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E-mail: acta@medfak.ni.ac.rs
Tel+381-18-4533001 lok. 122 fax. +381-18-4534336
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INCREASED BLOOD PRESSURE DURING PUBERTY

Božidar Stojiljković[1], Ljubiša Lilić[2], Sladjana Milošević[2]

Blood pressure is continuously increasing and increases with the child's age, that is, with the growth and development of the child.

The change in pressure value in puberty appears due to complex morphological and physiological changes in the organism under the influence of hormones.

With this work we have proven that blood pressure has a significant increase in children from 11 to 14 years compared to the previous period. We used the results of the systematic examination of school children of the 5th, 6th and 7th grade in the city of Niš. A total of 2333 children of both sexes were analyzed. The blood pressure was measured in a seated position three times at a time interval of 5 minutes. For statistical processing, the highest value was taken. The greatest differences in blood pressure, diastolic pressure, occurred in boys compared to girls in the fifth grade, while girls had a higher diastolic pressure than boys in the sixth and seventh grades of primary school ($p < 0.001$). Regarding morphological charac-teristics, girls had significantly higher body height values than boys in the sixth grade ($p < 0.005$), while boys had higher values of both body height and body weight in the seventh grade ($p < 0.001$). In the period of puberty, there are numerous changes in all systems and organs, blood pressure varies significantly, and therefore it has to be measured and controlled more frequently for the detection and prevention of arterial hypertension in children.

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Key words: *blood pressure, puberty, increase, sexes, arterial hypertension*

[1]General Hospital Leskovac, Departmetnt of Neurology, Serbia
[2]University of Kosovska Mitrovica, Faculty of Sport and Physical Education Leposavić, Serbia

Contact: Božidar Stojiljković
Rade Končara 9, 16000 Leskovac, Serbia
E-mail: drbozidarstojiljkovic@gmail.com

Introduction

Arterial blood pressure in children is a variable that increases with the age of the child, that is, with the growth and development of a child (1). From normal values at birth of 75/55 mmHg, blood pressure records a constant increase until puberty and adolescence. The highest increase of about 20 mmHg occurs during the first six weeks of life (5). A further rise in blood pressure is gradual and is approximately 5 mmHg for periods of three years to puberty and adolescence when for the same period blood pres sure increases about 8 mmHg (4, 6, 8). In the period of puberty there are complex morphological and physiological changes in the body of boys and girls that occur under the influence of hypophysis hormones and gonads. As the whole organism grows, the cardiovascular system develops normally, the heart, lungs and abdominal organs grow, and blood pressure rises to a value of 120/70 mmHg, which is the average of the blood pressure in adolescence (2, 3, 9).

The goal of the work

With this work we tried to prove that blood pressure in children from 11 to 14 years undergoes significant changes, that is, that its value significantly increases compared to the previous period.

Materials and methods

We conducted systematic examinations of school children in the three primary schools of the city of Niš, pupils of the fifth, sixth and seventh grades. There were in total 2333 children with approximately equal representation of both sexes. In addition to anthropometric measurements (body height and body mass), blood pressure measurements in the sitting position were also carried out, three times in a space of 5 minutes. The highest value was obtained for statistical processing. For comparison of the obtained values in boys and girls, Sudent's t-test was used. All results are presented in tabular and graphical form.

Results

The obtained results were statistically processed and graphically presented.

The mean values of the systolic (SV) and diastolic (FDIS) pressure, as well as anthropometric measurements of body height (AVIS) and body weight (AMAS) in boys of the fifth grade are shown in the Table 1. Also, the minimum (MIN) and maximum (MAX) values obtained in the systematic examination of children, as well as the values of the standard deviation (SD) and the coefficient of variation (CV) used in the student's t-test are presented.

Table 2 shows us the mean values of systolic and diastolic pressure in boys of the sixth grade, as well as the values of body height and mass.

Table 3 shows the mean values of systolic and diastolic pressure in boys of the seventh grade, mean systolic pressure is 117.2 mmHg, and diastolic 71.17 mmHg, as well as body height and body weight.

Table1. An overview of the blood pressure and anthropometric measures in the fifth grade boys

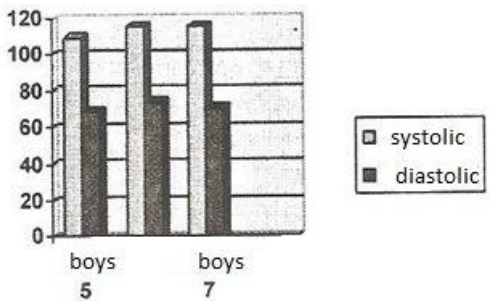
	SV	SD	MIN	MAX	CV
FSIS	109.46	11.64	80	155	10.63
FDIS	68.71	9.13	45	100	13.28
AVIS	150.94	7.37	134	179	4.88
AMAS	43.49	10.07	28	88	23.69

Table2. An overview of the blood pressure and anthropometric measures in the sixth grade boys

	SV	SD	MIN	MAX	CV
FSIS	115.51	9.7	90	170	8.39
FDIS	70.6	7.66	55	100	10.59
AVIS	156.32	8.42	130	179	5.38
AMAS	46.79	9.84	28	75	21.03

Table 3. An overview of the blood pressure and anthropometric measures in the seventh grade boys

	SV	SD	MIN	MAX	CV
FSIS	117.21	11.06	90	150	9.59
FDIS	71.17	10.18	50	100	14.5
AVIS	163.84	8.58	141	187	5.23
AMAS	53.02	12.41	30	90	23.4



Graph 1. An overview of the systolic and diastolic blood pressure values in boys from the fifth to the seventh grade

Graph 1 shows a graphical overview of the values of systolic and diastolic blood pressure in the boys from the fifth to the sixth grade.

Table 4 shows the mean values of systolic and diastolic pressure in girls of the fifth grade, mean systolic pressure is 107.28 mmHg, and diastolic 66.22 mmHg, as well as body height and body weight.

Table 5 shows the mean values of systolic and diastolic pressure in girls of the sixth grade, mean systolic pressure is 115.75 mmHg, and diastolic 74.1 mmHg, as well as body height and body weight.

Table 6 shows the mean values of systolic and diastolic pressure for the girls of the seventh grade, the mean value of systolic pressure is 116.5 mmHg, and of diastolic 75.07 mmHg, as well as the body height and body weight values.

Graph 2 shows a graphical overview of the values of systolic and diastolic blood pressure in the girls from the fifth to the sixth grade.

Table 4. An overview of the blood pressure and anthropometric measures in the fifth grade girls

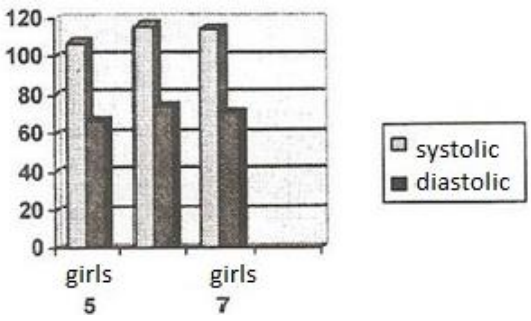
	SV	SD	MIN	MAX	CV
FSIS	107.28	11.17	80	145	10.41
FDIS	66.22	9.5	50	90	14.34
AVIS	150.71	7.1	132.5	172.5	4.71
AMAS	41.21	9.6	22	76	23.29

Table 5. An overview of the blood pressure and anthropometric measures in the sixth grade girls

	SV	SD	MIN	MAX	CV
FSIS	115.75	9.39	90	160	8.11
FDIS	74.1	7.18	50	90	9.68
AVIS	157.37	7.84	136	176	4.98
AMAS	48.58	9.44	28	72	19.84

Table 6. An overview of the blood pressure and anthropometric measures in the seventh grade girls

	SV	SD	MIN	MAX	CV
FSIS	116.5	12	85	180	10.48
FDIS	75.07	9.69	40	100	13.63
AVIS	161.42	6.4	140.7	182	3.96
AMAS	51.26	10.48	29	115	20.05



Graph 2. An overview of the systolic and diastolic blood pressure values in girls from the fifth to the seventh grade

In Tables 7, 8 and 9 we can see the values of the student's t-test (T-test) and the p-value, or calculated probability (p) in boys and girls according to grades for the tested parameters. It is noted that there were statistically significant differences in the values of systolic and diastolic pressure in boys and girls in the fifth grade. The boys had, statistically, significantly higher systolic pressure (109.41 mmHg), as well as diastolic blood pressure (68.71 mmHg) in comparison to girls, and this difference was statistically significant ($p < 0.001$). The boys of this age had statistically significantly higher body weight (43.49 kg) than girls and these differences were statistically significant ($p < 0.001$).

Girls in the sixth grade had statistically significantly higher values of diastolic pressure (74.1 mmHg) compared to boys of that age and these differences were statistically significant ($p < 0.001$).

Further, girls had statistically significantly higher body weight (48.58 kg) and height (157.35 cm) compared to boys and this difference was statistically significant ($p < 0.001$).

In pupils of the seventh grade, it is noted that the girls had statistically significant higher values of diastolic blood pressure (75.7 mmHg) compared to boys ($p < 0.001$). The boys of the seventh grade had statistically significantly higher values of body height (163.84 cm) and body weight (53.2 kg) than girls and this difference was statistically significantly higher.

Table 7. Statistical results of boys and girls in the fifth grade

	T-test	p
FSIS	3.631	$p < 0.001$
FDIS	5.078	$p < 0.001$
AVIS	0.64	$p = 0.546$
AMAS	2.472	$p < 0.001$

Table 8. Statistical results of boys and girls in the sixth grade

	T-test	p
FSIS	0.534	$p = 0.593$
FDIS	23.800	$p < 0.001$
AVIS	2.452	$p < 0.05$
AMAS	1.557	$p = 0.120$

Table 9. Statistical results of boys and girls in the seventh grade

	T-test	p
FSIS	1.169	$p = 0.243$
FDIS	5.504	$p < 0.001$
AVIS	12.957	$p < 0.001$
AMAS	10.224	$p < 0.001$

Discussion

In this paper, on the sample of 2333 boys and girls, pupils of the fifth, sixth and seventh grade of the primary school, the changes in arterial blood pressure during the puberty period were examined.

Tables 1, 2, 3, 4, 5 and 6 show the differences between the sexes when it comes to measured variables, that is, differences between boys and girls of the pubertal sample in systolic and diastolic pressure, body height and body mass. It can be seen that the biggest differences between boys and girls are observed when it comes to diastolic pressure.

Boys had significantly higher diastolic pressure than girls in the fifth grade ($t = 5.078$; $p < 0.001$), while girls had higher diastolic blood pressure than boys in the sixth ($t = 23.800$; $p < 0.001$) and the seventh grade of primary school ($t = 5.504$; $p < 0.001$). When it comes to systolic pressure, there were differences between boys and girls only during the fifth grade of primary school where boys have significantly higher values than girls ($t = 3.631$; $p < 0.001$). In terms of morphological characteristics, girls had significantly higher body height than boys in the sixth grade ($t = 2.452$; $p < 0.005$), while boys had higher body weight values in the fifth ($t = 2.472$; $p < 0.005$) as well as body height and body height mass in the seventh grade ($t = 12.957$; $p < 0.001$; $t = 10.224$; $p < 0.001$). The results obtained were expected and in accordance with previous research in this area, which supports the fact that there are significant differences in arterial blood pressure and morphological characteristics between boys and girls of pubertal age (4-6, 8).

It is possible to notice the tendency for boys to have higher values of systolic and diastolic blood pressure than girls during the fifth grade of the primary school, that is, at the onset of puberty, while later, during the growing up period, there is a sudden increase in pressure (especially diastolic) in girls, and they have significantly higher diastolic pressure values than boys, while in terms of systolic pressure boys and girls have roughly similar values in the sixth and seventh grade. It has also reen shown that the biggest difference between boys and girls is in the values of diastolic pressure in the sixth grade period. When it comes to morphological characteristics, it can be concluded that girls have higher values of body height than boys while boys have a greater body mass than girls at the beginning of puberty, and with aging, this difference in the boys' favor increases with respect to body height.

Conclusion

By comparing blood pressure values in boys and girls per grade, we have come to the conclusion that there is a statistically significant difference in blood pressure values of systolic and diastolic in boys and girls of the fifth and sixth grade, which proves that in the period between 11 and 13 years there is a significant increase in the value of blood pressure. Therefore, in the period of puberty, in addition to numerous changes in all systems and organs, there is a change in blood pressure, which is why blood pressure in this period must be measured and controlled more frequently to detect and prevent arterial hypertension in children.

References

1. Pejovic B, Peco-Antic A, Marinkovic-Eric J. Blood pressure in non-critically ill preterm and full-term neonates. Pediatr Nephrol 2007; 22(2):249-57. [CrossRef] [PubMed]
2. Dilon MJ. Secondary forms of hypertension in children. In: Portman RJ, Sorof JM, Ingelfinger JR, editors. Pediatric Hypertension. Totowa, New Jersey: Humana Press; 2004. p.159-79. [CrossRef]
3. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114(Suppl 2):555-76. [CrossRef] [PubMed]
4. Sorof JM. Definitions of Hypertension in Children. In: Portman RJ, Sorof JM, Ingelfinger JR, editors. Pediatric Hypertension. Totowa, New Jersey: Humana Press; 2004. p. 145-58. [CrossRef]
5. Hypertension. In: Rees L, Weebb NJA, Brogan PA, editors. Paediatric nephrology. Oxford: University Press, 2007. p. 317-51.
6. Stabouli S, Kotsis V, Toumanidis S, Papamicheael C, Constantopoulos A, Zakopoulos N. White-coat and masked hypertension in children: association with targetorgan damage. Pediatr Nephrol 2005; 20(8): 1151-5. [CrossRef] [PubMed]
7. Morgenstern BZ, Butani L. Casual blood pressure measurement methodology. In: Portman R, Sorof JM, Ingelfinger JR, editors. Pediatric hypertension. Totowa, New Jersey: Humana Press, 2004; p. 77-96. [CrossRef]
8. Muntner P, He J, Cutler JA, Wildman RP, Whelton PK. Trends in blood pressure among children and adolescents. JAMA 2004; 291(17):2107-13. [CrossRef] [PubMed]
9. Lurbe E, Sorof JM, Daniels SR. Clinical and research aspects of ambulatory blood pressure monitoring in children. J Pediatr 2004;144 (1):7-16. [CrossRef] [PubMed]

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POVIŠEN KRVNI PRITISAK TOKOM PUBERTETA

Božidar Stojiljković[1], Ljubiša Lilić[2], Slađana Milošević[2]

[1]Opšta bolnica Leskovac, Klinika za neurologiju, Leskovac, Srbija
[2]Univerzitet Kosovska Mitrovica Fakultet za sport i fizičko vaspitanje Leposavić, Srbija

Kontakt: Božidar Stojiljković
Rade Končara 9, 16000 Leskovac, Srbija
E-mail: drbozidarstojiljkovic@gmail.com

Krvni pritisak beleži neprestani porast i povišava se sa starošću deteta, odnosno sa rastom i razvojem deteta.

Do promene vrednosti pritiska u pubertetu dolazi zbog složenih morfoloških i fizioloških promena u organizmu pod uticajem hormona.

Ovim radom dokazali smo da krvni pritisak ima značajan porast od 11. do 14. godine deteta u odnosu na prethodni period. Koristili smo rezultate sistematskog pregleda školske dece grada Niša, učenika 5, 6. i 7. razreda. Analizirano je 2333 deteta oba pola. Krvni pritisak meren je u sedećem položaju tri puta u razmaku od 5 minuta. Za statističku obradu uzimana je najviša vrednost. Najveće razlike krvnog pritiska, dijastolni pritisak, sreću se kod dečaka u odnosu na devojčice u petom razredu, dok devojčice imaju veći dijastolni pritisak od dečaka u šestom i sedmom razredu osnovne škole ($p < 0,001$). Što se tiče morfoloških karakteristika, devojčice imaju značajno veće vrednsti telesne visine od dečaka u šestom razredu ($p < 0,005$), dok dečaci imaju veće vrednosti telesne visine i telesne mase u sedmom razredu ($p < 0,001$). U periodu puberteta postoje brojne promene u svim sistemima i organima, krvni pritisak značajno varira i zbog toga se mora češće meriti i kontrolisati radi detekcije i sprečavanja arterijske hipertenzije kod dece.

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Ključne reči: *krvni pritisak, pubertet, veći, pol, arterijska hipertenzija*

Original article

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THE ROLE OF MAST CELLS IN CARBON TETRACHLORIDE INDUCED RAT SKELETAL MUSCLE TISSUE DAMAGE

Ljubiša M. Lilić[1], Dragan Toskić[1], Rade Ž. Stefanović[1], Branimir B. Mekić[1], Ivan R. Ilić[2], Nikola M. Stojanović[3]

Animal models demonstrating skeletal muscle (SM) disorders are rarely investigated, although these disorders accompany liver disorders and can occur during prolonged exercise/training. It is speculated that mast cells, normally present in the interstitial SM tissue, are involved in the pathophysiology of different SM disorders. Thus, the present study aims to analyze, on the histopathological level, the involvement of mast cells in acute rat intoxication with carbon tetrachloride (CCl_4). Biceps and gastrocnemius muscle were obtained from male Wistar rats acutely exposed to CCl_4 (1 ml/kg) and the pathological analysis was performed on Toluidine blue stained tissue sections. The obtained results were statically compared with those from a control group using Student's t-test. In SM tissue obtained from the control group mast cells were found only in the interstitium, while in those that received CCl_4 they were located mainly near the blood vessels. Also, in the experimental group treated with CCl_4 mast cells were more abundant and were in percents more degranulated than those found in the control group. Thus, one can say that the herein presented model of CCl_4-induced SM damage is partially dependent on the activity of mast cells.

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Key words: *Carbon tetrachloride, Skeletal muscles, Mast cells*

[1]University of Priština, Faculty of Sport and Physical Education in Leposavić, Priština, Serbia
[2]University of Niš, Faculty of Medicine, Institute of Pathology, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Nikola M. Stojanović
Bulevard dr Zoran Djindjić 81, 18000 Niš, Serbia
E-mail: nikola.st90@yahoo.com

Introduction

Our body consisted of three distinct types of muscles, from which the skeletal muscles (SM) are the only ones that can contract under our will (deliberately) maintaining body posture and enabling locomotion. Skeletal muscle -specific anatomical and physiological organization allows SM to function (1). Each SM consisted of a large number of myofibrils (muscle cells) that represent the smallest functional unit (1). Body and SM tissue mass can be significantly affected under different underlying conditions and these changes occur due to tissue protein degradation (2). Changes in SM mass and function can be seen in several liver disorders, as well as under various physiological conditions that involve significant production of reactive oxygen species (ROS) (3, 4).

Carbon tetrachloride (CCl_4), a synthetic chemical often used in paints, solvents, and extinguishers (5), is known to be useful in inducing ROS mediated tissue damage in laboratory animals. When applied it causes liver, kidneys, brain, muscles, lungs, testis, etc. oxidative damage (6), via trichloromethyl free radicals generated in the liver (6, 7). The mechanism by which CCl_4 damages cell structures is relatively well studied. However, the role of mast cells in SM injury caused by CCl_4 is still not fully investigated.

Mast cells are the mesenchymal cells, stained metachromatically with some blue dyes, that contain numerous granules which contain the majority of the body's histamine. These cells play a crucial role in body inflammatory and allergic reaction (8). Before their final migration and differentiation in tissue, mast cells (the type of leukocytes) circulate in the blood as immature cells derived from hematopoietic progenitor cells. All tissues of the body possess different percent of mast cells, while they are more abundant in tissues that are coming in close contact with the external environment (skin, intestinal and airway mucosa) (8).

Aim of the study

Although the toxic effects of CCl_4 on SM tissue have been investigated, the specifics pathogenetic role of mast cells is not completely investigated. Thus, the goal of the present study was to detect and describe on the histopathological level the changes in mast cells occurring in rat SM after acute administration of CCl_4.

Material and methods

Animals and housing

Male Wistar rats, weighing 250-300 g, were housed in groups of 6 and obtained from the Vivarium of the Institute of Biomedical Research, Faculty of Medicine, University of Niš, Serbia. The animals were maintained under standard laboratory conditions: temperature 22 ± 2 ºC and humidity 60%, with food and water available *ad libitum*. All experimental procedures with the animals were conducted in compliance with the declaration of Helsinki and European Community guidelines for the ethical handling of laboratory animals (EU Directive of 2010; 2010/63/EU) and were also approved by the local Ethics Committee.

Muscle tissue damage induction

Before the experiment, all animals were divided into two groups of 6 rats each: the control group where the animals were administered only the vehicle (olive oil) in the dose of 10 ml/kg, and the experimental group with CCl_4-treated animals. Acute administration of CCl_4 (1 ml/kg), known to cause significant liver damage (6), was given to rats via an intraperitoneal injection 24 h before the animals were sacrificed by an overdose of ketamine. Skeletal muscle tissue samples collected, using scissors and tweezers, for histological analyses included the left gastrocnemius (GCM) and biceps (BM) muscles.

Histopathological observations

The GCM and BM tissue specimens separated for histopathological examination were fixed in buffered formaldehyde solution (10 %, w/v). The fixed tissues were then dehydrated with ethanol solutions of differing concentration (50-100 %, v/v), embedded in paraffin, cut into 4-5 μm thick sections, stained with Toluidine Blue (TB) and further examined under an Olympus BH2 light microscope. The average number of mast cells per high-power field (x40) was counted on 10 randomly selected fields for each muscle specimen stained with TB. Also, the percent of degranulated mast cells was counted on each examined high-power field.

Statistical analysis

The results were expressed as mean values ± SD. Statistically significant differences were determined by Student's t-test (Graph pad Prism version 5.03, San Diego, CA, USA) and the obtained results were tested for correlation as well. Probability values (p) less than or equal to 0.05 were considered to be statistically significant.

Results

The number and % of degranulated mast cells, found in GCM and BM tissue sections stained with TB, were found to be statistically significantly higher in both investigated muscles originating from animals exposed to CCl_4 compared to the control animals (Table 1 and Figure 1).

In the investigated TB stained tissue mast cells granules appeared red-purple (metachromatic granules), while their cytoplasm and background appeared pale blue (orthochromatic). The mast cell shape varied from ovoid to spindle-shaped and one could clearly distinguish preserved (not degranulated) and degranulated mast cells that appeared deep and light blue, respectively (Figure 1). In healthy (control group) animal mast cells were observed only in the interstitial connective tissue that normally separates skeletal muscle into separate fascicles. On the other hand, in animals treated with CCl_4 mast cells appeared in the interstitial connective tissue as well, however they were more frequently found around blood vessels.

Table 1. Number and % of degranulated mast cells obtained for each of the two studied muscles from the two different animal groups

Tracked parameter		Average number of mast cells/high power field (x40)	Degranulated mast cells (%)
Gastrocnemius muscle	Control	0.6 ± 0.2	0 ± 0
	CCl_4 treated	1.4 ± 0.2*	66.5 ± 16.1*
Biceps muscle	Control	0.9 ± 0.1	0 ± 0
	CCl_4 treated	1.2 ± 0.05*	62.7 ± 14.2*

The results are presented as mean ± SD (n = 6); *p < 0.001 vs. control group

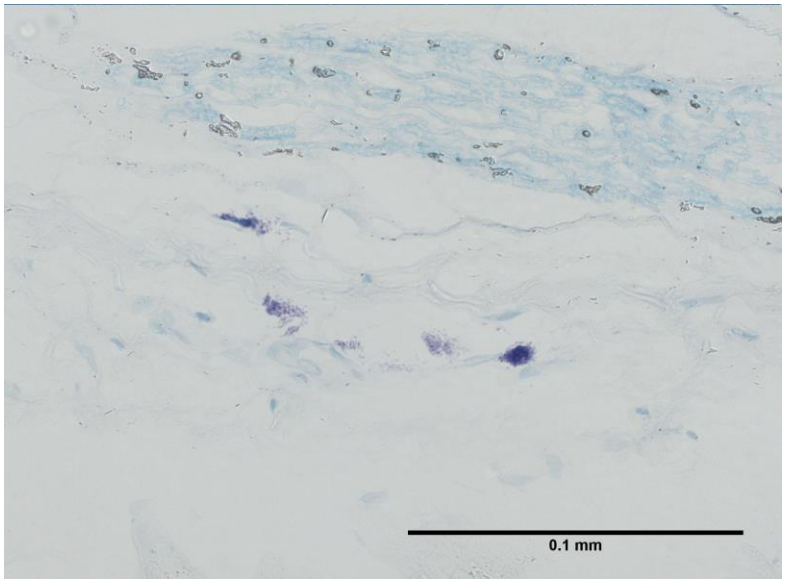

Figure 1. Microscopic appearance of degranulated and non-degranulated mast cells present in skeletal muscle tissue stained with Toluidine blue (x400).

Discussion

The two muscles evaluated in our study, GCM and BFM, represent important hindlimb muscles that enable animal movement. Biceps femoris muscle is the largest muscle in the hind limb and has multiple functions that involve thigh abduction, hip extension, and knee flexion, while GCM is responsible for plantar flexion (9).

Since their discovery, back in 1877, mast cells have been considered as normal constituents of interstitial tissue of different vertebrates (10). Later they were recognized as cells important in triggering and/or maintenance of different inflammatory and immunological processes (10). These cells represent the source of inflammatory mediators such as histamine, nitric oxide, proteases tryptase and chymase, and pre-formed tumor necrosis factor-alpha (TNF-α), as well as other toxic mediators by which the muscle tissue can be injured (8, 11). When mast cells are triggered to degranulate, the secretory granules located within the cell are released, exocytosis, resulting in the release of their internal contents (8).

The importance of mast cells in the pathophysiology of muscle disorders was previously shown in the ischemia/reperfusion muscle injury model (11) and the present research proves the involvement of these cells in acute CCl_4-induced muscle damage (Table 1, Figure 1), as well. Also, these cells were found to be significantly increased in different myopathic disorders, e.g. Duchenne muscular dystrophy, where it was suggested that grouped necrosis of extrafusal fibers occurs possibly due to the activity of these cells (10). Thus, the increase in both the number of mast cells and % of their degranulation indicates that their involvement in acute CCl_4-induced muscle damage should not be neglected. Additionally, there is a possibility that a much higher number of mast cells were involved in tissue damage, both resident and mobilized, however due to their degranulation TB staining might not have stained all of mast cells.

The consequence of degranulation can probably be brought in connection with inflammatory cell infiltrate, seen after CCl_4 application, that is comprised mainly of neutrophils. Namely, these neutrophils secrete an enzyme, myeloperoxidase, that causes the onset of degranulation (histamine release) form isolated mast cells (12). On the other hand, SM resident mast cells are known to provoke a significant increase in the number of neutrophils in injured SM tissue (13). Similar results were obtained in the study where mast cell membrane stabilizing agent, cromolyn, was administered prior to neutrophil- attracting agent, bupivacaine, thus causing a decrease in neutrophil infiltration by 70 % (14). All of the results, relating to the presence and state of mast cells, represent an important addition to the overall conclusion concerning the involvement of these cells in muscle tissue damage induced by an acute application of CCl_4.

Also, one can say that the damage caused by CCl_4 seems more significant in GCM than in BM (Table 1), and such slight differences in the extent of CCl_4 injury in GCM and BM are not completely unexpected. Namely, a previous study revealed different degrees of protein catabolism, estimated based on tyrosine release, in various muscle tissues after CCl_4 application (3). Thus, it is not surprising that the two muscles do not suffer identical damage, since their vascularisation, structure (fibers size, mitochondria amount, myoglobin concentration, etc.) and function are not the same.

Conclusion

The herein presented model of CCl_4-induced skeletal muscle damage can be considered a useful model that could mimic both mild ROS-mediated muscle damage seen in strenuous physical exercise and/or muscle damage (waste) that frequently accompanies liver diseases. The results unequivocally demonstrated the involvement of mast cells in skeletal muscle tissue damage caused by CCl_4 after an acute application.

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References

1. Thews G, Mutschler E, Vaupel P. Skelett. Muskulatur und Bindegewebe. In: Vaupel P, Schaible HG, Mutschler E, editors. Anatomie, Physiologie, Pathophysiologie des Menschen. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH; 1999. p. 553-603.
2. Wolfe, RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr 2006; 84(3):475-82. [CrossRef] [PubMed]
3. Weber FL Jr, Macechko PT, Kelson SR, Karajiannis E, Hassan MO. Increased muscle protein catabolism caused by carbon tetrachloride hepatic injury in rats. Gastroenterology 1992; 102(5):1700-6. [CrossRef] [PubMed]
4. Bentley DJ, Ackerman J, Clifford T, Slattery KS. Acute and chronic effects of antioxidant supplementation on exercise performance. In: Lamprecht M, editor. Antioxidants in sport nutrition. Boca Raton: CRC Press/ Taylor & Francis; 2015. p. 141-54. [PubMed]
5. Pope AM, Rall DP. Environmental Medicine. Washington D.C.: National Academy Press; 1995.
6. Radulović NS, Randjelović PJ, Stojanović NM, Ilić IR, Miltojević AB. Influence of methyl and isopropyl n-methyl antranilates on carbon tetrachloride-induced changes in rat liver morphology and function. FU Phys Chem Tech 2013; 11(1):67-73. [CrossRef]
7. Vural DF, Cebesoy S, Ozerkan D, Eyison HM. The effect of melatonine on rats gastrocnemius muscle applied with carbon tetrachloride (CCl_4). J Entomol Zool Stud 2017; 5(3):441-7.
8. Dawicki W, Marshall JS. New and emerging roles for mast cells in host defence. Curr Opin Immunol 2007; 19:31-8. [CrossRef] [PubMed]
9. Armstrong RB, Phelps RO. Muscle fiber type composition of the rat hindlimb. Am J Anat 1984; 171(3): 259-72. [CrossRef] [PubMed]
10. Nahirney PC, Dow PR, Ovalle WK. Quantitative morphology of mast cells in skeletal muscle of normal and genetically dystrophic mice. Anat Rec 1997; 247(3): 341-9. [CrossRef] [PubMed]
11. Bortolotto SK, Morrison WA, Han XL, Messina A. Mast cells play a pivotal role in ischaemia reperfusion injury to skeletal muscles. Lab Invest 2004; 84(9):1103-11. [CrossRef]
12. Stendahl O, Molin L, Lindroth M. Granulocyte-mediated release of histamine from mast cells. Effect of myeloperoxidase and its inhibition by antiinflammatory sulfone compounds. Int Arch Allergy Appl Immunol 1983; 70(3):277-84. [CrossRef] [PubMed]
13. Dumont N, Lepage K, Côté CH, Frenette J. Mast cells can modulate leukocyte accumulation and skeletal muscle function following hindlimb unloading. J Appl Physiol 2007; 103(1):97-104. [CrossRef] [PubMed]
14. Côte CH, Tremblay MH, Duchesne E, Lapoite BM. Inflammation-induced leukocyte accumulation in injured skeletal muscle: role of mast cells. Muscle Nerve 2008; 37(6):754-63. [CrossRef] [PubMed]

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ULOGA MASTOCITA U MODELU UGLJEN TETRAHLORIDOM INDUKOVANOG OŠTEĆENJA SKELETNOG MIŠIĆNOG TKIVA PACOVA

Ljubiša M. Lilić[1], Dragan Toskić[1], Rade Ž. Stefanović[1], Branimir B. Mekić[1], Ivan R. Ilić[2], Nikola M. Stojanović[3]

[1]Univerzitet u Prištini, Fakultet sporta i fizičke aktivnosti u Leposaviću, Priština, Srbija
[2]Univerzitet u Nišu, Institut za patologiju, Medicinski fakultet, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Nikola M. Stojanović
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: nikola.st90@yahoo.com

Animalni modeli koji imitiraju oštećenja skeletnih mišića (SM) retko su predmet istraživanja, iako poremećaji SM često prate druge poremećaje organa, kao što su oštećenja jetre. Takođe, oštećenja SM mogu da se jave nakon duge i intenzivne fizičke aktivnosti. Smatra se da su mastociti, deo normalne populacije ćelije intersticijuma SM, uključeni u patofiziološki mehanizam nastanaka oboljenja SM. Ova studija ima za cilj da na patohistološkom nivou pokaže ulogu mastocita u akutnom oštećenju SM pacova koji su izloženi ugljen-tetrahloridu (CCl_4). Od životinja koje su tretirane akutno CCl_4 (1 ml/kg) uzimani su uzorci m. bicepsa i m. gastrocnemiusa za dalju patohistološku obradu, bojenje (Toludin blue) i analizu. Dobijeni rezultati upoređeni su korišćenjem Studentovog t-testa. U uzorcima SM koji su dobijeni od životinja iz kontrolne grupe mastociti su bili prisutni najčešće u intersticijumu, dok kod životinja koje su bile izložene CCl_4 mastociti su se nalazili većinom u blizini krvnih sudova. Takođe, u eksperimentalnoj grupi životinja tretiranih CCl_4 mastociti su bili zastupljeniji nego u kontrolnoj grupi, a procenat onih koji su bili dagranulisani bio je statistički značajno veći. Na osnovu rezultata ove studije može se zaključiti oštećenje SM koje je indukovano CCl_4 delimično zavisi i od aktivnosti mastocita.

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Ključne reči: *ugljen-tetrahlorid, skeletni mišići, mastociti*

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INCIDENCE TREND OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Dejan Veljković[1], Zorana Deljanin[2]

Chronic Obstructive Pulmonary Disease (COPD) is associated with high morbidity and complications, decreased quality of life, increasing mortality. The objective of the paper was to determine incidence trends of COPD in the population of the Nišava District from 2002 to 2014. The descriptive study was used. Data about new cases of COPD were extracted from the Population Registry of COPD for the population of the Nišava District. Data about population originated from Censuses 2002 and 2011. Crude incidence, age, and gender specific rates were calculated per 100 000 inhabitants. Trend lines were estimated using linear regression. The total number of registered new cases were 7527 (4575 in males and 2952 in females). The average annual new cases was 627 and the annual average crude incidence rate was 164.3/100 000 inhabitants. The lowest annual incidence rate was in 2014 (36.7/100 000) and the highest one was in 2006 (232.6/100 000). Males represent 61 % and females 39 %. Females were significantly older than males (78.5 ± 2.0 vs 81.4 ± 1.8, $p < 0.001$). Patients older than 50 years of age represent 86% of all registered patients. Incidence rate increased with age. Incidence trend rapidly decreased in 2014 both in males and females; in males: $y = 3.874x + 160.3$, $R^2 = 0.038$, in females: $y = 0.631x+112.5$, $R^2 = 0.002$. There were 1.6 more new cases of COPD in males than in females but females were older compared with males. COPD wasn't common before the age of 40 and incidence increased with age. Under reporting and under registration of new cases of COPD was observed.

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Key words: *chronical obstructive pulmonary disease, trend, incidence*

[1]Department of Medical Security, Department of Gendarmerie, Kraljevo, Serbia
[2]Institute of Public Health Niš, Niš, Serbia

Contact: Veljković Dejan
Četvrti kraljevački bataljon 33/1, 36000 Kraljevo, Serbia
E-mail: drdejanveljkovic@gmail.com

Introduction

The World Health Organization (WHO) has ranked the chronic obstructive pulmonary disease (COPD) as one of the most prevalent long-term conditions worldwide (1). It is assessed that the global burden of COPD will rise, particularly in developing countries, because of the combination of ageing populations and increased smoking rates (2, 3). COPD is a chronic respiratory disease characterized by a decline in lung function over time and accompanied by respiratory symptoms, primarily dyspnea, cough, and sputum production (4). COPD is associated with a significant economic burden, including hospitalization, work absence, and disability (5).

Chronic obstructive pulmonary disease is a common, preventable, and treatable airflow limitation disease that is usually progressive and associated with enhanced inflammation in the airways and lungs (6). According to the Global Burden Studies (GBDs), COPD causes the death of at least 2.9 million people annually (7). The GBDs highlights that COPD was the sixth leading cause of death in 1990, has been the fourth since 2000 and is projected to be the third by 2020 (8).

It is estimated that about 210 million people have COPD worldwide (4). Overall, the prevalence of COPD in the general population is estimated to be around 1% across all ages, rising steeply to 8–10 % or higher among those aged 40 years or older (9).

The reported prevalence of COPD ranged from 0.2% in Japan to 37% in the USA, but the prevalence of CODP varies widely across countries and populations (10).

There are several well-known risk factors associated with the development and triggering of COPD exacerbations (11, 12). Tobacco smoking, includes many other environmental exposures, such as occupational exposures to dust and fumes in the developed and developing countries (13), and indoor

biomass fuel burning in many developing countries (14).

Factors that may worsen CODP include outdoor pollutants and passive smoke exposure. A number of factors associated with COPD development may not currently be possible to modify; these include the aging lung, sex, comorbidities, and child or adult repeated respiratory infections (15).

The aim

The objective of the paper was to determine the incidence trend of COPD in the population of the Nišava District from 2002 to 2014.

Materials and methods

The descriptive epidemiological study was used. Data about new cases of COPD were extracted from the Population Registry of COPD for the population of the Nišava District for the period 2002 to 2014. Data about population originated from Censuses 2002 and 2011. Crude incidence and age-specific were calculated per 100.000 inhabitants. Trend lines were estimated using linear regression.

Results

The total number of registered new cases was 7527 (4575 in males and 2952 in females). Males represented 61 % and females 39 %. The male to female incidence ratio was 1.6:1. The average annual number of new CODP cases was 627 and the annual average crude incidence rate was 164.3/100 000. Females were significantly older at the time of diagnosis of COPD than males (78.5 ± 2.0 vs 81.4 ± 1.8, $p < 0.001$).

Table 1. Number of new COPD cases and crude annual rates of incidence in the period 2002 to 2014

Year	Number of new cases	Crude incidence rates
2002	282	73.9
2003	378	99.0
2004	309	89.9
2005	622	162.9
2006	888	232.6
2007	873	228.9
2008	724	189.6
2009	580	151.9
2010	774	203.0
2011	671	175.8
2012	536	140.4
2013	750	196.5
2014	140	36.7
Total	7527	164.3*

*Annual average incidence rate

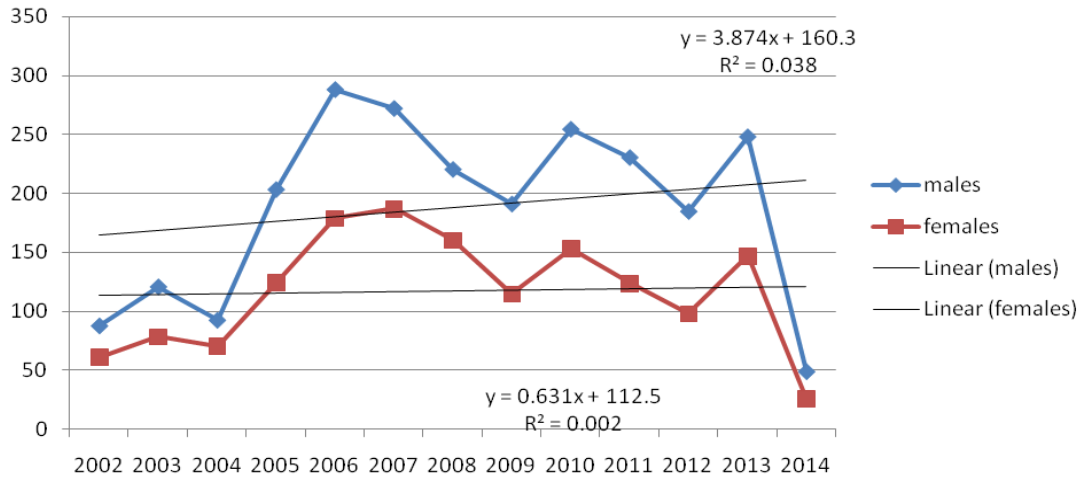

Figure 1. Incidence trend of COPD based on crude incidence rates in males and in females in the period 2002 to 2014

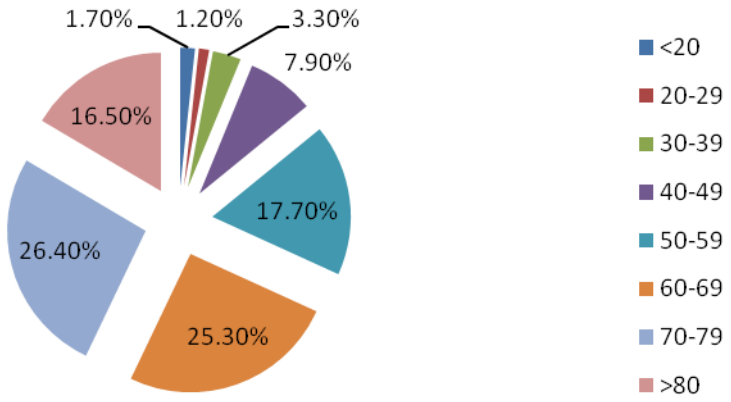

Figure 2. The Age-distribution of new cases of CODP in the period 2002-2014 on the Nišava District

COPD incidence trend, based on crude rapidly decreased both in males and in females.

Value of trend in males: $y = 3.874x + 160.3$, $R^2 = 0.038$

Value of trend in female: $y = 0.631x + 112.5$, $R^2 = 0.002$

The COPD wasn't common before 40 years of age. Patients older than 50 years of age represented 86% of all registered patients with CODP. The most registered new cases were in patients from the age-group 70 to 79 years of age. More than one quarter of registered patients was above 80 years of age.

Discussion

Chronic obstructive pulmonary disease is a common condition, associated with increasing age and smoking exposure. Only 10–15 % of all COPD cases are identified medically (16). For the last decade, an overall increase in COPD prevalence along with a decrease of incidence was reported in the Nišava District. Over time, all crude incidence rates were higher in males compared to females. In most of the studies, the incidence of COPD was greater in males than in females (16, 17).

The estimated prevalence of COPD is about 1% in the general population and rises sharply in persons aged ≥ 40 years (17). According to the presented results the incidence of COPD was greater in older patients, particularly in those aged 70 years and older. There are similar data in the medical literature (18-20).

The incidence of COPD varies between countries (21). Although COPD incidence has increased over the last 20 years, within the last 10 years, there has been an overall decrease in the USA, Canada (22, 23).

According to findings from Sweden incidence rates were a two- to three-times higher in smokers than non-smokers assessed by GOLD or BTS criteria (24, 25). One study also reported that COPD inci-

dence in former smokers was more than double that in nonsmokers (26).

There is estimated to be more than one billion smokers in the world, which is about a quarter of adults (27). Smoking prevalence is higher in males than in females worldwide. In Serbia in 2006, 33 % of the population, were smokers. There were more males than females who smoked on a daily basis (32.5 % vs 23.7 %), (28). About two-thirds of the population of Serbia (61.7 %) were exposed to tobacco smoke at home and 44.9 % were exposed at work (28).

According to WHO in many developing countries, the consumption of cigarettes is increasing rapidly in both sexes due to population growth and the increased targeted tobacco marketing in these areas (especially at young people) and in Serbia 50.5 % of young people do not have a desirable attitude to smoking (27, 28).

Other causes of airflow obstruction, such as biomass exposure, are not therefore required to explain any discrepancy between the prevalence of obstruction and the prevalence of smoking.

The rapid decrease of incidence trend of COPD in the Nišava District is the consequence of under reporting of COPD. Reporting of new cases of COPD isn't obligatory in Serbia since 2017 (29).

Conclusion

There were 1.6 more new cases of COPD in males than in females but females were older compared with males. COPD wasn't common before the age of 40 and the incidence increased with age. Under reporting and under registration of COPD was determined in the observed period.

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References

1. World Health Organization (WHO). The world health report 1998 - Life in the 21st century: A vision for all. Geneva: World Health Organization; 1998.
2. Högman M, Sulku J, Ställberg B, Johanes C, Bröms K, Hedenstorm H, et al. Global Initiative for Chronic Obstructive Lung Disease reclassifies half of COPD subjects to lower risk group. Int J Chron Obstruct Pulmon Dis 2018; 13:165-73. [CrossRef] [PubMed]
3. Simpson CR, Hippisley-Cox J, Sheikh A. Trends in the epidemiology of chronic obstructive pulmonary disease in England: a national study of 51 804 patients. Br J Gen Pract 2010; 60(576):277-84. [Crossref] [PubMed]
4. Global Strategy for the diagnosis, management, and prevention of chronic obstructive lung disease (updated 2014). Global Initiative for Chronic Obstructive Lung Disease (GOLD); 2014.
5. World Health Organization (WHO). European health for all database (HFA-DB). "cited 2019 Feb 21"; Available from: https://gateway.euro.who.int/en/datasets/european-health-for-all-database/
6. Rennard SI, Drummond MB. Early chronic obstructive pulmonary disease: definition, assessment, and prevention. Lancet 2015; 385(9979):1778-88. [Crossref] [PubMed]
7. World Health Organization (WHO). European mortality database (MDB). "cited 2019 Feb 21"; Available from: https://gateway.euro.who.int/en/datasets/european-mortality-database/
8. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet 1997; 349(9064): 1498-504. [Crossref] [PubMed]
9. Porter RS, Kaplan JL. The Merck manual of diagnosis and therapy. New Jersey: Merck Sharp & Dohme Corp.; 2011.
10. Rycroft EC, Heyes A, Lanza L, Becker K. Epidemiology of chronic obstructive pulmonary disease: a literature review. Int J Chron Obstruct Pulmon Dis 2012; 7:457-94. [Crossref] [PubMed]
11. Barnes PJ. Chronic Obstructive pulmonary Disease: effect beyond the lungs. Plos Med 2010; 7 (3): e1000220. [Crossref] [PubMed]
12. National Clinical Guideline Centre. Chronic obstructive pulmonary disease. Management of chronic obstructive pulmonary disease in adults in primary and secondary care. London: Royal College of Physicians; 2010. [PubMed]
13. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet 2007; 370(9589):765-73. [Crossref] [PubMed]
14. Hooper R, Burney P, Vollmer WM, McBurnie MA, Gislason T, Tan WC, et al. Risk factors for COPD spirometrically defined from the lower limit of normal in the BOLD project. Eur Respir J 2012; 39(6):1343-53. [Crossref] [PubMed]
15. Ford SE. Trends in mortality from chronic obstructive pulmonary disease among adults in the United States. Chest 2015; 148(4):962-70. [Crossref] [PubMed]

16. Soriano BJ. An epidemiological overview of chronic obstructive pulmonary disease: what can real-life data tell us about disease management? COPD 2017; 14 (Suppl 1):S3-S7 [Crossref] [PubMed]
17. Doucet M, Rochette L, Hame D. Incidence, prevalence, and mortality trends in chronic obstructive pulmonary disease over 2001 to 2011: a public health point of view of the burden. Can Respir J 2016; 2016: 7518287. [Crossref] [PubMed]
18. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013; 187(4):347-65. [Crossref] [PubMed]
19. World Health Organization (WHO). Global surveillance, prevention and control of chronic respiratory diseases. A comprehensive approach. Geneva: World Health Organization; 2007.
20. García Rodríguez LA, Wallander MA, Tolosa LB, Johansson S. Chronic obstructive pulmonary disease in UK primary care: incidence and risk factors. COPD 2009; 6(5):369-79. [Crossref] [PubMed]
21. Cerveri I, Accordini S, Verlato G, Corsico A, Zoia MC, Casali L, et al. Variations in the prevalence across countries of chronic bronchitis and smoking habits in young adults. Eur Respir J 2001; 18(1):85-92. [Crossref] [PubMed]
22. Lindgren B. Trends in obstructive lung disease in hospital registers in Sweden 1987–1996. Eur Respir Rev 2000; 10(75):423.
23. Lipton R, Banerjee A. The geography of chronic obstructive pulmonary disease across time: California in 1993 and 1999. Int J Med Sci 2007; 4(4):179-89. [Crossref] [PubMed]
24. Kabir Z, Connolly GN, Koh HK, Clancy L. Chronic obstructive pulmonary disease hospitalization rates in Massachusetts: a trend analysis. QJM 2010; 103(3): 163-8. [Crossref] [PubMed]
25. Lindberg A, Eriksson B, Larsson LG, Rönmark E, Sandstrom T, Lundbäck B. Seven-year cumulative incidence of COPD in an age-stratified general population sample. Chest 2006; 129(4):879-85. [Crossref] [PubMed]
26. Gershon AS, Wang C, Wilton AS, Raut R, To T. Trends in chronic obstructive pulmonary disease prevalence, incidence, and mortality in Ontario, Canada, 1996 to 2007: a population-based study. Arch Intern Med 2010; 170(6):560-5. [Crossref] [PubMed]
27. Kocic B, Petrovic B, Rancic N, Ilic M. Lung cancer trends in southeastern Serbia. Cent Eur J Public Health 2013; 21(1):17-21. [Crossref] [PubMed]
28. National Health Survey, Serbia, 2006. Key Findings. Belgrade: Ministry of Health of the Republic of Serbia; 2007.
29. Zakon o zdravstvenoj dokumentaciji i evidencijama u oblasti zdravstva. Službeni glasnik RS br. 123/2014 i 106/2015. "cited 2019 Feb 21"; Available from: http://www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/sgrs/skupstina/zakon/2014/123/2/reg

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TREND INCIDENCIJE HRONIČNE OPSTRUKTIVNE BOLESTI PLUĆA

Dejan Veljković[1], Zorana Deljanin[2]

[1]Odsek za sanitetsko obezbeđenje, Odred žandarmerije, Kraljevo, Srbija
[2]Institut za javno zdravlje Niš, Niš, Srbija

Kontakt: Veljković Dejan
Četvrti kraljevački bataljon 33/1, 36000 Kraljevo, Srbija
E-mail: drdejanveljkovic@gmail.com

Hroničnu opstruktivnu bolest pluća (HOBP) karakterišu visok morbiditet, česte komplikacije, visok mortalitet, snižen kvalitet života i veliki ekonomski troškovi. Rad je imao za cilj da prikaže trend incidencije HOBP-a na teritoriji Nišavskog okruga u periodu 2002—2014. Podaci o novoobolelima dobijeni su iz populacionog registra za HOBP i retrospektivno su analizirani za populaciju Nišavskog okruga. Izračunate su sirove stope, specifične stope prema polu i uzrastu na 100 000 stanovnika. Podaci o populaciji dobijeni su iz popisa iz 2002. i 2011. godine. Izračunat je linearni trend. U posmatranom 12-godišnjem periodu registrovano je 7527 novoobolelih (4575 muškaraca i 2952 žene). Prosečan godišnji broj novoobolelih iznosio je 627, a prosečna godišnja stopa incidencije bila je 164,3/100 000. Godišnje stope incidencije kretale su se od 36,7/100 000 (2014) do 232,6/100 000 (2006). Bilo je značajno više novoobolelih muškaraca nego žena (61 % prema 39 %). Žene su bile značajno starije od muškaraca (78,5 ± 2,0 prema 81,4 ± 1,8; $p < 0,001$). Stope incidencije povećavale su se sa starenjem, a stariji od 50 godina činili su 86 % svih novoobolelih. Trend incidencije i kod muškaraca i kod žena naglo je pao 2014, kod muškaraca: $y = 3,874x + 160,3$; $R^2 = 0,038$; a kod žena: $y = 0,631x + 112,5$; $R^2 = 0,002$. Muškarci su obolevali 1,6 puta više od žena, ali su žene bile starije u vreme postavljanja dijagnoze HOBP-a. Neredovno prijavljivanje imalo je za posledicu podregistraciju novoobolelih od HOBP-a u posmatranom periodu.

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Ključne reči: *trend, incidencija, hronična opstruktivna bolest pluća*

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PHYSICAL ACTIVITY AS AN IMPORTANT FACTOR FOR THE REDUCTION OF LIPID RISK FACTORS AT THE SECONDARY PREVENTION OF CORONARY HEART DISEASE IN MEN

Aleksandar Rajić[1], Vesko Milenković[1], Rade Stefanović[1], Milica Bojović[1], Sladjana Milošević[1], Jasmina Ranković[2], Branimir Mekić[1], Ljubiša Lilić[1], Lazar Toskić[1], Jadranka Kocić[1], Hadži Miloš Vidaković[1]

In people with normal or moderately elevated levels of cholesterol and triglycerides, a decrease in their values leads to positive health effects, and even at low levels of LDL-C, HDL-C levels represent an important predictor of coronary heart disease.

The aim of our study was to evaluate the changes in lipoprotein parameters and the reduction of cardiovascular risk in normolipidemic patients with myocardial infarction, under the influence of physical training.

The research included 38 normolipidemic men with coronary artery disease. The average age of the patients was 58.01 ± 5.31 years. All the subjects had elevated blood pressure, 93.38 % had a positive family history and 65.31 % of them were tobacco consumers. All patients were prescribed physical activity according to the recommendations of the EAS and the ACC/AHA and the diet by NCEP ATP III recommendations.

After the six months of implementation of physical activity in patients with normal values of lipids and myocardial stroke, the non-significant reduction in triglycerides of 9.81 %, VLDL-C of 9.91 %, LDL-C of 6.21 % and total cholesterol of 24.2 %, was recorded. Non-HDL-C was significantly reduced by 7.56 % ($p < 0.004$). There was a significant increase in protective HDL-C by 17.27 % ($pc < 0.003$); reduction in LDL-C/HDL-C relation of 20.81 % ($pc < 0.003$), the decrease of Hol/HDL-C by 17.34 % ($p < 0.002$) and a significant shift from the very high and high risk for new coronary incident to the moderate risk, by using coronary risk tables ($p < 0.05$).

Physical activity leads to a reduction of lipid risk factors and atherogenic index in males at the secondary prevention of coronary heart disease. The initial lipid screening indicates the presence of dyslipidemia in the majority of patients who were considered normolipidemic, so the lipid screening is recommended in all patients at the secondary prevention of coronary heart disease.

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Key words: *Physical activity, coronary heart disease, dyslipidemia*

[1]University of Priština, Faculty of Sport and Physical Education, Leposavić, Serbia
[2]Primary Health Care Centre Niš, Niš, Serbia

Contact: Aleksandar Rajić
Hilandarska 15/4, 18000 Niš, Serbia
E-mail: rajic.aleksandar89@gmail.com

Introduction

Paragh G. and associates pointed out the importance of lipid lowering therapy after myocardial infarction (MI), because it was shown that 83.3% of patients with previous IM, before the occurrence of the incident were hypercholesterolemia (1). In spite of statistical indicators, the level of lipid accumulation in the arteries is not necessarily caused by an increase of LDL-C in plasma (2), and border level of total cholesterol (Hol), below which there is no risk for the development of coronary incident, does not exist (3).

Many authors state that people with normal or moderately elevated levels of LDL and cholesterol, also have useful effects of lowering them. Also, significant prognostic potential, especially in men, have lowered levels of HDL-C, especially in states with low levels of LDL-C (4).

The interconnection of limit low levels of HDL-C and limit high levels of LDL-C has a cumulative risk of subsequent coronary events in patients at secondary prevention of coronary artery disease (CAD). These disorders in clinical practice often remain uni-

dentified and patients insufficiently acquainted with the importance of physical exercise, which is the first and often the only therapeutic step in treating these disorders (5).

Goal of work

The aim of our study was to determine the changes of lipid parameters and reduction of cardiovascular risk in normolipidemic respondents, with the preferred or limit values of total cholesterol and triglyceride in plasma, and with the coronary disease after organized and controlled six-month physical load.

Materials and methods

The study included 38 men with myocardial infarction, with the values of total cholesterol less than 5.2 mmol/l and triglycerides lower than 2.3 mmol/l. The mean age was 58.01 ± 5.31 years. The research was made in the Cabinet for lipids at the Institute for prevention, treatment and rehabilitation of rheumatic and cardiovascular diseases, Niška Banja. The methodological approach was based on the comparison of results obtained by a prospective analysis of the patients' medical history, clinical examination findings and determining biochemical parameters. All patients were prescribed physical activity and diet according to the NCEP ATP III recommenda tions (6).

Blood for the analysis of lipid parameters was taken after 12 hours of fasting, at the beginning and after (24 weeks) of the examination. Total cholesterol (Hol), triglycerides (TG) and HDL-cholesterol (HDL-C) were determined by standard methodology, and LDL-cholesterol (LDL-C), VLDL-cholesterol (VLDL-C), non-HDL cholesterol (nonHDL-C) and lipoprotein relations were determined by calculation. The preferred values for each of these parameters were given by recommendations of EAS and NCEP ATP III (3.6) and the level of physical activity was determined according to the AHA recommendations (7). The research results were analyzed by statistical methods of descriptive and quantitative analysis, with the use of appropriate tests, and the results are presented in tables and graphs.

Results

The frequency of nonlipid risk factors in the examined patients was very high. All patients had elevated blood pressure, positive family history of the existence of coronary disease had 93.38 % of patients, and 65.31 % of patients consumed tobacco.

All patients had normal levels of lipids in plasma, 88.13 % of them had the concentration of HDL-C < 1 mmol/l, and 35.02 % the concentration of HDL-C less than 0.91 mmol/l.

By analyzing the changes in lipid and lipoprotein parameters, as well as atherogenic relations at the beginning and at end of the study, made under the influence of the applied physical activity and diet regime, the non-significant reduction in triglycerides and VLDL-C for 9.91 %, LDL-C for 21.6 % and Hol for 14.2 %, was recorded. Although the reduction in these lipid parameters was insignificant, their cumulative effect was significant and it led to a significant reduction in the amount of non-HDL-C of 7.51 % ($p < 0.004$). A very important segment of physical activity, according to the AHA recommen dations, was manifested by an increase in the protective HDL-C, which increased by 17.28 % ($p < 0.003$).

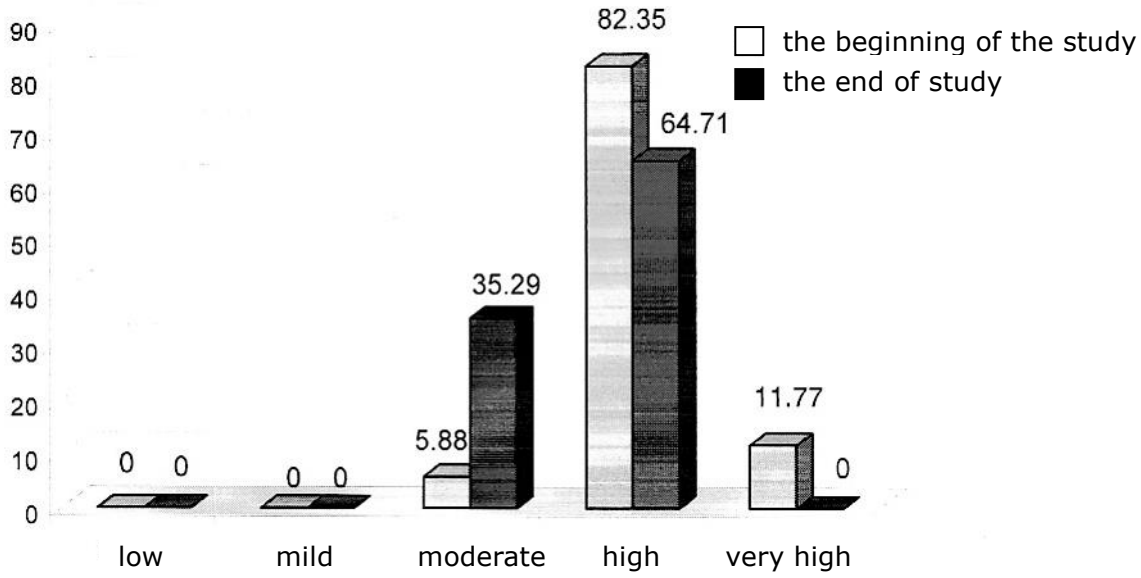
The changes in the concentration of lipids were also examined by the changes of atherogenic indexes. Thus, the tested men were found a significant reduction in relation LDL-C/HDL-C of 20.81 % ($p < 0.003$) and the reduction of relations Hol/HDL-C of 17.27 % ($p < 0.002$) (Table 1).

By the application of a diet and physical activity for a period of 6 months, favorable values of HDL-C and atherogenic indexes were achieved in all patients. The target values for triglycerides were achieved by 80 % and for LDL-C by 15 % of respondents.

Table 1. Change of lipid origin risk factors

Lipid origin risk factors	Total		Total difference (%)	P
	The beginning of testing	The end of testing		
total holesterol (Hol)	4.92 ± 0.26	4.81 ± 0.23	-2.25	NS
Triglycerides (TG)	1.91 ± 0.35	1.72 ± 0.32	-9.81	NS
VLDL-C	0.86 ± 0.16	0.78 + 0.14	-9.91	NS
LDL-C	3.02 ± 0.33	2.87 ± 0.33	-6.21	NS
non-HDL-C	3.96 ± 0.35	3.65 + 0.30	-7.51	0.004
HDL-C	0.97 ± 0.15	1,14 + 0.18	+17.28	0.003
LDL-C/HDL-C	3.23 ± 0.77	2.55 ± 0.52	-20.81	0,003
Hol/HDL-C	5.16 ± 1.00	4.271 + 0.67	-17.27	0.002

The absolute ten-year risk in subjects at the beginning of testing was very high in 11.75 %, high in 82.14 % and moderate in 5.88 % of the respondents. After six months of the applied therapy, there was a change in the distribution of risk in terms of reducing the very high for 11.77 % and high risk for 17.64 % ($p < 0.05$) on account of an increase in moderate risk (Graph 1).



Graph 1.

Discussion

Numerous studies have shown the clinical and angiographic benefits of reducing moderately or highly increased LDL-C in patients with the developed CAD. However, many patients with CAD have relatively normal values of total cholesterol. There are controversies concerning the attitude about the usefulness of therapy which alters the level of lipids in patients with CAD and mostly normal values of lipids. These concerns stem from the still insufficient knowledge of many pleiotropic effects of statin therapy as well as the insufficient motivation of the patients for continuous usage of these drugs. Bearing in mind that there are still no official recommendations for medicament therapeutic approach to patients, it remains to pay more attention to the struggle for the reduction of the major risk factors for atherosclerosis, which are primarily the implemen tation of hygienic dietary regime and increase of physical activity, regulation of hypertension, hyperglycemia and state of glucose intolerance, as well as to the fight against smoking (8).

LaRosa and colleagues report that people with normal or moderately elevated values of cholesterol (4.1 - 6.2 mmol/l) and LDL-C (2.6 - 4.1 mmol /l) have beneficial effects of lowering the same. The authors state that at low levels of LDL-C (< 2.84 mmol/l), the level of HDL-C presents an important predictor of CHD, especially in males (9).

By comparison of "normolipidemic" subgroup in FATS and HARP study, it was found that the low levels of HDL-C are the benchmark for the usefulness of therapy that alters lipids (8).

Some authors point out the importance of determining the HDL-C, and its subfractions for assessing the risk of recurrence of the CD events in normolipidemic patients (10). In accordance with these findings are also the results that after 12 weeks of cardiac rehabilitation with physical training, the patients with reduced levels of HDL-C and normal triglyceride levels had a significant increase of HDL-C and decrease of LDL-C/HDL-C relation (4).

Changes in the distribution of the ten-year risk for CHD in normolipidemic patients were created under the influence of chronic physical training, they show a reduction of very high and high risk and the increase of the prevalence of lower risk categories. There is a significant difference in the structure of risk between men and women, with a significantly higher risk in men. These results justify the view that, despite the normal levels of total cholesterol and triglycerides, after an estimated lipid risk levels and the ten-year risk for new coronary incidents, even in these patients the diet should be advised, that is the proper way of eating, physical activity, education about risk factors should be provided, and the analyses of lipoprotein should be repeated after a year (11). The information of FATS studies shows that subjects with levels of LDL-C < 4.14 mmol/l, have the same beneficial effects of lipid lowering as

well as those with LDL-C > 4.14 mmol/l. The values of HDL-C and triglyceride in the patients involved in the FATS study were: HDL-C = 0.88 mmol/l, Tg = 2.08 mmol/l (8).

