromboembolism (16, 17), while on the other hand, there are data indicating the absence of any connection between the genotypic variants with the aforementioned unwanted outcomes (18, 19). In addition, it was shown that in most cases the contribution of PAI-1 4G/5G polymorphism of the synergetic conditioned by the presence of other congenital and/or acquired conventional risk factors for the occurrence of arterial, and venous thrombosis, such as hypertension, diabetic disease, obesity, malignant diseases, chronic inflammatory diseases, hereditary hyper coagulopathy and similar (1-4), which also can have a significant impact on the level of PAI-1 in plasma. In contrast, none of the previous studies have established significant connection 4G/5G genotype and ischemic stroke (20, 21). Moreover, in contrast to the thrombosis of the coronary arteries which is dominated by allelic variant 4G with the increase of PAI-1 in plasma, cerebral artery thrombosis is significantly associated only with the homozygous genotype 5G/5G, accompanied by a reduced plasma levels of PAI-1 (8). This paradox suggests that specific polymorphism 4G/5G PAI-1 gene does not affect the mechanisms of cardiovascular risk solely dependent on the level of PAI-1 in plasma (8). When it comes to CVT, according to our knowledge so far only one type has been carried out of observational study "case-control", which has not shown that allelic variant of 4G/5G PAI-1 gene significantly affects the risk of this disorder, unlike mutations of prothrombin G20210A (22).

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Additionally, the PAI-1 4G/5G polymorphism was associated with the risk of the numerous and varied other clinical disorders such as diabetic mellitus and its chronic complications (23, 24), recurrent miscarriage (25), preeclampsia (26), polycystic ovary syndrome (27), bronchial asthma (28), cancer (especially colon and endometrium) (29), sepsis (including fatal outcome as a result) (30) etc, where due to unreconciled opinions, further studies on this are carried out intensively.

CONCLUSION:

In the case of our patient the available screening and confirmatory diagnostic tests excluded all other potential causes of venous sinus thrombosis. To our knowledge,this is the first described patient with the history of specific polymorphism 4G/5G (locus-675) in the promoter region of PAI-1 gene isolated a possible cause of thrombosis of the sagittal sinus, which should be confirmed in future prospective studies of adequate design.

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A CASE REPORT OF FEMALE PATIENT WITH LARYNGEAL GRANULOMA

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PRIKAZ SLUČAJA BOLESNICE SA LARINGEALNIM GRANULOMOM

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ABSTRACT

The aim of this case report is to present the laryngeal granuloma in 23 year old female patient. Case outline: The 23 year old female was admitted for examination, because of long lasting, progressive hoarseness. In anamnesis, we found that she has undergone general anesthesia for 8 times, in the early childhood. We performing direct laryngoscopy with complete otorhinolaryngologic examination, rigid endovideostroboscopy and the large granuloma of the larynx was found. Conclusions: Laryngeal granuloma of vocal cords affected mainly men, except for cases associated with laryngeal intubation. We should keep in mind that postintubation laryngeal granuloma might develop after tracheal intubation, so care must be taken to avoid the potential complication.

Keywords: *laryngeal granuloma, treatment, predisposing factors.*

SAŽETAK

Cilj ovog rada je da prikažemo slučaj granuloma larinksa kod bolesnice ženskog pola. Prikaz bolesnika: Bolesnica starosti 23 godine, javila se zbog dugotrajne, promuklosti. U anamnezi ove bolesnice dobijamo podatak da je kod nje, tokom ranog detinjstva 8 puta bila primenjena endotrahealna intubacija. Tokom dijagnostičke evaluacije primenjena je direktna laringoskopija, kompletan otorinolaringološki pregled i rigidnu endovideolaringostroboskopija i uočen je laringealni granulom. Zaključak: Laringealni granulom je benigni izraštaj koji je češći kod osoba muškog pola, osim u slučajevima kod kojih je primenjivana endotrahealna intubacija. Trebalo bi imati u vidu da bi laringealni granulom mogao da se razvije posle primene endotrahealne intubacije, pa treba preduzeti sve mere da bi se izbegla ova potencijalna komplikacija.

Ključne reči: *laringealni granulom, terapija, predisponirajući faktori.*

ABBREVIATIONS

LG- Laryngeal granuloma

INTRODUCTION

Laryngeal granuloma (LG), first described by Chevalier Jackson in 1928 as "contact ulcer of the larynx," is known in the literature by many names, including laryngeal contact ulcer, contact granuloma, vocal fold granuloma, postintubation granuloma, vocal process granuloma and arytenoid granuloma (1).

