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GOAT MILK - PREJUDICES AND FACTS

KOZJE MLEKO – PREDRASUDE I ISTINE

Olgica MILANKOV

Introduction

"Milk is the most nearly perfect food"
Hippocrates, 400 B. C.

Milk is the most significant and most complete food for infants. However, one should take care of what type of milk, when and how much milk to feed a newborn as a complementary food.

The start of complementary feeding of infants, introduction of animal milk feedings, and selection of foods mostly depend on the climate, culture and dietary practices of the people [1, 2].

Recently, there is a growing interest of both medical and non-medical public in goat milk and goat milk products. Goat milk has been consumed since the ancient times, especially in the Mediterranean countries. Today, in the developed European countries and the Unite States, there is a growing interest in this type of milk which has been unjustly neglected.

Health Benefits of Goat Milk

Over the centuries, people suffering from serious illnesses and children with malnutrition and rickets consumed goat milk as a "medicine for poor health". Recently, it has become more and more important how goat milk prevents liver diseases and at what degree; how it regenerates the liver tissue; and how it affects cirrhosis and other cases of damaged liver. Having fantastic biological and nutritional properties, goat milk is a strong immunostimulant and it boosts the immune system. Also, it is a good ally in fighting stress and allergies, and it reduces the lung cancer risks (it contains specific linoleic acid which has anti-cancer properties). Ancient peoples knew that goat milk "cures and strengthens the lungs" so they often gave it to the patients suffering from tuberculosis [3].

Goat milk contains vitamin B1, B2, B6, B12 - important for protection and boosting the nervous system. This milk has 47% more vitamin A compared to cow milk, four times more cuprum and 134% more potassium.

This milk is also rich with other minerals, calcium and phosphorus, which are significant for maintenance of strong bones. It contains zinc and selenium, strong anti-oxidants, vital for the maintenance of the immune system balance. This milk is recommended to persons with anemia who suffer from iron deficiency. Scientists believe that goat milk supports better iron utilization in the body and better processing of hemoglobin.

Since goats are healthy, clean and enduring animals who feed on bark of the trees, their milk is rich in silicon, a mineral which is beneficial for the quality of skin, nails, hair and nervous system.

Goat milk should be also consumed because it contains oligosaccharides which have anti-inflammatory properties. Whey, goat cheese and yogurt are powerful healthy natural foods. They boost and regulate bowel movement, because goat milk has low amount of lactose. In adults, it may help in preventing high blood pressure and atherosclerosis.

In fact, potassium is an essential mineral for maintenance of adequate heart function and regular blood pressure. It is worth noting that it has a beneficial effect on the osteoporosis therapy; it regulates cholesterol and improves renal function.

Proteins in goat whey are so called "fast proteins" which absorb quickly and directly supply muscle cells with energy and necessary amino acids. Therefore, goat milk is recommended to the athletes, recreational sportsmen, and all those engaged in physical activities who need prompt muscle revitalization following a challenging training. It is also beneficial for pregnant women, due to their increased requirements for proteins (increase by one third), it provides also "good health" [4].

It should be taken into account that goat whey is naturally rich in essential amino acid - tryptophan, so it acts as a natural sedative. Also, it influences

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the release of serotonin, the best known neurotransmitter, creating the feeling of drowsiness and relaxation. This milk reduces appetite, lowers cholesterol, cleans teeth plaque and prevents tooth decay.

Goat milk contains some forty probiotic bacterial cultures and large quantities of oligosaccharides, which help the bacteria in the intestines to stay in balance and prevent the development of pathogenic bacteria causing various intestinal infections. Therefore, it may well be said that goat milk acts both as a probiotic and a prebiotic [5].

Nutrition Problems with Goat Milk

The main problem with goat milk is its smell and taste. This "goat smell and taste" comes from caproic, caprylic and capric fatty acids which are, among other things, the result of the spontaneous decomposition of milk fat. The unpleasant smell of this milk may originate from the lack of sanitary conditions during the production and processing of raw goat milk. In any case, if strong, strange smell and taste of this milk appear, one should check out the following: whether the animal is healthy, if the sanitary conditions in the stall are adequate, the lactation phase (milk in the later lactation phase has stronger smell), the way of storing milk in the containers (the milk should ideally be stored in perfectly clean aluminium or stainless steel containers, or even in glass bottles), and if the milk is stored in the fridge immediately after milking the goat. Anyhow, each goat milk product of changed or poor taste should be checked out [6].

Another negative aspect of the goat milk is the fact that it is more expensive than cow milk. The reason for this is probably the fact that the daily milk yield of a goat is ten times less than the average daily yield of a cow.

Yet another significant fact is that goat milk contains less folic acid, and the deficiency of this vitamin may cause megaloblastic anemia. This type of anemia is related to slower maturation of erythrocytes in blood, which is followed by the decreased concentration of hemoglobin. The child with anemia is pale, nervous and prone to infections and diarrhea. Apart from playing an important role in the maturation of erythrocytes in the first four months of an infant, folic acid is also important for the maturation of brain functions. The deficiency in this vitamin may cause permanent consequences on child development [4, 7].

Beneficial Caracteristics of Goat Milk

In regard to the benefits of goat milk, it is necessary to discuss the following issues: medicinal, nutritional, immunological, and biological aspects of goat milk.

There are 480 million goats worldwide producing some 5 million tons of milk per year. An average goat produces 3 - 4 liters of milk per day, which is about 900 - 1.800 kg milk in 305 lactation days [5, 8]. The studies show that the average investment in buying and keeping a goat is lower than the investment in buying and keeping a cow. Also, the life expectancy of a goat is twice as long as the average cow's life expectancy. An average life expectancy of a goat and a cow is 8 - 10 years and 4 - 6, respectively. As for its size, a goat is significantly smaller than a cow (1/6 of cow's size) due to which it is more practical for handling and transport. Breeding goats requires smaller area of pasture. This is important because it shows that for the same quantity of produced milk less financial investment is required for their feeding [4, 9].

Physical and Chemical Aspects of Goat Milk

By its chemical composition, physical properties and organoleptic characteristics, goat milk is very similar to cow milk, although it has certain specific features [10, 11].

Goat milk is whiter in colour, compared to cow milk. Cow milk is yellowish because of the presence of carotene. Goat milk has a specific, strong smell, the so called "goat smell and taste" which come from caproic, caprylic and capric fatty acids which are the consequence of spontaneous decomposition of milk fat [8, 11].

Milk Fat

Milk fat determines the taste and consistency of milk and fat quantity in goat milk is 2.5 to 6% and it greatly varies. Fats are represented in the form of lipid droplets 2 - 3 μm in diameter (in cow milk the droplets are 3 - 8 μm). These droplets add to the milk homogeneity, and fast hydrolysis of fatty acids enables better digestion compared to cow milk [11]. This is a significant information which may be successfully used for children suffering from intestinal malabsorption problems. Due to fast enzyme decomposition of goat milk fat, it is better and faster resorbed. Digestion of goat milk fat lasts 40 minutes. When speaking about cow milk fat, its digestion lasts two and a half hours [8, 10]. Three significant characteristics of goat milk are better digestibility, alcality, and higher buffering capacity, which make it unique with regard to other types of milk [12]. Therefore, as earlier mentioned, this milk is recommended for patients with irregular intestinal absorption, patients with hyperlipoproteinemia, cardiac patients, children suffering from epilepsy, and patients with gallbladder and bile duct disorders.

It is of great importance that 28% of goat milk fat contains short-chain saturated fatty acids, so lipase decomposes them faster and more easily. The goat milk fat comprises 200 different fatty acids. Fifteen of them are present in over 1%, whereas others are found only in traces [8]. Milk fats contain conjugated linoleic acid for which it is proved to have anti-cancer properties. Also, phospholipids and liposoluble vitamins in goat milk contain choline, which is important for the oxidation of fat in the liver, and which maintains the balance of cholesterol levels. Goat milk is important as it contains three fatty acids: caproic, caprylic and capric fatty acid, which make 20% of all fatty acids in goat milk (in cow milk they make 6%) **(Table 1)**. These fatty acids are significant because they convert faster into energy,

Table 1. Comparative content of fatty acids in goat and cow milk
Tabela 1. *Komparativni sastav masnih kiselina kozjeg i kravljeg mleka*

Fatty acids/*Masne kiseline*	Goat milk/*Kozje mleko*	Cow milk/*Kravlje mleko*
Butyric C4/*Buterna*	2.99	3.30
Caproic C6/*Kapronska*	6.25	1.60
Caprylic C8/*Kaprilna*	2.52	1.3
Palmitic C16/*Palmitinska*	34.80	28.8
Stearic C18/*Stearinska*	6.84	14.6
Oleic/*Oleinska*	13.26	29.80
Linoleic/*Linolna*	3.60	2.50
Linolenic/*Linoleinska*	0.88	–

they do not deposit in body depots, nor they contribute to clogged arteries. Goat milk contains less cholesterol, about 10 mg/100 g compared to cow milk (13 mg/100 g) [10, 13].

Goat Milk Proteins

In regard to the protein quantity, goat milk is similar to cow milk. The only difference is in composition, type and proportion of amino acids. The nutritional value of proteins depends on the amount of amino acids in them. Goat milk proteins are easier to digest than cow milk proteins, and the absorption of amino acids is also better [9]. When comparing the quantity of milk which meets a child's requirements in proteins, it may be seen that 200 ml of goat milk secures 18% of the total daily protein requirements, whereas the same quantity of cow milk meets only 13% of a child's requirements [14].

The main milk protein is casein, which accounts for 80% of the total protein contents. It comprises alpha-S2-casein, beta-casein and k-casein. Whey proteins, as a non-casein fraction of milk proteins and as biologically most valuable proteins, comprise lactalbumin, lactoglobulin, and protease peptone [15].

The total quantity of amino acids, including the essential amino acids, is the same in both types of milk **(Table 2)**. Goat milk contains more cysteine, valine, leucine, and asparagine. The amount of serine, tyrosine and glutamine is lower. That means that goat milk contains more amino acids, especially the essential amino acids. It is important that it contains high levels of taurine, just like human milk. This essential amino acid, which is contained in bodily liquids and tissues, has a significant role in the growth and development of brain in infants and small children. In adults, its poor concentration influences the development of cardiomyopathy [8].

Goat Milk Lactose

Milk sugar, lactose, is an important source of energy. It comprises glucose and galactose and gives milk its sweet taste. Goat milk contains somewhat less lactose than cow milk, which is an advan-

Table 2. Comparative content of amino acids in goat and cow milk
Tabela 2. *Komparativni aminokiselinski sastav kozjeg i kravljeg mleka*

Amino acids/*Aminokiseline*	Goat milk/*Kozje mleko* g/100 g	Cow milk/*Kravlje mleko* g/100 g
Lysine/*Lizin*	7.78	7.86
Histidine/*Histidin*	3.47	2.35
Arginine/*Arginin*	3.54	2.43
Aspartic/*Asparaginska kiselina*	8.59	6.79
Serine/*Serin*	5.23	5.78
Glutamic/*Glutaminska kiselina*	19.82	22.86
Threonine/*Treonin*	4.77	4.81
Proline/*Prolin*	9.12	9.33
Glycine/*Glicin*	1.58	1.74
Alanine/*Alanin*	3.99	3.61
Cysteine/*Cistein*	1.04	0.83
Valine/*Valin*	6.07	5.64
Methionine/*Metionin*	2.51	2.63
Leucine/*Leucin*	10.17	9.92
Phenyl alanine/*Fenilalanin*	4.13	4.34
Tyrosine/*Tirozin*	3.64	4.75

Table 3. Comparative nutritional values of goat and cow milk
Tabela 3. *Komparativne nutritivne vrednosti kozjeg i kravljeg mleka*

Ingredients/*Sastojci*	Goat milk/*Kozje mleko*	Cow milk/*Kravlje mleko*
Dry matter/*Suva materija*	11.94%	12.89%
Fat/*Mlečna mast*	3.60%	4.10%
Protein/*Proteini*	3.10%	3.38%
Lactose/*Laktoza*	4.60%	4.60%
Free fatty acids/*Slobodne masne kiseline*	8.10 mg/lit	7.50 mg/lit
Cholesterol/*Holesterol*	10 mg/100g	13 mg/100g
Energetic value/*Energetska vrednost*	69.78 kcal/100ml	68.78 kcal/100ml
Ph value/*Ph vrednost*	6.72	6.68

tage for all those children who are diagnosed with lactose intolerance [16].

Goat milk contains oligosaccharides which have an anti-inflammatory effect on the intestinal mucosa and contribute to digestion, especially in case of intestinal malabsorption [17].

Goat Milk Vitamins and Minerals

The content of minerals in goat and cow milk is almost the same. Goat milk contains 13% more calcium, 134% more potassium, 4% more cuprum, 25% more vitamin B6, 47% more vitamin A, and three times more niacin. The quantities of vitamin C and D are insufficient, so all children who are fed with goat milk should obtain supplements of vitamins C and D [16].

One cup of goat milk provides 32.6% of a child's daily requirements of calcium and phosphorus. The same quantity of cow milk meets some 26% daily requirements in these minerals [18]. So, this milk plays an important role in the body, giving strength to the bones. With the quantity of zinc and selenium it contains, goat milk takes part in the maintenance of the immune system. Goat milk is rich in silicon, a mineral which positively affects the quality of skin, hair and nervous system [19].

Medicinal Aspects of Goat Milk

Many pediatricians recommend goat milk to children intolerant to cow milk proteins. Some 40% of patients intolerant to cow milk proteins tolerate goat milk proteins well. A probable cause to this is the fact that lactoalbumin is immunospecific in various types of milk. Goat milk is very useful for people who suffer from eczema, asthma, colitis, stomach ulcer, migraine, in people having various problems with digestion and liver disease, as well as stress-induced symptoms including insomnia, constipation, neurobiological food consumption, etc. [20].

Nutritional Aspects of Goat Milk

Nutritional positive effects of goat milk do not originate so much from proteins, minerals and vitamins it contains, but from lipids, i. e. specific fatty acids **(Table 3)**. Fats in goat milk are more easily digested than fats in cow milk, because the fatty droplets in goat milk are smaller, they have a larger surface, so intestinal lipase has a faster and more powerful effect on them. These all are the reasons for easier digestion of goat milk. In addition, the proteins in goat whey are the so called "fast proteins" because they are being absorbed fast, directly providing muscles with energy and necessary amino acids [13].

Immunological Aspects of Goat Milk

The scientific literature shows that opinions are divided in terms of using goat milk in infants and small children allergic to cow milk proteins. Some children may undoubtedly have a cross-allergic reaction, which, of course, excludes the possibility to replace cow milk with goat milk [21]. Today, an opinion prevails that goat milk is recommended for children extremely intolerant to cow milk proteins [10]. Alpha-S1-casein, responsible for allergic reactions in a lot of people, is found in goat milk only in traces; therefore this milk may be recommended for patients intolerant to cow milk proteins [22–24].

Biological Aspects of Goat Milk

Fatty droplets in goat milk are better dispersed, which enables better homogeneity, and faster hydrolysis of fatty acids contributing to better digestion. The faster the goat milk fat is decomposed with enzymes, the faster it is resorbed in the digestive system. Therefore, forty minutes are needed for a certain dose of goat milk to be digested, while for the same quantity of cow milk some two and a half hours are needed. It means that 20% less time is required to digest the same quantity of goat milk [9].

Goat Milk Products

Yogurt - is a drink enriched with probiotic bacteria, lactobacillus and bifidobacteria whose goal is to stimulate growth of beneficial bacteria in intestines, thus having a positive effect on homeostasis of the digestive system. This is especially important for children suffering from digestive disorders, whether they have diarrhea or constipation.

Elder persons are also recommended to consume dairy products of all types of milk. The Chinese believe that only children should drink sweet milk, and that adults should drink acidified milk. An ancient Indian proverb says: "Drink acidified milk and you will live long." In the countries whose people have the long-

est life expectancy, sour milk, yogurt, onion and small quantities of red milk, represent food responsible for their long life span. Hundred-year-old inhabitants of Caucasus and of some mountainous parts of Bulgaria are the best example which supports the claim that sour milk and yogurt have healing properties [13, 25].

Kefir - is very rich in valuable proteins, minerals and proteins. It contains no gluten and its low glycemic index is especially significant. It should be noted that aflatoxin in food, especially in milk, over time deposits in the body, and later may have mutagenic, teratogenic and cancerogenic effects. Due to the presence of many bacteria, sour milk and kefir bind to aflatoxin and thus take part in detoxification of the body [26].

Cheese - It is well known that goat cheese is particularly consumed in the Mediterranean region. The value of this cheese comes from whey rich in essential amino acids (lysine, methionine, and cysteine) [13]. It should also be mentioned that this milk is rich in fatty acids of the middle chain such as caproic, caprylic and capric fatty acids which give this milk its specific taste, smell and aroma. The bottom line is that these acids got their name after the Latin term "capra" meaning goat.

Whey - contains tryptophan, an important amino acid which has many beneficial effects on health. It is a natural sedative; it reduces the appetite, lowers cholesterol, prevents tooth decay and regenerates liver cells [8].

Conclusion

Goat milk is a good source of essential and high-quality substances. Because it is rich in proteins, calcium, phosphorus, potassium, sodium, selenium, and vitamins B2 and B6, but also due to a small quantity of lactose, and the presence of probiotic bacteria, goat milk is a "magical drink". It is easy to digest, and that is why it is ever more present in everyday nutrition.

As mentioned before, exclusive nutrition with non-modified cow milk may lead to certain conditions and illnesses in children, such as serious electrolytic disorders, metabolic acidosis, megaloblastic anemia and a series of accompanying allergic symptoms. In infant nutrition it is necessary to take goat milk proteins, but as they are present at high concentrations, it complicates digestion in this early age. If the goat milk is not pasteurized, the infants are at higher risk of bacterial infections and diarrhea. The scientific literature shows that opinions are still divided in terms of whether to give goat milk to infants and small children or not. Some nutritionists believe that there is no risk in consuming this type of milk, that it is easier digestible and rarely causes allergic symptoms, so it should be present in everyday diet.

In our opinion, non-modified goat milk should not be given to children before the age of two years, at least not in large quantities and on a daily basis. It is enough to give a child some 200 ml of goat milk, which will secure as much as 18% of daily requirements of proteins rich in essential amino acids. If there are family habits of consuming goat milk, parents should be advised to supplement this milk with folic acid, iron preparations and vitamins A and D, which are not present in goat milk in sufficient quantities.

Goat milk is highly nutritious and a valuable food of animal origin which has a whole range of benefits for human health. It is unjustly neglected in the diet of adults and children, due to the fear from its few side effects.

In general, milk is a very nutritious food, but one should keep in mind what type of milk to consume, when and in what quantities.

I would like to add that breast milk is the best choice for infants, and there is no alternative to it!

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EVALUATION OF DIFFICULTIES IN LAPAROSCOPIC CHOLECYSTECTOMY

PROCENA TEŽINE LAPAROSKOPSKE HOLECISTEKTOMIJE

Katarina ŠARČEV[1-3], Dimitrije DAMJANOV[1, 4], Dijana KOSIJER[1,4] and Dragomir DAMJANOV[1,4]

Summary

Introduction. For decades, laparoscopic cholecystectomy has been the gold standard in surgical treatment of patients with cholelithiasis all over the world. The main advantage of this approach is that it is a minimally invasive procedure for patients. Although this method is a routine in our country, there are certain cases where the presumed outcome of minimally invasive procedure is not achieved, and the surgery is converted to open surgery, or a subsequent laparoscopic surgery is performed, in order to deal with the complications. The aim of this study was to establish if it was possible to create a model for preoperative prediction of difficult laparoscopic cholecystectomies. **Material and Methods**. Two groups of patients were analyzed. Group A included patients with cholelithiasis who were studied in order to determine parameters associated with difficult laparoscopic cholecystectomies. Out of 16 analyzed parameters, 8 showed significant correlation with difficult laparoscopic cholecystectomies. Based on these parameters, a prediction model was established, consisting of five groups: I - easy (score 1), II - laparoscopic cholecystectomy with mild difficulties (score 2), III - laparoscopic cholecystectomy with major difficulties (score 3), IV - difficult (score 4), V - conversion to open surgery is expected (score 5). This model was preoperatively applied in patients with cholelithiasis included in group B. **Results**. The overall predictability of the model was 82%. The greatest prediction accuracy was achieved in groups II and III (98.3% and 100%, respectively). Conversion to open cholecystectomy was predicted in 76% of patients. **Conclusion**. Based on certain preoperative parameters it is possible to establish a model to predict a difficult laparoscopic cholecystectomy.

Keywords: Cholecystectomy, Laparoscopic; Surgical Procedures, Operative; Predictive Value of Tests; Treatment Outcome; Risk Factors; Decision Support Techniques; Risk Assessment

Sažetak

Uvod. Laparoskopska holecistektomija decenijama predstavlja zlatni standard u operativnom rešavanju problema bolesnika sa holelitijazom u mnogim zemljama sveta. Osnovni motiv uvođenja ovakvog načina je potreba za minimalno invazivnim pristupom lečenju. Iako se ova metoda rutinski izvodi u našoj zemlji, u određenom broju slučajeva ishod lečenja ne zadovoljava pretpostavljeni minimalno invazivni princip i operacija se završava bilo konverzijom u klasičnu operativnu proceduru, bilo ponovnim operacijama radi rešavanja komplikacija. Cilj istraživanja bio je da se proceni da li je moguće na osnovu postojanja preoperativnih parametara, koji su udruženi sa teškom laparoskopskom holecistektomijom, formirati model za preoperativnu procenu težine laparoskopske holecistektomije. **Materijal i metode**. Analizirane su dve grupe bolesnika. Grupa A bolesnika sa holelitijazom poslužila je za određivanje parametara koji su udruženi sa teškom laparoskopskom holecistektomijom. Od 16 analiziranih faktora, osam je pokazalo značajnu korelaciju sa teškom laparoskopskom holecistektomijom. Na osnovu ovih parametara formiran je model predikcije težine laparoskopske holecistektomije, koji ima pet grupa: I – laka (skor 1), II – laparoskopska holecistektomija sa manjim poteškoćama (skor 2), III – laparoskopska holecistektomija sa većim poteškoćama (skor 3), IV – teška (skor 4), V – očekuje se konverzija (skor 5). Ovakav model primenjen je na grupi B bolesnika sa holelitijazom preoperativno. **Rezultati**. Ukupna predikitabilnost modela iznosi 82%. Najviši stepen predikcije postignut je u grupi II i III (98,3% i 100%). Konverzija u klasičnu holecistektomiju predviđena je kod 76% bolesnika. **Zaključak**. Na osnovu postojanja određenih preoperativnih kriterijuma moguće je formirati model za preoperativnu procenu težine laparoskopske holecistektomije sa visokim stepenom pouzdanosti.

Ključne reči: laparoskopska holecistektomija; operativne hirurške procedure; prediktivna vrednost testova; ishod lečenja; faktori rizika; tehnike podrške pri odlučivanju; procena rizika

Introduction

The first laparoscopic cholecystectomy (LC) was performed by a German surgeon Erich Mühe in 1985, but his contributions were not recognized until 1992 [1]. Since then, laparoscopic approach has been accepted with great enthusiasm by the surgical society worldwide. It was a revolutionary shift in surgery

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Abbreviations
LC – laparoscopic cholecystectomy
BMI – body mass index

which initiated laparoscopic approach almost in all fields of general surgery. Obvious advantages are decreased hospital stay, increased patients' satisfaction (possibility to return to everyday routine within a week after surgery) and lower overall costs. However, as LC achieved wider acceptance, the number of complications began rising, especially those which were seldom seen with conventional (open) surgery. This mainly refers to bile duct injuries, bowel injuries, and bleeding. Nevertheless, LC is the most commonly performed abdominal procedure in Western countries [2]. For example, in the United States, about 90% of all cholecystectomies are performed laparoscopically [3]. Cholecystectomy, including the laparoscopic approach, is usually performed by a general surgeon, who has completed a five-year residency and received education in proper techniques including the use of a laparoscope. The learning curve is very important in this field, but equally important is the surgeon's knowledge of standard techniques. As it was mentioned before, laparoscopy, being a minimally invasive procedure is supposed to be easier both for the patient and the surgeon, but in some cases, it is not so. Reasons may be associated with the patient, or with the surgeon (disregarding problems with laparoscopic equipment). The aim of this study was to establish if it was possible to create a model for preoperative prediction of difficult LC.

Material and Methods

This case-control study analyzed the period from January 1, 2014 to December 31, 2015. The study was conducted at the Clinic for Abdominal, Endocrine and Transplantation Surgery of the Clinical Center of Vojvodina in Novi Sad. It included 200 consecutive patients with cholelithiasis in whom LC was indicated, regardless of sex, aged 18 years and over. The patients were divided into two groups, each of 100 patients: the first group (group A) allowed creation of a model for difficulty assessment of LC, while the second group (group B) was the control group to validate the prediction model.

Table 1. Difficulty parameters of laparoscopic cholecystectomy with values of reliability coefficients
Tabela 1. *Parametri težine laparoskopske holecistektomije sa vrednostima koeficijenta pouzdanosti*

№ Parameter/*Parametar*	Positive *Pozitivan* (N = 100)	Correlation R *Korelacija R*	T Values *Vrednosti T P=0,05*
1. Age over 65 years/*Starost preko 65 g.*	14	0.395	0.73
2. Previous surgery in the upper abdomen *Prethodne operacije u gornjem abdomenu*	15	0.504	0.81
3. Previous surgery in the lower abdomen *Prethodne operacije u donjem abdomenu*	19	0.840	2.69
4. History of cholelithiasis over 5 years *Anamneza holelitijaze duža od 5 godina*	35	0.371	0.68
5. Biliary colic in last tree preoperative weeks *Bilijarma kolika u poslednje tri preoperativne nedelje*	43	0.158	0.26
6. Obesity (BMI over 30)/*Gojaznost (BMI preko 30)*	26	0.852	2.62
7. Pain in the upper right abdominal quadrant *Bol u gornjem desnom kvadrantu abdomena*	40	0.830	2.56
8. Rigidity of the upper right quadrant *Rigiditet u gornjem desnom kvadrantu abdomena*	10	0.619	1.33
9. Body temperature over 38 °C/*Telesna temperatura preko 38° C*	1	0.319	0.56
10. Serum amylase level over 120 U/l/*Serumska amilaza preko 120 U/l*	0	0	0
11. Leukocytosis over 10 x 10^9/l/*Leukocitoza preko 10 x 10^9/l*	4	0.010	0.01
12. ES rate faster than 20/40/h/*SE brža od 20/40/h*	3	0.210	0.37
13. Calculi over 2 cm seen on ultrasonography *Kalkulusi veći od 2 cm na ultrasonografiji*	27	0.932	4.02
14. Thickness of the gallbladder wall of 5 mm or more seen on ultrasonography on admission *Debljina holeciste 5 mm ili veća na ultrasonografiji na prijemu*	10	0.841	2.69
15. Calculi inclavation in the cystic duct seen on ultrasonography *Inklavacija kalkula u duktusu cistikus na ultrasonografiji na prijemu*	9	0.523	1.02
16. Contracted gallbladder seen on ultrasonography *Skvrčena holecista na ultrasonografiji*	0	0	0

p=0.05, *BMI - indeks telesne mase*

The group A, including 100 patients with an indication for LC, was studied to establish the existence of certain parameters **(Table 1)** which were assumed to be associated with difficult laparoscopic cholecystectomy. After surgery, the surgeon classified the difficulty of LC in the surgical protocol as easy, medium difficult, very difficult. Difficulty criteria were determined for each individual stage of LC, as follows: 1. Installation of pneumoperitoneum; 2. Trocar ports placement; 3. Identification of anatomical structures; 4. Clips placement on the structures; 5. Gall bladder resection; 6. Evacuation of the gallbladder.

The LC was considered easy if the procedure, the course and the outcome were without any difficulties through all stages of surgery. Difficult LC was with problems in all, or some stages of surgery. Also, every surgery that lasted over 90 minutes was marked as difficult. Every conversion to open procedure was marked separately. Medium difficult LC was with minor problems during the course of surgery. On the other hand, routinely obtained preoperative parameters, necessary for every biliary procedure, were assessed. These parameters included patient history data, clinical examination, laboratory data and ultrasound findings. Sixteen parameters were observed: 1. age over 65 years; 2. previous operation in the upper abdomen; 3. previous operation in the lower abdomen; 4. history of cholelithiasis longer than 5 years; 5. biliary colic in the last three preoperative weeks; 6. obesity (Body mass index (BMI) over 30); 7. pain in the right upper abdominal quadrant on admission; 8. rigidity in the right upper abdominal quadrant; 9. body temperature over 38° C, 10. serum amylase level over 120 U/l; 11. leukocytosis over 10 x 10^9/l; 12. erythrocyte sedimentation rate of 20/40 on admission; 13. calculi over 2 cm seen on ultrasonography; 14. the thickness of the gallbladder wall of 5 mm or more seen on ultrasonography; 15. calculus inclavation in the cystic duct seen on ultrasonography; 16. contracted gallbladder seen on ultrasonography.

A parameter that was associated with difficult LC was marked by number 1, and if it was not associated - by 0. The correlation of factors was made using logistic regression analysis. $P < 0,05$ was considered statistically significant **(Table 2)**.

Parameters that have been statistically significantly associated with difficult LC were included into the evaluation of a prediction model. Five categories were defined by the scoring model: I - (score 1) easy LC; II - (score 2) LC with slight difficulties; III - (score 3) LC with major difficulties; IV - (score 4) difficult LC; V - (score 5 or more) - conversion to open surgery is expected.

This model was tested in group B, including patients in whom LC was indicated. The patients were preoperatively divided into 5 groups, according to the number of positive parameters established preoperatively. The presence of any parameter was scored 1, while their absence was scored 0. If the patient presented with three positive parameters, he was classified into group III, with four positive parameters into group IV, but if there were five or more positive parameters, the patient was classified into group V - conversion to open cholecystectomy was most likely. Accordingly, the difficulty of the LC was predicted for each patient as follows: group I (score 0 or 1) - easy LC was expected; group II (score 2) – LC with slight difficulties; group III (score 3) – LC with major difficulties; group IV (score 4) – difficult LC was expected; group V (score 5, or more) – conversion to open cholecystectomy was expected. Postoperatively, the surgeon classified LCc as easy, LCs with slight difficulties, LCs with major difficulties, difficult LC, or noted conversion to open surgery. Preoperative/predictive and postoperative/real data were compared in order to establish the reliability of the prediction model. The statistical package for the social sciences for Windows (version 16.0) software program was used for statistical analysis.