The low concentrations of HDL-C are accompanied with the increased risk for CHD even when levels of total cholesterol and triglycerides are not elevated (10). Large epidemiological studies suggest that any increase in HDL-C for 0.02 mmol/l, reduces the risk for the new coronary incident by 2-3 % (2).

In our study in patients with myocardial infarction and normal values of total cholesterol and triglycerides, in the beginning, a high risk (> 20 %) was present in eighty-three percent of patients. Low isolated HDL-C (ILHDL-C) was defined as level HDL-C < 0.91 mmol/l, LDL-C < 4.14 mmol/l and Tg < 2.82 mmol/l (2). In our study, 35.29 % of the examined men suited these criteria. Lien and colleagues determined that patients with CHD have the prevalence of ILHDL-C, which ranges from 17-36 % (2), which corresponds to our results.

The largest number of normolipidemic respondents had the desired and limited values of cholesterol, LDL-C, and triglycerides, while the high-risk HDL-C and abnormal HDL ratio had 87.20 % of the patients with coronary artery disease. More than two-thirds of normolipidemic patients with myocardial infarction (75.11 %) had high-risk values of atherogenic relations Hol/HDL-C and 17.64 % high risk values of atherogenic relation LDL-C/HDL-C. This kind of distribution of lipid risk levels in normolipidemic patients with IM, suits the fact that, despite relatively normal levels of total cholesterol and triglycerides, other lipoprotein fractions, non-HDL-C, and atherogenic relations may indicate the presence of dyslipidemia in these patients, which requires clinical evaluation and long-term monitoring in order to identify the subjects with an increased risk for new coronary events in the near or distant future.

Conclusion

1. Respondents with myocardial infarction and normal values of lipids, in a high percentage, belong to the group of patients with isolated low HDL cholesterol.

2. Normolipidemic patients with myocardial infarction and two non-lipid risk factors for coronary heart disease have a very high risk for new coronary events and require hypolipemic therapy.

3. The change of the lipoprotein profile in patients with myocardial infarction and normolipidemic dyslipidemia leads to a reduction of absolute ten-year risk for new coronary events, which is accompanied by a redistribution of risk profiles with a reduction of the very high and high risk on account of moderate.

4. Physical activity and diet measures signifi cantly affect the reduction of risks for new coronary incidents, which is followed by the decrease of patients with very high and high risk on account of the group of patients with moderate risk.

References

1. Pederson T. Statins trials and goals of cholesterol-lowering therapy after AMI. Am Heart J 1999; 138 (2): S177-82. [CrossRef][PubMed]
2. Harper CR, Jacobson TA. New perspectives on the management of low levels of high-density lipoprotein cholesterol. Arch Intern Med 1999;159(10):1049-57. [CrossRef][PubMed]
3. Prevention of coronary heart disease in clinical practice. Recommendations of the Second Joint Task Force of European and other Societes on coronary prevention. Eur Heart J 1998;19(10):1434-503. [PubMed]
4. Lavie CJ, Milani RV. Benefits of cardiac rehabilitation and exercise training. Chest 2000; 117(17):5-7. [CrossRef][PubMed]
5. Antić S, Lazarević G, Đinđić B, Jovanović V. Procena fizičke aktivnosti u obolelih od dijabetes melitusa tip 2. Acta Medica Medianae 2003; 42(4):5-10.
6. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285(19):2486-97. [CrossRef][PubMed]
7. Pollack CV Jr, Roe MT, Peterson ED. 2002 update to the ACC/AHA guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: Implications for emergency department practice. Ann Emerg Med 2003; 41(3):355-69. [CrossRef][PubMed]
8. Brown G, Stewart BF, Zhao XQ, Hillger LA, Poulin D, Albers JJ. What benefit can be derived from treating noncholesterolemic patients with coronary artery disease? Am J Cardiol 1995; 76(9):93C-7C. [CrossRef][PubMed]
9. LaRosa JC. Unresolved issues in early trials of cholesterol lowering. Am J Cardiol 1995;76(9):5C-9C. [CrossRef][PubMed]
10. Li XP, Zhao SP, Zhang XY, Liu L, Gao M, Zhou QC. Protective effect of high density lipoprotein on endothelium-dependent vasodilatation. Int J Cardiol 2000; 73(3):231-6. [CrossRef][PubMed]
11. Roberts CK, Chen AK, Barnard RJ. Effect of a short-term diet and exercise intervention in youth on atherosclerotic risk factors. Atherosclerosis 2007; 191 (1): 98-106. [CrossRef][PubMed]

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FIZIČKA AKTIVNOST KAO VAŽAN FAKTOR REDUKCIJE LIPIDNIH FAKTORA RIZIKA U SEKUNDARNOJ PREVENCIJI KARDIOVASKULARNE BOLESTI KOD MUŠKARACA

Aleksandar Rajić[1], Vesko Milenković[1], Rade Stefanović[1], Milica Bojović[1], Slađana Milošević[1], Jasmina Ranković[2], Branimir Mekić[1], Ljubiša Lilić[1], Lazar Toskić[1], Jadranka Kocić[1], Hadži Miloš Vidaković[1]

[1]Univerzitet u Prištini, Fakultet za sport i fizičko vaspitanje, Leposavić, Srbija
[2]Dom zdravlja Niš, Niš, Srbija

Kontakt: Aleksandar Rajić
Hilandarska 15/4, 18000 Niš, Srbija
E-mail: rajic.aleksandar89@gmail.com

Kod osoba sa normalnim ili umereno povišenim nivoima holesterola i triglicerida uočavaju se povoljni efekti prilikom njihovih smanjenja, pa čak i pri niskim nivoima LDL-C, HDL-C nivoi predstavljaju važan prediktor koronarne bolesti srca.

Cilj našeg istraživanja bio je da se pod uticajem fizičkog treninga procene promene parametara lipoproteina i smanjenje kardiovaskularnog rizika kod normolipidemijskih bolesnika sa infarktom miokarda.

Istraživanje je obuhvatilo 38 normolipidemijskih muškaraca sa koronarno-arterijskom bolešću. Prosečna starost bolesnika bila je 58,01 ± 5,31 godina. Svi ispitanici su imali povišen krvni pritisak, 93,38 % imalo je pozitivnu porodičnu istoriju, a 65,31 % bili su pušači. Svim bolesnicima propisana je fizička aktivnost prema preporukama EAS-a i ACC/AHA, a dijeta po preporuci NCEP ATP III.

Nakon šest meseci primene fizičke aktivnosti kod bolesnika sa normalnim vrednostima lipida i bolesnika sa moždanim udarom, zabeležena je značajna redukcija triglicerida od 9,81 %, VLDL-C od 9,91 %, LDL-C od 6,21 % i ukupnog holesterola od 24,2 %. Ne-HDL-C značajno je smanjen za 7,56 % ($p < 0,004$). Došlo je do značajnog povećanja zaštitnog HDL-C za 17,27 % ($pc < 0,003$); smanjenja LDL-C/HDL-C odnosa od 20,81 % ($pc < 0,003$), smanjenja Hol/HDL-C za 17,34 % ($p < 0,002$) i značajanog pomaka od veoma visokog i visokog rizika za novi koronarni incident do umerenog rizika, korišćenjem koronarnih tablica rizika ($p < 0,05$).

Fizička aktivnost dovodi do smanjenja vrednosti lipida i aterogenog indeksa kod muškaraca na sekundarnoj prevenciji koronarne bolesti srca. Početni skrining lipida ukazuje na prisustvo dislipidemije kod većine bolesnika koji su smatrani normolipidemičnim, tako da se skrining lipida preporučuje svima na sekundarnoj prevenciji koronarne bolesti srca.

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Ključne reči: *fizička aktivnost, koronarna bolest srca, dislipidemija*

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CARDIOVASCULAR RISK REDUCTION AND CORRECTION OF HYPERLIPIDEMIA IN PATIENTS AT PRIMARY PREVENTION THROUGH PHYSICAL ACTIVITY

Branimir Mekić[1], Dragan Toskić[1], Tatjana Popović-Ilić[1], Hadži Saša Ilić[1], Vesko Milenković[1], Aleksandar Rajić[1], Milica Bojović[1], Jasmina Ranković[2], Miroslav Živković[1], Hadži Miloš Vidaković[1]

Coronary heart disease is responsible for a high degree of morbidity and mortality in industrialized countries. Many countries have therefore adopted preventive measures to reduce the prevalence of major risk factors for coronary artery disease (CD): high serum cholesterol, high blood pressure, diabetes, and smoking.

The aim of this study was to determine the effects of physical training and diet on lipid and lipoprotein parameters in patients with hyperlipidemia without developed coronary heart disease.

The study included 45 patients, average age 56.1 ± 12.7 years. All patients had total cholesterol greater than 5.2 mmol/1, elevated blood pressure, and 16.42 % of respondents had used tobacco. Risk factors for the CD were evaluated through clinical history, anthropometric and cardiovascular parameters with the use of biochemical analysis. Lipid parameters included the determination of total cholesterol, triglycerides and HDL, LDL and VLDL cholesterol as well as lipid relations. All tested parameters were determined at the beginning and after the six months of testing. All patients were on a program of physical activity as recommended by ACC /AHA and on a diet according to NCEP ATP III recommendations for primary prevention of coronary heart disease.

After six months of testing, there was a change in the risk factors of lipid origin: triglycerides were reduced by 22.3 %, HDL-C was increased for 11.94 %, VLDL was reduced for 21.9 % and nonHDL-C for 8.09 %. By applying physical activity the target value of HDL-C > 1 mmol/1 was achieved in all patients. A high percentage of respondents achieved the target values for Tg < 2.3 mmol/1 (92.79 %) and for the atherogenic relation LDL-C/HDL-C < 5 (84.59 %). BMI was reduced for 8.06 % ($p < 0.05$) a systolic blood pressure for 12.8 % ($p < 0.03$).

In patients with hyperlipidemia without coronary heart disease and other risk factors for coronary heart disease, the achieved changes of lipoprotein levels are an indicator for the further implementation of non-pharmacological measures with the goal to reduce the risk for coronary events. Determination of lipoprotein profiles and atherogenic markers is very significant in the initial screening of dyslipidemia, monitoring the effects of physical activity and evaluating the risk for coronary heart disease.

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Key words: *physical activity, hyperlipidemia, primary prevention*

[1]Faculty of Sport and Physical Education, University of Priština, Leposavić, Serbia
[2]Health Centre Niš, Niš, Serbia

Contact: Branimir Mekić
Dositeja Obradovića bb, 38218 Leposavić, Serbia
E-mail: branimir.mekic@pr.ac.rs

Introduction

Coronary heart disease is responsible for large morbidity and mortality in industrialized countries.

Atherosclerotic process, the problem that has been studied even before 100 year, lies at the basis of this disease (1). In the last century the two very important hypotheses on the pathogenesis of this process have been made: thrombogenic and lipid.

The first link between cholesterol and atherosclerosis was established in 1841 when Vogel demonstrated the presence of cholesterol in atherosclerotic plaques.

In 1884 Carl VonRokitanskif made the thrombogenic theory, and in 1856 Virchov made the theory of response to endothelial damage.

In 1913, Anitschkov and Chalatov showed that a diet rich in cholesterol induced atherosclerotic

changes, and Goldstein and Brown identified LDL particles as main atherogenic lipoproteins. (2)

Based on the results of the Framingham Study, it became clear that lipid disorders, hypertension, diabetes, and obesity are major risk factors for CD.

Many countries have adopted the preventive measures in order to reduce the prevalence of major risk factors for coronary heart disease: high values of serum cholesterol, high blood pressure, diabetes and enjoying of tobacco. The coronary heart disease mortality rate in our country is increasing, while in developed countries this mortality rate of cardiovascular disease is significantly reduced below 50 % and in particular of myocardial infarction. (3) This was achieved by successful modification of risk factors for atherosclerosis (strict prohibition of smoking, aerobic physical activity, nutrition without cholesterol and saturated fatty acids, reduction of body weight in obese patients, treatment of hypertension and diabetes and lowering of elevated blood lipids by wide application of hypolipemics) (4).

The fact that two-thirds of the patients with coronary artery disease have a total cholesterol level in the serum of more than 5.17 mmol/1, LDL-C > 3.35 mmol/1 and HDL-C < 0.91 mmol/1.2 confirms that hyperlipidemia is one of the most important risk factors for CHD. (5)

Implementation of recommendations for changes in lifestyle, diet, and the increase of physical activity in patients with dyslipidemia is an initial measure in the treatment of patients with hyperlipidemia, which to a significant extent, reduces both non-lipid and lipid risk factors for CHD. This justifies the long-term use of non-pharmacological measures at primary and secondary prevention of coronary heart disease. If these measures do not show the desired results, antilipemic medications should be added to therapy. (6)

The aim of the research

To determine the effects of the use of physical training and diet on lipid parameters in patients with hyperlipidemia without a developed coronary heart disease.

Material and methods

In total, 45 persons of both sexes and average age 56.1 ± 7.12 years were examined. All the patients had total cholesterol greater than 5.2 mmol /1, elevated blood pressure, and 16.42 % of the respondents enjoyed the tobacco. The initial clinical examination of subjects included the registration of history data, based on questionnaires of the Cabinet for the disorder of fat metabolism at the Institute for prevention, treatment, and rehabilitation of rheumatic and cardiovascular diseases "Niška Banja", anthropometric measurements for calculating body mass index and registration of blood pressure.

Determination of total cholesterol, triglycerides, and HDL cholesterol, as well as non-lipid biochemical parameters (fibrinogen, uric acid, glucose), was performed by using standard methodology after twelve hours of fasting, and levels of LDL cholesterol, VLDL cholesterol, and lipid relations were determined by calculation. All of the examined parameters were determined at the beginning and after six months of testing. Based on the results of clinical examination and biochemical analysis, the lipid profile and risk factors of non-lipid origin for coronary heart disease were determined.

All patients were included in the outpatient program physical training program, which included physical activity for 45 minutes, five days a week, as recommended by the AHA for primary prevention (7).

In addition to physical training, the diet of the first degree was prescribed to the patients, (total fat intake < 30 %, energy intake < 10 %, saturated fatty acids, and cholesterol < 300 mg/a day) according to the recommendations of the NCEP ATP III (8). The research results were processed by using appropriate methods of descriptive statistics and were analyzed by using appropriate tests. The results are presented in tables and graphs.

Results

After a six-month physical training and diet, altered levels of lipid and non-lipid parameters were registered.

BMI was reduced for 8.06 % ($p < 0.005$), systolic blood pressure was reduced for 21.8 % ($p < 0.05$), diastolic blood pressure for 22.6 %.

Fibrinogen was lowered for 7.81 %, and uric acid for 9.71 %. There was a lowering of the glycemia for 9.89 %, by which the threshold of statistical significance was not reached (Table 1).

By implementation of physical activity and diet in patients with hyperlipidemia without coronary heart disease, the increase of HDL-C for 11.72 % ($p < 0.01$) was noted, the reduction of total cholesterol for 5.2 % was not significant, LDL-C was reduced for 31.4 % which also was not significant, triglycerides were reduced for 22.0 % ($p < 0.01$), VLDL was also reduced for 22.0 % ($p < 0.01$), while the nonHDL-C was reduced for 17.8 % ($p < 0.05$).

The regime of continuous physical activity and diet led to a significant reduction in atherogenic relation Hol/HDL-C (22.15 %, $p < 0.005$), the increase of HDL-ratio (21.45 %, $p < 0.005$) and reduction of atherogenic relation LDL-C/HDL-C (16.79 %, $p < 0.01$) (Table 2)

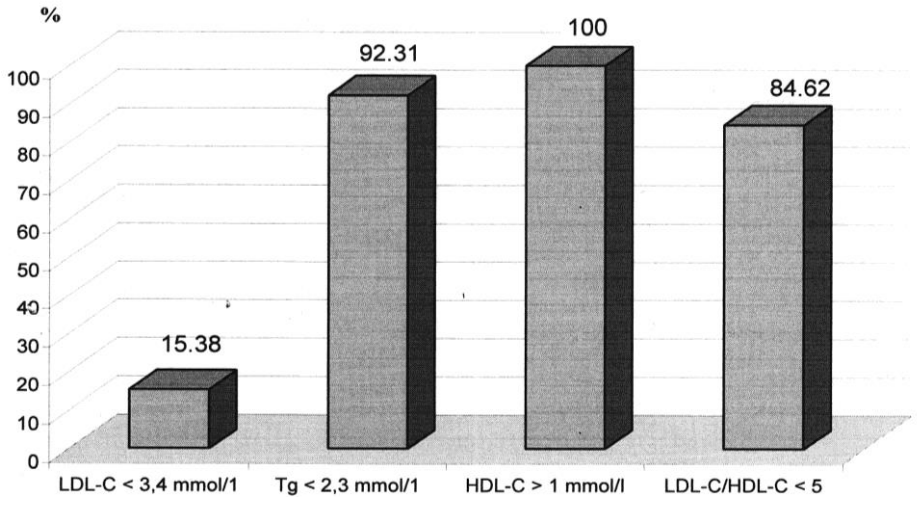
By applying the hygienic dietary therapy and physical activity in the duration of 6 months, all the participants have achieved the target values of HDL-C > 1 mmol/1. A very high percentage of respondents scored the target values for Tg < 2.3 mmol/1 (92.31 %) and for the atherogenic relation LDL-C/ HDL-C < 5 (84.62 %) (Graph 1).

Table 1. Changes of non-lipid origin risk factors during testing

Risk factors	The subjects with hyperlipidemia without coronary artery disease			
	Total		Difference	P
	The beginning	The end of testing	%	
BMI (kg/m^2)	29.54 ± 2.23	27.12 ± 1.53	-8.06	0.005
systolic blood pressure (mmHg)	147,6+ 15.36	135.28 + 12.16	-8.21	0.05
diastolic blood pressure (mmHg)	92.2 + 9.04	86.44 ± 6.88	-6.22	NS
fibrinogen (g/L)	3.73 + 0.72	3.42 ± 0.62	-7.81	NS
acidumuricum (umol /1)	265.4 ± 79.9	239.79 + 90.45	-9.71	NS
glycemia (mmol/1)	5.4 +1.1	4.8 + 0.5	-9,89	NS

Table 2. The change of lipid parameters values during testing

Lipid parametric relations	Subjects with hyperlipidemia without coronary artery disease			
	Total		Difference	P
	The beginning	The end of testing	%	
total cholesterol (Hol)	6.95 ± 0.45	6.58 ± 0.54	-5.2	NS
Triglycerides (TG)	2.23 ± 0.55	1.73 ± 0.29	-22.0	0.01
VLDL-C	1.01 ± 0.25	0.78 ± 0.13	-22.0	0.01
LDL-C	4.94 ± 0.57	4.67 ± 0.54	-4.31	NS
non-HDL-C	5.95 ± 0.49	5.47 ± 0.53 -	-8.17	0.05
HDL-C	1.00 +0.14	1.11 +0.07	±11.72	0.01
LDL-C/HDL-C	5.04 ± 1.01	4.18 ± 0.54	-16.79	0.01
Hol/HDL-C	7.08 ± 1	5.53± 1.51	-22.15	0.005
HDLratio *	0.16 ± 0.03	0.20 ± 0.02	+21.45	0.005



Graph1. Percentage of patients with achieved success

Discussion

Comparing the data of epidemiological studies of randomized clinical testings, it is revealed that it is highly possible to prevent coronary heart disease. Despite this fact, there is an alarming trend of prevalence and regulation of risk factors in women. The prevalence of obesity is increasing, and about 25 % of women do not practice regular physical activity. The rate of tobacco use is slightly increasing in women compared to men. Approximately 52 % of women > 45 years old have elevated blood pressure, and approximately 40 % of women > 55 years of age have elevated serum cholesterol levels (7).

Diabetes is a powerful risk factor in women, which leads to an increased risk for the occurrence of CHD 3 to 7 times as long as the increase in men 2 to 3 times. This difference between the sexes is present due to the extremely harmful effects of diabetes on lipids and blood pressure in women. Women tend to have higher levels of HDL-C compared to men, and triglyceride level may be a significant risk factor, especially in older women (9).

Physical activity in earlier sedentary respondents showed a small but significant drop in levels of cholesterol, LDL-C, and triglycerides which was accompanied by an increase in HDL-C level.

Long distance runners and other athletes, generally have high levels of HDL-C, especially HDL fractions. Sedentary men moderately increase the levels of HDL-C after 8-11 months of physical training. There is also evidence that physical training is associated with changes in the composition of LDL particles, transforming small dense atherogenic LDL particles to larger and lighter particles (10).

Physical training shows beneficial effects in those who adhere to the diet of first or second degree with the recommendations of the NCEP's.

Meta-analysis shows that physical exercise causes a greater reduction of total cholesterol, LDL-C and Tg, and the increase of HDL-C if it is accompanied by a low-fat diet. Stefanick and colleagues demonstrated that the subjects, with low HDL-C and high LDL-C, who were on a diet of the second degree, do not show a significant reduction in LDL-C without the use of physical training (10).

Bass and authors in the large study that evolved the role of HDL-C as a risk factor for cardiovascular disease in women, found that women with HDL-C < 50 mg/dl have nearly three times greater degree of mortality from cardiovascular disease. The negative correlation between the percentage of body fat or body mass and HDL-C was confirmed in premenopausal and postmenopausal women as well as in men. (11).

Physical training which is recommended for people at high risk for CHD, both at primary and secondary prevention, involves anaerobic, dynamic work with the aim to moderately increase the heart volume, ventilation, and consumption of oxygen.

Physical activity is a quick walk of 30-60 minutes, at least 3-4 times a week. It is a minimal level of physical activity in the prevention of CHD. Physical activity of high intensity and diet help to reduce cholesterol for 5-7 %, LDL-C for 7-12 % (12).

Physical activity, alone, leads to a reduction of triglyceride and LDL-C levels, and to a significant increase of HDL-C. The increase in HDL-C ranges from 5-16 % and is directly related to the levels of triglycerides, body mass, alcohol consumption, and smoking.

Intensive physical training leads to an increase in HDL-C among young people for 14 % and in the elderly, over 65years for 15 %, together with the reduction of other risk factors (13).

In ATP III recommendations, weight gain and obesity have been identified as major risk factors for CHD and are identified as direct targets that need to be regulated. Reduction of body weight affects the reduction of LDL-C and reduces all the risk factors in metabolic syndrome. Regular physical activity reduces the levels of VLDL, increases HDL-C, and in some patients reduces the levels of LDL-C. It can also reduce blood pressure, reduce insulin resistance and affect cardiovascular function. ATP III recommends physical activity as a routine component in the regulation of the high level of cholesterol in serum (8).

In our study of patients with hyperlipidaemia without CHD, there have been significant changes in the risk factors of lipid origin: triglycerides were reduced for 22.0 %, the increase of HDL-C for 11.72 % was recorded, VLDL was reduced for 22.0 % non-HDL-C 8.17 % . From risk factors of lipid origin, BMI pressure was significantly reduced for 8.17 %, and systolic blood pressure for 8.21 %.

The study on 3331 adults with physical activity at least 30 minutes, 3 times a week, showed that this group had significantly increased levels of HDL-C, lower triglyceride levels, significantly lower rates of smoking and lower BMI, compared to the group who led sedentary way of life, where the score of risk factors was significantly higher.

The group which practiced physical activity only once a week had a lower score for CHD risk factors, as compared to sedentary people.

Conclusion

1. Increased physical activity and dietary measures in patients with hyperlipidemia without coronary heart disease, lead to a reduction of lipid risk levels.

2. In patients with hyperlipidemia without coronary heart disease and other risk factors for coronary heart disease, the achieved changes of lipoprotein profiles are an indicator for the further implementation of non-pharmacological measures with the aim to reduce the risk for coronary events.

3. Determination of lipoprotein profiles and atherogenicmarkersis significant at the initial screening of dyslipidemia, monitoring of the effects of physical activity and estimation of the risk for coronary heart disease.

References

1. Ross R. The Pathogenesis of Atherosclerosis. In: Braunnwald E, editor. Heart Disease. A Text Book of Cardiovascular Medicine. London: W.B. Saunders Company; 2000. p. 1105-24.
2. Stehbens WE. Coronary Heart Disease, Hypercholesterolemia, and Atherosclerosis. Misrepresented Data. Experimental and Molecular Pathology 2001; 70:120-39. [CrossRef] [PubMed]
3. Ostojić M, Vukčević V, Dimković S, et al. Novine u prevenciji ishemijske bolesti srca. Balneoclimatologia 2003; 27:69-81.
4. Ilić S. Efekti Simvastatina u prevenciji koronarne bolesti. In: Savić T, editor. Evaluacija, dijagnostika i terapija dislipidemija. Zbornik radova Dislipidemija i ateroskleroza I izdanje; 2003 maj 5-7; Niš, Srbija. Niš: Punta; 2003. p. 334-43.
5. Đinđić B, Radić S, Damnjanović G, Savić T, Antić S, Sokolović D. Učestalost i udruženost faktora rizika za nastanak koronarne bolesti srca u dijabetes melitusu. Acta medica Medianae 2003; 42(l):21-7.
6. Antić S, Lazarević G, Đinđić B, Jovanović V. Procena fizičke aktivnosti u obolelih od dijabetes melitusa tip 2. Acta Medica Medianae 2003; 42(4):5-10.
7. Mosca L, Manson JE, Sutherland SE, Langer RD, Manolio T, Barrett-Connor E. Cardiovascular disease in women: Statement for helthcare professionals from the American Heart Association: writing group. Circulation 1997; 96:2468-82. [CrossRef] [PubMed]
8. Expert Panel on Detection, Evaluation, and Trealment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001; 285:2486-97. [CrossRef]
9. Manson JE, Spelsberg A. Risk modificalion in the diabetic patient. In: Manson JE Ridker PM, Gaziano JM, Hennekens CH, editors. Prevention of Myocardial infarction. New York: Oxford University Press; 1996: 241-73.
10. Stone NJ, Blum CB, Winslov E Management of Lipids in Clinical Practice. NY: Personal Comm. Ltd; 2000.
11. Warner JG, Brubaker PH, Zhy Y, Morgan TM, Ribisl PM, Miller HS, et al. Long-term (5-year) changes in HDL cholesterol in cardiac rehabilitation patients. Do sex differences exist? Circulation 1995; 92:773-7. [CrossRef] [PubMed]
12. Wood AJJ. Adjunctive Drug therapy of acute myocardial infarction - Evidence from Clinical Trials. N Engl J Med 1996; 335(22):1660-6. [CrossRef] [PubMed]
13. Schlierf G, Schuler G, Hambrecht R, Niebauer J, Hauer K, Vogel G, et al. Treatment of coronary heart disease by diet and exercise. J Cardiovasc Pharmacol 1995; 25 (Suppl4): S32-4. [CrossRef] [PubMed]

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SMANJENJE KARDIOVASKULARNOG RIZIKA I KOREKCIJA HIPERLIPIDEMIJE KOD BOLESNIKA NA PRIMARNOJ PREVENCIJI NAKON FIZIČKE AKTIVNOSTI

Branimir Mekić[1], Dragan Toskić[1], Tatjana Popović-Ilić[1], Hadži Saša Ilić[1], Vesko Milenković[1], Aleksandar Rajić[1], Milica Bojović[1], Jasmina Ranković[2], Miroslav Živković[1], Hadži Miloš Vidaković[1]

[1]Univerzitet u Prištini, Fakultet sporta i fizičke kulture, Leposavić, Srbija
[2]Dom zdravlja Niš, Niš, Srbija

Kontakt: Branimir Mekić
Dositeja Obradovića bb, 38218 Leposavić, Srbija
E-mail: branimir.mekic@pr.ac.rs

Koronarna bolest srca odgovorna je za visok stepen morbiditeta i mortaliteta u industrijalizovanim zemljama. Zbog toga su mnoge zemlje usvojile preventivne mere za smanjenje prevalencije glavnih faktora rizika za bolesti koronarne arterije (CD): visok nivo serumskog holesterola, visok krvni pritisak, dijabetes i pušenje.

Cilj ove studije je utvrditi efekte fizičkog vežbanja i ishrane na parametre lipida i lipoproteina kod pacijenata sa hiperlipidemijom bez razvijene koronarne bolesti srca.

Studija je obuhvatila 45 pacijenata prosečne starosti: 56,1 ± 12,7 godina. Svi pacijenti su imali ukupan holesterol veći od 5,2 mmol/l, povišen krvni pritisak, a 16,42 % ispitanika je koristilo duvan. Faktori rizika za CD procenjeni su kroz kliničku istoriju, antropometrijske i kardiovaskularne parametre uz korišćenje biohemijske analize. Parametri lipida uključuju određivanje ukupnog holesterola, triglicerida i HDL, LDL i VLDL holesterola, kao i odnosa lipida. Svi testirani parametri određeni su na početku i nakon šest meseci testiranja. Svi pacijenti bili su na programu fizičke aktivnosti prema preporuci ACC/AHA i na ishrani prema preporukama NCEP ATP III za primarnu prevenciju koronarne bolesti srca.

Posle šest meseci testiranja došlo je do promene faktora rizika lipidnog porekla: triglicerid su smanjeni za 22,3 %, HDL-C je povećan za 11,94 %, VLDL je smanjen za 21,9 %, a nonHDL-C za 8,09 %. Primenom fizičke aktivnosti ciljna vrednost HDL-C > 1 mmol/1 postignuta je kod svih pacijenata. Visok procenat ispitanika postigao je ciljane vrednosti za Tg < 2,3 mmol/l (92,79 %) i za aterogeni odnos LDL-C/HDL-C < 5 (84,59 %). BMI je smanjen za 8,06 % ($p < 0,05$) sistolni krvni pritisak za 12,8 % ($p < 0,03$).

Kod pacijenata sa hiperlipidemijom bez koronarne bolesti srca i drugih faktora rizika za koronarnu bolest srca, postignute promene nivoa lipoproteina indikator su za dalju primenu nefarmakoloških mera sa ciljem smanjenja rizika za koronarne probleme. Određivanje profila lipoproteina i aterogenih markera veoma je značajno u inicijalnom pregledu dislipidemije, praćenju efekata fizičke aktivnosti i proceni rizika za koronarnu bolest srca.

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Ključne reči: *fizička aktivnost, hiperlipidemija, primarna prevencija*

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CENTRAL CORNEAL THICKNESS MEASURED BY THE OCULYZER, BIOGRAPH, AND ULTRASOUND PACHYMETRY

Marko Zlatanović[1], Maja Živković[1,2], Aleksandra Hristov[3], Vesna Stojković[3], Saša Novak[1], Nevena Zlatanović[2], Mladen Brzaković[3]

Central corneal thickness (CCT) is an important parameter in selecting patients before any corneal refractive procedures and in postoperative follow-up, in monitoring the patients with corneal disorders such as keratoconus and Fuchs' dystrophy, as well as in adjusting the intraocular pressure according to central corneal thickness.

The aim of the paper was to compare the results of CCT measurements obtained by different measurement instruments: Oculyzer, BioGraph and ultrasound pachymetry.

CCT was measured in 64 eyes of 32 patients. The measurements were performed in a specialized Eye Hospital "Maja Clinic" in Niš, using the following instruments: WaveLight Allegro Oculyzer, WaveLight Allergo Biograph, ultrasound pachymeter DGH Pachette 3. The mean values of central corneal thickness obtained from different devices were compared.

The mean values of CCT (± standard deviation) obtained with Oculyzer were 552.94 μm ± 22.88 μm, 556.56 ± 25.32 μm obtained with BioGraph, and 559.46 ± 26.0 μm measured by ultrasound pachymeter. There were no statistically significant differences among different measurement devices.

The CCT measurements with the Oculyzer, BioGraph, and ultrasound pachymetry do not show statistically significant differences, so the results of CCT measurements obtained by using any of these devices may be considered valid.

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Key words: *corneal thickness, pachymetry, measurement devices*

[1]Ophthalmology Clinic, Clinical Center, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia
[3]Specialized Eye Hospital "Maja Clinic" Niš, Serbia

Contact: Aleksandra Hristov
Djordja Andrejevića Kuna 24, Niš
E-mail: alexandramiljkovic@gmail.com

Introduction

Central corneal thickness (CCT) is an important parameter in general ophthalmology. Average central corneal thickness is about 540 microns. The cornea varies in thickness, it is the thickest at the limbus and thinnest in the central part.

In refractive surgery CCT is the most important factor in preoperative selection of patients in addition to corneal topography, Preoperative corneal thickness is a factor that guides a surgeon in making decision on performing the intervention, on diopter value to be safely corrected, as well as on the choice of surgical method (1, 2).

Keratoconus is a progressive disorder graded from stage 1 to 4, resulting in the thinning of the cornea and its changing to irregularly shaped conus. CCT is the most important parameter in grading this disease, as well as in making decision on therapeutic options. One of the revolutionary discoveries in ophthalmology is corneal crosslinking method that might stabilize the disease, prevent the disease progression, and avoid the need for corneal transplantation, but unfortunately it is suitable only in patients with CCT of more than 450 microns (3, 4).

Corneal thickness indirectly reflects corneal endothelial integrity. An increase in central corneal thickness can be seen in Fuchs' dystrophy because of corneal edema caused by endothelial decompensation.

Glaucoma is one of the leading causes of blindness nowadays. Central corneal thickness measurement is an important part of ophthalmic examinations in patients with glaucoma, with higher correction factor being associated with thinner cornea. Protocol deviations and not incorporating pachymetry in ophthalmic examination may result in unnecessary antiglaucomatous treatment in patients with CCT values above the average. Also, potential glaucomatous values may be underestimated in patients with thinner cornea (2, 5).

A correlation with refractive surgery can also be made, in fact with intraocular pressure values in patients after refractive surgery. After correction in moderate to high myopia where significant central thinning occurs, the values of intraocular pressure may be interpreted as hypotonic if correction factor is not used, or thinner cornea may underestimate the true IOP value regarding potential glaucomatous damage, which is far more dangerous.

Central corneal thickness can be measured with various instruments available, such as: Oculyzer, BioGraph, ultrasound pachymeter, or OCT. Each of these types of measurements is supposed to be accurate, safe, with high repeatability of results, reproducible, easy and quick to perform.

Ultrasound pachymeter measures central corneal thickness by using ultrasound waves and a probe. This method offers the advantages of being economical and easy to perform, but since it is a contact method its main limitations include tissue indentation, placement of the probe on the center of the cornea, and its requirements for topical anesthesia

The BioGraph uses the optical low coherence reflectometry (OLCR) and thanks to highly sophisticated software it obtains data on anterior segment, including the CCT.

The Oculyzer is the most advanced and highly accurate device that allows a variety of diagnostic measures and enables obtaining a great deal of data on anterior chamber that are useful in making a final decision for a phakic intraocular lens implantation or refractive vision correction laser surgery. Its mechanism of action is based on improved Pentacam HR technology, non-contact measurement, and full analysis of the complete anterior eye segment (6).

Aim of the paper

The aim of the paper was to compare the results of central corneal thickness measurements obtained by various devices (Oculyzer, Biograph, and Ultrasound pachymeter) and draw conclusion on their statistical significance.

Material and methods

In this study, central corneal thickness (CCT) was measured in 64 eyes of 32 patients. Random selection resulted in a sample of 15 male and 17 female patients of average age being 33 ± 4.03 years. Patients with pathological changes that could affect corneal thickness and structure were excluded from the study. The voluntary, written informed consent for use of patients' data for medical and scientific purposes was obtained from each participant in the study.

The measurements were performed at the "Maja Clinic", a specialized hospital for eye diseases in Niš. The same patients underwent consecutive measurements on 3 different devices:

WaveLight Allergo Oculyzer, WaveLight Allergo Biograph and Ul-trasound Pachymeter DGH Pachette 3.

All measurements were taken on the same day. First, the measurements were performed using non-contact methods (Oculyzer and Biograph), followed by a contact method – ultrasound pachymetry. Before each ultrasound pachymetry measurement, topical anaesthetic eye drop of 0.5 % tetracaine-hydrochloride was applied. To avoid subjective biased evaluation in ultrasound pachymetry measurement, five consecutive CCT measurements were performed in all 64 eyes by two examiners.

Upon the completion of measurements all the data were statistically grouped and analyzed (SPSS version 20, Student's t test), then mean values of central corneal thickness obtained from different devices were compared.

Results

The obtained mean values of CCT (± standard deviation) were 552.4 ± 22.88 μm measured with Oculyzer, 556.56 ± 25.32 μm measured with Biograph, and 559.46 ± 26.0 μm measured with ultrasound pachymeter (Table 1). The measurement differences among the devices were not statistically significant ($p > 0.001$). The greatest difference was registered between central corneal thickness measured with Oculyzer and ultrasound pachymetry, but still not statistically significant. The highest value measured with Oculyzer was 576 μm, with Biograph it was 582 μm, while the highest value measured with ultrasound pachymetry was 584 μm.

Table 1. Mean values of CCT measured with three different instruments (Oculyzer, Biograph, ultrasound pachymetry)

Measurement device	CCT ± standard deviation (μm)
Oculyzer	552.94 ± 22.88
Biograph	556.56 ± 25.32
Ultrasound pachymeter	559.46 ± 26.00

Discussion

According to technical differences between aforementioned devices, the values of CCT were believed to be significantly different. Still, the results were surprisingly similar. Mean CCT values (± standard deviation) were 552.94 ± 22.88 μm as measured with Oculyzer, 556.56 ± 25.32 μm measured with Biograph, and 559.26 ± 26.00 μm measured with ultrasound pachymeter.

Comparison between central corneal thickness measurements obtained by different devices has been the subject of many studies. Those worth mentioning include CCT measurement with the Pentacam, OCT device, and ultrasound probe by J. Barkana et al. (7), then a study on comparison between central corneal thickness measurements by pentacam and ultrasound pachymetry by H.S.Al-Mezaine et al. (8), and a study on measuring central corneal thickness with pentacam, OCT device, and ultrasound pachymetry by I. Piotrowiak (6). The results of our study show the extent of correlation with the results obtained in previously mentioned studies and how relevant each measurement obtained by different devices is in making decisions on further therapeutic procedures.

It is well-known that refractive surgery is a procedure that requires laser removal of about 15 μm of corneal tissue per 1 diopter of vision corrected. The difference of 15 μm or more at measurement may be crucial in making decision on performing the intervention. A lot of papers on this subject may be found in available literature data. A great number of studies compared CCT results measured with different devices. Our results and conclusions are consistent with the majority of them. A study entitled "Comparison between central corneal thickness measurements by oculus pentacam and ultrasonic pachymetry" by H.S.Al-Mezaine et al. (8) suggested that differences in CCT measurements were not statistically significant. On the contrary, both the Oculyzer and Ultrasonic pachymetry measurements showed high correlation. Y. Barkana et al. (7) published a paper on central corneal thickness measured with Pentacam, Ultrasound pachymetry and BioGraph and concluded that the values obtained with these devices are highly correlated. Still, there are studies that observed statistically significant differences between the results of CCT measurements obtained using the Pentacam, Ultrasound pachymetry, or OCT. Thus, it is recommended that different devices cannot be used interchangeably without using correcting coefficient, as I. Piotrowiak et al. (6) observed in their study. A thorough analysis of the first two studies revealed that there was no statistical significance in CCT measurements with different devices, but the Pentacam slightly overestimated the CCT in comparison to ultrasound pachymetry. In the third study that registered statistically significant difference, that relationship is reversal. The authors of the first two studies explain it by tissue indentation since the probe requires corneal contact, while the authors of the third study explain higher values measured with ultrasound probe by corneal edema after topical anaesthetic had been applied, as well as by placement of the probe on the corneal center that is operator-dependent.

Feizi et al. evaluated CCT measurements by ultrasound pachymetry, Scheimpflug analyzer, and Orbscan in normal and keratoconic eyes. They found no statistically significant difference in the values of normal eyes, unlike keratoconic eyes (9). The discrepancy between CCT measurements in the keratoconus was observed in other studies as well (10, 11).

Since normal physiological corneal thickness is about 540 μm, the difference in the mean measurement values between the Oculyzer, BioGraph, and Ultrasound Pachymentry of only 6.32 μm from the lowest to highest value obtained in our study had no statistical significance in making decisions on further diagnostic or therapeutic procedures.

Conclusion

Based on the results of this study, it may be concluded that in healthy eyes there is no statistically significant difference in measurements obtained with WaveLight Allergo Oculyzer, WaveLight Allergo Biograph, and ultrasound pachymeter DGH Pachette 3. Each of these results may independently be considered valid and adequate in guiding therapeutic decisions. After all, each of these instruments has its place in ophthalmology, depending on the health status of the eye and further required procedures.

References

1. Sadoughi M, Einollahi B, Einollahi N, Rezaei J, Roshandel D, Feizi S. Measurement of Central Corneal Thickness Using Ultrasound Pachymetry and Orbscan II in Normal Eyes. J Ophthalmic Vis Res 2015; 10(1): 4-9. [CrossRef] [PubMed]
2. Rashid RF, Farhood QK. Measurement of central corneal thickness by ultrasonic pachymeter and oculus pentacam in patients with well-controlled glaucoma: hospital-based comparative study. Clin Ophthalmol 2016; 10:359-64. [PubMed]
3. Sanctis U, Missolungi A, Mutani B, Richiardi L, Grignolo FM. Reproducibility and repeatability of central corneal thickness measurement in keratoconus using the rotating Scheimpflug camera and ultrasound pachymetry. Am J Ophthalmol 2007; 144:712-8. [CrossRef] [PubMed]
4. Aghazadeh Amiri M, Hashemi H, Ramin S, Yekta A, Taheri A, Nabovati P, et al. Corneal thickness measurements with Scheimpflug and slit scanning imaging techniques in keratoconus. J Curr Ophthalmol 2017; 29(1):23-7. [CrossRef] [PubMed]
5. Ramesh PV, Jha KN, Srikanth K. Comparison of Central Corneal Thickness using Anterior Segment Optical Coherence Tomography Versus Ultrasound Pachymetry. J Clin Diagn Res 2017; 11(8):NC08-11. [CrossRef] [PubMed]
6. Piotrowiak I, Soldanska B, Burduk M, Kaluzny B, Kaluzny. Measuring Corneal Thickness with SOCT, the Scheimpflug System, and Ultrasound Pachymetry. ISRN Ophthalmology 2012; 2012:869319. [PubMed]
7. Barkana Y, Gerber Y, Elbaz U, Schwartz S, Ken-Dror G, Avni I, et al. Central corneal thickness measurement with the Pentacam Scheimpflug system, optical low-coherence reflectometry pachymeter, and ultrasound pachymetry. J Cataract Refract Surg 2005; 31(9):1729-35. [CrossRef] [PubMed]
8. Al-Mezaine H, Al-Amro S, Kangave D, Sadaawy A, Wehaib T, Al-Obeidan S. Comparison between central corneal thickness measurements by oculus pentacam and ultrasonic pachymetry. International Ophthalmology 2008;28(5):333-8. [CrossRef] [PubMed]
9. Feizi S, Jafarinasab MR, Karimian F, Hasanpour H, Masudi A. Central and peripheral corneal thickness measurement in normal and keratoconic eyes using three corneal pachymeters. J Ophthalmic Vis Res 2014; 9(3):296-304. [PubMed]
10. Dutta D, Rao HL, Addepalli UK, Vaddavalli PK. Corneal thickness in keratoconus: comparing optical, ultrasound, and optical coherence tomography pachymetry. Ophthalmology 2013; 120(3):457-63. [CrossRef] [PubMed]
11. de Sanctis U, Missolungi A, Mutani B, Richiardi L, Grignolo F. Reproducibility and repeatability of central corneal thickness measurement in keratoconus using the rotating Scheimpflug camera and ultrasound pachymetry. Am J Ophthalmol 2007; 144(5):712-8. [CrossRef] [PubMed]

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CENTRALNA DEBLJINA ROŽNJAČE MERENA OCULYZEROM, BIOGRAFOM I ULTRAZVUCNIM PAHIMETROM

Marko Zlatanović[1], Maja Živković[1,2], Aleksandra Hristov[3], Vesna Stojković[3], Saša Novak[1], Nevena Zlatanović[2], Mladen Brzaković[3]

[1]Očna klinika, Klinički centar Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Srbija
[3]Specijalna očna bolnica "Klinika Maja" Niš, Niš, Srbija

Kontakt: Aleksandra Hristov
Đorđa Andrejevića Kuna 24, 18000 Niš, Srbija
E-mail: alexandramiljkovic@gmail.com

Centralna debljina rožnjače (CCT) je parametar bitan u selekciji bolesnika kod svih kornealnih refraktivnih procedura i u postoperativnom praćenju, u praćenju pacijanata sa bolestima rožnjače kao što su keratokonus i Fuksova distrofija, kao i u korekciji izmerenog intraokularnog pritiska.

Cilj rada bio je uporediti rezultate merenja CCT dobijene različitim instrumentima merenja: oculyzerom, biografom i ultrazvučnim pahimetrom.

Merena je CCT kod 32 bolesnika kod 64 očiju. Merenje je vršeno u specijalnoj očnoj bolnici "Klinika Maja" u Nišu na instrumentima: WaveLight Allegro Oculyzer, WaveLight Allergo Biograph, ultrazvučni pahimetar DGH Pachette 3. Upoređivane su srednje vrednosti CCT merene različitim instrumentima.

Dobijene srednje vrednosti CCT (± standardna devijacija) iznosile su 552,94 μm ± 22,88 μm mereno Oculyzerom, 556,56 ± 25,32 μm mereno Biografom i 559,46 ± 26,0 μm mereno ultrazvučnim pahimetrom, pri čemu dobijene razlike među različitim aparatima nisu statistički značajne.

Rezultati merenja CCT oculyzerom, biografom i ultrazvučnim pahimetrom ne pokazuju statistički značajne razlike, te se rezultati CCT dobijeni na bilo kom od ovih instrumenata mogu smatrati validnim.

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Ključne reči: *debljina rožnjače, pahimetrija, instrumenti merenja*

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THREE MONTH FOLLOW UP OF THE EFFECTS OF CONTINUOUS POSITIVE PRESSURE DURING SLEEP (CPAP) ON THE VALUE OF GLYCATED HEMOGLOBIN HBA1C AND GLYCOREGULATION IN OBESE DIABETIC PATIENTS WITH CONFIRMED SEVERE OPSTRUCTIVE SLEEP APNEA

Nikola Mladenović[1], Zoran Stamenković[2], Lidija Ristić[2,3], Dane Krtinić[4,5], Nemanja Petrović[1], Andrej Preveden[1], Bogdan Okiljević[1,6], Iva Binić[7], Miodrag Djordjević[7,8]

Obstructive sleep apnea (OSA) is a serious disorder of breathing during sleep characterized by complete or partial interruption of breathing during sleep for 10 seconds and longer.

In this prospective longitudinal clinical study in obese patients with diabetes mellitus type II which is determined by polysomnography heavy degree of OSA is accompanied by the application of positive pressure during sleep in the course of three months and evaluated its impact on the value of glycated hemoglobin HbA1c as an indicator of long-term glycoregulation. A prospective clinical study in the quarterly monitoring included 98 patients (64 men and 34 women), who, after clinical, laboratory, spirometry and diffusion examination of lung function, made of respiratory gas analysis of arterial blood gases in rest, tests Epworth sleepiness scale, underwent polysomnography testing on Philips Respironics Alice PDX device.

Out of 98 obese patients suffering from diabetes mellitus type II and heavy degree OSA, average age 50.1, 23 of them were randomized in two groups: experimental - 11 patients with an average HbA1c of 9.9 %, the average BMI 37.1 and average AHI index of 36.7 (31 to 59) who used CPAP during sleep, and control group of 12 patients, with an average HbA1c was 9.1 %, average BMI 39.3 and AHI index was 39.7 (31 to 62). After three-month of using CPAP, the control of HbA1c in both groups was performed. Average HbA1c in the experimental group decreased with statistical significances ($p < 0.01$) from 9.9 % to 6.7 %, compared to the control group patients with no significant changes.

The results of this study indicated that in obese patients with diabetes mellitus II and severe OSA, long- term glycoregulation can be significantly improved using the CPAP during sleep.

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Key words: *obesity, diabetes mellitus type II, obstructive sleep apnea, CPAP*

[1]Institute for cardiovascular diseases of Vojvodina, Sremska Kamenica, Serbia
[2]Clinic for lung diseases, Clinical center Niš, Serbia
[3]University of Niš, Department for internal medicine, Faculty of medicine, Niš, Serbia
[4]Clinic for oncology, Clinical center Niš, Serbia
[5]University of Niš, Faculty of medicine Niš, Department for pharmacology and toxicology, Serbia
[6]University of Novi Sad, Department for surgery, Faculty of medicine, Novi Sad, Serbia
[7]University of Nis, Faculty of medicine, Niš, Serbia
[8]Clinic for general surgery, Clinical center Niš, Serbia

Contact: Nikola Mladenović
Bul.Nemanjića 59/20, 18000 Niš, Serbia
E-mail: nikolamlad89@gmail.com

Introduction

Obstructive sleep apnea (OSA) represents a disease of the heterogenic group of breathing disorders during sleep characterised by recurring episodes of breathing cessations or episodes (apnea) of shallow breathing (hypopnea), and it is increasingly being recognized as an important health issue in the last two to three decades (1). OSA is a prevalent condition in close association with obesity epidemic globally, and it is characterized by the repetitive, partial or complete collapse of the upper airway during sleep, causing impaired gaseous exchange and sleep disturbance (2, 3).

It is the most common form of sleep-disordered breathing (SDB) worldwide as shown in different epidemiological studies. It is characterised by frequent episodes of upper airway collapse during sleep, causing recurrent arousals, intermittent hypoxemia, sleep fragmentation, and poor sleep quality.

There is accumulating evidence that OSA is being considered as an independent risk factor for hypertension, glucose intolerance / diabetes mellitus, cardiovascular diseases and stroke, leading to increased cardiometabolic morbidity, and mortality (4, 5).

The prevalence rates of OSA have been estimated in the range of 2 to 10 per cent worldwide, and the risk factors for obstructive sleep apnea include advanced age, male sex, obesity, family history, craniofacial abnormalities, smoking and alcohol consumption. The common clinical presenting symptoms are heavy snoring, witnessed apneas and daytime hypersomnolence, which would help to identify the affected individuals (6).

With increasing awareness of this disease entity and associated complications in our society, there have been increased referrals to sleep physicians or expertise for further investigations and diagnostic evaluation.

Early recognition and treatment of obstructive sleep apnea may prevent from adverse health consequences. It has been associated with increased cardiovascular and cerebrovascular morbidity and mortality, although much of the causal role and mechanisms are still poorly understood. (6, 7, 8).

The interaction of obesity and sleep disorders, OSA typ, is presented in Figure 1.

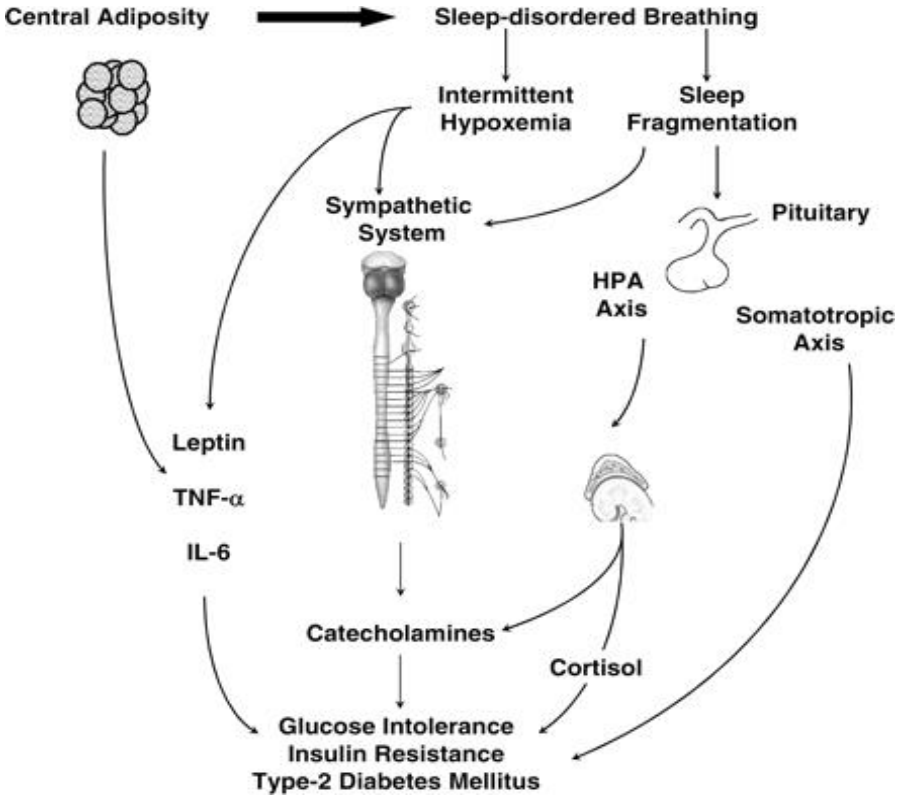

Figure 1. The interaction of obesity and sleep disorders

Aims

In this prospective, clinical, longitudinal study with three months of follow up, on consecutive obese patients with diabetes mellitus II in whom high degree of OSA by polysomography was confirmed, the authors examined the effects of using of continuous positive pressure CPAP during sleep on the value of glycated hemoglobin HbA1c as long–term indicator of glycoregulation.

Material and methods

The examinations were conducted at the Clinic for lung diseases Clinical Center Niš from October 2016. to December 2017.

In all patients at rest spirometry, clinical, laboratory, and gas analyses of arterial blood were conducted, Epworth scale of sleepiness, Berlin and STOP BANQ questionnaire were used, and polysomnographic examination performed using the Philips respironics Alice PDx device.

The gold standard diagnostic test for OSA is the overnight in-laboratory polysomnography. It involves multi-channel continuous polygraphic recording from surface leads for electroencephalography, electrooculography, electromyography, electrocardiodiography, nasal pressure transducer (supplemented by thermistor) for nasal airflow, thoracic and abdominal impedance belts for respiratory effort, pulse oximetry, tracheal microphone for snoring, and sensors for leg and sleep position. These recordings will identify different types of apnoeas and hyponoeas during sleep. An apnoea is defined as the complete cessation of airflow for at least 10 sec. There are three types of apnoeas: obstructive, central and mixed. In obstructive sleep apnoea, respiratory effort is maintained but ventilation decreases or disappears because of partial or total occlusion in the upper airway. Central sleep apnoea is defined as reduced respiratory effort resulting in reduced or absent ventilation. Mixed apnoea is often characterized by starting with central apnoeas and ending with obstructive events. A hypopnoea is defined as a reduction in airflow (30-50 %) that is followed by an arousal from sleep or a decrease in oxyhaemoglobin saturation (3-4 %) (5, 6). Sleep apnoea severity is assessed with apnoea-hypopnoea index (AHI), which is the number of apnoeas and hypopnoeas per hour of sleep. According to the American Academy of Sleep Medicine recommendations, OSA is defined with AHI > 5, and it is classified as mild OSA with AHI of 5 to 15; moderate OSA with AHI of 16 to 30; and severe OSA with AHI > 30 .

The study included 98 obese patients with OSA. Out of that number, 23 obese diabetic patients with severe OSA were randomized and divided into two groups: an experimental group of 11 patients who used three months continuous positive pressure during sleep (CPAP) and control group of 12 patients who did not use CPAP because of various reasons.

Results

Polysomnographic examination was done to 98 consecutive obese patients, and 58 (60,2 %) of them had severe OSA with AHI index > 30.

Out of 98 examined patients, 42 (42,85 %) had diabetes mellitus type II.

Out of 58 patients with high degree of OSA, diabetes mellitus type II had 37 patients (63,79 %), with average value of HbA1c of 11,7 %, indicating poor long-term glycoregulation in obese diabetic patients with severe OSA.

Out of 42 examined obese patients with diabetes mellitus type II, 37 of them had severe OSA (88 %).

In the experimental group of 11 obese diabetic patients with high degree OSA, we found an average HbA1c value of 8.9 %, average BMI value of 37.1 and an average AHI index of 36.7 (31 to 59) at the beginning of follow up period.

All of them 11 obtained devices for CPAP during sleep and its application began during sleep. After three months the results were summoned to control HbA1c values, just as for all patients from the control group.

In the control group consisting of 12 obese diabetic patients and high degree OSA in whom it was not possible to apply CPAP device during sleep, we found an average HbA1c value of 9.1 %, BMI of 39.3 and an AHI index of 39.7 (31 to 62).

There was no statistical difference between the examined groups in the value of glycated hemoglobin A1c as indicator of long term glucoregulation.

After three months of using CPAP devices during sleep statistically significant improvement in average values of HbA1c from 9.9 % to 6,7 % was found in the experimental group ($p < 0,001$).

In the control group we did not find any significant changes of average values of HbA1c from 9,1 to 9,7 %, Table 1.

Table 1. The representation of the values of glycosylated hemoglobin HbA1c in the experimental and control group after three-month use of CPAP in the experimental group

	Experimental group (No 11)		**Control group (12)**	
BMI index	37.1		39.3	n.s.
AHI index	36.7		39.7	n.s.
	Initial	**After 3 months of CPAPa**	**Initial**	**After 3 months**
The level of glycosylated hemoglobin HbA1c	9.9%	6.7%	9.1%	9.7
	(p < 0,001)		n.s	

Discussion

Obesity is a complex, multifactorial, and largely preventable disease (1), affecting, along with overweight, over a third of the world's population today (3). If secular trends continue, by 2030 an estimated 38 % of the world's adult population will be overweight and another 20 % will be obese (4).

Obstructive sleep apnea (OSA), a sleep-related breathing condition, is diagnosed based on a patient's apnea-hypopnea index from a sleep study, and the presence or absence of symptoms. Diabetes mellitus (DM) and OSA share a significant common risk factor, obesity, with all three conditions contributing to the risk of developing cardiovascular diseases. The pathophysiological links between OSA and DM are still unclear, but intermittent hypoxia may be an important mechanism. (8) More awareness of the possible link between OSA and DM is needed, given their increasing prevalence locally and worldwide. Continuous positive airway pressure is the standard treatment for OSA, while weight loss through dietary and lifestyle modifications is important to holistically manage patients with either condition. (10, 11) There is currently insufficient evidence to support the benefits of screening every diabetic patient for OSA. However, diabetic patients with symptoms suggestive of OSA should be referred to a sleep specialist for further evaluation. Epidemiological evidence has demonstrated a high prevalence of OSA in patients with Type 2 DM. In a cross-sectional study, up to 23% of a diabetic population were found to have OSA(13). In another study by Einhorn et al, 48 % of diabetic patients had OSA with AHI ≥ 10/hr. (14) The most common reason of breathing cessation is OSA, while in 10-15% of patients the reason is the lack of impulse from the brain – central sleep apnea- It is considered that approximately 5% of general population suffers from sleep apnea and its frequency is considered to be approximately the one of asthma, obesity and DM in general population. The main difference is that OSA remains unrecognised for a long period of time and is not treated until its consequences occur-daytime sleepiness, depression, obesity, insulin resistence, DM2, hypertension, atherosclerosis, coronary disease and sudden cardiac death. It is acknowledged that OSA is the cause of many traffic accidents, and in the developed world it is regulated by law that professional drivers must be polysomnographically examined (14).

As a basic consequence of breathing cessation during sleep intermittent hypoxy followed by tachycardia and arterial pressure rise occurs as a compensatory mechanism. As a long-term and cumulative consequence, the rise of leptin, TNF-a, IL-6 levels occur which have a definitive glucose intolerance, insulin resistence and DM2 as a consequence. Also, hypoxemy induced sympathetic stimulation and hypothalamo-hypophysis homeostatic disorder which by adrenal gland and cortisol secretion lead to DM2. (15, 16)

Many studies were conducted in obese patients with a different level of disorder with or without DM. Some of them show that the application of CPAP device during sleep may lead to a significant approvement in fat metabolism, fat loss, downregulation of sleepiness measured by the Epworth scale, glycoregulation approvement and a lower risk of sudden cardiac death which is undoubtedly confirmed by our investigation. (11, 14, 17)

Based on the goals set, applied methods and gathered data, it is evident that in obese patients with OSA and DM, that application of CPAP during sleep significantly approves values of HbA1c- This confirms that OSA is pathologically connected to glucose blood homeostasis and that CPAP application can be a significant therapeutic approach in these patients.

The results of our research are different from some of the previous ones. The explanation for this is the fact that we studied obesity diabetics only with severe OSA, where the use of CPAP over 3 months had undoubtedly enhanced long-term glycoregulation. For example, in another study subjects were recruited via the Oxford Sleep Clinic between June 2004 and August 2005. Eligible subjects were men aged 18-75 years with established type 2 diabetes (on diet, oral hypoglycemic agents or insulin therapy).This double-blind randomized controlled trial of therapeutic and placebo CPAP for 3 months in men with type 2 diabetes and OSA did not show any significant improvement in glycosylated hemoglobin. According to this study therapeutic CPAP did not significantly improve measures of glycogenic control or insulin resistance in men with type 2 diabetes and OSA. (10, 15, 18) While there is great interest in the question of whether CPAP treatment improves DM, the data have, unfortunately, been mixed. Two recent meta-analyses, which included non-randomized trials as well as trials with non-diabetic and diabetic OSA patients, demonstrated that CPAP treatment improved insulin sensitivity.(12, 13) However, in the only randomized controlled trial by West et al, which specifically evaluated the impact of CPAP treatment on glycaemic control in known Type 2 diabetic patients with newly diagnosed OSA, three months of CPAP intervention did not reveal any significant benefit for insulin resistance or HbA1c.(12) It is possible that longer durations of CPAP usage per night may be required to achieve improvements in glycaemic control. Gou and his co. comment on possible numerous reasons for the different effects of CPAP on glycoregulation (19). A recent study by Grimaldi et al. found that poor glycaemic control was associated with the frequency of obstructive respiratory events during rapid eye movement (REM) sleep, but not non-REM sleep (20). As REM sleep predominates in the latter part of the sleep period, the majority of the REM sleep period would have been left untreated in patients who only had four hours of CPAP.

Conclusion

In summary, there is strong epidemiological and pathophysiological evidence supporting the association between OSA and DM. The mechanism by which OSA impacts glucose homeostasis has yet to be fully elucidated, but multiple pathways are likely to play a role.

It is hoped that further research would be able to identify more specific pathophysiological pathways between the two diseases that may guide us in both the timing and nature of interventions to improve patient outcomes. Ultimately, it should be emphasized that diabetic medications are still the mainstay of treatment to achieve optimal glycaemic control, together with lifestyle modification and weight loss.