Laryngeal granuloma is benign growth that resembles tumor by its macroscopic appearance, but not by its biological-histological characteristics and that is why it is classified as pseudo tumor. Laryngeal granuloma is a nonspecific inflammatory process formed by granulation tissue that occurs primarily in the vocal process of arytenoids cartilage (2,3). Incidence and prevalence of LG in general population are not clearly evaluated. They occur in about 0,9-2,7% of adults with voice disorders (2). The localization and appearance of the LG is very characteristic. The main etiopathogenic factor associated is laryngopharyngeal reflux, followed by laryngeal intubation and vocal abuse. The correct diagnosis can be established only by clinical examination, but the histological examination is necessary in order to avoid misdiagnosis (4). Clinical treatment is based on the use of proton pump inhibitor, topical corticoid and speech therapy and also surgical therapy in laryngomicrosurgery (5). The aim of this case report is to present the LG in 23 year old female with a complex medical history.

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CASE REPORT

The 23 year old female student was admitted for examination to Department of Otorhinolaryngology Health Centre Kragujevac, Kragujevac, Serbia, because of hoarseness and also irritant and non-productive cough, globus pharingeal, throat cleaning and dysphagia. We performed detailed history and physical examination: anamnesis, indirect laryngoscopy with complete otorhinolaryngologic examination and referred patient to foniatric department of Clinic of Otorhinolaryngology, Clinical center Kragujevac, Kragujevac, Serbia to performed rigid endovideostroboscopy. In anamnesis, we found that she has undergone general anesthesia for 8 times, in the early childhood at the age of 3 and 4. The main reason for this invasive method was recurrence of polips in left nasal and maxillar cavities and for last time it was rabdomyosarcoma in epipharyngis, ethmoid cells, left orbit, left nasal and maxillar cavities. Previous clinical, computed tomography and pathohystological examination showed that it was embrional rabdomyosarcoma of botryoid type. Then, radical surgical resection of the tumor was performed, followed by chemotherapy and typically ending with standard course of radiation. Late effects of therapy in our patient were: problems with left eye, bony hypoplasia, orbital and facial asymmetry, direct cardio toxicity and hypertension caused by direct radiation. We must notice that the gonadal development was normal and her behavior was normal following the treatment. Also, she is a good student. It is important because she learns every day and repeats it loudly, so we can conclude that she uses her voice frequently, overuses and misuses.

The patient is directed to allergologist to undergo further analyses. Allergy testing found positive skin prick test to inhalant allergens (pollens of trees, grasses and weeds) and food allergens (banana, nut and peanut). In laryngeal pathology hypersensitive reactions may appear both as etiopathogenetic factors and factors predisponing laryngeal mucosa to effects of the other unfavorable factors such as voice overuse and misuse or laryngopharyngeal reflux (6). Throat and nasal bacterial and mycological examinations were negative. In gastroenterological examination the diagnoses of gastroesophageal reflux disease and laryngopharyngeal reflux were made. Our patient uses cardiological and anti-allergic drugs. Indirect laryngoscopy showed hyperemia of laryngeal mucosa, especially in posterior parts, the movement of the both sides of larynx was symmetric. Airway was not compromised. There is the insufficiency of glottis occlusion with noise in speech sound, accompanied by hyperkinesia of the phonation muscles. On the right vocal process area, tumorous lesion was found, which resembles granuloma by its macroscopics appearance. The dimension of the tumorous lesion was about 6 mm (Figure 1). Rrigid endovideostroboscopy showed asymmetric and irregular vocal folds vibrations and arising of traveling mucous wave.

We indicated microlaryngosurgery with pathohistological verification, but the general condition of our patient was not so good and the surgical treatment was delayed. Clinical treatment was initiated with proton pump inhibitors, inhalation therapy with corticosteroids and speech therapy (one session per week for two months). After the two months, we performed rigid endovideostroboscopy, and we found reduced hyperfunction, tumor lesion was smaller and the mucosal condition was much better. Clinical treatment was based on elimination of all causal and predisposing factors along with use of proton pump inhibitor (control laryngopharyngeal reflux), inhalation therapy with corticosteroids (have good responds in LG) and speech therapy (reduces hyperfunction) and the size oftumor lesion was slightly regressed (Figure 2).

DISCUSSION

Laryngeal granuloma is a disease whose etiopathogenesis is not well defined (7). Etiopathogenesis of LG is still undetermined and it is attributed to three predisposing

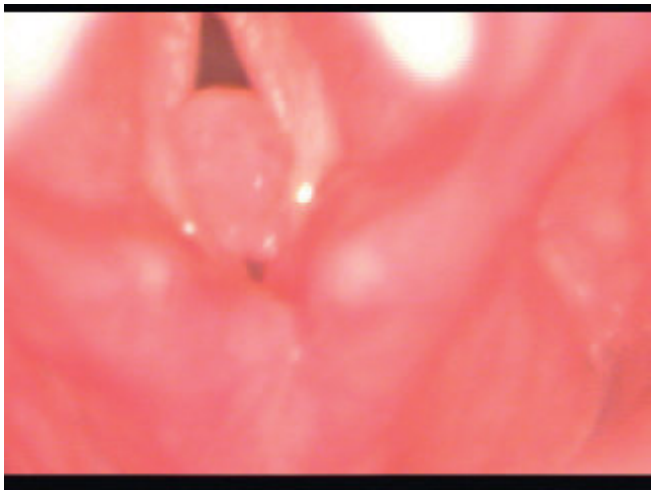

Figure 1. Laryngeal granuloma on the right vocal process area when patient came to examination, caught up from endovideostroboskopy.