Table 2. Number of positive parameters in group B patients
Tabela 2. *Broj pozitivnih parametara kod bolesnika grupe B*

№	Parameter *Parametar*	No of the patients *Broj pacijenata*	P
1.	Previous surgery in the upper abdomen/*Prethodne operacije u gornjem abdomenu*	14	p<0.01
2.	Previous surgery in the lower abdomen/*Prethodne operacije u donjem abdomenu*	17	p<0.01
3.	Obesity (BMI over 30)/*Gojaznost (BMI preko 30)*	18	p<0.05
4.	Pain in the upper right abdominal quadrant *Bol u gornjem desnom kvadrantu abdomena*	38	p<0.01
5.	Rigidity of the upper right quadrant/*Rigiditet u gornjem desnom kvadrantu abdomena*	5	p<0.01
6.	Calculi over 2 cm seen on ultrasonography/*Kalkulusi veći od 2 cm na ultrasonografiji*	26	p<0.01
7.	Thickness of the gallbladder wall of 5 mm or more seen on ultrasonography *Debljina zida holeciste 5 mm ili veća na ultrasonografiji na prijemu*	9	p<0.05
8.	Calculi inclavation in the cystic duct seen on ultrasonography *Inklavacija kalkula u duktusu cistikus u na ultrasonografiji na prijemu*	4	p<0.05

BMI - indeks telesne mase

Table 3. Predicted kind of surgery and treatment outcome of LC with regard to score - group B
Tabela 3. *Predviđena LH i njen ishod izvedena vrsta LH u odnosu na skor - grupa B*

Kind of surgery *Vrsta LH*	No of patients Preoperative prediction *Broj pacijenata - preoprativno predviđanje*	Score *Skor*	No of the patients - Operative results *Broj pacijenata- operativni nalaz*	Accurately assessed *Pravilno procenjeni* %
Easy LC/*Laka LH*	55	I	52	95.90%
LC with slight difficulties *LH sa manjim poteškoćama*	25	II	28	98.31%
LC with major difficulties *LH sa većim poteškoćama*	12	III	9	75%
Difficult LC/*Teška LH*	5	IV	9	85%
Conversion/*Konverzija*	3	V	2	76%

LH - laparoskopska holecistektomija

Results

The previously determined 16 parameters **(Table 1),** associated with difficult LC, were analyzed and after correlation, 8 parameters showed significance. The parameter that was associated with difficult LC was marked with 1, and if it was not associated, with 0. The correlation of these factors was made using logistic regression analysis. $P < 0,05$ was considered statistically significant **(Table 1)**. Significant correlation was determined for 8 parameters: 1. previous operation in the upper abdomen; 2. previous operation in the lower abdomen; 3. obesity (BMI over 30); 4. rigidity of the upper right abdominal quadrant; 5. calculus over 2 cm seen on ultrasonography; 6. thickness of the gallbladder wall of 5 mm or more seen on ultrasonography; 7. calculus inclavated in the cystic duct, 8. pain in the upper right abdominal quadrant **(Table 2)**.

The predictive model was created according to these results and it was tested in group B patients **(Table 3)**. This model has five categories: I - (score 1) easy LC; II - (score 2) LC with slight difficulties; III - (score 3) LC with major difficulties; IV - (score 4) difficult LC; V - (score 5 or more) conversion is expected. According to our results, 55 patients scored 1 (0 – 1 positive parameter), 25 patients scored 2 (2 positive parameters), 12 patients scored 3 (3 positive parameters), 5 patients scored 4 (4 positive parameters) and 3 patients scored 5 (5 or more positive parameters). Hence, we predicted that most of the patients in this group would have an easy LC, but we also predicted 5 difficult LCs, and 3 conversions. The actual results after surgery (group B), showed that 52 patients had an easy LC, 28 patients had slight difficulties, 9 patients had major difficulties, 9 patients had difficult LCs, and in 2 patients LC had to be converted to an open procedure.

Thus, the applied model in group B, showed that score 1 (easy LC) was determined in 95.90% of the patients, score 2 (LC with slight difficulties) in 98.31% of the patients, score 3 (LC with major difficulties) in 75% of the patients, score 4 (difficult LC) in 85% of the patients, and score 5 (conversion to open) was determined in 76% of the patients **(Table 3)**. Logistic regression analysis was used to verify the significance of the prediction model **(Table 4)**. The overall results showed that score 1 was properly assessed in 94% of the patients, score 2 in 89% of the patients, score 3 in 75 %, score 4 in 88%, and score 5 in 67% of the patients **(Table 5)**. The overall predictability of the model was 82%.

Discussion

The LC is one of the most frequently performed abdominal surgical procedures worldwide [2]. After initial enormous enthusiasm with this approach, both

Table 4. Results of regression analysis in group B
Tabela 4*. Rezultati regresione analize grupa B*

Kind of operation *Vrsta operacije*	WALD	χ^2	P	Odds ratio *Odds odnos*	Standard error *Standardna greška*	*Coeffic.* b_i Koefic. $b_{i/}$
Easy LC/*Laka LH*	0,0010	15,653	0,9812	0,6309	19,3508	-0,4605
LC with slight difficulties *LH sa manjim poteškoćama*	0,0265	9,3213	0,0228	0,9435	2,9094	-0,3789
LC with major difficulties *LH sa većim poteškoćama*	0,0189	5,4392	0,0446	0,9605	0,0201	-0,9608
Difficult LC/*Teška LH*	0,0009	2,1711	0,1406	1,0229	0,1064	-1,1337
Conversion/*Konverzija/*	0,0251	2,6566	0,1031	1,0185	0,7746	-0,9163

LH - laparoskopska holecistektomija

Table 5. Overall results of the model
Tabela 5. *Rezultat primene modela*

Assessement/*Procena*	Score I *Skor I*	Score II *Skor II*	Score III *Skor III*	Score IV *Skor IV*	Score V *Skor V*
Accurate assessment/*Pravilno procenjen*	94%	89%	75%	88%	67%

by the patients and surgeons, apparently, because of its advantages [4], in the following years many problems were reported [5–7]. The complications associated with LC, and conversion rate definitely depended on the difficulty of LC, and skills and experience of the surgeon [8, 9]. Nevertheless, as years passed by, surgical societies of the world have accepted LC as a gold standard for all patients in whom cholecystectomy was indicated [2, 10]. This was based on the fact that, according to many studies, there were no significant differences in the morbidity and mortality between LC and open cholecystectomy [11–13]. The estimated complication rate of LC generally varies from 0 – 8.6%, while the conversation rate is between 1.5 to 6%, with an acceptable rate up to 10% [12–17]. In order to minimize both complications and conversion rates, it is necessary to improve surgical skills and knowledge, for better selection of patients preoperatively, and establish those in whom LC could be difficult, or even unfeasible.

Factors that contribute to the difficulty of the procedure are reported in the literature: age over 65, previous abdominal operations, obesity, recurrent colic episodes, longstanding cholelithiasis, thickening of the gallbladder wall, male gender, inclavation of the calculus in the cystic duct, acute pancreatitis etc. In this study, in accordance with the literature, we observed the presence of 16 most frequent factors that contributed to the difficulty of LC [17–19].

This study was designed to establish the parameters of importance (patient-specific) in the prediction of the difficulty of LC, in order to avoid both complications and conversion. It means that if the predicted difficulty is higher, the most skillful surgeon chooses to perform LC. Surgeon-specific parameters were not included, observed or considered in this study. Some of these parameters were recognized in the literature as indicators of difficulty [15, 18–21]. Saber and coworkers estimated the difficulty of LC in a group of patients only with acute cholecystitis, but did not propose a prediction model [21]. Surge and coworkers offered a scoring system based on operative findings and the degree of cholecystitis in order to outline key operative findings [22]. Other authors were focused to establish predictive factors for difficult LC and conversion [23, 24], or only for conversion [25], assuming that nowadays all cholecystectomies are treated laparoscopically. We tried to establish a simple model of prediction, which was then validated. Our study established a model for preoperative prediction with the overall high predictability of 82%. The model is simple to use, as it takes only 8 parameters into account, which could be staggered into 5 groups.

Conclusion

It is possible to establish preoperative parameters associated with the difficulty of cholecystectomy, and based on them to create a model for preoperative difficulty assessment of laparoscopic cholecystectomy. This prediction model is easy to use and shows high predictability values. In order to achieve a hallmark of laparoscopy, it is also necessary to determine and to analyze surgeon-depended parameters, and to create an overall model of prediction for laparoscopic cholecystectomy.

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INCIDENCE OF FALLS IN THE ELDERLY POPULATION

UČESTALOST PADOVA KOD STARIH OSOBA

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Summary

Introduction. Falls and fall-related injuries are common in the geriatric population and may be associated with significant morbidity and mortality. The aim of this study was to determine differences in the incidence of falls and risk factors for falls between the community-dwelling and institutionalized elderly people. **Material and Methods**. The cross sectional study was conducted in the period from May 1, 2015 to December 1, 2015, including 300 community-dwelling elderly people and 110 nursing home residents. The research instruments were a sociodemographic questionnaire, Mini nutritional assessment, Katz index, and Lawton scale. **Results.** During the previous 12 months, at least one fall was reported in 17.1% of the total number of respondents. The incidence of falls was higher among nursing home residents ($c^2 = 13.550$; $p = 0.001$). The risk factors for falls were multifactorial. Community-dwelling elderly suffered from urinary incontinence more frequently compared to nursing home residents ($p = 0.004$), but a significantly worse nutritional status was found in community-dwelling elderly people ($p < 0.001$). Assistance in basic activities of daily living was required by 3.9% of nursing home residents, and 8.3% of community-dwelling elderly people ($p < 0.001$). Statistically significant difference was not found in the performance of instrumental activities of daily living ($p < 0.145$). **Conclusion.** The incidence of falls was higher in nursing home residents. Risk factors for falls in both examined groups were visual and hearing impairments, urinary incontinence, use of 3 medications per day, damaged functional status, and malnutrition. Prevention of falls requires modification of environmental hazards, reduction of risk factors, their evaluation and strategy implementation.

Key words: Accidental Falls; Aged; Risk Factors; Accident Prevention; Geriatric Assessment; Nursing Homes; Surveys and Questionnaires

Sažetak

Uvod. Padovi i povrede u vezi sa padovima uobičajeni su u gerijatrijskoj populaciji, a utiču na morbiditet i mortalitet. Cilj ovog istraživanja bio je da se utvrdi razlika u učestalosti padova i vodećih faktora rizika za padove kod osoba starijeg životnog doba koji su smešteni u gerijatrijskim ustanovama i starih osoba koje žive u zajednici. **Materijal i metode.** Studija preseka obuhvatila je 300 starih osoba koje su stanovale u zajednici i 110 stanovnika hospitalizovanih u staračkom domu, a istraživanje je sprovedeno u periodu od 1. maja 2015. do 1. decembra 2015. godine. Instrumenti istraživanja su sociodemografski upitnik, Mininutritivna skala; Kacov indeks i Lavtonova skala. **Rezultati.** Tokom prethodnih 12 meseci, najmanje jedan pad je imalo 17,1% ispitanika od ukupnog broja. Učestalost padova bio je veći kod ispitanika u staračkom domu ($c^2 = 13.550$; $p = 0,001$) a faktori rizika za padove su multifaktorski. Starije osobe koje žive u zajednici češće pate od urinarne inkontinencije u odnosu na ispitanike iz staračkog doma ($p = 0,004$). Značajno lošije stanje uhranjenosti je pronađeno kod starijih osoba u zajednici ($p < 0,001$) a potreba za pomoć pri obavljanju osnovnih aktivnosti svakodnevnog života je pronađeno kod 3,9% ispitanika u staračkom domu i 8,3% kod ispitanika u zajednici ($p < 0,001$). Statistički značajna razlika nije pronađena kod ispitivanja instrumentalnih aktivnosti svakodnevnog života ($p < 0,145$). **Zaključak.** Učestalost padova bila je veća kod ispitanika u staračkom domu. Faktori rizika za padove u obe ispitivane grupe su vizuelni i slušni poremećaji, inkontinencija, upotreba tri leka dnevno, oštećen funkcionalni status i neuhranjenost. Prevencija padova treba da sadrži modifikaciju opasnih faktora sredine, procenu i lečenje.

Ključne reči: slučajni padovi; stari ljudi; faktori rizika; prevencije nesreća; gerijatrijska procena; staračkih domovi; ankete i upitnici

Introduction

Contemporary societies are facing high increase of population over 65 years of age within the total population. It is a result of lifespan extension, advances in medicine and science in general, improvement of quality of life, etc. [1]. The elderly represent a vulnerable population group whose needs are numerous, diverse and highly specific. Health, social and economic needs of this category are intertwined and mutually connected as a whole, which requires specific approaches and adjustment of work in family medicine to these needs. High incidence of chronic and degenerative diseases leads to progressive decrease of functional skills in physical, psychological, but also in all other aspects of life [2, 3].

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Abbreviations
MNA – mini nutritional assessment
IADL – instrumental activities of daily living
ADL – activities of daily living
SPSS – statistical package for social sciences

Fall represents a sudden, unexpected change of position, during which static and mechanical mechanisms cannot function, and volitional mechanisms or reflexes responsible for maintenance of balance are not adequate [4]. The incidence of falls increases with age and frailty level. Older people who are living in nursing homes fall more often than those who are living in the community [5]. Numerous falls occur as an interaction of several factors, and they are divided into three basic groups: internal risk factors, external risk factors, and situational risk factors. Falls are the leading cause of lethal injuries in persons older than 65. In clinical-hospital institutions, falls are the most frequent unexpected incidents and make about 70% of all unwanted hospital occurrences [6]. Injuries which occur as a result of falls range from emotional disturbances to serious physical injuries. Falls may reduce the quality of life primarily because of the fear of repeated falling. Repeated falls extend hospitalization, increase the risk for repeated admission, and due to long-lasting care they increase the use of resources as well (financial funds). About half of adults with thigh fracture after a fall cannot go home, or establish a functional level they had before the injury [7]. In the United States, more than three fourths of deaths caused by falls occur in 13% of population older than 65, which indicates it is a primarily geriatric syndrome [7]. The incidence of falls within one year was 44%, whereas one kind of fall was present in 25% of cases, and recurrent falls (two or more) were present in 19% of cases. Women have statistically more individual falls (29% over 19%), than recurrent falls (22% over 12%). Falls at home were recorded in 41% of cases, and outdoor falls were recorded in 42% of cases, whereas in 17% of cases falls occurred both at home and outdoors. Serious injuries were recorded in 11.5% of those who fell (broken hips 1.4%, and other fractures in 6.6% of cases), whereas 49% had only minor inju-

Table 1. Data on the sex, age, marital status, spending time with friends, vision, hearing and incontinence
Tabela 1. *Podaci o polu, starosti, bračnom statusu, provođenju vremena sa prijateljima, vidu, sluhu i inkontinenciji*

	Nursing Home/*Staračka dom*	Health Center/*Dom zdravlja*	p/*p*
Sex/*Pol*			
Male/*Muški*	7.1%	31.5%	
Female/*Ženski*	19.5%	42.0%	0.003
Age/*Starost*			
70 - 79 years/*70–79 godina*	13.9%	5.4%	0.000
> 80 years/> *80 godina*	12.7%	68.0%	
Marital status/*Bračni status*			
Yes/*Da*	1.7%	31.0%	
No/*Ne*	24.9%	42.4%	0.000
Hobby/*Hobi*			
Yes/*Da*	1.2%	17.1%	
No/*Ne*	25.4%	56.3%	0.000
Spending time with friends/ *Provođenje vremena sa prijateljima*			
Yes/*Da*	25.9%	57.3%	
No/*Ne*	0.7%	16.1%	0.006
Visual disorders/*Vizuelne smetnje*			
Yes/*Da*	13.4%	25.6%	
No/*Ne*	13.2%	47.8%	0.000
Hearing disorders/*Poremećaji sluha*			
Yes/*Da*	3.2%	27.3%	
No/*Ne*	23.4%	46.1%	0.000
Urinary incontinence, defecation/ *Urinarna inkontinencija, defekacija*			
Yes/*Da*	8.8%	15.6%	
No/*Ne*	17.8%	57.8%	0.019

$p < 0.003$ related to the sex of examinees; $p < 0.000$ related to age, marital status, hobby, visual and hearing disorders
$p < 0,003$ u odnosu na pol ispitanika; $p < 0,000$ u odnosu na uzrast, bračni status, hobi, poremećaj vida i sluha

ries [8]. Almost one third of institutionalized persons fall at least once in a lifetime. Annual prevalence in persons older than 65 is 28% [9], and 15% of persons who fall belong to the group of healthy elderly people [10]. Within comprehensive geriatric assessment, physical health, functional capacity, cognitive functions, mental health, social status and environmental factors may be evaluated [11].

The aim of this study was to determine the incidence of falls and risk factors for falls between the community-dwelling and institution-dwelling elderly people. The basic research question was whether there was a difference in the incidence of falls and risk factors for falls between the elderly people living in the community and nursing homes in Bosnia and Herzegovina from the point of view of fall prevention.

Material and Methods

The cross sectional study included 300 community-dwelling elderly people and 110 nursing home residents older than 65 years of age.

Inclusion criteria for this study were orientation in time, place and person, and informed consent for taking part in the study. Elderly people with poor general health condition, disorientation and lack of cooperation were excluded.

All respondents were informed about the objective of the study, and their written informed consent was sought and obtained. The study was carried out in the period from May 1, 2015 to December 1, 2015. A general questionnaire, mini nutritional assessment (MNA), Katz index for the assessment of basic activities of daily living (ADL), and Lawton scale for instrumental activities of daily living (IADL) were used in this study. The incidence of falls assessment was based on the following question: ''Have you had any falls within the last 12 months?''

The MNA [12] was used for screening and estimation of nutrition status. The instrument consisted of 18 questions which included 4 parameters: anthropometric measurements, general estimation, eating habits, and subjective estimation of examinees. The total MNA score was between 0 and 30 points: the score 24 and over indicated a healthy nutritional status, whereas the score under 17 indicated undernourishment.

Lawton IADL was used for performance assessment for more complex activities and performance of instrumental activities (the use of phone, cooking, shopping, etc.). The activities were evaluated on an ordinal scale from 0 - 6 points, indicating independence to complete helplessness >11 [13].

Katz index of independence in ADL was used to rank 6 basic functions (taking a bath, dressing up, hygiene, movement, incontinence and nutrition) on an ordinal scale [14] also indicating persons without functional damage (6 points) to serious damage (2 points).

The statistical package for social sciences (SPSS) was used for data processing in this investigation. Statistically significant difference was established by c^2 test, and it was estimated based on the significance level $p < 0.05$. The gathered data are shown in tables.

All participants gave their signed informed consent. The authors used the provided data, but concealed the participants' identities.

Results

The study included 61.5% of females and 38.5% of males. Most of the respondents were not married (67.3%). More than a half of the respondents (81.7%) had no hobbies ($c^2 = 21.020$; $p = 0.001$). Thirty-nine percent stated that they had visual disorders, whereas 30.5% presented with impaired hearing ($c^2 = 24.136$; $p = 0.001$). The incidence of urinary incontinence was higher in community-dwelling elderly people compared to nursing home residents ($p = 0.004$) **(Table 1)**.

During the previous 12 months, at least one fall was recorded in 17.1% of the total number of respondents. The frequency of falls was higher among nursing home residents ($c^2 = 13.550$; $p = 0.001$). The risk factors for falls were multifactorial. Most of the respondents (98.3%) reported good access to medical help, whereas others stated that they could not get an appointment with their family practitioner easily. There was a statistically significant difference between the examined groups in use of medicines **(Table 2)**.

The need for assistance in basic activities of daily living was reported by 3.9 % of nursing home residents and 8.3% of community-dwelling people ($p < 0.001$). However, statistically significant difference was not found in the performance of IADL ($p < 0.145$) **(Table 3)**.

High risk of undernourishment was found in 46.5% of the respondents: 36.9% were undernourished, whereas only 16.6% had a normal nutritional status. A significantly worse nutritional status was found in community-dwelling elderly people ($p<0.001$) **(Table 4)**.

Discussion

Comprehensive geriatric assessment was carried out in 410 elderly people. More than half of the respondents (61.5%) were women. The study showed that the incidence of falls (17.1%) was high. Risk factors for falls among the nursing home residents included vision impairment, intake of more than 3 medicines a day, damaged functional status, whereas in the group of community-dwelling people risk factors were vision and hearing impairment, incontinence, previous positive history of falls, damaged functional status and malnutrition. More than half of the respondents had two or three risk factors for falls, which indicates that falls are a significant problem in the elderly. The results obtained in our study, related to risk factors and incidence of falls in elderly people are in accordance with other researches [15]. One of the problems in geriatric population is urinary and

Table 2. Data on used medicines, incidence of falls and access to health services
Tabela 2. *Podaci o korišćenim lekovima, učestalosti padova i pristup zdravstvenim uslugama*

	Nursing Home/*Starački dom*	Health Center/*Dom zdravlja*	p/*p*
Intake of more than 3 medicines *Uzimanje više od 3 leka*			
Yes/*Da*	16.3%	0.2%	0.001
No/*Ne*	10.2%	36.3%	
Falls during the last 12 months *Padovi tokom poslednjih 12 meseci*			
Yes/*Da*	7.6%	9.5%	0.001
No/*Ne*	19.0%	63.9%	
Access to health services *Dostupnost medicinske pomoći*			
Yes/*Da*	26.1%	72.2%	1.000
No/*Ne*	0.5%	1.2%	

$p < 0.001$ related to the number of medicines; $p < 0.001$ related to previous falls
$p < 0,001$ u odnosu na broj lekova; $p < 0,001$ u odnosu na prethodne padove

fecal incontinence. In our investigation, the incidence of fecal or urinary incontinence provided important results, since this problem is more common in outpatient examinees (15.6%), and in the group of the examinees who lived in nursing homes the incidence was 8.8%. According to the investigation of Perry et al., about 20% of elderly people who live in the community have urinary incontinence, and 30% - 60% of elderly people who live in institutions have problems with incontinence [16]. Frequent urinary infections, being the most frequent diseases among the users of Gerontology Centers, were reported by 9 (22.5%), 25 (62.5%) and 16 (40%) users, respectively [17]. It is clear that proper nursing care and application of preventive health measures for persons with incontinence, e. g. Kegel exercises, as well as avoidance of urinary catheter placement, unless it is really necessary, the number of persons with these problems can be greatly reduced. Our investigation showed that most of the examinees took more than 3 medicines per day, and that polypharmacy is more frequent in the examinees that lived in nursing homes, whereas 63.9% of outpatient examinees used less than 3 medications per day. Although comprehensive assessment was less applied in primary health care, the positive effects of work in the primary care were noticeable, as well as in the life of patients who lived in the community [17–20]. Contrary to the findings of other studies, the total number of medicines taken per day, showed an independent connection in relation to falls [21–25]. Nursing care requires collaborative care in all institutions, and it includes health promotion, prevention of diseases and injuries, care for diseased and elderly people. On average, the community-dwelling people take 3 medicines, and nursing home residents 8 medicines. Intake of more than 3 medicines a day causes a lot of side effects and interactions, which may increase the risk for falls [26–28]. Our investigation showed that the incidence of falls during the last 12 months was 7.6% in the examinees who lived in homes for elderly people, and 9.5% in the outpatient examinees. The total prevalence of falls in the examined group was about 20%, which is lower than reported by other authors [29]. The risk factors for falls in the elderly people include physical inactivity, muscular weakness, postural hypotension and polyphar-

Table 3. Functional status estimated by means of Katz index and Lawton scale
Tabela 3. *Funkcionalni status procenjen pomoću Kacovog indeksa i Lovtonovog indeksa*

	Nursing Home/*Starački dom*	Health Center/*Dom zdravlja*	p/*p*
Katz index/*Kacov indeks*			
0 - 3	3.9%	8.3%	0.000
4 - 7	14.4%	2.4%	
> 7	55.1%	15.9%	
Lawton index/*Lavtonov indeks*			
0 - 6	7.6%	3.7%	0.145
7 - 11	15.1%	7.3%	
> 11	50.7%	15.6%	

$p < 0.000$ related to the functional status - Katz index
$p < 0,000$ u odnosu na funkcionalni status – Kacov indeks

Table 4. Results obtained on the nutritional status
Tabela 4. *Rezultati ispitivanja nutritivnog statusa*

	Nursing Home/*Staračkidom*	Health Center/*Dom zdravlja*	p/*p*
Mini nutritional assessment/*Mini nutritivna procena*			
< 17	1.5%	35.5%	
17 - 23.5	18.6%	27.9%	0.000
> 24	6.6%	10.0%	

p < 0.000 related to the total nutritional status/*p < 0,000 u odnosu na ukupan status ishrane*

macy [30]. Many studies showed that the history of previous falls was not a risk factor for falls, but it should give medical workers a warning in order to examine physiological deficits and chronic diseases in the elderly and take appropriate measures in order to prevent repeated falls [31].

The results which are related to ADL and IADL showed various structures regarding the functional status. The examination of functional abilities by means of Katz and Lawton scale showed that many respondents needed assistance or they were dependent on others while doing their daily activities and IADL. We can see that the nursing home residents were more dependent on the help of others for all necessary activities. The respondents who needed help were mostly immobile or their movement was decreased to a minimum. Based on the results of this investigation, we analyzed the data related to the functional status which showed individual differences among the groups. There are a certain number of respondents older than 80 years, with a very good functional status, but, on the other hand, there are younger respondents with worse functional status because their ADL were limited. Among the elderly people, as well as among those who lived in nursing homes, there were a certain number of those with a satisfactory functional status, and they were completely independent, but also those with a different level of risk for lower functional status, weaknesses, and even death [32–34]. The nutritional status assessment in the studied two groups showed the presence of undernourishment, especially among community dwellers. Other studies also showed that malnutrition was common among the elderly people [35, 36]. Some investigations showed that malnutrition was more common as people got older, and some investigations found that the prevalence of this problem after the age of 65 years ranged from 16–85% [37, 38]. In Europe, Naber et al. found malnutrition in 45% of internal and gastrointestinal patients [39]. A Brazilian national study showed the presence of malnutrition in 48.1% of patients upon admission to hospitals or nursing homes [40]. Nutritional problems may affect the general health condition in elderly people. Various necessary measures for estimation of the nutritional status and undernourishment should become an integral component of comprehensive geriatric assessment, identifying the problems related to the nutritional status.

Conclusion

The frequency of falls was higher in nursing home residents. Risk factors for falls in both examined groups were visual and hearing disorders, incontinence, use of 3 medicines per day, damaged functional status and malnutrition. Prevention of falls should include modification of environmental hazards, evaluation of risk factors, and treatment options.

Comprehensive geriatric assessment of vulnerable and patients with chronic disease, may improve their adequate nursing care as well as clinical outcomes. One of the important tasks of the whole society is to put prevention from falls to a higher level and pay adequate attention to this great health problem in the elderly population.

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SPONTANEOUS CEREBELLAR ACTIVITY AND ITS ROLE IN NEUROTOXICITY

SPONTANA AKTIVNOST MALOG MOZGA I NJEGOVA ULOGA U NEUROTOKSIČNOSTI

Ljiljana MARTAĆ, Gordana STOJADINOVIĆ and Branka PETKOVIĆ

Summary

Introduction. In performed experiments, the parietal electrocortical activity of the cerebral cortex and the activity of the cerebellar cortex were simultaneously recorded. The main purpose was to compare their spontaneous activity, as the recording was conducted during anesthesia. **Material and Methods.** We used 2–3 months old rats weighing 200 – 350 g. Two groups of rats with same characteristics were made. The first group of 15 rats (control group) was recorded under anesthesia and there was a change in spectral power in accordance with frequency ranges. The second group of 30 rats was recorded under the same experimental conditions, but the rats were treated intraperitoneally by aluminium chloride hexahydrate solution or with 1.5% solution of aluminium chloride hexahydrate per os. **Discussion.** Changes in spontaneous activity of the cerebellum during aluminium intoxication were compared with control values. It was shown that lesion and neurotoxicity during stable anesthesia led to desynchronization of the cerebellar activity. This is described by the change in fractal dimension of cerebellar electrocortical activity. The assumption is that the inhibition caused by anesthetic is compensated during aluminium intoxication. **Conclusion**. The cerebellum plays a role in compensation through changes in spontaneous activity. This response involves an increase in the value of fractal dimension of cerebellar electrocortical activity which is reduced in neurotoxicity.

Key words: Aluminum; Cerebellum; Action Potentials; Neurotoxicity Syndromes; Neuronal Plasticity; Adaptation, Psychological; Rats; Anesthesia

Sažetak

Uvod. U eksperimentima je uporedo registrovana elektrokortikalna aktivnost parijetalne kore velikog mozga, kao i aktivnost kore malog mozga. Cilj je da se uporedi njihova spontana aktivnost jer se registrovanje vrši u anesteziji. **Materijal i metode**. U istraživanju smo koristili pacove starosti 2 - 3 meseca, telesne mase 200 - 350 g. Formirane su dve grupe pacova sa istim karakteristikama. Kod prve grupe, koju cinilo je 15 pacova (kontrolna grupa) ispitivanje je rađeno u anesteziji, zabeležene su promene spektralne snage u skladu sa obimom frekvencije. Druga grupa od 30 pacova je ispitivana pod istim eksperimentalnim uslovima, ali su pacovi tretirani rastvorom aluminijum hlorid heksahidrata, ili 1.5 % rastvorom aluminijum hlorid heksahidrata *per os*.**Diskusija**. Promena spontane aktivnosti malog mozga u uslovima intoksikacije aluminijumom je poređena sa kontrolnim vrednostima. Pokazano je da lezija i neurotoksičnost u uslovima stabilne anestezije dovode do desinhronizacije aktivnosti malog mozga. Ovo je opisano promenom fraktalne dimenzije elektrokortikalne aktivnosti. Pretpostavka je da se inhibicija izazvana anestetikom kompenzuje u uslovima intoksikacije aluminijumom. **Zaključak**. Mali mozak ima ulogu u kompenzaciji putem promene spontane aktivnosti. Ovakav odgovor podrazumeva povećanje vrednosti fraktalne dimenzije elektrokortikalne aktivnosti malog mozga koja je u uslovima neurotoksičnosti smanjena.