References

1. Grimaldi D, Beccuti G, Touma C, Van Cauter E, Mokhlesi B. Association of obstructive sleep apnea in rapid eye movement sleep with reduced glycemic control in type 2 diabetes: therapeutic implications. Diabetes Care 2014; 37:355-63. [CrossRef] [PubMed]
2. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet 2017; 390(10113):2627-42. [CrossRef] [PubMed]
3. Stevens GA, Singh GM, Lu Y, Danaei G, Lin JK, Finucane MM, et al. National, regional, and global trends in adult overweight and obesity prevalences. Popul Health Metr 2012; 10(1):22. [CrossRef] [PubMed]
4. West SD, Nicoll DJ, Stradling JR. Prevalence of obstructive sleep apnea in men with type 2 diabe-tes. Thorax 2006; 61:945-50. [CrossRef] [PubMed]
5. Einhorn D, Stewart DA, Erman MK, Gordon N, Philis-Tsimikas A, Casal E. Prevalence of sleep apnea in a population of adults with type 2 diabetes mellitus. Endocr Prac 2007; 13:355-62. [CrossRef] [PubMed]
6. West SD, Nicoll DJ, Wallace TM, Matthews DR, Stradling JR. Effect of CPAP on insulin resistance and HbA1c in men with obstructive sleep apnea and type 2 diabetes. Thorax 2007; 62:969-74. [CrossRef] [PubMed]
7. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014; 384(9945):766-81. [CrossRef] [PubMed]
8. Punjabi NM, Polotsky VY. Disorders of glucose metabolism in the sleep apnea. J Appl Physiol 2005; 99 (5):1998-2007. [CrossRef] [PubMed]
9. Foster GD, Sanders MH, Milman R, Zammit G, Borradaile KE, Newman AB, et al. Obstructive Sleep Apnea among Obese Patients with Type 2 Diabetes. Diabetes care 2009; 32:1015-7. [CrossRef] [PubMed]
10. Coughlin SR, Mawdsley L, Mugarza JA, Wilding JP, Calverley PM. Cardiovascular and metabolic effects of CPAP in obese men with OSA. Eur Respir J 2007; 29:720-7. [CrossRef] [PubMed]
11. Hoyos CM, Killick R, Yee BJ, Phillips CL, Grunstein RR, Liu PY. Cardiometabolic changes after continuous positive airway pressure for obstructive sleep apnoea: a randomised sham-controlled study. Thorax 2012; 67 (12):1081-9. [CrossRef] [PubMed]
12. Lindberg E, Theorell-Haglöw J, Svensson M, Gislason T, Berne C, Janson C. Sleep apnea and glucose metabolism: a long-term follow-up in a community-based sample. Chest 2012; 142(4):935-42. [CrossRef] [PubMed]
13. Pillai A, Warren G, Gunathilake W, Idris I. Effects of sleep apnea severity on glycemic control in patients with type 2 diabetes prior to continuous positive airway pressure treatment. Diabetes technol therapy 2011; 13(9):945-9. [CrossRef] [PubMed]
14. Chen L, Kuang J, Pei JH, Chen HM, Chen Z, Li ZW, et al. Continuous positive airway pressure and diabetes risk in sleep apnea patients: A systemic review and meta-analysis. Eur J Intern Med 2017; 39:39-50. [CrossRef] [PubMed]
15. Salord N, Fortuna AM, Monasterio C, Gasa M, Pérez A, Bonsignore MR, et al. A Randomized Controlled Trial of Continuous Positive Airway Pressure on Glucose Tolerance in Obese Patients with Obstructive Sleep Apnea. Sleep 2016; 39(1):35-41. [CrossRef] [PubMed]
16. Barceló A, Morell-Garcia D, Salord N, Esquinas C, Pérez G, Pérez A, at al. A randomized controlled trial: branched-chain amino acid levels and glucose metabolism in patients with obesity and sleep apnea. J Sleep Res 2017; 26(6):773-81. [CrossRef] [PubMed]
17. Ioachimescu OC, Anthony J Jr, Constantin T, Ciavatta MM, McCarver K, Sweeney ME. VAMONOS (Veterans Affairs' Metabolism, Obstructed and Non-Obstructed Sleep) Study: Effects of CPAP Therapy on Glucose Metabolism in Patients with Obstructive Sleep Apnea. J Clin Sleep Med 2017; 13(3):455-66. [CrossRef] [PubMed]
18. Martínez-Ceron E, Fernández-Navarro I, Garcia-Rio F. Effects of continuous positive airway pressure treatment on glucose metabolism in patients with obstructive sleep apnea. Sleep Med Rev 2016; 25:121-30. [CrossRef] [PubMed]
19. Guo LX, Zhao X, Pan Q, Sun X, Li H, Wang XX, et al. Glycemic Excursions and Insulin Sensitivity in Patients with Obstructive Sleep Apnea-hypopnea Syndrome and Type 2 Diabetes. Chin Med J 2015; 128(17): 2301-6. [CrossRef] [PubMed]
20. Pamidi S, Wroblewski K, Stepien M, Sharif-Sidi K, Kilkus J, Whitmore H, et al. Eight Hours of Nightly Continuous Positive Airway Pressure Treatment of Obstructive Sleep Apnea Improves Glucose Metabolism in Patients with Prediabetes. A Randomized Controlled Trial. Am J Respir Crit Care Med 2015; 192(1):96-105. [CrossRef] [PubMed]

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PRAĆENJE EFEKTA TROMESEČNE PRIMENE KONTINUIRANOG POZITIVNOG PRITISKA TOKOM SPAVANJA (CPAP) NA VREDNOSTI GLIKOLIZIRANOG HEMOGLOBINA HBA1C I DUGOTRAJNE GLIKOREGULACIJE KOD GOJAZNIH DIJABETIČARA SA DOKAZANOM TEŠKOM OPSTRUKTIVNOM APNEJOM TOKOM SPAVANJA

Nikola Mladenović[1], Zoran Stamenković[2], Lidija Ristić[2,3], Dane Krtinić[4,5], Nemanja Petrović[1], Andrej Preveden[1], Bogdan Okiljević[1,6], Iva Binić[7], Miodrag Đorđević[7,8]

[1]Institut za kardiovaskularne bolesti Vojvodine, Sremska Kamenica, Srbija
[2]Klinika za plućne bolesti, Klinički centar Niš, Srbija
[3]Univerzitet u Nišu, Katedra za internu medicinu, Medicinski fakultet, Niš, Srbija
[4]Klinika za onkologiju, Klinički centar Niš, Srbija
[5]Univerzitet u Nišu, Katedra za farmakologiju sa toksikologijom, Medicinski fakultet, Niš, Srbija
[6]Univerzitet u Novom Sadu, Katedra za hirurgiju, Medicinski fakultet, Novi Sad, Srbija
[7]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[8]Klinika za opštu hirurgiju, Klinički centar Niš, Srbija

Kontakt: Nikola Mladenović
Bul.Nemanjića 59/20, 18000 Niš, Srbija
E-mail: nikolamlad89@gmail.com

Opstruktivna apneja u spavanju(OSA) predstavlja ozbiljan poremećaj disanja tokom sna koji karakteriše potpuni ili delimični prekid disanja u trajanju od 10 sekundi i duže.

U ovoj prospektivnoj kliničkoj longitudinalnoj studiji kod gojaznih pacijenata sa dijabetes melitusom tipa II kojima je polisomnografskim ispitivanjem utvrđen težak stepen OSA, praćen je efekat primene pozitivnog pritiska tokom sna (CPAP) u toku tri meseca na vrednosti glikoliziranog hemoglobina HbA1c kao indikatora efikasne dugotrajne glikoregulacije.

U prospektivnu kliničku studiju sa tromesečnim, praćenjem uključeno je 98 pacijenata (64 muškaraca i 34 žene, prosečne starosti 50,1 godina. /31- 66 godina., i prosečnog BMI 39,9), kojima je nakon kliničke, laboratorijske, spirometrijsko difuzijske obrade plućne funkcije, načinjenih gasnih analiza respiratornih gasova arterijske krvi u miru, ispitivanja Epworthove skale pospanosti, načinjeno poli-somnografsko ispitivanje na Philips Respironics Alice PDx uređaju.

Polisomnografsko ispitivanje sprovedeno je kod 98 konsekutivnih gojaznih pacijenata od kojih je 42 (42,8 %) pacijenta imalo dijabetes II. Od ukupnog broja ispitanika kod 58 (60,2 %) nađena je OSA teškog stepena sa AHI indeksom većim od 30. Od ukupnog broja gojaznih pacijenata sa teškim stepenom OSA njih 37 (63,79 %) imalo je dijabetes melitus tip II sa prosečnom HbA1c 11,7 %, što ukazuje na lošu dugotrajnu glikoregukaciju kod dijabetičara sa teškim stepenom OSA.

Od 98 gojaznih pacijenata, koji su imali dijabetes melitus tip II i OSA teškog stepena, randomizovano je njih 23, od čega je njih 11 sa prosečnom vrednošću HbA1c 9,9 %, prosečnog BMI 37,1 i prosečnog AHI indeksa 36,7 (od 31 do 59), nabavilo aparate za primenu kontinuiranog pozi-tivnog pritiska (CPAP) tokom spavanja, te su nakon tromesečne primene CPAP obavili kontrolu vrednosti glikoliziranog hemoglobina HbA1c kao validnog indikatora dugotrajne glikoregulacije. Kontrolnu grupu ispitanika činilo je njih 12 u eksperimentalnoj grupi, čija je prosečna HbA1c bila 9,1 %, prosečna BMI 39,3 i AHI indeks bio je 39,7 (od 31 do 62), i bez statistički značajne razlike između grupa po stepenu težine OSA i vrednosti HbA1c. Nakon tromesečne primene kontinuiranog pozitivnog pritiska (CPAP) tokom spavanja ispitanici eksperimentalne grupe postigli su statistički značajno ($p < 0,001$) popra-vljanje vrednosti HbA1c sa prosečnih 9,9 % na 6,7 %, što nije utvrđeno kod pacijenata kontrolne grupe koji nisu koristili CPAP tokom sna dijabetesom tipa II i teš-kom OSA, primenom kontinuiranog pozitivnog pritiska tokom spavanja značajno popravlja dugotrajna glikoregulacija iskazana kroz vrednosti HbA1c.

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Ključne reči: opstruktivna sleep apnea, dijabetes melitus tip II, gojaznost, CPAP

Original article

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THE RELATIONSHIP BETWEEN BODY MASS INDEX AND THE QUALITY OF LIFE OF THE URBAN ADULT POPULATION OF THE CITY OF BELGRADE

Veroslava Stanković

Overweight has got a negative influence on the health but data about influence of overweight on the Health-Related Quality of Life (HRQoL) are contradict. The cross-sectional study included 105 men and women from the Health Center Clinicanova in Belgrade. The study was conducted from 1st February to 31st March 2018. Body height and body weight were measured by standard anthropometric methods. Body Mass Index (BMI) was calculated and correlation analysis was done. The questionnaire Obesity Related Well-Being 97 (ORWELL97) was applied. The response rate was 91 % (105 of 115). Men represented 43 % of all persons and women accounted for 57%. The average age was 29.5 ± 3.2. The average BMI was 25.4 ± 4.0 kg/m^2. There was strong positive correlation between BMI = 25-26.9 kg/m^2 and total scores in ORWELL97 questionnaire (r = 0.96), subquestion O (r = 0.98) and subquestion R (r = 0.97). Strong positive correlation was found between ITM = 27-29.9 kg/m^2 and total ORWELL97 score (r = 0.95), as well as with total scores on subquestion O (r = 0.95) and subquestion R (r = 0.98). Lower HRQOL had participants with BMI = 27-29.9 kg/m^2 (t = 6.866; p < 0.001) than participants with BMI = 25-26.9 kg/m^2. The difference between total scores in ORWELL97 questionnaire in participants who had BMI < 25 kg/m^2 and those with BMI > 25 kg/m^2 was not significant (t = 1.143, p > 0.05). The strong positive correlation between BMI higher than 25 kg/m^2 and the total ORWELL97 scores was determined. BMI higher than 25 kg/m^2 strongly correlates with lower quality of life.

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Key words: *Body Mass Index, quality of life, urban population*

Higher Education School of Profesional Health Studies Belgrade, Belgrade, Serbia

Contact: Veroslava Stanković
Pedje Milosavljevića St 10/14, 11077 New Belgrade, Serbia
E-mail: stankovicveroslava@gmail.com

Introduction

According to the World Health Organization (WHO) from 2016 there were 1.6 billions of adults who were overweight and at least 650 million obese persons worldwide (1). The number of adults who are overweight is constantly increasing, and the assessment of WHO is that up to 2020 overweight will be the most common cause of death (2). Majority of adults in the United States of America (USA), Australia and in most countries of West and East Europe are overweight (3, 4).

Nearly one third or 33% of the USA adults are overweight and 36% are obese (5), and in Europe overweight ranges from 32 to 79% in men and in women from 28 to 78%, respectively. Prevalence of obesity varies from 5 up to 23% in men and from 7 to 36% in women (6). According to the data from the National Survey about the health of the population in Serbia in 2013, there were 35.1% of adults who were older than 20 who were overweight and there were 21.2% of adults who were obese (7).

The overall quality of life is the overall quality of life which makes the union of all the factors which influence the life of an individual (8). The quality of life is a harmony inside a person and a harmony between a person and his world (9). Health-Related Quality of Life (HRQOL) makes union only of those factors which are part of someone's health (10). The HRQOL is a subjective assessment of health as well as the capability of a person to lead a life which fulfills him or her (11).

Overweight and obesity present the excess body weight which can damage health (12). Adults with higher than normal body mass index have significantly reduced physical quality of life (13).

The aim of the paper was to determine the correlation between body mass index and the Health-Related Quality of Life of adults.

Material and methods

The cross-sectional study involved 105 men and women from the Health Center Clinicanova in Belgrade. The study was conducted from 1st February to 31st March 2018. Body height and body weight were measured by standard anthropometric methods. Body Mass Index (BMI) was calculated and correlation analysis was done. BMI was calculated according to the formula: Body weight (kg)/Body height (m^2). The questionnaire Obesity Related Well-Being 97 (ORWELL97) was applied.

Table 1. Classification of nutritional status according to body mass index

Underweight	BMI < 18.5 kg/m^2
Normal weight	BMI from 18.5 to 24.9 kg/m^2
Overweight	BMI from 25 to 29.9 kg/m^2
Obesity of the first degree	BMI from 30 to 34.9 kg/m^2
Obesity of the second degree	BMI from 35 to 39.9 kg/m^2
Obesity of the third degree	BMI > 40 kg/m^2

Source: WHO Physical Status;The Use and Interpretation of Anthropometrics. WHO Tehnical Report 1993; Series No854, Geneva

The criteria for inclusion of participants in the study were: the participant older than 18 years of age from the territory of the City of Belgrade, and informed consent to participate in the study given by the participant.

The criteria for exclusion of participants from the study: obesity, pregnancy, the established diagnosis of some chronic disease such as diabetes mellitus, metabolic disorders, hypertension; insufficient cooperation of participants; refusal to give informed consent.

Permission to conduct this study was given by the Head of the Health Center Clinicanova in the City of Belgrade.

Questionnaires

Descriptive data and data on risk factors and comorbidities were collected by epidemiological questionnaire.

The questionnaire Obesity Related Well-Being 97 (ORWELL97) was applied in all examined adults. The questionnaire ORWELL97 consists of 18 questions divided into three groups. There are five questions in the first group and they refer to the presence of physical and mental disorders.

There are seven questions in the second group and they refer to the influence of the overweight and obesity on the emotional status. In the third group there are six questions which refer to the familiy and social relationships. Each question has two subquestions.

The first subquestion refers to the presence of the symptoms of the overweight and obesity and it is marked with Occurrence (O). The second subquestion refers to the personal experiance of the symptoms of overweight and obesity and it is marked with Relevance (R).

A total score of points was obtained by adding points from each given answer. The higher number of points in the questionnaire shows a lower quality of life in the examined participants. The total score on each subquestion was calculated. The respondents filled in the questionnaire by circling one of the given answers. The exact number of points for each question on the Linerts scale was determined (from 0 to 3).

The validity of ORWELL97 ($r = 0.92$, $p < 0.01$) questionnaire was examined in different studies worldwide (14).

Statistical analysis

Correlation analysis was done and Pearson's coefficient of correlation (r) was calculated. The estimation error of $p < 0.05$ was taken into account as the limit of statistical significance. The results are presented in tables and charts.

Results

Of 115 examined adults, 105 of them completed ORWELL97 questionnaire. Six (5.2 %) participants refused to complete the qusetionnaire (one man and four women) and four questionnaires were not completed. Finally, 105 ORWELL97 questionnaires were analyzed. There were 45 men and 60 women (43 %:57 %) (Figure 1).

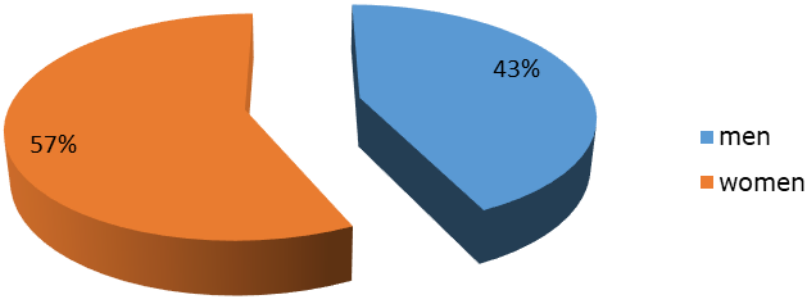
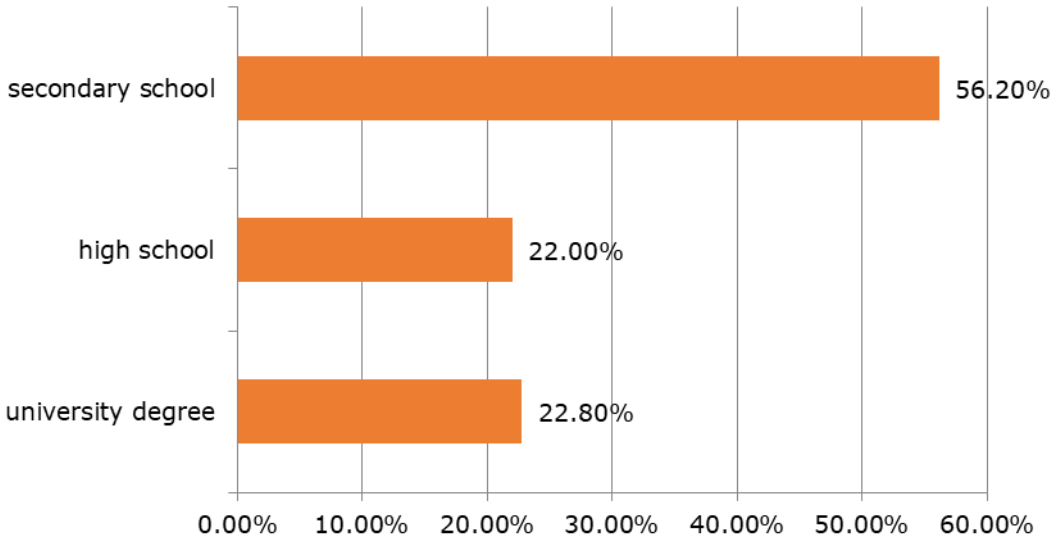
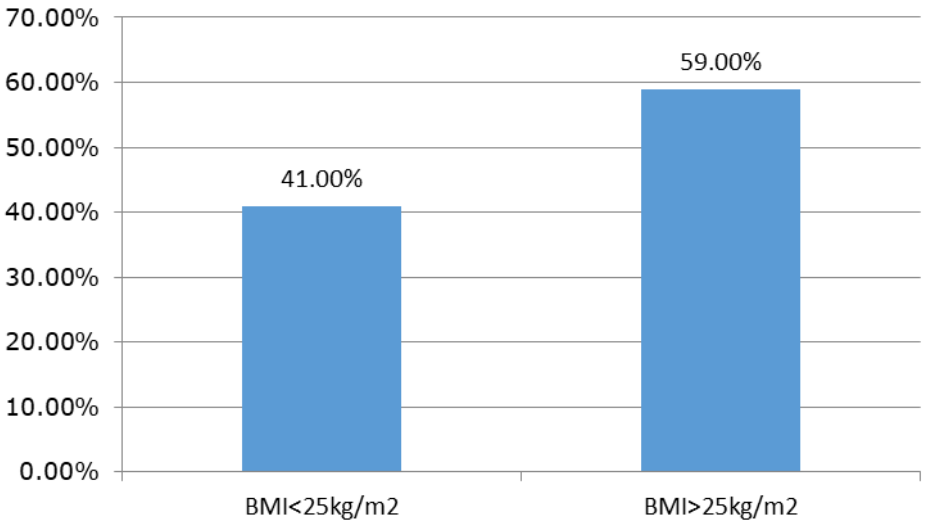


Figure 1. Distribution by gender



Graph 1. Disrtibution by level of education



Graph 2. Distribution by value of BMI

There were 24 (22.80 %) participants with university degree, 22 (21.00 %) with college degree, and 59 (56.20 %) of them who finished secondary school (Graph 1).

Fifty nine percent were from the City of Belgrade and 41% were from the surrounding regions of the city (Graph 2).

The average value of BMI of all examined participants was 25.60 ± 4.0 kg/m$^{2.}$ According to the values of BMI there were 43 (41.00 %) participants with normal weight and 62 (59.00 %) were considered overweight.

The participants who had BMI lower than 25 kg/m^2 had lower total scores relating to all questions in the ORWELL97 questionnaire, as well as to the subquestion O compaired with the participats who had BMI higher than 25 kg/m^2. The differance was not statistically significant ($t = 1.143$, $p > 0.05$).

Participants who had BMI 25-26.9 kg/m^2 had higher scores in ORWELL97 questionnaire than participants who had BMI = 27-29,9 kg/m^2 (Table 2). The difference was statistically significant ($t = 6.866$; $p < 0.001$).

Table 2. Correlation of Body Mass Index and scores in ORWELL 97 questionnaire

ORWELL 97 questionnaire	BMI = 25-26.9 kg/m^2	BMI = 27-29.9 kg/m^2
	Pearson's coefficient of correlation (r)	
All questions	0.98	0.95
Subquestion O	0.96	0.95
Subquestion R	0.97	0.98

Discussion

On the basis of the definition of health provided by WHO in 1946 (1) the interest about investigations of the quality of life of population began to grow. The research on the influence of overweight on the quality of life of adults is one of the frequent topics in the medical literature but findings of the association of overveight and lower HRQOL are still missing (12, 13, 14). The study of Zeller et al. found that depression, social support, the degree of overweight, and socio-economic status were significantly associated with HRQOL (15).

According to the presented results, participants who were overweight had higher scores in the ORWELL97 questionnaire compared with the participants who had a normal weight but the difference was not significant.

Similar to our results, researchers found that higher BMI was associated with physical, mental and emotional disorders (16).

Finkelstein et al. showed that quality of life was rapidly decreasing in persons with BMI higher than 25 kg/m^2 (17). Le Pen et al. did not find the association between the persons with BMI higher than 30 kg/m^{2}and the quality of life (18). Persons with BMI < 27 kg/m^2 and < 30 kg/m^2, had lower HRQOL than persons with normal weight (19). Recent investigations showed that overweight had no impact on the HRQOL (13, 20).

Obesity is a major public health problem and most researches investigate the HRQOL of persons with BMI > 30 kg/m^2 (11, 21). It has not been elucidated yet whether overweight according to actual BMI clasiffication (from25 to 29,9 kg/m^2) affects the HRQOL (20-23).

Some authors show that in their investigations there were no statistically significant difference between the HRQOL among persons who had BMI lower than 25 kg/m^2 and those whose BMI was equal or higher than 25 kg/m^2 (13). Overweight persons with BMI < 27 kg/m^2 and BMI < 30 kg/m^2 didn't have significantly lower HRQOL compaired with persons who had normal weight (22).

BMI is considered as a good indicator of nutritional status in the persons aged 20 to 65, especially when health risk is connected with the BMI (24, 25). Correlation between BMI and the percentage of the body fat in a body is on a degree that it allows an assessment of 70 to 80 % of the variation of fat in the Caucasian population (26).

The research conducted in America, China, Taiwan and Korea showed that obesity impaired physical health rather than mental health in urban population (27). Overweight persons in urban population had a higher risk of diabetes mellitus, high blood pressure, ischaemic heart disease and according to some epidemiological studies they had a higher risk of premature death (28, 29).

In urban population activities that formerly required high energy expenditure have been replaced by sedentary activities and eating habits that favor obesity due to the dissemination of refined and processed foods, rich in fat and simple sugars and served in ever-growing portions (30).

The strategies that improve HRQOL in an urban population with high BMI include changing lifestyle habits including working on the mental health of the population (1, 31).

Conclusion

The participants who were overweight had higher total scores in the ORWELL97 questionnaire compaired with those who had normal weight but the difference which was found was not statistically significat. The strong positive correlation between BMI higher than 25 kg/m^2 and the total ORWELL97 scores was determined. BMI higher than 25 kg/m^2 strongly correlates with lower quality of life.

Further research should be directed towards testing the quality of life after an interventional study in the overweight and obese population.

References

1. Available from: URL: http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight (access April 2017).
2. Gregg EW, Shaw JE. Global health effect of overweight and obesity. N Engl J Med 2017; 377:80-1. [CrossRef] [PubMed]
3. Grujic V, Draganic N, Jovanovic Mijatovic V, Ukropina S, Harhaji S, Radic I, et al. Predictors of overweight and obesity among adults aged 50 yers and above: Serbian national health survey. Vojnosanit Pregl 2017; 74(1):38-45. [CrossRef] [PubMed]
4. Flegal MK, Caroll DM, Kit KB, Ogden LC. Prevalence of obesity and trends in distribution of body mass index among US adults, 1999-2010. JAMA 2012; 307(5): 491-7. [CrossRef] [PubMed]
5. Rothberg EA, McEwen NL, Kraftson TA, Neshewat MG, Fowler EC, Burant FC, et at. The Impact of Weight Loss on Health-Related Quality-of-Life: Implications for Cost-effectiveness Analyses. Qual Life Res 2014; 23(4):1371-6. [CrossRef] [PubMed]
6. World Health Organization. Methodology and summary. Country profiles, physical activity and obesity in the 53 WHO European Region Member States, 2013. (access April 2017) Google Scholar
7. Ministry of Health od the Republic of Serbia and Institut Of Public Health of Serbia » Dr Milan Jovanović-Batut«. National Health Survy 2013-Key findings, 2014.
8. Wood-Dauphinee S. Assessing quality of life in clinical research: from where have we come and where are we going? J Clin Epidemiol 1999; 52:355-63. [CrossRef] [PubMed]
9. Elkington JP. Medicine and quality of life. Ann Intern Med 1966; 64:711-4. [CrossRef] [PubMed]
10. Testa MA, Simpson DC. Assessing quality-of-life outcomes. N Engl J Med 1996; 334:834-45. [CrossRef] [PubMed]
11. Schalock RL. Quality of life: what we know and do not know. J Intellect Disabil Res 2004; 48:203-16. [CrossRef] [PubMed]
12. Kolotkin LR, Andersen RJ. A systematic review of reviews: exploring the relationship between obesity, weight losse and health-related quality of life. Clinical Obesity 2017; 7:273-89. [CrossRef] [PubMed]
13. Ul-Haq Z, Mackay DF, Fenwick E, Pell JP. Meta-analysis of the association between body mass index and health-relatedquality of life among adults, assessed by the SF-36. Obesity (SilverSpring) 2013; 21:E322-7. [CrossRef] [PubMed]
14. Kroes M, Osei-Assibey G, Baker-Searle R, Huang J. Impact of weight change on quality of life in adults with Overwight/obesity in US: A systematic review. Curr Med Res Opin 2016; 32:485-508. [CrossRef] [PubMed]
15. Zeller HM, Modi CA. Predictors of Health-Related Quality of Life in Obese Youth. Obesity 2006; 14(1):122-30. [CrossRef] [PubMed]
16. Wang J. Factors Associated with Health-Related Quality of Life among Overweight or Obese Adult. J Clin Nurs 2013; 22(15-16):2172-82. [CrossRef] [PubMed]
17. Finkelstain MM. Body mass index and quality of life in as survey of primary care patients. J Fam Pract 2000; 49:734-7. [PubMed]
18. Le Pen C, Leavy E, Loos F, Banzet MN, Basdevant A. "Specific" scale compared with "generic" scale: a double measurement of the quality of life in a French community sample of obese subjects. J Epidemiol Community Health 1998; 52:445-50. [CrossRef] [PubMed]
19. Ghosh RK, Ghosh SM, Ganguly G. Health-related quality of life and its growing importance in clinical practice. N Z Med J 2010; 1313(123):99-101.
20. Panico A, Messina G, Lupoli AG, Lupoli R, Cacciapuoti M, Moscatelli F. Quality of life in overweight (obese)

and normal-weight women with polycystic ovary syndrome. Patient Prefer Adherence 2017; 11: 423-9. [CrossRef] [PubMed]
21. Keating CL, Peeters A, Swinburn BA, Magliano DJ, Moodie ML. Utility-based quality of life associated with overweight and obesity: the Australian diabetes, obesity, and lifestyle study. Obesity (Silver Spring) 2013; 21(3):652-5. [CrossRef] [PubMed]
22. McLaughlin L, Hinyard LJ. The relationship between health-related quality of life and body mass index. West J Nurs Res 2014; 36:989-1001. [CrossRef] [PubMed]
23. Dixon JB. The effect of obesity on health outcomes. Mol Cell Endocrinol 2010; 316:104–8. [CrossRef] [PubMed]
24. Vucenik I, Stains JP. Obesity and cancer risk: evidence, mechanisms, and recommendations. Ann N Y Acad Sci 2012; 1271:37-43. [CrossRef] [PubMed]
25. Zhang X, Brown CJ, Schmitz HK. Association between Body Mass Index and Physical Function among Endometrial Cancer Survivors. PLoS ONE 2016; 11(8): e0160954. [CrossRef] [PubMed]
26. Itani L, Calugi S, Grave DR, Kreidieh D, El Kassas G, El Masri D, et al. The Association between Body Mass Index and Health-Related Quality of Life in Treatment-Seeking Arab Adults with Obesity. Med Sci 2018; 6: 25. [CrossRef] [PubMed]
27. Wang R, Wu MJ, Ma XQ, Zhao YF, Yan XY, Gao QB, et al. Body mass index and health-related quality of life in adults: a population based study in five cities of China. Eur J Pub Health 2012; 22(4):497-502. [CrossRef] [PubMed]
28. Ojeda E, Lopez S, Rodriguez P, Moran L, Rodriguez JM, Delucas P. Prevalence of sleep apnea syndrome in morbidly obese patients. Chest 2014; 145 (3 Suppl.): 601A. [CrossRef]
29. Gilad TG, Afek A, Tzur DDE, Cukierman-Yaffe T, Hertzel C, Gerstein CH, et al. Diabetes Risk Among Overweight and Obese Metabolically Healthy Young Adults. Diabetes Care 2014; 37(11):2989-95. [CrossRef] [PubMed]
30. NCD-RisC. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet 2017; 390: 2627-42. [CrossRef]
31. USPSTF. Behavioral Counseling to Promote a Healthful Diet and Physical Activity for Cardiovascular Disease Prevention in Adults Without Cardiovascular Risk Factors. JAMA 2017; 318(2):167-74. [CrossRef] [PubMed]

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POVEZANOST INDEKSA TELESNE MASE I KVALITETA ŽIVOTA ODRASLOG STANOVNIŠTVA IZ URBANOG DELA GRADA BEOGRADA

Veroslava Stanković

Visoka zdravstvena škola strukovnih studija u Beogradu, Srbija

Kontakt: Veroslava Stanković
Peđe Milosavljevića 10/14, 11077 Novi Beograd, Srbija
E-mail: stankovicveroslava@gmail.com

Nema jedinstvenih stavova o negativnom uticaju povećane telesne mase na kvalitet života odraslih. Rad je imao za cilj da utvrdi korelaciju između indeksa telesne mase (ITM) i kvaliteta života odraslih. Studija preseka sprovedena je u Domu zdravlja Clinicanova u Beogradu u trajanju od 1. februara do 31. marta 2018. i obuhvatila je 105 ispitanika. Primenjena su antropometrijska merenja za određivanje telesne mase i visine, i izračunavan je ITM. Primenjena je korelaciona analiza. Kao instrument za istraživanje kvaliteta života primenjen je specifični upitnik Obesity Related-Well Being 97 (ORWELL 97). Od 115 distribuiranih upitnika, analizirano je samo 105 kompletno popunjenih. Muškog pola bilo je 45 (43 %) ispitanika, a 60 (57 %) ženskog pola. Prosečna starost ispitanika bila je 29,5 ± 3,2 godine, a prosečna vrednost ITM iznosila je 25,4 ± 4,0 kg/m^2. 54 (51,4 %) ispitanika bilo je normalno uhranjenio, a 51 ispitanik (48,6 %) imao je povećanu telesnu masu. Utvrđena je jaka pozitivna korelacija između ITM = 25-26,9 kg/m^2 i ukupnog skora u ORWELL97 (r = 0,96), ukupnog skora na potpitanje O (r = 0,98) i potpitanje R (r = 0,97). Postoji jaka pozitivna korelacija između ITM = 27-29,9 kg/m^2 i ukupnog skora u ORWELL97 upitniku (r = 0,95), kao i ukupnog skora na potpitanje O (r = 0,95) i potpitanje R (r = 0,98). Statistički značajno niži kvalitet života imali su ispitanici sa ITM = 27-29,9 kg/m^2 (t = 6,866; p < 0,001) od ispitanika sa ITM = 25-26,9 kg/m^2. Ispitanici sa ITM > 25 kg/m^2 nisu imali statistički značajno veći skor u ORWELL97 upitniku od ispitanika sa ITM < 25 kg/m^2 (t = 1,143; p > 0,05). Postoji izrazito jaka pozitivna korelacija između indeksa telesne mase većeg od 25 kg/m^2 i ukupnog skora u ORWELL97 upitniku, što ukazuje na niži kvalitet života ispitanika.

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Ključne reči: *indeks telesne mase, kvalitet života, urbana populacija*

Original article

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DIFFERENCE IN BODY MASS INDEX AND DIETARY HABITS OF STUDENTS OF FACULTY OF SPORT AND PHYSICAL EDUCATION

Tatjana Popović-Ilić, Veroljub Stanković, Igor Ilić, Saša Hadži Ilić

The modern way of life and technology development is often associated with negative factors that result in reduced physical activity, improper diet, and obesity. This is especially true for students who are exposed to a sedentary lifestyle, which in the sensitive period of adolescence has a significant impact on health. The goal of this research is to determine the difference between body mass index and dietary habits of students of the Faculty of Sport and Physical Education. The sample of examinees consisted of 41 students of both sexes who attend the third and fourth year of studies at the Faculty of Sport and Physical Education in Leposavić. Body mass index was determined by using the device InBody™ 720, while the questionnaire was used for the assessment of eating habits. The data were processed with the SPSS statistical package 20 by applying the t-test for independent samples and χ^2 test. No statistically significant difference ($p < 0.05$) of body mass index nor dietary habits between the groups of students were determined. Also, a positive trend of eating habits was noticed, where the presence of fruits and vegetables in the diet, as well as a regular meal intake, was observed in both groups of students. The presented results indicate that the third and fourth -year students at the Faculty of Sport and Physical Education have good eating habits, the influence of which is reflected in the body mass index, which can be interpreted as the result of their sport and academic education and activities.

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Key words: *body mass index, eating habits, students*

University of Priština, Faculty of Sport and Physical Education, Leposavić, Serbia

Contact: Tatjana Popović-Ilić
Kej kola srpskih sestara 13/80, 18000 Niš, Serbia
E-mail: tatjana.popovic.ilic@pr.ac.rs

Introduction

Obesity and physical inactivity are considered the most important risk factors for chronic non-infectious diseases, which are the leading public health problems in the world and in our country (1). According to National data related to the obesity epidemic in Serbia, 40 % of people have normal body weight, 3.2 % are undernourished, 35 % are in the pre-obese state, and 21 % are obese (2). One of the leading factors with a negative influence on the human organism is hypokinesis, with daily exposure to the effects of numerous forms of stress. Nutrition of children and adolescents is one of the important indicators of youth health, their psycho-physical abilities and potential for normal and healthy growth and development. The research on the nutritional habits of young people in Serbia indicates a widespread distribution of irregular patterns related to nutrition, such as skipping snacks, insufficient consumption of milk, fruit, and vegetables, consuming white flour bread, insufficient fish consumption and other (3).

When considering students of the Faculty of Sport and Physical Education, having in mind their program of study, we eliminate insufficient movement as a cause of possible obesity. Bad eating habits remain as a risk factor, which is, in the young population, largely based on fast food and use of energy and fizzy drinks. Having in mind that they're future pedagogues of physical culture, we consider their state of nourishment, as well as their knowledge and respect for principles of healthy eating a very influential factor to the state of broad layers of the population to work with.

According to the attitude of the World Health Organization, a body mass index (BMI) can be used (4) in order to estimate the prevalence of obesity. Numerous studies indicate the correlation between BMI and pathological changes on blood vessels, cholesterol levels, arterial pressure increase, physical inactivity (5, 6).

Incorrect diet, along with hypokinesis, is an equally important causative factor of spreading obesity epidemic. Proper planning, good habits, choos-

ing quality food are inevitabilities of proper growth and development of children and youth, as well as adults' health protection. The modern way of life, along with the fast tempo, brought new demands for fast, irregular, poor quality food containing saturated fats, refined sugars, emulsifiers, salts. Thus, it is necessary, even within the family environment, and then during schooling and personal engagement, to influence the gaining of proper information and good eating habits.

Aim of paper

The goal of research is to determine differences between BMI and diet habits of students of the Faculty of Sport and Physical Education.

Material and methods

The sample size consisted of 41 students of both sexes. Students of the third and fourth year of basic academic studies at the Faculty of Sport and Physical Education in Leposavić were examined. They were divided into two groups, according to the currently attended year of studies. The condition was regular attendance of both theory and practice classes. The sample of variables for obesity condition assessment consisted of body height, body mass and body mass index (BMI). The categories of body mass index limit values of the sample size were determined in accordance with the World Health Organization criteria. Morphological variables were measured by using InBody™ 720 device in accordance with standards of the International Biological Program (IBP).

For eating habits assessment a questionnaire was used applied in a few studies dealing with similar issues (7-9) and it consisted of 12 closedended questions. The first six questions related to the number of main meals during the working week and weekends, while questions 7-12 determined the frequency of intake of fruits, vegetables, sweet drinks, and alcohol during the working week.

The data were processed using a statistical package SPSS 20 (SPSS Inc., Chicago, IL.), in which t-test for independent samples and χ^2 test with significance level of $p < 0.05$ in both tests were applied

Results

The results of the basic morphological characteristics of the sample size show that there is no statistically significant difference between students of the third and the fourth year (Table 1). This is in accordance with the previous researches, as well as with the assumed theoretical models since it is a group being selected by the same standards while enrolling at the studies. It is also noticed that the value of the height variable is very close to the one statistically significant ($p = 0.55$).

Table 1. Differences of students' morphological characteristics

Variable	Min	Max	AS	SD	p
Height	158.9	205.6	179.5	10.4	.055
Weight	51.2	97.0	75.9	10.8	.676
BMI	19	29.0	23.5	2.3	.073

Legend: Min - minimum, Max - maximum, AS - Mean, SD - standard deviation, p – statistical significance

When eating habits in the questionnaire are concerned it was shown that most students have healthy eating habits. More than 60 % of students regularly consume all three meals, both during working week and weekends. A great number of students consumes fruits (53.7 %) and vegetables (41.5 %) twice to four times a week, but also consume sweets (39 %). The reason for the increased consumption of sweets is the increased need for energy, caused by activities that students are exposed to during studies and sports activities. It is also encouraging that sweet drinks are consumed by a large number of students only once a week (34.1 %), while most of them (68.3 %) consume alcohol once or less than once a week. The cause of proper habits can be found in the fact that the respondents had regular subjects during studies concerning the issue of adequate nutrition. On the basis of this, it can be concluded that the proper education of young people about healthy eating habits is very important and contributes to manifesting changes in the quality of their lives. Supporting this fact, the determined BMI results show that as many as 68.3 % of respondents are in the optimal range of values for this population.

Examining differences in eating habits by using the χ^2 test (Table 2) indicates that there are no statistically significant differences in eating habits between groups of samples. The conclusion is that the third and fourth - year students at the Faculty of Sport and Physical Education have stable health habits when it comes to nutrition, which is a product of knowledge gained during academic studies and sports careers.

Table 2. Differences in eating habits of students

Question 1	Value	p	Question 2	Value	p	Question 3	Value	p
χ^2	4.92	.179	χ^2	.81	.369	χ^2	.40	.941
Kendall tau	.074	.624	Kendall tau	-.140	.390	Kendall tau	-.094	.539
Question 4	Value	p	Question 5	Value	P	Question 6	Value	P
χ^2	.13	.715	χ^2	2.50	.645	χ^2	1.72	.633
Kendall tau	-.057	.722	Kendall tau	-.076	.611	Kendall tau	.094	.537
Question 7	Value	p	Question 8	Value	P	Question 9	Value	P
χ^2	3.22	.666	χ^2	3.29	.771	χ^2	5.64	.465
Kendall tau	-.144	.313	Kendall tau	.006	.967	Kendall tau	.054	.703
Question 10	Value	P	Question 11	Value	p	Question 12	Value	P
χ^2	1.49	.960	χ^2	5.15	.398	χ^2	.50	.778
Kendall tau	-.002	.989	Kendall tau	.228	.105	Kendall tau	.077	.605

Legend: p - statistical significance, χ^2 - Chi square test

Discussion

Physical activity has positive effects on numerous changes in the human organism. The essence of these changes is reflected in the increase of functional, motoric abilities of organic systems, as well as the resistance of the human organism, and at the basis of functional abilities improvement, there are the structural changes of organs and systems (10).

The biggest problem with obesity is registered in the US territory with even 25 % overweight female student population (11). A worrying rise in obesity is also registered in research of Australian National Center for Epidemiology and Human Health among students of the University of Bangkok, with focus on factors such as unhealthy diet and physical inactivity. Test results in European countries also show an in-crease in the prevalence of obesity among students (12).

Student population entering the Faculty of Sport and Physical Education is heterogeneous, and their growth and development are influenced by numerous endogenous and exogenous factors (13). Physical exercise as an exogenous factor plays an important role in forming body matter and obesity prevention. The balance of BMI with the third and fourth - year students determined by this study is considered to be the result of uniform physical exercises, which lead to gradually less manifestation of differences among students. Similar results were gained in an analysis of anthropological characteristics of the third year students of the Faculty of Sport and Physical Education in Novi Sad. The results showed a favourable anthropometric profile in the majority of students (14).

In the same way, as the overweight has a great negative impact on all aspects of life and vital functions, the ideal body weight makes it possible to develop quality morphological and functional parameters. Normal distribution of BMI values with the third and fourth - year students of the Faculty of Sport and Physical Education represents a positive interaction with their mental, motor and functional abilities. A similar conclusion about better results of respiratory, motor and dynamic functions of students with BMI in normal frames was deducted by the researchers at the Beijing University of Sport (15).

Along with regular physical activity as the first precondition, proper nutrition with adequate participation of macro and micronutrients is another prerequisite for proper growth and development, preservation of health and prevention of cardiovascular, endocrine, digestive, rheumatic, carcinogenic diseases. Modern lifestyle contributes to quantitative and qualitative eating disorders. Fast food, especially popular among young people as a tasty, quick, simple diet, is considered as one of the main culprits for spreading epidemic of obesity.

The period of studying inevitably brings changes in nutrition due to separation from parent's home, changes in dynamics of taking meals, altered material status. Proper and timely information is important part of forming healthy habits. It is obtained first in the family, then during schooling, as well as on internet portals. Researches on the level of knowledge about principles of proper nutrition showed satisfactory results among students of the Faculty of Sport in Leposavić. They provide information on proper nutrition through the programme of studies in the first and second year, so there is no statistically significant difference considering adopted habits between students of the third and fourth year. Similarly, positive results on the level of knowledge about principles of proper nutrition were gained in researches among students of the Faculties of Sport in Italy (66.7 %) and those in Serbia (63 %) (16).

Regular meals consumption is an inevitable condition for proper development and functioning of children and young people. The survey shows that 60 % of students at the Faculty of Sports regularly consume at least three meals a day; a large part of them consumes fruits and vegetables at least twice to four times a week. Also, majority of student population in Serbia and the world show good eating habits considering number of meals, which is not the case when it comes to consuming fruits and vegetables (17-19).

Consumption of alcohol is also a factor that affects physical, mental and social health, and contributes to increased calories intake with the ten-

dency of BMI increase. 68.3 % of students of the Faculty of Sport in Leposavić consume alcohol at most once a week, which is in positive correlation with normal BMI. In contrast to these results, researches at US colleges show a worrying presence of alcohol consumption among students, and even among active athletes in colleges, with the tendency of increased consumption during competition period (20). A similar situation is found in European area. During researches at the Faculty of Sport in Timisoara, positive results of alcohol screenings were found mostly in the first - year students (16.39 %), and the majority of them (11.48 %) were male (21).

Conclusion

The results show that there is no statistically significant difference between the third and fourth-year students at the Faculty of Sport and Physical Education, at the level of examined morphological characteristics and eating habits. It was also found out that respondents developed good eating habits, which is interpreted as a result of their sports and academic education and activities.

References

1. Radić I. Gojaznost i fizička neaktivnost kao javnozdravstveni problemi odraslog stanovništva Vojvodine. Novi Sad: Faculty of Medicine; 2016.
2. Pekmezović T. Nacionalni podaci vezani za epidemiju gojaznosti u Srbiji: Projektni ciklus konferencija "Problemi javnog zdravlja u Srbiji": Epidemija gojaznosti i Srbija, 1. sesija; 27. oktobar 2016.
3. Vlaški J, Katanić D. Zdravstveni i socijalni značaj epidemije gojaznosti kod adolescenata u Srbiji. Medicinski glasnik Specijalne bolnice za bolesti štitaste žlezde i bolesti metabolizma "Zlatibor" 2010; 15(34):43-6.
4. World Health Organization. Obesity: preventing and maniging the global epidemic. WHO Tech Rep Ser 2000; 894:1-253.
5. Sporišević L, Skelin A, Musić M, Šečić D. Prehrambene navike i čimbenici rizika za kardiovaskularne bolesti u djece. Paediatr Croat 2014; 58(1):253-8.
6. McGill HC Jr, McMahan CA, Gidding SS. Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. Circulation 2008; 117(9):1216-27. [CrossRef] [PubMed]
7. Rožek-Mitrović T, Petrović V. Body mass index and eating habits of secondary schoolchildren in Inđija. Timočki medicinski glasnik 2015; 40(4):231-6. [CrossRef]
8. Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Division of Adolescent and School Health. The National Youth Physical Activity and Nutrition Study (NYPANS). "cited 2016 Dec 25". Available from: URL: https://www.cdph.ca.gov/programs/cpns/Documents/Compendium%20of%20Surveys.pdf
9. School Nutrition and Physical Activity Advisory, Student Nutrition and Physical Activity Questionnaire. "cited 2016 Dec 26". Available from: URL: http://www.surveymonkey.com/s/fspsstudent
10. Đurašković R. Sportska medicina. Niš: M KOPS Centar; 2009.
11. Centers for Disease Control and Prevetnion. Youth Risk Behavior Surveillance: National College Health Risk Behavior Survey - United States, 1995. MMWR Surveillance Summary 1997; 46(6):1-56.
12. Srdić B, Dimitrić G, Obradović B. Anthropological characteristics of students of Faculty of sports and physical education. Glasnik Antropološkog društva Srbije 2009; 44:463-70.
13. Pavlović R, Simeonov A, Radić Z, Raković A, Idrizović K. Trend of the change of the anthropometric characteristics of students of physical education and sport in the period from 2008. to 2012. Research in Physical Education, Sport & Health 2014; 3(2):83-91.
14. Budakov N, Bokan D, Rakić D, Bokan D. Body mass index and physical activity of students of University of Novi Sad. SEEHSJ 2012; 2(1):8-14.
15. Wu XY, Fu XC. The Research of the Relationship Between the Body Mass Index and the Physical Health Variables of the College Students. Journal of Beijing Sport University 2006; 8:1087-93.
16. Trabucco G, Nikolić M, Mirković BV. Nutritional Knowledge and Behavior Among Students Practicing Sports: Comparison Between Two Countries. Acta Facultatis Medicae Naissensis 2013; 30(4):201-8. [CrossRef]
17. Dimoski Z, Majstorović B, Kocić S, Radovanović S. Nutritive habits of students. Materia medica 2011; 27 (2):310-7. [CrossRef]
18. Yahia N, Achkar A, Abdallah A, Rizk S. Eating habits and obesity among Lebanese university students. Nutrition Journal 2008; 7(1):32. [CrossRef] [PubMed]
19. Al-Rethaiaa AS, Fahmy AEA, Al-Shwaiyat NM. Obesity and eating habits among college students in Saudi Arabia: a cross sectional study. Nutrition Journal 2010; 9(1):39. [CrossRef] [PubMed]
20. Selby R, Weinstein HM, Bird TS. The health of university athletes: Attitudes, behaviors, and stressors. Journal of American College Health 1990; 39(1):11-8. [CrossRef] [PubMed]
21. Gligor Š, Koroiu F, Petrakovšči S, Negrea K, Amanar-Tabara S, Voiku S. Epidemiological aspects of tobacco, alcohol and Internet use addictions among Physical Education and Sport Faculty students from Timişoara, Romania. Fizička kultura 2011; 65(2):42-50. [CrossRef]

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RAZLIKE U INDEKSU TELESNE MASE I NAVIKAMA U ISHRANI STUDENATA FAKULTETA ZA SPORT I FIZIČKO VASPITANJE

Tatjana Popović-Ilić, Veroljub Stanković, Igor Ilić, Saša Hadži Ilić

Univerzitet u Prištini, Fakultet za sport i fizičko vaspitanje, Leposavić, Srbija

Kontakt: Tatjana Popović-Ilić
Kej kola srpskih sestara 13/80, 18000 Niš, Srbija
E-mail: tatjana.popovic.ilic@pr.ac.rs

Savremeni način života i razvoj tehnologije često je povezan sa negativnim faktorima koji za posledicu imaju smanjenu fizičku aktivnost, nepravilnu ishranu i gojaznost. Ovo je posebno izraženo kod studenata koji su izloženi sedentarnom načinu života, što se u osetljivom periodu adolescencije značajno odražava na zdravlje. Cilj rada je utvrđivanje razlika indeksa telesne mase i navika u ishrani studenata Fakulteta za sport i fizičko vaspitanje. Uzorak ispitanika sastojao se od 41 studenta oba pola treće i četvrte godine studija na Fakultetu za sport i fizičko vaspitanje u Leposaviću. Indeks telesne mase utvrđen je upotrebom uređaja InBody™ 720, dok je za procenu navika u ishrani korišćen anketni upitnik. Podaci su obrađeni u okviru statističkog paketa SPSS 20 primenom t-testa za nezavisne uzorke i χ^2 testa. Utvrđeno je da ne postoje statistički značajne razlike ($p < 0,05$) između grupa studenata na nivou indeksa telesne težine, kao i u navikama u ishrani. Takođe, primećen je pozitivan trend u navikama u ishrani, gde je zastupljenost voća i povrća u ishrani, kao i redovnost obroka registrovana kod obe grupe studenata. Prikazani rezultati ukazuju na činjenicu da studenti treće i četvrte godine studija Fakulteta za sport i fizičko vaspitanje imaju dobre navike u ishrani, čiji se uticaj reflektuje i na indeks telesne mase, što se može tumačiti rezultatom njihove sportske i akademske edukacije i aktivnosti.

Acta Medica Medianae 2019;58(2):51-55.

Ključne reči: *indeks telesne mase, navike u ishrani, studenti*

Case report

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WHEN ELECTRIC SHOCK CAN MEAN LIFE - IMPLANTABLE CARDIOVERTER DEFIBRILLATOR AND ITS EFFECT: A CASE REPORT

Tomislav Kostić[1,4], Zoran Perišić[1], Dragana Stanojević[1], Goran Koraćević[1,2], Vladimir Mitov[2], Mladjan Golubović[3], Predrag Cvetković[1], Mirko Krstić[1], Aleksandar Stojković[1], Sonja Šalinger[1], Stefan Momčilović[4], Sanja Banković[4]

Sudden cardiac death (SCD) caused by ventricular fibrillation (VF) remains a major cause of mortality in the Western world. The majority of VF and cardiac arrest occur outside the hospital. Implantation of an ICD is strongly supported by evidence from many randomized trials for the primary and secondary prevention of sudden cardiac death. Current ICDs have multiple features to enhance diagnostics, minimize unnecessary pacing, conserve energy use, and deliver pain-free therapy such as anti-tachycardia pacing (ATP). The most important of above-mentioned features remains the ability of pacemaker to recognize a life-threatening ventricular arrhythmia and terminate it with shock. The benefit and significance of an ICD shock are dependent on the type of heart disease, and the presence of structural heart disease. These concepts are illustrated in a brief overview of ICD trials in patients with ischemic and non-ischemic heart disease. We present a patient with dilated cardiomyopathy and a reduced left ventricular ejection fraction (LVEF) who had an ICD implanted 1 year ago, as a primary prevention of SCD. The patient was admitted to the Clinic for cardiovascular diseases after a brief loss of consciousness. The electrical control of the device showed that a VF suddenly occurred and was successfully interrupted by a shock as a type of ICD-based therapy.

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Key words: *implantable cardioverter defibrillator, shock, sudden cardiac death*

[1]Clinic for Cardiovascular diseases, Clinical Centre Niš, Serbia
[2]Health Centre Zaječar, Zaječar, Serbia
[3]Clinic for cardiothoracic and transplantation surgery, Clinical Centre Niš, Niš, Serbia
[4]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Tomislav Kostić
Blvd. dr Zoran Djindjić 48, 18000 Niš, Serbia
E-mail: tomislav.kostic1977@gmail.com

Introduction

Sudden cardiac death (SCD) due to ventricular fibrillation (VF) remains a major cause of mortality in the Western world. The majority of VF and cardiac arrest occur outside of the hospital. Implantation of an ICD is strongly supported by the evidence from many randomized trials for the primary and secondary prevention of SCD. In these high - risk patients from industrialized countries, coronary artery disease is the most common cause of left ventricular dysfunction and heart failure (1, 2).

Ventricular fibrillation occurs when an electrical wave-break induces re-entry which results in the propagation of new wave-breaks. Tissue heterogeneity in patients with underlying cardiomyopathy results in a predisposition to wave-break, reentry and ultimately to VF. These wandering wavelets of VF are generally self-sustaining once they are initiated (3). There have been significant advances in the technology required for defibrillation of VF. However, less is known about the basic underlying mechanism of defibrillation. Contemporary theories about the mechanisms of electrical shock that terminates VF are similar. These theories are broadly defined as "critical mass", "the upper limit of vulnerability", "progressive depolarization", and "virtual electrode depolarization". The theories underscore the two most import ant components of an ICD shock. First, the shock must successfully terminate the arrhythmias. Second, the ICD shock must not restart the arrhythmia in the mentioned process.

Current ICDs have multiple features to enhance diagnostics, minimize unnecessary pacing, conserve energy use, and deliver pain-free therapy such as anti-tachycardia pacing (ATP). Of all of these features, the most important remains the ability to recognize a life-threatening ventricular arrhythmia

and terminate it with a shock. The benefit and significance of an ICD shock are dependent on the type of heart disease, and on the presence of structural heart disease. These concepts are illustrated in a brief overview of ICD trials in patients with ischemic and nonischemic heart disease (4).

Case report

A 62 year- old patient was admitted to the Clinic for Cardiovascular Diseases, Clinical Centre Niš, due to a short-term loss of consciousness. The patient had an ICD implanted 1 year ago due to dilated cardiomyopathy with LVEF 25 %. During the PM implantation procedure, coronarography was also performed. The patient had normal coronary arteries, and therefore, ischemic heart disease was excluded as a cause of heart failure. On the 24-hour ECG Holter prior to pacemaker implantation, malignant arrhythmias were verified: frequent ventricular extrasystoles, couplets, and non sustained VT. Laboratory analyses did not indicate a myocardial inflammatory process. According to these findings, as part of the primary prevention of sudden cardiac death, the implantation of ICD pacemaker was performed. The patient came now due to the sudden loss of consciousness and electric shock caused by the current generated by the ICD. At the admission patient was conscious, oriented, blood pressure 110/70 mmHg, on the ECG sinus rhythm was recorded, and heart rate 78 beats per minute, without rhythm and conduction disturbances, without changes in the ST segment. Echocardiography finding showed a globally reduced contractile function, LVEF 25 %, without regional wall motion abnormalities. An emergency electrical control of the implanted ICD was performed. It showed that the detection and therapy of VF were successfully performed. Registered VF was 37 seconds in duration, heart rate was 227 bpm, and it was successfully resolved by the first delivered electrical shock with 34J (Figure 1 and 2). Cardiospecific enzymes were at the upper limit - 0.040 ng/mL. This was caused by the minimal myocardial necrosis due to electrical shock. After successful electrical control of the device (ICD control), we changed drug therapy. We introduced Amiodarone as an antiarrhythmic, as part of secondary prevention now, and the patient was discharged from our clinic.

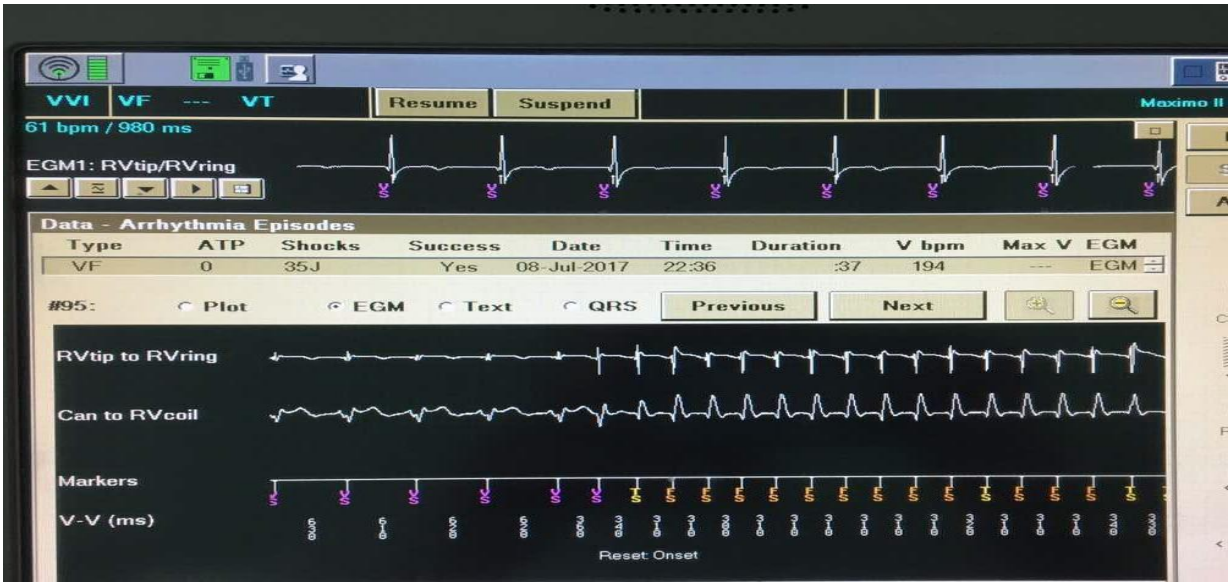

Figure 1. Start of VF recorded on intracardial ECG

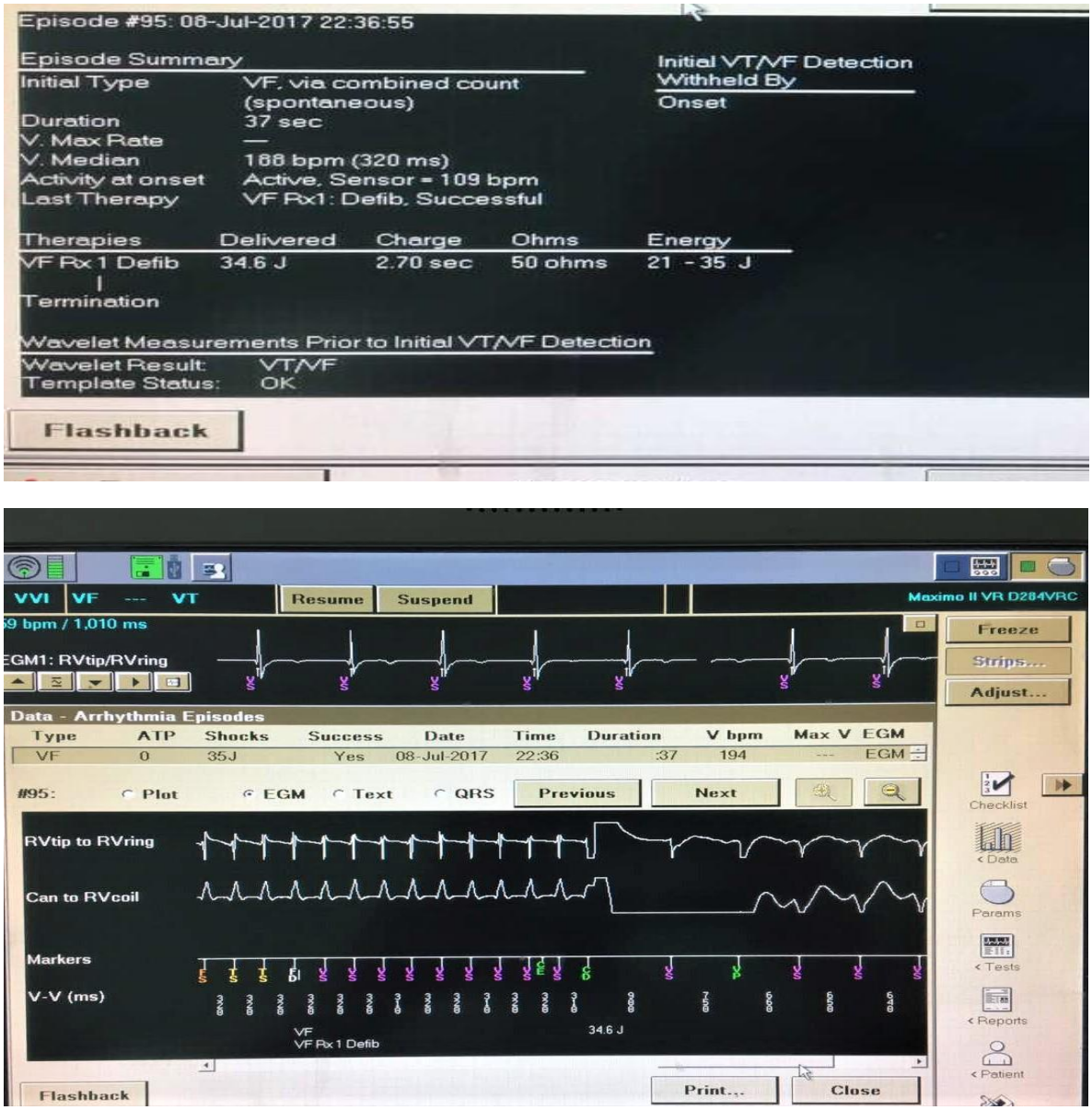

Figure 2. Detection of VF and DC therapy

Discussion

Implantable cardioverter defibrillators (ICDs) are devices that are designed primarily for the therapy of life-threatening heart rhythm disorders. In cooperation with electrical engineers, Mirovski started the project of creating an implantable device in 1980. The purpose of those devices was the ability to recognize and to stop the heart rhythm disorders, whose outcome could lead to SCD. In the period from 1980 to 1985, the device was called the Automatic Implantable Defibrillator and in 1985 its use was approved by the FDA. In Serbia, the first device with that purpose was implanted by Prof. Dr. Milan Bane Djordjevic in 1986 at the Clinical Center of Serbia. Today, modern ICD devices are very similar to standard bradycardic pacemakers regarding the size and purpose. They use a lithium-vanadium battery due to the reliability of the energy source and the need to deliver a higher amount of energy over a short period of time.

For earlier defibrillators, we used epicardial patch electrodes and that required operation with a thoracotomy approach. Today the implantation of the pacemaker's electrode is performed endovenously, practically as well as for standard anti-bradycardia pacemakers.