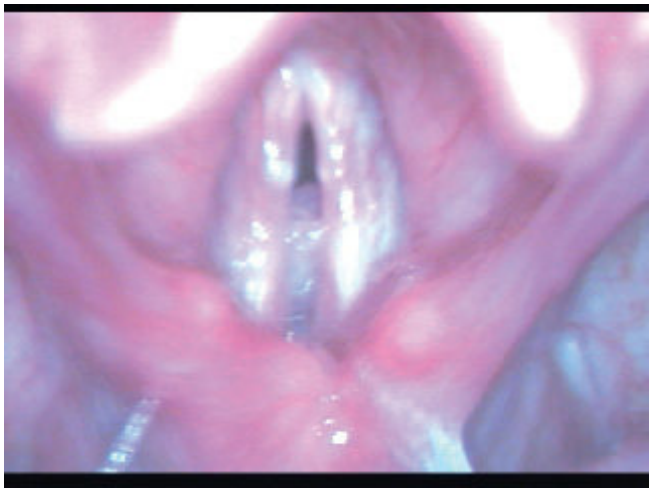

Figure 2. Laryngeal granuloma on the right vocal process area after the treatment, caught up from check up endovideostroboskopy.

factors: laryngopharyngeal reflux disease, laryngeal intubation and vocal abuse. If none of these causes are found, it is considered idiopathic (8). It is predominant in male subjects, except in cases associated with laryngeal intubation, which has higher incidence in female cases (7,5). Pontes et al. concluded that the higher frequency of intubation-related LG in women is related to lower glottis proportion (9).

As it is known from literature, initially treatment for LG is clinical (10). The surgical treatment is recommended in case of post-intubation related LG. Distribution of remissions after clinical treatment in different etiopathogenesis is: laryngopharyngeal reflux 46.2%, postintubation 44.4% and vocal abuse 80% (5). Some authors recommend an observation period of at least six months, but in persistent cases or in differential diagnostic doubts (lupus, sarcoidosis, Wegener's granulomatosis, larynx malignancy...) the surgical treatment with pathohistological verification is necessary, as well as for treating airway insufficiency (11, 12, 13, 14, 15). But, do not forget LG is recurrent in about 50% of cases subjected tosurgical removal. Surgery should be followed by clinical treatment also in the postoperative period to reduce risk of LG recurrence (5).

CONCLUSION

Laryngeal granuloma affected mainly men, except for cases associated with laryngeal intubation. In our patient etiopathogenic factors were overlapping: laryngopharyngeal reflux, laryngeal intubation and vocal abuse. The clinical treatment in our patient is well responded and general condition is not allow the surgical treatment which was recommended in case of post-intubation related LG. We should keep in mind that postintubation LG might develop after tracheal intubation, so care must be taken to avoid this potential complication.

Conflict of Interest

There is no financial interest and no other conflict of interest.

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Johnson, A. (2000). *Abstract Computing Machines*. Springer Berlin Heidelberg. Retrieved March 30, 2006, from SpringerLink http://springerlink.com/content/w25154. DOI: 10.1007/b138965.

f. Thesis

Format:

Author(s). (Year of publication). *Title*. Information, Place of publication.

Example:

Begg, M. M. (2001). *Dairy farm women in the Waikato 1946-1996: Fifty years of social and structural change*. Unpublished doctoral dissertation, University of Waikato, Hamilton, New Zealand.

g. Report

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Author(s). (Year of publication). *Title*. Place of publication: Publisher. (Report number)

Example:

Osgood, D. W., & Wilson, J. K. (1990). *Covariation of adolescent health problems*. Lincoln: University of Nebraska. (NTIS No. PB 91-154 377/AS)

h. Government publication

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Institution name. (Year of publication). *Title.* Place of publication: Publisher.

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Ministerial Council on Drug Strategy. (1997). *The national drug strategy: Mapping the future*. Canberra: Australian Government Publishing Service.

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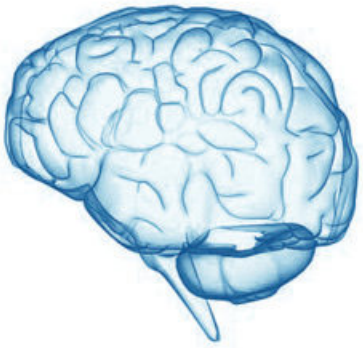

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