Ključne reči: aluminijum; mali mozak; spontana aktivnost; neurotoksični sindromi; neuroplastičnost; adaptacija; pacovi; anestezija

Introduction

Cerebellar electrocorticographic (ECoG) activity is characterised by the presence of synchronized oscillations. Synchronization involves oscillation of two or more different structures that are at the same phase. There are two hypotheses on the role of synchronized oscillations: a role of testing conditions, or in the preparation of motor activities [1]. It is known that the cerebellum has other roles as well, such as: regulation of motor activities, cognitive processes, role in motor studying, and cognitive function.

A group activity of neurons of the cerebral and cerebellar cortex was recorded. The recording of the parietal cortex was carried out in order to monitor changes due to anesthesia. The electrocortical activity of the cerebellar cortex was recorded at different depths (0.5 - 1.5 mm) for the purpose of getting an insight based on local potentials of the cortex field, of the entire cortex activity in coordination with other cerebellar structures (cerebellar network).

Previous research of parietal cerebral cortex lesion revealed the specific role of the cerebellum in reparation of the damaged function - plasticity [2]. Also, the

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Abbreviations

ECoG – electrocorticography
FD – fractal dimension
DC – direct current
GABA-A – gamma-aminobutyric acid type A

tested activity was monitored in different experimental conditions and types of anesthesia [3] in order to cover all changes in the activity of various layers of the cerebellar local circuit. During stable anesthesia (the same relative spectral power of the cerebellum and delta activity of the cerebrum of approximately 45%) we recorded a spontaneous activity of rats' cerebellum under controlled conditions and after aluminium treatment. The neurotoxic effect of aluminium can be described by spectral and fractal analysis [4]. The oscillating cerebellar activity is associated with fluctuations between excitation and inhibition of the cerebellar structures [5].

Changes in spontaneous activity during anesthesia and aluminium intoxication led to desynchronization of the rhythm and change in the balanced state**.** During anesthesia, this change is offset by a change in neurotransmission, while in aluminium intoxication, this activity has been altered. Since anesthesia and aluminium intoxication have different mechanisms of action and affect the entire cerebellar network, it is possible to monitor the role of the cerebellum, that is, to monitor the adaptability and plasticity as a difference in the spontaneous activity in anesthetized and intoxicated units. It is possible also, by comparing their activity, to quantify the change by calculating values of the fractal dimension (FD) of electrocortical activity. The anesthetic effect is reversible, while in aluminium intoxication there is an adaptable response. An increase in FD of electrocortical activity during anesthesia suggests a change in the activity of cerebellum, which is related to neurons' inhibition and excitatory connections blockade. Variable values of FD electrocortical activity in aluminium intoxication indicate changes in neurotransmission which differ from mechanisms of anesthesia and are responsible for the change in the state of the cerebellar network. The difference in FD of electrocortical activity between these two conditions (intoxication and anesthesia) is in compliance with the adaptive ability of the cerebellum.

Material and Methods

In this experiment, male Wistar and Sprague Dawley rats were used. By using these different sorts of rats, it was possible to monitor stereotyped functions and individual variability that changes due to change in physiological parameters. The animals were kept in cages, with 12-hour exposure to light and 12-hour exposure to dark, with an unlimited access to briquetted food and water, at a temperature of 18 to 21ºC. These uniform breeding conditions allowed us to compare different sorts of rats. All experiments were carried out in compliance with the Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes and was approved by the Ethics Committee of the Institute for Biological Research "Siniša Stanković", University of Belgrade.

We used 2–3 months old rats weighing 200 – 350 g. Two groups of rats with same characteristics were made. The first group of 15 rats (control group) was recorded under anesthesia and there was a change in spectral power in accordance with frequency ranges. The second group of 30 rats was recorded under the same experimental conditions, but the rats were treated intraperitoneally by aluminium chloride hexahydrate solution or with 1.5% solution of aluminium chloride hexahydrate per os. The procedure of aluminium intoxication lasted at least 4, and at most for 6 weeks, so the rats were just over 3 months old at the time of recording. The surgical procedure for setting-up the electrodes was carried out on animals anesthetized with nembutal (Pentobarbital Sodium, SERVA, Heidelberg, Germany) or by zoletil (Virbac S., A. Carros, France), which was given intraperitoneally (i. p.) at a dose of 45 mg/kg of nembutal i. e. 60 mg/kg of zoletil. Even though the molecular mechanisms of these two anesthetics are different, their effects are similar and involve an increase of the relative spectral power in the delta range. During the experiment, anesthesia was added at a dose of about 8 mg/kg. The anesthetized animals were fixed in a stereotaxic frame. Afterwards, a craniotomy was carried out, and electrodes were set up. Craniotomy was carried out on parietal bones, by drilling round holes 2 mm in diameter, with coordinates: 2 - 2,5 mm posterior to the bregma and 2 mm lateral to the sagittal suture, and 10,5 mm posterior to the bregma and 1 - 1,5 mm lateral to the sagittal suture [6]. The activities in the parietal and cerebellar cortex were recorded simultaneously. Changing the position and the insertion depth of electrodes showed that there was an overlap of the recorded local field potentials, describing the parietal activity during anesthesia, as well as in the entire cerebellum together with all cerebellar structures. Delta activity is predominant in anesthesia and its activity in the parietal cortex is variable in anesthesia. Signals recorded at 5 - 15 min were selected, after anesthesia and in stable anesthesia, and a value of about 45% of the relative spectral power of delta range of the cerebral cortex was selected. During stable anesthesia, changes in the delta and theta rhythm of the parietal cortex were monitored, showing the level of intoxication. During stable anesthesia, as well as during exposition to neurotoxicity, there was a selection of signals in the cerebellar cortex. We compared the activity in the cerebellar cortex during stable anesthesia with the changes of slow-wave components in intoxication. Recording of the activity in the cerebral parietal cortex and cerebellar cortex was performed by tungsten electrodes which were positioned at 0,5 - 1,5 mm within the cerebral cortex. The recordings were done at different depths, in order to describe the average activity of the local circuit of the cerebellum, including all the structures. By selecting a signal with a certain relative spectral power, we achieved uniformity, thus avoiding variability due to anesthesia and intoxication that vary due to various applications or doses of aluminium. Only neurotoxicity in experimental models was monitored by increasing the delta and theta rhythms, thus avoiding differences due to uneven application of aluminium, as

well as dose dependency and length of the treatment. Signals were observed on an oscilloscope (Textronix, USA). Amplification of the signal was carried out using the Multi Channel Processor-Plus (Alpha Omega Engineering, Israel) amplifier**.** Filtering of the signals was performed as well. The filter parameters were direct current (DC) for high-pass filter and 150 Hz for low-pass filter. The entire experiment of recording the cerebral cortex was performed in 30 - 120 min. The recording of brain's activity was performed at intervals, every 5 – 10 min, and lasted for 121 sec. In this way, we have made a signal file recorded at every 5 min for 120 min with signals lasting 121 seconds, which is optimal time frequency of 128 Hz.

Analogue digital conversion was carried out at a sampling frequency of 256 Hz. Recording was performed with 4 i. e. 2 tungsten electrodes, with one electrode for grounding. All data were recorded with a program for signals' acquisition SIGVIEW [7].

The analysis of the recorded electrocortical activity in the cerebral and cerebellar cortex was carried out in FORTRAN programs under DOS operating system and in the Matlab 6.5. Program, Windows operating system. We used programs designed by the associates of our neurophysiological laboratory (Janković B, Kalauzi A). The recorded signals were filtered at 50, 60 and 101 Hz and any possible irregularities (drift, interferences and movements) were excluded from the analysis.

The recorded 121 seconds-long signals were saved as binary files. For each recorded signal, Fourier transformation was performed for 15 epochs that lasted 8 sec. The obtained power spectra (relative spectral power) were compared and the mean value of the entire recording experiment was calculated, and a standard deviation was determined. Within analysis of spectra, we monitored the relative spectra power at frequency ranges: delta (0,1 - 4 Hz), theta (4,1 - 8 Hz), alpha (8,1 - 15 Hz), beta (15,1 - 32 Hz) and gamma range (above 32 Hz).

Fractal analysis belongs to a group of nonlinear ECoG analysis methods. The value of FD of electrocortical activity was calculated using the Higuchi's algorithm [8, 9]. The size of non-overlapping window was 200 points, which corresponds to an epoch of 0,781 seconds at a 256-Hz sampling frequency. In regard to k_{max} parameters, an optimal value of k_{max}=8 was established [10]. Based on the obtained FD electrocortical activity of individual windows, the mean value and standard deviation of the FD electrocortical activity of the entire signal was estimated. The FD values of electrocortical activity range from 1,000 – 2,000.

The power spectrum was described by statistical parameters, i. e. mean value and standard deviation for 15 registered epochs. Also, the entire procedure of recording was done in each experimental animal, determining the mean value and standard deviation. This kind of descriptive analysis provides data on the distribution of relative spectral power across frequency ranges. The mean value indicates grouping of the variable value, while the standard deviation indicates its variations. In a group of similar population with a group of individuals with similar characteristics of the delta rhythm, the hypothesis tested changes in biological parameters by t-test with a level of significance of 0,05. These tests indicate changes in the activity of a group of neurons within the neurotransmitter size, or with respect to the connection between the cerebral regions and the change in functionality of this connection. This experiment includes both changes in the activity of the brain due to aluminium intoxication.

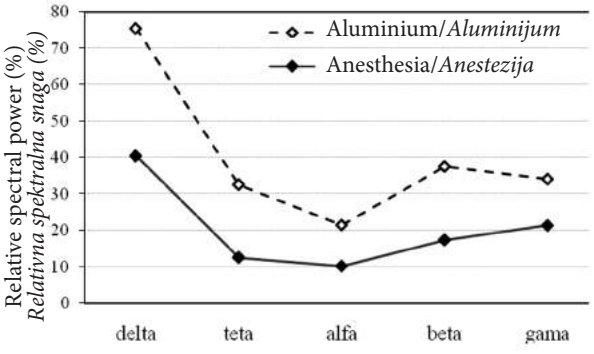
Results

Table 1 shows results obtained by comparing rats treated with aluminium (2 mg/kg i. p.) and rats exposed only to the effects of anesthesia, i. e. they were not treated with aluminium. Spectral analysis showed the change in the relative spectral power of the signal in different frequency ranges (delta, theta, alpha, beta and gamma range). Increase in the relative spectral power due to anesthesia effects (Zoletil) in a model of aluminium neurotoxicity in the delta range is an indicator of neurotoxicity and compared to the group of anesthetized rats (dominant delta range), it differs in the cerebrum and cerebellum. Namely, due to inhibition and lack of sensory-motor functions, we can talk about spontaneous activity of neurons of cerebral and cerebellar cortex in the anesthetic state, as well as change of the spontaneous activity due to neurotoxicity. Both anesthesia and neurotoxicity showed increased relative spectral power in the delta range, but mechanisms of action are different. By comparing activities during anesthesia and during intoxication, we obtained a full picture on spontaneous activity and its changes.

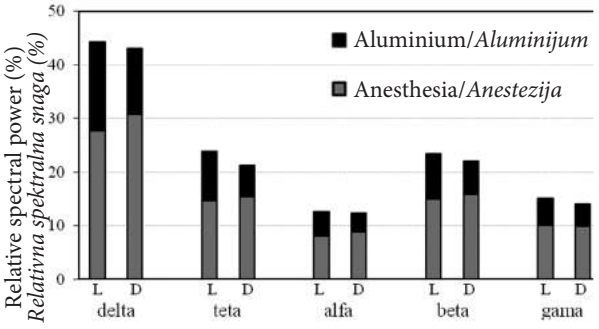
In the experiments, we have simultaneously recorded the parietal electrocortical activity of the cerebral cortex activity in order to monitor changes after anesthesia, based on the relative spectral power in the delta range, as well as different cortex layers of chemosphere of the back cerebellar lobe **(Table 1)**. The ob-

Table 1. Changes in the relative spectral power in frequency ranges of the cerebrum and cerebellum, anesthesia vs. treatment with aluminium (2 mg/kg i. p.)
Tabela 1. *Promena relativne spektralne snage po frekventnim područjima za veliki i mali mozak, anestezija vs. tretman aluminijumom (2 mg/kg i.p.)*

p value/*p vrednost*	Delta/*Delta*	Theta/*Teta*	Alpha/*Alfa*	Beta/*Beta*	Gamma/*Gama*
Cerebrum/*Veliki mozak*	0.0016	0.4913	0.0002	0.0072	< 0.0001
Cerebellum/*Mali mozak*	0.0075	0.1624	0.0378	0.0246	< 0.0001



Graph 1. Relative cerebellar spectral power in anesthetized rats (10 rats) and rats treated with aluminium (1.5 % solution of aluminium chloride hexahydrate per os, 10 rats).
Grafikon 1. *Relativna spektralna snaga malog mozga kod pacova pod anestezijom (10 jedinki) i pacova tretiranih aluminijumom (1,5% rastvor aluminijum-hlorid heksahidrata per os, 10 jedinki*)



Graph 2. Changes in the cerebellar relative spectral power – spontaneous activity in rats under anesthesia (10 rats) and in rats treated with aluminium (2 - 6 mg/kg i. p., 5 rats)
Grafikon 2. *Promena u relativnoj spektralnoj snazi malog mozga – spontana aktivnost kod jedinki pod anestezijom (10 jedinki) i kod pacova tretiranih aluminijumom (2 – 6 mg/kg i.p., 5 jedinki)*
Legend: L – left hemisphere, D – right hemisphere
Legenda: L – leva hemisfera, D – desna hemisfera

jective was to compare their spontaneous activity because recording was carried out under anesthesia. As a rule, the cerebrum is more susceptible to changes, while the cerebellum exhibits adaptive ability, and also has a role in the reparation of the damaged function - plasticity. During anesthesia, the cerebellum has a dominant delta activity, while during neurotoxicity, the delta activity fluctuates. The criterion for this change can be described by an increase in the relative spectral power within the delta range and by changing the FD of the neurotransmitting activity of the cerebellum. The role of the cerebellum in adaptability and plasticity is presented through spontaneous activity in the absence of stimuli and higher functional connections.

Spectral analysis shows changes in the relative spectral power across the frequency ranges. The present changes in relative spectral power are evident in all frequency ranges, indicating that anesthesia and aluminium have an effect on the entire neural network. Desynchronization of the rhythm is more exhibited in rats treated with aluminium than in anesthetized ones.

We presented the maximum change in relative spectral power in the group of rats treated with aluminium in frequency ranges, compared to the mean change in relative spectral power in the group of anesthetized rats.

Graph 1 shows the maximum change in individual rhythms of the relative spectral power in relation to the control group (anesthetized rats). All changes above the control level indicate intoxication. Both groups were recorded under anesthesia, with absence of sensory-motor reactions. The control group included 10 rats with stable anesthesia, and a minor change in the delta rhythm. The treated group also included 10 adult rats, exposed to solution of 1.5 % aluminium chloride hexahydrate per os, showing signs of intoxication. It appeared that there was a similar distribution of relative spectral power in the frequency ranges in the first and the second group of animals. It can be concluded that the spontaneous activity of the cerebellum cortex during anesthesia, as well as neurotoxicity, show the same distribution of relative spectral power, but under two different conditions of adaptation.

This kind of response in terms of changes in frequency ranges compared with spontaneous activity shows an increase in spectral power with symmetric distribution. This confirms the initial assumption that activity in a group of neurons was stimulated or inhibited simultaneously.

Graph 2 shows spontaneous activity of the cerebellum in rats treated with aluminium (2 mg/kg i. p.) during stable anesthesia (Zoletil). **Graphs 2 and 3** represent brain activity during anesthesia and neurotoxicity. The neurotoxicity of aluminium in anesthesia has a double effect. Anesthesia itself leads to an increase in relative spectral power within the delta range, while inhibition can affect glutamatergic transmission that occurs during toxicity. **Graph 3** shows the activity of the rat brain in the frequency ranges, and effects of anesthetic were described by standard deviation of the relative spectral power within the delta range**.** Changes in delta and theta ranges are related to plasticity, while in alpha and beta range they refer to higher sensory-motor performances.

Graph 4 shows an increase in the FD of electrocortical activity corresponding to the plasticity of the cerebellar cortex and the compensated toxicity, i. e. the adaptability of spontaneous activity of the cerebellum. The change in spontaneous activity of the cerebellum can be described by FD of electrocortical activity. An increase in FD of electrocortical activity during anesthesia suggests a change in the activity of the cerebellum, which is related to the inhibition of neurons and blocking of excitatory connections. This effect is reversible and indicates restoring the balance to reduce the effect of anesthesia. During aluminium intoxication, no changes were recorded, but the system oscillated around the balanced state. Even though anesthesia and

aluminium intoxication has different mechanisms, their effects are expressed on the entire local neural network of the cerebellum. Under controlled conditions (anesthesia), variability and differences between activities of the left and the right hemisphere were recorded, which points to the plasticity and connectivity of the local networks of the cerebellar cortex. However, this feature is partially lost during aluminium intoxication, which was illustrated by FD values of electrocortical activity in **Graph 4**. During intoxication, there was a stable change of FD electrocortical activity in time, indicating that aluminium poisoning affects also the functional activity of the cerebellar cortex. But unlike the cerebrum, the cerebellum has a greater plasticity of neural network, so the reduction in FD of electrocortical activity is slightly smaller. This is related to inhibition due to the effect of anesthetics and glutaminenergic differences in the activity of these different parts of the brain. In relation to the control group of anesthetized animals, the increase in the relative spectral power within the delta range and the decrease in FD of electrocortical activity is the extent of intoxication of the cerebellum.

Discussion

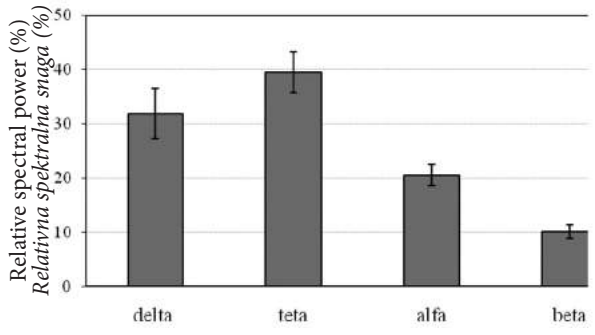
The cerebellar electrocorticographic activity is characterized by the presence of synchronized oscillations. Synchronization involves oscillation of two or more different structures at the same phase. There are two hypotheses on the role of synchronized oscillations: in testing conditions or in the preparation of motor activities [1].

In non-anesthetized rats, theta rhythm activity is dominant in cerebellum. It occurs in the granular layer and in an inert state of hemisphere it produces sensory information [11]. Theta activity of the granular cells occurs due to the repolarisation of the slow K^+ current [12]. K^+ and Na^+ current are sufficient for the occurrence of theta oscillations. Theta activity of the cerebellum is significant for synchronization and learning in the cerebellum. Theta oscillations of the cerebellum are also related to the activity of the neurons of the lower olive core or nucleus and its projections in the form of crawling fibres [13], as well as moss-like fibres that stimulate and inhibit grain cells.

In vivo stimulation of the lower olive nucleus induces oscillations over 10 Hz which are modulated by blockers of Ca^{2+} channels.

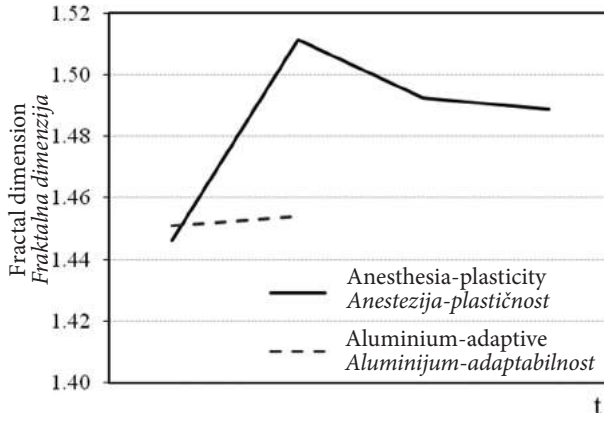
This activity is related to the Ca^{2+} transport [14]. The moss fibres stimulate the granular layer cells (granular and Goldgies) to oscillate with a frequency of 10 - 40 Hz. Oscillations depend on the excitation of the moss-shaped fibres during reducing the activation of parallel fibres [15]. During this process, desynchronization is caused by very weak activity of the moss fibres, a strong excitation of Goldgie cells, and activation of gamma-aminobutyric acid type A (GABA-A) receptors of granular cells. The mossy fibres are increasing the excitation of the grain cells, while Goldgie cells are inhibiting it. During desynchronization, the relation of excitation and inhibition is changing.

It has been shown that in lesions [16] and neurotoxicity [4] there is a desynchronization of the cerebellar activity. In case of lesions or neurotoxicity, the oscillatory activity is generated under the influence of input and output paths [5]. Spontaneous activity of the cerebellum is recorded in the absence of sensory-motor performances, and it describes the adaptive-plastic function in pathological conditions such as lesions or brain intoxication. Adaptability implies a change in the activity of the local cerebellar circuit, while plasticity implies a change in neurotransmitter activity under the influence of other structures. This activity in younglings involves compensation of changes and establishment a new balance, which has an adaptive value. In adult rats, the adaptability is associated with changes in the molecular and physiological neurotransmitter function which represents the plasticity



Graph 3. Average change in the biological rhythms of the cerebellum in 5 rats treated with aluminium (2 mg/kg i. p.) during anesthesia – relative spectral power in the frequency range

Grafikon 3. *Prosečna promena bioloških ritmova malog mozga kod 5 pacova tretiranih aluminijumom (2 mg/kg i.p.) u uslovima anestezije - relativna spektralna snaga po frekventnim oblastima*



Graph 4. Fractal dimension of electrocortical activity in rats under anesthesia (15 rats) and treated with aluminium (2 mg/kg i. p., 11 rats) – plasticity and adaptive change

Grafikon 4. *Fraktalna dimenzija elektrokortikalne aktivnosti kod pacova pod anestezijom (15 jedinki) i tretiranih aluminijumom (2 mg/kg i.p., 11 jedinki) – plastičnost i adaptivna promena*

of the neurological network. It has been shown that occurrence of dark neurons in the cerebrum is due to intoxication and indicates a pathological condition, i. e. neurotoxicity [17].

In the model of aluminium neurotoxicity, changes caused by anesthesia are reversible, while toxicity implies a change in spontaneous activity – plasticity and adaptability. These changes imply that desynchronized activity strives to establish a balance in terms of increasing slow-wave/sporadic components. Particularly, an increase in the delta range is an indicator of neurotoxicity, while change in the delta/theta relation is a criterion of intoxication [18]. The FD of electrocortical activity during neurotoxicity has a lower value [19]. The change in FD of electrocortical activity in anesthetized rats and those treated with aluminium, describes rhythm desynchronization. An increase in FD of electrocortical activity in some rats in aluminium-treated group indicates a change in the oscillatory activity of the cerebellum. The increase is based on the change in the spontaneous cerebellar activity.

Conclusion

The cerebellum plays a role in compensating for neurotoxicity by changing spontaneous activity. The change in spontaneous activity indicates a dual role of the cerebellum: plasticity and adaptability. This kind of response implies an increase in fractal dimension of electrocortical activity which is otherwise reduced during neurotoxicity. The change in the delta rhythm in anesthesia or during intoxication is associated with a reduction in fractal dimension of electrocortical activity of the cerebellum. In case of reduction of fractal dimension of electrocortical activity, there is a change in the rhythm of spontaneous activity. It can be concluded that the fractal dimension of electrocortical activity can describe the role of the cerebellum in neurotoxicity (plasticity and adaptability).

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KNOWLEDGE, ATTITUDES AND PRACTICES AMONG THE YOUTH IN BOSNIA AND HERZEGOVINA RELEVANT FOR REPRODUCTIVE HEALTH

ZNANJE, STAVOVI I PRAKSA MLADIH U BOSNI I HERCEGOVINI RELEVANTNI ZA REPRODUKTIVNO ZDRAVLJE

Azra HADŽIMEHMEDOVIĆ[1], Mahira JAHIĆ[2] and Selma MURATOVIĆ[3]

Summary

Introduction. The aim of this study was to investigate the sexual behavior, attitudes and knowledge on contraception, sexually transmitted infections and reproductive health among the youth in Bosnia and Herzegovina. **Material and Methods.** A prospective study included a sample of 6.000 subjects, aged 19 - 24, of both sexes, investigating the sexual behavior of the youth in Bosnia and Herzegovina in the period 2007 - 2009. **Results.** Of the interviewed examinees, 61% were sexually active, of which 56.4% had sexual intercourse with one partner ($z = 28.51$; $p < 0.001$). The average age of sexarche was significantly lower in males 17.34 ± 1.77 years, than in females 18.20 ± 1.84 years ($z = -14.44$; $p < 0.001$). Contraception was used by 67.6% at first intercourse, and by 70.4% at the last intercourse. The most common method of contraception was male condom (74%), coitus interruptus (11.1%), and oral hormonal contraceptives (14.9%). The examined subjects had inadequate knowledge (51.6%) about contraceptive methods, and it was significantly higher in males (64.6%) than in females (42.2%) ($z = 10.17$; $p < 0.001$). Some 4.9% of the sexually active females had intentional abortions. Up to 51.7% of females had never had a pelvic examination, and Pap test was done by 37.2%. The examinees obtained information about contraception and sexually transmitted infections from peers 50.7% and parents 9.7%. Only 28% of females had a positive attitude towards oral hormonal contraceptives. **Conclusion.** The interviewed young people in Bosnia and Herzegovina keep traditional sexual behavior patterns, attitudes and practices. However, more effort should be put into the improvement of knowledge on contraception, sexually transmitted infections, and healthcare protection.

Key words: Health Knowledge, Attitudes, Practice; Adolescent; Reproductive Health; Sexual Behavior; Contraception Behavior; Sexually Transmitted Diseases; Surveys and Questionnaires; Papanicolaou Test

Sažetak

Uvod. Cilj ove studije bilo je ispitivanje polnog ponašanja, stavova i znanja o kontracepciji i polno prenosivim infekcijama kod mladih u Bosni i Hercegovini u vezi sa reproduktivnim zdravljem. **Materijal i metode**. U prospektivnoj studiji na uzorku od 6.000 ispitanika, starosti 19 – 24 godine života oba pola, sprovedena je anonimna anketa o polnom ponašanju omladine u Bosni i Hercegovini u periodu od 2007. do 2009. godine. **Rezultati.** Polno aktivne omladine je bilo 61% od ispitanih, od čega je 56,4% polne odnose imalo samo sa 1 partnerom ($z = 28,51$; $p < 0,001$). Srednje doba seksarhe je statistički značajno manje kod mladića (17,34 ±1.77 godine) nego kod devojaka (18,20 ±1.84 godine) u Bosni i Hercegovini ($z = -14.44$; $p<0,001$). Kontracepciju pri prvom polnom odnosu koristilo je 67,6%, a pri poslednjem 70,4% ($z = -5.31$; $p < 0,001$). U najvećem broju 74% navodi se kondom kao metoda kontracepcije, 11,1% prekinuti snošaj i 14,9% oralna hormonska kontracepcija. Omladina (51,6%) nedovoljno poznaje metode kontracepcije, a proporcija mladića 64,6% sa nedovoljnim poznavanjem metoda kontracepcije značajno je veća u odnosu na devojke 42,2% ($z = 17.10$; $p < 0,001$). Namerni prekid trudnoće je imalo 4,9% polno aktivnih ispitanica. Devojke u 51,7% slučajeva nisu nikada obavile ginekološki pregled, a Papanikolau test je uradilo 37,2%. Informacije o kontracepciji i polno prenosivim infekcijama omladina dobija i traži od vršnjaka 50,7%, a 9,7% od roditelja. Ispitanice su u 28% slučajeva imale pozitivan stav prema oralnoj hormonskoj kontracepciji. **Zaključak.** Anketirana omladina u Bosni i Hercegovini u izvesnoj meri zadržava tradicionalni obrazac polnog ponašanja, stavova i praksi, ali treba raditi na poboljšanju znanja o kontracepciji, polno prenosivim infekcijama i zdravstvenoj zaštiti.

Ključne reči: znanje o zdravlju, stavovi, praksa; adolescenti; reproduktivno zdravlje; seksualno ponašanje; kontraceptivno ponašanje; polno prenosive bolesti; ankete i upitnici; Papanikolau test

Introduction

Due to the economic transition process, the youth in Bosnia and Herzegovina (BiH) has been burdened with the socio-economic stratification and uncertainty, undefined new social roles, the "crisis of the family" and low moral values. Although the sexual behaviors of the youth in our country differ from those in other countries, in relation to the beginning of sex life, use of efficient contraception, specific fertility and the number of intentional abortions, their common de-

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Abbreviations

BiH	– Bosnia and Herzegovina
STI	– sexually transmitted infection
UNDP	– United Nations Development Programme
Pap test	– Papanicolaou test

nominator is that they are reluctant to accept the model of reproductive health protection [1, 2].

The contemporary reproductive health concept was established at the International Conference on Population and Development held in Cairo in 1994, as a state of complete physical, mental, and social well-being in all matters relating to the reproductive system in all stages of life [3]. The early beginning of sexual activity, accompanied by later marriage, is resulting in a prolonged period of sexual activity before realization of the reproductive function. This consequently increases the risk of reproductive health disorders [1, 4].

Risky sexual behavior among the young population, intentional abortions, and sexually transmitted infections (STIs), cause infertility and a decrease in the biotic potential of the population [4, 5].

The primary reasons for high incidence of pregnancies, abortions, and STIs at this age lie in the insufficient use of contraception, limited access to the services for reproductive health, high biological vulnerability, and negative social reactions towards youth sexuality [6, 7].

Apart from the research on the sexual behavior of high school students of the Tuzla Canton and a questionnaire on the knowledge on contraception among high school students in Mostar [8–11], there are no data on the reproductive health in the youth of BiH. There is also a report on the research on the youth conducted by the United Nations Development Programme (UNDP) in BiH in 1999, but the data are rather brief and general [12].