Detection of heart rhythm disorders is a specific and basic function of ICD. This detection is based on cardiac rhythm, frequency, but requires individual programming, practically for every patient. The detection criteria in ICD evolved as much as therapeutic. Initially, the only detection criterion was

the number of detected R-R intervals, however, today complex detection algorithms were designed. Those detection algorithms prevent un-detection of VT and VF, false detection of VT/VF (instead of atrial arrhythmias or sinus tachycardia). The greatest advancement in technology was the introduction of a gradual tiered therapy which implies that the detected VT is treated with the least aggressive therapy, with the antitachycardic burst stimulation of the different duration of the V-V stimulus. After a series of progressively more aggressive ATP options, synchronous cardioversion with lower power is applied, and finally defibrillation with the maximum current (30-40J). In the case of VF, the maximum DC shock strength is immediately applied, with the possibility of polarity changes (3, 4).

For example, the benefit of ICD therapy in patients with ischemic heart disease is directly related to the time from myocardial infarction (MI) to its implantation. DINAMIT trial randomized patients to receive an ICD or standard medical therapy, 4 to 40 days after MI, and it showed no difference in overall mortality between the two treatment groups. This study was in contrast to the majority of prior trials with the patients with an ICD. That included patients with a recent MI (MUSSTT, MADIT II). The dramatic difference in outcomes with an ICD in these studies likely reflects the underlying significance of ventricular arrhythmias after MI. Early after MI, ventricular arrhythmias are surrogate markers of worsening myocardial function, recurrent ischemia, and infarction. By comparison, late ventricular arrhythmias are often related to alterations of the electrophysiological substrate in the setting of compensated heart failure due to scar and re-entry mechanism.

In comparison, those patients who have nonischemic cardiomyopathy have a benefit of an ICD implantation which depends on time. In the DEFINITE trial, 458 patients with nonischemic dilated cardiomyopathy were randomized to an ICD versus conventional therapy. In this trial, there was a significant reduction in sudden death and a trend toward a reduction in total mortality. In a subtrial of DEFINITE study, the benefit of ICD therapy was seen predominantly in those who had nonischemic dilated cardiomyopathy less than 3 months compared to those with longer duration of the disease. The findings of this study were opposite with those in the population with ischemic cardiomyopathy in which the benefit of ICD therapy is seen in late course of the disease (5-8).

Antitachicardia pacing, when appropriately programmed can significantly reduce ICD shocks as well as any reduced quality of life that results from shock therapy. Reduction in shocks has an important role in terms of patient acceptance and potentially the reduction of any deleterious effect of shock therapy. The routine use of ATP for the treatment of VT, including fast VT, should be considered in all patients with implantable cardioverter defibrillators. Although there are many ways to program ATP, it is important that the clinicians become familiar with the different algorithms and aspects of programming. The two most commonly applied and studied ATP schemes are burst and rump pacing. Burst pacing is a stimulation pattern whereby a train of pacing pulses is delivered with an equal interstimulus interval. Some manufacturers permit an increased duration of the train with each successive train. Ramp pacing is where the train of pacing pulses have an automatically decrementing interstimulus interval. Initially, avoidance of inappropriate ICD shocks was heralded to improve quality of life and improve device longevity. However, additional studies have shown that these shocks are also significant for both alterations in morbidity and mortality. In both patients who received an ICD for primary or secondary prevention, an inappropriate shock was associated with an increase in total mortality. The most common cause of inappropriate shock was atrial fibrillation, followed by supraventricular tachycardia, and abnormal sensing. The significance of new onset atrial fibrillation after ICD implantation has been investigated as well (7, 9-12).

Conclusion

As a conclusion, the use of ICD in primary and secondary prevention of SCD is the most effective therapy in patients with ischemic or non-ischemic cardiomyopathy. Unwanted effects of DC shock as syncope are rare, but the benefits of implanted device outweigh rare occurrence of uncomfortable feeling in patients.

References

1. Bardy GH, Lee KL, Mark DB, Poole JE, Packer DL, Boineau R, et al. Amiodarone or an implantable cardioverter defibrillator for congestive heart failure. N Engl J Med 2005; 352(3):225-37. [CrossRef] [PubMed]
2. Bunch Tj, Hohnloser SH, Gersh BJ. Mechanism of sudden cardiac death in myocardial infarction survivors: insights from the randomized trials of implantable cardioverter defibrillators. Circulation 2007; 115(18): 2451-7. [CrossRef] [PubMed]
3. Weiss JN, Qu Z, Chen PS, Lin SF, Karagueuzian HS, Hayashi H, et al. The dynamics of cardiac fibrillation. Circulation 2005; 112(8):1232-40. [CrossRef] [PubMed]
4. Bunch TJ, Hayes DL, Friedman PA. Clinically relevant basics of pacing and defibrillation. In: Hayes DL, Friedman PA, editors. Cardiac pacing, defibrillation, and resynchronization. A practical approach. Vol II. West Sussex:Wiley-Blackwell; 2008. p. 1-42. [CrossRef]
5. Wilber DJ, Zareba W, Hall WJ, Brown MW, Lin AC, Andrews ML, et al. Time dependence of mortality risk and defibrillator benefit after myocardial infarction. Circulation 2004; 109(9):1082-4. [CrossRef] [PubMed]
6. Daubert JP, Zareba W, Cannom DS, McNitt S, Rosero SZ, Wang P, et al. Inappropriate implantable cardioverter defibrillator shocks in MADIT II: frequency, mechanism, predictors, and survival impact. J Am Coll Cardiol 2008; 51(14):1357-65. [CrossRef] [PubMed]
7. Bunch TJ, Day JD, Olshansky B, Stolen KQ, Mulin CM. Newly detected atrial fibrillation in patients with an implantable cardioverter defibrillator is a strong risk marker of increased mortality. Heart Rhythm 2009; 6(1):2-8. [CrossRef] [PubMed]
8. Maron BJ, Spirito P, Ackerman MJ, Casey SA, Semsarian C, Estes NA 3rd, et al. Prevention of sudden cardiac death with implantable cardioverter-defibrillators in children and adolescents with hypertrophic cardiomyopathy. J Am Coll Cardiol 2013; 61(14): 1527-35. [CrossRef] [PubMed]
9. Earley A, Persson R, Garlitski AC, Balk EM, Uhlig K. Effectiveness of implantable cardioverter defibrillators for primary prevention of sudden cardiac death in subgroups a systematic review. Ann Intern Med 2014; 160(2):111-21. [CrossRef] [PubMed]
10. Satake H, Fukuda K, Sakata Y, Miyata S, Nakano M, Kondo M, et al. Current status of primary prevention of sudden cardiac death with implantable cardioverter defibrillator in patients with chronic heart failure--a report from the CHART-2 Study. Circ J 2015; 79 (2): 381-90. [CrossRef] [PubMed]
11. Pun PH, Al-Khatib SM, Han JY, Edwards R, Bardy GH, Bigger JT, et al. Implantable Cardioverter Defibrillators for Primary Prevention of Sudden Cardiac Death in CKD: A Meta-analysis of Patient-Level Data from 3 Randomized Trials. Am J Kidney Dis 2014; 64(1):32-9. [CrossRef] [PubMed]
12. Konstantinou DM, Efthimiadis GK, Vassilikos V, Paraskevaidis S, Pagourelias E, Maron BJ, et al. Implantable cardioverter defibrillators for primary prevention of sudden death in hypertrophic cardiomyopathy. J Cardiovasc Med (Hagerstown) 2016; 17(6):433-9. [CrossRef] [PubMed]

Prikaz bolesnika

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KADA ELEKTRIČNI ŠOK ZNAČI ŽIVOT - IMPLANTABILNI KARDIOVERTER DEFIBRILATOR I NJEGOVI EFEKTI: PRIKAZ SLUČAJA

Tomislav Kostić[1,4], Zoran Perišić[1], Dragana Stanojević[1], Goran Koraćević[1,2], Vladimir Mitov[2], Mlađan Golubović[3], Predrag Cvetković[1], Mirko Krstić[1], Aleksandar Stojković[1], Sonja Šalinger[1], Stefan Momčilović[4], Sanja Banković[4]

[1]Klinika za kardiovaskularne bolesti, Klinički centar Niš, Srbija
[2]Zdravstveni centar Zaječar, Zaječar, Srbija
[3]Klinika za kardiotorakalnu hirurgiju i transplantaciju, Klinički centar Niš, Niš, Srbija
[4]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Tomislav Kostić
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: tomislav.kostic1977@gmail.com

Iznenadna srčana smrt (SCD) uzrokovana ventrikularnom fibrilacijom (VF) ostaje glavni uzrok smrtnosti u zapadnom svetu. Većina VF i iznenadnih srčanih smrti javljaju se izvan bolnice. Implantacija inplantabilnog kardioverter defibrilatora (ICD) potkrepljena je dokazima iz mnogih randomizovanih studija za primarnu i sekundarnu prevenciju iznenadne srčane smrti. Aktuelni ICD-ovi imaju više mogućnosti za poboljšanje dijagnostike, smanjivanje nepotrebnog pejsinga, očuvanje upotrebe energije i pružanje terapije bez bolova, kao što je antitahikardni pejsing (ATP). Najvažnija od gore pomenutih osobina ostaje sposobnost pejsmejkera da prepozna po život opasnu ventrikularnu aritmiju i da je prekine isporučujući struju. Pogodnost i značaj ICD šoka zavisi od vrste srčanih bolesti i prisustva strukturnih bolesti srca. Ovi koncepti ilustrovani su u kratkom pregledu ispitivanja ICD-a kod bolesnika sa ishemijskom i neishemijskom bolesti srca. Predstavljamo bolesnika sa dilatativnom kardiomiopatijom i smanjenom ejekcionom frakcijom leve komore (LVEF) koji je imao ugrađeni ICD pre godinu dana, kao primarnu prevenciju SCD. Bolesnik je primljen u Kliniku za kardiovaskularne bolesti nakon kratkog gubitka svesti. Električna kontrola uređaja pokazala je da se VF iznenada desila i uspešno ju je prekinuo šok kao tip terapije ICD.

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Ključne reči: *implantabilni kardioverter defibrilator, šok, iznenadna srčana smrt*

Case report

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ORTHODONTIC TREATMENT OF ANKYLOSED PERMANENT TEETH AFTER SURGICAL LUXATION

Branislav Vidović[1], Ana Todorović[2], Marko Igić[3]

Tooth ankylosis is the process of the fusing of a tooth root to the surrounding alveolarbone whereby the periodontal ligament is lost, and in later stages, a tooth root isreplaced by the bone, which eventually results in the tooth loss. The etiology of thiscondition includes trauma, genetic factors, metabolic anomalies, local anomalies,endocrine anomalies, iatrogenic causes as well as idiopathic factors. Theconsequences are evident and involve prevented ankylosed tooth growth causing non-occlusion,adjacent teeth inclination, opponent teeth eruption and cause malocclusion.Therapy can be surgical, conservative, orthodontic or combined. The case presentedin this paper illustrates the efficacy of the surgical luxation of ankylosed teethfollowed by the immediate application of orthodontic elastic force. The outcome maybe debatable, but considering a poor prognosis for ankylosed teeth, the suggested treatment represents a better therapeutic choice than facing potential consequences ofadministering or not administering the usual treatment of ankylosed permanent teeth.

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Key words: *permanent tooth ankylosis, etiology, prognosis, therapy*

[1]Specialist Orthodontic Dental Pracice Ortis, Novi Sad, Serbia
[2]Clinic for Dentistry, Department of Orthodontics, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Department of Prosthodontics, Niš, Serbia

Contact: Branislav Vidović
Vojvode Putnika 11, 21000 Novi Sad, Serbia
E-mail: ortis.co@gmail.com

Introduction

Ankylosed permanent teeth are clinically most commonly noticed by the lack of orincomplete eruption (not reaching the occlusal plane) of one or a group of teeth. The harmful effect of this condition can be more serious of that causing the possible loss of the ankylotictooth. Its most common consequences are the inclination of adjacent and the extrusion ofopposing teeth in the space which should be filled with insufficiently erupted teeth, thusnormal occlusal relation becomes permanently disturbed. In addition, due to the absence ofankylotic tooth eruption, the reduction or the absence of full growth of surrounding alveolarprocess occurs. The prognosis of ankylotic teeth is poor. The cementum tissue of dental roottransforms into bone tissue, which is followed by instability and permanent tooth loss within a period of 3 to 7 years (1).

The tooth and the alveolar bone form the gomphosis joint (Figure 1). Joint surfacesrepresent cementum and bone tissue. As these are two histologically similar tissues, bone andcementum cells fusion would be a natural phenomenon. However, this process does not occurunder physiological conditions due to the presence of periodontal ligament which represents aphysical barrier for the fusion of these two tissues. If the lesion of fibrous tissue which createsthe periodontal ligament occurs (Figure 2), the barrier between the dental root cementum cellsand alveolar bone cells becomes disturbed. In this place, bone and cementum cells penetrationoccurs and a bone bridge is formed, i.e. tooth ankylosis occurs (Figure 3). Any absence of PDLintegrity creates the space for the fusion of periodontium cells by which the naturalmorphological and functional surrounding of the tooth is disturbed.

Diagnosis

As a result of bone and cement tissue fusion, a solid connection is formed between the tooth and the alveolar bone. Therefore, the absence of physiological mobility typical for healthy teeth is clinically noticed. An ankylotic tooth very often does not reach the occlusal plane or even remains impacted in the alveolar ridge. This occurs as a result of impossible mobility of the ankylotic tooth, so it cannot follow the vertical growth of the alveolar bone and adjacent teeth. Percutaneously, muffled sounds are heard (as in hitting the bone) due to the fusion of cementum and bone tissues. The most visible sign of tooth an-

kylosis is the lack of its mobility after forces being applied by orthodontic appliance. X-rays can show spots of periodontal ligament obliteration although such an occurrence may remain unnoticed due to relatively small surfaces of cells fusion, as well as the localisation of newly-formed bone bridges in locations which interpone the very dental root (2). The advancement of radiography and the appearance of the three-dimensional computed tomography enable measuring the extent of dental root ankylosis and its precise localisation (3). Anamnestically, patients often indicate the injury of ankylotic tooth.

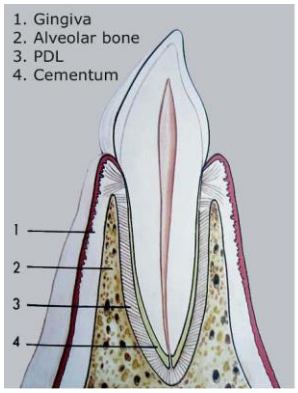

Figure 1.

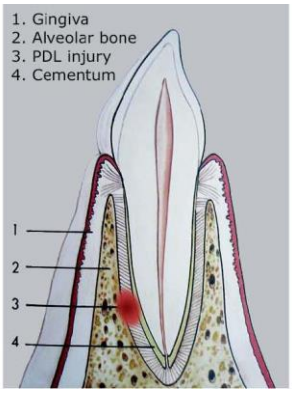

Figure 2.

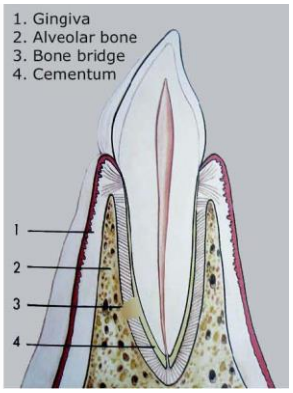

Figure 3.

Etiology

Generally, it is believed that tooth injuries are the most common cause of ankylosis (4) as they are often followed by the injuries of periodontium and even ruptures of periodontal ligament. The next most frequent cause of tooth ankylosis is tooth replantation, especially if performed unprofessionally. Iatrogenic factors, such as traction of impacted teeth by a steel ligature in the shape of a loop positioned on the enamel – cementum junction also frequently lead to ankylosis as a result of periodontal ligament injury with steel ligature. There are suggestions that ankylosis may occur for completely unknown reasons changing the fibrous of the periodontal ligament with the adjacent bone tissue (5, 6). Many papers discuss the occurrence and causes of ankylosis (7-10). A connection has been observed between the endocrine disruptions, metabolism disorders and tooth ankylosis. It is also related to genetic predispositions, local dysfunctions such as infections, trauma to the given area, hard pressure on soft tissues or the deficit in bone growth. We have to say, however, that we lack the full understanding of the essence of this process and that the idiopathic ankylosis is very common, especially in those cases where the visible external signs such as trauma or pathological lesion of periodontal ligament are missing.

Therapy

In the literature, conflicting opinions can be found about the possibilities for ankylosed permanent teeth treatment (11) which reflect insufficient knowledge of etiological processes behind this condition. Some authors believe that the prognosis of such teeth is hopeless and suggest their surgical removal as the treatment of choice (12, 13). However, those treatments are often connected with bone fractures and massive bone defects which disable the quality of prosthetic rehabilitation. This is why tooth decoronation is suggested as an alternative to this method, which implies mucoperiosteal flap elevation and surgical removal of the tooth crown while the root remains to be replaced with bone tissue. Distraction osteogenesis is one of the methods of placing the tooth in the dental arch (14, 15) although in those cases we have to closely monitor patient's growth because his vertical dimension can be highly compromised. Others believe that ankylotic teeth, even if they have a longer term prognosis, cannot be moved by orthodontic forces (16), thus they recommend conservative, prosthetic or some other non-orthodontic therapy which often collides with biological needs of growing patients. The third suggest surgical luxation of the ankylotic tooth and its extraction unless a spontaneous eruption occurs following this intervention (17). Extraction and replantation of the ankylotic tooth is also suggested. This method is connected to vitality loss and frequent re-ankylosis unless the tooth is immediately moved from the replantation place by orthodontic appliance. A minor segmental osteotomy with repositioning of the tooth and the adjacent alveolar bone as a whole is recommended (18-20). The disadvantage of this method is that it does not affect the ankylosis itself but it changes the position of the tooth. Tooth luxation is a method of mechanical ankylotic contacts breaking preserving vasculature and innervation of the ankylotic tooth. Orthodontic movement of the tooth following luxation enables the restoration of periodontal area so that a functional tooth within healthy bone surrounding is obtained as a result of this method.

Aim

The aim of this paper is to indicate the causes and possible preventive measures, as well as to present therapeutical possibilities that are available to practitioners when they encounter the problem of ankylotic tooth in their clinical practice. The case presented in this paper illustrates a successful treatment of ankylotic upper central incisor. Immediately after the surgical luxation, the method of ankylotic tooth traction was applied with orthodontic appliance after which occlusal problems that occurred as a result of the tooth malposition were solved. Simultaneously, the restitution of the periodontal area occurred and the mobility of the treated tooth was enabled which persisted for even nine months after the surgical luxation.

Case report

A female patient, 21 years old, with maxillary retrognathism, open bite, bilateral crossbite and crowding in upper dental arch, came with the problem of unerupted upper left central incisor. (Figure 4, 5). Anamnestically, the information was gained that the patient had already been orthodontically treated for the existing problem, and could not recall any trauma. Percutaneously, the tooth makes a muffled tone. There is no physiological mobility. Periodontal ligament obliteration is noticed on retroalveolar X-ray (Figure 6).

Figure 4.

Figure 5.

Figure 6.

Figure 4, 5. Patient before the beginning of the therapy
Figure 6. The arrow marks the place of periodontal ligament obliteration

Therapy plan

◇ Creating space for positioning the tooth 21 in the dental arch

◇ Ankylotic tooth undergoing surgical luxation to retain mobility

◇ Vertical traction of the luxated tooth immediately upon surgical intervention

◇ Correction of sagittal and vertical jaw relations, extension of upper dental arch, alignment of tooth arch midlines

Creating the space for positioning of the tooth 21 in dental arch was done by fixed orthodontic appliance, using steel springs (Figure 7). Regardless of the space secured for the tooth eruption, there was no reaction at all. Frontal elastic traction was also applied but the reaction occurred only in the sense of lower frontal teeth extrusion due to the traction of ankylotic tooth 21 and intrusions of upper frontal teeth, whereas tooth 21 remained unmoved. (Figure 8). Surgical luxation was performed, followed im-mediately by the traction of the tooth 21 with elastic bands and round .014 NiTiarchwires (Figure 9). It took 4 months to reach its occlusal surface (Figure 10).

Nine months upon performed luxation and after more than five months of absolute inactivity, tooth 21 was still reacting to orthodontic forces which indicates that it was set in a completely morphologically and physiologically normal and healthy surrounding. Due to this fact, we were able to move it medially by 1.5 mm which aligned midlines (Figure 10, 11, 12).

Fixed orthodontic appliance was removed twenty-three months after the performance of surgical luxation. Figures 13, 14, 15 show normal incisaloverjet, incisal overbite andmidline alignment. Occlusion is in class I. Tooth 21 shows signs of physiological mobility and percutaneouslyproduces a resonant sound. The vitality of the tooth is entirely intact. Retroalveolar X-ray film does not show signs of periodontal ligament obliteration and welldevelopedtrabecular bone structure can be seen (Figure 16).Figure 16 shows resorption of apex 11 developed in stage I of the therapy due to the intrusion of upper frontal teeth.

Figure 7.

Figure 8.

Figure 9.

Figure 10.

Figure 11.

Figure 12.

Figure 13.

Figure 14.

Figure 15.

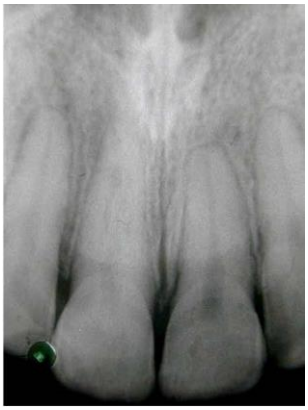

Figure 16.

Discussion

In respect to poor prognosis of ankylotic teeth, it is important to take a preventive action. This primarily refers to the prevention of injuries (orthodontic treatment of class II/1, protectors, etc.) and after that to a satisfactory recovery of traumatised teeth as the most common cause of ankylosis. In this respect, the focus of the problem is shifted from the fight against root canal inflammation more towards procedures for the preservation of physiological integrity of the periodontal ligament. This is why it is important to conduct the procedure of tooth replantation correctly if the avulsion occurred after the injury. Two procedures are suggested in this case depending on whether they are performed less or more than 60 minutes from the time of avulsion:

Treatment < 60 min

• Rinse the root canal with physiological solution
Remove the coagulum with physiological solution jet
• Examine the presence of injuries in surrounding tissues and perform
Treatment for their recovery
Replant the tooth with gentle finger pressure

Treatment > 60 min

• Remove necrotic tissue (pdl included)
• Remove the coagulum with physiological solution jet
• Examine the presence of injuries in surrounding tissues and perform
Treatment for their recovery
• Submerge the tooth in 2.4% sodium fluoride pH5.5 for at least 5 minutes
Or, if possible, in emdogain
• Replant the tooth with gentle finger pressure

After such a treatment it is necessary to:

• Treat the woundand verify the position of the tooth with x-ray
• Apply flexible splint (only for 7 days)
• Administer antibiotics
• Refer the patient to the doctor
• Administer anti-tetanus serum
• Perform endodontic treatment after 7-10 days if necessary
• Carry out a soft food diet
• Recommend cleaning teeth with a soft tooth brush
• Rinse mouth twice a day with octenidol mouth rinse for 7 days

The tooth and the alveola which surrounds it represent a joint. To avoid ankylosis afterluxation or subluxation of the tooth it is necessary to perform a "tilting" exercise, i.e. minimal movement of the injured tooth within 1 mm range. This is why placing an elasticsplint is necessary. Partial and full fixed appliances with round archwires can serve thepurpose perfectly. Exercise needsto be performed immediately after placing the elasticsplint, 7 days at the latest following the injury. Continuous shearing of the tissue in thehealing stage leads to fibrous-like instead of bone-like fusion, which is exactly the way inwhich joints should heal. In the end of the process, periodontium is the most similar to itsanatomic structure, with fibrous tissue between the bone and cementum (Figure17). Rigidimmobilisation is undesirable as it leads to re-ankylosis with all the potential consequencesand whose final stage is the substitution of dental root for the bone tissue, which begins fromthe very first moment of establishing the communication through the injured periodontalligament and ends in instability and tooth loss (Figure 18).

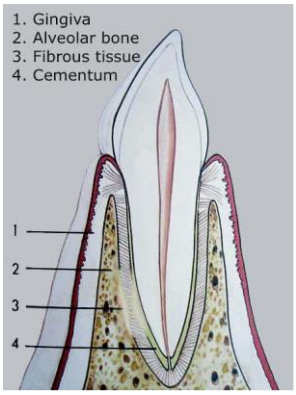

Figure 17.

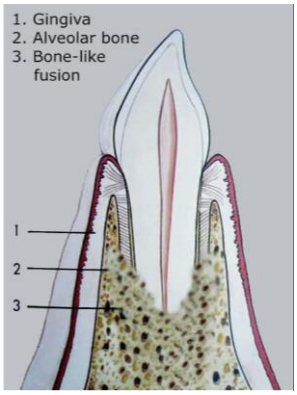

Figure 18.

Figure 19.

Figure 20.

Antibiotic protection is necessary to be administered during 7-10 days in order to avoidthe infection of periodontal ligament. The antibiotic of choice is tetracycline. Unlikeamoxicillin, it not only reduces the risk of infection but has the inhibitory effect onosteoclasts which lead to dental root resorption (21). In order to minimise the initialinflammatory stage and therefore reduce the activation of osteoclasts, local application ofsteroids (dexamethason) is recommended (22). The use of emdogain is recommendable as it formsa matrix for fibrocytedevelopment. Tilting exercises or continuous orthodontic force, if needed, can be applied 1 day after the tooth replantation (4). The prevention of ankylosis impliesavoiding any therapeutic methods that lead to lesions of periodontal ligament.

If ankylosis still occurs, the chance for ankylotic teeth to survive without undergoingorthodontic treatment is little. Rhisolysis, which has the form of external inflammatoryrhisolysis, includes the dental root and the bone substitutes the dental root cementum.Granulation tissue, which is present in the condition of ankylosis, resorbs the root in the samemanner. In both cases the loss of dental root occurs and the tooth falls out (Figure 18). Thisprocess is not irreversible unless the necrosis of periodontal ligament exceeds 20% of theradicular surface (23).

The therapy that we administered was recommended by Proffit (18): anesthesia andmild luxation of the ankylotic tooth in order to break bone fibres between the dental root andalveolar bone. If this procedure is performed, it is extremely important for the orthodonticforce to be applied immediately after luxation. Otherwise, it is only the matter of time whenre-ankylosis will occur. Orthodontic forces must be reactivated every 10 days until themoment when the tooth is positioned in its place in the dental arch (4).

The method of tooth luxation and its orthodontic movement is risky. It can lead toroot fracture, especially in multi rooted teeth. Apart from that, external and internal rhisolysiscan follow such a procedure. Pulp devitalisation is also one of the potential problems. Analternative to this method is the survival of the ankylotic tooth in the jaw with all the consequences that go with it. Prosthetic, conservative or surgical treatment can reducepatient's difficulties, but only to a certain extent. With all the risks they take, combinedorthodontic-surgical treatments are the only ones that can lead to full recovery of theankylotic tooth and preservation of the natural conditions inpatient's mouth, which providehim or her with comfortable dental future (Figure 19, 20).

References

1. Andersson L. Dentoalveolarankylosis and associated root resorption in replanted teeth. Experimental and clinical studies in monkeys and man. Swed Dent J Suppl1988; 56:1-75.[PubMed]
2. Geiger A,Bronsky M.Orthodontic management of ankylosed permanent posterior teeth: A clinical report of three cases. Am J OrthodDentofacialOrthop1994; 106 (5):543-8. [PubMed]
3. Paris M, Trunde F, Bossard D, Farges JC, Coudert JL. Dental ankylosis diagnosed by CT with tridimensional reconstructions. J Radiol2010; 91(6):707-11. CrossRef][PubMed]
4. Graber MT, Vanarsdall LR. Orthodontics: current principles and techniques. 2nd ed. St. Louis: Mosby; 1994.
5. Line SE, Polson A, Zander HA.Relationship between periodontal injury selective cell repopulation and ankylosis. J Periodontal 1974; 45(10):725-30. [CrossRef][PubMed]
6. Melcher AH. Repair of wounds in the periodontium of the rat: influence of periodontal ligament on osteogenesis. Arch Oral Biol1970; 15(12):1183-204. [CrossRef][PubMed]
7. Biederman W. The problem of the ankylosed tooth. Dent Clin North Am 1968; 409-24.[PubMed]
8. Kronfeld R. Bony union (ankylosis) of roots and alveolus. In: Histopathology of the teeth and their surrounding structures, 2nd ed. Philadelphia: Lea & Febiger; 1939.
9. Noyes FB. Submerging deciduous molars. Angle Orthod1932; 2:77-87.
10. Vanarsdall RL. Management of ankylosedteeth: Special Interest Clinics.Washington DC: American Association of Orthodontists annual session; 1979.
11. Alruwaithi M, Jumah A, Alsadoon S, Berri Z, Alsaif M. Tooth Ankylosis And its Orthodontic Implication. IOSR-JDMS 2017; 16(2): 108-12.[CrossRef]
12. Moyers RE. Handbook of orthodontics. 4th ed. Chicago: Year Book; 1988.
13. Schafer WB, Hine MK, Levy BM. A textbook of oral pathology. 3rd ed. Philadelphia: WB Saunders; 1983.
14. Dolanmaz D, Karaman IA, Pampu A,Topkara A. Orthodontic Treatment of an Ankylosed Maxillary Central Incisor through Osteogenic Distraction. Angle Orthod2010; 80(2):391-5. [CrossRef][PubMed]
15. Aludden H, Jensen T. Dentoalveolar Segmental Osteotomy Combined with Orthodontic Treatment for an Impacted and Ankylosed Upper Canine: A Case Report.Open Journal of Stomatology 2016; (6): 97-102.
16. Andreasen JO. Traumatic injuries of the teeth. 2nd ed. Philadelphia: WB Saunders; 1981.
17. Biederman W. Etiology and treatment of tooth ankylosis. Am J Orthod 1962; 49: 670-84. [CrossRef]
18. Proffit WR. Contemporary orthodontics. 2nd ed. St Louis: Mosby Year Book; 1993.
19. Chae JM, Paeng JY. Orthodontic treatment of an ankylosed maxillary central incisor through single-

tooth osteotomy by using interdental space regained from microimplant anchorage. Am J OrthodDentofacial Orthop. 2012; 14(2): 39-51.[CrossRef][PubMed]
20. Metin-Gürsoy G, Okur B, Türkyilmaz F, Bozkaya S. Corticotomy assisted orthodontic treatment of ankylosed and impacted upper canine. J Case Rep Images Dent 2017; 3: 20-24.
21. Sae Lim V, Wang CY, Choi GW, Trope M. The effect of systematic tetracycline on resorption of dried replanted dogs' teeth. Endod Dent Traumatol1998; 14(3): 127-32.[CrossRef][PubMed]
22. Sae Lim V, Metzger Z, Trope M: Local dexamethasone improves periodontal healing of replanted dogs' teeth. Endod Dent Traumatol1998; 14(5): 232-6. [CrossRef][PubMed]
23. Tronstad L. Clinical endodontics. 3rd ed. New York: Georg ThiemeVerlag;2009.

Prikaz bolesnika

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ORTODONTSKI TRETMAN ANKILOZE STALNIH ZUBA NAKON IZVEDENE HIRURŠKE LUKSACIJE

Branislav Vidović[1], Ana Todorović[2], Marko Igić[3]

[1]Specijalistička stomatološko-ortodontska ordinacija Ortis, Novi Sad, Srbija
[2]Klinika za stomatologiju, Niš, Služba za ortopediju vilica, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Katedra za stomatološku protetiku, Niš, Srbija

Kontakt: Branislav Vidović
Vojvode Putnika 11, 21000 Novi Sad, Srbija
E-mail: ortis.co@gmail.com

Ankiloza zuba je proces srastanja korena zuba za okolno koštano tkivo u kome dolazi do gubitka periodontalnog ligamenta, a u kasnijim fazama do pretvaranja korena zuba u koštano tkivo i do gubitka samog zuba. Etiologija ovakvih stanja kreće se od traume, genetske predispozicije, poremećaja metabolizma, lokalnih poremećaja, endokrinih poremećaja, jatrogenih uzroka pa do idiopatskih faktora. Posledice su evidentne i odnose se na izostanak rasta ankilotičnih zuba usled čega dolazi do nonokluzije, inklinacije susednih zuba, erupcije zuba oponenata i narušavanja cele okluzije. Terapija može biti hirurška, konzervativna, ortodontska ili kombinovana. Slučaj prikazan u ovom radu svedoči o efikasnosti hirurške luksacije ankilotičnih zuba praćene neposrednom primenom ortodontske elastične vuče. Ishod može biti diskutabilan, ali s obzirom na lošu prognozu ankilotičnih zuba, hirurška luksacija predstavlja bolji terapeutski izbor od suočavanja sa mogućim posledicama izostanka ili primene uobičajenih tretmana ankilotičnih zuba.

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Ključne reči: *ankiloza stalnih zuba, etiologija, prognoza, terapija*

Original article

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HYPOTHENSIVE AND ANTIOXIDANT EFFECTS INDUCED BY POLYPHENOL RICH BLACK CHOKEBERRY (*ARONIA MELANOCARPA* [MICHX.] ELLIOTT) JUICE

Milica Milutinović[1], Suzana Branković[2], Katarina Šavikin[3], Gordana Zdunić[3], Milica Kostić[1], Bojana Miladinović[1], Dušanka Kitić[1]

Chokeberry (*Aronia melanocarpa* (Michx.) Elliott) has been traditionally used for centuries in the herbal medicine of the Native North Americans considering the numerous pharmacological activities. The aim of this research was to analyze the effects of the chokeberry juice on the cardiovascular activity, in order to authenticate the use of *Aronia melanocarpa* as a functional food. Nevertheless, the antioxidative properties of chokeberry juice were determined by DPPH method and the β-carotene/linoleic acid model system, to prove the estimated activity of the juice. The significant antioxidative activity was confirmed. The concentration of chokeberry that inhibited 50 % of DPPH free radicals (IC_{50}) was 1.25 ± 0.08 mg/ml. In β-carotene/linoleic acid model system IC_{50} was achieved by concentration of 1.73 ± 0.07 mg/ml. The effects of chokeberry juice on the blood pressure and heart rate in anaesthetized rabbits were performed. The results demonstrated the reduction of the blood pressure (EC_{50} value of 195.63 ± 14.45 mg/kg, the concentration which elicited 50 % of maximal response) and heart rate (EC_{50} value of 171.71 ± 11.21 mg/kg) in the animals. The administration of the chokeberry juice could produce hypotension and negative chronotropic effects. However, it is necessary to conduct the study in human population to confirm those findings.

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Key words: *Aronia melanocarpa (Michx.) Elliott, chokeberry juice, antioxidant effects, blood pressure, heart rate*

[1]University of Niš, Faculty of Medicine, Department of Pharmacy, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Department of Physiology, Niš, Serbia
[3]Institute for Medicinal Plants Research "Josif Pancic", Belgrade, Serbia

Contact: Milica Milutinovic
Bulevard dr Zoran Djindjić 81, 18 000 Niš, Serbia
E-mail: milica.milutinovic@medfak.ni.ac.rs

Introduction

The berries of *Aronia melanocarpa* (Michx.) Elliott (black chokeberry, aronia, aronia berry), a woody shrub of the Rosaceae family, have been used in the Native North American herbal medicine for centuries. In the last decades, the interest of food and medicinal science has been focused on chokeberry's secondary metabolites with highly expressed antioxidant activity and their possible use in the prevention and treatment of many diseases (1, 2).

The chemical and nutritive composition of *A. melanocarpa* berries have been determined by previous research. Chokeberries are the rich source of polyphenolic compounds, as phenolic acids, proanthocyanidins, anthocyanins, flavonols, and flavanones (2-5). Anthocyanins are the dominating flavonoids in chokeberry, representing about 25 % of total polyphenols (6-9). Studies have also confirmed the presence of dietary fiber in *A. melanocarpa* berries (10) and several other constituents such as microcrystalline cellulose, pectins, lignins, cutin-like polymers and condensed tannins (11). The content of fat and proteins in fruits was analyzed, and there was found to be 0.14 g/100 g FW of fat and 0.7 g/ 100 g FW of proteins in fresh chokeberries (10). Fresh chokeberries also contain 16-18% of reducing sugar, which is often used in a diet as a weak laxative (9). The mineral content (ash values) of fresh chokeberry has also been confirmed and it was 440 mg/100 g 13 and 580 mg/100 g. During processing, the mineral content of juices was between 300 and 640 mg/100 mL (12). Chokeberry fruits contain relatively high average amounts of K and Zn. Nevertheless, they contain small amounts of Na, Ca, Mg and Fe (10). In fresh pressed juice vitamins B1, B2,

B6, C, pantothenic acid and niacin (13) were found. Some studies have confirmed the presence of carotenoids, ß-caroten and ß-cryptoxanthin, in comparatively high amounts of chokeberry fruits (10, 14).

A. melanocarpa fruits contain mostly cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, cyanidin-3-O-xyloside and cyanidin-3-O-glucoside, among anthocyanins (15). The high content of phenolic compounds, especially anthocyanins with their highly expressed antioxidant activity, correlates with the biological activity of these berries and their use in health promoting and well-being purposes (16-18). Anthocyanins have an active role in cardio and neuroprotection, and as anticancer, hepatoprotective, gastroprotective, anti-inflamatory, normolipidemic and normo-glicemic agents and intracellular antioxidants (19-22).

We performed this study to examine the effects of chokeberry juice on the blood pressure and heart rate in anaesthetized rabbits, in addition to its antioxidant properties.

Materials and methods

Plant material and juice preparation

Fruits of black chokeberry were collected from a plantation field on the mountain Suvobor (750 m.a.s.l.), Serbia, in August 2011. The berries were stored at +5°C for 24 h. Fresh berries were crushed and squeezed. The yield of the juice with respect to the weight of the fresh fruits was 73 %. The juice was filtered, pasteurized at 80°C for 10 min and stored at 0°C. For the experiment the juice was diluted with distilled water to the appropriate concentration.

Determination of antioxidant capacity

The free radical scavenging activity of the juice on the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was carried out according to the procedure described previously (23), with slight modifications. The juice sample (2 g) was diluted with distilled water up to 10 ml, centrifuged (2500^x g; 10 min) and a supernatant was used for analyses. The antiradical capacity of the sample was evaluated using a dilutions series, in order to obtain a large spectrum of sample concentrations. The juice (100 µl) was mixed with 1400 µl of 80 mM methanol solution of DPPH. The absorbance at 540 nm was measured after 20 min. The percentage of inhibition was calculated us-ing the equation:

Inhibition % = $[(A0-Ai)/A0]^x$ 100, where A0 is absorbance of the control and Ai is absorbance of the sample. The concentration of the juice that inhibited 50% of DPPH radicals (IC_{50}) was calculated from the concentration/% inhibition curve. Trolox was used as a positive control.

The assessment of the lipid peroxidation inhibitory activity was carried out according to the β-carotene/linoleic acid model system described by Koleva and others (2002) (24), with modifications. The juice sample (2 g) was diluted with distilled water up to 10 mL, centrifuged (2500^x g; 10 min) and supernatant was used for analyses. A solution of β-carotene was prepared by dissolving 2 mg of crystalline β-carotene in 10 ml of chloroform. One millilitre of the solution, 180 mg of Tween 20 and 25 µl of linoleic acid were pipetted into a round-bottom flask. After all chloroform had evaporated, 50 ml of oxidized distilled water were added to the flask with gentle shaking. Aliquots (200 µl) of this aqueous emulsion were pipetted into each of the wells of 96-well microtiter plates containing 40 µl of chokeberry juice in different concentrations. The microtiter plates had been shortly shaken before zero time absorbance was measured by Elisa reader at 450 nm (A0) (ELISA microplate reader Multiskan Ascent No354 (Thermo Labsystems, Finland).

Microtiter plates were then incubated at 55ºC for 2 hours and absorbances were read again (A120). A blank, deprived of β-carotene, was prepared as a negative control. Antioxidant activity was calculated using the following equation (25):

Inhibition % = $(A120/ A0)^x$ 100.

The concentration of the juice that provided protection of 50 % β-carotene (IC_{50}) was calculated from the concentration / % inhibition curve. Trolox was used as a positive control.

Blood pressure and heart rate measurement

Artery blood pressure in the anaesthetized rabbits was measured as described previously (26). The rabbits were anesthetized intravenously with urethane (750 mg/kg). The catheter filled with heparinized saline (60 IU ml^{-1}) was implanted into left carotid artery. This catheter was connected to a blood pressure transducer (P-1000-A) coupled with a Narcophysiograph (NARCO Bio system, Houston, USA) for measurement arterial pressure. Arterial blood pressure was expressed in mmHg, as systolic and diastolic blood pressure, and then the mean arterial blood pressure was calculated using the following formula:

Mean arterial pressure = diastolic pressure + (systolic pressure-diastolic pressure) / 3

The measurements of the blood pressure and heart rate were made before and after the administration of the chokeberry juice. Arterial pressure was allowed to return to the resting level between injections. Changes in blood pressure were recorded as the difference between the steady state values before and after the injection.

Animals were treated with 0.2 ml of black chokeberry juice, which was administered in rising concentrations (0.45 - 150 mg/kg) at intervals of 15 - 20 min. The effects of the black chokeberry were followed during 10 minutes of continuous registration of the arterial blood pressure. All results were based on the data obtained in six different sets of experiment. All experimental procedures with animals were in compliance with the European Union Directive (2010/63/EU) for animal experiments and were also approved by the Animal Ethics Board of the Medical Faculty in Nis (number 01-206-7).

Statistical analysis

The experimental results were presented as mean ± SD of the mean of 6 determinations. Statistical evaluation was performed using the Student`s t-test. A probability value of $p < 0.05$ was considered to be significant. The effective concentrations EC_{50}, that is the concentration which elicited 50 % of maximal response, were established by regression analysis. Statistical analyses were performed using SPSS statistical software package (ver. 20.0; Chicago, IL, USA).

Results

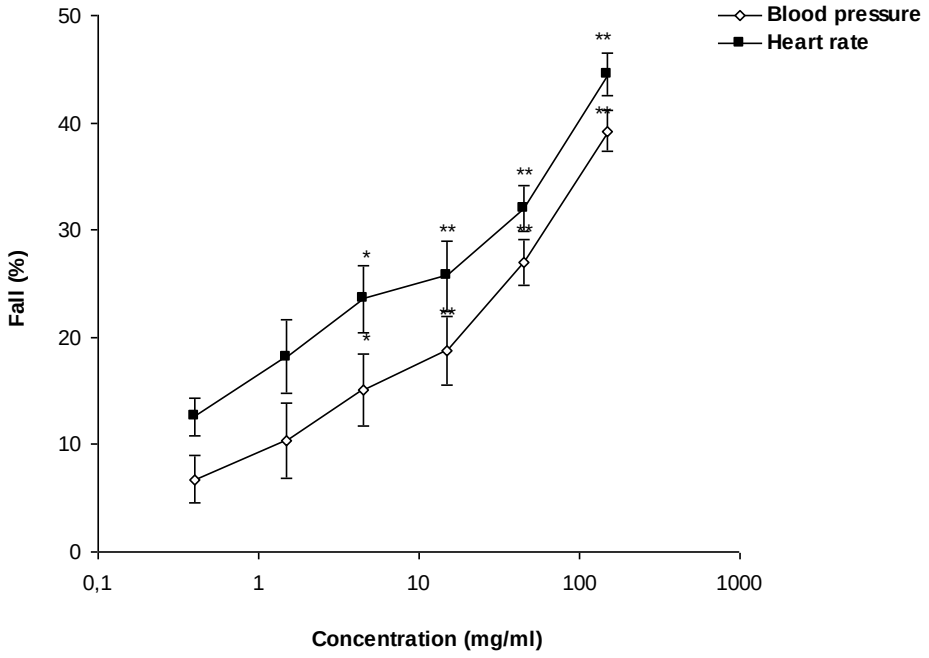
Antioxidant assessment

The assessment of antioxidant potency was conducted by two in vitro complementary methods. The concentration of chokeberry juice that inhibited 50% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (IC_{50}) was 1.25 ± 0.08 mg/ml. In β-carotene/linoleic acid model system IC_{50} was achieved by concentration of 1.73 ± 0.07 mg/ml. Trolox showed better activity in β-carotene/linoleic acid model system than in DPPH (1.69 ± 0.11 and 6.15 ± 0.64 mg/ml, respectively).

Hypotensive and bradycardic effects of black chokeberry juice

The intravenous injection of the chokeberry juice, which was administered in rising concentrations, immediately induced significantly and dose-dependent decrease of the systolic, diastolic and mean arterial blood pressure and heart rate of rabbits. The chokeberry juice at doses of 150 mg/kg decreased arterial blood pressure of rabbits for 39.23 ± 4.58 % ($p < 0.01$), with EC_{50} value of 195.63 ± 14.45 mg/kg.

In vivo intravenous application of juice at doses of 150 mg/kg (Graph 1) caused a significant decrease in heart rate in anesthetized rabbits for 44.48 ± 5.02 % ($p < 0.01$), with EC_{50} value of 171.71 ± 11.21 mg/kg. After the hypotensive peak, the blood pressure and heart rate increased progressively and reached the basal value in about 3-5 min.



values shown are mean ± standard deviation of six separate experiments
*p < 0.05 and ** p < 0.01 compared to baseline values before treatment

Graph 1. Dose-dependent effects of intravenous injection of the black chokeberry (*Aronia melanocarpa* [Michx.] Elliott) juice on the mean arterial blood pressure and heart rate of anaesthetized rabbits

Discussion

Black chokeberry fruits are the one of the richest plant sources of phenolic substances, mainly anthocyanins-glucosides of cyanidin (27). Numerous studies have shown that the polyphenolic compounds present in fruit and vegetable exhibit a wide range of biological effects. Well-known are their role as free radical scavengers and their potentially significant interactions with biological systems (28). Anthocyanins exhibit lipid-lowering and anti-aggregative action in patients with metabolic syndrome (29). Yang et al. (2011) (30) showed that a dietary supplementation with the blueberry extracts and cyanidin-3-O-galactoside from blueberry in the aged mice might induce changes of the endogenous plasma and brain metabolic profiles which improves cognitive impairment and neurodegenerative diseases. Previous studies showed that chokeberry juice and products demonstrate strong antioxidative activity (31, 32) and that was confirmed by our results.

Strong antioxidant activities of chokeberry products qualify them to be used in prevention and treatment of numerous non-communicable diseases, such as cardiovascular diseases (33–38). The present study also showed that black chokeberry juice produced significant decrease in blood pressure. Blood pressure is determined by cardiac output and total peripheral resistance; hence chokeberry juice was investigated on heart rate for its possible inhibitory effects. Our study also confirms that the chokeberry juice has hypotensive and bradicardic effects on cardiovascular system. Intravenous injection of the black chokeberry juice induced the short-term and dose-dependent hypotensive and negative chronotropic effects in anesthetized rabbits. Results showing that the chokeberry juice caused a decrease of blood pressure with bradicardic effect indicate that the chokeberry juice might have effect at the cardiovascular regulation region of the central nervous system and/or effect at the heart. These findings are consistent with the literature data. The blood pressure-lowering properties of the juice used in our study are in accordance to those obtained by Hellstrom et al. (2010) (39) with a lyophilized chokeberry juice (*Aronia mitchurinii*) and polyphenols in spontaneously hypertensive rats. Dietary supplementation with extract of the black chokeberry reduced systolic and diastolic blood pressure in patients after myocardial infarction (38), as well as in patients with metabolic syndrome (40, 41).

In vivo studies in animal models, as well as in humans, have confirmed that consumption of the chokeberry preparations could improve the blood pressure and lipid status. (35, 38, 42-44). It can be explained by the inhibition of the activity of angiotensin l-converting enzyme by the chokeberry extracts (39, 45). It is known that the chokeberry preparations could protect the coronary arteries (46) and heart muscle (38) from the oxidative damage. Literature data confirmed its anti-inflammatory properties which are connected with the protection of the human aorta and aortic endothelial cells *in vitro* (34, 47).

Conclusion

The present study demonstrated that black chokeberry juice (*Aronia melanocarpa*) was able to reduce the blood pressure and heart rate in anesthetized rabbits. Intravenous administration of the chokeberry juice could produce hypotension and negative chronotropic effects. Hence, the chokeberry fruit could be used as a functional food ingredient for the control functions of the cardiovascular system. However, it would be necessary to carry out clinical studies to demonstrate its hypotensive effects in humans.

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References

1. Kulling SE, Rawel HM. Chokeberry *(Aronia melanocarpa)* – A Review on the Characteristic Components and Potential Health Effects. Planta Med. 2008; 74: 1625-34. [CrossRef][PubMed]
2. Kokotkiewicz A, Jaremicz Z, Luczkiewicz M. Aronia Plants: A Review of Traditional Use, Biological Activities, and Perspectives for Modern Medicine. J Med Food. 2010; 13: 255-69. [CrossRef][PubMed]
3. Koponen J, Happonen A, Mattila P, Torronen R. Contents of anthocyanins and ellagitannins in foods consumed in Finland. J Agric Food Chem. 2007; 55: 1612-9. [CrossRef][PubMed]
4. Mattila P, Hellstrom J, Torronen R. Phenolic acids in berries, fruits, and beverages. J Agric Food Chem. 2006; 54: 7193-9. [CrossRef][PubMed]
5. Slimestad R, Torskangerpoll K, Nateland H, Johannessen T, Giske N. Flavonoids from black chokeberries, *Aronia melanocarpa*. J Food Compos Anal. 2005; 18: 61-8. [CrossRef]
6. Wu X, Gu L, Prior RL, McKay S. Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. J Agric Food Chem. 2004; 52: 7846-56. [CrossRef][PubMed]
7. Oszmianski J, Wojdylo A. *Aronia melanocarpa* phenollics and their antioxidant activity. Eur Food Res Technol. 2005; 221: 809-13. [CrossRef]
8. Jakobek L, Seruga M, Medvidovic-Kosanovic M, Novak I. Anthocyanin content and antioxidant activity of various red fruit juices. Dtsch Lebensmitt Rundsch 2007; 103: 58-64.
9. Benvenuti S, Pellati F, Melegari M, Bertelli D. Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus*, *Ribes*, and *Aronia*. J Food Sci. 2004; 69: FCT164-FCT9. [CrossRef]
10. Strigl AW, Leitner E, Pfannhauser W. Die schwarze Apfelbeere (*Aronia melanocarpa*) als natürliche Farbstoffquelle. Dtsch Lebensmitt Rundsch 1995; 91: 177-80.
11. Tanaka T, Tanaka A. Chemical components and characteristics of black chokeberry. J Jpn Soc Food Sci. 2001; 48: 606-10. [CrossRef]
12. Wawer I. In *The power of nature: Aronia melanocarpa*, 1st edition; Nature's Print Ltd.: London, 2006; pp 1-168.
13. Nawirska A, Kwasniewska M. Dietary fibre fractions from fruit and vegetable processing waste. Food Chem. 2005; 91: 221-5. [CrossRef]
14. Lehmann H. Die Aroniabeere und ihre Verarbeitung. Flüssiges Obst. 1990; 57: 746-52.
15. Ara V. Schwarzfruchtige *Aronia*: Gesund – und bald "in aller Munde". Flüssiges Obst. 2002; 10: 653-8.
16. Razungles A, Oszmianski J, Sapis JC. Determination of carotenoids in fruits of *Rosa* sp. (*Rosa canina* and *Rosa rugosa*) and of chokeberry (*Aronia melanocarpa*). J Food Sci. 1989; 54: 774-5. [CrossRef]
17. Oszmianski J, Sepia JC. Anthocyanins in fruits of *Aronia Melanocarpa* (Chokeberry). J Food Sci. 1988; 53: 1241-2. [CrossRef]
18. Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, and pelargonidin. J Agric Food Chem. 2002; 50: 166-71. [CrossRef][PubMed]
19. Chong MF, Macdonald R, Lovegrove JA. Fruit polyphenols and CVD risk: a review of human intervention studies. Br J Nutr. 2010; 104: S28-39. [CrossRef][PubMed]
20. Tarozzi A, Morroni M, Merlicco A, Bolondi C, Teti G, Falconi M, et al. Neuroprotective effects of cyanidin 3-O-glucopyranoside on amyloid beta (25-35) oligomer-induced toxicity. Neuroscience Lett. 2010; 473: 72-6. [CrossRef][PubMed]
21. Bornsek SM, Ziberna L, Polak T, Vanzo A, Poklar Ulrih N, Abram V, et al. Bilberry and blueberry anthocyanins act as powerful intracellular antioxidants in mammalian cells. Food Chem. 2012; 134: 1878-84. [CrossRef][PubMed]
22. Kahkonen MP, Hopia AI, Vuorela HJ. Antioxidant activity of plant extracts containing phenolics compounds. J Agric Food Chem. 1999; 47: 3954-62. [CrossRef][PubMed]
23. Blois MS. Antioxidant determinations by use of a stable free radical. Nature. 1958; 181: 1199-200. [CrossRef]
24. Koleva II, Van Beek TA, Linssen JPH, Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Analysis. 2002; 13: 8-17. [CrossRef][PubMed]
25. Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. Total phenols, ascorbic acid, *β*-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem. 2007; 103: 413-9. [CrossRef]
26. Kitic D, Brankovic S, Radenkovic M, Savikin K, Zdunic G, Kocic B, et al. Hypotensive, vasorelaxant and cardiodepressant activities of the ethanol extract of *Sideritis raeseri* spp. Boiss & Heldr. J Physiol Pharmacol. 2012; 63: 531-5. [PubMed]
27. Valcheva-Kuzmanova SV, Belcheva A. Current knowledge of *Aronia melanocarpa* as a medicinal plant. Folia Med. 2006; 48: 11-7. [PubMed]
28. Mazza G, Kay C, Cottrell T, Holub B. Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. J Agric Food Chem. 2002; 50: 7731-7. [CrossRef][PubMed]
29. Sikora J, Broncel M, Markowicz M, Halubinski M, Wojdan K, Mikuciuk-Olasik E. Short-term supplementation with *Aronia melanocarpa* extract improves platelet aggregation, clotting and fibrinolysis in patients with metabolic syndrome. Eur J Nutr. 2012; 51: 549-56. [CrossRef][PubMed]
30. Yang H, Pang W, Lu H, Cheng D, Yan X, Cheng Y, Jiang Y. Comparison of metabolic profiling of cyanidin-3-O-galactoside and extracts from blueberry in aged mice. J Agric Food Chem. 2011; 59: 2069-76. [CrossRef][PubMed]
31. Oszmianski J, Wojdylo A. *Aronia melanocarpa* phenollics and their antioxidant activity. Eur Food Res Technol. 2005; 221: 809-13. [CrossRef]
32. Denev PN, Kratchanov CG, Ciz M, Lojek A, Kratchanova MG. Bioavailability and antioxidant activity of black chokeberry (*Aronia melanocarpa*) polyphenols: *in vitro* and *in vivo* evidences and possible mechanisms of action: a review. Compr Rev Food Sci. 2012; 11: 471-89. [CrossRef]
33. Hun Kim J, Auger C, Kurita I, Anselm E, Rivoarilala LO, Lee HJ, et al. *Aronia melanocarpa* juice, a rich source of polyphenols, induces endothelium-dependent rela-

xations in porcine coronary arteries via the redox-sensitive activation of endothelial nitric oxide synthase. Nitric Oxide. 2013; 35: 54-64. [CrossRef][PubMed]
34. Daskalova E, Delchev S, Peeva Y, Vladimirova-Kitova L, Kratchanova M, Kratchanov C, et al. Antiatherogenic and cardioprotective effects of black chokeberry (Aronia melanocarpa) juice in aging rats. Evid based Complement Alternat Med. 2015; 2015. [PubMed]
35. Ciocoiu M, Badescu L, Miron A, Badescu M. The involvement of a polyphenol-rich extract of black chokeberry in oxidative stress on experimental arterial hypertension. Evid based Complement Alternat Med. 2013; 2013. [PubMed]
36. Borowska S, Brzóska MM. Chokeberries (*Aronia melanocarpa*) and Their Products as a Possible Means for the Prevention and Treatment of Noncommunicable Diseases and Unfavorable Health Effects Due to Exposure to Xenobiotics. Compr Rev Food Sci Food Saf. 2016; 15: 982-1017. [CrossRef]
37. Jurikova T, Mlcek J, Skrovankova S, Sumczynski D, Sochor J, Hlavacova I, et al. Fruits of Black Chokeberry Aronia melanocarpa in the Prevention of Chronic Diseases. Molecules. 2017; 22: E944. [CrossRef][PubMed]
38. M. Naruszewicz, Laniewska I, Millo B, Dłuzniewski M. Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infarction. Atherosclerosis. 2007; 194: e179-e184. [CrossRef][PubMed]
39. Hellstrom JK, Shikov AN, Makarova MN, Pihlanto MA, Pozharitskaya NO, Ryhanen E, et al. Blood pressure-lowering properties of chokeberry (*Aronia mitchurinii*, var. Viking). J Funct Foods. 2010; 2: 163-9. [CrossRef]
40. Broncel M, Kozirog-Kolacinska M, Adryskowski G, Duchnowicz P, Koter-Michalak M, Owczarczyk A, et al. Effect of anthocyanins from *Aronia melanocarpa* on blood pressure, concentration of endothelin-1 and lipids in patients with metabolic syndrome. Pol Merkur Lekarski. 2007; 23: 116-9. [PubMed]
41. Broncel M, Kozirog M, Duchnowicz P, Koter-Michalak M, Sikora J, Chojnowska-Jezierska J, et al. Aronia melanocarpa extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with methabolic syndrome. Med Sci Monit. 2010; 16: CR28-34. [PubMed]
42. Skoczynska A, Jedrychowska I, Poreba R, Affelska-Jercha A, Turczyn B, Wojakowska A, et al. Influence of chokeberry juice on arterial blood pressure and lipid parameters in men with mild hypercholesterolemia. Pharmacol Rep. 2007; 59:177-82.
43. Park, JM, Park JB. The preventive and therapeutic effects of Aronox extract on metabolic abnormality and hypertension. J Korean Soc Hyper. 2011; 17: 95-102. [CrossRef]
44. Kardum N, Milovanovic B, Savikin K, Zdunic G, Mutavdzin S, Gligorijevic T, Spasic S. Beneficial effect of polyphenol-rich chokeberry juice consumption on blood pressure level and lipid status in hypertensive subjects. J Med Food. 2015; 18: 1231-8. [CrossRef][PubMed]
45. Sikora J, Broncel M, Mikiciuk-Olasik E. *Aronia melanocarpa* Elliot reduces the activity of angiotensin I-converting enzyme – *in vitro* and *ex vivo* studies. Oxid Med Cell Longev. 2014; 2014: 739721. [CrossRef][PubMed]
46. Bell DA, Gochenaur K. Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. J Appl Physiol. 2006; 100: 1164-70. [CrossRef][PubMed]
47. Zapolska-Downar D, Bryk D, Małecki M, Hajdukiewicz K, Sitkiewicz D. *Aronia melanocarpa* fruit extract exhibits anti-inflammatory activity in human aortic endothelial cells. Eur J Nutr. 2012; 51: 563-72. [CrossRef][PubMed]

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HIPOTENZIVNI I ANTIOKSIDATIVNI EFEKTI POLIFENOLIMA BOGATOG SOKA ARONIJE (*ARONIA MELANOCARPA* [MICHX.] ELLIOTT)

Milica Milutinović[1], Suzana Branković[2], Katarina Šavikin[3], Gordana Zdunić[3], Milica Kostić[1], Bojana Miladinović[1], Dušanka Kitić[1]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za farmaciju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Katedra za fiziologiju, Niš, Srbija
[3]Institut za proučavanje lekovitog bilja "Dr Josif Pančić", Beograd, Srbija

Kontakt: Milica Milutinovic
Bulevar dr Zoran Đinđić 81, 18 000 Niš, Srbija
E-mail: milica.milutinovic@medfak.ni.ac.rs

Aronija (*Aronia melanocarpa* (Michx.) Elliott) se vekovima tradicionalno koristila u biljnoj medicini drevnih naroda Severne Amerike, s obzirom na brojne farmakološke aktivnosti koje ispoljava. Cilj ovog istraživanja bilo je ispitivanje efekata soka ploda aronije na aktivnost kardiovaskularnog sistema, kako bi se potvrdila upotreba soka kao funkcionalne hrane. Antioksidativna aktivnost soka procenjivana je pomoću DPPH metode i β-karoten / linolna kiselina model sistema. Potvrđena je značajna antioksidativna aktivnost soka aronije. Koncentracija soka aronije koja je inhibirala 50 % DPPH radikala (IC_{50}) bila je 1,25 ± 0,08 mg/ml. U β-karoten / linolna kiselina model sistemu IC_{50} vrednost iznosila je 1,73 ± 0,07 mg/ml. Efekti soka na krvni pritisak i frekvenciju srčanog rada ispitivani su na anesteziranim kunićima. Rezultati su pokazali smanjenje krvnog pritiska (EC_{50} vrednost bila je 195,63 ± 14,45 mg/kg, koncentracija koja je izazvala 50 % maksimalnog odgovora) i frekvencije srca (EC_{50} vrednost bila je 171,71 ± 11,21 mg/kg) kod kunića. Primena soka aronije može izazvati hipotenzivni i negativni hronotropni efekat. Neophodno je sprovesti studije na ljudima kako bi se ovi efekti i potvrdili.

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Ključne reči: *Aronia melanocarpa (Michx.) Elliott, sok aronije, antioksidativna aktivnost, krvni pritisak, frekvencija srčanog rada*

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DIFFERENTIAL DIAGNOSIS BETWEEN BULBOSPINAL MUSCULAR ATROPHY – KENNEDY'S DISEASE AND AMYOTROPHIC LATERAL SCLEROSIS

Elena Simeonovska-Joveva[1], Marija Karakolevska–Ilova[2], Stefan Petrovski[3]

Amyotrophic lateral sclerosis (ALS) includes a number of disorders causing degeneration of lower and upper motor neurons and findings in the bulbar region and at least two spinal regions or UMN and LMN in three spinal regions. ALS is typically presented with bulbar or asymmetric limb weakness, loss of ability to speak, to swallow and to breathe. Kennedy disease is a form of MND that is associated with bulbar involvement and X linked recessive inheritance. The symptoms include muscular cramps, a limb-girdle distribution of muscle weakness, bulbar symptoms and distinguishing clinical features include facial and perioral fasciculations in particular. This is a case study of a 43-year- old man who suffered from recurrent muscle cramping and progressive symmetric lower extremity weakness, with a prominent fatigable component to this weakness, shoulder weakness, difficulty swallowing and facial twitching. EMNG showed widespread reinnervation changes and fasciculations in arms, legs, tongue, and thoracic paraspinals, with minimal fibrillation potentials. Motor NCS were normal or borderline in amplitude, and sensory responses were absent in upper and lower limbs. Genetic testing was positive (45 CAG). Kennedy disease may be underdiagnosed, owing in part to misdiagnosis and to the mild symptoms exhibited by some patients. The electro-physiological examinations are the key point to the diagnosis of Kennedy disease. In our case, we found the symmetric weakness, sensory abnormalities on electrophysiological testing, prominent facial fasciculations, and gynecomastia which were not characteristic of the ALS, and indicated that was one of MND syndromes such as Kennedy disease and that was confirmed by genetic testing.