The recent studies indicate that the youth who receive information on sexual issues (discussion and education – traditional, through the media as well as the Internet) are more responsible and their exposure to sexual risks is significantly lower compared to their uneducated and sexually uninformed peers [13, 14].

The aim of this study was to investigate the sexual behavior, attitudes and knowledge on contraception, reproductive health, and STIs among the youth in BiH.

Material and Methods

A prospective study included a representative sample of 6.000 subjects, aged 19 - 24, of both sexes, and investigated the sexual behavior among the youth in BiH in the period 2007 - 2009.

The survey was conducted among the youth of both sexes in four largest university cities in BiH: Sarajevo, Tuzla, Mostar, and Banja Luka. The total sample included 6.000 subjects aged 19 - 24, of both sexes, with various education background and professions, from different places and types of residence. Out of the total number, 5.000 were full time students at five public universities in BiH (University in Sarajevo, University in Tuzla, University in Banja Luka, and two Universities in Mostar) enrolled at different schools and study groups. They were at different years of studies, living with their parents, at dormitories, or renting a flat. The remaining 1.000 subjects were high school graduates, of various professions or unemployed, living with their parents or renting a flat. None of the subjects were married or had children.

This prospective study included the research instruments (questionnaire) previously designed for a PhD thesis at the Medical Faculty in Tuzla, adapted to our research.

The first part of the questionnaire included data on the subjects' demographic profile (time and place of birth, gender, profession, university, year of study and type of school, as well as the place and type of residence).

The second part of the questionnaire included data on the subjects' sexual behavior (beginning of sexual activity, usage and knowledge on contraceptive methods, counseling on contraception and STIs.

The third part of the questionnaire was intended only for the female subjects (3.492) gathering information on gynecological examinations, frequency of visits to the gynecologist and types of institutions at which they performed them, the number of intentional abortions, and the number of Pap smear tests.

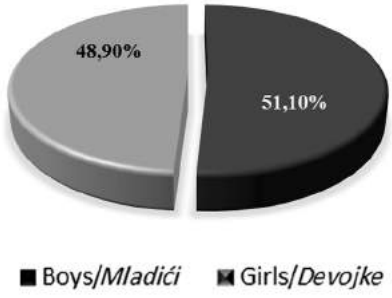
The questions related to the knowledge on contraceptive methods were scored as follows:

- knowledge on one or no methods (insufficient) - 0 points;
- knowledge on two to three methods (weak) - 1 point;
- knowledge on more than three methods (satisfactory) - 2 points.

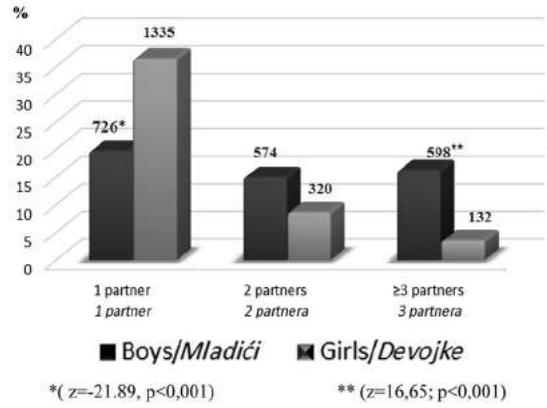
The sources of information on contraception and STIs were defined through nine offered options (school, friends, parents, healthcare workers, teachers, partner, TV, print media, Internet).

While conducting the survey, the subject sample included all female and male students who were present at lectures during the survey procedure and who accepted to participate. The survey was conducted by students – volunteers, previously educated on the procedure and the type of the survey. They first held a brief introductory session on the type and content of the questionnaire and gave instructions on how to fill in the questionnaire. The procedure typically lasted from 15 to 20 minutes. The survey was anonymous and approved by the university at which it was conducted.

The subjects who belonged to the group of non-students completed the questionnaire offered by volunteers of the AIDS Association, Youth Associations in Mostar and Sarajevo, Outpatient Clinic Banja Luka and Outpatient Clinic Tuzla. They were previously informed on the procedure and type of survey. The volunteers held a brief introductory session on the type and content of the questionnaire and gave instructions on how to fill in the questionnaire. The survey was anonymous and approved by the institution where it was conducted.



Graph 1. Gender distribution and sexual activity among the youth in BiH
Grafikon 1. *Polna distribucija u polna aktivnost omladine u Bosni i Hercegovini*



Graph 2. Gender distribution and the number of sexual partners in BiH
Grafikon 2. *Polna distribucija ispitanika i broj polnih partnera u Bosni i Hercegovini*

Standard methods of descriptive and inferential statistics were used. Quantitative data for sexarche were analyzed by ANOVA statistical procedure. Rank and range were used for quantitative data. Qualitative data were tested by the c^2 test and z-score to determine the statistical significance of differences. While testing the statistical hypotheses, the $p < 0.05$ was used as the criterion of significance. The programs Microsoft Office Excel (graphic presentation and statistical package) and Arcus QuickStat biomedical were used for statistical data processing.

Results

Out of the total of 6.000 subjects, there were 2.508 (41.8%) males and 3.492 (58.2%) females aged 19 to 24, with the average age of 21.5 years **(Table 1)**. In terms of education, there were 2.128 male students and 2.872 female students, along with 380 male and 620 female high school graduates. The subjects mainly lived with their parents (N = 3.042), rented a flat (N = 2.000), or lived in dormitories (N = 948).

Out of 6.000 young participants in BiH, 3.659 (61%) were sexually active, without a statistical significance regarding gender **(Graph 1)**. There were 2.062 sexually active subjects who had sexual relations only with one partner (56.3%) and this number was significantly higher ($z = 28.51$; $p < 0.001$) compared to those with two 23.7% (N = 867), and those with more than three partners 20% (N = 730) **(Graph 2)**.

There were 16.3% (N = 598) males who had more than three sexual partners, which is significantly higher ($z = 16.65$; $p < 0.001$) when compared to the females 3.6% (N = 132).

The average age of sexarche in males was 17.34 ± 1.77, and 18.20 ± 1.84 in females, and it was statistically significantly lower in males in the entire BiH ($z = -14.44$; $p < 0.001$) when compared to females.

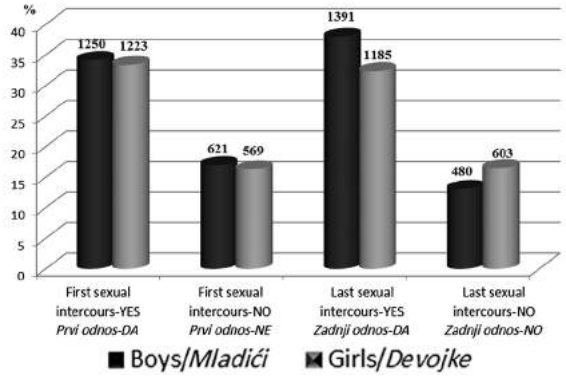
Out of the total number of sexually active participants (N = 3.659), 67.6% (N =2.437) used contraception at the first sexual intercourse, out of whom 34.2% (N = 1.250) were males and 33.4% (N = 1.223) were females. However, the usage of contraception at the last sexual intercourse was 70.4% (N = 2.576), out of whom 38% (N = 1.391) were males and 32.4% (N = 1.185) were females **(Graph 3)**.

At the level of the entire BiH, the percentage of males who used protection at their last sexual intercourse was significantly higher than in females ($z = -5.31$; $p < 0.001$).

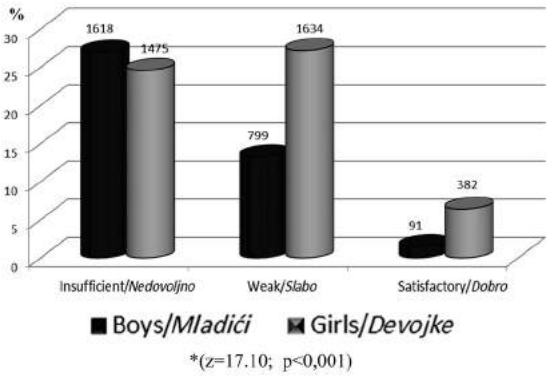
Most subjects, 74% (N = 2.707) used condoms as the method of contraception (40% of males (N = 1.463) and 34% (N = 1.244) of females). About 26% (N = 952) of examined subjects used some other method of contraception: 11.1% (N = 408) used coitus interruptus, and 14.9% (N = 544) of females used oral hormonal contraception. At the level of all cities of BiH, the proportion of males who used condoms was significantly higher than females who used condoms ($z = 17.10$; $p < 0.001$).

Table 1. Distribution of subjects by gender in BiH
Tabela 1. *Distribucija ispitanika po polu u Bosni i Hercegovini*

City/*Grad*	Girls/*Devojke*	Boys/*Mladići*
Sarajevo	790	460
Tuzla	679	571
Mostar	1247	1003
Banja Luka	776	474
Total/*Ukupno*	3492	2508



Graph 3. Use of contraceptive methods during the first and last sexual intercourse with gender distribution of examinees in BiH
Grafikon 3. *Primena kontraceptivnih metoda pri prvom i poslednjem polnom odnosu i polna distribucija ispitanika u Bosni i Hercegovini*



Graph 4. Gender distribution of examinees and their knowledge on contraceptive methods in BiH
Grafikon 4. *Polna distribucije ispitanika i njihovo poznavanje metoda kontracepcije u Bosni i Hercegovini*

More than a half of the investigated youth in BiH, 51.6% (N = 3.097) showed insufficient knowledge on contraceptive methods, while only 7.9% (N = 473) showed adequate knowledge.

The proportion of males in BiH who showed insufficient knowledge on contraceptive methods was 64.6%, and it was significantly higher when compared to females, 42.2% ($z = 17.10$; $p < 0.001$) **(Graph 4)**.

Among the examined sexually active females in BiH (N = 1.788) there were 4.9% (N = 88) registered intentional abortions. Out of the investigated young females in BiH (N = 3.492) 51.7% (N = 1.805) had never visited a gynecologist, 23.7% (N = 829) only visited a gynecologist when they had problems, while 24.6% (N = 858) regularly visited their gynecologists. One third of the sexually active young females had a Pap smear test, which is 37.2% (N = 666).

The young seek and get most information on contraception and STIs from their peers (50.7%) and from their partners (33.7%), then from healthcare workers (14.9%), while the lowest percentage (9.7%) of them turn to their parents for information. Only one fourth of females (24.6%) knew about the Pap smear test and its purpose, while as many as 75.4% of females gave incorrect answers.

In regard to their attitude towards contraception and oral hormonal contraceptives, 28% of females had a positive attitude, 19% of them had a negative attitude, while more than half of them (53%) were indecisive and their answer was "I do not know".

Discussion

One of the characteristics of the modern society is the increase in sexual activity among the youths. It may be assumed that in accordance with the contemporary world tendencies, sexual activity of the youths in BiH is also on the increase. Our data indicate that 61.0% of the young people in BiH have had a sexual experience, without a significant difference in gender, mainly with one sexual partner. Sexually active young persons in BiH most frequently have their first sexual experience at the age of 17, and 18, in males and females, respectively.

The first registered data on the sexual activity of the young people in BiH are given in the report made by the UNDP in 2000, and they show that there are 62% of sexually active young people, which is almost identical to our results (61%).

The earlier study conducted among the adolescents in the Tuzla Canton indicated that sexarche in young males is at the age of 16, and 17 in young females [9], which is somewhat earlier when compared to our results. The study among the students in Mostar showed that there were 61% of sexually active subjects, and that females have their first sexual experience at the age of 18 and males at the age of 17, which is identical to our results [10].

The data on sexual activity of the young people in the neighboring countries (Croatia, Serbia, Slovenia, Montenegro) are similar to our data and are in the range of 42% to 84.0% [1, 5, 7, 15–17].

Also, the sexually active young people in these countries show a tendency to initiate sexual activities at an earlier age, young males at the age of 16, and young females at the age of 17, while the percentage of those who had their first sexual intercourse before the age of 15 ranges from 23.4% to 28.5% [1, 7, 14–20]. The study conducted in 44 countries indicates variations in the age of the first sex from 16.2 to 16.5 years of age (Norway, Sweden, and Finland) to 22.1 to 22.9 years of age (India and China) [4]. The number of female adolescents who had sex before the age of 15 in the United States accounts for 14%, in Canada, France and Great Britain for 40.9%, in Sweden 12%, Hungary 32%, Germany 36%, and in Switzerland for 40% [21–23].

The studies conducted at university cities both in the world and in the neighboring countries indicate the sexual activity of students ranges from 67.0% to 85.0% [6, 10, 16, 19, 24], which is similar to the results of the study conducted in BiH. Some authors believe that more educated young persons

have their first sexual intercourse later than the less educated [15, 25–27].

The data on the number of sexual partners differ for the neighboring countries, but most studies conducted in these countries as well as in the world show that 10.2% to 29.8% of the sexually active young persons have had more than three sexual partners [1, 7, 16, 22, 23, 28].

When it comes to contraception, two thirds of the sexually active youth (67.6%) in BiH used contraception at their first sexual intercourse, while 70.4% of them used contraception at their last sexual intercourse. Condom is used by 74% of sexually active youth in BiH, while 26% of them use some other methods (11.2% of the young males use coitus interruptus, and 14.9% of the young females use oral hormonal contraceptives). The earlier studies done by the UNDP (2000) in BiH on the sexual activity of the youth found that 40% of the young people aged 15 – 25 used contraception, while studies conducted two years later showed that the incidence is 48%. Our data indicate a positive trend and an increased usage of contraception among the youth in BiH. However, it is significant that more than half of the examined youth (51.6%) did not have sufficient knowledge on contraceptive methods, males more than females. Also, data on the usage of contraception by high school students in the Tuzla Canton in 2003 showed a high usage of condoms (females 59.8% and males 63%), oral hormonal contraceptives (12.6% of the examined females) and coitus interruptus by 2.3% [9], as well as the study conducted among the students in Mostar, showing that 52.6% of subjects used contraception [10].

On the other hand, a large study performed by United Nations placed BiH into the group of countries with the lowest rate of contraceptive use among the women of reproductive age [29]. This study indicated a rather high rate of contraceptive use in the Scandinavian countries and the United States [29].

The situation in the neighboring countries is similar. The use of contraceptives at the first sexual intercourse ranged from 45.5% to 80% [5, 7, 14 –17]. Our data on the use of condoms are rather similar to results of studies conducted in four countries in the Balkans (BiH, Macedonia, Serbia, and Montenegro) among the youth aged 12–24. Condoms were used by 73.7% of young male persons at the first sexual intercourse, and by 69% of young female persons, while religion, age or type of school had no significant effects [30]. The condom use in the neighboring countries ranged from 40% to 80% [1, 5, 7, 16–18, 24].

The results of an international study on the first sexual intercourse in France, Spain, and Belgium, showed that 75% of subjects aged 20 – 24 used some kind of protection, in Italy 74.0% and in the Netherlands 85% of students used protection and around 55.0% in Poland, Hungary and Latvia [28, 31]. On the other hand, in Romania, only 15% of the young female subjects aged 15 – 24 used contraception at the first sexual intercourse [32].

A high level of condom use (67.5% of the young female subjects and 70.7% of the male subjects) at the first sexual intercourse and 52% at their last sexual intercourse was registered in the United States [22].

Our data show that 4.9% of the sexually active females had an intentional abortion. The exact number of intentional abortions in BiH is not known, because a significant number of abortions are not registered and there is no unique system of registration. The results of the population cross sectional study conducted in BiH in 1998 indicate that every third woman (aged 15 to 49) had an intentional abortion, and 7% of them had three or more abortions [33]. The data about the Tuzla Canton can mainly be found in the papers, for example on the incidence of adolescent pregnancies, which was between 3% and 5% [34, 35].

The rate of abortions among the females aged 20 – 24 in the European countries varies (Croatia 1.4%, Slovenia 1.6%, Hungary 3%, Italy 1.9%, Finland 2.9%, the Netherlands 8%), while the highest rate is still in Romania 78% [36, 37].

More than a half of the investigated youth (51.6%) have insufficient knowledge of contraceptive methods, whereby young male persons have significantly lower knowledge than females do. The earlier studies conducted in BiH also showed low knowledge on contraceptive methods, bearing in mind that the female subjects had better results than the males [9, 10]. The studies in the neighboring countries also found low level of knowledge on contraceptive methods, with female subjects having better scores, which is in accordance to our results [5, 10, 14, 17, 20]. The world studies also confirm that the knowledge on contraceptive methods is insufficient worldwide [23, 29, 38–40]. More than a half of the females surveyed (51.7%) in BiH never visited a gynecologist, while 24.6% of them regularly visited their doctors. The Pap smear test was done by 37.2% or one third of the sexually active females.

The findings of various studies on visits to gynecologist and Pap smear tests are rather diverse [28, 38, 41, 42]. However, all the studies pointed out the importance of visiting gynecologists for sexually active young girls, as well as doing medical tests such as Pap smear test, counseling on the use of contraception, and protection against STIs (Chlamydia infections and infections caused by human papillomavirus (HPV)) [39, 42, 43].

Significant indicators of reproductive health as well as the ability to assess future fertility are the pathological Pap smear test results, gynecological exams, and the frequency of Chlamydia infections, most spread STIs among the youth today.

The young population seeks and gets most information on STIs from their peers (50.7%), while only 9.7% turn to their parents. The results similar to ours are reported by other authors [1, 2, 11, 14, 40, 46]. When it comes to the attitude towards contraception and oral hormonal contraceptives, somewhat more than a quarter of the female subjects

(28%) had a positive attitude while more than a half (53.0%) were indecisive (their answer to this question was "I do not know").

Such attitudes of the young people and the ways they obtain information on sexual behavior and contraception are evident in other studies as well, which shows that young people mainly get information from their peers, whereas the least of them talk to their parents [1, 4, 29, 40, 46]. It is also important to mention that the negative attitude towards hormonal contraceptives is often the result of insufficient knowledge on contraception which results in fear and prejudice [20, 45].

Sometimes the most frequent sources of knowledge about contraception (peers, parents, media) are inadequate and may cause numerous consequences (harmful effects of modern contraception and the reliability of certain methods of contraception such as coitus interruptus). Most studies reveal that the knowledge the young people obtain about family planning often remains purely informational, while the knowledge on fertility and practical skills in applying certain methods are lacking [4, 46].

The obtained data indicate the increased sexual activity among the youth in BiH, high levels of contraceptive use at first and last sexual intercourse, and condom as a method of contraception for more than two thirds of the subjects, and only one sexual partner. However, the knowledge of contraceptive methods and STIs is still insufficient, as well as the awareness of the female subjects on the use of oral hormonal contraceptives, the need for regular visits to gynecologists and doing Pap smear tests. Therefore, appropriate education should be implemented through counseling centers and the media.

The earlier studies among the youth in BiH (performed by the United Nations Population Fund and UNDP) showed that the examinees had little information on STIs and how to prevent them, as well as limited knowledge or complete lack of understanding pregnancy and personal sexuality. Hence, regardless of the knowledge of contraceptive methods, they did not have enough knowledge on their use and role in preventing STIs.

The lack of counseling centers and a National strategy for the protection of reproductive health in BiH is the greatest problem. Young people obtain knowledge on reproductive health through general school programs, media, Internet, and their peers, while very little information are provided by the healthcare and education system. Some cities have counseling centers for the young, non-governmental organizations (XY Association) and student polyclinics within the gynecology departments.

We believe that the National plan for the protection of reproductive health of the youth needs to be adopted. However, this should be done after studying the reproductive health and risky sexual behavior among the youth, which may help in planning, education, and actions.

The fact that more than nine years have passed since the study was conducted may be seen as its limitation. Also, this period may be a limiting factor in validating the questionnaire used in the study.

Conclusion

The results of the research about the sexual behavior of youth in Bosnia and Herzegovina indicate an increase in sexual activity, a high rate of contraceptive use at the first and last sexual intercourse, condom as a method of contraception in more than two thirds of subjects, as well as only one sexual partner. However, the knowledge of contraceptive methods and sexually transmitted infection is still insufficient, as well as the awareness of the females on the use of oral hormonal contraceptives and the need for regular visits to gynecologist and doing Pap smear tests. Therefore, appropriate education should be implemented through counseling centers and the media.

Our study showed that the young people in Bosnia and Herzegovina, unlike the young in the countries of the Western Europe and the United States, do keep the traditional behavior patterns, attitudes and practices to a certain extent.

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RISK ASSESSMENT OF DRINKING WATER FROM PUBLIC WELLS

PROCENA RIZIKA ISPRAVNOSTI VODE ZA PIĆE IZ JAVNIH BUNARA

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Summary

Introduction. The safety of drinking water should be considered in the context of managing the risk from hazards that may compromise it. The aim of this study was to identify microbiological, chemical and physical hazards of drinking water from public wells which may impact human health, and to evaluate the results of routine drinking water analysis, not taking into account the national legislation, but the risk management approach. **Material and Methods.** Drinking water was sampled from 20 public wells in South Bačka District and analyzed at the Institute of Public Health of Vojvodina according to accredited national standards during 2016. The drinking water hazards were defined according to international recommendations. Risk assessment was done using a semi-quantitative approach, which assesses the likelihood and consequences of a hazard, rating the risks as low, medium, high and very high. **Results.** Of 218 drinking water samples, according to national regulations, only 10% were healthy for consumption. The recognized hazards were thermotolerant coliforms (especially Escherichia coli), enterococci (genus Streptococcus), Pseudomonas aeruginosa, Proteus species and nitrates above legal limits. The risk was rated high, with an impact on the morbidity of sensitive populations in 2/3 of controlled public wells, in 1/3 as medium, leading to abandonment of drinking water sources. **Conclusion.** The proposed risk assessment methodology is a tool that provides easily understandable and clear information on the possibility of using public wells with a consequent impact on human health. The management of drinking water safety is the greatest challenge, systematically prioritizing risk assessment of drinking water from public wells for the health of the population in the Republic of Serbia.

Key words: Risk Assessment; Drinking Water; Water Wells; Public Health; Environmental Medicine; Escherichia coli; Nitrates

Sažetak

Uvod. Utvrđivanje zdravstvene ispravnosti vode treba posmatrati kroz proces analize rizika, koji podrazumeva procenu, upravljanje i komunikaciju u vezi sa rizikom. Procena rizika definiše aktivnosti kao što su prepoznavanje i karakterizacija opasnosti koje mogu usloviti obolevanje ljudi, procenu izloženosti i karakterizaciju rizika. Cilj rada bio je prepoznavanje mikrobiološke, hemijske i fizičke opasnosti u vodi za piće javnih bunara koje mogu uticati na zdravlje ljudi i sagledati rezultate analiza zdravstvene ispravnosti vode za piće javnih bunara analizom rizika, a ne primenom nacionalnih propisa. **Materijal i metode.** Uzimanje i analiza uzoraka vode za piće iz 20 javnih bunara na teritoriji Južnobačkog upravnog okruga tokom 2016. obavio je Institut za javno zdravlje Vojvodine u skladu sa akreditovanim, standardizovanim i propisanim metodama. Opasnosti su definisane u skladu sa međunarodnim preporukama. Rizik je procenjen semikvantitavnom metodom, koja ukrštanjem verovatnoće pojavljivanja i posledica po zdravlje ljudi, kategoriše rizik kao mali, srednji, visok i veoma visok. **Rezultati.** Od ukupno 218 analiziranih uzoraka vode za piće, samo 10% je zdravstveno ispravno u odnosu na propisane normative. Prepoznate opasnosti u vodi za piće javnih bunara su termotolerantni mikroorganizmi (posebno *Escherichia coli*), eneterokoke (genus *Streptococcus*), *Pseudomonas aeruginosa, Proteus* vrste i koncentracija nitrata iznad propisanih graničnih vrednosti. Rizik upotrebe vode za piće koji doprinosi obolevanju osetljive populacije, procenjen kao visok, utvrđen je u 2/3 kontrolisanih javnih bunara, dok u 1/3 upotreba vode za piće predstavlja srednji rizik, uslovljavajući promenu izvorišta vode za piće među stanovništvom. **Zaključak.** Procena rizika predstavlja alatku koja obezbeđuje lako razumljive i jasne informacije o mogućnosti upotrebe vode JB sa posledičnim uticajem na zdravlje ljudi, te je sistemska primena principa procene rizika kao načina utvrđivanja zdravstvene ispravnosti vode za piće najveći izazov za upravljanje zdravstvenom bezbednošću vode za piće u Republici Srbiji.

Ključne reči: procena rizika; voda za piće; javni bunari; javno zdravlje; humana ekologija; Escherichia coli; nitrati

Introduction

Water is essential to sustain life of all living beings [1]. Adequate amount of safe and easily accessible water must be available to all, as a basic human right [2]. According to the World Health Organization (WHO) [1], safe drinking-water does not represent any significant risk to health over a lifetime of consumption, including different sensitivities (infants, young children, chronically ill and immu-

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Abbreviations

WHO – World Health Organization
WSP – Water Safety Plan
E. coli – Escherichia coli
HPC – heterotrophic plate count
IOS – International Organization for Standardization
SMEWW – Standard Methods for the Examination of Water and Wastewater
APV – Autonomous Province of Vojvodina

nocompromised people, the elderly) that may occur during one's lifespan. The WHO recommends that the safety of drinking water should be considered in the context of managing the hazard risks that may compromise it.

Hazards (any biological, chemical or physical agent that has the potential to cause harm) [1] may occur or be introduced throughout the water system, from catchment to consumer. Therefore, effective risk management requires identification of all potential hazards, their sources, possible hazardous events, and assessment of the risks they may occur [1]. Mainly, for organized drinking water systems, these activities are defined by the Water Safety Plan (WSP), but for public wells, WSP is usually not sufficient. In the Republic of Serbia, there is a National Bylaw which defines the microbiological, chemical and physical parameters and guidelines, as well as frequency and minimal number of controls of the drinking water safety [3], but it is not based on risk management approach.

If routine analysis of drinking water in the Republic of Serbia is concerned [3] (so called "A" volume for analysis according to national legislations), the biological and chemical hazards are represented as the thermotolerant coliforms, especially Escherichia coli (E. coli), Enterococci (genus Streptococcus), Pseudomonas aeruginosa, Proteus species and excessive concentrations of nitrates/nitrites.

Thermotolerant coliforms, with predominant genus of Escherichia, but also with some types of Citrobacter, Klebsiella and Enterobacter, are coliform bacteria that ferment lactose at 44 – 45 °C. E. coli is present in human and animal feces and thus it is most commonly used as an indicator of fecal contamination. E. coli or, alternatively, thermotolerant coliforms, is the first organism of choice in monitoring programs for verification of drinking water safety. These organisms are also used as disinfection indicators [1, 4–6].

Intestinal enterococci are a subgroup of the fecal streptococci, which comprise species of the genus Streptococcus. They are typically excreted in the feces of humans and other warm-blooded animals. Intestinal enterococci tend to survive longer in water environments than E. coli or thermotolerant coliforms; they are more resistant to chlorine, so they are used as an indicator of fecal pollution, especially genus Streptococcus. Intestinal enterococci are also used as the indicator of water quality after repairs to distribution systems or after new mains have been laid [1, 7].

Pseudomonas aeruginosa, a member of the family Pseudomonaceae (aerobic, Gram-negative rods), is a common environmental organism and it can be found in feces, soil, water and sewage. It multiplies in water environments and also on the surface of suitable organic materials in contact with water. It has been isolated from a range of moist environments such as sinks, water baths, hot water systems, showers and spa pools. Pseudomonas aeruginosa in drinking water can be considered as an indicator of secondary fecal pollution of water distribution systems caused by lack of disinfection after new mains have been laid, inadequate water flow, old and damaged pipes and taps where the precipitation of organic matter is high and tends to form biofilms. The main route of infection among humans is by exposure of susceptible tissue, notably wounds and mucous membranes, to contaminated water, instruments and other surfaces. Ingestion of drinking water is not an important source of infection [1, 8–11].

The genus Proteus includes Gram-negative, facultative anaerobic, heterotrophic, and proteolytic rods being human opportunistic pathogens. Human and animal feces are probably an important source of these rods in natural environments. The presence of Proteus spp. bacteria in water and soil may indicate a fecal pollution of the environment, being a threat of poisoning if the contaminated water is consumed. The health risk may also be connected with drug resistant strains sourcing from intestines. Proteus spp. strains are able to adapt to different environmental conditions, such as high concentrations of heavy metals or toxic substances, which may be exploited as sources of energy and nutrition by the bacteria and could be used in bioremediation and environmental protection [12].

Heterotrophic plate count (HPC) bacteria represent a wide spectrum of heterotrophic microorganisms among which are some sensitive to disinfection processes (coliform bacteria), some are resistant to disinfection (spore formers) and some rapidly proliferate in treated water in the absence of residual disinfectants. The HPC can be a useful tool in monitoring, treatment and as a disinfectant indicator, with the objective to keep numbers as low as possible. The HPC tests can also be used in assessing the cleanliness and integrity of distribution systems and the presence of biofilms. The HPC can include potentially "opportunistic" pathogens such as Acinetobacter, Aeromonas, Flavobacterium, Klebsiella, Moraxella, Serratia, Pseudomonas and Xanthomonas, for which there is no evidence of an association with gastrointestinal infection through ingestion of drinking-water in the general population [1, 4, 8].

The chemicals of greatest health concern in some natural waters are usually found in excessive amounts of natural nitrates/nitrites. Nitrates and nitrites are frequently present in drinking water due to sewage contamination or agricultural runoff, so they can be defined as chemical indicators of fecal water contamination. The great concentration of nitrates are typical for untreated and non disinfected underground waters, while the nitrites, which are

more toxic, are present in non treated, but disinfected waters. Excessive nitrate/nitrite exposure of infants up to approximately 3–6 months of age represents a risk factor for methaemoglobinaemia. Nitrite can react with nitrosatable compounds, primarily secondary amines, in the body to form N-nitroso compounds. A number of these are considered to be carcinogenic to humans. The formation of N-nitroso compounds is greatest among individuals who are achlorhydric (have very low levels of hydrochloric acid in the stomach) [1, 13].

An incident or situation that can lead to a hazard is defined as a hazardous event [1]. It can be caused by natural (drought, flood, earthquake, fires and emergency situations) or anthropogenic factors (contamination of drinking water sources by outflow of untreated waste water, by animals in catchment, by ammonia from uncontrolled use of fertilizers in agriculture; construction defects of wells and pipes causing microbiological, chemical and physical contamination from the soil and surroundings; lack of disinfections).