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Key words: *Kennedy disease, Amyotrophic lateral sclerosis, muscular atrophy, motor neuron disease*

[1]Clinical Hospital Štip, Department of neurology, Štip, RN Macedonia
[2]Clinical Hospital Štip, Department of oncology, Štip, RN Macedonia
[3]Clinical Hospital Štip, Department of surgery, Štip, RN Macedonia

Contact: Elena Simeonovska-Joveva
ASNOM 5, 2000 Štip, RN Macedonia
E-mail: elena.joveva@ugd.edu.mk

Introduction

The differential diagnosis of amyotrophic lateral sclerosis (ALS) includes a number of disorders causing degeneration of lower and upper motor neurons. It is important to consider these diagnoses because the prognosis is often better and in certain situations, specific treatments may be available. (1)

ALS is defined on clinical evidence and requires both upper motor neuron - UMN (such as spastic tone, hiperreflexia, and Babiski sign) and lower motor neuron - LMN (including muscle atrophy, fasciculations and weakness) and findings in the bulbar region and at least two spinal regions (cervical, thoracic, lumbosacral) or UMN and LMN in three spinal regions. A patient with ALS typically presents with bulbar or asymmetric limb weakness, more prominent in distal than in proximal muscles. Most patients with ALS will lose their ability to control their limbs, to speak, to swallow and to breathe (1). Without mechanical ventilation, death from respiratory failure typically ensues within 3 to 5 years of the onset of symptoms. The typical age of ALS onset is 60 years. Some patients have comorbid frontotemporal dementia and strong family history - familial ALS accounts for 3 to 10 % of all ALS forms while at least 90% have none -sporadic ALS. In recent years, 14 different genes have been identified that cause various types of motor neuron disease. The best evidence for this involves the protein TDP-43 in the intraneuronal cytoplasmatic protein aggregates of

SALS and in some forms of FALS. Another example, within FALS 1 has a mutation in SOD1 (2).

ALS continues to be diagnosed and followed almost entirely on the basis of clinical findings. There is no laboratory test to prove or disapprove the diagnosis. For patients with classic presentation routine chemistries, complete blood count, serum kinases , electrophysiologic testing, and imaging studies of the spine/brain are performed. More extensive laboratory testing should be reserved or more atypical presentations (pure UMN or LMN syndromes, the disease of early onset under 40 years of age or prolonged duration, coexistent systemic illness or the presence of sensory or urinary symptoms). Diagnostic CSF biomarkers may allow for improved diagnosis of ALS, may be leading to the ability to make diagnosis definitively in early cases. Routine testing for heavy metals in the serum or urine is not indicated unless there is a high suspicion for exposure. Another laboratory study that may support the diagnosis of ALS is muscle biopsy.

Multiple studies have shown that a multidisciplinary approach may prolong survival and improve the quality of life of patients with ALS (3). Only one drug has ever been shown to prolong survival in patients with ALS: riluzole. Riluzole affects neurons by three mechanisms: inhibiting excitatory amino acid release, inhibiting events after stimulation of excitatory amino acid receptors and stabilizing the inactivated state of voltage-dependent sodium channels (3). Palliative care and symptomatic therapy play an integral part in the management of patients with ALS. That included exercises, hydrotherapy, and the drugs baclofen, gabapentin, amitriptyline when there is comorbid sleep disturbance, depression or pseudobulbar affect (4).

The motor neuron disease (MND) that may present clinically with progressive dysfunction of motor neurons are included in the differential diagnosis ALS. MND includes the spectrum of clinical syndromes that result from degeneration of upper motor neurons, lower motor neurons or both. One of the syndromes is Kennedy disease.

Kennedy disease is a form of MND that is associated with bulbar involvement and X linked recessive inheritance (5).

The disease affects only males, usually at the beginning of the third or fourth decade of life. The initial symptoms include muscular cramps, a limb-girdle distribution of muscle weakness, and bulbar symptoms. Distinguishing clinical features include facial and perioral fasciculations, in particular, which are present in more than 85 % of patients, hand tremor, and tongue atrophy associated with a longitudinal midline furrow. There is no evidence of UMN involvement and sensory examination is typically normal. Other systemic manifestations include gynecomastia in 60-90 % of patients due to elevated gonadotropin levels associated with testicular atrophy, feminization, impotence, and infertility (6). Diabetes mellitus is seen in 10-20 % of patients. Genetic testing can be performed to confirm the presence of an abnormal trinucleotide repeat expansion (CAG) in the androgen receptor gene on the X chromosome. In healthy individuals, the repeats range from 17 to 26 in this coding area, whereas in Kennedy disease, the number of repeats ranges from 40 to 65. The number of the enlarged CAG repeats is significantly correlated with the age of onset but has no correlation with severity of weakness, degree of sensory neuropathy, of gynecomastia or impotence (7).

Materials and methods

This is a case study of a 43 - year-old man who was referred for a second opinion regarding the diagnosis of ALS. He reported recurrent muscle cramping since he was 25 and progressive symmetric lower extremity weakness 4 years ago. He noted a prominent fatigable component to this weakness, with worsening associated with prolonged use of the muscles. He had noted shoulder weakness, difficulty swallowing, and facial twitching. He denied a family history of neurologic illness.

The examination has shown bifacial weakness with continuous facial fasciculations, proximal extremity weakness, areflexia, and normal sensation. There was also weakness and atrophy of the tongue, mild nasal dysarthria, and significant axial weakness with lumbar lordosis. Postural tremor was noted in the upper extremities and frequent fasciculations were observed in the arms and legs. He had gynecomastia.

Serum CK level was elevated at 1800 IU/L. EMNG showed widespread reinnervation changes and fasciculations in arms, legs, tongue, and thoracic paraspinals, with minimal fibrillation potentials. Motor NCS were normal or borderline in amplitude, and sensory responses were absent in upper and lower limbs. SEP (n. medianus) showed that cortical responses and spinograms are morphologically severe altered indicating a defect in the conduction in the central and peripheral roads SS bilaterally. SEP (n. tibialis) showed morphologically altered cortical responses with prolonged latencies. Spinograms were low volted with prolonged latencies. The finding suggests a defect in the conduction in the central and peripheral roads SS bilaterally.

Muscle biopsy showed inequality in the size of muscle fibers due to the presence of atrophic fibers with elongated, angular and rounded contours that meet in small groups. Increased core presents with centralization and rare fibers under phagocytosis. Interstitially, connective tissue was neat and vessels were with a neat wall and lumen. Genetic testing was positive for an expanded allele in CAG repeat region of the androgen receptor gene (45 CAG). MRI of the brain and spine were normal.

Conclusion and discussion

Kennedy's disease, also known as spinal and bulbar muscular atrophy (SBMA), is a rare, adult-onset, X-linked recessive neuromuscular disease with an estimated incidence of approximately 1 case in 40,000 men, but the general impression is that Kennedy disease may be underdiagnosed, owing in part to misdiagnosis and to the mild symptoms exhi-

bited by some patients. SBMA is caused by expansion of a CAG repeat sequence in exon 1 of the androgen receptor gene (AR) encoding a polyglutamine (polyQ) tract. The polyQ-expanded AR accumulates in nuclei, and initiates degeneration and loss of motor neurons and dorsal root ganglia. While the disease has long been considered a pure lower motor neuron disease, recently, the presence of major hyper-creatine-kinase (CK)-emia and myopathic alterations on muscle biopsy has suggested the presence of a primary myopathy underlying a wide range of clinical manifestations (8).

The electrophysiological examinations are the key point to the diagnosis of Kennedy disease. Electroneurography shows normal conduction velocity in peripheral nerves, but the sensory nerves usually show axonal degeneration, which causes only very mild or subclinical neurological deficits. Electromyography shows chronic anterior horn cell degeneration in skeletal muscles. The molecular genetic diagnosis was introduced in 1991 when on the abnormal expansion of CAG repeat was found in the first exon of the androgen receptor gene on chromosome X with a frequency of 100 % in the affected population. Since the progression is very slow and these patients can expect a normal life span, it is essential to this syndrome from other, often more severe diseases, such as ALS.

In our case, we found symmetric weakness, sensory abnormalities on electrophysiological testing, prominent facial fasciculations, and gynecomastia which were not characteristic of the ALS, and indicated that is one of MND syndromes such as Kennedy disease and that was confirmed by genetic testing. Various endocrine abnormalities including decreased fertility and gynecomastia are common and among the first features of KD, what was the finding in our case also.

Animal models of KD have demonstrated improvement on withdrawal of testosterone, indicating that this agonist of the androgen receptor is required for the toxic effect. Potential therapies based on testosterone withdrawal in humans have shown some promise, but efficacy remains to be proven (9).

Androgen deprivation, to gene silencing, an expanding repertoire of peripheral targets, including muscle and advancement of these strategies into the clinic, can be reasonably anticipated that the landscape of treatment options for SBMA and other neuromuscular conditions will change rapidly in the near future (10).

To date, the abnormal expansion of CAG repeat has been identified to cause nine neurodegenerative diseases including SBMA, Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA) and six forms of spinocerebellar ataxia (11). Although the causative gene varies with each disease, these polyglutamine disorders share common pathways of molecular pathogenesis, such as accumulation of abnormal proteins, transcriptional dysregulation and disruption of axonal transport (12). Additionally, several lines of evidence suggest that mitochondrial impairment and elevated oxidative stress are also implicated in the pathogenesis of Kennedy's disease (13).

Despite several therapeutic attempts made in mouse models, no effective disease-modifying therapy has been available yet, although symptomatic therapy is beneficial for the management of the weakness, fatigue and bulbar symptoms (8). The disease typically lasts at least 2-3 decades and life expectancy does not appear to be compromised (14).

References

1. Goutman SA. Diagnosis and Clinical Management of Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders. Continuum (Minneap Minn) 2017; 23(5): 1332-59. [PubMed] [CrossRef]
2. Van Damme P, Robberecht W, Van Den Bosch L. Modelling amyotrophic lateral sclerosis: progress and possibilities. Dis Model Mech 2017; 10(5):537-49. [PubMed] [CrossRef]
3. Landfeldt E, Edström J, Lindgren P, Lochmüller H.J. Patient Preferences for Treatments of Neuromuscular Diseases: A Systematic Literature Review. J Neuromuscul Dis 2017; 4(4):285-92. [PubMed] [CrossRef]
4. Ng L, Khan F, Young CA, Galea M. Symptomatic treatments for amyotrophic lateral sclerosis/motor neuron disease. Cochrane Database Syst Rev 2017; 1: CD011776. [PubMed] [CrossRef]
5. Querin G, Sorarù G, Pradat PF. Kennedy disease (X-linked recessive bulbospinal neuronopathy): A comprehensive review from pathophysiology to therapy. Rev Neurol 2017; 173(5):326-37. [PubMed] [CrossRef]
6. Szabó A, Mechler F. Kennedy syndrome-bulbo-spinal muscular atrophy. Ideggyogy Sz 2002; 55(9-10):323-9. [PubMed]
7. Finsterer J, Soraru G. Onset Manifestations of Spinal and Bulbar Muscular Atrophy (Kennedy's Disease). J Mol Neurosci 2016; 58(3):321-9. [PubMed] [CrossRef]
8. Querin G, Sorarù G, Pradat PF. Kennedy disease (X-linked recessive bulbospinal neuronopathy): A comprehensive review from pathophysiology to therapy. Rev Neurol 2017; 173(5):326-37. [PubMed] [CrossRef]
9. Zajac JD, Fui MN. Kennedy's disease: clinical significance of tandem repeats in the androgen receptor. Adv Exp Med Biol 2012; 769:153-68. [PubMed] [CrossRef]
10. Pennuto M, Rinaldi C. From gene to therapy in spinal and bulbar muscular atrophy: Are we there yet? Mol Cell Endocrinol 2018; 465:113-21. [PubMed] [CrossRef]
11. La Spada AR, Taylor JP. Repeat expansion disease: progress and puzzles in disease pathogenesis. Nat Rev Genet 2010; 11:247-58. [PubMed] [CrossRef]
12. Beitel LK, Alvarado C, Mokhtar S, Paliouras M, Trifiro M. Mechanisms mediating spinal and bulbar muscular atrophy: investigations into polyglutamine-expanded androgen receptor function and dysfunction. Front Neurol 2013; 4:53. [PubMed] [CrossRef]
13. Ranganathan S, Harmison GG, Meyertholen K, Pennuto M, Burnett BG, Fischbeck KH. Mitochondrial abnormalities in spinal and bulbar muscular atrophy. Hum Mol Genet 2009; 18:27-42. [PubMed] [CrossRef]
14. Finsterer JJ. Perspectives of Kennedy's disease. Neurol Sci 2010; 298(1-2):1-10. [PubMed] [CrossRef]

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DIFERENCIJALNA DIJAGNOZA SPINOBULBARNE MIŠIĆNE ATROFIJE - KENEDIJEVE BOLESTI I AMIOTROFIČNE LATERALNE SKLEROZE

Elena Simeonovska-Joveva[1], Marija Karakolevska–Ilova[2], Stefan Petrovski[3]

[1]Klinička bolnica Štip, Departman za neurologiju, Štip, Republika Severna Makedonija
[2]Klinička bolnica Štip, Departman za onkologiju, Štip, Republika Severna Makedonija
[3]Klinička bolnica Štip, Departman hirurgije, Štip, Republika Severna Makedonija

Kontakt: Elena Simeonovska-Joveva
ASNOM 5, 2000 Štip, RS Makedonija
E-mail: elena.joveva@ugd.edu.mk

Amiotrofična lateralna skleroza (ALS) uključuje višestruke poremećaje koji uzrokuju degeneraciju gornjeg i donjeg motoričnog neurona kao i promene u bulbarnom regionu i u najmanje dva spinalna područja ili GMN i DMN u tri spinalna područja. ALS se obično manifestuje bulbarnom ili asimetričnom slabošću ekstremiteta, gubitkom govorne sposobnosti, gutanja i disanja. Kenedijeva bolest je oblik MNB koji je povezan sa bulbarnim oštećenjem i X recesivnim naslednim oboljenjem. Simptomi uključuju mišićne spazme, mišićnu slabost ekstremiteta, bulbarne simptome, a klinički simptomi uključuju facijalnu i posebno perioralnu fascikulaciju. Ovo je studija slučaja 43-godišnjeg čoveka sa rekurentnim mišićnim spazmama i progresivnom simetrijskom slabošću donjih ekstremiteta, praćen umorom, slabošću ramenog pojasa, teškoćama gutanja i grčevima lica. EMNG je pokazao promene u inervaciji i fascikulaciji ruku, nogu, jezika i torakalno paraspinalno sa minimalnim potencijalima na fibrilaciju. Motorna brzina sprovodljivosti bila je normalna do granice amplitude, sa odsustvom senzornog odgovora gornjih i donjih ekstremiteta. Genetičko testiranje bilo je pozitivno (45 CAG). Kenedijeva bolest može da bude subdijagnostifikovana, kao rezultat propuštene dijagnoze ili kao rezultat blagih simptoma kod bolesnika. Najvažniji deo dijagnoze su elektrofiziološki pregledi. U našem slučaju pokazale su se simetrična slabost, senzorne abnormalnosti u elektrofiziološkom testiranju, facijalne fascikulacije i ginekomastija, koje nisu karakteristične za ALS, što zauzvrat ukazuje na to da se radi o nekom od MNB sindroma kao što je Kenedijeva bolest, što je potvrđeno genetskim testiranjem.

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Ključne reči: *Kenedijeva bolest, amiotrofična lateralna skleroza, atrofija mišića, bolest motornog neurona*

Case report

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ASYMPTOMATIC "GIANT" PHEOCHROMOCYTOMA DISCOVERED AS ADRENAL INCIDENTALOMA-CASE REPORT AND LITERATURE REVIEW

Danijela Radojković[1,2], Milan Radojković[1,3]

Adrenal gland tumours, revealed during radiological procedures in patients without previous suspicion for adrenal disease, are known as adrenal incidentalomas (AI). Asymptomatic pheochromocytoma-incidentaloma is usually smaller than 10 mm. Incidentally found large pheochromocytoma without any clinical signs is the rarity.

Herein, a young woman is presented, with a tumour in the right adrenal gland, size 60x70 mm, discovered on the abdominal sonogram, performed during a regular systematic examination. Computed tomography confirmed "giant" tumour, size 70x74 mm, with cystic and necrotic areas and inhomogeneous contrast captivity. Besides discrete elevated vanillymandelic acid (VMA) level in 24 hours dieresis, all the results of the endocrine evaluation were in normal range. The patient underwent [131I]-meta-iodobenzylguanidine ([131I]-MIBG) scintigraphy which was indicative for right pheochromocytoma. After adequate preoperative preparation, right adrenalectomy was performed. The procedure and postoperative course went without complications, and pheochromocytoma was confirmed by histopathological examination.

It appears that frequency of AI is constantly rising in the last few decades, thanks to widely used radiological diagnostic techniques. Even though the most of AI are nonfunctional, we should always keep in mind that under clinical "mute" adrenal tumours, malignant or secreting lesions could hide. A thorough examination of each incidentally revealed adrenal mass can prevent potential oversight and provide proper treatment.

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Key words: *asymptomatic pheochromocytoma, incidentaloma*

[1]University of Niš, Facultyof medicine, Niš, Serbia
[2]Clinic of Endocrinology, Diabetes and Metabolic Disorders, Clinical Centre Niš, Niš, Serbia
[3]Surgery Clinic, Clinical Centre Niš, Niš, Serbia

Contact: Danijela Radojković
Sestre Baković 14/28, 18000 Niš, Serbia
E-mail: dr.danijela3@gmail.com

Introduction

Tumours localised in adrenal gland, revealed during radiological procedures in patients without previous suspicion for adrenal disease are known as adrenal incidentalomas (AI).

Along with technology advances and frequent radiological imaging, detection of AI is significantly increased (1). After diagnosed AI, two crucial tasks are:

1. to distinguish functional from nonfunctional adrenal tumour and

2. to conclude whether it is benign or malignant one. Usually, AI are hormonal inactive adenomas (74-82.2 %), followed by Cushing syndrome (CS 6-7 %), pheochromocytoma (4.7-7.2 %), aldosteronomas (1.2-4.6 %), adrenocortical carcinoma (ACC 4.8 %), and metastatic lesions (2.3 %) (2, 3).

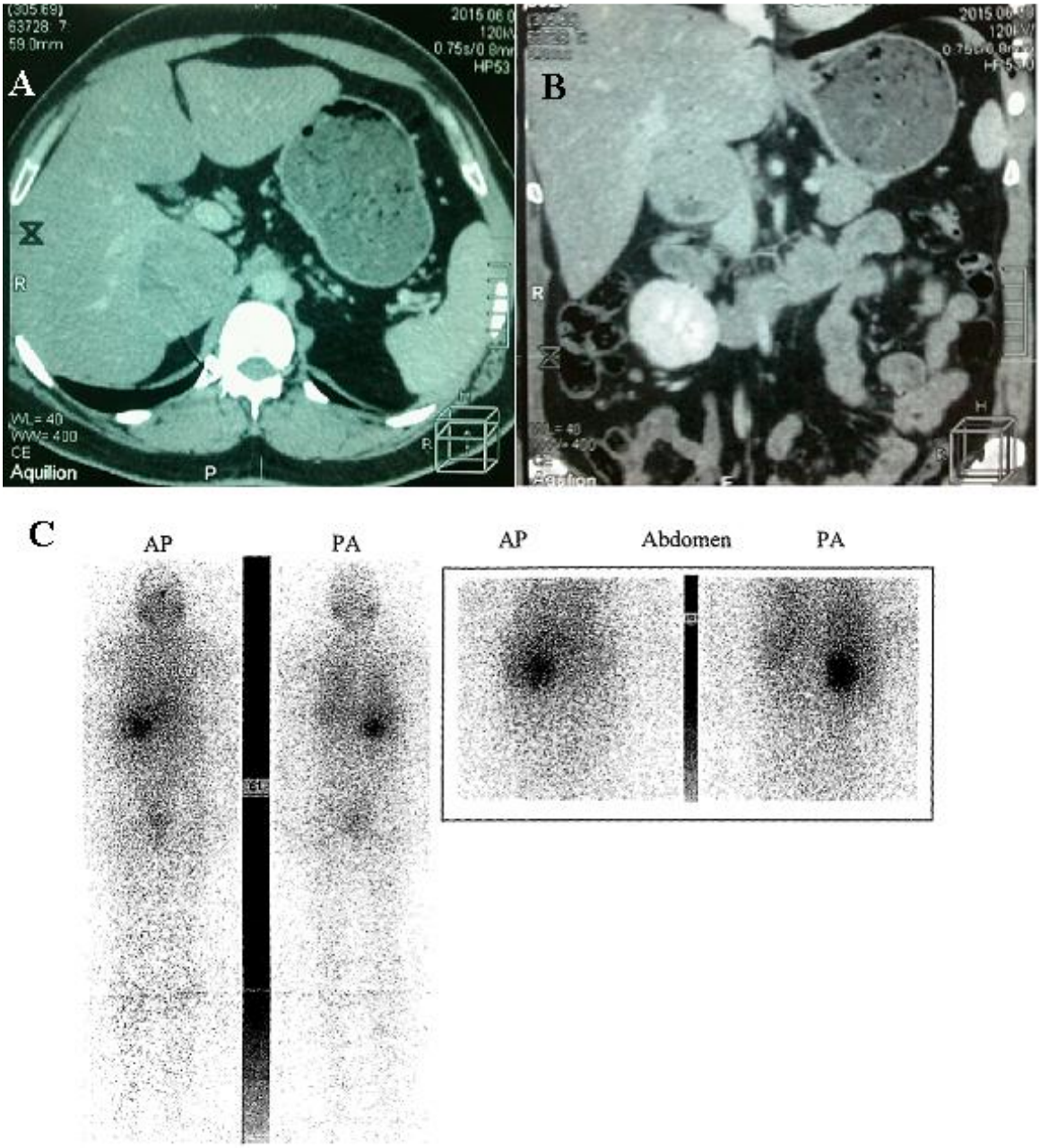
Pheochromocytomas are tumours that arise from the adrenal medulla. The most common localization is the abdomen, where adrenal glands are target places for 90 % of these tumours, usually unilateral. Since the majority of pheochromocytomas produce and secrete catecholamines, hallmarks of clinical presentation are symptoms associated with catecholamine excesses like hypertension, headache, sweating, tachycardia, pallor and panic attacks. Therefore, each incidentally revealed adrenal mass should be thoroughly examined and screened for pheochromocytoma.

Herein, we present a young woman with clinically "silent" pheochromocytoma and discuss diagnostic procedures along with treatment.

The patient has given informed consent that all data about her diagnostic procedures and treatment could be collected and published. All procedures performed in the study were in accordance with the ethical standards of the institutional and national research committee.

Patient case report

During regular systematic examination in a young woman (32 years old) an abdominal sonogram was performed, which revealed the right adrenal mass, size 60x70 mm. Computed tomography (CT) confirmed "giant" tumour, size 70x74 mm, with cystic and necrotic areas and inhomogeneous contrast captivity (Figure 1A and 1B).



A. CT scan of right adrenal tumour, transverse plane. B. CT scan of right adrenal tumour, coronal plane.
C. [131I]-MIBG scan of right adrenal pheochromocytoma

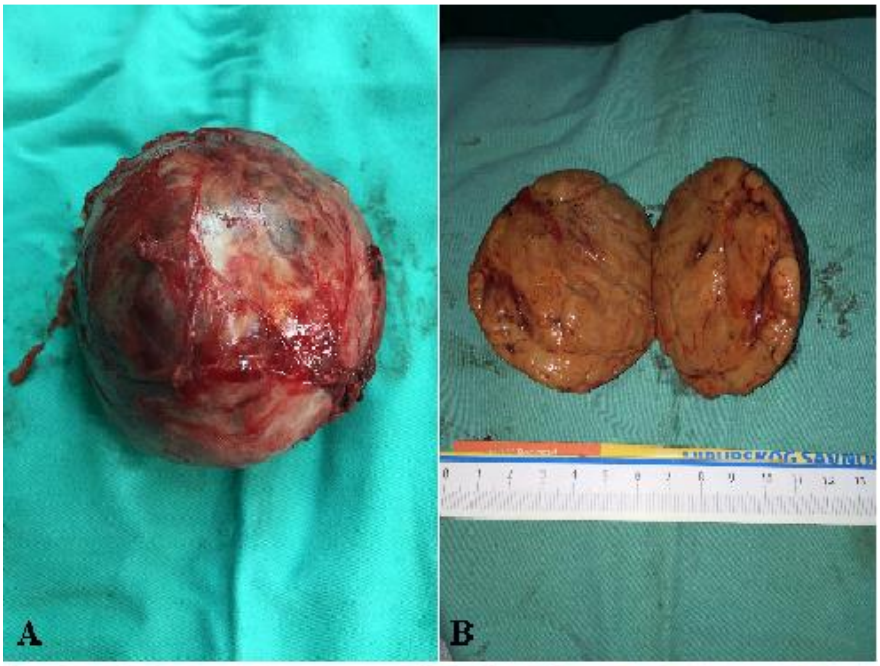
Figure 1. Visualization diagnostic procedures

She was admitted to Endocrinology Clinic, Clinical Centre, Niš for further examination. The patient's medical history of any relevant illnesses, including hypertension, was negative. Even though physical examination reviled obesity (BMI 32) body weight was stable, without weight gain. There were no symptoms and signs such as hirsutism, purple striae, hair loss, or oedema. The blood pressure (120/80 mmHg) heart rate (80/min) and respiratory rate (18/min) were normal. Family history was positive for diabetes mellitus type 2, hypertension and obesity (patient's parents). Initial laboratory testing (blood cell count, electrolytes, urea, creatinine, hepatogram, amylase, lipids, and glycaemia) was normal, except for increased fasting plasma glucose (6.4 mmol/l). Performed oral glucose tolerance test (OG-TT) revealed impaired glucose tolerance (IGT) with hyperinsulinemia in the second hour of the OGTT (plasma glucose level was 8.4 mmol/l and insulin concentration was 172.7 uU/ml; reference value: 2.6-24.90 uU/ml). Further endocrine evaluation included plasma cortisol assay, adrenocorticotropic hormone (ACTH), plasma catecholamines (adrenaline, noradrenaline and dopamine), 24 hours urinary catecholamine metabolite (vanillymandelic acid – VMA), sodium, potassium, calcium, phosphorus, parathyroid hormone (PTH), calcitonin, chromogranin A (CgA), thyroid stimulating hormone (TSH) and insulinemia during OGTT. Beside discrete elevated VMA level, all the results of the endocrine evaluation were in normal range and shown in Table 1. Basal cortisol level was significantly suppressed after low-dose dexamethasone (1 mg) test (cortisol level was 470.2 nmol/l before DST and 52 nmol/l after; reference range 150-638 nmol/l).

Table 1. Initial endocrine evaluation

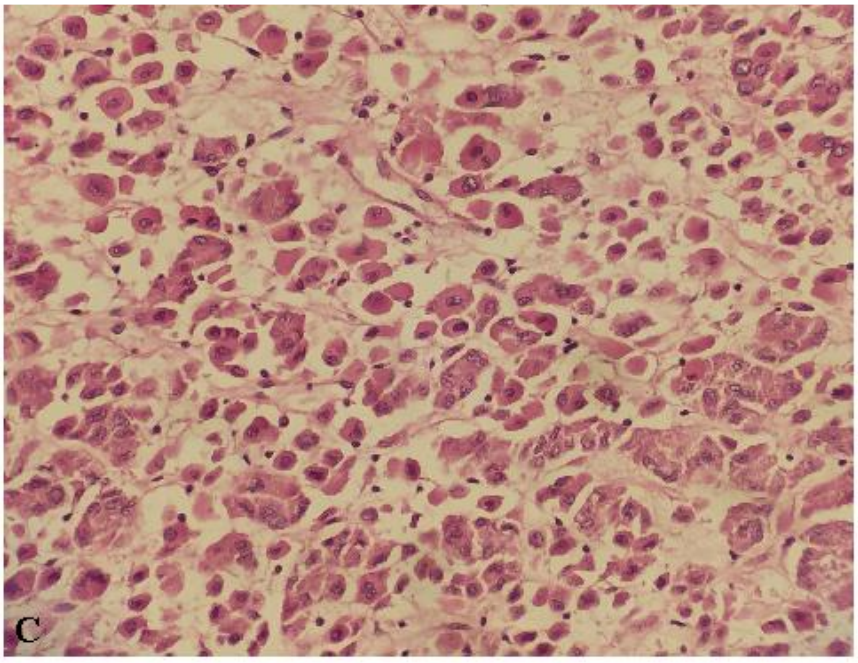
Parameter	Result	Reference range
Plasma Cortisol at 08, 16 and 23h (nmol/L)	470.2/ 225.6/ and 96.0	150 - 638, 80 - 388
VMA (µmol/D24h)	68.7	0 - 68.6
plasmaAdrenalin (pg/mL)	78.0	<100
plasmaNoradrenalin (pg/mL)	523.3	<600
plasmaDopamine (pg/mL)	102.7	<100
ACTH (pg/mL)	31.52	7.2 - 63.3
PTH (pg/mL)	42	8 - 76
TSH (mIU/L)	4.296	0.4 - 4.60
Calcium (mmol/L)	2.43	2.2 – 2.65
Phosphorus (mmol/L)	0.98	0.80 – 1.55
Potassium (mmol/L)	4.3	3.5 – 5.5
Calcitonin (pg/mL)	0.1	<10
Chromogranin (ng/mL)	12.0	19 - 98

Further investigation included [131I]-meta-iodobenzylguanidine ([131I]-MIBG) scintigraphy which was indicative for right adrenal pheochromocytoma. (Figure 1C). According to the institution protocol, which we reported in our previous publication standard preoperative preparation was started with 10mg phenoxybenzamine daily (4). The dose was gradually increased up to 60 mg (30 mg two times a day). Following two weeks, during thorough blood pressure monitoring, nor hypertension, neither orthostatic hypotension was verified. Performed ECG and echocardiography were normal. Right adrenalectomy was performed, with no significant change in blood pressure during the procedure. The tumour was firm and well encapsulated. Areas of haemorrhages and necrosis within the tumour, typical for larger pheochromocytomas, gave spotted and dark red appearance. There were no intraoperative signs of tumour growing into nearby areas or spreading to lymph nodes. The surgical diagnosis was localized benign adrenal tumour, highly suspicious of pheochromocytoma (Figure 2 A and B). The procedure and postoperative period went without complications, and pheochromocytoma was confirmed by histopathological examination (Figure 2C).



A. Adrenal gland with the tumour. B. Intersected adrenal gland with tumour

Figure 2A, 2B. Macroscopic and histopathological findings



C. Histopathological examination: pheochromocytoma

Figure 2C. Macroscopic and histopathological findings

Discussion

A number of newly diagnosed patients with AI is growing along with technological advances and available radiology imaging for various reasons. Once verified, AI requires appropriate diagnostic approach. Biochemical and endocrine evaluation should reveal if the tumour has hormonal activity, and further imaging examination will estimate its size and potential malignancy. Based on these features, the decision about surgical removal should be made.

Imaging examination

Abdominal ultrasonography (USG) is usually the first radiology procedure which discovers AI. Enlargement of adrenal glands greater than 2 cm can be detected by USG, but this radiological technique cannot characterize adrenal lesions with high precision (5). Based on the USG findings adrenal tumour was suspected in our patient and CT was performed. This radiological method is much more informative than USG. Unenhanced adrenal CT can provide data about tumour features such as size, shape, radiation attenuation coefficient (in Hounsfield units - HU) and structure homogeneity. Benign tumours are usually smaller than 4cm, with smooth borders, which are well-distinguished from adjacent organs and attenuation value less than 10 (6). Higher attenuation values (30-40 HU) due to low lipid component are indicative for pheochromocytoma or ACC, as well as heterogeneous cystic areas caused by necrosis and/or bleeding (1, 7). Adrenal CT with contrast administration assesses the absolute and relative washout values, features of the surrounding tissues infiltration, as well as the presence of liver metastases. Adenomas attenuation coefficient will return quickly to basic values, ACC attenuation coefficient will remain elevated and pheochromocytoma may present various washout characteristics (7). In our patient, after contrast administration, CT verified solid tumour, with well-defined borders, localized in right suprarenal gland, size 70x74 mm, with inhomogeneous contrast captivity which could be the result of necrosis and cystic degeneration, pathognomonic for pheochromocytoma.

Hormonal examination

It is a common opinion that incidentally found pheochromocytomas, size less than 1 cm, are usually clinically "mute". Even though our patient had "giant" adrenal tumour highly suspicious of pheochromocytoma on CT, there were no clinical symptoms or signs whatsoever. A possible explanation for this discrepancy could be the fact that large tumours gain in the size due to haemorrhagic necrosis and cystic formation and not always due to tissue that can produce catecholamines.

Recommended diagnostic procedures for pheochromocytoma always include measurements of urinary or plasma metanephrines (8). These tests have higher sensitivity and specificity than measurement of unfractionated catecholamines, since pheochromocytomas may secret catecholamines episodically, but its degradation will be continuous. Unfortunately, those tests are not available in our institution.

Determination of the VMA is an old test, easy to perform, with specificity that goes up to 95 %. On the other hand, it has low sensitivity (64 %) which why it is no longer recommended for pheochromocytoma screening (5). However, increased VMA concentration in our patient, along with CT presentation, was helpful to be more certain that this AI was pheochromocytoma, even though serum unfractionated catecholamines were normal.

It is still unclear why some pheochromocytomas are "silent". It is possible that chronically elevated catecholamine levels lead to down regulation of the adrenergic receptors making them more tolerable. Also, besides catecholamines, pheochromocytomas may produce a variety of biologically active peptides. The majority will cause vasoconstriction such as neuropeptide Y, but some will show vasodilatation effects, which will prevent hypertension (6).

In the last decade CgA has become valuable diagnostic test for pheochromocytoma with reported sensitivity and specificity from 74 % - 96 % (9). False positive results could appear in patients with atrophic gastritis type A, in chronic therapy with proton pump inhibitors or H2 receptor blockers, or in the patients with prostate cancer and neuroendocrine tumours (9). More important is an awareness that CgA could be normal or false negative in patients with pheochromocytoma as it was in our case. Chromogranin is known to inhibit catecholamine secretion by the adrenal medulla. The CgA level correlates with catecholamine level during sympathetic or adrenal medulla stimulation, but it is not significant at rest and may be afflicted by various tissue factors (10). If we assume that clinically silent pheochromocytoma is due to the absence of increased catecholamine production and secretion, or presence of vasodilatation components produced in the same tumor, it is possible that silent pheochromocytoma produces smaller amounts of CgA.

Screening tests for multiple endocrine neoplasias (MEN) were also in the referent range (calcitonin and PTH). Cortisol-producing adenoma was excluded since cortisol levels, ACTH concentration and overnight DST were normal. Plasma aldosterone concentration and plasma renin activity were not determined, because potassium levels were in referent range.

Scintigraphy imaging

A further diagnostic procedure included [131I]-MIBG scan. It is useful for determining if the adrenal lesion is pheochromocytoma, to screen patients for metastases and to confirm if the extra-adrenal mass is paraganglioma or neuroblastoma. On the other hand, [131I]-MIBG can accumulate in normal adrenal medulla making the false-positive diagnosis of the pheochromocytoma (11). In our patient, together with characteristic CT findings and elevated VMA concentration MIBG was a very useful tool.

Histopathological examination

Histopathological examination of the specimen confirmed pheochromocytoma composed of large cells that are pink to mauve, arranged in cords or nests with capillaries in between (HE x 200).

Conclusion

Hormonally inactive asymptomatic pheochromocytomas are very rare. Even though diagnostic algorithms are useful, physicians should always keep in mind that clinical signs could be absent and diagnostic tests false negative. Pheochromocytoma diagnosis should be made as stratification of all available radiological and hormonal tests in order to prevent potential oversight and provide proper treatment.

References

1. Young WF Jr. Clinical practice. The incidentally discovered adrenal mass. N Engl J Med 2007; 356:601-10. [PubMed] [CrossRef]
2. Andoulakis II, Kaltsas G, Piaditis G, Grossman AB. The clinical significance of adrenal incidentalomas. Eur J Clin Invest 2011; 41: 552-60. [PubMed] [CrossRef]
3. Kim J, Bae KH, Choi YK, Jeong JY, Park KG, Kim JG, et al. Clinical Characteristic for 348 patients with adrenal incidentaloma. Endocrinol Metab 2013; 28:20-5. [PubMed] [CrossRef]
4. Radojkovic D, Stojanovic M, Pesic M, Radojkovic M, Radenkovic S, Radjenovic Petkovic T, et al. Clinically „silent" giant pheochromocytoma, case report. Acta Endocrinol (Buc) 2013; 9(1):121-30. [CrossRef]
5. Kanagarajah P, Ayyathurai R, Manoharan M, Narayanan G, Kava BR. Current concepts in the management of adrenal incidentalomas. Urology Ann 2012; 4(3): 137-44. [PubMed] [CrossRef]
6. Kasperlik-Załuska AA, Otto M, Cichocki A, Rosłonowska E, Słowinska-Srzednicka J, Zgliczyński W, et al. 1161 patients with adrenal incidentalomas: indications for surgery. Langenbecks Arch Surg 2008; 393(2): 121-6. [CrossRef]
7. Sugawaea T, Hashimoto T, Owada M, Kawamura M. CT features of early pheochromocytoma. Inte Med 2011; 50:787. [PubMed] [CrossRef]
8. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol 2016; 175(2): 1-34. [PubMed] [CrossRef]
9. Unger N, Hinrichs J, Deutschbein T, Schmidt H, Walz MK, Mann K, et al. Plasma and Urinary Metanephrines Determined by an Enzyme Immunoassay, but not Serum Chromogranin A for the Diagnosis of Pheochromocytoma in Patients with Adrenal Mass. Exp Clin Endocrinol Diabetes 2012; 120(8):494-500. [PubMed] [CrossRef]
10. Louthan O. Chromogranin A in physiology and oncology. Folia Biologica (Praha) 2011; 57:173-81.
11. Ilias I, Pacak K. Current approaches and recommend ed algorithm for the diagnostic localization of pheochromocytoma. J Clin Endocrinol Metab 2004; 89: 479-91. [PubMed] [CrossRef]

Prikaz bolesnika

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ASIMPTOMATSKI "GIGANTSKI" FEOHROMOCITOM OTKRIVEN KAO ADRENALNI INCIDENTALOMA – PRIKAZ PACIJENTA I PREGLED LITERATURE

Danijela Radojković[1,2], Milan Radojković[1,3]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Klinika za endokrinologiju, dijabetes i bolesti metabolizma, Klinički centar Niš, Niš, Srbija
[3]Hirurška klinika, Klinički centar Niš, Niš, Srbija

Kontakt: Danijela Radojković
Sestre Baković 14/28, 18000 Niš, Srbija
E-mail: dr.danijela3@gmail.com

Adrenalni incidentalomi (AI) definišu se kao tumori otkriveni radiološkim procedurama kod bolesnika kod kojih prethodno nije postojala sumnja na bolest nadbubrežnih žlezdi. Asimptomatski feohromocitom, otkriven na ovaj način, obično je manji od 10 mm. Slučajno otkrivanje feohromocitom većih dimenzija, koji je uz to bez ikakvih kliničkih znakova, prava je retkost.

U ovoj studiji prikazana je mlada bolesnica sa tumorom desne nadbubrežne žlezde, promera 60x70 mm, otkrivenim ultrazvučnim pregledom abdomena u okviru sistematskog pregleda. Kompjuterizovanom tomografijom (CT) potvrđeno je prisustvo "gigantskog" tumora, veličine 70x74 mm, sa cističnim i nekrotičnim poljima i nehomogenom distribucijom kontrasta. Sem diskretno povišene koncentracije vanilmandelične kiseline (VMA) u 24-časovnom urinu, svi ostali rezultati endokrinološkog ispitivanja bili su u referentnom opsegu. Urađena je [131J] MIBG scintigrafija ([131J] meta-jod-benzil-gvanidin) koja je išla u prilog feohromocitoma desne nadbubrežne žlezde. Nakon adekvatne preoperativne pripreme, urađena je desnostrana adrenalektomija. Sama operacija i postoperativni tok protekli su uredno, a histopatološkim ispitivanjem potvrđen je feohromocitom.

Veća učestalost AI, koja se beleži u poslednjih nekoliko decenija, posledica je široke rasprostranjenosti brojnih radioloških dijagnostičkih tehnika. Iako je većina AI nefunkcionalna, uvek treba imati na umu da se iza klinički "nemog" nadbubrežnog tumora može kriti maligni process ili hormonski aktivan tumor. Detaljno ispitivanje svakog AI sprečiće previde ovakve vrste, a bolesniku obezbediti adekvatnu terapiju.

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Ključne reči: *asimptomatski feohromocitom, incidentalom*

Review article

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CLINICOPATHOLOGIC ANALYSIS OF "IDIOPATHIC" SCLERITIS AND SCLERITIS ASSOCIATED WITH RHEUMATHOD ARTHRITIS -MINI REVIEW

Jasmina Djordjević-Jocić[1,2], Ljubinka Janković-Veličković[1,3], Sonja Cekić[1,2], Marija Radenković[1,2], Maja Živković[1,2]

Scleritis is a chronic, painful, and potentially blinding inflammatory disease that is characterized by edema and cellular infiltration of the sclera tissues. It can occur as isolated ("idiopathic") or associated with systemic immune-mediated diseases of connective tissue. We present two different cases of scleritis: one case was that of a female patient with diffuse "idiopathic" scleritis, and the other – that of a female patient with nodular scleritis associated with rheumatoid arthritis. Both patients underwent detailed clinical, laboratory, and immune examinations, as well as the pathohistological analysis of the biopsy sample. The first patient had all laboratory tests within normal limits. The pathomorphological substrate of diffuse scleritis showed considerable edema of the episcleral and scleral tissues, the presence of inflammatory infiltrates with abundant lymphoid cells, histiocytes, accompanied by active hyperemia of capillaries with unusual relationships of blood vessels of the sclera and episclera. The second patient had nodular scleritis associated with rheumatoid arthritis. The pathomorphological substrate of sclera showed multiple foci of mononuclear infiltration with the domination of lymphocytes, the inner zone of polymorphonuclears and histiocytes, epithelioid and foreign body type giant cells and the outer zone of lymphocytes and plasma cells. Compared to the normal sclera, the number of inflammatory cells was 10-15 times elevated in scleritis with the domination of lymphocytes and plasma cells.

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Key words: *scleritis, rheumatoid arthritis, scleromalacia perforans*

[1]University of Nš, Faculty of medicine, Niš, Serbia
[2]Ophthalmology Clinic, Clinical Center Niš, Niš, Serbia
[3]Centre of Pathology, Clinical Center Niš, Niš, Serbia

Contact: Jasmina Djordjević-Jocić
Bulevard dr Zoran Djindjić 81, Niš, Serbia
E-mail:jdjordjevic.jocic@gmail.com

Introduction

Scleritis is a chronic, painful, and potentially blinding inflammatory disease characterized by edema and cellular infiltration of the sclera tissues (1-4). It can occur as isolated ("idiopathic") or associated with systemic immune-mediated diseases of connective tissue (Rheumatoid arthritis-RA, Systemic lupus erythematosus-SLE, Wegner's granulomatosis, polyarteritis nodosa-PAN) (5-7). RA is the most common systemic disease associated with scleritis. The classification system first proposed by Watson and Hareh is still used today: I - anterior scleritis: diffuse, nodular, necrotizing (with inflammation), and scleromalacia perforans (without inflammation) and II - posterior scleritis (1, 2). Pain and erythema are the most characteristic subjective symptoms. Pain is severe and it is intensified during the eye movement and reading, thus making any work impossible. Most cases of scleritis (diffuse, nodular, necrotizing) affect the front part of the sclera. A special characteristic of scleritis is its recurrence. The diffuse form is described in the literature as more frequently bilateral, while the nodular one as more frequently unilateral. In literature, it can be found that nodular scleritis is more frequent in patients with rheumatoid arthritis than in patients with other systemic immune-mediated diseases (5, 6). Precise pathogenesis has not been discovered yet. The presence of microangiopathy in most cases of scleritis indicates an associated immuno-complex reaction – III-type of hypersensitive reaction, and partly the influence of the late IV-type of the hypersensitive reaction. Recent studies show a clear morphological difference between substrates in the analyzed forms of scleritis. In the diffuse form of scleritis, a strong infiltration of lymphoid cells is present.

In the nodular type of scleritis, the pathohistological finding has a formation similar to or even the same as nodules in rheumatoid arthritis with a granulomatous inflammatory reaction.

Materials and methods

This paper presents the case studies of two female patients: I – N.Z., who had diffuse scleritis of unknown etiology and II – G.M., who had nodular scleritis along with rheumatoid arthritis. Both patients underwent detailed clinical, laboratory, immune examinations as well as the pathological analysis of the biopsy material. The ophthalmological examination included: visual acuity by Snellen signs, biomicroscopy of the anterior segment, applanation tonometry and indirect ophthalmoscopy. The rheumatological examination included relating to the general and local clinical findings as well as standard biohumoral and radiological examinations. The RA diagnosis was consistent with the criteria of the American College of Rheumatology.

Table 1. Diagnostic laboratory testing and scleritis

Laboratory test	Identified condition
CBC	Non-specific: infection, tumor, other
Chemistry panel: includes BUN, Creatine, CO_2	Non-specific for vasculitis-induced renal disease
ESR	Non-specific for systemic inflammation
Urinalyses	Kidney or liver dysfunction, metabolic disease
Rheumatoid factor	Rheumatoid arthritis
ANA	Systemic lupus erythematosus
ANCA	Specific for Wegener's granulomatosis, PAN, and related vasculitis – associated diseases
Cryoglobulins	RA, SLE
ACE	Sarcoid
C-reactive protein	Non-specific for systemic inflammation
Circulating immune	RA, SLE, Cogan's syndrome complexes
Scleral biopsy	Infectious diseases and rare causes
Chest radiography	RA, Tuberculosis, Wegener's granulomatosis
Sacroiliac radiography	Ankylosing spondylitis
ELISA	Lyme disease, HIV
HLA-typing	HLA-related inflammatory disease, such as SLA

ESR-erythrocyte sedimentation rate, ANA-antinuclear antibody, ANCA-antineutrophil cytoplasmic antibody, ACE-angiotensin converting enzyme, ELISA enzyme-licensed immunoassay, HLA-human lymphocyte antigen
Diagnostic laboratory testing was performed in the Central Biochemical Laboratory of the Clinical Center in Niš (Serbia).

The pathohistological examination was done at the Centre of Pathology, Clinical Centre of Niš (Serbia). The biopsy samples were taken after the local application of an anesthetic (cystocain). The tissue samples were taken from the clinically most affected inflammatory area above the scleral nodule. The samples were fixed in 10 % buffered formalin and processed with a modified method for small samples in the automatic tissue processor. The samples 3-5 microns thick were colored with the hematoxylin-eosin-method, Trichrome by Mallory and Woerchef-Fe hematoxylin method.

The patients gave a written informed consent.

Results

CASE I

A 36-year-old woman came in with a two-month history of painful red eyes and an "achy" pain on the temporal sides of her left globe. The pain was

present during the day. She had experienced five similar monocular episodes that affected the left eye. The pain became stronger at each movement of the eye, at reading, so any kind of activity was impossible. The patient's ocular history was positive for diffuse episcleritis of the left eye a year before. The slit–lamp examination revealed chemosis, and deep, diffuse 3+ hyperemia of the conjunctiva vasculature was noted, being most prominent in the temporal areas. There was a bluish tinge to the sclera superior on the left eye. The cornea was clear, and the anterior was 16 mmHg. A dilated fundus examination revealed normal optic nerve, macula, and fundus. These were treated to resolution with 1 % prednisolone ophthalmic suspension four times a day.

On the one-week follow-up visit, pain symptoms somewhat resolved. Hyperemia worsened to 4+, and engorged vasculature was noted. The cornea, anterior chamber, and optic nerve were still unchanged. The diagnosis was changed to recurrent episcleritis versus anterior diffuse scleritis. The patient has been using 1.0 % topical prednisolone acetate ophthalmic suspension six times a day and systemic 80 mg prednisone per day with 150 mg ranitidine once a day.

All laboratory tests (Table 1) (CBC, chemistry panel, urinalysis, ANA, ANCA, ESR, circulating immune complex, Complement, C reactive complement, Rheumatoid factor and uric analysis, ELIA, HLA typing) were within normal limits, and the final diagnosis was idiopathic diffuse scleritis. On the four-week follow- up visit, the patient showed remarkable improvement.

The pathomorphological substrate of diffuse scleritis showed considerable edema of the episcleral and scleral tissues, Presence of inflammatory infiltrates with abundant lymphoid cells, histiocytes, accompanied by active hyperemia of capillary vessels with an unusual relationship of blood vessels of the sclera and episclera (Figure 1). The margination of erythrocytes up to the formation of microthrombosis indicated a disrupted blood flow (Figure 2). Repetitive regenerative processes were noticed with significant deposits of collagen fibers in the form of imprisoned whirlpools, which resulted in a change of the usual distribution of these and elastic fibers in the sclera. The thickening of the capillary basal membrane of focal nodular nature was also noticed (Figure 3).

CASE II

A 57-year-old female was referred to the Ophthalmology Clinic in Nis (Serbia). Her symptoms were: pain, decreased visual acuity and photophobia in the left eye. The patient had a history of left eye redness and pain, which had begun a year before, treated with local steroids by her local ophthamollogist. The slit-lamp examination revealed a large scleral nodule, with a 4+ injection of the overlying conjunctiva and episclera. Ten percent of phenylephrine did not affect the deep vascular engorgement, thus confirming the diagnosis of nodular scleritis. The slit-lamp examination revealed the congestion and tortuosity of the superficial and deep episcleral plexuses overlying the nodule. The cornea was clear and the anterior chamber was without cells. Intraocular pressure was 20 mmHg OD and 20 mmHg OS. The dilated fundus examination revealed normal optic nerves, maculae, and fundi.

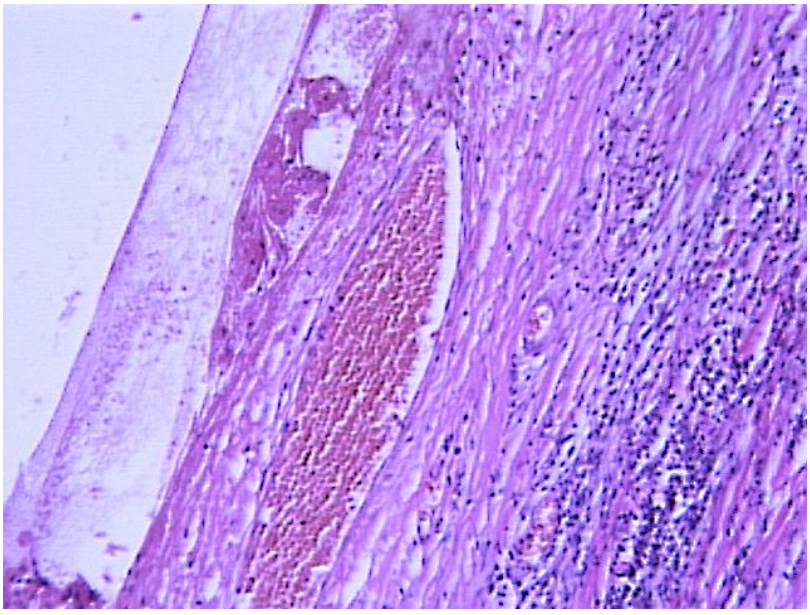

Figure 1. Inflammatory infiltrates and active hyperemia of capillary vessels with an unusual relationship of blood vessels of the sclera and episclera (HE, x 200)

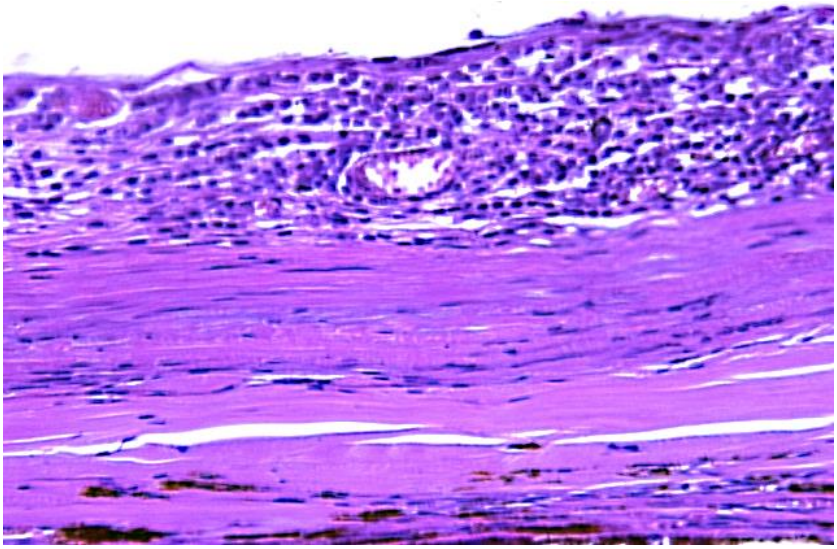

Figure 2. Significant deposits of collagen fibers in the form of imprisoned whirlpools, with distribution of these and elastic fibers in the sclera (HE, x 200)

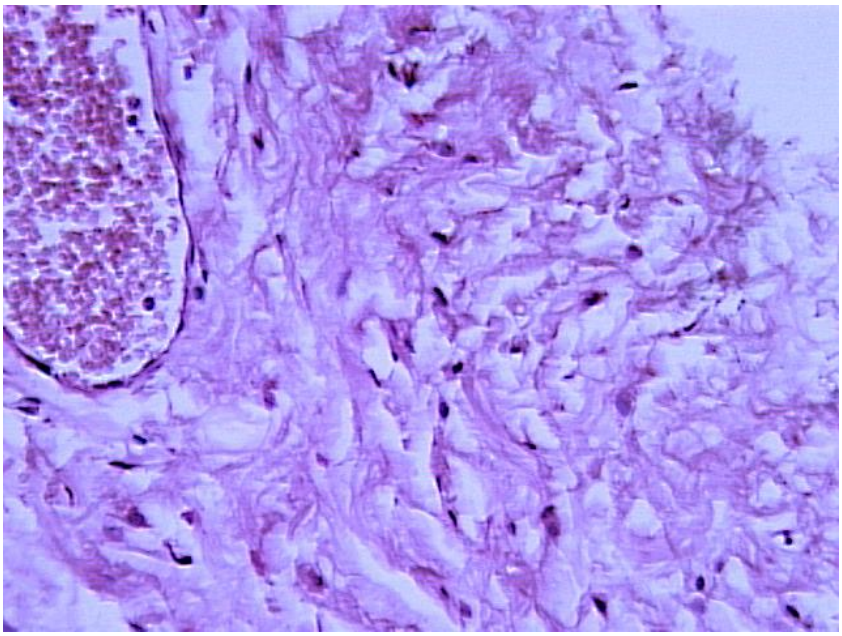

Figure 3. The thickening of the capillary basal membrane (HE, x 200)

Family history: The patient's mother and aunt had rheumatoid arthritis. Past medical history revealed rheumatoid arthritis of 10-year duration. This patient had III stage of the rheumatic disease according to the criteria of the American College of Rheumatology. The clinical laboratory test was positive for rheumatoid factor (600U; normal 40U), showed an accelerated erythrocyte sedimentation rate of 50 mm/h (Westergreen). Hand radiographs showed juxtaarticular osteopenia, marginal erosion. The topical prednisolone acetate was used four times a day. On the four-day follow-up visit, there was no improvement in symptoms. The rheumatologist offered the patient the treatment options of hydroxychloroquine or methotrexate for systemic control. The rheumatologist also recommended that we should initiate a regimen of oral steroids if an additional control of scleritis was needed. This treatment course led to slow symptomatic improvement approximately over the next 4 to 6 weeks, but scleral erythema remained.

The pathomorphological substrate of the sclera with the clinical form of nodular scleritis showed multiple foci of mononuclear infiltration with the do-

minance of lymphocytes, the inner zone of polymorphonuclears and histiocytes, epithelioid and foreign body type giant cells, and the outer zone of lymphocytes and plasma cells (Figure 4, 5). Compared to the normal sclera, the number of inflammatory cells was 10-15 times elevated in scleritis with the domination of lymphocytes and plasma cells.

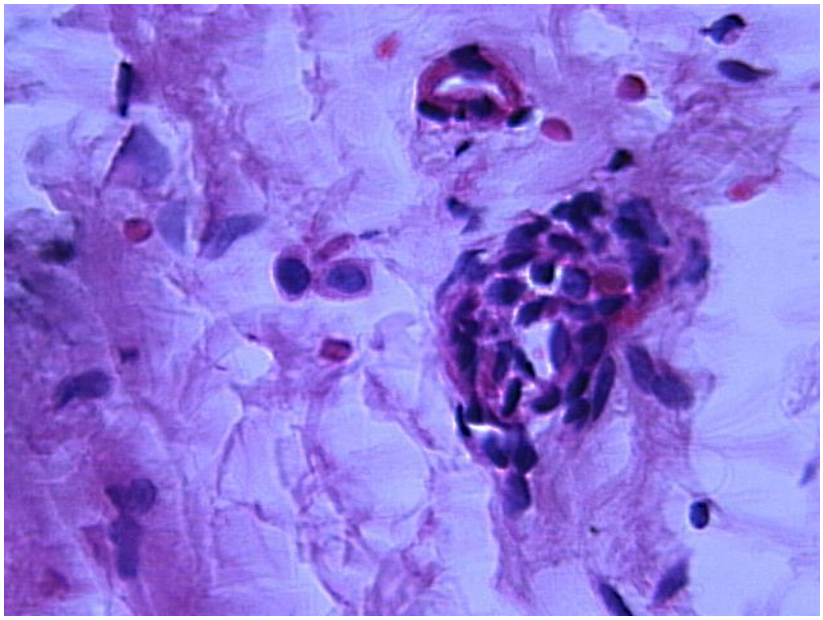

Figure 4. Nodular scleritis with multiple foci of mononuclear infiltration (HE, x200)

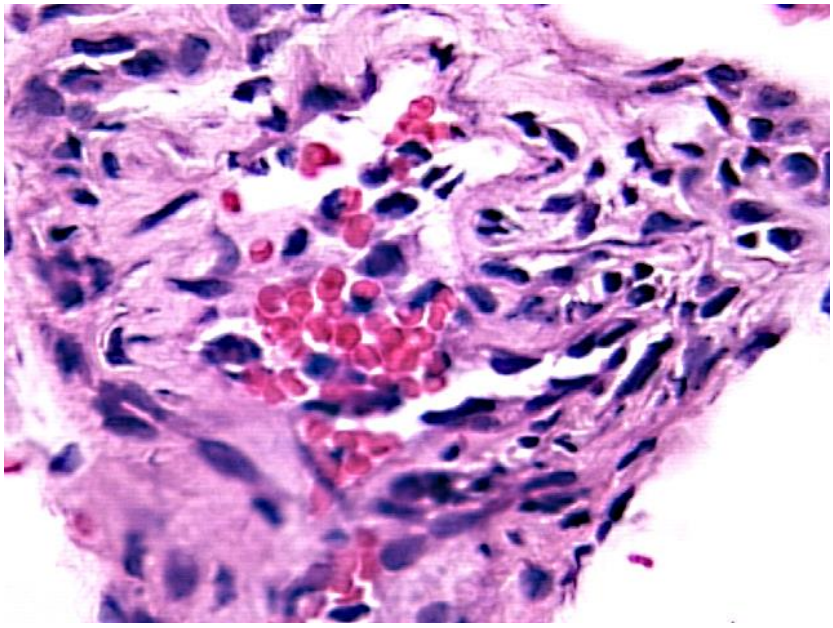

Figure 5. Elevated number of inflammatory cells in scleritis with the domination of lymphocytes and plasma cells (HE x 200)

Discussion

Scleritis is a severe inflammatory condition that is characterized by edema and inflammatory cell infiltration of the sclera. The most common symptoms present are pain and redness (1). In most cases, scleritis affects the front part of the sclera. Diffuse and nodular scleritis are equally frequent,

usually bilaterally and more common in females. While diffuse scleritis is usually bilateral with an unknown etiology, nodular scleritis and necrotizing scleritis are usually unilateral and frequently associated with systemic diseases (6).

Recent studies show that the percentages of patients manifesting scleritis can be classified as follows: diffuse anterior scleritis – 39 % to 45 %, nodular – 23 % to 45 %, necrotizing with inflammation – 10 % to 23 %, scleromalacia perforans – 3 % to 4 %, and posterior scleritis – 2 % to 12 % (9). Diffuse anterior scleritis manifests diffuse or sectoral hyperemic vascular congestion that includes deep scleral blood vessels and is associated with chemosis. This is the least severe class of scleritis. Nodular anterior scleritis is characterized by localized inflammation, with swelling in the form of a scleral nodule which predominantly appears in the intrapalpebral region, close to the limbus. The non-necrotizing classifications of diffuse scleritis and nodular anterior scleritis are usually far less destructive than the necrotizing types of scleritis and present a minimal risk of vision loss. However, 20 % of cases of nodular scleritis may progress to necrotizing scleritis.

Necrotizing scleritis is far more serious than non-necrotizing one due to the severity of potential sequel, including vision loss. Scleromalacia perforans usually involves the destruction of the scleral tissue with minimal to no visible inflammation. It is the most destructive form of scleritis, as it can lead to globe perforation in an asymptomatic fashion, although globe perforations are uncommon (6-8, 9).

Scleritis may be idiopathic or associated with systemic immune-mediated diseases. Rheumatoid arthritis is the most common systemic condition associated with scleritis. Several studies have demonstrated that patients with rheumatoid arthritis associated with scleritis usually have advanced joint disease and extra-articular manifestations; many of these extra-articular manifestations reflect an underlying systemic vasculitis. The most common extra-articular manifestations are subcutaneous nodules (50 %) and skin vasculitis ulcers (25 %). Other extra-articular manifestations include pulmonary disorders, cardiac abnormalities, neurological involvement, amyloidosis. Several studies have shown that the prognosis for life is poorer in patients with rheumatoid arthritis complicated by scleritis than in those without (9-11). McGavin found a 3-year mortality rate of 45.5 % for patients with rheumatoid arthritis and scleritis versus 18.2 % for patients with the disease but without scleritis (12). Foster and coworkers found rheumatoid arthritis and necrotizing scleritis in their series of 20 patients, while 7 patients died of vascular-related events within a 10-year period (13, 14).

The correct and rapid diagnosis and appropriate systemic therapy can halt the relentless progression of both ocular and systemic processes, thus preventing the destruction of the globe and prolonging survival. The treatment of scleritis requires systemic therapy such as nonsteroidal anti-inflammatory drugs (NSAID's), corticosteroids, or DMARDs. (15, 16). In the cases of necrotizing scleritis, therapy includes immunosuppressive drugs, and new data suggest that new biotech therapies, such as Rituximab, provide significant efficacy and safety (17).

The true pathogenesis of scleritis is still unknown. The presence of microangiopathy in most scleritis specimens suggests an underlying immune-complex reaction (Type I hypersensitive reaction), in which vascular injury is the result of antigen-antibody conjugation within and outside the vessel wall, with the subsequent activation of the complement, attraction of neutrophils and fibrinoid necrosis of vessels and the surrounding tissue. The antigen is usually the aberrant expression of HLA-DR on scleral fibroblasts, induced by interferon gamma (11).

Sainz de la Maza points out that microangiopathy developed through the deposits of immunocomplex, and revealed by the immunohistochemical examination of scleral biopsy, the finding of fluorescein angiography, as well as a good response to corticosteroids and immunosuppressives, suggest the autoimmune nature of scleritis. The analysis of pathomorphological substrates and establishing a clinical morphological correlation represent the basis for the understanding of disease pathogenesis (5).

The pathohistological appearance is not specified. In the diffuse form of scleritis, there is a strong infiltration of lymphoid cells. Nodules are similar to or even of the same composition as subcutaneous nodules in rheumatoid arthritis with a granulomatous inflammatory response, central necrosis, surrounded by epithelioid and giant cells as well as lymphocytes and plasma cells. In the necrotic form, prominent processes are the infiltration of mast cells and the degeneration of scleral collagen accompanied by a fibroblastic reaction.