A risk is the likelihood of harm by identified hazards in exposed populations in a specified timeframe, including the magnitude of that harm and/or the consequences [1]. The semi-quantitative approach, known as a simple scoring matrix, relies to a significant extent, on expert opinion, evaluating the likelihood and consequence/impact of identified hazard suitable for risk assessment and rating it as low, medium, high and very high. The results of risk rating can be further used for managing and ensuring the drinking water safety.

The objectives of the study were to identify microbiological, chemical and physical hazards in drinking water from public wells which may have an impact on human health, and to evaluate, for the first time, the results of routine analysis of drinking water safety from public wells using the risk management approach, not the national legislations.

Material and Methods

During 2016, 20 most frequently used public wells in South Bačka District **(Figure 1)** were chosen for analysis. These are situated in Novi Sad (8), Petrovaradin (1), Bukovac (3), Sremska Kamenica (3), Stari Ledinci (4) and Ledinci (1).

Sampling and transport of 218 drinking water samples was done in line with International Organization for Standardization (IOS) standards described in 5667 series part 1, 3 and 5. The cold chain transport for drinking water samples from the sampling point to laboratory was ensured. The sanitary inspection data, which were recorded on site, were also gathered.

The "A" volume analysis was applied for all gathered drinking water samples, which are defined by microbiological, physical and chemical parameters, according to national regulations [3].

Among microbiological parameters, we analyzed the presence and number of HPC, coliforms, thermotolerant coliforms (especially E. coli), enterococci (genus Streptococcus), Pseudomonas aeruginosa, Proteus species, and Clostridium perfringens. Standard methods of analysis were applied. Membrane filter technique was used to estimate the number of suspected organisms present in 100 ml of water. An amount of 100 ml of water was filtered through nitrocellulose membrane of 0.45 μm pore-size, the membrane was transferred onto Endo agar for coliforms and Proteus species, Slanetz-Bartley agar for enterococci, sulphite-polimixin agar for Clostridium perfringens, all incubated at 37ºC for 24 - 48 hours. King A agar for Pseudomonas aeruginosa was incubated at 42ºC for 24 – 48 hours, and MacConkey agar for thermotolerant coliforms (especially E. coli) was incubated at 44ºC for 24 – 48 hours. The presence of typical colonies referred to a positive reaction. For aerobic plate count, 1 ml portion of water was mixed with 16 ml of nutrient agar medium (cooled to 44 – 46ºC), sample of water was immediately mixed with agar medium thoroughly and uniformly, allowing agar to solidify and then incubated at 35 – 37°C for 48 hours. It was assumed that each visible colony was the result of multiplication of a single cell on the agar surface.

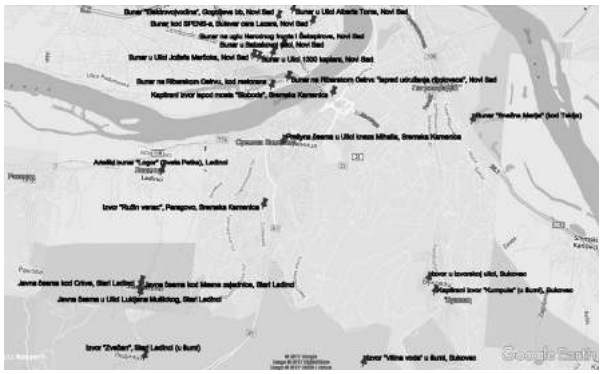

Figure 1. Map of public wells in the South Bačka District
Slika 1. *Mapa javnih bunara na teritoriji Južnobačkog upravnog okruga*

The physical parameters were analyzed by applying a standard methodology (SRPS EN IOS 7887:2013 method C, Standard methods for the Examination of Water and Wastewater (SMEWW) 20th Edition, 1998; 2150B and 2130B] examining the color, odor and turbidity.

The following chemical parameters were analyzed: pH value (by standard electrochemical IOS method number 10523), the consumption of potassium permanganate (by volumetric method based on IOS 8467), evaporation residue (by Standard Methods for the Examination of Water and Wastewater - SMEWW 20th Edition: 2540B and 2540C), conductivity (by standard IOS method number 27888 using electrodes), the concentration of ammonia (by spectrophotometric method based on reaction with Nessler reagent), nitrates (by standard spectrophotometric method: SMEWW 20th Edition; 4500-NO_3B), nitrites (by standard spectrophotometric method: SMEWW

20th Edition; 4500-NO_2B), chlorides (by standard volumetric method: SMEWW 20th Edition; 4500-Cl B), total iron and manganese (by standard atomic absorption spectrophotometric method: SMEWW 20th Edition; 3111B) and free residual chlorine (by spectrophotometric method with orthotolidine).

Risk assessment was performed according to WHO semi quantitative methodology [1], assessing whether there were microbiological, chemical or physical hazards which might cause harm (severity of consequences on human health) in exposed populations in a specified timeframe (likelihood), known as a risk (**Table 1**). Risk assessment was based both on likelihood and severity, and 4 categories of risk were low (< 6), medium (6 – 9), high (10 - 15) and very high (> 15).

Risk likelihood was defined for each controlled public well, specified by dynamic of sampling. Severity was set according to the type and scientifically defined impact on human health caused by microbiological and chemical hazards. For the purpose of risk rating among the analyzed microbiological parameters, known and defined microbiological hazards were taken into account, such as the presence and number of thermotolerant coliforms (especially E. coli), enterococci (genus Streptococcus), Pseudomonas aeruginosa and Proteus species. The concentrations of nitrates and nitrites above the national guideline values, according to WHO recommendations [1], were recognized as chemical hazards.

The microbiological, physical and chemical parameters which exceeded the guideline values, but are not categorized as hazards according to the scientific knowledge (exceeding the guideline values for HPC, iron, manganese, ammonia, organic meter, changes in odor, turbidity and color), were also used for risk assessment.

Results

During 2016, considering national legislation, of 218 samples of drinking water from 20 public wells, microbiological, physical and chemical analysis showed that the drinking water was safe for use only in 23 (10.55%) controlled samples and none (0%) of the controlled public wells, [1]. Results of each controlled public wells are presented in **Table 2**.

Table 1. Risk scoring matrix for risk rating
Tabela 1. *Matrica za rangiranje rizika*

Likelihood with scores/*Verovatnoća sa ocenom*	Severity of consequences and hazard scoring/*Posledice po zdravlje ljudi sa ocenom*				
	Insignificant (no impact), score - 1 *Bez značaja (nema uticaja), ocena 1*	Minor* (compliance impact), score - 2/*Minimalan (nije dokazan negativan uticaj), ocena 2*	Moderate** impact, score - 3 *Umeren (senzorne promene), ocena 3*	Major*** impact, rating, score - 4 *Velike (preko propisanih vrednosti), ocena 4*	Catastrophic§ (public health impact), score - 5 *Katastrofalan (uticaj na zdravlje ljudi), ocena 5*
Almost certain (once per day), score 5/*Skoro sigurno (jednom/dnevno) ocena 5*	5	10	15	20	25
Likely (once per week), score 4/*Verovatno (jednom nedeljno), ocena 4*	4	8	12	16	20
Moderately likely (once per month), score 3/*Moguće verovatno (jednom mesečno), ocena 3*	3	6	9	12	15
Unlikely (once per year), score 2/*Malo verovatno (jednom godišnje), ocena 2*	2	4	6	8	10
Rare (once in a 5 years), score 1/*Retko (jednom u 5 godina), ocena 1*	1	2	3	4	5

*Minor: Minor impact (color, odor…) causing dissatisfaction, but not likely leading to the use of alternative water source; ** Moderate: Moderate impact (color, odor…) possibly resulting in the use of alternative water source; ***Major: Morbidity expected from consuming water; § Catastrophic: Mortality expected from consuming water

**Bez značaja: Beznačajne senzorne izmene koji mogu usloviti nezadovoljstvo korisnika, ali ne i potrebu nalaženja drugog alternativnog izvora vode za piće; ** Minimalan: Izmenjene senzorne osobine vode koje će usloviti potrebu nalaženja drugog alternativnog izvora vodosnabdevanja; ***Velike: Promene u morbiditetu uzrokovane konzummiranjem vode; § Katastrofalan: Izmene mortaliteta zbog upotrebe kontaminirane vode*

Microbiological hazards were found in 13 (65%) public wells (**Table 2**), 2 located in Novi Sad (at the Fishing Island, near the restaurant, and in Jožefa Marčoka Street), 1 in Petrovaradin („Snežna Marija“), 3 in Bukovac (“Kumpula” (in the forest), in Izvorska Street and “Vilina vodica” (in the forest)), 3 in Sremska Kamenica (in Kneza Mihaila Street, “Ružin venac” and under the “Sloboda” bridge), 4 in Stari Ledinci (near the Local Community, in front of the Church, in Lukijana Mušickog Street and “Zvečan”). The microbiological hazards were thermotolerant coliforms (E. coli especially, as Citrobacter and Klebsiella), enterococci (genus Streptococcus), Pseudomonas aeruginosa and Proteus spp.

Chemical hazards (exceeding the national guidelines levels for nitrates) were found in 4 (20%) public wells **(Table 2)**, located in Petrovaradin („Snežna Marija“), Bukovac (in Izvorska Street), Sremska Kamenica (under the bridge “Sloboda”) and Ledinci (“Sveta Petka”).

The analyzed sanitary inspection data showed that causes of hazardous events due to microbiological hazards with high or moderate likelihood of severe morbidity from consuming water were: untreated sewage, close septic systems, animals in catchment, uncontrolled human access, lack of disinfection, construction defects of wells and pipes and area surrounding the well tap, and entry of contaminated water.

Hazardous events associated with chemical hazards, also according to the analysis of the sanitary inspection data with moderate and major likelihood of severe morbidity from consuming water, rating the risks as high or medium were: untreated waste waters, close septic systems, uncontrolled use of ammonia fertilizers in agriculture, uncontrolled human access, and natural characteristics of the soil where the water sources are located.

The likelihood of moderate health risk for each controlled public well was scored - 3. The severity qualified by the presence of microbiological hazards impacting the public health was defined as major and scored - 4, as were the exceeding levels of nitrates impacting the sensitive population. The microbiological, physical and chemical parameters which exceeded the guideline levels, but according to scientific knowledge were not rated as hazards, were qualified as moderate hazards, scored - 3, having impact on humans, but not causing population morbidity.

By combining the likelihood and severity levels for each controlled public well, based on one year analysis, the final risk scoring **(Table 3)** shows that using drinking water from public wells in Petrovaradin („Snežna Marija“), Bukovac (“Kumpula” and “Vilina vodica” in the forest and in Izvorska Street), Sremska Kamenica (in Kneza Mihaila Street, “Ružin venac” and under the bridge “Sloboda”), Stari Ledinci (Near to the Local Community, in front of the Church, in Lukijana Mušickog Street and “Zvečan”), Ledinci (“Sveta Petka”) and in 2 objects in Novi Sad (at „The Fishing Island“, near to restaurant and in Jožefa Marčoka Street) represents a high public health risk, scored - 12, while drinking water from other 6 controlled public wells located in Novi Sad, represents a medium public health risk, scored - 9.

Discussion

Risk assessment showed that drinking water from all controlled public wells located in Petrovaradin, Sremska Kamenica, Bukovac, Stari Ledinci and Ledinci presents a high risk for the population and may cause illness, especially among sensitive population. Furthermore, among controlled public wells in Novi Sad, there are 2 wells which represent a high health risk, one of them situated near the river Danube and the other in the city center. In about 1/3 of controlled public wells, mainly situated in Novi Sad, the health risk is medium, causing people to change their source of drinking water due to water odor, color, or turbidity. Presented results are well known among specialist who are managing the drinking water safety in public health institutes, but without their knowledge and ability to explain whether the drinking water from public wells is or is not safe for consumption, the information is not useful to the population. That is why risk assessments represent easy to understand and complete information concerning whether it is safe to use drinking water from certain public wells.

Analyses have shown that coliforms, thermotolerant coliforms, enterococci (genus Streptococcus), Pseudomonas aeruginosa and Proteus species were also found in drinking water from controlled public wells in other parts of Autonomous Province of Vojvodina (APV), as in 2015 and years before, just like in the South Bačka District in the previous period [14–16]. Unfortunately, the lack of unique method of sampling, analysis and evaluation, disables risk assessment of all controlled public wells in APV. Rapid assessment of drinking water quality in rural areas of the Republic of Serbia performed in 2016 showed that E. coli was present in 32% of 182 controlled individual supplies and represented a high risk in 33.5% and a very high risk in 7.1% individual drinking water supplies, concerning the amount of E. coli and results of sanitary inspection [17]. Data from the Republic of Serbia do not differentiate E. coli strains, like in 1989 in Missouri, United States, in 1991 in Grampian, Scotland, United Kingdom, in 1993 in South Africa, in 1994 in Saitama, Japan, in 1995 in Ontario, Canada, in 1997 in Grampian, Scotland, United Kingdom and in Fuerteventura, Canary Islands, in 1999 and 2002 in Highland, Scotland, United Kingdom, E. coli strain O157, found in private and other unchlorinated drinking water sources caused drinking water-associated outbreaks [18].

When it comes to chemical parameters in the drinking water from public wells in APV, nitrates are above the guideline values and present a hazard mainly in South Bačka District [14, 15]. In contrast, in Eastern and Western parts of Serbia, of

Table 2. The number of controlled drinking water samples from public wells in South Bačka District in 2016 with microbiological, physical and chemical test results and hazard scoring
Tabela 2*. Broj kontrolisanih uzoraka vode za piće iz javnih bunara na teritoriji Južnobačkog upravnog okruga tokom 2016. sa rezultatima mikrobioloških, fizičkih i hemijskih analiza i prisustvom opasnosti*

No. *R.b.*	Drinking water source/*Izvor vode za piće*	Number of controlled samples/year *Broj kontrolisanih uzoraka godišnje*	Microbiological analyses *Mikrobiološke analize*		Physical/chemical analyses *Fizičko-hemijske analize*	
			Number and %* of samples which were not safe/*Broj i % neispravnih mikrobioloških uzoraka*	Reasons of microbiological unsafety of drinking water quality-number and %** / *Uzroci mikrobiološke neispravnosti – broj i %*	Number and % of samples which were unsafe for physical and chemical reasons/*Broj i % fizičko-hemijski neispravnih uzoraka*	Reasons of physical/chemical unsafety of drinking water quality-number and %** / *Uzroci fizičko-hemijske neispravnosti – broj i %*
1.	Novi Sad, in 1300 kaplara Street/ *u Ulici 1300 kaplara*	12	0 (0.00%)	-#	12 (100.00%)	Turbidity/*Zamućenost* >GV† in 12 (100.00%); Total iron/*Ukupno gvožđe* >GV in 12 (100.00%) Manganese/*Mangan* >GV in 12 (100.00%) Odour/*Miris* >GV in 8 (66.67%); Color/*Boja* >GV in 3 (25.00%)
2.	Novi Sad, at the corner of Narodnog fronta and Šekspir Street / *na uglu Narodnog fronta i Šekspirove ulice*	12	0 (0.00%)	-	12 (100.00%)	Total iron/ *Ukupno gvožđe* >GV in 12 (100.00%); Manganese/*Mangan* >GV in 12 100.00%); Turbidity/*Zamućenost* >GV in 11 (91.67%); Odour/*Miris* >GV in 8 (66.67%); Color/*Boja* >GV in 4 (33.33%)
3.	Novi Sad, near to „SPENS“ (Sport and Recreate Center)*/u blizini SPENS-a*	11	0 (0.00%)	-	11 (100.00%)	Total iron/ *Ukupno gvožđe* >GV in 11 (100.00%); Manganese/*Mangan* >GV in 11 100.00%); Turbidity/*Zamućenost* >GV in 10 (90.91%); Odour/*Miris* >GV in 4 (36.36%); Color/*Boja* >GV in 1 (9.09%)
4.	Novi Sad, at „The Fishing Island“, near to restaurant / *na Ribarskom ostrvu, u blizini restorana*	4	3 (75.00%)	TC‡ in 2 (66.67%); FC§ (Citrobacter freundii, Citrobacter spp., Klebsiella pneumonia) in 2 (66.67%); Ent.¶ in 1 (33.33%); Proteus spp. in 1 (33.33%)	4 (100.00%)	Manganese/*Mangan* >GV in 4 (100.00%); Turbidity/*Zamućenos*t >GV in 1 (25.00%); Total iron/ *Ukupno gvožđe* >GV in 1 (25.00%)
5.	Novi Sad, at „The Fishing Island“, in front of the Fishermen's Association / *na Ribarskom ostrvu, ispred Udruženja ribolovaca*	12	0 (0.00%)	-	12 (100.00%)	Manganese/*Mangan* >GV in 12 (100.00%); Total iron/ *Ukupno gvožđe* >GV in 11 (91.67%); Turbidity/ *Zamućenos*t >GV in 9 (75.00%); Color/*Boja* >GV in 3 (25.00%); Odour/*Miris* >GV in 2 (16.67%)
6.	Novi Sad, in Alberta Tome Street / *u Ulici Alberta Tome*	10	0 (0.00%)	-	10 (100.00%)	Total iron/ *Ukupno gvožđe* >GV in 10 (100.00%); Manganese/*Mangan* >GV in 10 100.00%); Turbidity/ *Zamućenost* >GV in 9 (90.00%); Color/*Boja* >GV in 6 (60.00%); Color/ *Boja* >GV in 2 (20.00%)

7.	Novi Sad, in Jožefa Marčoka Street/*u Ulici Jožefa Marčoka*	12	1 (8.33%)	TC in 1 (100.00%); FC (Klebsiella oxytoca, Klebsiella pneumonia) in 1 (100.00%); Ent.in 1 (100.00%)	12 (100.00%)	Turbidity/*Zamućenos*t >GV in 12 (100.00%); Conductivity/*Provodljivost* >GV in 12 (100.00%); Total iron/*Ukupno gvožđe* >GV in 12 (100.00%); Ammonia/*Amonijak* >GV in 10 (83.33%)
8.	Novi Sad, in Balzakova Street/*u Balzakovoj ulici*	12	0 (0.00%)	-	12 (100.00%)	Total iron/*Ukupno gvožđe* >GV in 12 (100.00%); Manganese/*Mangan* > GV in 11 (91.67%): Turbidity/ *Zamućenost* >GV in 10 (83.33%); Color /Boja >GV in 2 (16.67%); Odour/*Miris* >GV in 2 (16.67%)
9.	Petrovaradin, „*Snežna Marija*“	12	8 (66.67%)	TC in 8 (100.00%); FC (Citrobacter freundii, Citrobacter spp., Klebsiella pneumonia) in 8 (100.00%); Proteus spp. in 2 (25.00%); Pseudomonas aeruginosa in 2 (25.00%)	9 (75.00%)	Nitrates/*Nitrat*i >GV in 9 (100.00%); Conductivity/ *Provodljivost* >GV in 2 (22.22%)
10.	Bukovac, “Kumpula” (in forest)/ (*u šumi)*	7	2 (28.57%)	TC in 1 (50.00%); FC (E.coli, Citrobacter freundii) in 1 (50.00%); Ent. in 1 (50.00%)	0 (0.00%)	-
11.	Bukovac, in Izvorska Street/u Izvorskoj ulici	12	9 (75.00%)	FC (Citrobacter freundii, Klebsiella pneumonia, E.coli, Citrobacter spp.) in 8 (88.89%); TC in 6 (66.67%); HPC†† in 3 (33.33%); Ent.in 2 (22.22%); Proteus spp. in 1 (11.11%)	11 (91.67%)	Nitrates/*Nitrati* >GV in 11 (100.00%); Turbidity/*Zamućenos*t >GV in 1 (9.09%)
12.	Bukovac, “Vilina vodica” (in forest)/ *(u šumi)*	7	6 (85.71%)	TC in 3 (50.00%); FC (Citrobacter freundii, Klebsiella pneumonia) in 3 (50.00%); Ent. in 2 (33.33%); Proteus spp. in 1 (16.67%)	0 (0.00%)	-
13.	Sremska Kamenica, in Kneza Mihaila Street/*u Ulici kneza Mihaila*	12	7 (58.33%)	TC in 6 (85.71%); FC (E.coli, Citrobacter freundii, Citrobacter spp., Klebsiella pneumonia) in 6 (85.71%); Ent. in 3 (42.86%)	1 (8.33%)	Conductivity/ *Provodljivost* >GV in 1 (100.00%)
14.	Sremska Kamenica, “Ružin venac”	12	7 (58.33%)	TC in 7 (100.00%); FC (Citrobacter freundii, Klebsiella pneumonia, E.coli) in 6 (85.71%); Pseudomonas aeruginosa in 3 (42.86%); Ent. in 1 (14.29%); Proteus spp. in 1 (14.29%)	1 (8.33%)	Turbidity/*Zamućenost* >GV in 1 (100.00%)
15.	Sremska Kamenica, under the bridge “Sloboda”/*ispod mosta Sloboda*	11	10 (90.91%)	TC in 9 (90.00%); FC (E.coli, Citrobacter freundii, Klebsiella pneumonia) in 9 (90.00%); Ent.in 5 (50.00%) HPC in 3 (30.00%)	5 (45.45%)	Turbidity/*Zamućenost* >GV in 5 (100.00%); Nitrates/*Nitrati* >GV in 1 (20.00%);

16.	Stari Ledinci, near to the Local Community/*u blizini Mesne zajednice*	12	12 (100.00%)	FC (E.coli, Klebseialla pneumonia, Citrobacter freundii, Citrobacter spp., Klebsiela oxytoca, Klebseilla spp.) in 12 (100.00%); TC in 11 (91.67%); Ent.in 11 (91.67%); HPC in 6 (50.00%); Pseudomonas aeruginosa in 3 (25.00%)	0 (0.00%)	-
17.	Stari Ledinci, in front of the Church / *ispred crkve*	12	12 (100.00%)	FC (E.coli, Klebseilla pneumonia, Citrobacter freundii, Klebseilla oxytoca) in 12 (100.00%); Ent. in 12 (100.00%); TC in 11 (91.67%); HPC in 7 (58.33%); Pseudomonas aeruginosa in 3 (25.00%)	0 (0.00%)	-
18.	Stari Ledinci, in Lukijana Mušickog Street/*u Ulici Lukijana Mušickog*	12	12 (100.00%)	TC in 12 (100.00%); FC (E.coli, Klebsiella pneumonia, Citrobacter freundii, Klebsiella oxytoca) in 12 (100.00%); Ent. in 11 (91.67%); HPC in 7 (58.33%); Pseudomonas aeruginosa in 3 (25.00%)	0 (0.00%)	-
19.	Stari Ledinci, *Zvečan*	12	4 (33.33%)	TC in 4 (100.00%); FC (E.coli, Citrobacter freundii, Citrobacter spp., Klebseilla pneumonia) in 3 (75.00%); HPC in 3 (75.00%); Ent. in 3 (75.00%)	0 (0.00%)	-
20.	Ledinci, *Sveta Petka*	12	0 (0.00%)	-	11 (91.67%)	Nitrates/*Nitrati* >GV in 11 (100.00%); Conductivity/ *Provodljivost* >GV in 1 (9.09%)

Legend: *% - percent; **- number and % are defined according to total number of controlled samples either for microbiological or for physical and chemical analyses; # - Unsafety was not defined; † GV – guideline value; ‡ TC – Total Coliforms; § FC – Fecal Coliforms; ¶ Ent. – Enterococcus (genus Streptococcus); †† HPC – Heterotrophic Plate Count

*Legenda: *% - procenat; **- broj i % je određen u odnosu na ukupan broj neispravnih mikrobioloških i/ili fizičko-hemijskih analiza; #-Neispravnost nije utvrđena; †GV – granična vrednost; ‡TC – ukupan broj koliformnih mikroorganizama; §FC – fekalni koliformni mikroorganizmi; ¶Ent. – Enterokoke (genus Streptococcus); ††HPC – Aerobni mezofilni mikroorganizmi*

182 controlled individual supplies, in 21% nitrates were above the guideline levels [17]. Based on the European Environmental Agency data from 1989 to 2000, there was a rising trend of nitrate concentrations in the groundwater in Europe, but information are currently available mainly from France and Sweden [19]. It is assumed that agriculture is the main source of groundwater nitrate concentrations [19]. The results of studies performed in 2009 in the United States, including about 2.100 private wells, showed that nitrates were the most common inorganic contaminant that was found at concentrations higher than the Federal Drinking Water Standard for Public Water Supplies (45 mg/L) in USA [20].

Risk assessment is not mandatory, but in many European countries and in the United States, specialists in public health sector and managers in drinking water supply systems, use it, and it is almost incorporated in WSP [1, 21, 22]. It provides identification of hazards, evaluation of hazard sources, hazardous events, and impact on human health and, with respect to the results, taking adequate measures, preventive or interventional. It is known that WSP with risk analysis approach for managing the drinking water supply and distribution system is similar to Hazard Analysis Critical Control Point procedures or IOS 9001 in food production or management [1, 21], so it is advisable to adopt risk analysis approach in national policy.

Table 3. Public health risks caused by drinking water from public wells in South Bačka District
Tabela 3. *Rizik po zdravlje ljudi uzrokovan vodom za piće poreklom iz javnih bunara na teritoriji Južnobačkog upravnog okruga*

Public well/*Javni bunar*	Risk Assessment (Likelihood/Severity)/*Rangiranje rizika (Verovatnoća/Uticaj na zdravlje)*			
	Low/*Nizak* <6	Medium/*Srednji* 6-9	High/*Visok* 10-15	Very high/*Veoma v*isok >15
Novi Sad*	–	9	–	–
Novi Sad**	–	–	12	–
Petrovaradin, S*nežna Marija*	–	–	12	–
Bukovac***	–	–	12	–
Sremska Kamenica†	–	–	12	–
Stari Ledinci‡	–	–	12	–
Ledinci, *Sveta Petka*	–	–	12	–

Legend: * In 1300 Kaplara Street, at the corner of Narodnog fronta and Shakespeare Street, near the „SPENS" (Sport and Recreate Center), at „The Fishing Island" in front of the monument of the Fishermen's Association and Public Health Institute, in Alberta Tome Street, in Balzakova Street; ** At „The Fishing Island", near to restaurant and in Jožefa Marčoka Street; ***In Izvorska Street, "Vilina vodica" (in forest), "Kumpula" (in forest); † In Kneza Mihaila Street, "Ružin venac" and under the bridge "Sloboda"; ‡ Near to the Local Community, in front of the Church, in Lukijana Mušickog Street and "Zvečan".
Legenda: ** Ulica 1300 Kaplara, ugao Narodnog fronta i Šekspirove ulice, u blizini „SPENS"-a, na Ribarskom ostrvu ispred spomenika "Udruženja ribolovaca i Higijenskog Zavoda", u Ulici Alberta Tome i u Balzakovoj ulici; ** Na Ribarskom ostrvu blizu restorana i u Ulici Jožefa Marčoka ; ***U Izvorskoj ulici, "Vilina vodica" (u šumi), "Kumpula" ((u šumi); †U Ulici kneza Mihaila, "Ružin venac" i ispod mosta "Sloboda"; ‡ U blizini Mesne zajednice, ispred crkve, u Ulici Lukijana Mušickog i "Zvečan".*

The risk analysis considers hazard identification and risk assessment, which are mainly done by researchers and experts in drinking water systems; a multidisciplinary approach for risk management and risk communication [23], should engage drinking water producers, technicians, in health and environment sector, politicians and population for managing risks in a systematic way.

Conclusion

This study showed that microbiological and chemical hazards were found in the drinking water from public wells in the South Bačka District. After rating the likelihood and severity of health risks, 2/3 of controlled public wells showed high risk levels of multiple contaminants with an impact on the morbidity of sensitive population. It also showed that the proposed risk assessment methodology provides information that are easy to understand and clearly rates the risks caused by drinking water from public wells. The greatest challenge in managing the drinking water safety is to prioritize risk assessment in the Republic of Serbia systematically.

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VISION RELATED PROBLEMS AFTER CATARACT SURGERY

VIDNA FUNKCIJA NAKON HIRURŠKOG LEČENJA KATARAKTE

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Summary

Introduction. Senile cataract is an age-related eye disease and one of the most common causes of visual impairment. It is one of the most important causes of reversible blindness in the elderly persons, with a steady increase in prevalence with advanced age. The resulting loss of visual acuity has important implications for physical functions and the overall quality of life of affected individuals. However, cataract related visual loss is completely reversible with modern cataract surgery techniques. The purpose of the study was to evaluate the visual outcomes (visual acuity and visual function) before and after cataract surgery, and to assess patient satisfaction with final surgical outcomes. **Material and Methods**. This prospective study included 176 consecutive patients with senile cataract who underwent phacoemulsification with a foldable intraocular lens implantation. Evaluation of vision related problems of daily living was done in all patients using a vision related quality of life questionnaire, before and 10 days after the surgery. All patients self-rated their vision-related problems into 4 categories: no problems, small problems, big problems, and very big problems. The results were presented as a vision-related quality of life index. **Results.** There were 176 patients included in this study, with an average age of 65.4 ± 12 years. The operated patients had significantly better visual acuity than before cataract surgery ($p < 0,001$), with a median postoperative visual acuity of 0.8. Before cataract surgery, the average vision-related quality of life index was 53.7 (range 21.3 – 87.7). The self-rated vision related problems were present in 59.8% patients before senile cataract surgery, and only in 12.8% of patients undergoing phaco cataract surgery ($p<0.001$). Ten days after cataract surgery vision-related quality of life index improved to 92.9 (range 57.1 – 98.7). **Conclusion.** Visual function, self-estimated vision related problems and patients satisfaction are significantly higher after the phaco cataract surgery. We believe that vision related quality of life index is a valid and sensitive tool for visual function and vision-related quality of life evaluation.

Key words: Cataract; Aging; Visual Acuity; Vision, Low; Cataract Extraction; Phacoemulsification; Quality of Life; Patient Satisfaction; Activities of Daily Living; Surveys and Questionnaires

Sažetak

Uvod. Senilna katarakta je bolest oka povezana sa starenjem i jedan je od najčešćih uzroka slabljenja vida. Spada u najvažnije faktore izlečive onesposobljenosti kod starije populacije, sa stalnim porastom prevalencije sa godinama starosti. Posledica gubitak vidne oštrine što ima značajne konsekvence na fizičko funkcionisanje, kao i na sveukupni kvalitet života pacijenata. Gubitak vida prouzrokovan kataraktom je u potpunosti izlečiv uz primenu savremenih hirurških tehnika za njeno lečenje. Cilj ovog rada bio je da se izvrši procena vida (vidna oštrina i vidna funkcija) pre i posle operacije katarakte i da se sagleda zadovoljstvo pacijenata krajnim ishodom hirurškog lečenja. **Materijal i metode.** Ova prospektivna studija je obuhvatila 176 konsekutivnih pacijenata sa senilnom kataraktom koji su operisani metodom fakoemulzifikacije uz ugradnju savitljivog intraokularnog sočiva. Procena problema u vezi sa vidom pri izvođenju dnevnih aktivnosti svih pacijenata izvršena je pomoću upitnika za procenu kvaliteta života u vezi sa vidom pre i 10 dana nakon zahvata. Svi pacijenti su odredili svoje probleme u vezi sa vidom u četiri grupe: bez problema, manji problem, veliki problemi i veoma veliki problemi. Resultati su iskazani kroz indeks kvaliteta života u vezi sa vidom. **Rezultati.** Osamdeset osam pacijenta je uključeno u našu studiju, sa prosečnom starošću od 65,4 ± 12 godina. Pacijenti su nakon operacije imali statistički značajno poboljšanje vidne oštrine u poređenju sa nalazom pre operacije ($p<0,001$), sa medijanom od 0,8 postopeartivno. Prosečan VQOL indeks pre operacije katarakte iznosio je 53,7 (21,3 – 87,7). Problemi sa vidom, na osnovu lečne procene ispitivanih pacijenata bili su prisutni kod 58,9% pacijenata preoperativno, a kod samo 12% pacijenata nakon hirurgije katarakte ($p<0,001$). Deset dana nakon operacije VQOL indeks je porastao na 92,9 (57,1– 98,7). **Zaključak.** Vidna funkcija je poboljšana znatno, a zadovoljstvo pacijenata je veće nakon operacija katarakte metodom fakoemulzifikacije. Indeks kvaliteta života u vezi sa vidom predstavlja validan i osetljiv pokazatelj u proceni vidne funkcije i kvaliteta života u vezi sa vidom.

Ključne reči: katarakta; starenje; vidna oštrina; slab vid; uklanjanje katarakte; fakoemulzifikacija; kvalitet života; zadovoljstvo pacijenta; aktivnosti svakodnevnog života; ankete i upitnici

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Abbreviations
GBD – global burden of disease
WHO – World Health Organization
VA – visual acuity
VQOL – vision-related quality of life

Introduction

Senile cataract is an age related eye disease and a common cause of visual impairment. It is the leading cause of blindness and the second cause of moderate and severe vision impairment according to the Global Burden of Disease (GBD), Injuries and Risk Factors Study [1–3]. According to GBD and World Health Organization (WHO) data, it accounts for 33% and 48%, respectively. The WHO has estimated that this number will increase to 40 million by 2025, as populations grow and age, with greater life expectancies [4]. The GBD showed that in 2010 there were 10.8 million cataract blind people, 1/3 of blind people worldwide [1].

Senile cataract is one of the most important causes of reversible visual disability in elderly persons. The prevalence is on steady rise with age, from 16% in 65 – 69 year olds, to 24% in persons aged 70 – 75 years.

The most common complaint of patients with senile cataract include decreased visual acuity (VA), decrease in contrast sensitivity in brightly lit environments, or disabling glare during the day or to oncoming headlights at night, myopic shift and monocular diplopia.

The resulting loss of VA has important implications for physical functions [5]. Visual impairment caused by cataract affects the patient in several ways and leads to perceived difficulties in performing vision related everyday activities. Daily activities abandoned because of low vision decrease the quality of life [6]. Poor vision caused by cataracts may also result in an increased risk of falling and depression [7].

More attention is now paid to the functional impact of the lens opacities and the requirements of the patient as an individual. This has led to additional weight being given to the patient's perceived difficulties in their daily life and their visual needs.

Cataract extraction is the most common surgical procedure in ophthalmology and continues to be one of most commonly performed elective surgical procedures in medicine [8]. Over the years, various surgical techniques have evolved from the ancient method of couching to the present-day technique of modern phacoemulsification. Advances in new technology have lead to the development of small incision phaco surgery, minimizing complications arising from improper wound closure, affording more rapid wound healing, faster visual rehabilitation, and reintegration in daily life activities. It uses a relatively closed system during both phacoemulsification and aspiration with better control of intraocular pressure providing safeguards against positive vitreous pressure and choroidal hemorrhages. A closed system also minimizes fluid turbulence within the anterior chamber, reducing endothelial and trabecular meshwork trauma. However, more sophisticated and expensive machines, disposables instruments are required to perform phacoemulsification.

Phaco cataract surgery is a clinically effective and safe surgical procedure, and the incidence of sight threatening complications is low [9]. High surgical success rates have created high patient expectations regarding the vision outcome and improvement in vision related everyday activities.

Therefore, the goal of cataract extraction and intraocular lens implantation is to improve visual acuity and visual function, with an implicit assumption that it will improve the overall quality of life [10]. The second eye surgery confers significant additional gains in visual function in everyday activities and quality of life above and beyond those achieved after surgery to the first affected eye.

The purpose of this study was to evaluate the visual outcomes (visual acuity and visual function) before and after cataract surgery, and to assess patient satisfaction with final surgical outcome.

Material and Methods

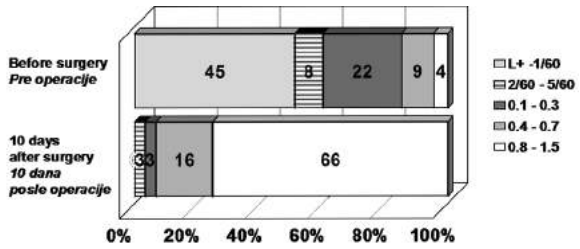
The prospective study included 176 consecutive patients with senile cataract, who underwent surgery at the Eye Clinic of the Clinical Center of Vojvodina in Novi Sad.

The VA scores were obtained with Snellen charts and expressed as a decimal number.

In order to evaluate vision related problems of daily living, all patients were interviewed individually using the vision-related quality of life (VQOL) questionnaire before and 10 days after the surgery. The questionnaire contained questions about perceived difficulties in daily activities, basic visual functions necessary for activities, independent living, and activity level in daily living and self-reported vision satisfaction. It was developed based on the experience and results of a retrospective study including a sample of 10% of 10.000 operated patients, and on results of a prospective study "Quality of life after intraocular surgery" performed at the University Eye Clinic in Novi Sad [11, 12].

All patients self-estimated their vision related problems into 4 categories: no problems, small problems, big problems, and very big problems. The answers from the questionnaire were coded and summarized into a VQOL-index. "No problems" was coded as 3, "small problems" as 2, "big problems" as 1, and "very big problems" as 0.

In patients with cataract, the VQOL was measured using a VQOL-index. VQOL-index is an average numeric value of coded answers from the VQOL questionnaire pondered to a 0 - 100 scale, multiplied by a constant of 33.3 (0 represents complete disability, and 100 represents a status with no problems regarding vision related daily life activities). All questions were treated equally.



Graph 1. Preoperative and postoperative visual acuity
Grafikon 1. *Preoperativna i postoperativna vidna oštrina*

Results

The questionnaire responses on visual function and clinical data of 176 patients with senile cataract were recorded pre-operatively and 10 days postoperatively. Patients with coexisting eye and general diseases were excluded. The average age of patients was 65.4 ± 12.1; 61% of patients were females and 39% were males. After cataract surgery, the patients had significantly better VA than before surgery ($p < 0{,}001$). The median VA (decimal notation) of the operated eye was 0.8 postoperatively, and postoperative astigmatism was 0.6 D. The preoperative and postoperative VA is shown in **Graph 1**.

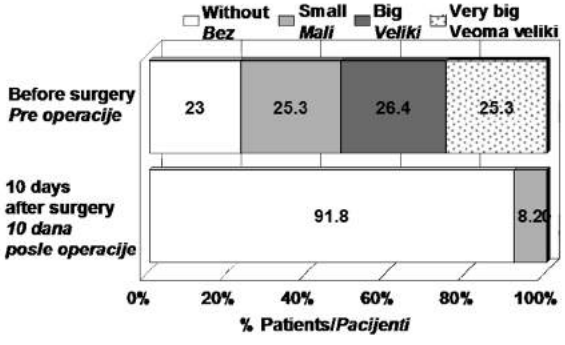
Out of the examined patients, 51.7% patients reported big or very big problems with reading books and newspapers before cataract surgery. Ten days after cataract surgery 91.8% reported no problems, or small problems **(Graph 2)**.

Before surgery, reading TV subtitles and watching the TV was a big or very big problem for 47.7% of patients. Ten days after surgery, 8.1% had small problems and 91.9% had no problems at all **(Graph 3)**.

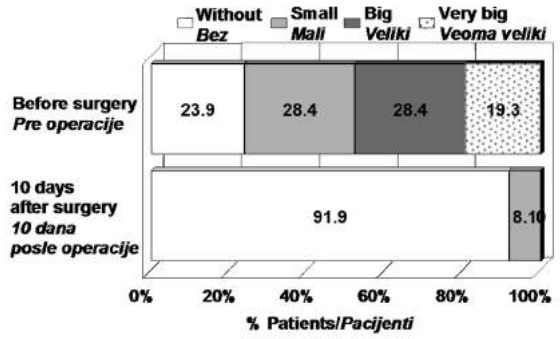
Outdoor and indoor mobility was a big or very big self-estimated problem in 28.41% of patients with cataract. Ten days after cataract surgery, 98.8% were without or had small problems outdoors **(Graph 4)**.

The average VQOL index before cataract surgery was 53.7 (range 21.3 – 87.7). More than 50% of patients with cataracts had big or very big problems in VQOL. Only 5.39% of patients had no problems in daily life activities **(Graph 5)**. Phaco cataract surgery significantly improved the VQOL-index in a period of ten days after surgery (53.7 vs. 92.9, $p < 0.00l$).

Self-estimated vision related problems were present in 59.8% patients before the senile cataract surgery, versus 12.8% after phaco cataract surgery ($p < 0.001$). Self-estimated vision related problems before and 10 days after surgery are shown in **Graph 6**.



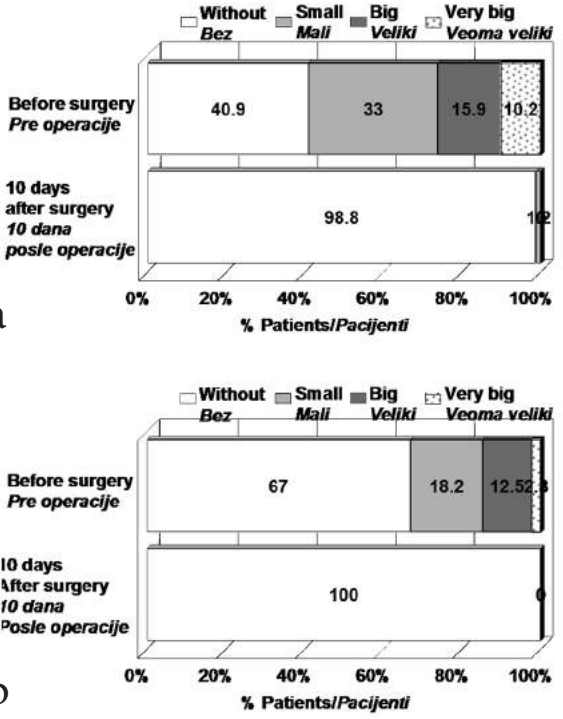
Graph 2. Reading books and newspapers
Grafikon 2. *Čitanje knjiga i novina*



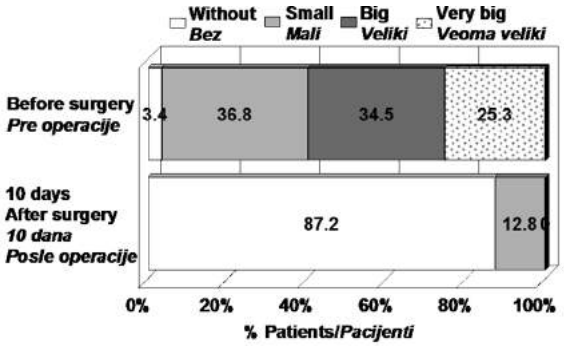
Graph 3. Watching TV and reading subtitles
Grafikon 3. *Čitanje TV teksta/gledanje TV programa*

Discussion

Cataract impairs vision and reduces the quality of life. This is particularly significant and important in the elderly, as they are likely to experience difficulties in daily life activities.



Graph 4. a. Outdoor mobility, b. Indoor mobility
Grafikon 4. a. *Pokretljivost napolju, b. Kretanje u stanu*



Graph 5. Self-estimated vision related problems
Grafikon 5. *Samoprocena problema u vezi sa vidom*

Visual morbidity caused by cataract is reversible. It can be treated successfully by corrective surgical procedures. Cataract surgery is proven to be safe and effective, with a low risk of serious complications [13]. It is one of the safest and most effective surgical procedures performed today. The volume of cataract surgery has already increased dramatically around the world. Therefore, cataract surgery is the most commonly performed operation, majority occurring in the elderly population. High correlations between national socioeconomic development with the quantity and quality of cataract has been documented [14].

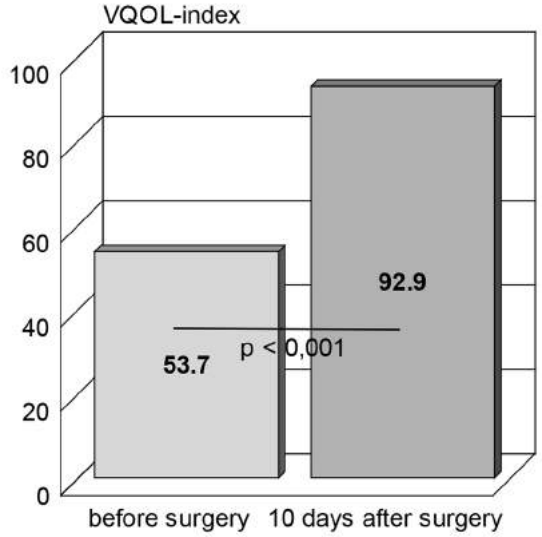
The European Registry of Quality Outcomes for Cataract and Refractive Surgery provide information on cataract surgery clinics in 15 European countries, and they are available for analysis [14]. According to the investigators, excellent visual outcomes were found in more than 60% of patients in the study, vision was unchanged in 5.7%, and 1.7% of patients experienced a decrease in VA. A corrected VA of 0.5 or better was achieved in 94.3%, and of 1.0 in 61.3% of cases. The best visual outcomes were achieved in the age groups between 40 to 74 years, and men showed a higher percentage of excellent vision than women.

In our study, the best corrected visual acuity of 0.5 or better was achieved in 82%, and 0.8 or better in 66% of operated patients.

The results of a large database study by Lundström et al. indicate that poor visual outcome following cataract surgery is most strongly determined by ocular comorbidity and surgical complications [15]. The importance of outcome evaluation of cataract surgery from the standpoint of patients is now widely recognized.

Conventional clinical measures such as VA, contrast sensitivity, visual field testing etc. do not fully capture the influence of visual disability on daily visual functioning and on abilities to perform activities of daily living that are valued by patients.

A significant contribution in the development of a methodology for measuring the quality of life in patients with cataracts was done by Lundström [16].



Graph 6. VQOL index
Grafikon 6. *VQOL indeks*

A relation was found between VA before surgery and the number of problems experienced ($p < 0.001$). After cataract extraction, a reduction in problems was closely associated with an increase in VA ($p < 0.001$) and also, in the patients' opinion, a better life situation ($p < 0.001$).

Our study shows good outcomes after cataract surgery. The mean VQOL-index in patients with senile cataract was 53.7 compared with mean VQOL-index of 92.9 after phaco cataract surgery ($p < 0.001$).

An important measure in cataract surgery is the patients' self-assessed visual function [16]. Self-reported vision related problems in our study were also significantly lower after the surgery.

Our findings suggest that improvement in visual function and self-reported vision related problems in daily activities are valuable parameters in evaluation of surgical results. Therefore, we recommend evaluation of such parameters following routine phaco cataract surgery.

Conclusion

Cataract impairs vision and reduces the quality of life. Phaco cataract surgery with intraocular lens implantation improves visual acuity and vision related daily activities of operated patients.

Vision function, self-estimated improvement in vision related problems, and patients satisfaction are significantly higher after the phaco cataract surgery.

The researchers at the Eye Clinic of the Clinical Center of Vojvodina in Novi Sad, Serbia, have come to the conclusion that vision related quality of life index provides valid and responsive data on vision-related quality of life for tracking therapeutic outcomes.

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PREVALENCE, PREVENTION AND RISK FACTORS FOR LUMBAR SPINE PAIN IN DENTAL PRACTITIONERS

PREVALENCIJA, PREVENCIJA I FAKTORI RIZIKA ZA POJAVU LUMBALNOG BOLA KOD ZAPOSLENIH U STOMATOLOŠKOJ PRAKSI

Elvis MAHMUTOVIĆ[1,2], Radoslava DODER[2,3], Zana DOLIĆANIN[1], Božana RADANOVIĆ[2], Tanja JANKOVIĆ[2,4] and Ksenija BOŠKOVIĆ[2,5]

Summary

Introduction. Lumbar spine pain is among the most common conditions affecting daily activities in modern-day societies, as well as the second most common cause of absenteeism from work. The aim of the paper was to determine the incidence of lumbar spine pain in dental professionals of the Dental Clinic of Vojvodina, the effects of lumbar spine pain on their general health and work ability, their attitude to treatment options, as well as potential etiological factors for the appearance of lumbar spine pain. **Material and Methods.** A prospective study included 45 employees of the Dental Clinic of Vojvodina. We investigated the association between the appearance of lumbar spine pain and the work of dental professionals. The data were gathered via a questionnaire analyzing musculoskeletal disorders, and the probable risk factors for their appearance. **Results.** There were 75.6% (34/45) of women and 24.4% (11/45) of men, aged from 22 to 64 years, with a total work experience of 13±9.6 years. Lumbar spine pain was recorded in 60% of examinees. They were mostly localized in the lower back (52.2%), but also spreading down along both legs (17.4%) and into the right hip (13%) ($p < 0.01$). The incidence of low back pain was higher in dentists (72.4%) than in dental assistants (45.5%) and dental technicians (20%) ($p < 0.05$). **Conclusion.** Lumbar spine disorders are among the most frequent occupational diseases in dental practice, associated with multifactorial causes. Ergonomics and ergonomic education are primary factors in the prevention and therapy of lumbar spine conditions.

Key words: Dentistry; Occupational Diseases; Work Performance; Risk Factors; Low Back Pain; Safety Management; Treatment Outcome

Sažetak

Uvod. Tegobe u slabinskoj kičmi među najjzastupljenijim su činiocima koji remete savremenog čoveka u svakodnevnim aktivnostima, a i drugi su najčešći razlog odsustvovanja sa radnog mesta. Cilj ove studije bio je da se utvrdi zastupljenost tegoba u slabinskoj kičmi kod stomatološkog osoblja na Klinici za stomatologiju Vojvodine, uticaj tegoba u slabinskoj kičmi na opšte zdravlje i radnu sposobnost, stavovi u vezi sa lečenjem ovih tegoba, kao i potencijalni etiološki faktori za nastanak ovih tegoba. **Materijal i metode.** Prospektivna studija je obuhvatila 45 zaposlenih u stomatološkoj praksi na Klinici za stomatologiju Vojvodine. Analizirana je povezanost pojave tegoba u slabinskoj kičmi sa načinom i vrstom rada. Podaci u vezi sa ovim tegobama prikupljeni su upitnikom za analizu mišićno-koštanih tegoba. Analizirani su mogući faktori rizika za njihov nastanak. **Rezultati.** Bilo je 75,6% (34/45) žena i 24,4% (11/45) muškaraca, uzrasta od 22 do 64 godine, ukupnog radnog staža 13 ± 9,6 godine. Tegobe u slabinskoj kičmi zabeležene su kod 60% zaposlenih. Najčešće su lokalizovane samo u krstima (52,2%); ukoliko se bolovi šire, najčešće su prisutni duž obe noge (17,4%) i u desnom kuku (13%) ($p < 0,01$). Stomatolozi (72,4%) češće od stomatoloških sestara (45,5%) i zubnih tehničara (20%) imaju tegobe u predelu krsta ($p < 0,05$). **Zaključak.** Tegobe u slabinskoj kičmi su među najučestalijim bolestima u vezi sa radom zaposlenih u stomatološkoj praksi, čiji su uzroci multifaktorski. Ergonomija i ergonomska edukacija su primarni činioci u prevenciji i terapiji tegoba u slabinskoj kičmi.

Ključne reči: stomatologija; profesionalna oboljenja; radna sposobnost; faktori rizika; lumbalni sindrom; sigurnosne mere; ishod lečenja

Introduction

The main characteristic of lumbar pain syndrome (LPS) is pain in the lower part of the spine, which differs in intensity, character and duration, and with progression it decreases the functional capacity of the lumbosacral part of the spine [1]. Due to their occupational activities, even dental professionals are not spared, because of the exposure to additional and often improper spinal overload [2]. According to the literature data, there are about 130 different causes of LPS, such as inflammatory diseases, neurological diseases, vascular disorders, tumors, and even psychogenic bone pain; still, around 80% of bone pain is caused purely by mechanical reasons [2]. The main mechanism of mechanical bone pain are torsional movements, espe-

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Abbreviations
LPS – lumbar pain syndrome
DCV – Dental Clinic of Vojvodina

cially when connected to compression [3]. Most dentists lean down towards their patients during work, with their right side rested upon the patient's right side. When they need to reach for their tools or materials from the working desk, they have to turn in the direction opposite from their working position to reach them. During the process of performing torsional movements, they turn the upper part of the body with only partial leg turning, and all that just to save time. Frequent repetition of this type of movements, together with strained, so called S-position of cervical part of the spine during work, leads to overstrain in the lumbar part [4]. In dentists, overstrain during work may cause discomfort and occasional pain in the lumbar part of the spine. If the pain is constant in the lower part of the back, reducing the work ability, the condition is probably in the acute phase and requires certain therapeutic measures [4]. It is considered that 80% of adults experience acute lumbar pain at least once in their lifetime, and in 30% the acute lumbar pain evolves into chronic lumbar pain [5]. The results of clinical examinations show that efficient treatment with ergonomic measures decrease the recurrence rate of bone pain, which calls for therapy optimization and ergonomic working conditions and education.

The paper aimed to determine the presence of LPS in dental healthcare workers, the effects of LPS on quality of life, views connected to treating these disorders, as well as potential etiological factors for appearance of these conditions. Gathered information will help raise the awareness about the significance of correct approach to lumbar syndrome, its prevention and fast recovery in working age population, especially in dentistry.

The Ethics Committee of the Dental Clinic of Vojovdina has approved the research.

Material and Methods

The research sample included 45 employees of the Dental Clinic of Vojvodina (DCV): dentists, dental technicians and dental assistants. The volontary and anonymous analysis of LPS was performed by using a modified "Standardized Nordic questionnaire for the analysis of musculoskeletal symptoms", which included 9 sections of questions related to presence of pain in specific areas of the body: neck, shoulders, elbows, wrists, hands, upper back, lower back, hips, knees, ancles and feet [6]. In addition, another questionnaire was made, including socio-demographic questions related to potential etiological factors, pain and its treatment if it was present, as well as questions related to gender, age, weight, height, profession, years of service, working hours during the day and week, body position during work, presence and intensity (visual-analog scale of pain) of pain, intensity of pain during different periods of the day; functional, psychological and emotional problems caused by the pain; factors causing the increase of pain; use of drug and/or physical treatment; and effects and outcome of treatment. The questionnaires were self-administered and delivered in person. For each department of DCV one poll taker was determined to collect filled questionnaires in a closed box.

All the gathered information were processed by statistical program International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistics 20. The results were displayed via tables and graphs alongside textual comments. Stand-

Table 1. Characteristics of patients
Tabela 1. *Karakteristike pacijenata*

Characteristics/*Karakteristike*		Number/*Broj* (%); $\overline{X}$±SD
Gender/*Pol*	Male/*Muški*	11 (24.4)
	Female/*Ženski*	34 (75.6)
Age (years)/*Starost* (*godine)*	Mean/*Srednja vrednost*	37.8±9.3
Dentist staff/*Stomatološko osoblje*	Dentists/*Stomatolog*	29 (64.5)
	Dental assistant/*Stomatološka sestra*	11 (24.4)
	Dental technician/*Zubni tehničar*	5 (11.1)
Body weight/*Telesna težina (kg)*	Male (mean)/*Muškarci (srednja vrednost)*	80.8±8.9
	Female (mean)/*Žene (srednja vrednost)*	64.2±10.7
Body height/*Telesna visina (cm)*	Male (mean)/*Muškarci (srednja vrednost)*	182.3±5.8
	Female (mean)/*Žene (srednja vrednost)*	170.5±7
Years of service/*Radni staž*	Mean/*Srednja vrednost*	13.2±9.4
Working hours per day/*Dnevno radno vreme*	Mean/*Srednja vrednost*	7.3±1.5
Breaks during work/*Trajanje dnevne pauze (min)*	Mean/*Srednja vrednost*	30±9
Body position during work *Položaj tela u toku rada*	Standing/*Stojeći*	9 (20)
	Sitting/*Sedeći*	8 (17.8)
	Changing/*Promenljivo*	28 (62.2)

Table 2. The results of the questionnaire used in the study
Tabela 2. *Rezultati upitnika korišćenih u istraživanju*

Characteristics *Karakteristike*			Number *Broj (%)*	p-value *vrednost*
Low back pain *Tegobe u donjem delu leđa*	Dentists *Stomatolozi*	No/*Ne*	8 (27.6)	0.046
		Yes/*Da*	21 (72.4)	
	Dental assistant *Stomatološka sestra*	No/*Ne*	6 (54.5)	
		Yes/*Da*	5 (45.5)	
	Dental technician *Zubni tehničar*	No/*Ne*	4 (80)	
		Yes/*Da*	1 (20)	
Low back pain in the past 12 months *Tegobe u donjem delu leđa u poslednjih 12 meseci*	Male/*Muškarci*	No/*Ne*	5 (45.5)	0.671
		Yes/*Da*	6 (54.5)	
	Female/*Žene*	No/*Ne*	13 (38.2)	
		Yes/*Da*	21 (61.8)	
Most common complaints during the day *Najizraženije tegobe u toku dana*	Morning/*Ujutru*		7 (25.9)	0.127
	Afternoon/*Popodne*		8 (29.6)	
	Evening/*Uveče*		9 (33.3)	
	All day/*Celog dana*		3 (11.1)	
Functional problems caused by low back pain *Funkcionalni problemi uzrokovani tegobama u donjem delu leđa*	No complaints/*Bez smetnji*		14 (31.2)	0.004
	With temporary pain during full work time *Sa privremenim bolom tokom punog radnog vremena*		29 (64.4)	
	With difficulties and therapy during full work time *Sa poteškoćama uz terapiju u punom radnom vremenu*		2 (4.4)	
	With difficulties and therapy during part time work *Sa poteškoćama uz terapiju u skraćenom radnom vremenu*		0 (0.0)	
The pain is localized in… *Tegobe su lokalizovane u…*	Lower back/*Krstima*		15 (55.5)	<0.01
	Lwer back and spreading towards the right hip *Krstima i šire se prema desnom kuku*		3 (11.1)	
	Lower back area and spreading alongside the left leg *Krstima i šire se niz levu nogu*		2 (7.4)	
	Lower back area and spreading alongside the right leg *Krstima i šire se niz desnu nogu*		2 (7.4)	
	Lower back area and spreading alongside both legs *Krstima i šire se niz obe noge*		5 (18.5)	
Low back pain is intensified with.../*Tegobe u donjem delu leđa se intenziviraju pri…*	Coughing/*Kašljanju*		2 (7.4)	<0.001
	Strain/*Naporu*		22 (81.5)	
	All the above/*Sve pomenuto*		3 (11.1)	
Low back pain and associated symptoms *Tegobe u donjem delu leđa i prateći simptomi*	Anxiety/*Nervoza*		4 (28.6)	0.174
	Bad sleep/*Lošiji san*		1 (7.1)	
	Bad concentration/*Slabija koncentracija*		1 (7.1)	
	Problems with everyday activities *Smetnje u svakodnevnim aktivnostima*		5 (35.7)	
	Anxiety and problems in everyday activities *Nervoza i smetnje u svakodnevnim aktivnostima*		1 (7.1)	
	Anxiety, low threshold tolerance and problems in everyday activities/*Nervoza, snižen prag tolerancije i smetnje u svakodnevnim aktivnostima*		1 (7.1)	
	Bad sleep and problems in everyday activities *Lošiji san i smetnje u svakodnevnim aktivnostima*		1 (7.1)	

ard descriptive methods were used in analysis of the information – determining the average value (X) and standard deviation (SD). For testing the differences of average values between results, Students t-test and t-test of pairs, non-parameter method Pearson's χ^2 test (test of independence and matching) were used. Statistical significance was defined at $p < 0.01$.

Results

The research included 45 healthcare workers from 6 different dental departments: Department of Dental Prosthetics (26.7%), Department of Dental Diseases with Endodontics (22.2%), Department of Child and Preventative Dentistry (15.6%), Departments of Periodontics and Oral Medicine (13.3%), Department of the Jaw Orthopedics (13.3%), Department of Oral Surgery (8.9%). Out of 45 examinees, 34 were women (75.6%) and 11 were men (24.4%). The average age was 37 years. The youngest examinee was 22, and the oldest 64. Most examinees were aged from 30 - 34 and 35 – 39 years.

The average body weight was 80.8 ± 8.9 kg in male examinees and 64.2 ± 10.7 kg in the female examinees. The average height of males was 182.3±5.8 cm, and 170.5±6.9 cm of females. The questionnaires were filled out by 29 (64.5%) dentists, 11 (24.4%) dental assistants, and 5 (11.1%) dental technicians. On average, the employees were at current position for at least 13 years. Most employees had a work experience of 6 - 10 and 11 - 15 years.