The application of the morphological analysis shows clear differences between substrates in the analyzed forms of scleritis. These differences in the morphologic changes in scleritis associated with systemic autoimmune diseases, compared with morphological changes occurring in idiopathic scleritis, could be caused by differences in the pathogenesis of the two types of scleral inflammations (8, 9). Rheumatoid arthritis is an autoimmune vasculitis disease associated with a circulating immune-complex. The histopathological findings of scleritis associated with a systemic immune disease - that is, the presence of vasculitis associated with zonal granulomatous inflammation surrounding the central necrotic sclera - support an immune-complex mediated immunopathogenesis for scleritis associated with a systemic autoimmune disease (14-20). Idiopathic scleritis, on the other hand, is less likely to be an immune-complex mediated process; its immunopathological findings are rather consistent with a delayed type of the hypersensitivity reaction. This is supported by the absence of vasculitis and the presence of the reactive proliferation of connective tissue (21, 22).

Conclusion

There are numerous abnormalities in the immune status of patients with both idiopathic scleritis and scleritis associated with rheumatoid arthritis. The analysis of the pathomorphological substrate shows clear differences in analyzed forms of scleritis.

Acknowledgments

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Conflict of Interest

There are no conflicts of interest.

References

1. Watson PG. The diagnosis and managament of scleritis. Ophthalmology 1980; 87:716-20. [CrossRef]
2. Watson P, Hayreh S. Scleritis and episcleritis. Br J Ophthalmol 1976; 60:163-91. [PubMed] [CrossRef]
3. Denk PO, Thiel HJ, Zierhut M. Episcleritis and scleritis. An overview of modern diagnostic and therapeutic concepts. Klin Monatsbl Augenheilkd 1997; 211(30): 140-50. [CrossRef]
4. Denk PO, Roshrbash JM, Thiel HJ. New clinical and epidemiologic aspects of episcleritis and scleritis. Ophthalmologe 1997; 94(3):202-5. [PubMed] [CrossRef]
5. Sainz de la Maza M, Foster CS, Jabbur NS. Scleritis associated with systemic vasculitis diseases. Ophthalmology 1995; 102(4):685-92. [PubMed]
6. Sainz de la Maza M, Foster CS, Jabbur NS. Scleritis associated with rheumatoid arthritis and with other systemic immune-mediated diseases. Ophthalmology 1994; 101(7):1281-6. [PubMed] [CrossRef]
7. Reddy SC, Rao UR. Ocular complications of adult rheumatoid arthritis. Rheumatol Int 1996; 16(2):49-52. [PubMed] [CrossRef]
8. Widiarti P, Riono MD, Ahmed A, Hidayat NA, Rao A. Clinicopathologic Study of 55 cases. Ophthalmology 1999; 106(7):1328-33.
9. Rao NA, Marak GE, Hidayat AA. Necrotizing scleritis. A clinicopathologic study of 41 cases. Ophthalmology 1995; 102:687-92. [PubMed]
10. Akpek EK, Uy HS, Christen W, Gurdal C, Foster CS. Severity of episcleritis and systemic disease association. Ophtalmology 1999; 106(4):723-31. [PubMed] [CrossRef]
11. McGavin D, Williamson J, Forrester J, Foulds W, Buchanan W, Dick W, et al. Episcleritis and scleritis. A study of theirclinical manifestations and association with rheumatoid arthritis. Br J Ophtalmol 1976; 60: 192-226. [PubMed] [CrossRef]
12. Jabs D, Mudun A, Dunn J, Marsh M. Episcleritis and scleritis: clinical features and treatment results. Am J Ophthalmol 2000; 130:469-76. [PubMed] [CrossRef]
13. Foster CS, Forstot SL, Wilson LA. Mortality rate in rheumatoid arthritis patients developing necrotiying scleritis or peripheral ulcerative keratitis. Effect of systemic immunsuppression. Ophthalmology 1984; 91: 1253-63. [PubMed] [CrossRef]
14. David C. Anterior scleritis: three case reports and review of the literature. Optometry 2004; 75(7):430-44. [PubMed] [CrossRef]
15. Ashok D, Ayliffe W, Kiely P. Necrotizing scleritis associated with rheumatoid arthritis: long-term remission with high-dose infliximab therapy. Rheumatol 2005; 44:950-1. [PubMed] [CrossRef]
16. Ahn S, Oh J, Kim M, Wee W. Treatment of refractory scleritis with infliximab. Jpn J Ophtalmol 2009; 53: 286-7. [PubMed] [CrossRef]
17. Chauhan S, Kamal A, Thompson R, Estrach C, Moots R. Rituximab for the treatment associated with rheumatoid arthritis. Br J Ophtalmol 2009; 93:984-5. [CrossRef]
18. Keystone E, Kavanaugh A, Sharp J, Tannenbaum H, Hua Y, Teoh L, et al. Radiographic, clinical, and functional outcomes oftreatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. Arthritis Rheum 2004; 50:1400-11. [PubMed] [CrossRef]
19. Zlatanović G1, Veselinović D, Cekić S, Zivković M, Dorđević-Jocić J, Zlatanović M. Ocular manifestation of rheumatoid arthritis-different forms and frequency. Bosn J Basic Med Sci 2010; 10(4):323-7. [PubMed] [CrossRef]
20. Sokolović S, Kasumagić S, Mackić-Durović M, Aganović-Musinović I. The impact of Rituximab therapy on the chromosomes of patients with rheumatoid arthritis. Bosn J Basic Med Sci 2010; 10(2):121-4. [PubMed] [CrossRef]
21. Artifoni M, Rotchild PR, Brezin A, Gullevin L, Puéchal X. Ocular imflammatoty diseases associated with rheumatoid arthritis. Nature Reviews Rheumatology 2014; 10:108-16. [PubMed] [CrossRef]
22. Levy-Clarke G, Jabs DA, read RW, Rosenbaum JT, Vitale A, Van Gelder N. Expert panel recommendations for use of anti-tumor necrosis factor biologyc agents in patients with ocular inflammatory disorders. Opthalmology 2014; 3:785-96. [PubMed] [CrossRef]

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KLINIČKO PATOHISTOLOŠKA ANALIZA SKLERITISA – PRIKAZ DVA SLUČAJA

Jasmina Đorđević-Jocić[1,2], Ljubinka Janković-Veličković[1,3], Sonja Cekić[1,2], Marija Radenković[1,2], Maja Živković[1,2]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Klinika za očne bolesti, Klinički centar Niš, Niš, Srbija
[3]Klinika za patologiju, Klinički centar Niš, Niš, Srbija

Kontakt: Jasmina Đorđević-Jocić
Bulevar dr Zoran Đinđić 81, Niš, Srbija
E-mail:jdjordjevic.jocic@gmail.com

Skleritis je hronična bolest sklere koja može biti povezana sa sistemskim bolestima vezivnog tkiva, a koju karakterišu bol, otok, crvenilo i potencijalni gubitak vida. Skleritis se može pojaviti kao samostalan ("idiopatski") ili kao udružen sa sistemskim bolestima vezivnog tkiva. U radu su prikazana dva različita slučaja skleritisa: prvi prikaz predstavio je ženu koja je imala "idiopatsku" formu sa difuznim tipom, i drugi ženu sa nodularnim tipom skleritisa udruženim sa reumatoidnim artritisom. Obe bolesnice podvrgnute su detaljnom kliničkom, laboratorijskom i imunološkom ispitivanju, urađena je patohistološka analiza biopsiranog uzorka. Prva bolesnica koja je imala difuznu formu skleritisa, imala je laboratorijske analize sa normalnim parametrima. Patomorfološki supstrat pokazao je edem episkleralnog i skleranog tkiva i prisustvo inflamatornih infiltrata sa obilnim limfocitima i histiocitima praćenih aktivnom hiperemijom i kapilarima sa neuobičajnim vezama između krvnih sudova sklere i episklere. Druga pacijenkinja imala je nodularni skleritis udružen sa reumatoidnim artritisom. Patomorfološki supstrat sklere pokazao je multiple fokuse mononuklearne infiltracije sa dominacijom limfocita, unutrašnjom zonom od polimorfonuklearnih ćelija i histiocita, gigantskih ćelija tipa stranog tela, i spoljašnom zonom sačinjenom od limfocita i plazma ćelija. U poređenju sa normalnim tkivom sklere, broj inflamatornih ćelija bio je 10-15 puta veći kod ove vrste skleritisa sa dominacijom limfocita i plazma ćelija.

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Ključne reči: *skleritis, reumatoidni artritis, scleromalacia perforans*

Original article

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MINIMALLY INVASIVE AORTIC VALVE REPLACEMENT VS AORTIC VALVE REPLACEMENT THROUGH MEDIAL STERNOTOMY: PROSPECTIVE RANDOMIZED STUDY

Igor Živković[1], Petar Vuković[1,4], Staša Krasić[2], Aleksandar Milutinović[1], Dragan Milić[3,5], Predrag Milojević[1,4]

Standard surgical approach in an aortic valve treatment is medial sternotomy. In recent years minimally invasive procedures have been used more toward decrease of trauma and faster recovery of patients.

The aim of our study is a comparison of the preoperative, perioperative results and early mortality of patients in whom aortic valve replaced through mini- (Mini-AVR) or medial-sternotomy.

The study included 70 patients. Preoperative, intraoperative and early postoperative patients, and characteristics were analysed. Preoperative variables were homogenous. The euro-score value was significantly higher in medial sternotomy group ($p = 0.037$). The cross-clamp and cardiopulmonary bypass time were longer in mini-AVR group ($p < 0.001$). There was no difference in the incidence of postoperative myocardial infarction, stroke and acute renal failure. One patient in each group underwent surgical revision because of bleeding. There was no difference in hospital mortality between two groups. Postoperative blood loss was insignificantly lower in mini-AVR group ($p = 0.69$). Three patients had suffered from wound infection after medial sternotomy: 2 superficial infections and 1 deep infection ($p = 0.4$). The length of intensive care unit was similar in both groups. Patient in mini-AVR group had shorter hospital stay when compared with patient operated thought medial sternotomy (8 days (IQR 7-11) vs 7 days (IQR 7-9)).

The mini-AVR reduces tissue trauma and hospital stay and also promotes a patient's recovery. In high- risk patients with comorbidities like obesity, diabetes and elder patients reduces the prevalence of infection.

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Key words: *aortic valve, ministernotomy, aortic valve replacement, minimally invasive approach*

[1]Institute of Cardiovascular Diseases "Dedinje", Clinic of Cardiac surgery, Belgrade, Serbia
[2]Mother and Child Health Care Institute "Dr Vukan Čupić", Belgrade, Serbia
[3]Clinical Center Niš, Clinic of Cardiovascular surgery, Niš, Serbia
[4]University of Belgrade, Faculty of Medicine, Belgrade, Serbia
[5]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Igor Živković
Heroja Milana Tepića 1, 11000 Belgrade, Serbia
E-mail: igor88zivkovic@gmail.com

Introduction

Standard surgical approach in aortic valve treatment is median sternotomy, which means complete longitudinal transection of sternal bone. Nowadays, minimally invasive procedures are in the focus of patients as well as physicians because of reduced trauma and faster recovery of patients (1). In aortic valve surgery, a lot of different approaches can be used: parasternal, infra-axillar, lower hemisternotomy, transverse sternotomy, but the most commonly used is partial upper "J" sternotomy. This technique implies small skin incision 8-10 cm long and sternal transection from jugular notch to 3th or 4th intercostals space, dependent on a patient's constitution (2). Many different studies showed that with this approach we achieve smaller skin incision which improves cosmetics effect. This includes less bleeding, faster respiratory function recovery after surgery, reduced wound and mediastinal infection, as well as the length of hospital stay (3). Other studies showed that minimally invasive aortic valve replacement (mini-AVR) has some disadvantages such as longer duration of cardio-pulmonary bypass and cross-clamp, less visibility of heart structures, worse myocardial protection and heart deareation (removal

of air from the heart chambers) (4). Newer studies about mini-AVR presented different results and conclusions which are still unclear.

Aim

The aim of our study is a comparison of the pre- and perioperative results and early mortality in patients in whom mini-AVR and conventional AVR were performed.

Materials and methods

This prospective randomized study was performed at the Cardiovascular Institute "Dedinje" from 11.02.2016. to 24.05.2017. The study included 70 patients in whom isolated aortic valve replacement was performed. The patients were randomized in two groups. In both groups, we had 35 patients. Surgical approach in the first group of patients was partial upper "J" sternotomy, in another group conventional sternotomy was performed. All the patients in whom aortic valve surgery was performed concomitant with other cardiac procedures were excluded from the study. Re-do AVR procedures were not included in the study.

Surgical technique

We performed mini-sternotomy through an 8 to 10 cm skin incision. The sternum is transected horizontally at the level of the 3rd or 4th intercostal space, taking care to avoid injury to the right internal mammary artery. Thymol fat is dissected, and pericardial sutures are placed and retracted to the dermis while the sternal retractor is temporarily removed, thereby exposing the aorta and operative field. The patient is fully heparinized, and the ascending aorta assessed for a safe cannulation site with manual palpation. We use a 22 or 24 Fr standard plastic right angle tip aortic cannula. For venous drainage, double stage venous cannula is used. Cardiopulmonary bypass is initiated, and the aorta is directly cross-clamped. Antegrade cardioplegia is given in ascending aorta.

Basic (descriptive) statistics included mediana, mean values and standard deviations. Furthermore, frequencies of certain features inside each group were determined. The difference in the distribution of frequencies of certain features among the tested groups was determined using the χ^2 or Fisher's Exact test. After the normality of the distribution of numerical variables had been tested using the Shapiro Wilk and Kolmogorov Smirnoff tests, the comparison between the groups was done using the Student T- and Mann Whitney test. A starting point of statistical significance was determined at the level $p \leq 0.05$. Data processing was done using statistical software SPSS 25.0 for Windows 10.

Results

The study included 70 patients, 28 males and 42 females, mean age 64.75 ± 8.05 years.

Table 1. Preoperative caracteristic of patients devided in two groups

Preoperative caracteristics	Medial Sternotomy n = 35	Mini Sternotomy n = 35	P value
Age	69.3 ± 7.7	65.71 ± 7.9	0.12
Female gender	19	23	0.9
BMI	27.8 ± 4.2	27.1±3.3	0.75
Hypertension	25	29	0.25
Hyperlipidemia	16	20	0.33
NYHA clasification	2	2	
Smoking history	16	14	0.63
Diabetes mellitus	10	7	0.4
Cerebrovaculare disease	3	3	1.0
Vascular disease	6	5	0.74
Ejection fraction	50.14 ± 11.4	54.4 ± 9.9	0.09
EF <30%	3	1	0.12
EF 30-50%	13	7	0.12
EF >50%	19	27	0.12
Aortic valve area (AVA)	0.66 ± 0.25	0.68 ± 0.20	0.77
Aortic valve pressure gradient	54.4 ± 23.7	63.1 ± 20.6	0.1
EuroScore	2.33 ± 2.03	1.53 ± 0.9	**0.037**

*BMI - body mass index, † EF- ejection fraction

The mean value of Euro-score in all patients was 1.93 %. Comparing gender, age and risk factors in groups we found that groups were homogenous ($p \geq 0.05$). Preoperative characteristic of the patients divided into separate groups were presented in Table 1.

In all of the patients sick aortic valve was replaced with a mechanical or biological prosthesis. Different types of artificial valve were used equally in both groups (mechanical valve - 20; biological valves - 15). The cardio-surgical procedures were performed by using the cardiopulmonary bypass support.

Analysis of the intraoperative data in groups showed statistically significantly longer duration of cardiopulmonary bypass and cross-clamp in mini - AVR group ($p < 0.001$). The other intraoperative results are presented in Table 2. Regarding the data statistically significant difference between groups was not registered.

Table 2. Intraoperative and postoperative characteristics of patients divided in two groups

Intraoperative and postoperative Caracteristics	Coventional Sternotomy n = 35	Mini sternotomy n =35	P value
CBP time, minutes	72.14 ± 14.63	94.11 ± 20.1	**0.001 <**
Aortic cross clamping time	52.17 ± 11.5	69.40 ± 13.3	**0.001 <**
Prothesis size	21	21	
Blood drainage (ml)	454.2 ± 458	415.71 ± 355	0.69
Ventilator time (hours)	13.03±4.4	13.46 ± 3.72	0.9
ICU length of stay, days	2	2	
Reintubation	1	0	1.0
Reexploration for bleeding	1	1	1.0
Blood transfusion, ml	164.2±208.2	235.43 ± 276.6	0.22
Postoperative IM	0	0	
Postoperative CVI	0	0	
New onset of AF	9	14	0.2
Atrioventricular block	0	0	
Hemodialysis	1	0	1.0
Wound infection	2	0	0.4
Mediastinitis	1	0	1.0
Intrahospital mortality	1	1	1.0

*CBP time- cardiopulmonary bypass time, †ICU- intensive care unit, ‡IM- infarction myocardial, ¥CVI- cerebrovascular insult, £AF- atrial fibrilation

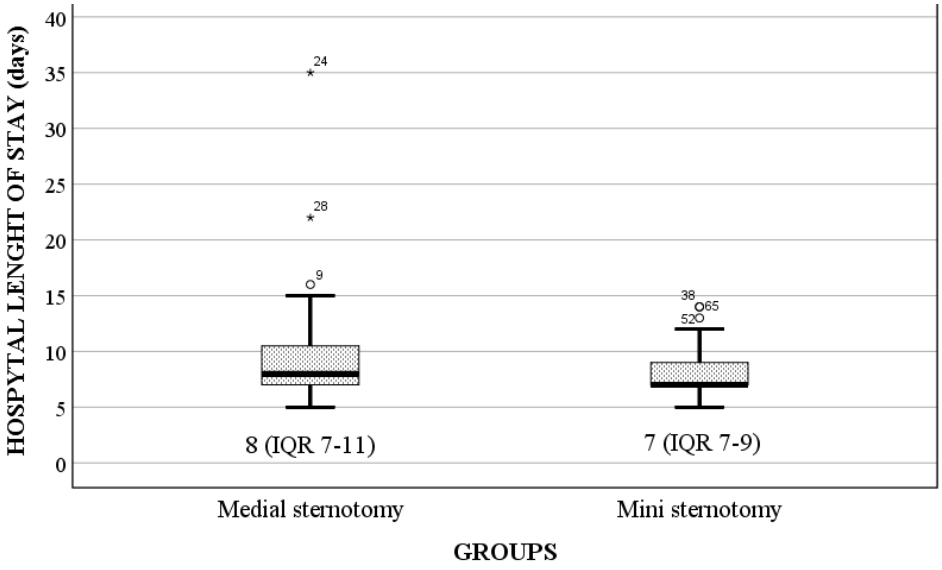

Figure 1. Hospital lenght of stay of our patients dependent on the used operative technique

One patient in whom AVR was done through the medial sternotomy, was reintubated and died during the early postoperative period. Conversion in full sternotomy was performed in one case because the patient was hemodynamically unstable and this patient also died. Presternal infection was detected in two cases in whom medial sternotomy was made but without statistically significant difference between groups. Mediastinitis was registered in one case in the same group. Presternal infection and mediastinitis weren't registered in mini-AVR group.

Patients in whom aortic valve was replaced through medial sternotomy were longer in hospital than patients in mini-AVR group (Figure 1).

Discussion

The standard surgical approach for aortic valve surgery is medial sternotomy. In recent years standard surgical approach has been replaced by the minimally invasive procedures. Mini-AVR represents a safe and efficient option for aortic valve treatment because it reduces the number of complications (5). The aim of this procedure is a smaller scar with less of bleeding, hospital stay, and cost of treatment (6). The special benefit can be seen in patients with significant comorbidities, like obesity, diabetes, chronic obstructive pulmonary disease and in seniors. In these high risk patients, mini-AVR reduces the incidence of wound infection and promotes respiratory function recovery (7). In our study, we followed an intraoperative and early postoperative period of patients in whom mini-AVR and conventional AVR was performed.

Our results showed no statistically significant difference in age, gender, NYHA and ejection fraction (EF) and indicate that groups were homogenous because of the performed randomization. The euroscore values had higher statistical significance in conventional sternotomy group. Cardiopulmonary bypass and cross-clamp times were statistically significant longer in mini -AVR group. The similar results were obtained in the other studies (8, 9). Longer duration of cardiopulmonary bypass didn't influence the morbidity and mortality rate during the early postoperative period in our patients, Ghanta et al. showed similar results in their study (4). By the time, we expect that duration of the cardiopulmonary and cross-clamp will be shorter with the better trained surgical team. Although there was less blood loss in the mini-AVR group, we could not demonstrate a statistically significant difference between groups. The most available studies showed less blood drainage in the mini-AVR group (9). Also, it should be noted that measurement of blood lost through drainage tube isn't precise, because fluid collection in the chest tube may be a combination of blood and other fluids, and therefore values can be variable. Bakir at al. showed that mini-AVR reduced rate of blood transfusion (10). In our study, less blood transfusion was used in the group of a medial sternotomy, but without statistically significant difference. We found many different results about the requirements for blood transfusion. Some studies showed that blood transfusion requirements were equal in both groups (11). Other studies report an important reduction of blood transfusion in the mini-AVR group (12). Murtaza et al. in their large study reported important reduction of blood loss in mini-AVR group (13). We didn't see any difference in reoperations because of bleeding. We found similar results in other studies (2, 10, 13). One of the problems of mini-AVR is difficult deaeration at the end of surgical procedures (14). Although, it is more difficult to make deaeration than in the procedures which were performed through the medial sternotomy, in our study, clinical signs of the bad preformed deaeration weren't noticed. Namely, postoperative CVI and increase blood rate of CK-MB were not registered during hospitality in our patients. Through our experience we can perform successful deareation in case of mini-sternotomy. Good results in deareation are being reached with the fulfillment of operative area with carbon dioxide because carbon dioxide is more soluble in blood than air.

Robert et al. said that in a group of patients with convention sternotomy higher rate of postoperative atrial fibrillation (POAF) was detected (2). Our results showed a higher number of POAF in mini-AVR group, but without significance. The results about POAF rate must be taken with reserve because their etiology is multifactorial (15). A significant advantage of the mini-sternotomy in comparison to the other minimally invasive procedures is the possibility of fast conversion in full sternotomy (2). The conversion is associated with significant morbidity and mortality, and the usual reasons for conversion are bleeding, ventricle dysfunction and bed exposition (9, 13). During our research, only one conversion was done because hemodynamic instability and this patient died in hospital. While some studies wrote about a shorter duration of the mechanical ventilation in a mini-AVR group (9, 13, 16), the analysis of our results showed no statistically significant difference.

Total intensive care unit stay was similar in our groups, but Brown et al. presented shorter intensive care stay in the mini-AVR group. Same studies presented that total intra-hospital stay was shorter for one day in patients in whom mini-AVR was performed (9). Our results were the same (Figure 1). Statistical significance difference between groups was not found when we were analysing presternal infection and mediastinitis, although in mini-AVR group presternal infection and medistinistis were not registered. We expect that a statistically significant difference will be proved in our further studies with higher number of included patients. One of the ideas, why we are doing this procedure at all, is better sternum healing and reduced frequency of SSI (surgical site infection). Santana et al. showed less frequency of wound infection in obese patients in whom partial upper sternotomy was performed (17). Welp et al. showed that partial upper sternotomy in high -risk patient didn't have significant benefit and the similar results were presented in the studies which included patients with lower risk (18).

The mortality rate was equal in both groups. Namely, in each group, only one patient died. Our results are similar with results in earlier presented studies which concluded that difference in mortality

between mini-AVR and conventional AVR group did not exist (9, 10). On the other hand, Raja et al. Presented less mortality rate in the mini-AVR group than in the medial sternotomy group but without statistical significance.

Conclusion

Our research showed that aortic valve replacement might be safely and effectively performed through the partial upper sternotomy. This surgical approach reduces tissue trauma and hospital stay, and also promotes the patient's recovery. In high-risk patients with comorbidities like obesity, diabetes and elder patients reduces the prevalence of infection. Partial upper sternotomy prolongs the duration of the operative treatment and cardiopulmonary bypass, and does not influence the morbidity and mortality rate. Mini-AVR is a good approach for aortic valve treatment but it requests perfect trained and experienced surgical team.

References

1. Fudulu D, Lewis H, Benedetto U, Caputo M, Angelini G, Vohra HA. Minimally invasive aortic valve replacement in high risk patient groups. J Thorac Dis 2017; 9:1672-96. [CrossRef] [PubMed]
2. Neely RC, Boskovski MT, Gosev I, Kaneko T, McGurk S, Leacche M, et al. Minimally invasive aortic valve replacement versus aortic valve replacement through full sternotomy: the Brigham and Women's Hospital experience. Ann Cardiothorac Surg 2015; 4:38-48. [CrossRef] [PubMed]
3. Gilmanov D, Bevilacqua S, Murzi M, Cerillo AG, Gasbarri T, Kallushi E et al. Minimally invasive and conventional aortic valve replacement: a propensity score analysis. Ann Thorac Surg 2013; 96:837-43. [CrossRef] [PubMed]
4. Ghanta RK, Lapar DJ, Kern JA, Kron IL, Speir AM, Fonner E, et al. Minimally invasive aortic valve replacement provides equivalent outcomes at reduced cost compared with conventional aortic valve replacement: A real-world multi-institutional analysis. J Thorac Cardiovasc Surg 2015; 149:1060-5. [CrossRef] [PubMed]
5. Schmitto JD, Mokashi SA, Cohn LH. Minimally-invasive valve surgery. J Am Coll Cardiol 2010; 56:455-62. [CrossRef] [PubMed]
6. Lehmann S, Merk DR, Etz CD, Seeburger J, Schroeter T, Oberbach A et al. Minimally invasive aortic valve replacement: the Leipzig experience. Ann Cardiothorac Surg 2015; 4:49-56. [CrossRef] [PubMed]
7. Young CP, Sinha S, Vohra HA. Outcomes of minimally invasive aortic valve replacement surgery. Eur J Cardiothorac Surg 2018; 53 Suppl 2:19-23. [CrossRef] [PubMed]
8. Borger MA, Moustafine V, Conradi L, Knosalla C, Richter M, Merk DR et al. A randomized multicenter trial of minimally invasive rapid deployment versus conventional full sternotomy aortic valve replacement. Ann Thorac Surg 2015; 99:17-25. [CrossRef] [PubMed]
9. Brown ML, McKellar SH, Sundt TM, Schaff HV. Ministernotomy versus conventional sternotomy for aortic valve replacement: a systematic review and meta-analysis. J Thorac Cardiovasc Surg 2009; 137: 670-9. [CrossRef] [PubMed]
10. Bakir I, Casselman FP, Wellens F, Jeanmart H, De Geest R, Degrieck I. Minimally invasive versus standard approach aortic valve replacement: a study in 506 patients. Ann Thorac Surg 2006; 81:1599-604. [CrossRef] [PubMed]
11. Yamada T, Ochiai R, Takeda J, Shin H, Yozu R. Comparison of early postoperative quality of life in minimally invasive versus conventional valve surgery. J Anesth 2003; 17:171-6. [CrossRef] [PubMed]
12. Young CP, Sinha S, Vohra HA. Outcomes of minimally invasive aortic valve replacement surgery. Eur J Cardiothorac Surg 2018; 53 Suppl 2:19-23. [CrossRef] [PubMed]
13. Murtuza B, Pepper JR, Stanbridge RD, Jones C, Rao C, Darzi A, et al. Minimal access aortic valve replacement: is it worth it? Ann Thorac Surg 2008; 85:1121-31. [CrossRef] [PubMed]
14. Doll N, Borger MA, Hain J, Bucerius J, Walther T, Gummert JF, et al. Minimal access aortic valve replacement: effects on morbidity and resource utilization. Ann Thorac Surg 2002; 74:1318-22. [CrossRef] [PubMed]
15. Hogue CW Jr, Hyder ML. Atrial fibrillation after cardiac operation: risks, mechanisms, and treatment. Ann Thorac Surg 2000; 69:300-6. [CrossRef] [PubMed]
16. Phan K, Xie A, Di Eusanio M, Yan TD. A meta-analysis of minimally invasive versus conventional sternotomy for aortic valve replacement. Ann Thorac Surg 2014; 98:1499-511. [CrossRef] [PubMed]
17. Santana O, Reyna J, Grana R, Buendia M, Lamas GA, Lamelas J. Outcomes of minimally invasive valve surgery versus standard sternotomy in obese patients undergoing isolated valve surgery. Ann Thorac Surg 2011; 91:406-10. [CrossRef] [PubMed]
18. Welp HA, Herlemann I, Martens S, Deschka H. Outcomes of aortic valve replacement via partial upper sternotomy versus conventional aortic valve replacement in obese patients. Interact Cardiovasc Thorac Surg 2018; 27(4):481-6. [CrossRef] [PubMed]
19. Raja SG, Benedetto U, Amrani M. Aortic valve replacement through J-shaped partial upper sternotomy. J Thorac Dis 2013; 5 Suppl 6:662-8. [CrossRef] [PubMed]

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MINIMALNO INVAZIVNA ZAMENA AORTNE VALVULE NASUPROT ZAMENI AORTNE VALVULE KROZ MEDIJALNU STERNOTOMIJU: PROSPEKTIVNA RANDOMIZOVANA STUDIJA

Igor Živković[1], Petar Vuković[1,4], Staša Krasić[2], Aleksandar Milutinović[1], Dragan Milić[3,5], Predrag Milojević[1,4]

[1]Institut za kardiovaskularne bolesti "Dedinje", Klinika za kardiohirurgiju, Beograd, Srbija
[2]Institut za zdravstvenu zaštitu majke i deteta Srbije "Dr Vukan Čupić" , Beograd, Srbija
[3]Klinički centar Niš, Klinika za kardiohirurgiju, Niš, Srbija
[4]Medicinski fakultet Beograd, Beograd, Srbija
[5]Univerzitet u Nišu, Medicinski fakultet Niš, Niš, Srbija

Kontakt: Igor Živković
Heroja Milana Tepića 1, 11000 Beograd, Srbija
E-mail: igor88zivkovic@gmail.com

Standardni pristup u lečenju aortne valvule je medijalna sternotomija. Proteklih godina se minimalno invazivne procedure sve češće upotrebljavaju u cilju smanjenja traume i bržeg oporavka bolesnika.

Cilj ove studije bio je upoređivanje preoperativnih, perioperativnih reziltata i ranog mortaliteta kod bolesnika kojima je učinjena zamena aortne valvule kroz ministernotomiju (Mini-AVR) i medijalnu sternotomiju.

Studija je uključivala 70 bolesnika. Analizirani su preoperativni, intraoperativni i rani postoperativni rezultati. Preoperativni rezultati bili su homogeni. Vrednosti euro-skora bile su statistički veće u grupi sa medijalnom sternotomijom ($p = 0,037$). Trajanje kardiopulmonalnog bajpasa i kleme bilo je duže u grupi sa mini-AVR ($p < 0,001$). Nije bilo razlike u pojavi postoperativnih infarkta miokarda, slogova i renalne insuficijencije. Po jedan bolesnik iz svake grupe hirurški je revidiran zbog krvarenja. Nije bilo razlike u hospitalnom mortalitetu između dve grupe. Postoperativni gubitak krvi beznačajno je bio niži u mini-AVR grupi ($p = 0,69$). Tri bolesnika imala su infekciju rane nakon medijalne sternotomije: dve površinske infekcije i jedna duboka infekcija ($p = 0,4$). Dužina boravaka u intenzivnoj nezi bila je slična u obe grupe. bolesnici sa mini-AVR imali su kraći boravak u bolnici u poređenju sa bolesnicima operisanim kroz medijalnu sternotomiju (8 dana (IQR 7-11) nasuprot 7 dana (IQR 7-9)).

Mini-AVR redukuje traumu tkiva, boravak u bolnici i takođe ubrzava oporavak bolesnika. Kod visokorizičnih bolesnika sa komorbiditetima, kao što su gojaznost i dijabetes i kod starijih osoba smanjuju prevalenciju infekcija.

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Ključne reči: *aortna valvula, ministernotomija, zamena aortne valvule, minimalno invazivni pristup*

Review article

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POTENTIAL TREATMENTS OF TOOTH EXTRACTION WOUNDS: A REVIEW

Kosta Todorović[1], Marija Bojović[2], Vladimir Mitić[3], Milan Spasić[1], Ana Todorović[3]

Wound healing is a complex process occurring in injured tissue with an aim to restore its homeostasis. Depending on the type of wound the closure can be defined as either primary or secondary. The process of wound healing is divided into several precisely programmed (defined) phases that mutually overlap and include (I) hemostasis, (II) inflammation, (III) proliferation, (IV) maturation and in some cases (V) bone regeneration. Tooth extraction represents a very common dental procedure which involves the extraction of decayed, periodontally affected or impacted teeth. After the extraction procedure, the formation of the wound is inevitable, as well as the pain and discomfort that follow it. In this review, we addressed the influence of low-level lasers, polarised light, curcumin and coenzyme Q_{10} on the tooth extraction wound healing process. It seems that there might be potential candidates which might enhance wound healing, after tooth extraction, by modulating different phases in the process. Thus, new and more in-depth clinical and pre-clinical studies need to be conducted in order to estimate the real efficacy and safety levels in humans before introducing them in every day clinical practice.

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Key words: *Wound, Tooth extraction, Low energy laser, Polarised light, Curcumin, Coenzyme Q_{10}*

[1]Department of Oral Surgery, Faculty of medicine, Niš, Serbia
[2]University of Niš, Faculty of Medicine Niš, Department of Oral Medicine and Periodontology, Niš, Serbia
[3]University of Niš, Faculty of Medicine Niš, Department of Jaw and Dental Orthopaedics, Niš, Srbija

Contact: Kosta Todorović
Bulevard dr Zoran Djindjić 81, 18000 Niš, Serbia
E-mail: kosta_todorovic@yahoo.com

The process of extraction wound healing

The wound is defined as the breakage in tissue continuity occurring after either physical, chemical and/or mechanical trauma. Regardless of the etiology of wound occurrence, every mammalian organism is trying to maintain tissue homeostasis, trying to preserve (regenerate) tissue integrity and function, by preventing hemorrhage and infection. Some conditions affecting the human organism, such as diabetes, cachexia, vitamin deficiency, exposure to radiation, etc., can significantly affect the process of wound healing. The wound healing process can be either primary or secondary closure.

Primary closure (*santaio per primam intentionem*) is referred to when the wound edges are smooth, relatively close and when there is no significant tissue damage or infection (1). Thus, one can expect that the primary closure is occurring when the wound is aseptic and fresh, which is mainly related to some small surgical wounds where the edges are sutured together. This type of wound is rarely affected by a significant degree of inflammation and is generally closing in up to 8 days, forming a linear scar and leaving no defect of the surrounding tissue.

Secondary closure (*sanatio per sekundam intentionem*) occurs in wounds with major tissue damage and defect, where it is impossible to bring the wound edges in close contact. The risk of infection, frequently seen immediately after trauma, in this type of closure is significantly higher due to a larger loss of tissue. This type of closure is slower, prolonged and can last up to a few months, and is often followed by a larger amount of granulation tissue and irregular scaring process (2). After surgical intervention in the oral cavity, post-extraction wound or wound involving bone loss, the wound closure is predominantly secondary.

The process of wound healing is divided into several precisely programmed (defined) phases that mutually overlap. They include (I) haemostasis, (II) inflammation, (III) proliferation, (IV) maturation and in some cases (V) bone regeneration. Each change in the line of their occurrence (e.g. prolongation of one of the phases) can lead to significant complications and to chronification of the process, chronic

wound. The changes between the phases are mainly depending on the maturation and differentiation of mastocytes, fibroblasts, keratinocytes, and macrophages, which play a key role in the wound healing process (3).

(I) Hemostasis

Haemostasis occurs after an initial trauma in order to prevent excessive blood loss or capture of blood within the damaged blood vessel. This relatively short phase (lasting around 15 min) involves vasoconstriction, thrombocyte adhesion, and aggregation, as well as coagulum formation. There are numerous systems that are participating in this process, such as injured walls of blood vessels, thrombocytes, all factors involved in the coagulation cascade, as well as fibrinolytic and phagocytic system (4).

Endothelial cells of the blood vessels are playing a key role in clot formation, which under physiological condition secrete thrombomodulin and heparin-like molecule causing prevention of blood coagulation (5). When the trauma of the blood vessel occurs, these endothelial cells decrease the secretion of the before mentioned molecules (coagulation inhibitors) and start to produce/secrete von Willebrand's factor.

Blood vessel damage acts as a switch for thrombin production, which further converts intravascular fibrinogen to insoluble fibrine allowing the formation of a provisory matrix comprised of fibrin, fibronectin (from plasma) and some components of the extracellular matrix (ECM) (4). Minutes after the trauma immune system cell activation occurs, followed by thrombocyte degranulation and bacterial products degradation. These processes cause a release of pro-inflammatory cytokines and numerous growth factors (TGF-β, PDGF, EGF, FGF) from the surrounding tissue and cloth (4).

(II) Inflammation

One of the first researchers that recognized the importance of inflammation in the wound healing process was John Hunter almost 200 years ago (6). The initial stimulus for this process is trauma itself, where after hemostasis, 5 to 6 h after the trauma, the acute inflammatory reaction is developed in the injured tissue. This reaction is characterized by the release of the different substance from necrotic cells that lead to local, regional and systemic inflammatory response (6). The main feature of the local inflammatory response is inflammatory cell migration mainly neutrophils, macrophages, and lymphocytes (7).

The key role of neutrophils is the removal of the microorganism, necrotic tissue and other cell debris that is formed after tissue damage. However, these cells are also responsible for the generation of reactive oxygen species (ROS) and protease production, which lead to additional tissue damage and potentially can prolong the regeneration process (8).

There are numerous phases in the wound healing process that involve macrophage function. In the early phase, they release cytokines that stimulate an immune response, which is reflected in leukocyte activation and migration to the site of trauma. Addition, macrophages also eliminate necrotic cell material, which mainly consists of necrotic neutrophils, allowing the transition for the inflammatory to the proliferative phase of the wound healing. At the same time, the change in their phenotype stimulates keratinocytes, fibroblast and angiogenesis, which irretrievably leads to the proliferation phase (9).

This phase is a crucial step that further leads to the finalization of the wound healing process and it can directly be related to the development of numerous complications (10). It is obvious that attitude towards inflammation needs to be changed from a simple reaction to injury or infection to a process that can be used in the diagnosis and can represent a therapeutic target (11).

The posttraumatic acute inflammatory response consists of three phases: nerve, immune and endocrine phase.

1. Never phase (immediate, initial, progressive oxygenation)

This phase is dominated by sensory and motoric reactions of the injured tissue. Sensory reactions involve pain which represents an efferent neural response of somatic motoneuron and autonomous nervous system to painful sensation (12). In the earliest phases of the inflammatory response numerous substances are released into the bloodstream and accumulate into the intestinal tissue, these include cortisol, aldosterone, biogenic amines, and glucagon (13). Motoric reactions involve the contraction of smooth muscle cells that are part of blood vessels and their contraction significantly contribute to the development of ischemia and local/systemic blood redistribution. The intensity of tissue ischemia is in direct correlation with a further inflammatory response (14), where the formed edema significantly affects cell metabolism and gene expression and is consider to initialize cell anabolic activity (14).

After ischemia, vasodilation and reperfusion of the injured tissue are responsible for the interstitial edema formation, as well as for the production of ROS and nitrogen reactive species. These molecules increase lipid peroxidation, cause cell membrane permeabilization which further aggravates the edema process (15). Besides the aforementioned roles of edema, this process also affects tissue morphogenesis, cell migration and differentiation, as well as the extracellular matrix (ECM) remodeling during wound healing. Intensified tissue perfusion can also activate fibroblasts, causing ECM remodeling and fibrous tissue formation (16). The role of mast cells should not be neglected as well, where besides vasodilating substance (histamine, serotonin) these cells liberate proteolytic enzymes contributing to the formation of interstitial edema (16). Thus, effective control of edema can be significant for tissue regeneration, since the damaged tissue can't be regenerated without a complete absence of edema.

Cell response to hypoxia leads to the expression of hypoxia-dependent and independent induci-

ble factor (HIF). These two pathways, HIF dependent and independent, allow the cell to survive by inducing the transcription genes that are involved in cell metabolism, migration, invasion, and angiogenesis (17). Damaged tissue is going through the phase of metabolic hypoxia, where although there is a sufficient oxygen concentration they are not able to use it for respiratory processes. The activation of HIF-independent pathway enables the cell to survive extremely low oxygen concentrations and the formation of new tissue, which is necessary for wound healing. On the other hand, the activation of the HIF-dependent pathway, during the states with mild and moderate hypoxia, promotes vascularisation and enable cell survival as well (18).

2. Immune phase (leucocytic, intermediary)

Following ischemia and reperfusion phase the infiltration of the damaged tissue by both inflammatory cells and bacteria is occurring. During low oxygen concentration, numerous cells (fibroblasts, macrophages, mast cells, lymphocytes) migrate to the interstitium probably in order to provide the tissue with necessary energy (14). One can say that the "true" inflammatory cells that migrate to the damaged tissue are neutrophils, followed by mast cells and macrophages and some of these cells arrive from blood cloth where they were previously trapped. Activated neutrophils are secreting a large number of substances (ROS, peptides, leukotrienes, prostaglandins) and enzymes (elastase, cathepsin G-proteinase, urokinase), with a role to remove necrotic material. These molecules are one of the reasons why the wound healing can be prolonged since they act on other healthy cells as well.

Before migrating from circulation to tissue monocytes are going through differentiation into tissue macrophages. There are two types of macrophages, M1 (classically activated macrophages) and M2 (alternatively activated macrophages), where the first one secretes inflammatory cytokines (IL-1, -6 and -23) and ROS, while the second ones are involved in angiogenesis and tissue remodeling (19). Additionally, T-helper lymphocytes play a key role in the modulation of macrophages differentiation. Type 1 T lymphocytes (Th1) produce different cytokines (INF-γ and TNF-α) that lead to the transformation of macrophages to M1 subtype, while type 2 T lymphocytes (Th2) induce M2 polarization by secreting IL-4, -5 and -13 (19). Additionally, B lymphocytes are found to be involved in the development of tissue fibrosis (19).

Besides the leucocytes, thrombocytes are found to be enrolled in the initiation and propagation of the inflammatory response as well. Thrombocytes are known to be rich in different secretory granules (alpha, delta and lambda type) and lysozymes (4). These granules contain various signaling molecules that allow other cells adhesion or act as chemokines or growth factors. Among the most recognized growth factors found in these granules is platelet-derived growth factor (PDGF) which even found its potential use as a healing agent (20). Apart from PDGF other growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), haptic growth factor (HGF), etc. could be found (20).

In the inflammatory response, the activation of Toll-like receptor (TLR) plays an important role since they lead to the transcription of nuclear factor kappa B (NF-(k)B), AP-1 and interferon regulating factor (IRF). This TLR (TLR-2 and TLR-4) activation during ischemia and reperfusion phase of the wound healing can be seen during both sterile and unsterile wounds (8). This activation of NF-(k)B, one of the major steps in inflammatory phase, leads to a cascade reaction which results in specific gene expression which in turn cause cytokine and chemokine, as well as their receptor, synthesis (6, 21). Besides its role as immune phase stimulator NF-(k)B is very important for the inflammation resolution and tissue reparation.

In every day clinical practice this phase is followed by wound infection, where a different type of secretions due to bacterial colonization appears. One of the most common pathogens that infect wounds is *Staphylococcus aureus*, which due to the production of numerous enzymes (nucleases, proteases, lipases, collagenases, etc.) prolong wound healing process (22).

3. Endocrine phase (late, angiogenesis)

The formation of new blood vessels (angiogenesis) in this final phase of the inflammatory process appears to be a dominant feature (23). Interestingly newly formed endothelial cells play an important immunomodulatory role at the beginning of the inflammation, thus affecting the final resolution and progression of tissue regeneration (23). The process of angiogenesis is based on endothelial proliferation (microvascular growth) and can be divided into five clear phases (23):

1) disintegration of basement membrane and the formation of new blood vessels that penetrate into perivascular stroma;

2) migration of endothelial cells to the newly formed blood vessel;

3) endothelial cell proliferation;

4) canal formation, branching and formation of vascular loops;

5) perivascular apposition of pericytes and smooth muscles around blood vessels, as well as "de novo" synthesis of basement membrane proteins.

The process of angiogenesis is regulated by numerous factors, such as VEGF, TGF, FGF-2, PDGF, angio-protein, angiotensin II, endothelin, andromeduin, adipokines (leptin, adiponectin), neuropeptide-Y and vasoactive intestinal peptide (24). Angiogenesis is tightly connected to the process of granulation tissue formation since it is necessary to enable cell survival in newly formed tissue (25). The process of granulation (granulation phase) starts three to four days after tissue trauma, and under the impact of macrophages, fibroblast, keratinocytes, and endothelial cells. In parallel to granulation tissue formation, vascular cells react with the provisional matrix comprised of fibrin, fibronectin and vibronectin leading to change in a fibrous matrix.

The resolution of the inflammatory phase is mainly guided by local mediators that are synthe-

sized from fatty acids and these include eicosanoids, docosanoids, resolvins, and lipoxins. They mainly act as ROS production inhibitors, leading to a decrease in blood vessels permeability, blockage of adhesion molecules for leucocytes and endothelial cells, as well as to a decrease in chemokine synthesis (25). The progression of inflammation resolution stimulates the process of epithelization, which occurs only a few days after tissue trauma by migration of keratinocytes to the edge of the wound. This migration and organization of keratinocytes is a complex process that is guided by a number of small mediators such as growth factors (EGF, TGF-α, PDGF), integrins, metalloproteinases (MMP-1, -9 and -10), plasminogen and structural proteins. All these molecules affect the provisional matrix organization and collagen degradation and at the end of this phase the change in keratinocyte phenotype from mesenchymal to epithelial (26).

Fibroblasts also play an important role in inflammation resolution, where besides ECM formation they can influence the process of angiogenesis by secreting different growth factors. Some growth factors, such as PDGF and TGF-β, can influence the differentiation of fibroblast to myofibroblasts, thus helping the wound to contract (27, 28). Also, these cells mainly influence the formation of ECM by synthesizing collagen, elastin, glycoproteins, etc. During this phase, many cells, macrophages, endothelial cells, myofibroblasts are going through apoptosis which leaves the tissue comprised mainly of collagen fibers and ECM proteins. The remodulation phase starts 2-3 weeks after tissue injury and sometimes lasts up to a year, while in some cases this phase can last much longer (19, 27, 28).

(III) Proliferation

The proliferation phase is taking place between the 4th and 21st day after the trauma, and since it overlaps with inflammatory phase, there is a thin line between them, it makes it almost impossible to clearly separate one phase from another. This phase is characterized by fibroblast and keratinocyte proliferation, which will further lead to growth factor secretion, angiogenesis stimulation, ECM formation and epithelization (27, 29). The formation of ECM is mainly depending on fibroblast presence and their ability to produce collagen (maximum deposition seen 21 day after trauma), glycosaminoglycans and proteoglycans (27). Besides these roles' fibroblasts act as one of the main cellular components of granulation tissue formed at the bottom of the wound. Keratinocyte differentiation is also a very important step in the wound healing process since the change in creatine production enables tissue elasticity and easier migration (30).

(IV) Maturation

Following proliferation and ECM synthesis the wound healing process in entering its final phase of remodeling, which starts 2-3 weeks after initial tissue trauma and can last up to a year (27). Complete reepithelization stimulates fibroblast, myofibroblasts and keratinocytes to produce fibrin, fibronectin and collagen III. Fibroblasts are the key cells of this phase since they are responsible for transformation from collagen type III to collagen type I (Tracy et al., 2016). This process is lasting for around 30 days, while the maximum is expected somewhere between 42 and 60 days after an initial injury (27). The change in ECM is occurring in order to form a new tissue (27) and represent a change in provisional ECM into the mature matrix, comprised mainly of collagen type I fibers and molecules such as actin and myosin within the cells (31).

The scar tissue is going through the physiological contraction, that is dependent on myofibrils, which is occurring through the process of wound healing (27). In the end stage of successful tissue reparation apoptosis of cells that contain myofibrils is starting, followed by deactivation and differentiation of keratinocytes (27). Maturation phase is also characterized by regression of newly formed capillary vessels and the density of the vessels within the tissue is returning to the level before the injury.

(V) Bone regeneration

Some wounds, such as those after tooth extraction, are going through an additional phase which involves bone tissue regeneration. This rather complex mechanism acquires the involvement of different types of cells and biological agents that stimulate cell proliferation, differentiation and tissue organization (32). The most important cells in this type of regeneration are osteoblast which is responsible for the migration of bone cells (33). An additional characteristic of this regeneration is the process of ECM mineralization which starts around 7th day which starts from the edge of the defect and goes towards the middle, while in the case of extraction defects from the apical third and edge of the alveolar ridge. The newly formed bone tissue and trabeculae are resorbed 14 days after the injury and this is considered the begging of the bone remodeling and maturation (34). Around 21 day after tissue injury, the number of young blood vessels is drastically lower, and the defect is a field with bone trabeculae (35). The final step of mineralization begins after 4 weeks and involves consolidation of bone tissue and formation of regular bone trabecules, as well as differentiation of osteoblasts into osteocytes. This phase is known to last for the very long time period and in most cases, it is finished after a year.

Therapeutic approaches in tooth extraction wound healing

Having in mind all previously described processes, as well as factors that can influence them, one cannot oversee a number of places for different therapeutic approaches. Standard, everyday clinical approaches involve the removal of local causes, wound revision and addressing of systemic disorders. Faster wound healing leads to pain elimination, decrease in swelling and infection, as well as to an increase in life quality in patients (22, 36).

A great number of studies evaluated the course of the wound healing process, where the experimental model in rats represents a perfect model for

the evaluation of this process and the obtained results give a clear insight into processes happening in humans (28). Different studies applied various procedures (chemical and physical) in order to estimate their potential in accelerating the wound healing process.

(I) Physical procedures

Among the most popular physical procedures applied for the treatment of wounds after tooth extraction are low-level lasers (LLL) (37). In a group of subjects, LLL therapy was reported to remarkably decrease trismus, swelling and intensity of pain on the first and the seventh postoperative days after third molar extraction (38). The LLL (helium-neon and argon laser) were found to be able to increase collagen synthesis, estimated based on hydroxyproline concentration in scar tissue (39). Also, LLL induce the formation of new blood vessels (angiogenesis) and modulate the expression of MMP-2 in the newly formed granulation tissue (40). In patients with extracted molars, LLL increased salivary IgA and albumin concentrations and decreased subjective feelings during treatment (41). Besides the effects of LLL on soft tissue healing it also enhances the bone tissue regeneration by increasing cell proliferation and the number of osteoclasts (37), while other studies found no effect of LLL on ossification process (41). However, one should not neglect the possible carcinogenic potential of laser radiation therapy (39).

On the other hand, polarised light, which involves more wavelengths (polychromatic), with small energy as well, found its application for the treatment of almost all complications that follow tooth extraction wound (37, 42-44). This light is obtained through the system of specially designed crystals which transduce light at wave lengths between 400 and 2000 nm (42). The results of several studies conducted suggest that polarised light treatment affects all phases of wound healing that follow tooth extraction (42-44). In the inflammatory phase, polarized light increases the number of lymphocytes present in wound tissue and induces a faster transition from monocytes to macrophages. The function of macrophages is also affected and it is found that this type of treatment increases their number, as well as the release of biologically active substance (42). In an animal model six-day exposure to this type of light lead to a faster epithelisation process of extraction wound, where narrower defect with two-layered cubicle epithelium was found in treated animals while in those from the control group only granulation tissue was seen at this time point (43). Up to know no undesired effects were observed during the usage of polarised light.

(II) Chemical procedures – pharmacological treatment

There is a large number of compounds both synthetic and naturally occurring ones that were assayed for their potential in enhancing the tooth extraction wound healing process. However, due to the inability to include all the research conducted we will focus our attention on two well-studied antioxidants curcumin and coenzyme Q_{10}.

Curcumin (Cur) is a naturally occurring compound, found in *Curcuma longa*, which has a long history of ethnomedicinal usage (45). Besides its antitumor, antibacterial, antiviral, antifungal, antiulcer and anti-inflammatory properties, it is proven to possess a wound healing property as well (45-47). Its traditional usage suggests that it can be applied to wounds in a form of ointment (46), where it enhances the process of healing. The proposed mechanism of action involves the inhibition of inflammatory processes, which leads to faster and better healing of acute and chronic wounds (47). These include downregulation of TNF-α, IL-1, NF-kB and MMP-9 transcription and upregulation of IL-10 and catalase, superoxide dismutase and glutathione peroxidase (21, 47). In a model of tooth extraction wound healing, it is found that Cur enhances epithelization process and increases the density of newly formed blood vessels in soft tissue that surrounds wound. Also, measured biochemical parameters that reflect tissue inflammatory reaction (NO and myeloperoxidase (MPO)) were found to be decreased (suppressed) in the group that received Cur (45). In other models of inflammation Cur inhibited cyclooxygenase and 5-lipooxygenase, decreased histamine liberation and production, enhanced the activity of cortisol and increased tissue blood flow (47).

Coenzyme Q_{10} (CoQ_{10}) is a ubiquitous lipophilic molecule mainly present in its *trans* form (48). In cells it is located in the membrane of different organelles, especially in mitochondria, in two forms: oxidized and reduced (49). Its concertation depends on cell type, where cells which have higher energy (cardiomyocytes, hepatocytes, skeletal muscle cells) needs the concentration of CoQ_{10} is higher. It acts as a radical scavenger in cells and is incorporated in several mitochondrial enzymes (respiratory chain enzymes), thus its deficiency is tightly related to a decrease in tissue oxidative defenses (49). Among others, CoQ_{10} protects ROS-induced DNA damage and together with tocopherol protects lipids from peroxidation (50). In gingival biopsies obtained from patients with damaged periodontal tissue, researchers found a decrease in CoQ_{10} and it is suggested that its application in oral mucosa, in a form of different supplements, lead to faster wound healing (51). Local application of CoQ_{10} on rat tooth extraction wounds significantly faster lead to wound healing, possibly by decreasing tissue inflammation estimated through NO and MPO levels (48). Also, the same work showed that encapsulation of CoQ_{10} in nanoparticles increases the effectiveness of this compound by increasing its solubility, concentration and delivery in target tissue after its application (48). On a molecular level, the application of CoQ_{10} to oral cavity wounds was proven to decrease proinflammatory cytokines expression (IL-1 and TNF-α), as well as of some molecules involved in inflammation (NF-kB and hypoxia factor-1) (52). Here again, besides soft tissue CoQ_{10} was shown to be able to affect bone regeneration in a similar animal model of tooth extraction (52).

Conclusion

It seems that there is a number of potential candidates which might enhance wound healing, after tooth extraction, by modulating different phases in the process. Over the years researchers collected a vast amount of information regarding the potential mechanisms by which these candidates modulate the wound healing process and it is not so clear which of them might be the best one for the treatment. Thus, new and more in-depth clinical and preclinical studies need to be conducted in order to estimate the real efficacy and safety levels in humans before introducing them in every day clinical practice.

References

1. Kujath P, Michelsen A. Wounds-from physiology to wound dressing. Dtsch Arztebl Int 2008; 105(13): 239-48. [CrossRef]
2. Nwomeh BB, Yager DR, Cohen IK. Physiology of the chronic wound. Clin Plast Surg 1998; 25(3):341-56. [PubMed]
3. Rodero MP, Khosrotehrani K. Skin wound healing modulation by macrophages. Int J Clin Exp Pathol 2010; 3(7):643-53. [PubMed]
4. Wozniak P, Kontek B, Rozanski W, Olas B. Evaluation of hemostasis parameters and the role of oxidative damage to plasma proteins in the modulation of hemostasis in patients with nephrolithiasis before and after extracorporeal shock wave lithotripsy. PloS ONE 2017; 12:e0185157. [PubMed] [CrossRef]
5. Novak P, Olas B, Wachowicz B. Oxidative stress in hemostasis. Post Bioch 2010; 56(3):239-47.
6. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. Clin Dermatol 2007; 25(1):9-18. [PubMed] [CrossRef]
7. Campos AC, Groth AK, Branco AB. Assessment and nutritional aspects of wound healing. Curr Opin Clin Nutr Metab Care 2008; 11(3):281-8. [PubMed] [CrossRef]
8. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature 2008; 453: 314-21. [PubMed] [CrossRef]
9. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008; 8: 958-69. [PubMed] [CrossRef]
10. Del Rosso J. Wound care in the dermatology office: Where are we in 2011? J Am Acad Dermatol 2011; 64:S1-7. [PubMed] [CrossRef]
11. Singh RK, Rai D, Yadav D, Bhargava A, Balzarini J, De Clercq E. Synthesis, antibacterial and antiviral properties of curcumin bioconjugates bearing dipeptide, fatty acids and folic acid. Eur J Med Chem 2010; 45(3): 1078-86. [PubMed] [CrossRef]
12. Oschman LJ, Chevalier G, Brown R. The effects of grounding (earthing) on inflammation, the immune response, wound healing, and prevention and treatment of chronic inflammatory and autoimmune diseases. J Inflamm Res 2015; 8:83-96. [PubMed] [CrossRef]
13. Slavich GM, Irwin MR. From stress to inflammation and major depressive disorder: A social signal transduction theory of depression. Psychol Bull 2014; 140 (3): 774-815. [PubMed] [CrossRef]
14. Aller MA, Arias JL, Arias JI, Sanchez-Patan F, Arias J. The inflammatory responserecapitulates phylogeny through trophic mechanisms to the injured tissue. Med Hypotheses 2007; 68(1):202-9. [PubMed] [CrossRef]
15. Reed RK, Rubin K. Transcapillary exchange: role and importance of the interstitial fluid pressure and the extracellular matrix. Cardiovasc Res 2010; 87(2):211-7. [PubMed] [CrossRef]
16. Wiig H. Pathophysiology of tissue fluid accumulation in inflammation. J Physiol 2011; 589(Pt 2):2945-53. [PubMed] [CrossRef]
17. Elvidge GP, Glenny L, Appelhof RJ, Ratcliffe PJ, Ragoussis J, Gleadle JM. Concordant regulation of gene expression by hypoxia and 2-oxoglutarate-dependent dioxygenase inhibition. The role of HIF-1*α*, HIF-2*α*, and other pathways. J Biol Chem 2006; 281(22): 15215-26. [PubMed] [CrossRef]
18. Luo H, Gary O, Rankin GO, Daddysman MK, Jiang BH, Chen YC. Kaempferol inhibits angiogenesis and VEGF expression through both HIF dependent and independent pathways in human ovarian cancer cells. Nutr Canc 2009; 61(4):554-63. [PubMed] [CrossRef]
19. Eming SA, Hammerschmidt M, Krieg T, Roers A. Interrelation of immunity and tissue repair on regeneration. Semin Cell Dev Biol 2009; 20(5):517-27. [PubMed] [CrossRef]
20. Blair P, Flaumenhaft R. Platelet α-granules: Basic biology and clinical correlates. Blood Rev 2009; 23(4): 177-89. [PubMed] [CrossRef]
21. Akbik D, Ghadiri M, Chrzanowski W, Rohanizadeh R. Curcimin as a wound healing agent. Life Sci 2014; 116(1):1-7. [PubMed] [CrossRef]
22. Edwards R, Harding KG. Bacteria and wound healing. Curr Opin Infect Dis 2004; 17(2):91-6. [PubMed] [CrossRef]
23. Kurz H, Burri PH, Djonov VG. Angiogenesis and vascular remodeling by intussusception: from form to function. News Physiol Sci 2003; 18:65-70. [PubMed] [CrossRef]
24. Bochaton-Piallat ML, Gabbiani G, Hinz B. The myofibroblast in wound healing and fibrosis: answered and unanswered questions. F1000Res 2016; 5 (F1000 Faculty Rev):752. [PubMed]
25. Ridiandries A, Bursill C, Tan J. Broad-spectrum inhibition of the CC-chemokine class improves wound healing and wound angiogenesis. Int J Mol Sci 2017; 18 (1):55. [PubMed] [CrossRef]

26. Martinotti S, Ranzato E. Dynamic interplay between cell types during wound healing. In: Ranzato E, editors. Keratinocytes: Structure, Molecular Mechanisms and Role in Immunity. Hauppauge: Nova Publishers Inc; 2013. p. 1-12.
27. Tracy LE, Minasian RA, Caterson EJ. Extracellular matrix and dermal fibroblast function in the healing wound. Advances in wound care 2016; 5(3):119-36. [PubMed] [CrossRef]
28. Robinson PM, Blalock TD, Yuan R, Lewin AS, Schultz GS. Hammerhead ribozyme - mediated knockdown of mRNA for fibrotic growth factor s: transforming growth factor - beta 1 and connective tissue growth factor. Methods Mol Biol 2012; 820:117-32. [PubMed] [CrossRef]
29. Borena BM, Martens A, Broeckx SY, Meyer E, Chiers K, Duchateau L, et al. skin wound healing in mammals: state-of-the-art on growth factor and stem cell based treatments. Cell Physiol Biochem 2015; 36(1):1-23. [PubMed] [CrossRef]
30. Freedberg IM, Tomić-Canić M, Komine M, Blumenberg M. Keratins and the keratinocyte activation cycle. J Invest Dermatol 2001; 116(5):633-40. [PubMed] [CrossRef]
31. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 2002; 3(5):349-63. [PubMed] [CrossRef]
32. Ferreira CL, Abreu FA, Silva GA, Silveira FF, Barreto LB, Paulino Tde P, et al. TGF-β1 and BMP-4 carried by liposomes enhance the healing process in alveolar bone. Arch Oral Biol 2013; 58(8):646-56. [CrossRef]
33. El- Amin SF, Hogan MV, Allen AA, Hinds J, Laurencin CT. The indications and use of bone morphogenic proteins in foot, ankle, and tibia surgery. Foot Ankle Clin 2010; 15(4):543-51. [PubMed] [CrossRef]
34. Farina R, Trombelli L. Wound healing of extraction sockets. Endodontic Topics 2012; 25(1):16-43. [CrossRef]
35. Mendes RM, Silva GAB, Caliari MV, Silva EE, Ladeira LO, Ferreira AJ. Effects of single wall carbon nanotubes and its functionalization with sodium hyaluronate on bone repair. Life Sci 2010; 87(7-8):215-22. [CrossRef]
36. Gupta N, Singh K, Sharma S. Hematoma - A complication of posterior superior alveolar nerve block. J Dent Probl Solut 2015; 2(1):15-6.
37. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. Lasers Surg Med 2000; 26(3):282-91. [PubMed] [CrossRef]
38. Ferrante M, Petrini M, Trentini P, Perfetti G, Spoto G. Effect of low-level laser therapy after extraction of impacted lower third molars. Lasers Med Sci 2013; 28(3):845-9. [PubMed] [CrossRef]
39. Kana JS, Hutschenreiter G, Haina D, Waidelich W. Effect of low-power density laser radiation on healing of open skin wounds in rats. Arch Surg 1981; 116 (3):293-6. [PubMed] [CrossRef]
40. de Medeiros ML, Araújo-Filho I, da Silva EM, de Sousa Queiroz WS, Soares CD, de Carvalho MG, et al. Effect of low-level laser therapy on angiogenesis and matrix metalloproteinase-2 immunoexpression in wound repair. Lasers Med Sci 2017; 32(1):35-43. [PubMed] [CrossRef]
41. Kucerová H, Dostálová T, Himmlova L, Bártová J, Mazánek J. Low-level laser therapy after molar extraction. J Clin Laser Med Surg 2000; 18(6):309-15. [PubMed] [CrossRef]
42. Todorović K, Katić V. Dejstvo polarizovane svetlosti na zarastanje postekstrakcione rane – literaturni podaci. Acta Stom Naissi 2002; 39-40:51-2.
43. Todorović K, Katić V. Uticaj Bioptron lampe na organizaciju i epitelizaciju rana nastalih ekstrakcijom zuba – eksperimentalna studija. Acta Stom Naissi 2003; 19(42):67-75.
44. Todorović K, Katić V, Janev J, Stanković S. Morfološki efekt na polarizirana svetlina na zarasnuvanje rana po vadenje na zab eksperimentalen model na glušec. Makedonski Stomatološki Pregled 2003; 27(1-4):23-8.
45. Mitić A, Todorović K, Stojiljković N, Stojanović N, Ilić S, Todorović A, et al. Beneficial effects of curcumin on the wound-healing process after tooth extraction. Nat Prod Comun 2017; 12(12):1905-8.
46. Anamika B. Extraction of curcumin. J Environ Sci Toxicol Food Technol 2012; 1(3):1-16.
47. Mehrbani D, Farjam M, Geramizadeh B, Tanideh N, Amini M, Panjehshahin MR. The healing effect of curcumin on burn wounds in rat. World J Plast Surg 2015; 4(1):29-35. [PubMed]
48. Todorovic K, Jovanovic G, Todorovic A, Mitic A, Stojiljkovic N, Ilic S, et al. Effects of coenzyme Q_{10} encapsulated in nanoliposomes on wound healing processes after tooth extraction. J Dent Sci 2018; 13(I2):103-8. [CrossRef]
49. Bentinger M, Tekle M, Dallner G. Coenzyme Q-biosynthesis and functions. Biochem Biophys Res Commun 2010; 396(1):74-9. [PubMed] [CrossRef]
50. Cordero MD, Alcocer-Gómez E, Culic O, Carrión AM, de Miguel M, Díaz-Parrado E, et al. NLRP3 inflammasome is activated in fibromyalgia: The effect of coenzyme Q_{10}. Antioxid Redox Signal 2014; 20(8):1169-80. [PubMed] [CrossRef]
51. Sale ST, Parvez H, Yeltiwar R, Vivekanandan G, Pundir AJ, Jain P. A comparative evaluation of topical and intrasulcular application of coenzyme Q_{10} (Perio Q TM) gel in chronic periodontitis patients: A clinical study. J Ind Soc Periodont 2014; 18(4):461-5. [PubMed] [CrossRef]
52. Yoneda T, Tomofuji T, Kawabata Y, Ekuni D, Azuma T, Kataoka K, et al. Application of coenzyme Q10 for accelerating soft tissue wound healing after tooth extraction in rats. Nutrients 2014; 6(12):5756-69. [PubMed]

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POTENCIJALNI TERAPEUTSKI PRISTUPI U TRETMANU RANA NAKON EKSTRAKCIJE ZUBA: PREGLEDNI RAD

Kosta Todorović[1], Marija Bojović[2], Vladimir Mitić[3], Milan Spasić[1], Ana Todorović[3]

1Univerzitet u Nišu, Medicinski fakultet, Odeljenje za oralnu hirurgiju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Odeljenje za oralnu medicinu i parodontologiju, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Odeljenje za ortopediju vilica, Niš, Srbija

Kontakt: Kosta Todorović
Bulevar dr Zoran Đinđić 81, 18000 Niš, Srbija
E-mail: kosta_todorovic@yahoo.com

Zarastanje rana je kompleksan proces koji se dešava nakon povrede tkiva sa ciljem povraćaja njegove homeostaze. U zavisnosti od tipa rane, može biti primarno ili sekundarno zarastanje. Sam proces zarastanja rane podeljen je u nekoliko precizno definisanih faza koje se međusobno vrlo često preklapaju, a one uključuju: (I) hemostazu, (II) inflamaciju, (III) proliferaciju, (IV) maturaciju, a u nekim slučajevima i (V) koštanu regeneraciju. Ekstrakcija zuba je veoma česta procedura u stomatologiji i predstavlja ekstrackiju pokvarenih, periodontalno izmenjenih ili impatkiranih zuba. Nakon ekstrakcije, nastanak rane je neizbežan, a vrlo često je ovaj proces praćen bolom i osećajem nelagodnosti. U ovom revijalnom radu osvrnućemo se na mogućnost primene lasera male snage, polarizovane svetlosti, kurkumina i koenzima Q_{10} u terapiji rana nastalih ekstrakcijom zuba. Na osnovu pregleda literature može se zaključiti da postoje potencijalni kandidati koji mogu da poboljšaju zarastanje rane nakon ekstrakcije zuba, jer moduliraju različite faze u procesu zarastanja. Takođe, smatramo da su neophodne nove i detaljnije kliničke i pretkliničke studije koje bi odredile pravi uticaj i potencijalne neželjene efekte ovih terapeutskih mogućnosti kod ljudi pre no što se krene sa njihovom primenom u svakodnevnoj kliničkoj praksi.