The most of examinees (62.2%) answered that they changed their body position during work. The clinical characteristics of patients are shown in **Table 1**.

Dentists (72.4%) experienced low back pain more frequently than dental assistants (45.5%) and dental technicians (20%). Significant statistical difference was determined in the occurrence of low back pain in regard to the type of work done by healthcare workers ($\chi^2 = 6.2$, df = 2, p=0.046) **(Table 2)**.

During the previous 12 months, women (61.8%) reported more low back pain episodes (pain, irritation) than men (54.5%) (χ^2=0.005, df=1, p=0.671) **(Table 2)**. Due to low back pain in the last 12 months, women (32.4%) experienced more pain and discomfort than men (27.3%) in performing their usual activities at home or elsewhere (χ^2=0, df=1, p=1).

Almost half of the examinees (48.2%) suffered from low back pain up to 5 years. Approximately ¼ of examinees had low back pain up to 10 years (25.9%), four patients (14.8%) suffered for more than 10 years, and three of them up to 1 year (11.1%).

Regardless of profession, LPS is always temporary, appearing in 94.4% of dentists, in 50% of dental assistants, and 100% of dental technicians. There is no statistically significant difference in the incidence of low back pain in relation to the type of work of the employees (χ^2=5.856, df=2, p=0.053). In men (80%) and women (88.9%) LPS was most commonly temporary (χ^2=0.273, df=1, p=0.602).

Regardless of position, LPS is most commonly temporary. In standing position, LPS is present in 71.4% of the cases, 100% in sitting position, and 93.3% in interchanging positions (χ^2=2.176, df=2, p=0.337).

Low back pain intensity was rated via visual-analog scale for pain. The score „0“ meant absence of pain, whereas the score „10“ stood for the most severe pain. None of the examinees rated their pain with „10“. The pain was most commonly rated as „4“, which was also the average score (X = 4 ± 1.5).

Functional problems due to low back pain in the 65 examinees were of such type that working activities were performed with temporary pain during the full work period, while 31% of the examinees declared they performed activities without problems. None of the examinees declared they performed activities with difficulties and using therapy during part time work **(Table 2)**.

The chi-square test indicated that the number of the examinees who performed activities with temporary pain during full work period (64.4%) was significantly different ($\chi^2 = 10.9$, df = 2, p = 0.004) from the number of examinees who declared that they performed activities without problems (31.2%) and using therapy (4.4%)

Low back pain is most commonly localized only in the lower back region (55.5%). If the pain spreads, it is most commonly localized along both legs (18.5%) and in the area of the right hip (11.1%). It is statistically significant ($\chi^2 = 15.478$, df = 4, p = 0.004) that low back pain is localized in the lower back area more frequently compared to the pain spreading from the lower back to hips and legs **(Table 2)**.

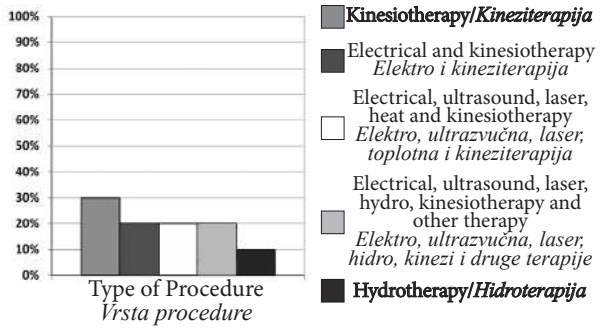
In case of low back pain, the examined employees most commonly (57.7%) did not see a doctor. Those who did, mostly visited a physician (70%). About 1/3 of the employees with LPS do not take medications (33.3%), and if they do, they (81.2%) occasionally take non-steroidal anti-inflammatory drugs (NSAIDs) (37.5%) or some other medications (29.2%). In most cases (63%) examinees take medications for LPS, on their own, without physical therapy (63%). The most common reason for not having physical therapy was the lack of time. Ten employees (37%) said that they had physical therapy in case of LPS and kinesitherapy was the most frequent (90%). Kinesitherapy is most commonly used in combination with electrotherapy (60%) **(Graph 1)**.

Most of the employees (50%) stated they did not know whether physical therapy decreased low back pain, 40% of examinees answered that physical therapy had positive effects, and 10% that it had no effects on reducing symptoms of the low back pain **(Graph 2)**.

Discussion

The research and analysis of LPS was done using a “Standardized Nordic questionnaire for the analysis of musculoskeletal symptoms”, as well as an additional questionnaire on socio-demographic, etiological factors, and factors related to pain and its treatment, if there was any. The “Standardized Nordic questionnaire for the analysis of musculoskeletal symptoms” is internationally acclaimed and one of the most commonly used standardized tests for evaluation of musculoskeletal pain [7–10].

The prevalence of LPS among the examinees was 60%. A similar prevalence rate was recorded in Australia, Denmark, Izrael, United States and Pakistan [11, 12]. Lower prevalence rate was recorded in Southern Iran (54.6%), Belgium (54%),



Graph 1. Physical therapy procedures used in low back pain
Grafikon 1. *Fizikalne procedure koje se koriste kod bola u donjem delu leđa*

and China (52.5%), while higher prevalence rate was recorded in Taiwan (66%), Nigeria (77.1%) and Lithuania (91%) [2, 7, 8, 13–15].

The majority of examinees in our research were women (75.6%) with a lumbar syndrome prevalence of 61.8% that was higher compared to men (54.5%). Also, due to LPS, women had more difficulties (32.4) in doing everyday activities (at home or elsewhere) than men (27.3%). The most examinees were aged from 30 to 39 indicating that the biggest percentage of examinees with LPS was in this age group. These results are in agreement with researches conducted in United States, Pakistan and Taiwan [7, 12, 16].

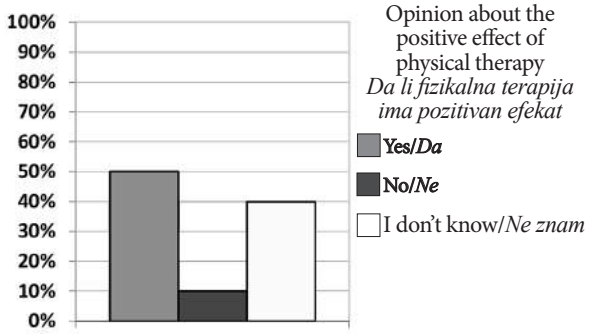
According to some researches, the risk factors for LPS include the type of profession, years of service, age of dentists, and number of patients treated per day [8, 12]. The prevalence of low back pain is affected by the dentists' body weight and body height [17]. Our research showed that LPS is more common in dentists (72.4%) than dental assistants (45.5%) and technicians (20%), because during work dentists spend time in forced positions and positions straining spinal structures and thus indirectly affecting appearance of pain which is proven by researches of Thornton et al., Åkesson, Balogh and Hansson, as well as two case series of Sanders and Turcotte [18–20].

The usual position of dentists is sitting or standing and it is associated with extreme flexion of the neck above 30 degrees with rotation and/or side flexion to the right or left, flexion of shoulders in protraction and abduction greater than 45 degrees and flexion of torso in kyphosis [20]. This position of dentists causes increased strain on spinal unit which along with movement, rotation, flexion and torsion of torso, in time leads to instability of spinal unit and consequential changes on spinal discs such as herniation and compressions on nerve roots, and development of lumbar radiculopathy [18, 20]. Along with body position, another risk factor is length of intervention [17]. It is proven that low degree of strain that lasts for a long time can cause muscle fatigue and chronic pain [21].

Some studies suggest taking 10 minute brakes during interventions [7, 20]. A greater number of shorter brakes during working hours (not longer than 5 seconds) is much more practical and provides full recovery to fatigued muscles. During these micro brakes muscles receive more blood and time to recover [17]. Static positions that distort neutral posture must be avoided; it is helpful to adjust the seat and have a rest from time to time, so that the strain can be transferred from one tissue to another, minimizing the micro trauma [17].

The ideal posture during work means: neck in flexion from 0 to 10 degrees, without rotation or side flexion; shoulders relaxed and in retraction; elbows in the level of patient's mouth and in flexion of up to 90 degrees; lumbar part of the spine in neutral position with preserved physiological lordosis with leg support in mild abduction and outside rotation; knees slightly above the hip level; the feet should be flat on the floor, or rested upon the seat bar to decrease the strain on the lumbar spine [20].

The results have shown that in dental practice adequate ergonomic conditions are the most important for the prevention of LPS. From the ergonomic point of view, the most important thing is posture during work in the office, both in sitting and standing positions. For a dentist, the most important thing in his office is the chair [17, 20]. The chair should keep the body in a neutral position. The back support should keep physiological lumbar lordosis with its concavity during sitting, and is thus called lumbar support. When it comes to large back supports, the upper part should not press the thoracic spine and push it forward. The back supports that don't cross the bottom edge of a shoulder blade can decrease the effect of lumbar support by transferring the pressure to the shoulder blades, and they should be 6 cm bellow the bottom edge of the shoulder blade. Wide back supports should be avoided, because they can obstruct lateral movements of torso and upper limbs [22]. Due to the nature of the profession, dentists cannot always keep contact with lumbar support of the chair; occasionally they lean forward to see a specific surface of the teeth. The pressure on intervertebral disc is highest in this position, which requires dentists to properly stabilize and protect lumbar spine with stabilizing muscles (primarily transverse abdominal muscles) whenever they leave the



Graph 2. Opinions about the effects of physical therapy in low back pain
Grafikon 2. *Mišljenje o uticaju fizikalne terapije u slučaju tegoba u donjem delu leđa*

chair support. When used properly, these muscles prevent and decrease the low back pain [22].

Conclusion

The prevalence of lumbar pain syndrome is greater in dentists than in dental assistants and technicians. During work, dentists spend long periods of time in forced positions which create a risk for appearance of spinal issues. The low back pain is of higher intensity in women who have more difficulties in doing everyday activities than men. Low back pain affects the quality of life in the employees of the Dental Clinic of Vojvodina. The results of this study showed that adequate (ergonomic) working conditions are required, as well as education of dentists and dentistry students, on the risk factors, prevention of lumbar pain syndrome, and therapeutic modalities. Timely and adequate prevention of lumbar pain syndrome ensures longer and more comfortable working life, and a nice and painless retirement.

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CASE REPORTS
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Case report
Prikaz slučaja
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THE OUTCOME OF PNEUMOCYSTIS JIROVECI PNEUMONIA COMPLICATED WITH VENTILATOR - ASSOCIATED PNEUMONIA IN A PATIENT SUFFERING FROM ACQUIRED IMMUNODEFICIENCY SYNDROME - A CASE REPORT

ISHOD PNEUMOCYSTIS JIROVECII PNEUMONIJE KOMPLIKOVANE PNEUMONIJOM UDRUŽENOM SA MEHANIČKOM VENTILACIJOM KOD PACIJENTA OBOLELOG OD SINDROMA STEČENE IMUNODEFICIJENCIJE – PRIKAZ SLUČAJA

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Summary

Introduction. The respiratory tract is the most common site of opportunistic infections in patients with acquired immunodeficiency syndrome, and respiratory failure is the leading cause of death in these cases. In addition, the use of mechanical ventilation in the treatment of respiratory failure carries a high risk of nosocomial pneumonia caused by multidrug-resistant strains of bacteria. **Case report.** We present a human immunodeficiency virus-positive patient with positive outcome of Pneumocystis jiroveci pneumonia whose condition progressed to acute respiratory distress syndrome with the development of complications associated with mechanical ventilation. As soon as an opportunistic infection was suspected, trimethoprim-sulfamethoxazole and corticosteroid therapy were initiated, with a short application of mechanical ventilation. Despite a good initial response to treatment, during the mechanical ventilation, a relapse of fever occurred, as well as development of productive cough, new zones of consolidation on chest X-ray and Acinetobacter spp. was isolated in a swab culture. According to the literature recommendations, colistin-meropenem combination was conducted with adequate response. The patient was discharged after 22 days of treatment, in good general condition, afebrile, with normal laboratory parameters and gas exchange, without subjective symptoms. **Conclusion.** Early identification of Pneumocystis jiroveci pneumonia, timely initiation of prophylaxis and treatment, along with antiretroviral therapy, reduced the risk of severe clinical events and respiratory failure. Also, timely recognition of complications of mechanical ventilation, identification of infection, early extubation and application of appropriate antibiotic therapy, reduce the mortality from nosocomial pneumonia caused by multi-drug resistant hospital strains of microorganisms.

Keywords: Pneumonia, Pneumocystis; Pneumonia, Ventilator-Associated; Acquired Immunodeficiency Syndrome; Pneumocystis carinii; Respiratory Distress Syndrome, Adult; Opportunistic Infections; Treatment Outcome; Diagnosis; Risk Factors

Sažetak

Uvod. Respiratorni trakt je najčešće ishodište oportunističkih infekcija kod pacijenata obolelih od sindroma stečene imunodeficijencije, a respiratorna insuficijencija vodeći uzrok smrti u tim slučajevima. Dodatno, primena mehaničke ventilacije u lečenju respiratorne insuficijencije nosi rizik od razvoja nozokomijalnih pneumonija uzrokovanih sojevima multirezistentnih bakterija. **Prikaz slučaja**. Prikazali smo bolesnika pozitivnog na virus humane imunodeficijencije sa pozitivnim ishodom *Pneumocystis jirovecii* pneumonije čije je stanje progrediralo do akutnog respiratornog distres sindroma uz razvoj komplikacije udružene sa mehaničkom ventilacijom. Odmah po postavljanju kliničke sumnje na ovu oportunističku infekciju započeto je lečenje trimetoprim-sulfametoksazolom i kortikosteroidnom terapijom uz kratkotrajnu primenu mehaničke ventilacije. Uprkos inicijano dobrom terapijskom odgovoru, tokom primene mehaničke ventilacije došlo je do ponovne pojave febrilnosti, produktivnog kašlja, novih zona konsolidacije na radiografiji pluća uz izolaciju *Acinetobacter spp.* iz brisa aspirata. Prema preporukama iz literature sprovedeno je lečenje kombinovanom antibiotskom terapijom, kolistin i meropenem, uz adekvatan odgovor. Bolesnik je otpušten kući nakon 22 dana lečenja, u dobrom opštem stanju, afebrilan, urednih laboratorijskih parametara, dobre gasne razmene, bez subjektivnih tegoba. **Zaključak**. Rano prepoznavanje *Pneumocystis jirovecii* pneumonije, pravovremeno započinjanje profilakse i lečenja ove infekcije, uz antiretrovirusnu terapiju, smanjuje rizik od razvoja teške kliničke slike sa nastankom respiratorne insuficijencije. Takođe, pravovremeno prepoznavanje komplikacija mehaničke ventilacije, identifikacija infekcije, rana ekstubacija i primena odgovarajuće kombinovane antibiotske terapije smanjuju mortalitet od nozokomijalnih pneumonija uzrokovanih multirezistentnim bolničkim sojevima mikroorganizama.

Ključne reči: pneumocistis pneumonija; pneumonija udružena sa mehaničkom ventilacijom; AIDS; Pneumocystis carinii; respiratorni distres sindrom; oportunističke infekcije; ishod lečenja; dijagnoza; faktori rizika

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Abbreviations

HIV – human immunodeficiency virus
AIDS – acquired immune deficiency syndrome
PJP – Pneumocystis jiroveci pneumonia
ARDS – acute respiratory distress syndrome
MV – mechanical ventilation
PCR – polymerase chain reaction
VAP – ventilator-associated pneumonia
TMP-SMX – trimethoprim/sulfamethoxazole
ART – antiretroviral therapy
pO_2 – partial pressure of oxygen
pCO_2 – partial pressure of carbon dioxide
SaO_2 – oxygen saturation

Introduction

Pneumocystis jiroveci pneumonia (PJP), previously known as Pneumocystis carinii pneumonia (PCP), is one of the leading causes of death among human immunodeficiency virus (HIV)-positive individuals [1].

The PJP clinically presents with subfebrile fever, nonproductive cough, dyspnea, malaise and a significant loss of body weight. At first the symptoms may be subtle, but may also rapidly progress causing acute respiratory failure. The physical examination is usually nonspecific. The pulmonary auscultatory findings may vary from normal to inspirium crackles, and chest X-ray usually shows symmetrical, bilateral, perihilar interstitial infiltrates. It is estimated that PJP is the cause of respiratory failure in HIV/ acquired immune deficiency syndrome (AIDS) patients in 35% of cases treated in intensive care units [2]. The development of ARDS during this opportunistic infection requires mechanical ventilation (MV) [3, 4], in spite of which a high mortality rate is still recorded in these patients [5].

Application of invasive mechanical respiratory support may be complicated by a variety of conditions, such as barotrauma, oxygen toxicity damaging the airways, tracheal stenosis and nosocomial pneumonia caused by multidrug-resistant strains of bacteria [6, 7]. The mentioned complications increase the mortality rate of these patients, as well as the length of hospitalization, and treatment costs.

Ventilator-associated pneumonia (VAP) is defined as pneumonia that occurs after 48 - 72 h of endotracheal intubation, and it is characterized by the appearance of a new infiltrate or progression of the existing, signs of systemic infection, changes in sputum characteristics and isolation of the agent [8].

In this paper, we present a patient with positive outcome of PCJ, whose condition progressed to ARDS with the development of complications associated with MV.

Case Report

A 44-year-old HIV-positive patient was hospitalized at the Clinic for Infectious Diseases, Clinical Center Kragujevac, presenting with fever, cough, shortness of breath, fatigue, loss of appetite, and a 15-kg weight loss. The symptoms started four weeks before hospitalization, gradually, with subfebrile fever up to 37.4°C, malaise, loss of appetite and weight loss. A week before admission, the symptoms intensified, with high fever up to 40°C, appearance of dry cough, accompanied by chest pain that intensified with cough and deep breathing, and fatigue.

The patient was confirmed to be HIV-positive in 2012, when he was still asymptomatic. In August 2013, the number of $CD4^+$ T lymphocytes amounted to 196/mm^3, therefore the treatment with antiretroviral therapy (ART) started. Since July 2014, the patient has not come to control examinations and stopped using ART.

On admission, the patient was conscious, oriented, febrile (38.4° C), tachypneic, dyspneic, oxygen saturation (SaO_2) 88%, with pale skin, without rash, without signs of peripheral lymphadenopathy, cachectic. Seborrheic dermatitis was observed on his head skin. The tongue was covered with whitish layer. The thorax was cylindrical, symmetrical respiratory mobile. Diffusely decreased breath sounds with inspiratory crackles. The abdomen was below the level of the chest, soft, painless. Other physical findings were normal.

The laboratory test results on admission showed positive biohumoral inflammatory syndrome **(Table 1)** and chest X-ray showed diffuse changes in the lung parenchyma that corresponded to ARDS **(Fig-**

Table 1. Laboratory findings
Tabela 1. *Laboratorijski nalaz*

	Le	Ne	Er	Hgb	Tr	CRP	PCT	LDH
Day 1/*Dan 1.*	6.8	93.8	3.00	90	217	155.6	2.66	1653
Day 5/Dan 5.	5.7	92.5	2.94	87	166	23.4	0.306	1257
Day 8/Dan 8.	4.4	90.3	3.34	100	168	43.6	0.283	699
Day 13/Dan 13.	3.9	81.8	3.43	102	201	10.1	0.195	398
Reference range *Referentni opseg*	3.7 - 10.0 x 10^9/L	44 - 72%	4.34 - 5.72 x 10^{12}/L	138 - 175 g/L	135 - 450 x 10^9/L	< 5.0 mg/L	< 2.0 ng/mL	220 – 450 IU/L

*Legend/*Legenda*: Le – leukocytes/*leukociti*, Ne – neutrophils/*neutrofili*, Er – erythrocytes/*eritrociti*, HGB – hemoglobin/*hemoglobin*, Tr – thrombocytes/*trombociti*, CRP – C-reactive protein/*C-reaktivni protein*, PCT – procalcitonin/*prokalcitonin*, LDH - Lactate dehydrogenase/*laktat dehidrogenaza*; IU – international unit/*internacionalne jedinice*

ure 1A). Initial arterial blood gas analysis, without oxygen therapy, indicated existence of hypoxia, hypocapnia and low oxygen saturation (pH 7.45; partial pressure of carbon dioxide (pCO_2) 3.9 kPa; partial pressure of oxygen (pO_2) 6.9 kPa; SaO_2 88%).

According to the protocol, blood, urine and swabs were sampled for microbiological analysis; apart from fungal isolates from the tongue swab (Candida spp.), the results indicated no presence of other microbiological agents. The serological tests registered IgG class of antibodies to Cytomegalovirus, Epstein-Barr virus, Herpes simplex virus type 1 and Toxoplasma gondii, and excluded acute Influenza virus type A (H1N1), Mycoplasma pneumoniae and Candida albicans infections. By determining the number of T lymphocytes, a significant quantitative disorder of $CD4^+$ T lymphocytes with only 6 lymphocytes/mm^3 was registered.

Considering that at the time of admission, according to Centers for Disease Control and Prevention (CDC) classification system, this was a C3 category of AIDS, with opportunistic lung infection, polymerase chain reaction (PCR) analysis on Cytomegalovirus, Mycobacterium tuberculosis, Mycobacterium avium complex and PJP was performed on bronchoalveolar lavage. The results of PCR analysis excluded Cytomegalovirus pneumonitis and pulmonary tuberculosis, but confirmed PJP.

From the second day of hospitalization, due to clinical presentations and deterioration of the patient's general condition, despite the use of oxygen therapy of 5L/min oxygen, through a mask, a satisfactory gas exchange was not achieved (pH 7:46; pCO_2 3.2 kPa; pO_2 6.0 kPa; SaO_2 84%) and invasive mechanical ventilation was applied. On the eighth day of treatment, after achieving adequate gas exchange, the patient was extubated.

Immediately after the clinical suspicion of PJP pneumonia was confirmed, on the day of admission, parenteral treatment with trimethoprim-sulfamethoxazole (TMP-SMX, 4 amp of 480 mg three times a day), with antifungal (fluconazole) combined with broad spectrum antibiotics was initiated. In addition to antimicrobial therapy, the patient was also administered oral corticosteroid therapy for seventeen days, initially at a dose of 80 mg per day, which was then successively decreased. Treatment with TMP-SMX was conducted for a period of twenty-one days. The patient had a favorable response to the initial treatment. He became afebrile from the second day of hospitalization, with gradual auscultation and radiographic regression of lung findings **(Figure 1B)**, and inflammation parameters also decreased **(Table 1)**.

However, during the period of MV application, on the seventh day of hospitalization the patient became febrile again, up to 38° C, with a rise of biohumoral inflammatory syndrome markers **(Table 1)**, as well as the occurrence of abundant purulent secretion from the lower respiratory tract and radiographically verified new consolidation of lung parenchyma **(Figure 1C)**. With the microbiological examination of the aspirate, Acinetobacter spp. was isolated, therefore in the further course of newly developed ventilator-associated pneumonia, the treatment was conducted according to antibiogram (meropenem and colistin), from the ninth day of hospitalization. The combined antimicrobial treatment of VAP was conducted over ten days. After a ten-day treatment of VAP, control laboratory tests indicated a significant reduction in neutrophil levels, C-reactive protein and procalcitonin **(Table 1)**.

On the thirteenth day of hospitalization, the patient restarted highly active antiretroviral therapy (HAART) treatment (abacavir, lamivudine, lopinavir and ritonavir) and was discharged after twenty-two days, in good general condition, afebrile, with normal laboratory parameters and gas exchange, without subjective symptoms **(Figure 1 D).**

Discussion

The PJP is the most common opportunistic infection in AIDS. The spectrum of clinical manifestations ranges from mild symptoms to severe clinical picture with the development of respiratory failure. It is estimated that in 35% of cases, PJP is the cause of respiratory failure in HIV/AIDS patients treated in intensive care units [2]. The first-line therapy in the treatment of severe forms of PJP is intravenous administration of high doses of TMP-SMX, at least for 21 days. In patients with poor gas exchange, corticosteroid therapy showed the most favorable effects, reducing the risk of death by 50% [9, 10]. Also, the optimal

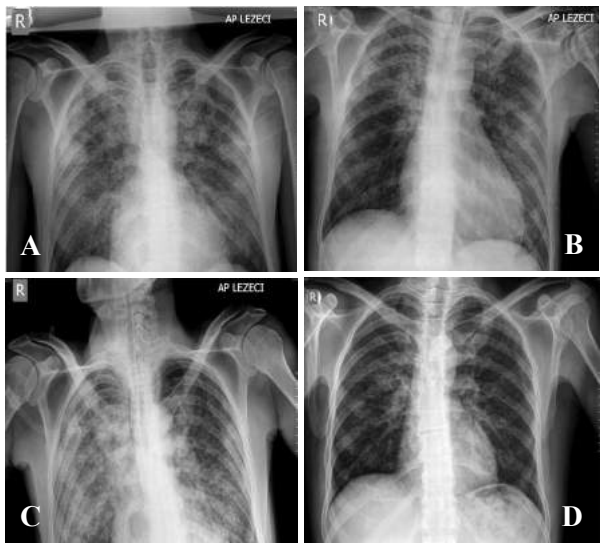

Figure 1. Chest x-ray **A.** on admission; **B.** regression of initial findings as a response to therapy (TMP-SMX, corticosteroids); **C.** development of new pulmonary changes during application of mechanical ventilation; **D.** on discharge.

Slika 1. *Radiografije grudnog koša:* ***A.*** *na prijemu;* ***B.*** *regresija inicijalnog nalaza kao odgovor na terapiju (TMP-SMX, kortikosteroidi);* ***C.*** *razvoj novih plućnih promena tokom primene mehaničke ventilacije;* ***D.*** *na otpustu*

moment for the introduction of ART in these patients is not clearly defined. Zolapa and associates compared the effect of early (within the first 14 days of antibiotic treatment) and deferred (after completion of antibiotic treatment) administration of ART. This group of authors showed that the introduction of ART in the first 14 days of antibiotic treatment in patients with opportunistic infections reduces the risk of developing complications and death [11]. On the other hand, it has been shown that in some cases, early introduction of ART therapy can lead to the formation of immune reconstitution inflammatory syndrome, which is paradoxically clinical deterioration, and correlates with an increase in the number of $CD4^+$ T-lymphocytes [12].

At the beginning of the HIV/AIDS epidemic, PJP has been one of the leading causes of death, but now, with new antiretroviral drugs and the use of prophylaxis in patients at increased risk, the mortality has been drastically reduced [13]. However, in severe forms of PJP, which require the use of MV, mortality remains high, up to 60% [14]. In addition, intubation and MV further increase the the risk of nosocomial bacterial pneumonia by 6 to 20 times, with a mortality rate of 20 to 50% [8, 15].

The VAP is defined as pneumonia that occurs after 48 – 72h of endotracheal intubation, and it is characterized by the appearance of a new infiltrate or progression of the existing, signs of systemic infection, changes in sputum characteristics, and agent isolation [8]. They are commonly caused by the multidrug-resistant Gram-negative pathogens, such as Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli and Klebsiella pneumoniae, but there is also an increased incidence of multi-resistant Staphylococcus aureus (MRSA) infections [8, 16]. The occurrence of these infections is difficult to control and they are associated with prolonged stay in the intensive care units, prolonged hospital stay, and high cost of treatment [17].

The choice of adequate antimicrobial treatment of VAP is limited by the fact that there is a high resistance rate to many available antibiotics [18]. In our case, VAP was caused by Acinetobacter spp. So far, it has been shown that combination antibiotic therapy, colistin with carbapenems (imipenem or meropenem) or rifampicin, is the treatment of choice in treating severe forms of VAP caused by this pathogen, preventing the development of colistin-resistant strains of bacteria [17, 19].

Conclusion

Early recognition of signs and symptoms of Pneumocystis jiroveci pneumonia, timely initiation of prophylaxis and treatment of this opportunistic infection, as well as introduction of antiretroviral therapy, with adequate compliance, reduces the risk of severe clinical picture and the development of acute respiratory failure. Also, timely recognition of mechanical ventilation complications, identification of infection, early extubation and application of appropriate antibiotic combination therapy, may reduce the mortality from nosocomial pneumonia caused by multi-drug resistant hospital strains of microorganisms.

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DETECTION OF HUMAN EMBRYO ANEUPLOIDY IN THE PROCEDURE OF IN VITRO FERTILIZATION AFTER PREIMPLANTATION GENETIC SCREENING USING ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

DETEKCIJA ANEUPLOIDIJA U HUMANIM EMBRIONIMA U POSTUPKU IN VITRO FERTILIZACIJE NAKON PREIPLANTACIONOG GENETIČKOG SKRININGA ARRAY KOMPARATIVNOM GENOMSKOM HIBRIDIZACIJOM

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Summary

Introduction. Numerical aberrations (whole chromosomal aneuploidy) have been considered one of the most important factors leading to implantation failure and early miscarriages in patients undergoing assisted reproductive procedures. Embryo selection is mainly based on morphological assessment; however, embryos produced from aneuploid gametes cannot be distinguished from euploid based on morphological characteristics. **Detection of aneuploidy in human embryos**. Thanks to the introduction of molecular-genetic screening of embryos, it is possible to identify aneuploid embryos via preimplantation genetic screening/diagnosis and thus select the best embryos based on their ploidy. Array comparative genomic hybridization is a molecular technique which allows ploidy analysis of the entire genome amplification from a single cell, within 24 hours after polar body, blastomere or trophectoderm cell biopsy. Trophectoderm cell biopsy is considered the most reliable screening approach given the lower mosaicism appearance at the blastocyst stage. **Conclusion**. This paper points to the importance and necessity of molecular analysis in embryo selection. Further investigations and improvements are required, because this technology has only recently become available in clinical practice in the in vitro fertilization procedure.

Key words: Aneuploidy; Chromosome Aberrations; Embryo Research; Embryo Transfer; Fertilization in Vitro; Preimplantation Diagnosis; Genetic Testing; Comparative Genomic Hybridization

Sažetak

Uvod. Numeričke hromozomske aberacije (aneuploidije) smatraju se jednim od najvažnijih faktora koji dovodi do neuspešnih implantacija i ranih pobačaja među pacijentima u postupcima asistirane reprodukcije. Selekcija embriona za transfer najčešće se sprovodi na osnovu morfologije embriona, međutim, embrioni koji su nastali od aneuploidnih gameta ne mogu se razlikovati od euploidnih na osnovu morfoloških karakteristika. **Detekcija aneuploidija u humanim embrionima**. Zahvaljujući uvođenju molekularno-genetičkog skrininga ploidnosti embriona, danas je moguće identifikovati aneoploidne embrione u postupku preimplantacione genetičke dijagnostike/skrininga i na taj način izvršiti selekciju embriona za transfer. *Array*-komparativna genomska hibridizacija je molekularna metoda koja omogućava analizu ploidnosti čitavog genoma iz samo jedne ćelije i to za samo 24 časa nakon biopsije polarnog tela, blastomere ili ćelija trofoektoderma. Biopsija ćelija trofoektoderma smatra se najpouzdanijim pristupom za skrining, s obzirom na manju zastupljenost mozaicizama na stadijumu blastociste. **Zaključak.** U ovom radu ukazano je na važnost i potrebu molekularnog pristupa pri selekciji embriona za transfer. Budući da je ova tehnologija tek nedavno započela svoju kliničku primenu u postupcima *in vitro* fertilizacije, neophodna su dalja istraživanja i usavršavanja ove procedure.

Ključne reči: aneuploidija; hromozomske aberacije; istraživanja na embrionima; embriotransfer; in vitro fertilizacija; preimplantaciona dijagnoza; genetsko testiranje; komparativna genetička hibridizacija

Introduction

It is estimated that 15% of spontaneous clinical pregnancies are miscarried in the first trimester (7th to 10th gestational week), mostly as a result of chromosomal aberrations, particularly aneuploidies [1]. According to evidence based observations, less than half of all conceptions result in childbirth, while this

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Abbreviations

ART	– assisted reproductive technology
IVF	– in vitro fertilization
PGD	– preimplantation genetic diagnosis
PGS	– preimplantation genetic screening
FISH	– fluorescent in situ hybridization
DNA	– deoxyribonucleic acid
PCR	– polymerase chain reaction
CGH	– comparative genomic hybridization
TE	– trophectoderm
WGA	– whole genome amplification
ICSI	– intracytoplasmic sperm injection
ICM	– inner cell mass
ESHRE PGD Consortium	– European Society of Human Reproduction and Embryology Preimplantation Genetic Diagnosis Consortium

percentage is reduced even further with advanced maternal age [2–4]. These negative consequences of chromosomal imbalance are especially noticeable in assisted reproductive technology (ART) procedures, leading to an early arrest in cell division, embryo implantation failure or pregnancy loss, immediately after implantation, before it can be ultrasonically confirmed [1]. Out of the total number of embryos with sustained viability after in vitro culture, only 31% lead to a clinical pregnancy and live birth [5].

In in vitro fertilization (IVF) procedures, since the birth of Louise Brown in 1978, more than 5,4 million children have been born, and nearly 600.000 ART cycles are performed each year only in Europe [6]. Embryo selection for transfer is mainly done according to morphological criteria and the developmental characteristics of the embryo. However, even after the transfer of the best quality embryos, the implantation potential is relatively low. Researches have shown that more than 50% of in vitro derived embryos contain aneuploid cells. Extended cultivation until the blastocyst stage somewhat contributes to increased implantation rates, but it has been proven that aneuploid embryos can reach high-quality blastocyst stage as well as euploid embryos [7, 8].

Understanding the mechanisms which cause changes in chromosomal constitution and their detection is of great clinical significance. In addition to traditional cytogenetic methods for the identification of chromosomal abnormalities, many IVF centers have introduced preimplantation genetic diagnosis/screening, based on molecular methods for the analysis of genetic material.

Detection of Aneuploidy in Human Embryos

Human embryo development begins with the fertilization process which simultaneously triggers the completion of the second oocyte meiosis. Fertilization is followed by the fusion of the male and female pronuclei into a zygote. Over the next several days, certain mitotic divisions take place until blastocyst formation, but these events are unsuitable for observations, as they occur in the oviducts and the uterus. Improvements in ART have enabled these events to be viewed, thus the growth and development of the embryo can be monitored and studied during all developmental phases [9].

It has been proven that the majority of trisomies and/or monosomies causing miscarriages are the result of errors in the first maternal meiotic division [10]. Furthermore, it has been observed that the maternal meiotic process is more prone to errors than the paternal meiotic process. This is most likely caused by prolonged meiotic arrest at the dictyotene stage during fetal life and ends upon ovulation, i. e. several decades later [11]. Unfertilized oocyte karyotyping after IVF has disclosed two leading mechanisms for the origin of aneuploidy. The first is homologues chromosome segregation towards the same axis during meiosis I, leading to disomic or nulisomic daughter cells. The second mechanism indicates that chromosome imbalance may occur due to untimely centromere division before anaphase I, where sister chromatids are distributed randomly towards both axis [11]. This and other researches have also confirmed a direct correlation between advanced maternal age and increase in meiotic errors [9, 11, 12].

Although the incidence of chromosomal aberrations is ten times higher in infertile male population compared to the fertile population, 95% of infertile men have normal karyotypes [13]. It is obvious that men with a diagnosed chromosomal imbalance have an increased risk of producing aneuploid spermatozoa; however, researches indicate that men with normal karyotype, yet with abnormal sperm morphology, are also in the risky zone. In the male population aneuploidies appear due to meiotic synapse abnormalities resulting in the creation of bivalent sister chromatids which lead to abnormal sperm morphology production, or meiotic arrest. Traditional karyotyping cannot detect meiotic synapse abnormalities [13].

In addition to uniformly abnormal embryos, more than 50% of human embryos contain diploid cells and aneuploid cells in addition to normal cells, and this phenomenon is called mosaicism [14, 10]. Mosaicism can greatly affect false positive or false negative results for preimplantation genetic diagnosis/screening, since the biopsy gives a small number of cells for analysis which does not represent the genetic content of the entire embryo. Clinical researches have confirmed that if an embryo contains a small portion of mosaicism (only a few abnormal cells) the implantation potential can be very good [13]. If more than half cells in the embryo are aneuploid, the implantation potential and the survival of the implanted embryo are minimal. The most common mosaicism is of the diploid/aneuploid type - an embryo which starts its fetal life as a normal diploid and aneuploidy appears during early mitotic divisions [13]. It is considered that the incidence of mosaicism varies depending on multiple factors, where intrafollicular and changes in laboratory conditions may increase the incidence of mosaicism [7].

The most common aneuploidies in the human population are trisomy 16, 21, 22 and X chromosome monosomy. Although trisomy of the 13th and 18th

chromosome is not very common, these aneuploidies have a high survival rate. In general, the other imbalances are not compatible with life and result in early miscarriages or are so lethal that these embryos don't even implant [14]. The introduction of preimplantation genetic diagnosis into clinical practice revealed that Robertsonian translocation often leads to aneuploidy. In this case, parts of acrocentric chromosomes are exchanged in a way in which the satellite region is lost and two chromosomes remain coupled, so that this embryo contains 45 chromosomes. In preimplantation genetic diagnosis, identification of this aberration is possible since specific commercial probes for Robertsonian translocation are available [15].

It should be noted that the greatest contribution to the studies of aneuploidy came from the treatment of embryos from infertile patients during in vitro procedures. It is in this respect that opinions are divided regarding weather high incidence of aneuploidy exists only among infertile patients or the entire population. Although embryos in ART occur after aggressive hormonal stimulation and in vitro controlled laboratory cultivation, which may affect the appearance of aneuploidy, preimplantation genetic diagnosis showed that aneuploidies are present in fertile population as well [14].

Preimplantation Genetic Diagnosis/Screening

Preimplantation genetic diagnosis (PGD) is a technique which allows polar body/cell genetic status assessment obtained from an embryo/oocyte biopsy in order to detect aneuploidy or mutated alleles of monogenic diseases [16]. It was first applied in the early 1900s for identification of a genetically normal embryo in the case of an X-linked recessive mutation [16, 17]. The most important indications for PGD are high risk of transmission of hereditary diseases, several unsuccessful IVF cycles (at least 3 cycles or ≥10 transferred embryos without implantation), advanced maternal age (most often over 35 years of age) and several miscarriages [14]. PGD is considered justified when it can contribute to an increase in implantation rates, decrease miscarriages, and reduce aneuploid conception [7]. PGD can be divided into two categories: high- risk PGD in fertile patients with a diagnosed genetic disorder, and low-risk PGD for infertile patients where the aim is to increase implantation rates after the transfer of a genetically normal embryo. The second category is actually preimplantation genetic screening (PGS) [13]. Therefore, the difference between these two categories lies in appropriate application. PGD is advised in cases where a specific aberration is isolated (single-gene disorders, inherited genetic diseases, and X-linked mutations), while PGS is carried out in infertile patients for the purpose of selecting the most viable euploid embryo [16]. Currently, PGD is successfully used for more than 200 disorders, such as cystic fibrosis, Tay-Sach disease, Huntington disease, hemophilia, sickle cell anemia, Fragile-X syndrome, etc. [18].

The whole chromosome set karyotyping would be the ideal analyzing method. Due to the small amount of available genetic sample and other limiting factors (contamination, high risk of cultivation arrest, chromosome shortening due to mitotic inhibition, etc…) traditional cytogenetic methods required molecular replacement. The first available technology was fluorescent in situ hybridization (FISH) [1], still in use in many IVF centers. The procedure consists of the hybridization of fluorescently labeled deoxyribonucleic acid (DNA) probes (fluorochromes), with complementary target DNA, and the detection of the fluorescent signals derived after hybridization under a fluorescent microscope. This process implies cytoplasm digestion for nucleus isolation and allows the analysis of 6 - 9 chromosomes, whereby probes can be used during multiple rounds with a precaution due to decreasing binding efficiency of the probes with each round. While many clinics prefer this method owing to its simplicity and time-convenient procedure, FISH technology has numerous limitations. The main deficiency of this method lies in the restricted number of analyzed chromosomes, so that many aneuploidies may go undetected. In addition, this technology requires nuclei fixation which comes with at least two negative consequences: if isolated blastomere does not contain a nucleus or it is damaged during fixation, the lack of genetic material disables analysis, or if there are cytoplasmic residues after fixation, fluorescent signal evaluation will be inappropriate [14].

To complete analysis of all chromosomes in one blastomere, the FISH method is replaced with polymerase chain reaction (PCR) based methods. This approach enables multiplication of DNA material obtained in one cell which is sufficient for further analysis [19]. However, PCR methods have restrictions as well, such as foreign DNA contamination, amplification failure and allele dropout. All of these limitations can be avoided by process optimization, educated personnel and strict protocols [17].

Comparative Genomic Hybridization

The new technology of comparative genomic hybridization **(**CGH) bridges the gap between molecular genetics and cytogenetics. It is based on PCR amplification (most often) of the entire genome presented from only one cell, which makes this method equally efficient for polar body, blastomere or trophectoderm (TE) cell analysis [1]. Ploidy analysis is performed as a comparison of a test sample with a reference sample, avoiding cell cultivation. After DNA isolation of the test and reference samples, isolated DNA is labeled with two different fluorescent colors (fluorophores) followed by denaturation and hybridization with normal cell lines metaphase chromosomes (m-CGH approach) or DNA clones fixed to array (a-CGH approach). Finally, fluorescent signal is evaluated using fluorescent microscope and software [16].

Before the hybridization process, it is crucial to amplify a sufficient amount of genetic material for

a sustained and valid analysis, especially for very small samples such as one cell obtained from biopsy. In CGH cases, the whole genome amplification (WGA) method is applied, i.e. the entire genome of one cell is nonspecifically amplified. WGA modes may be PCR-based and non PCR-based [16]. Non PCR-based WGA is multiple displacement amplification, while for the CGH purpose, PCR-based WGAs are most common: primer extension preamplification and degenerate oligonucleotide primed-PCR [17].

Although microarray-CGH technology turned out to be more efficient than FISH in aneuploidy detection, it is limited by low resolution and prolonged analysis time (about 4 days). However, by introducing microarrays into clinical practice the main disadvantage of m-CGH has been overcome. The performance principle of array-CGH is basically the same as m-CGH with the difference being the use of a platform-array containing immobilized DNA fragments cloned with various vectors (bacterial artificial chromosome, yeast artificial chromosomes or P1-derived artificial chromosome), instead of metaphase chromosomes [20]. Every microplate contains 100 – 200 kb clones, representing the entire genome, allowing for a more detailed analysis than m-CGH. The procedure is implemented with a laser scanner and advanced software displaying the results in the form of an algorithm. Considering the complete automatization of the process, it takes 24h for the full procedure, meaning that the biopsy can be done on the third cultivation day and blastocyst embryo transfer can be performed on the fifth cultivation day without cryopreservation required [16]. Given that an analysis of the whole set of chromosomes can be done with an array-CGH technology many clinical trials indicate that if examinations were to be done by FISH alone, there would a high percentage of undetected aneuploidies [21–23].

Polar Body, Blastomere and Trophectoderm (TE) Cells Biopsy

The biopsy procedure starts with ablation, a process corresponding to zona pellucida, opening most widely with diode laser, enabling scroll and determination of laser path as well as the number and size of holes. Following ablation, polar body/cell biopsy is carried out via special biopsy pipette. In blastomere biopsy, prior to blastomere aspiration, embryo is exposed to the biopsy media containing no $Ca2^{+}$ i $Mg2^{+}$ ions, which loosens tight cell junctions leading to relieved cell isolation. This effect is reversible, and cell connections are reestablished after displacement of an embryo into the cultivation media [7].

Polar body biopsy is the removal of one or both polar bodies, independently or simultaneously. Biopsy of the first polar body is performed prior intracytoplasmic sperm injection (ICSI) procedure providing insight into maternal genome only. The IVF centers performing polar body biopsy, almost always practice isolation and analysis of both polar bodies in this order: the first polar body is removed before ICSI, the second polar body after fertilization, or both polar bodies are simultaneously removed after ICSI. The biopsy of both polar bodies provides more DNA material than one polar body biopsy, but tremendous limitation still persist - information about complete genetics of an embryo is unknown regarding the developmental stadium of analyzed cell, and the fact that maternal genome controls embryonic genome until the 8-cell developmental stage [13]. Some IVF centers still run polar body biopsy mainly due to law restrictions where embryo/blastocyst biopsy is banned (Italy, Germany, Austria) or because of potential destructive effect of cell removal on the embryo integrity [16].

Until recently, blastomere biopsy of an early embryo has been the most commonly used method for PGD/PGS. It is performed on the 3rd cultivation day at the 8-cell stage when one or two blastomeres with visible nucleus are removed for analysis. Biopsy at this developmental stage ensures avoidance of cryopreservation if genetic analysis doesn't take more than 48h, meaning that embryos can have extended cultivation until day 5 after biopsy and embryo transfer of euploid blastocyst in current cycle. Nevertheless, even in undisrupted culture conditions, only 60% of embryos reach blastocyst stage and this percentage decreases with disturbing embryo development in some way, by biopsy for instance [13].

The trophectoderm (TE) cells biopsy at blastocyst stage and the popularity of this approach constantly increases in accordance with improved culture conditions, thus the percentage of blastocyst formation is higher. At this stage, biopsy can be performed in two ways: zona pellucida is opened at 3rd cultivation day, and during the next two days TE cells progress towards the hole considering their hatching tendency, so biopsy of TE cells is simplified. Negative consequence of these methods lies in great possibility of inner cell mass (ICM) extrusion. As a precaution, many IVF centers apply a different approach: zona pellucida is opened in the morning of the 5th cultivation day at the opposite side of ICM. After several hours, hatching process has started and removal of TE cells can be performed. Blastocyst biopsy provides significantly greater cell amount compared to polar body/blastomere biopsy, but this method demands cryopreservation owing to the time required for genetic analysis. However, improvement of cryopreservation protocols, especially vitrification which ensures up to 90% survival of warmed blastocysts [24–27], makes TE cells for PGD/PGS the most promising method at this moment [16].

Clinical Experiences in the Detection of Aneuploidy

The European Society of Human Reproduction and Embryology Preimplantation Genetic Diagnosis Consortium (ESHRE PGD Consortium) was established in 1997, with the aim of collecting prospective and retrospective data about the accuracy, efficiency and safety of preimplantation genetic diagnosis/screening, defining specific guidelines for good clinical practice in PGD laboratories, and promoting

continuous education. Since 1999, ESHRE PGD Consortium has published data obtained from member centers of the PGD Consortium. According to the latest report, the published data cover ten years of PGD/PGS utilization in 60 PGD centers [28].

Until 2009, there were 33.271 PGD/PGS cycles reported, of which 12.388 were PGD, 20.207 were PGS, and 676 were PGD with PGS. With respect to FISH technology, as the first applied method in aneuploidy detection, the number of FISH cycles was 26.052 and 6.054 were PCR. From 2009 to 2010, 6.160 PGD/PGS cycles were reported, among them 2.580 were PGD, 3.551 were PGS, and 29 were PGD with PGS. An increasing trend of PCR preimplantation genetic diagnosis has been noticed in comparison to FISH (2009 – 2010: 946 FISH cycles vs. 1.435 PCR cycles; 1999 – 2008 5.851 FISH cycles vs. 5.869 PCR cycles). For the preimplantation genetic screening purpose, most centers of the 60 members still prefer FISH than PCR technology (1999 – 2008, FISH 19.723 cycles vs. PCR 3 cycles; 2009 – 2010, FISH 3526 cycles vs. PCR 6 cycles). Until 2010, early embryo biopsy was the most frequent aspect of genetic analysis (1999 - 2009: 26.284 cycles; 2009 - 2010: 4.918 cycles) compared to polar body (1999 - 2009: 3.750 cycles; 2009 - 2010: 997 cycles) and blastocyst biopsy which was used the least often (1999 - 2009: 105 cycles; 2009 - 2010: 6 cycles) [28].

The newest report of the PGD Consortium is being prepared and it is expected to emphasize the recent trends in regard to the stage of embryo biopsy, particularly on the usage of advanced PCR based technologies, such as array-CGH [28]. Defining the optimal biopsy stage depends on multiple factors. Primarily, determining the timing of biopsy, in order to precisely identify chromosomal aberrations, is essential: too early screening may leave genetic errors which affect implantation potential unidentified [10]. It is estimated that 33% of embryo aneuploidies occur due to genetic errors in meiosis I, meaning maternal errors [29]. These aneuploidies can be identified by polar body biopsy; however most aneuploidies appear due to improper separation of sister chromatids after meiosis II, so that polar body biopsy will not detect them [30]. Recent papers on serial embryo biopsies (biopsy of the same embryo at all stages) indicate that little information was obtained by polar body biopsy in comparison to the blastocyst stage. Even after a biopsy of both polar bodies, 1 of 4 aberrations remains unidentified [30]. Compared to polar body biopsy, blastomere biopsy is more invasive, but overcomes polar body biopsy limitations. In this case, the biopsy occurs after meiosis completion leading to the detection of meiosis errors regardless of their maternal or paternal origin [10].

Apart from accurate identification of aberrations, when choosing the timing of the biopsy, the question is whether abnormalities identified in the specimen accurately and consistently predict a corresponding abnormality in the embryo. Namely, if an embryo has the ability of self-correction, then aneuploidies detected in a biopsied sample could lead to a misdiagnosis and discarding of normal embryos. According to the latest data, mosaicism accounts for 29% of all human embryos [10]. Since screening of one blastomere at the 3rd cultivation day is most often the case, there is a considerable risk of imprecise mosaicism detection resulting in a false positive diagnosis [10]. Only one false positive aneuploidy is sufficient for a false positive mosaicism diagnosis. For example, assuming that genetic testing is done in one blastomere, resulting in 10% false positive diagnosis, then the analysis of 6 genetically normal blastomeres would result in 50% false positive mosaicism detections or even 70% if the analysis includes 10 blastomeres. Therefore, it is no surprise that research referring to third day biopsy (FISH on one blastomere) showed that 50 - 90% early embryos were mosaic, considering previous statistical analysis where 75% of false positive results were expected (8 blastomere analysis revealed 15% of false positive results). Consequently, an appropriate strategy obtaining valid mosaicism incidence in embryo is crucial [30]. A false positive diagnosis of mosaicism could explain recent reported clinical cases of mosaic embryo implantation and the birth of healthy children [31]. Capalbo et al. indicated that the group of authors who published these cases did not provide detailed information, i.e. original diagnosis of mosaicism turned out incorrect causing an assessment of euploid embryos as mosaic [30].

With respect to biopsy timing, the developmental potential of the embryo shouldn't be compromised [10]. It has been estimated that the implantation rate of early embryos declines by 39%, in other words, 2 of 5 biopsied early embryos on which a biopsy was performed on the third day, will have negative consequences on their developmental and implantation potential due to the biopsy [10].

In cases of blastocyst biopsy, a negative effect on implantation and delivery rate of biopsied blastocysts compared to untouched blastocysts hasn't been found [10]. Parallel progress in embryo cultivation to the blastocyst stage and vitrification introduction in routine practice of ARTs, has enabled efficient biopsy of TE cells with a minimal risk [31]. In addition, significantly lower mosaicism incidence has been found after blastocyst biopsy versus early embryo biopsy, merely 4–5% [30]. Clinical trials revealed that about 20% aneuploidies, detected by polar body biopsy and confirmed by blastomere biopsy, turned out nonexistent at blastocyst stage due to self-correction [32]. A recently published paper noted that embryo derived from reciprocal aneuploid oocyte (untimely sister chromatid division during meiosis I, revealed after polar body biopsy) resulted in the birth of a healthy child after correct chromatid segregation during meiosis II [33]. ESHRE PGS Task Force published that 4% embryos are euploid, despite detected aneuploidies after polar body biopsy [34]. Another advantage of a blastocyst biopsy is reflected in the amount of obtained genetic material: 5 – 10 TE cells [35] isolated

after blastocyst biopsy and successfully amplified at approximately 98% [36] versus 1 – 2 cells after blastomere biopsy and unsuccessful amplification at 20% [37]. According to clinical data, 1 of 10 oocytes remains unexamined due to unsuccessful amplification after polar body biopsy [32]. It is still unclear if blastocyst morphology and developmental characteristics reflect chromosome constitution. Only one study confirmed a slight correlation between poor blastocyst morphology and incidence of aneuploidy [2].

Although an official opinion from the PGD Consortium is still pending, thus far, biological and clinical research indicates that TE cell biopsy at the blastocyst stage followed by advanced molecular technologies in aneuploidy detection is the best choice for PGD/PGS and consequently this management has encountered increased application around the world [3, 10, 12, 38, 39].

Conclusion

In this paper we have shown that comparative genomic hybridization provides completely different insight into chromosomal aberrations in preimplantation embryos. The information obtained in this manner is extremely significant for patients undergoing assisted reproduction. During the in vitro fertilization process, a great number of high-quality embryos are produced, so genetic selection of healthy embryos instead of morphological selection would mean avoiding emotional and financial stress, as well as loss of time in the process of assisted reproduction, that may be completed unsuccessfully due to undetected aneuploidies.

Nevertheless, comparative genomic hybridization is still a technical challenge, especially the microarray approach which has found application in a few laboratories equipped with advanced equipment and personnel educated and experienced in molecular biology. Despite limitations of detecting structural chromosomal aberrations, that has no effect on changes in the chromosome number (balanced chromosome translocations and inversions) and mosaicism detection, the research is going towards finding a simpler and more valid comparative genomic hybridization performance, particularly when it comes to reduction of time required for analysis, and obtaining appropriate amounts of genetic material samples.

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IN MEMORIAM
IN MEMORIAM

Prim. dr DOBROSLAV ULIĆ
(27.03.1930 – 14.10.2017.)

Početna i završna stanica putovanja, kroz dobar deo vremena dva veka, jednog svetskog i jednog civilnog rata, dva razaranja država koje je voleo i smatrao svojim. I pored toga, u roditeljskoj i njegovoj porodici nije bilo mesta *beloj kugi*. Generacijsko vreme dve porodice Ulića izrodilo je njih dvadeset dvoje – otac Jovan – petnaest, plus Dobroslav – sedam. Neka se zna. Istorija jednog izuzetnog čoveka, ljudine, gorostasa, humaniste, lekara koji je bio više od toga, obrazovanog pravnika i nesuđenog slikara.

Izrastao i izgradio se u umetnika života, i to kakvog života – koji ga je gazio gde god da je stigao. U detinjstvu ga je stigla surova stvarnost odrastanja bez oca, u dečačko doba imanja pa nemanja pod okriljem sejanja smrti na sve strane i spasavanja žive glave po pećinama reke Morače. Tokom narednih vremena neljudski je izigravan mnogo puta – nakon mature kada je nezasluženo odbijen na Akademiji za slikarstvo, odmah potom i na upisu studija medicine u Beogradu (prosleđen je u Zagreb kao vojni pitomac), a nakon specijalizacije ginekologije i akušerstva u Beču kada nije primljen na obećano mesto lekara na novosadskoj klinici. Mnogo i za običnog čoveka.

Dobroslav je bio u životu mnogo toga, samo nije bio običan. Nakon svakog posrtanja izlazio je pobednički na nove megdane, naučio kako treba sa ljudima, prema svima koji su mu se obratili bio je predusretljiv, stvarao i otvarao nove horizonte i u porodici, i u medicini i u umetnosti. Pisao je priručnike i stručne knjige iz svoje specijalnosti, a čim se kod nas pojavio izazov sa uvođenjem kineske akupunkture u zdravstvenu praksu, ostvaruje pionirski rad knjigom „Od tradicionalne ka savremenoj akupunkturi“. Nije bio koristan samo našima, hrabro odlazi da pomaže i siromašnim Afrikancima. Nakon rada u Gvineji Bisao rezultati koje je postigao unapređuju ga u zvanje primarijusa. Odmah potom je i doktorirao iz oblasti planiranja porodice, kontracepcije i prevencije u trudnoći i porodiljstvu.

Po mnogim svojstvima, prirodnim i dakako stečenim, po vokaciji, nadarenosti i pregalaštvu, ne da je bio slikar, već izuzetan – više nego slikar, iskonski umetnik. Ne čudi onda skromnost u zahtevima, bogatstvo duhom i neobuzdana ljubav za životom i ljudima, roditeljska odanost i odgovornost. Pre nekoliko dana nas je napustio, a već nam nedostaje jer za nekoliko dana otvaramo ovogodišnju, 26. po redu, smotru umetničkih radova lekara i drugih zdravstvenih poslenika kojoj neće i lično prisustvovati, a na svim dosadašnjim jest – zato što je to želeo i neraskidivo osećao pripadnost zajednici entuzijasta okupljenih pri Umetničkoj sekciji Društva lekara Vojvodine Srpskog lekarskog društva.

Gde god da je bio, vredno je radio posao ginekologa-akušera, a kad je imao više slobodnog vremena – slikao je. Kako je nalazio vremena i uspevao, samo je on znao: da obiđe sve najbolje opremljene muzeje i galerije na ovoj planeti; da bude i u Aleksandriji sa akademcima likovnjacima, njihovim profesorima i dekanom; u Parizu sa Vladom Veličkovićem; u Beču sa Kokoškinim asistentom Ungarom; na slikanju sa Titom i Jovankom Broz i našom intelektualnom dijasporom u Beču; sa predsednikom Republike Gvineje Bisao; kod nas sa istaknutim slikarima, od Milana Konjovića do Milića od Mačve, sve do onih slikara sa Petrovaradinske tvrđave i, što je posebno voleo, gradio i razvijao, poznanstva i druženja sa umetnicima, našim zdravstvenim radnicima.

Ne znam na koliko je samostalnih i kolektivnih izložbi izlagao, bilo ih je mnogo, ali znam da su mu

sve bile jednako važne i drage. Rado se odazivao svuda gde je pozivan, a kada je slikao, radio je to dajući sebe u potpunosti. Portretisao je mnoge istaknute likove naše istorije, kulture i umetnosti, posebno književne i pozorišne – broja im se ne zna. Nesebično je poklanjao svoje radove odgovarajućim ustanovama. U autorskoj knjizi „Slikarsko stvaralaštvo jednog lekara i pravnika“ priložio je 466 reprodukcija. Posebno treba istaći da se nikada na izložbama nije pojavio bez brojnih članova porodice, dece i unuka, a posebno ne bez njemu odane i drage saputnice i saradnice, Branke Đurović Oklješa, zaslužne umetnice operske scene.

Otišao je iz naših redova čovek velikog formata, visoki intelektualac, neumorni i valjani stvaralac na poljima medicine, društvene zajednice i umetnosti. Neka mu je zato večna slava i ogromno hvala za sve čime nas je darivao za života.

Prof. dr Milorad Žikić
Predsednik Umetničke sekcije DLV-SLD

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U časopisu *Medicinski pregled* objavljuju se: uvodnici, originalni članci, prethodna ili kratka saopštenja, pregledni članci, stručni članci, prikazi slučajeva, članci iz istorije medicine i drugi članci.

1. Uvodnici – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

2. Originalni članci – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

3. Pregledni članci – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

4. Prethodna ili kratka saopštenja – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

5. Stručni članci – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

6. Prikazi slučajeva – do 6 strana. Opisuju retke slučajeve iz prakse. Slični su stručnim člancima. U ovim radovima prikazuju se neuobičajeni oblici i tokovi oboljenja, neočekivane reakcije na primenjenu terapiju, primene novih dijagnostičkih procedura ili retke i nove bolesti.

7. Članci iz istorije medicine – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

8. Ostali članci – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

Priprema rukopisa

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

Propratno pismo:

– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

– autori svojim potpisom preuzimaju odgovornost da rad ispunjava sve postavljene uslove i da ne postoji sukob interesa i

– autor mora navesti kategoriju članka (originalni rad, pregleni rad, prethodno saopštenje, stručni rad, prikaz slučaja, rad iz istorije medicine, itd.).

Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings*, *MeSH*.

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al*.

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ Organizacija kao autor

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ Bez autora

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ Volumen sa suplementom

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ Sveska sa suplementom

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ Sažetak u časopisu

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije

∗ Jedan ili više autora

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ Urednik (urednici) kao autor (autori)

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ Poglavlje u knjizi

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ Zbornik radova sa kongresa

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

∗ Disertacija

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ Monografija u elektronskom formatu

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ Kompjuterska datoteka

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

Manuscript submission should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/

A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

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

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.