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Ključne reči: *rana, ekstrakcija zuba, laseri male snage, polarizovana svetlost, kurkumin, koenzim Q_{10}*

Review article

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CURRENT ACHIEVEMENTS OF DENDRITIC CELL-BASED IMMUNOTHERAPY

Tanja Džopalić[1], Marija Topalović[1], Marko Bjelaković[1,2]

Dendritic cells (DCs) are the key antigen-presenting cells and stimulators of the immune response. Numerous studies have proven DC-based tumor vaccines as the most effective form of tumor vaccines with good results in clinical trials. Due to the marked disproportion in the results of complete tumor curing in some patients using DC-based vaccines and modest results achieved in other patients, there is a need for improvement of preparation methods. This review summarizes the current protocols in creating DC-based tumor vaccines and future perspectives as well.

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Key words: *dendritic cells, immunotherapy, cancer vaccines*

[1]University of Niš, Faculty of medicine, Niš, Serbia
[2]Clinical Center Niš, Pulmonary Diseases Clinic, Niš, Serbia

Contact: Tanja Džopalić
Blvd. Dr Zoran Djindjić 81, 18000 Nis, Serbia
E-mail: tanja.dzopalic@medfak.ni.ac.rs
tanjche80@gmail.com

Introduction

Dendritic cells (DCs) have been occupying the attention of immunologists all over the world for more than 40 years. During the last two decades, DCs have been shown as a useful tool in boosting the antitumor immune response (1-3).

In their resting state, DCs are considered to be immature but prepared to recognize and acquire antigens through numerous pattern-recognition receptors (PRRs). There are several groups of PRRs, including Toll-like receptors (TLRs), C-type lectins (CLRs), cytoplasmic retinoic acid–inducible gene-I-like receptors (RLRs) and nucleotide oligomerization domain-like receptors (NLRs) (4-6). Upon PRRs activation, DCs undergo phenotypic and functional maturational changes (7). They include the upregulation of chemokine receptors (CCR7), adhesion molecules (CD54), co-stimulatory molecules (CD80 and CD86), immunoproteosomes and major histocompatibility complex (MHC)-MHC class I and II molecules, all important for DCs migration to the lymphoid tissues and activation of the immune response (8). Co-stimulatory molecules and cytokines expressed by the DCs determine the immune response towards T helper 1 (Th1), Th2 or Th17 profile. Thanks to the production of different cytokines (IL-12, IL-15, type I IFNs), DCs also activate B cells (9), natural killer (NK) cells (10) and NKT cells (11).

Upon recognition, DCs take up antigens through pinocytosis, endocytosis, and receptor-mediated phagocytosis by Fc receptors, integrins, apoptotic cell receptors, CLRs and scavenger receptors (8). Antigens are further processed into peptides that are presented on MHC class I and II molecules via the endogenous pathway or exogenous pathway, respectively (12, 13). Endogenous pathway includes processing and presentation of intracellular antigens to $CD8^+$ T cells, whereas exogenous peptides are presented to $CD4^+$ T cells via exogenous pathway. In addition, DCs can process the exogenous antigens on MHC I molecules stimulating $CD8^+$ T cells via cross-presentation.

Following maturation, DCs migrate to the secondary lymph organs (lymph nodes, spleen, Peyer's patches) where they interact with T cells (14). T cells specifically recognize antigens expressed on MHC molecules through their T cell receptor (TCR). Duration and intensity of DC-T cell interaction seem o be of great importance in T cell activation. B cells are activated by DCs through their ability to stimulate T follicular helper cells and induction of B cell growth and antibody production. DCs process glycolipid antigens and present them on the CD1d molecule and activate NKT cells (11). Concluding, DCs are an important part in the orchestration of innate and adaptive arm of immune responses.

In humans, myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) are considered as two main types of DCs in the blood (2). Both mDCs and pDCs express distinct TLRs and respond differently to pathogenic stimuli, suggesting their specific direction of the immune responses (15). The main role of mDCs

refers to immunity against fungi and bacteria (16) and detection and uptake of necrotic cells as well (17). pDcs produce high levels of type I IFNs in response to viral products, suggesting their important part in preventing viral infections (18). There are some studies indicating a synergistic act of mDcs and pDCs in antitumor response (19).

DC immunotherapy

In order to eliminate the malignant tumors by immune mechanisms, modern chemotherapeutic protocols are supplemented with immunotherapeutic procedures. Within active specific immunotherapy, the best clinical results are achieved using DC-based tumor vaccines (2, 8). DCs are key antigen-presenting cells and immune response stimulators. On the other hand, they are also important for immunological tolerance and maintaining the immune system nonreactive to its own antigens. One of the mechanisms by which the tumor evades the effector functions of the immune system is the stimulation of the tolerant properties of the DCs. Immature DCs are potentially tolerogenic and might even promote antigen-specific tolerance when used in DC vaccines (20). Therefore, the latest studies of tumor vaccine development are based on the preparation of mature DCs with immunogenic properties (21-23).

Methods of generating DCs

Current protocols for generating DCs include differentiation from monocyte precursors or $CD34^+$ hematopoietic precursors, *in vivo* expansion of circulating DCs and isolation and enrichment of circulating blood DC subsets (8).

Since the circulating number of human DCs is very low, most of the studies exploit *in vitro*-generated monocyte-derived DCs (MoDCs) obtained by cultivation of monocytes from peripheral blood mononuclear cells (PBMCs) (24, 25). Monocytes are induced to differentiate into immature MoDCs after 6 days in the presence of GM-CSF and IL-4, upon which the cells are stimulated to mature into the immunogenic MoDCs.

Another way of the DCs generation involves mobilization of $CD34^+$ precursors from the bone marrow upon which the cells are expanded *in vitro* in the presence of GM-CSF, Flt3L, TNF-α, TGF-β, and SCF. Such cells represent the mixture of MoDCs and myeloid cells at different stages of differentiation (26).

Maturation of DCs

Since immature DCs are not able to induce an mmune response, only mature DCs are included in clinical trials (23, 27). Maturation of DCs can be achieved in many different ways, but still, there is no consensus on adequate maturation stimuli. Gold standard for maturation of DCs used to include a well-known cocktail of proinflammatory cytokines TNF-α, IL-1β and IL-6 with PGE_2 (28). This way matured DCs highly express CD40, CD80, CD86, MHC class I and II, but fail to induce IL-12 production, important for the development of Th1 antitumor response. TLR agonists have also been used to mature DCs. Poly I:C (TLR3), LPS (TLR4), loxoribine and resiquimod (TLR7) are well-known TLR agonists and activators of DCs (29-31). Simultaneous engagement of different TLRs potentiates more effective antitumor immune response through the maturation of DCs (32, 33). Additionally, single endosomal TLR agonists could be effectively delivered to DCs by nanomaterials (carbon nanotubes) inducing very potent immunostimulatory effect (34). Co-activation of TLRs and CLRs by TLR3 and Dectin-1 agonists can also be used as a good maturation DC stimulus. Such treated DCs express mature phenotype and polarize immune response in Th1 and Th17 direction (35).

Tumor Antigens and Loading of DCs

In order to exert good immune response, MHC molecules of DCs should be loaded with adequate tumor antigens. For the induction of a strong and sustained antitumor T-cell response, it would be of great interest to activate both, $CD4^+$ and $CD8^+$ T cells (36, 37). Several methods of loading of DCs with tumor epitopes have been described.

DCs are usually loaded through incubation with peptides, proteins, RNA or autologous/ allogeneic tumor cells before maturation process (8, 38). Peptides loading do not require antigen processing, therefore they can be directly placed on the MHC molecules on the surface of the DCs. However, such an approach is HLA-type dependent and antigen identification is required for these specific haplotypes. Clinical trials using peptides as a loader, exerted satisfactory results in patients with cervical (39, 40), ovarian (41) and colorectal cancer (42) where $CD4^+$ and $CD8^+$ T cell responses were induced.

DCs loading with proteins and tumor cell lysates does not need antigen identification, thus multiple epitopes can be presented on MHC molecules of different haplotypes (43). The best results were achieved in multiple myeloma and ovarian cancer patients where DCs were fused with tumor lysates and both $CD4^+$ and $CD8^+$ T cell responses occurred (44, 45). A study in the mouse model of high-grade glioma exerted an increased survival accompanied by the immunostimulatory switch from regulatory T cells to Th1 and Th17 (46).

Currently, RNA transfection of DCs is a very useful method in achieving potent antitumor immunity by encoding specific antigens and maturation factors (47, 48). Clinical benefit among patients with metastatic clear cell renal cell carcinoma and non-small cell lung cancer was achieved by RNA transfected DCs (47, 49). Recent investigations using neoantigen-loaded DCs showed promising results by the promotion of neoantigen-specific T-cell response (50).

Administration and migration of DCs

The biological potential of the DCs to stimulate the antitumor immune response also depends on their migration to the lymph nodes (2). Therefore, the optimal route of administration of DCs needs to be established. Current immunization strategies include subcutaneous, intradermal, intranodal, intrave-

nous and intratumoral administration (8). So far, the best systemic response was achieved via a combination of the intradermal and intravenous application of DC vaccines in the treatment of patients with melanoma (51, 52).

In Vivo Targeting of DCs

In vivo DC targeting represents a novel approach that involves targeting specific receptors, such as Fc receptors, CD40, and CLRs (3). CLRs are the most attractive targets since DCs express a variety of CLRs (Clec9a, DEC205, Langerin) involved in recognition of glycosylated antigens (53). Targeting of Clec9a in animal models induced good antitumor response through cellular and humoral immunity (54, 55). Clinical trials of DEC205 targeting are currently ongoing in ovarian cancer, acute myeloid leukemia, and melanoma (8).

Delivery of tumor antigens via RNA lipoplexes is the newest approach for *in vivo* DC targeting (8). Such combination protects RNA from degradation, while the RNA itself activates pDCs and induces the release of IFN type I. There is an ongoing clinical trial with melanoma patients where high amounts of IFNα production and antigen- specific T cell response were observed (56).

DC-derived exosomes

Another new approach in cancer immunotherapy involving DCs refers to DC-derived exosomes (DC-Exo). It has been shown that DC-Exo turns cancer cells into more immunogenic targets, which can contribute to the effectiveness of DC vaccines (3, 57).

Future approaches

Combination of DCs with immune checkpoint inhibitors (CTLA-4 and PD-1 blocking antibodies), should direct T-cell response in a specific manner. This novel approach is still under investigations, where the increase in the number of circulating T cells is expected (58, 59).

There are ongoing studies using new technologies in the differentiation of DCs from human pluripotent stem cells and embryonic stem cells with the addition of growth factors, BMP-4, VEGF, GM-CSF, SCF, Flt3L and IL-4 at key intervals of differentiation (60).

Additionally, new genetic engineering technologies using Cas9 endonuclease, can modulate DCs and delete expression of inhibitory molecules (PD-L1) and cytokines (IL-10). Such modulated DCs express improved efficiency *in vivo* and drive $CD8^+$ T cell differentiation (8).

A recent study by Woo et al. has demonstrated that activation of the STING (Stimulator of Interferon Genes) pathway in tumor-resident host DCs is required for induction of a spontaneous $CD8^+$ T cell response against tumor-derived antigens *in vivo* (61). Activation of this pathway and the subsequent production of IFNβ lead to potent and systemic tumor regression and immunity, which can be potentiated with co-administration of a natural STING agonist (62).

Schematic presentation of current and future approaches in DC-based immunotherapy is pictured in Figure 1.

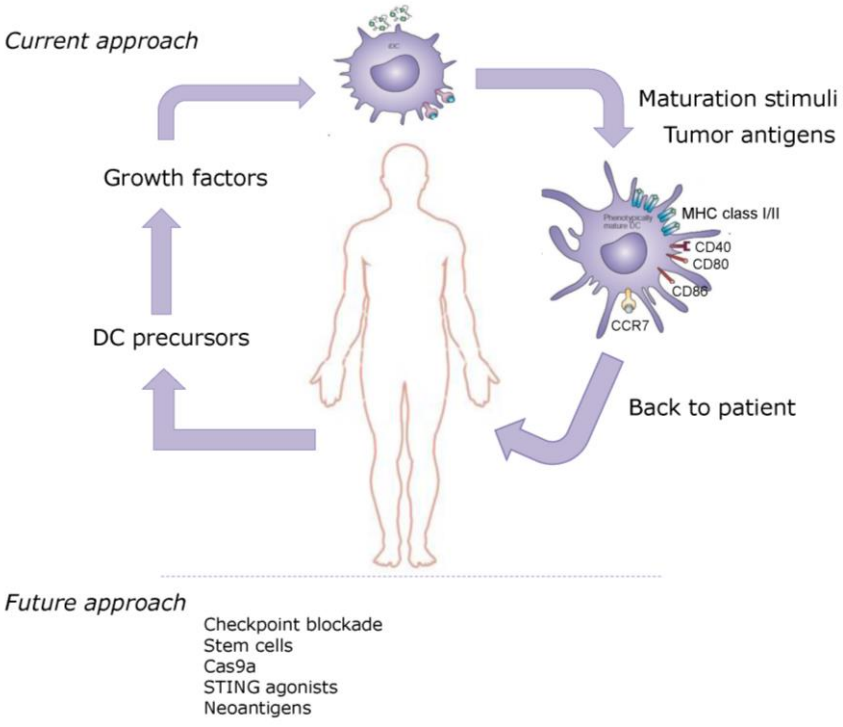

Figure 1. Current and future approaches in dendritic cell-based immunotherapy

Conclusion

Recent studies have shown the treatment of malignant tumors by tumor vaccines as an acceptable and harmless way of immunotherapy along with surgical treatment, radiotherapy, and chemotherapy. DC-based tumor vaccines, as the most potent tumor-specific immune response stimulants, are the most effective form of tumor vaccines. There are great expectations of numerous ongoing clinical studies involving DCs as cancer vaccines, as Karolina Palucka and Jacques Banchereau stated: *Just as immunotherapy is moving to the forefront of cancer therapy, DC-based therapy is moving to the forefront of cancer immunotherapy.*

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References

1. Sabado RL, Bhardwaj N. Directing dendritic cell immunotherapy towards successful cancer treatment. Immunotherapy 2010; 2:37-56. [PubMed] [CrossRef]
2. Bol KF, Schreibelt G, Gerritsen WR, de Vries IJ, Figdor CG. Dendritic Cell-Based Immunotherapy: State of the Art and Beyond. Clin Cancer Res 2016; 22:1897-906. [PubMed] [CrossRef]
3. Constantino J, Gomes C, Falcao A, Neves BM, Cruz MT. Dendritic cell-based immunotherapy: a basic review and recent advances. Immunol Res 2017; 65: 798-810. [PubMed] [CrossRef]
4. Medzhitov R, Janeway CA Jr. Decoding the patterns of self and nonself by the innate immune system. Science 2002; 296:298-300. [PubMed] [CrossRef]
5. Figdor CG, van Kooyk Y, Adema GJ. C-type lectin receptors on dendritic cells and Langerhans cells. Nat Rev Immunol 2002; 2:77-84. [PubMed] [CrossRef]
6. Underhill DM. Collaboration between the innate immune receptors dectin-1, TLRs, and Nods. Immunol Rev 2007; 219:75-87. [PubMed] [CrossRef]
7. Reis e Sousa C. Dendritic cells in a mature age. Nat Rev Immunol 2006; 6:476-83. [PubMed] [CrossRef]
8. Sabado RL, Balan S, Bhardwaj N. Dendritic cell-based immunotherapy. Cell Res 2017; 27:74-95. [PubMed] [CrossRef]
9. Jego G, Pascual V, Palucka AK, Banchereau J. Dendritic cells control B cell growth and differentiation. Curr Dir Autoimmun 2005; 8:124-39. [PubMed] [CrossRef]
10. Munz C, Dao T, Ferlazzo G, de Cos MA, Goodman K, Young JW. Mature myeloid dendritic cell subsets have distinct roles for activation and viability of circulating human natural killer cells. Blood 2005; 105:266-73. [PubMed] [CrossRef]
11. Fujii S, Shimizu K, Kronenberg M, Steinman RM. Prolonged IFN-gamma-producing NKT response induced with α-galactosylceramide- loaded DCs. Nat Immunol 2002; 3:867-74. [PubMed] [CrossRef]
12. Palucka K, Banchereau J. Cancer immunotherapy via dendriticcells. Nat Rev Cancer 2012; 12:265-77. [PubMed] [CrossRef]
13. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature 2007; 449:419-26. [PubMed] [CrossRef]
14. Benvenuti F. The dendritic cell synapse: a life dedicated to Tcell activation. Front Immunol 2016; 7:70. [PubMed] [CrossRef]
15. Schreibelt G, Tel J, Sliepen KH, Benitez-Ribas D, Figdor CG, Adema GJ, et al. Toll-like receptor expression and function in human dendritic cell subsets:implications for dendritic cell-based anti-cancer immunotherapy. Cancer Immunol Immunother 2010; 59:1573-82. [PubMed] [CrossRef]
16. Liu YJ. Dendritic cell subsets and lineages, and their functions in innateand adaptive immunity. Cell 2001; 106:259-62. [PubMed] [CrossRef]
17. Sancho D, Joffre OP, Keller AM, Rogers NC, Martinez D, Hernanz-Falcon P, et al. Identification of a dendritic cell receptor that couples sensing ofnecrosis to immunity. Nature 2009; 458:899-903. [PubMed] [CrossRef]
18. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes andproduce large amounts of type I interferon. Nat Med 1999; 5: 919-23. [PubMed] [CrossRef]
19. Piccioli D, Sammicheli C, Tavarini S, Nuti S, Frigimelica E, Manetti AG, et al. Human plasmacytoid dendritic cells are unresponsive to bacterial stimulationand require a novel type of cooperation with myeloid dendritic cellsfor maturation. Blood 2009; 113:4232-9. [PubMed] [CrossRef]

20. Dhodapkar MV, Steinman RM, Krasovsky J, Munz C, Bhardwaj N. Antigenspecific inhibition of effector T cell function in humans after injection ofimmature dendritic cells. J Exp Med 2001; 193:233-8. [PubMed] [CrossRef]
21. Draube A, Klein-Gonzalez N, Mattheus S, Brillant C, Hellmich M, Engert A, et al. Dendritic cell based tumor vaccination in prostate and renal cellcancer: a systematic review and meta-analysis. PLoS One 2011; 6: e18801. [PubMed] [CrossRef]
22. Jonuleit H, Giesecke-Tuettenberg A, Tuting T, Thurner-Schuler B, Stuge TB, Paragnik L, et al. Acomparison of two types of dendritic cell as adjuvants forthe induction of melanoma-specific T-cell responses in humans followingintranodal injection. Int J Cancer 2001; 93:243-51. [PubMed] [CrossRef]
23. de Vries IJ, LesterhuisWJ, Scharenborg NM, Engelen LP, RuiterDJ, Gerritsen MJ, et al. Maturation of dendritic cells is a prerequisite for inducingimmune responses in advanced melanoma patients. Clin Cancer Res 2003; 9:5091-100. [PubMed]
24. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen bycultured human dendritic cells is maintained by granulocyte/macrophagecolony-stimulating factor plus interleukin 4 and downregulated by tumornecrosis factor alpha. J Exp Med 1994; 179(4): 1109-118. [PubMed] [CrossRef]
25. Colic M, Stojic-Vukanic Z, Pavlovic B, Jandrić D, Stefanoska I. Mycophenolatemofetilinhibits differentiation, maturation and allostimulatory function of human-monocyte-derived dendritic cells. Clin Exp Immunol 2003; 134(1):63-9. [PubMed] [CrossRef]
26. Redman BG, Chang AE, Whitfield J, Esper P, Jiang G, Braun T, et al. Phase Ib trial assessingautologous, tumor-pulsed dendritic cells as a vaccineadministered with or without IL-2 in patients with metastaticmelanoma. J Immunother 2008; 31:591-8. [PubMed] [CrossRef]
27. Dhodapkar MV, Steinman RM, Sapp M, Desai H, Fossella C, Krasovsky J, et al. Rapid generationof broad T-cell immunity in humans after a single injecttionof mature dendritic cells. J Clin Invest 1999; 104:173-80. [PubMed] [CrossRef]
28. Lee AW, Truong T, Bickham K, Fonteneau JF, Larsson M, Da Silva I, et al. A clinical grade cocktailof cytokines and PGE2 results in uniform maturation ofhuman monocyte-derived dendritic cells: implications for immunotherapy. Vaccine 2002; 20 Suppl 4:A8-22. [PubMed] [CrossRef]
29. Rouas R, Lewalle P, El Ouriaghli F, Nowak B, Duvillier H, Martiat P. Poly(I:C) used for humandendritic cell maturation preserves their ability to secondarily secretebioactive IL-12. Int Immunol 2004; 16(5):767-73. [PubMed] [CrossRef]
30. Colic M, Mojsilovic S, Pavlovic B, Vucićević D, Majstorović I, Bufan B, et al**.** Comparison of two differentprotocols for the induction of maturation of human dendritic cells in vitro. Vojnosanit Pregl 2004; 61(5): 471-8. [PubMed] [CrossRef]
31. Dzopalic T, Dragicevic A, Vasilijic S, Vucevic D, Majstorovic I, Bozic B, et al**.** Loxoribine, a selective Toll-likereceptor 7 agonist, induces maturation of human monocyte-deriveddendritic cells and stimulates their Th-1- and Th-17-polarizing capability. Int Immunopharmacol 2010; 10(11):1428-33. [PubMed] [CrossRef]
32. Napolitani G, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A. Selected Toll-like receptoragonist combinations synergistically trigger a T helper type 1-polarizingprogram in dendritic cells. Nat Immunol 2005; 6(8):769-76. [PubMed] [CrossRef]
33. Dzopalic T, Rajkovic I, Dragicevic A, Colic M. The response of humandendritic cells to co-ligation of pattern-recognition receptors. Immunol Res 2012; 52(1-2):20-33. [PubMed] [CrossRef]
34. Čolić M, Džopalić T, Tomić S, Rajković J, Rudolf R, Vuković G, et al. Immunomodulatory effects of carbonnanotubes functionalized with a Toll-like receptor 7 agonist on humandendritic cells. Carbon 2014; 67: 273-87. [CrossRef]
35. Dragicevic A, Dzopalic T, Vasilijic S, Vucevic D, Tomic S, Bozic B, et al. Signaling through Toll-likereceptor 3 and Dectin-1 potentiates the capability of human monocytederiveddendritic cells to promote T-helper 1 and T-helper 17 immuneresponses. Cytotherapy 2012; 14(5):598-607. [PubMed] [CrossRef]
36. Bos R, Sherman LA. CD4þ T-cell help in the tumor milieu is required forrecruitment and cytolytic function of CD8þ T lymphocytes. Cancer Res 2010; 70:8368–77. [PubMed] [CrossRef]
37. Aarntzen EH, De Vries IJ, Lesterhuis WJ, Schuurhuis D, Jacobs JF, Bol K, et al. Targeting CD4(þ) T-helper cells improves the induction of antitumorresponses in dendritic cell-based vaccination. Cancer Res 2013; 73:19-29. [PubMed] [CrossRef]
38. O'Neill D, Bhardwaj N. Generation of autologous peptideandprotein-pulsed dendritic cells for patient-specific immunotherapy. Methods Mol Med 2005; 109: 97-112. [PubMed]
39. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, et al. Phase I immunotherapeutictrial with long peptides spanning the E6 and E7sequences of high-risk human papillomavirus 16 in end-stagecervical cancer patients shows low toxicity and robust immunogenicity. Clin Cancer Res 2008; 14:169-77. [PubMed] [CrossRef]
40. Welters MJ, Kenter GG, Piersma SJ, Vloon AP, Löwik MJ, Berends-van der Meer DM, et al. Induction of tumor-specific $CD4^+$ and $CD8^+$ T-cell immunity in cervicalcancer patients by a human papillomavirus type 16 E6 and E7 long peptides vaccine. Clin Cancer Res 2008; 14:178-87. [PubMed] [CrossRef]
41. Leffers N, Lambeck AJ, Gooden MJ, Hoogeboom BN, Wolf R, Hamming IE, et al. Immunization witha P53 synthetic long peptide vaccine induces P53-specific immuneresponses in ovarian cancer patients, a phase II trial. IntJ Cancer 2009; 125:2104-13. [PubMed] [CrossRef]
42. Zeestraten EC, Speetjens FM, Welters MJ, Saadatmand S, Stynenbosch LF, Jongen R, et al. Addition ofinterferon-α to the p53-SLP(R) vaccine results in increasedproduction of interferon-gamma in vaccineted colorectalcancer patients: a phase I/II clinical trial. Int J Cancer 2013; 132:1581-91. [PubMed] [CrossRef]
43. Schnurr M, Chen Q, Shin A, Chen W, Toy T, Jenderek C, et al. Tumor antigen processingand presentation depend critically on dendritic cell type andthe mode of antigen delivery. Blood 2005; 105:2465-72. [PubMed] [CrossRef]
44. Chiang CL, Kandalaft LE, Tanyi J, Hagemann AR, Motz GT, Svoronos N, et al. A dendritic cell vaccinepulsed with autologous hypochlorous acid-oxidized ovarian-cancer lysate primes effective broad antitumor immunity:from bench to bedside. Clin Cancer Res 2013; 19:4801-15. [PubMed] [CrossRef]
45. Rosenblatt J, Vasir B, Uhl L, Blotta S, Macnamara C, Somaiya P, et al. Vaccination with dendriticcell/tumor fusion cells results in cellular and humoral antitumor-immune responses in patients with multiple myeloma. Blood 2011; 117:393-402. [PubMed] [CrossRef]
46. Garg AD, Vandenberk L, Koks C, Verschuere T, Boon L, Van Gool SW, et al. Dendritic cell vaccinesbased on

immunogenic cell death elicit danger signalsand T cell-driven rejection of high-grade glioma. Sci Transl Med 2016; 8:328ra327. [PubMed] [CrossRef]
47. Muller MR, Tsakou G, Grunebach F, Schmidt SM, Brossart P. Induction of chronic lymphocytic leukemia (CLL)-specificCD4- and CD8-mediated T-cell responses using RNA-transfecteddendritic cells. Blood 2004; 103:1763-69. [PubMed] [CrossRef]
48. Milazzo C, Reichardt VL, Muller MR, Grunebach F, Brossart P. Induction of myeloma-specific cytotoxic T cells using dendriticcells transfected with tumor-derived RNA. Blood 2003; 101:977-82. [PubMed] [CrossRef]
49. Amin A, Dudek AZ, Logan TF, Lance RS, Holzbeierlein JM, Knox JJ, et al. Survival with AGS-003, an autologous dendritic cell-based immunotherapy, incombination with sunitinib in unfavorable risk patients withadvanced renal cell carcinoma (RCC): phase 2 study results. J Immunother Cancer 2015; 3:14. [PubMed] [CrossRef]
50. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, et al. Cancer immunetherapy. A dendritic cell vaccine increases the breadth and diversity of melanomaneoantigen-specific T cells. Science. 2015; 348(6236):803-8. [PubMed] [CrossRef]
51. Verdijk P, Aarntzen EH, Lesterhuis WJ, Boullart AC, Kok E, van Rossum MM, et al. Limitedamounts of dendritic cells migrate into the T-cell area oflymph nodes but have high immune activating potential inmelanoma patients. Clin Cancer Res 2009; 15:2531-40. [PubMed] [CrossRef]
52. Lesterhuis WJ, de Vries IJ, Schreibelt G, Lambeck AJ, Aarntzen EH, Jacobs JF, et al. Route ofadministration modulates the induction of dendritic cell vaccine-induced antigen-specific T cells in advanced melanomapatients. Clin Cancer Res 2011; 17:5725-35. [PubMed] [CrossRef]
53. van Kooyk Y. C-type lectins on dendritic cells: key modulatorsfor the induction of immune responses. Biochem Soc Trans 2008; 36:1478-81. [PubMed] [CrossRef]
54. Kato Y, Zaid A, Davey GM, Mueller SN, Nutt SL, Zotos D, et al. Targeting antigen to Clec9Aprimes follicular Th cell memory responses capable of robustrecall. J Immunol 2015; 195:1006-14. [PubMed] [CrossRef]
55. Li J, Ahmet F, Sullivan LC, Brooks AG, Kent SJ, De Rose R, et al. Antibodies targeting Clec9Apromote strong humoral immunity without adjuvant in miceand non-human primates. Eur J Immunol 2015; 45:854-64. [PubMed] [CrossRef]
56. Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, Reuter KC, et al. Systemic RNA deliveryto dendritic cells exploits antiviral defence for cancer immunetherapy. Nature 2016; 534:396-401. [PubMed] [CrossRef]
57. Romagnoli GG, Zelante BB, Toniolo PA, Migliori IK1, Barbuto JA. Dendritic cell-derived exosomes may be a tool for cancer immunotherapy by converting tumor cells into immunogenic targets. Front Immunol 2014; 5:692. [PubMed]
58. Zarour HM. Reversing T-cell dysfunction and exhaustion in cancer. ClinCancer Res 2016; 22(8):1856-64. [PubMed] [CrossRef]
59. Wilgenhof S, Corthals J, Heirman C, van Baren N, Lucas S, Kvistborg P, et al. Phase II study of autologousmonocyte-derived mRNA electroporated dendritic cells (TriMixDCMEL)plus ipilimumab in patients with pretreated advanced melanoma. JClin Oncol 2016; 34(12):1330-8. [PubMed] [CrossRef]
60. Senju S, Hirata S, Motomura Y, Fukuma D, Matsunaga Y, Fukushima S, et al. Pluripotent stem cellsas source of dendritic cells for immune therapy. Int J Hematol2010; 91:392-400. [CrossRef]
61. Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MY, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity 2014; 41:830-42. [PubMed] [CrossRef]
62. Corrales L, Glickman LH, McWhirter SM, Kanne DB, Sivick KE, Katibah GE, et al. Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity. Cell Rep 2015; 11:1018-30. [PubMed] [CrossRef]

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SAVREMENA DOSTIGNUĆA U IMUNOTERAPIJI TUMORA ZASNOVANOJ NA DENDRITSKIM ĆELIJAMA

Tanja Džopalić[1], Marija Topalović[1], Marko Bjelaković[1,2]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Klinički centar Niš, Klinika za plućne bolesti, Niš, Srbija

Kontakt: Tanja Džopalić
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: tanja.dzopalic@medfak.ni.ac.rs
tanjche80@gmail.com

Dendritske ćelije (DĆ) glavni su antigen prezentujuće ćelije i pokretači imunskog odgovora. Brojne studije i klinička ispitivanja pokazali su da vakcine zasnovane na DĆ predstavljaju najefikasniji vid imunoterapije tumora. Zbog izrazite disproporcije u odličnim rezultatima potpunog izlečenja tumora kod nekih bolesnika primenom DĆ vakcina i skromnih rezultata koji se postižu kod drugih bolesnika, nameće se potreba da se priprema DĆ vakcina unapredi. U ovom preglednom članku navedeni su savremeni protokoli generisanja DĆ i kreiranja vakcina, kao i buduća razmišljanja.

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Ključne reči: *dendritske ćelije, imunoterapija, tumorske vakcine*

Review article

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MEDICAL NEGLIGENCE IN THE REPUBLIC OF SERBIA

Vladimir Miletić[1], Iva Zdravković[2]

In the area of protection and improvement of human health, it is of particular importance to provide legal, effective, correct, professional and timely medical assistance; performing other healthcare activities, or providing medical assistance or care. In this way, a significant social function is achieved, as well as the protection of the proclaimed right by the Constitution on the inviolability of the physical and psychological integrity of man (human health). However, due to the physician's or other medical activity, it is possible that the health of the person according to which the corresponding activity is undertaken is deteriorating. In the case of serious mistreat of physician's or another medical profession, or a gross violation of the rules of the profession, resulting in a more serious consequence of the health of people, then all modern legislation provides for criminal responsibility and punish-ability for a particular criminal offense - inadequate medical assistance. A similar situation exists in the Republic of Serbia. In this paper, this crime is analyzed from the aspect of the concept, characteristics, features, forms of expression and other grounds for determining criminal responsibility and punishment of its perpetrator.

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Key words: *health, protection, unconsciousness, criminal act, law, responsibility*

[1]Magistrate court of Belgrade, Belgrade, Serbia
[2]Magistrate court of Niš, Niš, Serbia

Contact: Iva Zdravković
Studenička 48/70, 18000 Niš, Serbia
E-mail : ivaduke.ia@gmail.com

Introduction

People's healthcare, along with life protection and bodily integrity, represents a social function which every country has performed since the early ages until this day. This is manifested in numerous crimes from which these personal and social values are protected, since the first written legal codes (e.g. Dušan's code) (1). Naturally, not only that the care was incomplete, inefficient, unevenly distributed, but also had minor or greater differences in incriminations of violating or imperiling these social values, depending on characteristics and type of a state organization.

Starting from France's bourgeois revolution, the protection of these social values has gained significance, considering the declared human rights and freedoms. Naturally, among basic, fundamental, universal, natural and general human rights, there is the right to live, the right of inviolability of the physical and mental (psychological) integrity, and the right of an individual to be healthy. The protection of these human rights was proclaimed as a part of universal (UN) and regional (CoE) international documents and constitutions of states as the highest legal acts (2).

All positive obligations in Criminal law in this specific area regulate various forms and aspects of manifestations of the crimes against all of these human and social rights, as well as against people's health. The situation in Serbia is similar. Serbia acknowledged the first crimes against the general welfare just in 1929 altogether with the Declaration of the Criminal law of the Kingdom of Yugoslavia. All future criminal law acts: Criminal law of the Federal People's Republic of Yugoslavia from 1959, just as the Criminal law of the Socialist Republic of Serbia (as well as the laws of other socialist republics and socialist autonomous provinces) from 1977, acknowledged crimes against people's wellbeing (3). It is the same nowadays in the Republic of Serbia, where starting from January 1st, 2006 the Criminal law has been in use. This law in chapter 23 predicts more felonies against people's welfare (known as the Crimes against Human Life and Health).

The Protection of People's Health in the Criminal Law

The Criminal law has been in use since January 1st, 2006 in the Republic of Serbia. It predicts more criminal acts in chapter 23 "Criminal Acts against People's Health" which, as the object of pro-

tection, has people's welfare (4). Namely, these are the crimes against not only the people's wellbeing but also the right to protect one's health, which is guaranteed in article 68 of the Republic of Serbia's Constitution. The crimes against general health had been earlier systematized (according to the law from 1977) into a unique group of acts together with "ecological" acts against the environment (5). Health is the psychophysical state of a human being, including all organs and body parts functioning harmoniously and processes, which enables one to lead a normal life and labor (6).

As opposed to health there is illness, the psychophysical state, which is created under the effect of germs, bacteria and viruses on the human body and which leads to a dysfunction in the built and function of particular organs, tissues or the whole body as a whole unit, because of which a normal life and labor are weakened or interrupted. Among illnesses, the most dangerous of all are infectious diseases, characterized by spreading fast among great number of people in large areas, causing severe and permanent consequences, and , finally, death (7).

Therefore, the object of protection from crimes is welfare. However, the object is determined in different ways, depending on the type of the felony, and so it appears as: a) the health of a subjectively unspecified number of people affected by the transmission of infectious diseases; b) the health of a specific individual affected by impetuous medical assistance (8).

These criminal acts are naturally blanketed. In most cases, the act of implementation consists of acting against the regulations, in fact, violating law or by-law regulations, or orders given by competent state bodies which belong to the field of healthcare, which means that for knowing the content and characteristics of a structure responsible for these acts, it is essential first to determine the content of the law, other regulations and the extent of the competent state bodies (restrictions or orders) which are being violated (9). Moreover, felonies, as drug abuse, are systematized as well in this chapter, and are a part of the scope and structure of the crimes against health with approximately 90 % representing the actual violation of international documents, universal or regional, which regulate actions of preventing and undoing illegal activities related to production, processing and usage of narcotics and psychoactive substances (10).

These felonies result in endangering people's health or creating the danger of arrival, or spreading a disease or deteriorating other individual's health (11). That danger as a consequence appears in two forms: concrete and abstract danger. The concrete danger is real, direct; it can suddenly affect the life, bodily integrity and the health of an individual. This means that the possibility of violating these values is definite and yet there is no such doing. This type of danger is included in the form of a felony and it must be proved in the legal procedure. Abstract or general danger can occur in a particular action, thus endangering life, bodily integrity or health, but does not occur in some particular incidence. To carry out a legal process, it is considered that the consequence or the abstract danger compromising the protected has been caused. This danger is not a part of a crime's form, therefore in a legal procedure, it does not necessarily have to be proved in a particular case. The solely existence of the abstract danger is enough to undertake the procedure because the moment the danger occurred it is considered to be a consequence of a felony (12).

However, some criminal activities in this chapter can result in a violation (creating a new illness, making the already existent condition deteriorate or a simple trauma) (13). Still, these are not held against unspecified individuals, but against a specific individual. Such felonies are: transmitting an infectious disease, negligent medical assistance and medicine preparation and distribution. If undertaking the procedure for the basic form of a crime in this chapter causes a violation, such as: a) severe physical trauma, b) actions taken to severely endanger health, c) death of one or multiple individuals, then, these actions are formed as a specific crime- serious crime against people's health.

Many of these crimes can be committed by anyone, but some of them can only be performed by certain individuals: a doctor of medicine or any other health officer (negligent medical assistance), a doctor of medicine (not providing medical care), an individual responsible for providing medicines (negligent behavior during making and distributing medicines), or an individual responsible for medical examination of livestock for slaughter (negligent examination of provisions).

In terms of fault, many of these crimes are premeditated, while others can be qualified as premeditated or not premeditated.

Among crimes against human welfare, there is a crime which by its significance, nature, characteristics, the perpetrator, the type and scope of the caused consequence is singled out, and it is the crime of Medical Negligence from article 251 in the Criminal law, although since 1951 until today it has been named as Patient's Medical Negligence. Anyway, this crime appeared for the first time in the Serbian law system in 1929 along with the declaration of the Criminal law of the Kingdom of Yugoslavia, which determined two unnamed types of this very crime, nature, and character, in regulations of articles 263 and 264 (14).

Medical negligence

The crime of Medical Negligence was put in the positive criminology in the Criminal law of Serbia in 2005 in chapter 23 under the name of Crimes against People's Welfare according to regulation in article 251. It is used to inculpate incompetent, immoral, illegal acts of doctors of medicine and other medical officers, by which there is a great deviation from the medical field, knowledge, and skill, and causing severe health issues in the sense of "deteriorating" the condition of an individual who is receiving certain medical assistance.

The crime itself consists of Medical Negligence, malpractice of medical care or assistance, by physicians or other health workers, which results in deterioration of one's health (15). The object of protection from this crime - Medical Negligence, the

malpractice of medical workers, or failing in other medical treatments, is individuals' welfare. Therefore, the type of an individual's medical condition is not of great importance.

In historical terms, this crime appears for the first time in domestic Criminal law together with the Criminal law of the Kingdom of Yugoslavia in 1929 (16). Reportedly, this law's chapter 23, "Crimes against the General Welfare" distinguishes two types of this crime. Since they are unnamed crimes, their nature, characteristics, and content are excluded from legal descriptions.

The first act is formulated in article 263. This crime is committed by a physician who, while treating some individual, without any intention harms the individuals' health or greatly deteriorates it. The act is determined by consequential damage, which means that in the process of performance, there is an occurrence of performing or not performing, which is a reason enough for causing a consequence: a) affecting health, b) greatly deteriorating one's condition. It is of major importance for the crime to be committed: a) by a specific individual- a physician and b) at the right time- while providing medical assistance. A perpetrator is a specific person- a medical doctor, and when it comes to the fault, negligence is necessary. This crime receives a cumulative punishment, incarceration and a fine of 30.000 dinars. A more serious form of this crime is punishable by incarceration for at least two years only if the procedure caused a severe consequence- death.

The other crime of this sort is formed in regulation of article 264. This act is committed by a physician who uses a treatment which has still not been used, or performs a surgery which has not been tried before, without a patient's consent or the patient's legal representative's consent if the patient is unconscious or still is not 16 years old, which causes the death of the patient. This crime, for which the implemented punishment is at least 5 years of imprisonment and definite restriction of fulfilling medical duties in time of 2 to 5 years, is consisted of: a) implementation, dually and alternatively distinguished as: 1) usage of new, unused or simply "evidently unsuitable treatment" and 2) performing a surgery which has not been done before, b) only a doctor can perform a treatment, c) treatment is given to a specific person- to a patient, so, in the field of performing medical duty, d) assistance is done in a particular way- unauthorized, without a patient's consent or the patient's legal representative's consent (when the patient is not able to give the consent because of mental or physical disabilities) and e) the consequence of the performance is death.

Then, this crime, under many names, is acknowledged by our after-war criminal law system, such as the Criminal law of The Federal People's Republic of Yugoslavia from 1951, article 203 under the name of Patient's Medical Negligence, and the Criminal law of the Socialist Republic of Serbia from 1977, article 126, under the name of Patient's Medical Negligence which had been in use until January 1st, 2006.

1. The Term and the Elements of a Crime

In the protection of people's health, it is of paramount importance to provide medical assistance or any kind of healthcare to patients. People who are authorized to provide this kind of help- physicians or medical workers, along with the appropriate professional skills, should behave responsibly according to the rules of medicine, medical skills, and knowledge. Because of this, there is the need for a strict criminal law to protect the health of patients (17). As a matter of fact, there are opinions in the legal theory that this is actually an occupational crime, or a crime of the negligence of work duty (article 361, Criminal law) (18).

The crime from article 251 in the Serbian Criminal law under the name of Medical Negligence has replaced the already existent crime from article 126 in the Criminal law of Serbia from 1977 which was called Patient's Medical Negligence (19). Apart from the change in the name, this crime kept its old content, characteristics, forms of occurrence and implemented punishments. As a matter of fact, even this term does not fully reflect its nature, character, and content. Namely, this act doesn't consist solely of violating the regulations of patient treatment by a medical examiner, but also of general illegal behavior of medical workers while performing any kind of a medical procedure. Hence, a more logical and more appropriate term of this act would be Negligent Behavior in the Field of Medicine (20).

Now, this crime consists of negligent medical assistance by a doctor of medicine, or negligent medical assistance, care or any other medical treatment by a medical worker, which results in deteriorating a condition of an individual (21, 22). A medical doctor who declines to assist a patient medically also behaves in a negligent way, but in this case, it is not this particular crime but crime from article 253 which is named Refusal of Medical Assistance (23).

As the object of a protection in this particular crime, there is the health of a specific individual which is protected from negligent medical assistance, or medical workers while giving medical assistance, care or any other medical treatment. Hereby, it is not necessarily important to know the type of medical condition. Also, it is not important to have the consent of a patient for some medical procedure. Such consent of the injured party does not exclude the unlawfulness of the person at fault, or their field.

The consequence of an act appears as a violation in terms of causing the deterioration of one's health condition, to whom medical care by a medical doctor was already delivered, or by any other medical worker while delivering medical treatment, care or any other medical treatment. Other individuals' health condition can deteriorate when a healthy individual gets ill or an already ill person's condition deteriorates, so, when the injured party gets into a condition which is not as good as it was before treatment or any other medical procedure (24).

The perpetrator of the first act can only be a medical doctor. That is a person with a medical or dental degree who works in medical and scientific institutions or has a private practice, as well as an intern. In legal theory, whether a medical doctor who is unemployed can be a perpetrator of this crime has been discussed (25, 26). There are two opinions as to the answer to the question. According to the first opinion, for the occurrence of the crime, it is necessary for a medical doctor to be in a medical institution at the time of the committing of the crime, therefore the patient is the injured party delivered to the doctor because of the needed medical assistance (27). The other opinion states that whether the medical doctor is unemployed or not, does not affect the existence and legal qualification of this crime because that does not represent the legal element of the act. The first opinion prevails as not suitable for the law, referring to this matter (28).

As the perpetrator of the second form of the act is another medical worker, apart from doctor, dentist, or pharmacist (29). This is a person who graduated from a medical or dental school: nurses, X-ray technicians, laboratory workers, health technicians, medical equipment technicians, midwives, and others.

In terms of fault in both of the forms premeditation is possible (usually eventual) or negligence.

Premeditation acts in any of the two forms is punishable by incarceration, 3 months to 3 years, and for unpremeditated acts, a fine or a year in prison.

2. Basic Crime forms

The crime of Medical Negligence from article 251, Criminal law, depending on the characteristics of the perpetrator and type, character and nature of the act, appears in two basic forms and they are: a) medical negligence by a physician and b) medical negligence, care negligence or any other medical treatments, by other medical worker (30).

The first basic form of this crime is a doctor who, while providing medical care, uses an unsuitable tool or evidently unsuitable treatment, or does not apply specific hygienic measures or completely behaves irresponsibly, by which the doctor deteriorates other person's health condition.

Judicial Practice: In dealing with medical assistance, the doctor acted negligently when he received the patient as an emergency officer as a duty officer, and failed to look at the first page of the health booklet, which indicated that she was allergic to certain medications, and ordered the nurse to her injection that contained the component of the medicine to which she was allergic and thus caused her health deterioration (31). Implementing consists of three alternatively chosen practices: a) using an evidently unsuitable instrument, or evidently unsuitable treatment, b) not applying hygienic measures and c) total medical negligence (32, 33).

For the existence of an act, it is important that these are committed by a doctor while providing medical care (34). Although different in content, all of these acts represent only forms of medical negligence, not following the rules and common practice in the treatment of a patient (35). Delivering medical care relating to this incrimination should have a broader meaning. That means performing or not performing has to relate to the procedure of the treatment. The treatment is considered to be the measures directed to the determination of the existence, nature, type, and gravity of the disease, as well as measures which are used with the goal of alleviating or curing the existing disease. In legal theory, but in court practice as well, the question of whether vaccinating classifies as a crime, general examination (the monitoring of health) of residents, as well as in the case of surgeries for aesthetic reasons, unrelated to health, has remained unresolved (36).

Medical Negligence can be manifested in giving the wrong diagnosis, not using remedies properly, and giving the wrong treatment (37). The wrong diagnosis is a result of the negligent, reckless and incompetent examination, the omission of medical search and gathering findings, roentgen rays, analyses, etc. The right diagnosis influences the treatment and the process of it. The wrong diagnosis leads to the usage of the wrong remedies and treatment (38). Remedies are substances and devices which are taken into the organism or put on the body in order to diagnose the disease, or because of treatment or prevention. Medical treatment is a method and a type of medical assistance and a process of any kind of a disease which is scientifically confirmed and proved in practice. Remedies and treatments are connected to each other because there are specific treatments which require specific remedies and vice versa (39).

For the occurrence of the crime, it is necessary that a medical doctor uses remedies or treatments which are evidently unsuitable, by which there is a great deviation from medical practice or medical rules violation (40, 41). Evidently unsuitable remedies are those which shouldn't be used for a certain disease or at least not in a great amount (dose) or not in a specific manner or process. Evidently unsuitable manner of treatment exists if an inapplicable treatment, not approved by medical practice and science, is used for a certain kind of a disease or is unnecessary and harmful. Not applying suitable hygienic measures is reflected in applying or not applying, by which a deviation from medical practice and rules is made, in terms of applying suitable hygienic measures whether these measures are violated completely, partly or are applied insufficiently and inconveniently. Negligent behavior, in general, includes various manners of performing or not performing, by which a deviation from obligational behavior in medical assistance is created.

Judicial practice: A nurse taking care of a sixteen -year -old patient failed to control the temperature of the water prior to therapy. As a consequence of overheated water, 40% of the surface of the patient`s body was burnt causing health deterioration (42).

The other basic form of the crime is performed by a medical worker, who, while performing medical assistance, care or any other medical treatment, evidently behaves irresponsibly and causes deterioration of an individual's health condition (43). The very action is evidently irresponsible behavior of

a medical worker in the process of the medical assistance, care or any other health -related process (44). This kind of behavior consists of omissions, lack of performance or insufficient performance, inappropriate performance from the domain of medical assistance, care or other health - related process (45). For the existence of the act, it is necessary to encounter quite irresponsible behavior, which means a behavior which roughly, to great extent or longer duration, deviates from the rules of performing certain medical duties.

3. Serious Crime Forms

Serious crime forms of medical negligence from article 251 in the Criminal law of Serbia are specified in a separate regulation- article 259, named Serious Crimes against People's welfare (46). Severe punishments are implemented in this regulation of various crimes against people's health, depending on the form of the fault which a perpetuator used to commit the crime, and the scope and intensity of the caused consequences by negligence.

Severe crimes against people's welfare are qualified as grave manifestations of various crimes for which strict punishments implemented, because of the specially presented gravity and degree of danger, or degree of the perpetrator's fault. The following acts may become severe crimes against people: a) transmitting an infectious disease, b) negligent medical assistance, c) unlawful medical experiments and medication examination, d) medical quackery and pharmaceutical quackery, e) irresponsible behavior in producing and distributing medications, f) the production and distribution of harmful products and g) contamination of drinking water and provisions (46).

A serious consequence qualified as an occurrence, which is the result of an action of the basic negligence crimes, comes in two forms: a) serious physical trauma or serious health damage, and b) death of one or more individuals. The other occurrence that influences the gravity of these acts, qualified as the one with serious consequences, is a form of fault with which the act itself was done (it appears as not premeditated and premeditated) (47).

If we take a) the scope and intensity of a consequence as the result of negligence and b) the form of fault with which the basic crime is committed into consideration, there are four forms of serious crimes against people's health, and they are: 1) when the basic act is premeditated and the serious consequence is a serious physical trauma or health endangering of another individual, the punishment for this act is incarceration of 1 to 8 years, 2) when the basic act is done with premeditation and the consequence is death of one or more individuals, for this act the punishment is incarceration of 2 to 12 years, 3) when the basic act is done in pure neglect, but the consequence is a serious physical trauma or health endangering of another individual, the punishment is incarceration for up to three years and 4) when the basic crime is done in pure neglect and the severe consequence is the death of one or more individuals, the punishment for this act is incarceration of 1 to 8 years (48, 49).

Conclusion

Among the crimes against people's welfare (crimes against health,) there is a crime which by its significance, nature, characteristics, the perpetrator, the type and scope of the caused consequence is singled out, and it is the crime from article 251, the Criminal law of Serbia. Although since 1951 until today it has been named as Patient's Medical Negligence, nowadays it is called the crime of Medical Negligence, as it is known by this name some other modern Criminal laws (e.g. Bosnia and Herzegovina, Croatia and Macedonia).

Anyway, this crime appeared for the first time in the Serbian law system in 1929 along with the declaration of the Criminal law of the Kingdom of Yugoslavia, which determined two unnamed types of this very crime, nature and character, in regulations of articles 263 (when a medical doctor while giving medical assistance harms other individual's health or deteriorates the already existent disease) and 264 (when a medical doctor uses unsuitable treatment or performs surgery without a patent's consent which leads to the patient's death).

Then, this crime, under many names, is acknowledged by our after-war Criminal law system, such as the Criminal law of Federal People's Republic of Yugoslavia in 1951, article 203 under the name of Medical Negligence, and the Criminal law of the Socialist Republic of Serbia 1977, article 126 under the name of Patient's Medical Negligence which had been in use until January 1st, 2006.

The crime from article 251 in the Serbian Criminal law under the name of Medical Negligence (which is under the same name in the laws of Republika Srpska, Montenegro, and Slovenia) has replaced the already existent crime from article 126 in the Criminal law of Serbia from 1977. This act does not consist solely of violating the regulations of the patient treatment by a medical examiner (medical doctor or dentist), but also of general illegal behavior of medical workers while performing any kind of a medical procedure. Hence, a more logical and more appropriate term of this act would be Negligent Behavior in the Field of Medicine.

Now, this crime consists of negligent medical assistance by a doctor of medicine, or negligent medical assistance, care or any other medical treatment, by a medical worker, which results in deteriorating a condition of an individual. A physician who declines to assist a patient medically also behaves in a negligent way, but in this case it is not this particular crime but crime from article 253 which is named Refusal of Medical Assistance.

The object of protection related to this crime is health or the right of an individual to be healthy which is guaranteed in article 68 in the Constitution of the Republic of Serbia, of medical negligence by medical doctors, or medical workers failing to provide medical assistance, care or other medical treatment. Hereby, the type of condition of some individual is not of great importance. The existence of a patient's consent for a certain medical treatment is not necessary, either. The crime Medical Negligence

is formulated in the Criminal law of Serbia in 2005 in chapter 23, called "Crimes against People's Welfare" according to regulation in article 251. It incriminates incompetent, irresponsible, illegal behavior of doctors and other medical workers, by which there is a great deviation from the rules of medicine, skills and knowledge and creation of harmful consequences related to health in the sense of deterioration of the condition of an individual who is treated medically.

The crime itself consists of medical negligence by a medical doctor or malpractice of medical care or assistance, or some other medical treatment by other health workers, which results in the deterioration of one's health.

References

1. Stojičić S. Dušanovzakonik. Leskovac: Kulturnicentar; 1971.p.19.
2. Srzentić N, Stajić A, LazarevićLj. Krivično pravo. Opšti deo. Beograd: Savremena administracija; 1978. p. 117-9.
3. Stojanović Z, Perić O. Krivičnopravo. Posebnideo. Beograd: Pravna knjiga;2000.p.151.
4. Criminal Code, Official gazette of the Republic of Serbia, No. 85/2005, 88/2005, 107/2005, 72/2009, 111/2009, 121/2012, 104/2013, 108/2014 i 94/2016.
5. Jovašević D. Komentar Krivičnog zakona Republike Srbije sa sudskom praksom. Belgrade: Nomos; 2003. p. 278-82.
6. 6Jovašević D. Leksikonkrivičnogprava. Belgrade: Službeni glasnik; 2011. p. 378-81.
7. Mijatović E. Krivična dela protiv zdravlja ljudi. Proceedings of XLII redovno godišnje savetovanje, Nove tendencije u savremenoj nauci krivičnog prava i naše krivično zakonodavstvo; 2005; Zlatibor, Serbia. Belgrade: Udruženje za krivično pravo i kriminologiju Srbije i Crne Gore; 2005. p. 203-26.
8. Law on Health Care (Official Gazette of the Republic of Serbia, No. 25/2019).
9. Jovašević D. Krivično pravo - Opštideo. Belgrade: Dosije; 2016. p. 76-8.
10. Đorđević M, Đorđević, Đ. Krivično pravo. Beograd: Projuris; 2016. p.189.
11. Mrvić Petrović N. Krivično pravo. Beograd: Službeni glasnik; 2009. p.213.
12. Ćirić J. Krivično pravna zaštita zdravlja ljudi. In: Jugoslovensko krivično zakonodavstvo i slobode i prava čoveka i građanina. Belgrade: Udruženje za krivično pravo i kriminologiju Jugoslavije; 2001. p. 142-51.
13. Jovašević D. Leksikon krivičnog prava. Belgrade: Službeni glasnik; 2011. p. 420-3.
14. SelinšekLj. Kazensko pravo. Splošni del in osnove posebnega dela. Ljubljana: Založga GV; 2007. p.213.
15. Čubinski M. Naučni i praktični komentar Krivičnog zakonika Kraljevine Jugoslavije. Belgrade: GecaKon; 1934. p. 452-6.
16. Lazarević Lj, Vučković B, Vučković V. Komentar Krivičnog zakonika Crne Gore. Cetinje; 2004. p. 702-3.
17. Radovanović M, Đorđević M. Krivično pravo – Posebni deo. Belgrade: Savremena administracija; 1977. p. 213.
18. Ćirić J. Krivično delo nesavesnog lečenja bolesnika. Zbornik Instituta za kriminološka i sociološka istraživanja 1991; 2:142-9.
19. Pavlović Z. Saradnja pacijenta pri pregledu i krivično delo nesavesnog lečenja bolesnika. Zbornik radova i pravnih stavova. Knjiga četvrta. Novi Sad: 1998. p. 15-8.
20. Pejaković S, Lukić M. Značaj sudsko medicinskog veštačenja nesavesnog lečenja bolesnika. Pravniživot 1980; 3:47-54.
21. Jovašević D. Krivični zakonik Republike Srbije sa uvodnim komentarom. Belgrade: Službeni list; 2007. p. 78.
22. Ćirić J. Nesavesno lečenje bolesnika. Pravni život 1995; 9:211-9.
23. Nesavesno pružanje lekarske pomoći može da izvrši I stomatolog (presuda Okružnog suda u Beogradu Kž. 553/2009 od 12.3.2009.).
24. Đorđević M, Đorđević Đ. Krivično pravo, Belgrade: Projuris; 2016. p. 181.
25. Simonović B. Teret dokazivanja dijagnostičkih grešaka i njihovih posledica. Glasnik prava 1997; 6:23-39.
26. Simić I, Petrović M. KrivičnizakonRepublikeSrbije – praktična primena. Belgrade; Službeni glasnik; 2002. p. 126.
27. Tomić Z. Krivična odgovornost liječnika i drugih zdravstvenih djelatnika za delicta propria protiv zdravlja ljudi. Proceedings of 1. Symposium Medicinaipravo; 2008; Mostar, Bosnia and Herzegovina. Mostar: Liječnička/Ljekarska komora; 2008. p. 21-45.
28. Stojanović Z, Perić O. Komentar Krivičnog zakona Republike Srbije i Krivični zakon RepublikeCrne Gore sa objašnjenjima. Belgrade: Službeniglasnik; 1996. p. 192-4.

29. Ćirić J. Krivično delo nesavesnog lečenja bolesnika. Zbornik Instituta za kriminološka i sociološka istraživanja 1991; 1:11-6.
30. Ćirić J. Radnja izvršenja krivičnog dela nesavesnog lečenja bolesnika od strane lekara, Jugoslovenska revija z akriminologiju i krivično pravo 1991; (1):79-86.
31. Presuda Vrhovnog suda Srbije Kž. 136/92 od 18.4.1992.
32. Marjan R. Kaznena odgovornost liječnika. Hrvatska pravna revija 2008; 8(6):78-86.
33. Lazarević Lj. Krivičnopravo – Posebni deo. Belgrade: Savremena administracija; 1993. p. 110-1.
34. Pejaković S, Lukić M. Hirurške intervencije I krivično delo nesavesnog lečenja bolesnika. Pravni život 1981; (8-9):65-72.
35. Čejović B, Miladinović V. Krivičnopravo - Posebnideo. Niš: 1995. p. 264-6.
36. 36. Law on Drugs and Medical Devices, Official Gazette of the Republic of Serbia, No. 30/2010 and 107/2012.
37. Simonović B. Problem I dokazivanja krivično pravne odgovornosti lekara za krivično delo nesavesno lečenje bolesnika. Proceedings of Pravoimedicina; 1997 Mar 27-30; Kopaonik, Serbia; Belgrade: Srpsko udruženje za krivično pravo; 1997. p. 265-86.
38. Marjan R. Kaznena odgovornost liječnika. Proceedings of Aktualna pitanjaka kaznenog zakonodavstva; 2008; Zagreb, Croatia; 2008. p. 185-201.
39. Mrvić Petrović N. Krivično pravo. Belgrade: Službeni glasnik; 2005. p. 290-1.
40. Stojanović Z, Perić O. Komentar Krivičnog zakona Republike Srbije i Krivični zakon Republike Crne Gore sa objašnjenjima. Belgrade: Službeni glasnik; 1996. p. 193-4.
41. Radovanović M, Đorđević M. Krivično pravo – Posebni deo. Belgrade: Savremena administracija; 1977. p. 216.
42. Presuda Okružno gsuda u Beograd uKž. 1335/94 od 17.9.1994.
43. Tomić Z, Pajić D. Krivično djelo nesavjesnog liječenja (uporedno pravna analizakrivičnih zakonodavstava u BosniiHercegovini). Proceedings of 2. Symposium Medicina i pravo; 2012; Mostar, Bosnia and Herzegovina. Mostar: Liječnička/Ljekarska komora; 2008. p 11-23.
44. Simić I, Trešnjev A. Krivični zakonk sakraćim komentarom. Beograd: Ing pro; 2010. p. 186.
45. Jovašević D. Komentar Krivičnog zakona Republike Srbije sasudskompraksom. Beograd: Nomos; 2003. p. 242-3.
46. Ćirić J. Nesavesno lečenje bolesnika [Magistarski rad]. Beograd: Pravnifakultet; 1985. p. 78-82. Čejović B, Miladinović V. Krivičnopravo - Posebnideo. Niš: 1995. p. 273-4.
47. Jovašević D. Krivično pravo - Posebnideo. Beograd: Dosije; 2017. p. 256-7.
48. Đorđević Đ. Krivičnopravo - Posebnideo. Beograd: Kriminalističko-policijska akademija; 2011. p. 144.

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NESAVESNO PRUŽANJE LEKARSKE POMOĆI U REPUBLICI SRBIJI

Vladimir Miletić[1], Iva Zdravković[2]

[1]Prvi osnovni sud u Beogradu, Beograd, Srbija
[2]Osnovni sudu u Nišu, Niš, Srbija

Kontakt: Iva Zdravković
Studenička 48/70, 18000 Niš, Srrbija
E-mail : ivaduke.ia@gmail.com

U oblasti zaštite i unapređenja zdravlja ljudi od posebnog su značaja zakonito, efikasno, pravilno, stručno i blagovremeno pružanje lekarske pomoći, vršenje druge zdravstvene delatnosti, odnosno pružanje medicinske pomoći ili nege. Na taj način, ostvaruje se značajna društvena funkcija, kao i zaštita Ustavom proklamovanog prava na nepovredivost fizičkog i psihičkog integriteta čoveka (zdravlja ljudi). No, moguće je da usled preduzete lekarske ili druge medicinske delatnosti nastupi pogoršanje zdravlja lica prema kome je odgovarajuća delatnost preduzeta. Ako se radi o teškim povredama lekarske ili druge zdravstvene profesije, odnosno o grubom kršenju pravila struke, usled čega nastupi teža posledica po zdravlje drugog ili drugih lica, tada sva savremena zakonodavstva predviđaju krivičnu odgovornost i kažnjivost za posebno krivično delo – nesavesno pružanje lekarske pomoći. Slična je situacija i u Republici Srbiji. U radu se ovo krivično delo analizira sa aspekta pojma, karakteristika, obeležja, oblika ispoljavanja i drugih osnova za utvrđivanje krivične odgovornosti, i analizira se kažnjavanje njegovog počinioca.

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Ključne reči: *zdravlje, zaštita, nesavesnost, krivično delo, odgovornost*

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RADIOMORPHOMETRIC ANALYSIS OF HUMAN PITUITARY GLAND DURING THE AGING PROCESS

Sonja Janković[1,3], Sladjana Ugrenović[2], Isidora Janković[1], Ivan Jovanović[2], Dragana Ilić[3], Filip Petrović[1,3], Dušan Radomirović[1], Sladjana Petrović[1,3], Dragan Stojanov[1,3], Zoran Radovanović[1,3]

During life, there are significant changes in the function of the hypothalamic-pituitary axis and its relationship to other endocrine glands in the body, which can affect the external and internal gland morphology. Also, the wide range of pathological changes shows the change in pituitary volume. The knowledge of dimensions and volume of the pituitary gland is very important for the correct diagnosis and prognosis of pituitary diseases.

The aim of our study was to perform radiomorphometric analysis of size and volume of human pituitary gland according to age and gender on MRI.

Our study represents a retrospective study which included 144 subjects (60 male (41.6 %) and 84 female (58.3 %)) with age range 20-80 years, who underwent magnetic resonance imaging (MRI) of the endocranium. Subjects were divided based on age: first (20-39), second (40-59) and the third group (60-80). Three pituitary diameters were measured: anteroposterior (AP), latero-lateral (LL) and craniocaudal (CC). Pituitary volume (V) was calculated based on the formula: V = AP x LL x CC /2.

Our study showed a statistically significant difference for the parameters AP-H ($p < 0.037$) and AP-A ($p < 0.040$) in patients of the first and second age group. In addition, the parameter CC showed a statistically significant difference between the first and third age group ($p < 0.031$). The value of AP-H parameter in females was 10.99 ± 1.16 mm, while in males it was 10.43 ± 1.31 mm, indicating a statistically significant difference for AP-H parameter in both genders ($p < 0.008$). The AP-N parameter in female subjects was 2.21 ± 0.72 mm, and in male subjects 1.91 ± 0.67 mm, which also indicates that there was a statistically significant difference of AP-N parameter between genders ($p < 0.012$).

The pituitary gland parameter values that were obtained in our study show normal values of pituitary gland dimensions in the Serbian population. During the aging process, the morphology of the pituitary gland changes, AP parameter increases, and parameter CC decreases. In addition, differences between genders were recorded which should be kept in mind during the analyses of pathological conditions or injury of the pituitary gland.

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Key words: *pituitary gland, MRI, radiomorphometric analysis, aging, volume*

[1]University of Niš Faculty of medicine, Niš, Serbia
[2]University of Niš, Faculty of medicine, Department of Anatomy, Niš, Serbia
[3]Center for Radiology, Clinical Center Niš, Niš, Serbia

Contact: Sonja Janković
Blvd dr Zoran Djindjić 48, 18000 Niš, Serbia
E-mail: sonjasgirl@gmail.com

Introduction

Pituitary gland (PG) is an endocrine gland that has a central role in body growth, metabolism, and reproductive function. PG is located in a special osteofibrous lodge – pituitary fossa within the sphenoid bone and is surrounded by the Turkish saddle (sella turcica), covered by a dural fold (diaphragma sellae). The pituitary gland consists of two separate parts, different in origin, histological structure and function: neurohypophysis and adenohypophysis (1).

The pituitary gland size depends on age, gender and other conditions. During life, there are significant changes in the function of the hypothalamic-pituitary axis and its relationship to other endocrine glands in the body, which can affect the external and internal gland morphology (2, 3). Age and some physiological conditions can change pituitary morphology as well as its physiology, for example, pituitary enlargement during hormonally active conditions – puberty, pregnancy etc. (4-8). Also, some pathological changes show the change in pituitary

volume such as psychiatric disorders (9, 10). There is not enough literature data which show normal pituitary gland dimensions and volume depending on age and gender.

The modality of choice for visualization of pituitary morphology and detection of pathological changes of the pituitary gland is the magnetic resonance imaging (MRI). The optimal MRI technique includes thin section T1–weighted sequences with and without contrast enhancing medium (i.e. gadolinium) in the sagittal and coronal planes (11). The most common pituitary gland pathological changes that can be diagnosed with magnetic resonance imaging are congenital anomalies, dysontogenetic expansive formations, inflammatory and vascular lesions, and tumors, of which the most common are pituitary adenomas and craniopharyngiomas (11). The aim of our study was to perform radiomorphometric analysis of size and volume of human pituitary gland according to age and gender on MRI.

Materials and methods

Our research is a retrospective study which included patients who underwent the MRI examination of the endocranium in the Center for Radiology, Clinical Center Niš, in the period between January 2017 and December 2017. This study involved all examined subjects, whose MRI examination did not show any pathological findings. Patients with any kind of pituitary gland pathology were excluded from the study, as well as the patients with pathological process of endocranium, endocrinologic abnormalities, acute trauma patients, patients with any type of systemic diseases or other abnormal MRI findings. None of the examined female patients were pregnant and lactating. The total number of examinations was 144 patients, 60 males (41.6 %) and 84 females (58.3 %). The average age of all examined patients was 46.44 ± 15.58 years (range: 20-80 years). Study groups were divided according to age into group I (20-39 years), group II (40-59 years) and group III (60-80 years).

MRI examinations were performed on the Siemens Avanto 1.5 T MRI scanner (Siemens Medical Systems, Erlangen, Germany). The processing of reconstructed MRI scans was done at the Leonardo workstation in the Syringe program. The measurement was performed on MRI tomograms in coronal, sagittal and axial plane. The sequences that were used were a T1-weighted (T1w) sequences before and after Gadolinium contrast enhancement. Sequence parameters included TR – 550 ms, TE – 11 ms, FoV – 150 mm, slice thickness – 3 mm. All measurements were performed by the same radiologist.

The analysis parameters included diameters of the pituitary gland as well as the pituitary volume. Three diameters of the pituitary gland were measured: anterior-posterior (AP), latero-lateral (LL) and cranio-caudal (CC) diameter. AP diameter (length) determined the maximum distance between the anterior and posterior points of the pituitary gland at the sagittal level. In this projection, the diameters of adenohypophysis and neurohypophysis (AP-A and AP-N, respectively) were also measured (Figure 1a). LL diameter (weight) was determined by the maximum distance of the lateral points of the pituitary gland in the coronal plane (Figure 1b). CC diameter (height) was determined as the maximum distance of the points in the mediosagittal line, measured in the coronal plane (Figure 1c). Patients were analyzed by gender and age. The pituitary volume (V) was calculated according to the formula V = AP x LL x CC / 2 (12).

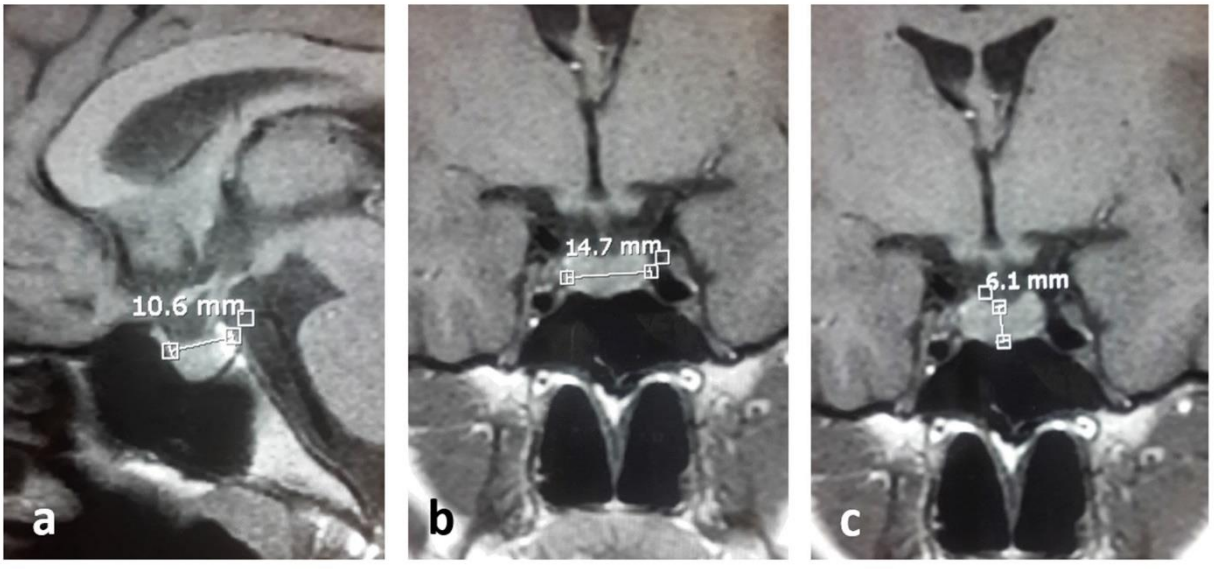
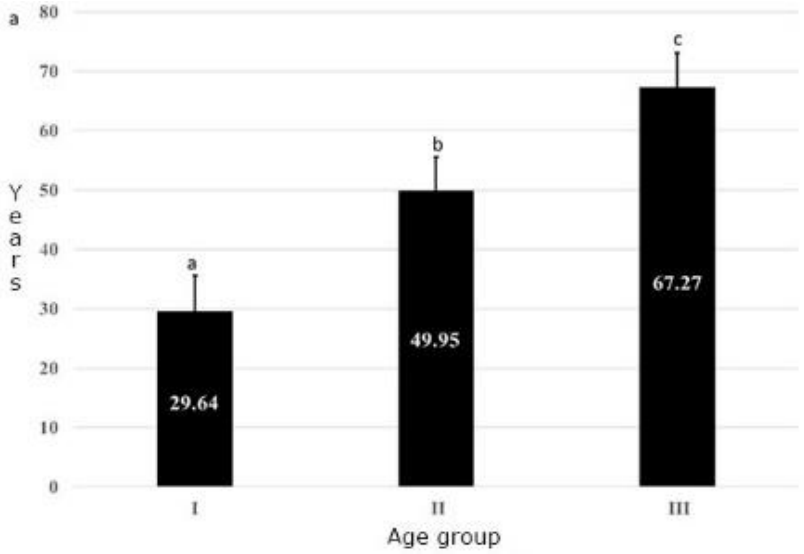


Figure 1. a - AP diameter of the hypophysis, measured in T1w sequence at the sagittal level; b - LL diameter of the pituitary gland, measured in T1w sequence at the coronal level, and c - CC diameter of the pituitary, measured in T1w sequence at the coronal level.

Statistical analysis. The Microsoft Excel program (Windows 10; Microsoft Office Excel, version 2016) was used to collect data, and statistical processing was done in the Statistical Package for the Social Sciences - SPSS version 22.0 (SPSS Inc, Chicago, IL, USA). The relationship between pituitary diameters was observed according to the age and sex of the patients and half of the subjects were analyzed by a Tukey-Kramer post hoc assay; $p < 0.05$ was considered statistically significant.

Results

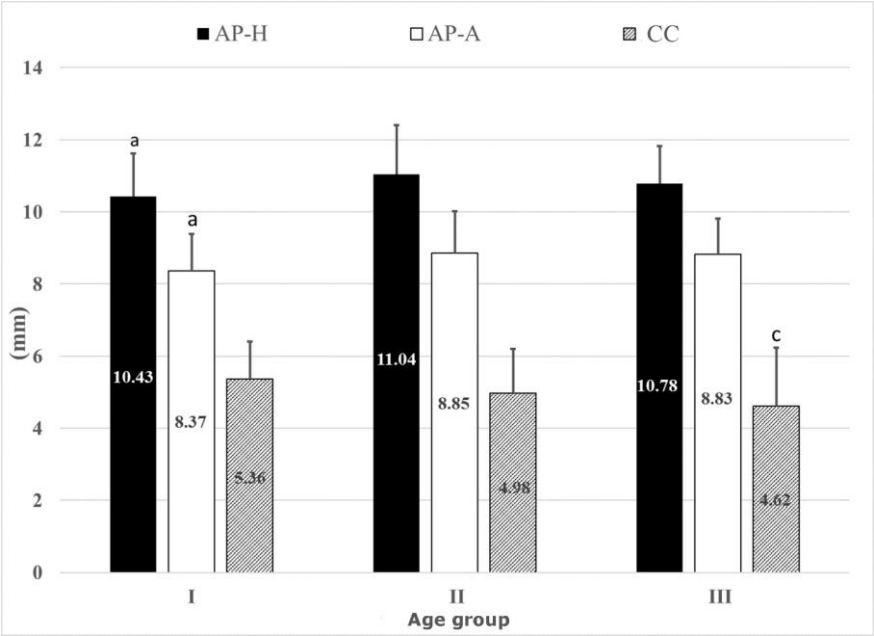
Our study included 144 patients; 60 male (41.6 %) and 84 female patients (58.3 %). The average age of all examined subjects was 46.44 ± 15.58 years (range: 20-80 years). The average age in group I was 29.64 ± 5.96 years; in group II 49.95 ± 5.58 years and in group III 67.27 ± 5.83 years (Graph 1, Table 1). There was a statistically significant difference in the age groups I, II and III (a, b, c).



Graph 1. Age groups of the patients presented with the mean value of years

Table 1. Distribution of patient groups by age: group I (20-39 years), II (40-59 years) and III (60-80 years) in relation to the diameter and volume of the pituitary gland

Parameter	Age	N	Average	SD	SE	95% CI (DG)	95% CI (GG)	F	p	Tukey - Kramer post hoc
Age	I	53	29.64	5.96	0.82	28.00	31.28	448.58	<0.0001	a
	II	58	49.95	5.58	0.73	48.48	51.42			b
	III	33	67.27	5.83	1.02	65.20	69.34			c
AP-H (mm)	I	53	10.43	1.19	0.16	10.11	10.76	3.37	0.037	a
	II	58	11.04	1.36	0.18	10.68	11.40			/
	III	33	10.78	1.04	0.18	10.41	11.15			/
AP-A (mm)	I	53	8.37	1.02	0.14	8.09	8.65	3.31	0.040	a
	II	58	8.85	1.17	0.15	8.54	9.16			/
	III	33	8.83	0.98	0.17	8.48	9.18			/
AP-N (mm)	I	53	2.07	0.64	0.09	1.89	2.24	2.52	0.084	/
	II	58	2.22	0.76	0.10	2.02	2.42			/
	III	33	1.88	0.71	0.12	1.62	2.13			/
LL (mm)	I	53	13.99	2.21	0.30	13.38	14.59	0.70	0.498	/
	II	58	13.95	2.30	0.30	13.35	14.56			/
	III	33	13.44	2.19	0.38	12.67	14.22			/
CC (mm)	I	53	5.36	1.04	0.14	5.07	5.64	3.58	0.031	/
	II	58	4.98	1.22	0.16	4.66	5.30			/
	III	33	4.62	1.60	0.28	4.05	5.19			c
Volume (mm^3)	I	53	391.67	111.01	15.25	361.07	422.27	2.27	0.107	/
	II	58	377.17	109.72	14.41	348.32	406.02			/
	III	33	336.17	144.60	25.17	284.90	387.44			/
a – I:II, p<0.05; b – II:III, p<0.05; c – I:III, p<0.05										



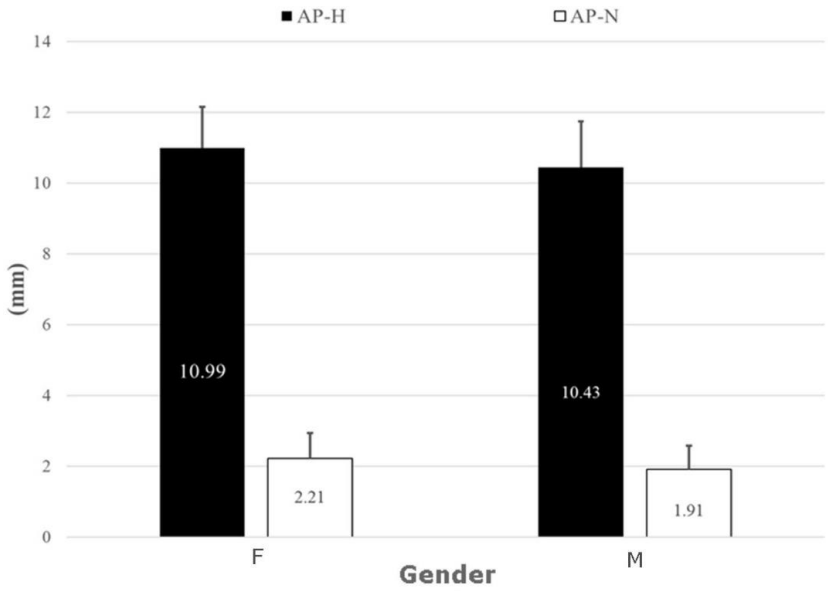
Graph 2. Relation of the pituitary gland parameters according to the age groups

Table 2. Relation of the pituitary gland dimensions in three projections and pituitary gland volume according to the genders

Parameter	Gender	N	Mean	SD	SE	T	SS	p
Age	female	84	45.18	15.45	1.69	-1.16	142	0.250
	male	60	48.22	15.72	2.03			
AP-H (mm)	female	84	10.99*	1.16	0.13	2.68	142	0.008
	male	60	10.43	1.31	0.17			
AP-A (mm)	female	84	8.80	1.08	0.12	1.77	142	0.079
	male	60	8.48	1.08	0.14			
AP-N (mm)	female	84	2.21*	0.72	0.08	2.55	142	0.012
	male	60	1.91	0.67	0.09			
LL (mm)	female	84	13.96	2.44	0.27	0.69	142	0.492
	male	60	13.70	1.92	0.25			
CC (mm)	female	84	5.05	1.41	0.15	0.14	142	0.887
	male	60	5.02	1.09	0.14			
Volume (mm^3)	female	84	383.24	125.68	13.71	1.20	142	0.232
	male	60	358.92	111.14	14.35			

In our work, a statistically significant difference was obtained for the parameters AP-H ($p < 0.037$) and AP-A ($p < 0.040$) in patients from group I and group II (a). Also, for the CC parameter, there was a statistically significant difference between I and III age groups (c, $p < 0.031$) (Table 1, Graph 2). Other parameters (AP-N, LL, and volume) did not show a statistically significant difference in age ($p > 0.05$).

The values of AP-H parameter in female patients was 10.99 ± 1.16 mm, while in male patients it was 10.43 ± 1.31 mm, which indicated a statistically significant difference in the AP-H parameter between genders ($p < 0.008$). The value of AP-N parameter in female patients was 2.21 ± 0.72 mm, and in male patients 1.91 ± 0.67 mm, which also indicated a statistically significant difference in the AP-N parameter between the genders ($p < 0.012$) (Table 2, Graph 3). Other parameters (AP-A, LL, CC, and volume) did not show a statistical significance between the genders ($p > 0.05$).



Graph 3. The relation between total AP pituitary gland diameter and AP diameter of neurohypophysis according to the gender distribution

Discussion

Knowledge of dimensions and volume of the pituitary gland is very important for the diagnosis and prognosis of pituitary diseases in everyday clinical practice. Magnetic resonance imaging is a reliable, non-invasive and highly sensitive method for the visualization of the pituitary gland. During the aging process, there is a physiological change in the size and volume of the pituitary gland due to an increase or decrease in the secretory function (3). The increase in the size of the pituitary gland occurs during puberty, pregnancy, lactation and early postpartum period (4-8, 13, 14). In the early stages of traumatic brain injury, there is an increase in the pituitary gland volume that persists in the chronic phase (15). Also, some drugs such as antipsychotic drugs can increase the pituitary size in the first 12 months of therapy due to dysregulation of hypothalamus-pituitary-adrenal (HPA) axis function (16). In addition, some recent studies showed the presence of pituitary volume enlargement in first-episode psychosis (10). The reduction occurs in a wide number of pathological conditions, as well as in psychiatric diseases such as anorexia nervosa, bipolar disorders and established psychosis (16-18). Also, the reduction of pituitary volume has been noticed in women who take oral hormonal therapy (13). The aging process involves all the endocrine glands, including a pituitary gland. During aging, the size of the gland itself is reduced, as well as its secretory activity. There is proliferation of connective tissue, pituitary fibrosis, and vascular network reduction (19). It is very important to know these oscillations (peaks) in order to give the right diagnosis and treatment.

An increasing number of various studies have been done for the evaluation and establishment of normal pituitary gland range values and have found a wide variation of pituitary gland size and volume according to age and gender. In literature, we did not find any morphometric study of this type in the Serbian population. This study attempted to gain further insight into the range of normal dimensions of the human pituitary gland in the Serbian population. This study can also be used in further larger studies.

In our study, the value of the pituitary length (AP-H) was statistically significantly lower in the age group of 20 to 39 years (10.43 mm) compared to patients aged 40-59 years (11.04 mm). Our data are consistent with other literature data (20, 21). In addition, the value of adenohypophysis length (AP-A) was statistically significantly lower in subjects of the same age groups (8.37 mm and 8.85 mm, res-

pectively). According to these results, we consider that the length of the pituitary gland changes depending on the length of the adenohypophysis. The main peak of the increase in the length of the pituitary gland occurs between the age of 40 and 59 years, which we consider as the result of a negative feedback due to a decrease in the levels of hormones of certain organs. Literature data show a similar peak in the length of the pituitary gland in the sixth decade of life (22).

By comparing the length of the pituitary gland between the genders, a statistically significant difference was obtained in the length of the pituitary gland (AP-H) and in the length of neurohypophysis (AP-N) in both males and females. The mean pituitary height (CC parameter) in our study was 5.02 mm in men and 5.05 mm in women. Our values correspond to the height of the pituitary gland from the literature: in the work of Lurie et al. (5.18 mm and 5.25 mm, respectively) (23), Ikram et al. (6.3 mm and 5.9 mm, respectively) (2), Denk et al. (5.7 mm and 5.6 mm, respectively) (24), Elster et al. (25) and Tsunoda et al. (3) (5.33 mm and 4.93 mm, respectively), while the values in Ibinaiye et al. (22) were slightly higher (7.62 mm and 7.81 mm, respectively). The pituitary height values in our work showed a statistically significant reduction in older patients (60-80 years) compared to the patients aged 20-39 years, which corresponds to the literature data (23).

Depending on hormonal status, pituitary gland volume changes during life. The pituitary gland volume was slightly higher in females - 383.24 mm^3, while in men it was 358.92 mm^3 and did not show statistical significance between genders. Our data correspond to the most publications data which reported a larger pituitary gland in females (3, 8, 16, 24-26). There was no statistically significant difference in pituitary volumes among age groups, but overall gland volume decreases with age. The development of the human body is followed by pituitary gland changes. The results of the study by Ibinaiye et al. (22) are in agreement with our results (29, 3), while Lurie at al. (23) state that there was a statistically significant difference in the pituitary volume in younger patients compared to the older ones. The differences between the measured pituitary volumes can be explained considering different volume examinations due to different volume formulas and technical parameters, such as the strength of the magnetic field, sequence parameters etc. Depending on the pituitary shape, different formulas should be used as well as correction factors for these formulas (30). The evaluation of pituitary measurements can be assessed using 2D and 3D-MRI examination. In the present study, we used 2D-MRI examination, but we consider that it would be useful to compare both methods in future studies. Pituitary gland volumes are individual and can vary depending on age and gender, but it is the most important to compare the volume in the context of clinical manifestations and all factors that can lead to pituitary volume change.

Comparison of the pituitary parameters on the cadaver with the results of our study showed that the data coincide (31) with the study of Rahman et al. where the values of all the parameters (length, height and width) of the pituitary were much lower, with the author suggesting that this was a possible reason for variation among different races (32). Determining the size of the pituitary post-mortem is, in the opinion of Ju et al. more precisely (31), but certainly, morphometry on MRI is more significant for diagnostics and prognosis in everyday clinical practice.

We consider that our study data are limited to a Serbian adult population and further studies are needed to assess pituitary measurements in pediatric patients. In addition, the number of examined subjects should be increased in order to have more precise and accurate sample data. Further evaluation with better technical improvements such as the higher strength of the magnetic field or thinner slice thickness in larger patients sample is necessary for the establishment of a normal range of pituitary gland values in everyday clinical diagnostics.

Conclusion

Based on statistically significant differences in the parameters that were analyzed, it can be concluded that the pituitary morphology changes over the course of life in terms of an increase in the anterior-posterior and reduction of the cranio-caudal dimension, with the overall gland volume showing a downward trend but without statistical significance. The differences between the genders showed that the anterior-posterior diameter was significantly higher in females than in the male patients, based on a statistically significant difference in the dimensions of the neurohypophysis. The identified differences in age and gender should be kept in mind when diagnosing pathological changes of this gland.

References

1. Amar AP1, Weiss MH. Pituitary anatomy and physiology. Neurosurg Clin N Am 2003; 14(1):11-23. [CrossRef] [PubMed]
2. Ikram MF, Sajjad Z, Shokh IS, Omair A. Pituitary height on magnetic resonance imaging observation of age and sex related changes. J Pak Med Asso 2008; 58:261-5. [PubMed]
3. Tsunoda A, Okuda O, Sato K. MR height of the pituitary gland as a function of age and sex: Especially physiological hypertrophy in adolescence and in climacterium. Am J Neuroradiol 1997; 18:551-4. [PubMed]
4. Hayakawa K, Konishi Y, Matsuda T, Kuriyama M, Konishi K, Yamashita K, et al. Development and aging of brain midline structures: assessment with MR imaging. Radiology 1989; 172(1):171-7. [CrossRef] [PubMed]
5. Argyropoulou M, Perignon F, Brunelle F, Brauner R, Rappaport R. Height of normal pituitary gland as a function of age evaluated by magnetic resonance imaging in children. Pediatr Radiol 1991; 21(4):247-9. [CrossRef] [PubMed]
6. Takano K, Utsunomiya H, Ono H, Ohfu M, Okazaki M. Normal development of the pituitary gland: assessment with three-dimensional MR volumetry. Am J Neuroradiol 1999; 20(2):312-5. [PubMed]
7. Marziali S, Gaudiello F, Bozzao A, Scirè G, Ferone E, Colangelo V, et al. Evaluation of anterior pituitary gland volume in childhood using three-dimensional MRI. Pediatr Radiol 2004; 34(7):547-51. [CrossRef] [PubMed]
8. Dinç H, Esen F, Demirci A, Sari A, Resit Gümele H. Pituitary dimensions and volume measurements in pregnancy and post partum. MR assessment. Acta Radiol 1998; 39(1):64-9. [PubMed]
9. Pariante CM, Vassilopoulou K, Velakoulis D, Phillips L, Soulsby B, Wood SJ, et al. Pituitary volume in psychosis. Br J Psychiatry 2004; 185:5-10. [CrossRef] [PubMed]
10. Cullen AE, Day FL, Roberts RE, Pariante CM, Laurens KR. Pituitary gland volume and psychosocial stress among children at elevated risk for schizophrenia. Psychol Med 2015; 45(15):3281-92. [CrossRef] [PubMed]
11. Evanson J. Radiology of the Pituitary. In: De Groot LJ, Chrousos G, Dungan K, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279161/ [PubMed]
12. Roldan-Valadez E, Garcia-Ulloa AC, Gonzalez-Gonzalez-Gutierrez O, Martinez-Lopez M. 3D volumetry comparison using 3T magnetic resonance imaging between normal and adenoma-containing pituitary glands. Neurol India 2011; 59:696-9. [CrossRef] [PubMed]
13. Grams AE, Gempt J, Stahl A, Förschler A. Female pituitary size in relation to age and hormonal factors. Neuroendocrinology 2010; 92(2):128-32. [CrossRef] [PubMed]
14. Fink AM, Vidmar S, Kumbla S, Pedreira CC, Kanumakala S, Williams C, et al. Age-related pituitary volumes in prepubertal children with normal endocrine function: volumetric magnetic resonance data. J Clin Endocrinol Metab 2005; 90(6):3274-8. [CrossRef] [PubMed]
15. Craciunas SC, Cirstea CM, Yeh HW, Hutfles L, Lierman JA, Schmitt A, et al. Longitudinal volumetric MRI study of pituitary gland following severe traumatic brain injury. Romanian Neurosurgery 2012; 19(3):193-202. [CrossRef] [PubMed]
16. MacMaster FP, El-Sheikh R, Upadhyaya AR, Nutche J, Rosenberg DR, Keshavan M. Effect of antipsychotics on pituitary gland volume in treatment-naïve first-episode schizophrenia: a pilot study. Schizophr Res 2007; 92(1-3):207-10. [CrossRef] [PubMed]
17. Pariante CM, Vassilopoulou K, Velakoulis D, Phillips L, Soulsby B, Wood SJ, et al. Pituitary volume in psychosis. Br J Psychiatry 2004; 185(1):5-10. [CrossRef] [PubMed]
18. Clark IA, Mackay CE, Goodwin GM. Pituitary gland volumes in bipolar disorder. J Affect Disord 2014; 169:197-202. [CrossRef] [PubMed]
19. Pavlović M, Jovanović I, Pavlović S, Stojanović V, Živković V, Bakić M, et al. Morphometric study of connective tissue in the human pituitary during aging process. Acta facultatis medicae Naissensis 2013; 30(2):79-84.
20. Ibinaiye PO, Olarinoye-Akorede S, Kajogbola O, Bakari AG. Magnetic resonance imaging determination of normal pituitary gland dimensions in Zaria, Northwest Nigerian population. J Clin Imaging Sci 2015; 5(2):29. [CrossRef] [PubMed]
21. Sinclair J, Kanodia AK, Schembri N, Sudarshan T, Guntur P. MRI Measurement of Normal Pituitary Size Using Volumetric Imaging in Scottish Patients. Curr Trends Clin Med Imaging 2017; 1(3): CTCMI.MS.ID.55563.
22. Lamichhane TR, Pangeni S, Paudel S, Lamichhane HP. Age and Gender Related Variations of Pituitary Gland Size of Healthy Nepalese People Using Magnetic Resonance Imaging. Am J Biomed Engineering 2015; 5 (4):130-5.
23. Lurie SN, Doraiswamy PM, Husain MM, Boyko OB, Ellinwood EH Jr, Figiel GS, et al. In vivo assessment of pituitary gland volume with magnetic resonance imaging: the effect of age. J Clin Endocr Metab 1990; 71 (2):505-8. [CrossRef] [PubMed]
24. Denk CC, Onderoğlu S, Ilgi S, Gürcan F. Height of normal pituitary gland on MRI: Differences between age groups and sexes. Okajimas Folia Anat Jpn 1999; 76:81-7. [CrossRef] [PubMed]
25. Elster AD, Chen MY, Williams DW, Key LL. Pituitary gland: MR imaging of physiologic hypertrophy in adolescence. Radiology 1990; 174:681-5. [CrossRef] [PubMed]
26. Doraiswamy PM, Potts JM, Axelson DA, Husain MM, Lurie SN, Na C, et al. MR assessment of pituitary gland morphology in healthy volunteers: age- and gender-related differences. Am J Neuroradiol 1992; 13(5):1295-9. [PubMed]
27. Kato K, Saeki N, Yamaura A. Morphological changes on MR imaging of the normal pituitary gland related to age and sex: main emphasis on pubescent females. J Clin Neurosci 2002; 9(1):53-6. [CrossRef] [PubMed]
28. Singh AKC, Kandasamy D, Garg A, Jyotsna VP, Khadgawat R. Study of Pituitary Morphometry Using MRI in Indian Subjects. Indian J Endocrinol Metab 2018; 22(5):605-9. [CrossRef] [PubMed]
29. Terano T, Seya A, Tamura Y, Yoshida S, Hirayama T. Characteristics of the pituitary gland in elderly subjects

from magnetic resonance images: relationship to pituitary hormone secretion. Clin Endocrinol 1996; 45(3):273-9. [CrossRef] [PubMed]
30. Lukyanyonok PI, Doubrovin AV, Kologrivova IV. Determination of hypophysis volume by saggital slices data obtained by low field magnetic resonance tomography. International Journal of Applied and Fundamental Research 2011; 1:11-7. [CrossRef]
31. Ju KS, Bae HG, Park HK, Chang JC, Choi SK, Sim KB. Morphometric study of the Korean adult pituitary glands and the diaphragma sellae. J Korean Neurosurg Soc 2010; 47:42-7. [CrossRef] [PubMed]
32. Rahman M, Ara S, Afroz H, Nahar N, Ara Sultana A, Fatema K. Morphometric study of the human pituitary gland. Bangla J Anat 2011; 9(2):79-83. [CrossRef]

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RADIOMORFOMETRIJSKA ANALIZA HIPOFIZE ČOVEKA U PROCESU STARENJA

Sonja Janković[1,3], Slađana Ugrenović[2], Isidora Janković[1], Ivan Jovanović[2], Dragana Ilić[3], Filip Petrović[1,3], Dušan Radomirović[1], Slađana Petrović[1,3], Dragan Stojanov[1,3], Zoran Radovanović[1,3]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet Institut za anatomiju, Niš, Srbija
[3]Centar za radiologiju, Klinički centar Niš, Niš, Srbija

Kontakt: Sonja Janković
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: sonjasgirl@gmail.com

Tokom života postoje značajne promene u funkcionisanju hipotalamo-hipofizarne ose i ostalih endokrinih žlezdi u organizmu, što utiče na unutrašnju i spoljašnju morfologiju same žlezde. Takođe, veliki broj patoloških stanja pokazuje promenu u volumenu hipofize. Poznavanje dimenzija i volumena hipofize veoma je značajno za dijagnostiku i prognozu bolesti hipofize.

Cilj našeg istraživanja bio je izvršiti morfometrijsku analizu hipofize osoba različite starosti i različitog pola.

Naše istraživanje predstavlja retrospektivnu studiju koja obuhvata 144 ispitanika (60 muškaraca (41,6 %) i 84 žene (58,3 %)), starosti od 20 do 80 godina, koji su upućeni na pregled endokranijuma na magnetnoj rezonanci (MR). Ispitanici su podeljeni na osnovu godina starosti na: I grupu (20-39 godina), II grupu (40-59 godina) i III grupu (60-80 godina). Merena su tri dijametra hipofize: anteroposteriorni (AP), latero-lateralni (LL) i kranio-kaudalni (KK). Zapremina hipofize (V) izračunavana je na osnovu formule V = AP x LL x KK/2.

U našem radu dobijena je statistički značajna razlika za parametre AP-H ($p < 0,037$) i AP-A ($p < 0,04$) kod bolesnika I i II grupe. Takođe, kod parametra KK postoji statistički značajna razlika između I i III starosne grupe ($p < 0,031$). Kod ispitanika ženskog pola vrednost AP-H parametra iznosi 10,99 ± 1,16 mm, dok kod ispitanika muškog pola iznosi 10,43 ± 1,31 mm, što ukazuje na postojanje statistički značajne razlike AP-H parametra u odnosu na pol ($p < 0,008$). AP-N paremetar kod ispitanika ženskog pola iznosi 2,21 ± 0,72 mm, a kod ispitanika muškog pola 1,91 ± 0,67 mm, što takođe ukazuje na postojanje statisti-čki značajne razlike AP-N parametra u odnosu na pol ($p < 0,012$).

Vrednosti parametara hipofize dobijene u našem radu ukazuju na normalne vrednosti dimenzija hipofize u srpskoj populaciji. Tokom života dolazi do promene morfologije hipofize u smislu povećanja AP dimenzije, a smanjenja KK dimenzije. Takođe su utvrđene razlike prema polu što sve zajedno treba imati na umu prilikom ispitivanja patoloških promena ili povrede hipofize.

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Ključne reči: *hipofiza, MR, radiomorfometrijska analiza, starenje, zapremina*

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EFFECT OF CURCUMIN AND P53 SIGNALING PATHWAY IN RAT THYMOCYTES TOXICITY INDUCED BY MANCOZEB

Voja Pavlović[1], Snežana Cekić[1], Maja Petrović[2]

Mancozeb, as a dithiocarbamate fungicide, is widely used in agriculture due to its low acute toxicity and short environmental persistence. We examined the protective role of curcumin on Mancozeb-induced toxicity in rat thymocytes and potential mechanisms involved. Rat thymocytes were exposed to Mancozeb (0.01 μg/ml) and/or curcumin (0.3, 1, 3 μM) and levels of cell viability, caspase-3, caspase-9 activity, cytochrome C oxidase, catalase activity, reactive oxygen species (ROS) production and p53 signaling involvement were evaluated after 24 h of incubation. Cells treated with Mancozeb showed increased toxicity, caspase-3, 9 activity and ROS production with decreased cytochrome C oxidase and catalase activity. Inhibition of caspase-3 and 9 activity resulted with reduced rat thymocytes toxicity while inhibition of p53 signaling pathway suppressed caspase-3 activity in cells. Co-treatment with curcumin (1, 3 μM) displayed significantly reduced toxicity, caspase-3, 9 activity and ROS production, together with increased cytochrome C and catalase activity in cells. These findings propose that Mancozeb-induced apoptosis in rat thymocytes is caspase dependent and is partially attributed to p53 signaling pathway. Certain curcumin concentrations may modulate Mancozeb-induced rat thymocytes toxicity, due to its anti-oxidative effect, and may be useful for providing potential therapeutic strategy in immunomodulation induced by Mancozeb.

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Key words: *Mancozeb, curcumin, p53, thymocytes, toxic*

[1]University of Niš, Faculty of medicine, Institute of Physiology Niš, Serbia
[2]Clinic of OphatImology, Clinical Center Niš, Niš, Serbia

Contact: Pavlović Voja
Blvd dr Zoran Djindjić 81, 18000 Niš, Serbia
E-mail: vojapav@yahoo.com

Introduction

The application of pesticides represents the most effective means of protecting plants without causing much damage to non-target species. However, humans are often exposed to pesticides through persistent bio-accumulative residues in the environment (1) which may lead to increased risk of adverse health effects, including genotoxicity and cancer (2). Mancozeb is a broad-spectrum fungicide of the ethylene-bis-dithiocarbamate (EBDC) family. Despite various studies which reported toxic effects of Mancozeb in different immune cells (1, 3, 4, 5), this fungicide has been widely used globally due to its low acute toxicity and short environmental persistence (6). Continuous exposure to pesticides raises the risk of immunomodulation (7). Earlier studies showed that occupational exposure to Mancozeb resulted with modulated T cell functional response and alterations in Th1 and Th2 cytokines profiles (6, 8). *In vitro* experiments suggested that Mancozeb mainly targets mitochondrial enzymes (9) and induces reactive oxygen formation with resulted cytotoxicity (3).

Curcumin is a polyphenol derived from the rhizome of the plant Curcuma longa and has been a commonly used seasoning spice and medical plant in Asia for thousands of years. Due to its anti-inflammatory and anti-oxidant properties, curcumin has been proposed as a potential candidate for the prevention and treatment of different diseases (10, 11). Moreover, several reports demonstrated that curcumin, under in vitro conditions, stimulated apoptosis and inhibited proliferation in different cancer cells (12, 13). It has been considered that mechanism for anti-cancer effect of curcumin includes its inhibition of multiple signaling pathways and anti-oxidant property (14). On the other hand, some studies demonstrated cytotoxic effects of curcumin (15), suggesting that the specific mechanisms of curcumin induced cytotoxicity remains controversial due to the variable anti and pro-apoptotic signaling pathways in

different cell types (16). Therefore, in the present study we evaluated the effect of Mancozeb in rat thymocytes and tested whether there is any preventive role of curcumin, along with underlying mechanisms involved.

Materials and methods

Animals

Experiments were performed on adult male Wistar rats (190-220 g), 9-11 weeks old, bread at the Vivarium of the Institute of Biomedical Research, Medical Faculty, Niš, under conventional laboratory conditions and in accordance with national animal protection guidelines. All procedures were performed in line with the recommendations for the proper use and care of laboratory animal and confirmed to the European Communities Council Directive of November 1986 (86/609/EEC).

Materials

Culture medium (CM) was prepared using RPMI 1640 (Sigma-Aldrich, St. Louis, 16 Mo., USA) according to the manufacturer's instructions. CM contained 25 mM HEPES, 2 mM glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml) and 10 % fetal calf serum (FCS).

Cell Counting Kit (CCK-8), Cytochrome C Oxidase Assay kit, Catalase Assay, Pifithrin-α hydrobromide (PFT-α Kit), Z-VAD-FMK, Z-LEHD-FMK and Curcumin were purchased from Sigma-Aldrich (St. Louis, Mo., USA). Caspase-3 colorimetric assay and caspase-9 colorimetric assay were obtained from R&D Systems (Minneapolis, USA). Mancozeb was purchased from Galenika-Fitofarmacija a.d., Belgrade, Serbia.

Preparation of thymocytes

Rat thymocytes were isolated as described previously (17). The viability of the isolated cells, as determined by trypan blue dye exclusion test, was always over 94 %. Isolated thymocytes were counted and adjusted to a density of 1x106 cells/ml.

Cell culture

Isolated rat thymocytes were cultivated in 96-well round-bottom plates (NUNC, Aarhus, Denmark), containing a 100 μl of cell suspension (1x105 cells) in each well. Cells were cultured with Mancozeb (0.01 μg/ml) without or with increasing concentrations (0.3, 1, 3 μM) of curcumin. Control cells were treated with appropriate amounts of vehicle alone, diluted in CM. All cell cultured are done in triplicates and cultivated for 24 h in an incubator (Galaxy, Wolf Laboratories, USA) with 5 % CO_2 at 37°C. When indicated, rat thymocytes were cultured in either the presence or absence of Cyclic Pifithrin-α hydrobromide (PFT-α), an inhibitor of p53 protein, at a final concentration of 20 μM (18), Z-VAD-FMK, a pan-inhibitor of caspases, at final concentration of 10 μM (19) or Z-LEHD-FMK, caspase-9 inhibitor, at final concentration of 20 μM (20). Mancozeb solutions were prepared immediately before use in dimethyl sulfoxide (DMSO) and diluted in CM. Control cells were treated with the same amount of vehicle alone. The final DMSO concentration never exceeded 0.5 % (v/v). Based on the results in our revious study (4), regarding the dose dependent toxicity in rat thymocytes induced by Mancozeb, as well as on proposed acceptable daily intake (0.05 mg/kg body weight) of Mancozeb in humans (21), in our experiment we used 0.01 μg/ml of Mancozeb which corresponds to an in vivo exposure 0.1 mg/kg body weight (3, 22).

Curcumin was dissolved in DMSO as a stock solution. The stock solution was stored at -20°C and diluted in CM before use. The final concentration of DMSO, applied to the cells, was less than 0.5 %. Incubation of increasing concentrations of curcumin (0.3, 1, 3 μM) was chosen due to our recently published findings (5) and previous study results in rat thymocytes (23), which showed that 3 μM was the lowest concentration which was not able to induce any cytotoxic actions in rat thymocytes.

Analysis of cell viability

Cell viability of rat thymocytes, after cultivation period, was evaluated by CCK-8 assay as it was previously described (24). Ten microliter of reaction mixture was added in each well. After 2h of incubation, the solubilized formazan product was quantified spectrophotometrically. Absorbance was measured at 450 nm. For each sample, basal intensity values were subtracted from those obtained after different treatments. Absorbances were presented as a ratio of control for further comparison.

Caspase-3 and caspase-9 activity assay

The enzymatic activity of the caspases were determined by a colorimetric assay (by using the chromogenic substrate DEVD-pNA and LEHD-pNA), according to the manufacturer's protocol. The reaction was measured by determining the change in absorbance at 405 nm. The activity was expressed as fold change of treated cell over the non-treated cells. The background values were subtracted from the experimental results before calculation the fold induction.

Cytochrome C oxidase activity

The change in cytochrome c oxidase activity was assayed by using colorimetric kit following the manufacturer's protocol and as previously reported (25). The method is based on observing the decrease in absorbance at 550 nm of ferrocytochrome c, which is caused by its oxidation to ferricytochrome c by cytochrome c oxidase. The activity was expressed as fold change of treated cell over the non-treated cells (25). The background values were subtracted from the experimental results before calculation of the fold induction.

Catalase assay

Catalase (CAT) enzyme activity was analyzed with Catalase Assay Kit. The assay was performed following the manufacturer's instructions. The CAT degrades H_2O_2 to water and molecular oxygen and the amount of degraded H_2O_2 is proportional to the enzymatic activity. The color change of reaction mixture was evaluated spectrophotometrically at 240 nm and activity was expressed as fold change of treated cell over the non-treated cells.

Measurement of intracellular reactive oxygen species (ROS) production

A redox-sensitive probe (H2DCF-DA) was used to determine changes in overall cellular ROS levels, as described previously (4). The change in fluorescence was measured using a Epics XL flow cytometer (Coulter, Krefeld, Germany). Basal inten-sity values were subtracted from the experimental results before calculation the fold induction.

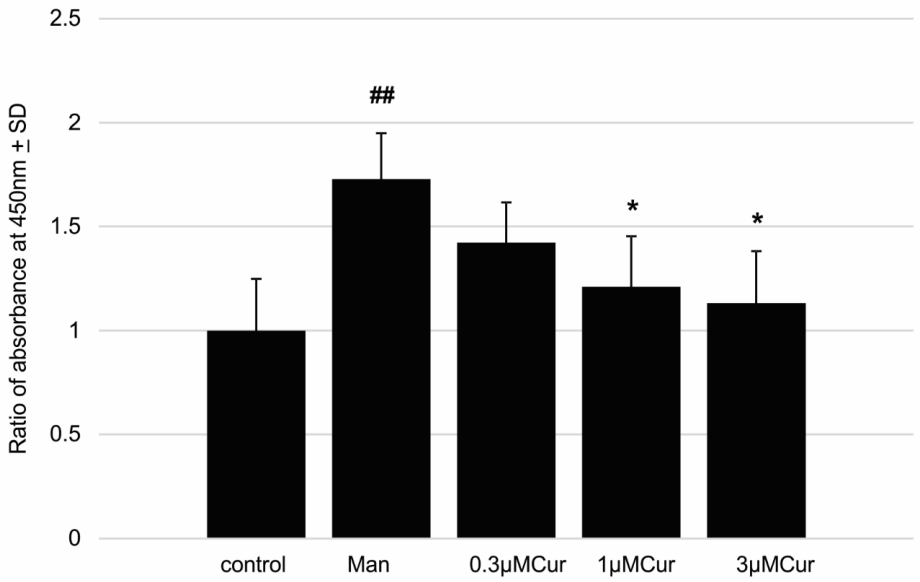
Statistical analysis

Results are presented as mean ± SD. The comparisons among groups were carried out using the analysis of variance (ANOVA) coupled to the Dunnett's post hoc test and student's t test. A p value < 0.05 was considered significant.

Results

Based on the results of our recently published study (5) where we optimized curcumin dose and previous study results in rat thymocytes (23), we found that 3 μM was the lowest concentration which was not able to induce any cytotoxic actions in rat thymocytes. Therefore, we used 0.3, 1 and 3 μM curcumin in all of the experiments in this study.

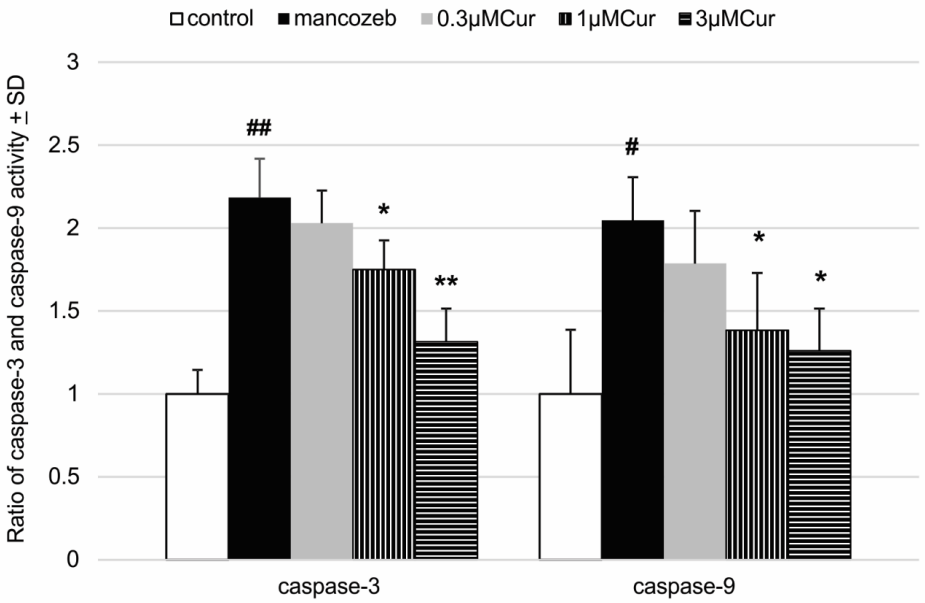
In an attempt to determine the effect of curcumin on Mancozeb treated rat thymocytes, cells were exposed to the increasing curcumin concentrations (0.3, 1, 3 μM) and/or Mancozeb (0.01 μg/ml) for 24 h and assayed for cell viability. The obtained results showed that cells treatment with Mancozeb resulted with significantly reduced cell viability ($p < 0.01$), compared to the control cells (Graph 1). Co-treatment with curcumin, at concentrations of 1 and 3 μM significantly ($p < 0.05$) inhibited cell toxicity induced by Mancozeb. On the other hand, lowest curcumin concentrations (0.3 μM), used in our study, failed to restore rat thymocytes viability after treatment with Mancozeb (Graph 1).



Graph 1. Effect of Mancozeb (Man) and curcumin (Cur) on rat thymocytes toxicity

Cells were treated with Man (0.01 μg/ml) without or with increasing Cur (0.3, 1 and 3 μM), for 24 hours. Data were expressed (mean ± SD) as the absorbance ratio of control for further comparison. Man-cells treated only with Man;
0.3 μMCur-cells treated with Man and Cur (0.3 μM);
1 μMCur-cells treated with Man and Cur (1 μM); 3 μMCur-cells treated with Man and Cur (3 μM); ##-$p < 0.01$ vs. control cells; *-$p < 0.05$ vs. Man treated cells.

It has been shown that caspase is a key executioner of apoptosis (26). Since our previous results (4, 5) demonstrated that Mancozeb-induced toxicity in rat thymocytes involves apoptotic cell death, we next investigated whether caspases play an important role in Mancozeb-induced toxicity. As shown in Graph 2, caspase-3 ($p < 0.01$) and caspase-9 ($p < 0.05$) activity were markedly increased by a 24h Mancozeb treatment, suggesting that caspases are intimately involved in this model of cytotoxicity. Also, co-treatment cells with curcumin (1, 3 μM) significantly downregulatedcaspase 3 ($p < 0.05$; $p < 0.01$) and caspase-9 ($p < 0.05$) activity, indicating the protective role of curcumin in Mancozeb-induced toxicity in rat thymocytes. Application 0.3 μM of curcumin in rat thymocytes culture resulted in no significant reduction of caspase-3 and caspase-9 activity (Graph 2).

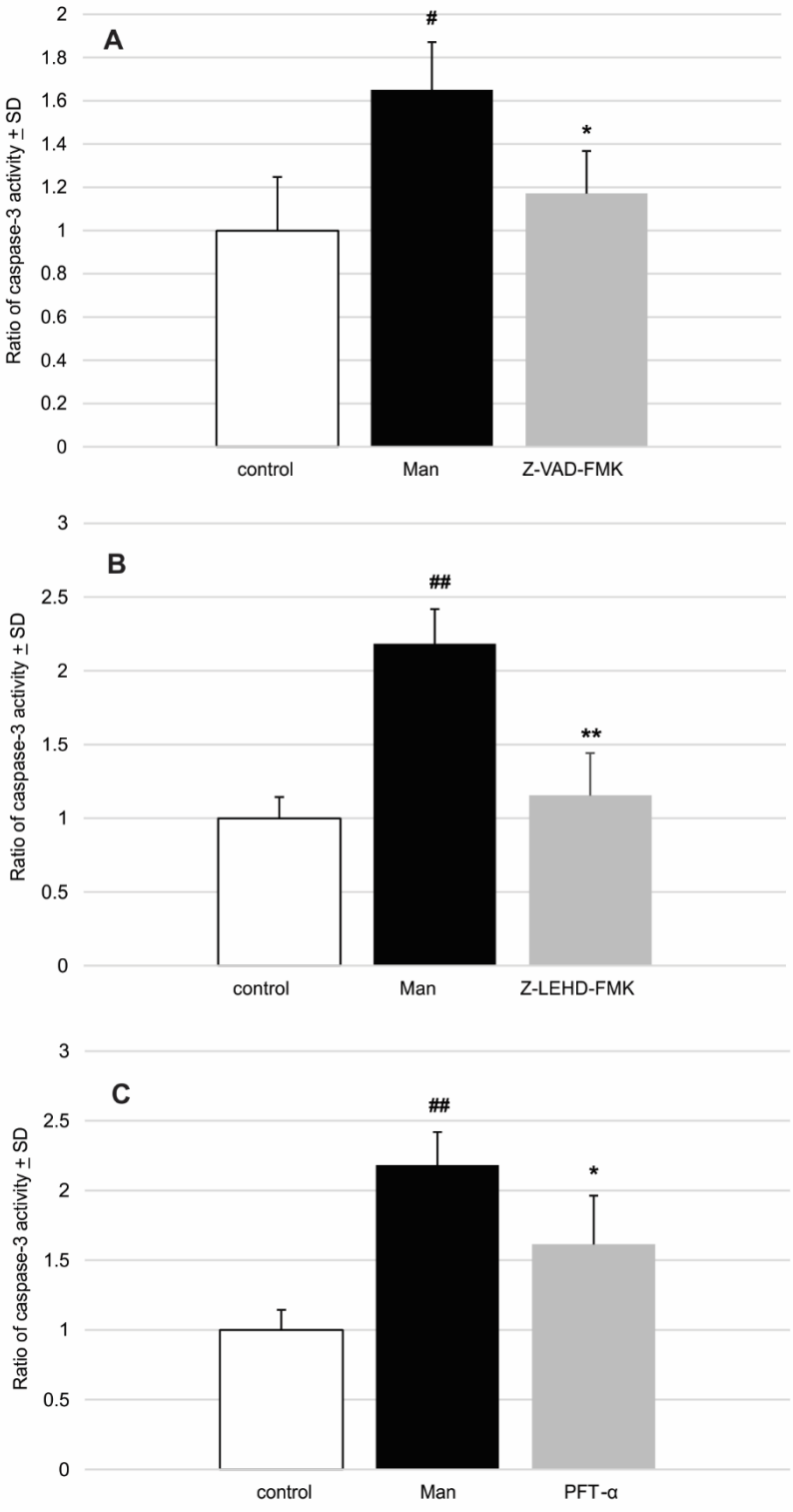


Graph 2. Effect of Mancozeb (Man) and curcumin (Cur) on caspase-3 and caspase-9 activity in rat thymocytes

Cells were treated with Man (0.01 μg/ml) without or with increasing Cur (0.3, 1 and 3 μM), for 24 hours.
Data were expressed (mean ± SD) as the absorbance ratio of control for further comparison.
Man-cells treated only with Man; 0.3 μMCur-cells treated with Man and Cur (0.3 μM); 1 μMCur-cells treated with Man and Cur (1 μM); 3 μMCur-cells treated with Man and Cur (3 μM); #-$p < 0.05$; ##-$p < 0.01$ vs. control cells; *-$p < 0.05$; **-$p < 0.01$ vs. Man treated cells.

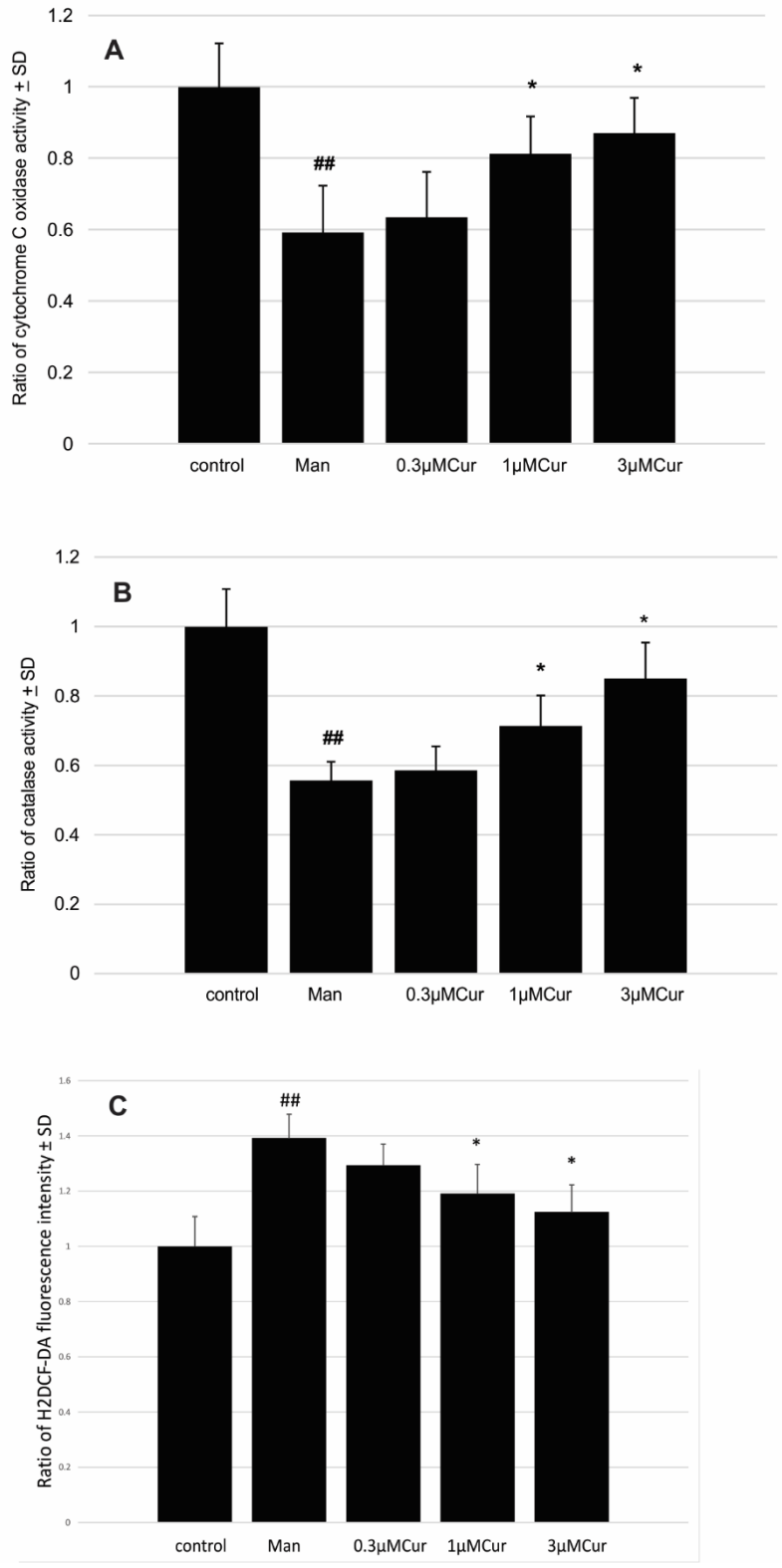
To further elucidate the involvement of caspase-3 and caspase-9 in Mancozeb-induced toxicity in rat thymocytes, cells were simultaneously treated with Mancozeb and Z-VAD-FMK (a pan-inhibitor of caspases) or Z-LEHD-FMK (specific caspase-9 inhibitor). Caspase-3 activity was markedly inhibited in cell treated with Z-VAD-FMK ($p < 0.05$) and Z-LEHD-FMK ($p < 0.01$), proposing that Mancozeb-induced toxicity in ratthymocytes involves caspase dependent toxicity (Graph 3A and 3B). Since caspase inhibitors were not able to completely inhibit caspase-3 activity in rat thymocytes, further analyses are required to evaluate the possibility that Mancozeb-induced toxicity may be caspase independent.

The tumor suppressor p53 has been related to different key cellular processes, including the regulation of apoptotic cell death (27, 28). Different reports propose crosstalk between p53 and caspases in apoptosis induction in various cells (26, 29). Therefore, we next examined the potential role of p53 in Mancozeb-induced cytotoxicity. In response to co-treatment with Mancozeb and PFT-α (an inhibitor of p53 protein) caspase-3 activity was significantly downregulated ($p < 0.05$), indicating the involvement of p53 in Mancozeb-induced toxicity in rat thymocytes (Graph 3C). Taking into account that the rescue was not complete, we are not able to neglect the activation of other multiple signaling pathways.



Graph 3. Effect of Mancozeb (Man) and Z-VAD-FMK (pan-inhibitor of caspases) (A), Z-LEHD-FMK (caspase-9 inhibitor) (B), PFT-α (inhibitor of p53 protein) (C) on caspase-3 activity and toxicity in rat thymocytes

Cells were treated with Man (0.01 μg/ml) without or with Z-VAD-FMK, Z-LEHD-FMK, PFT-α, for 24 hours.
Data were expressed (mean ± SD) as the absorbance ratio of control for further comparison.
Man-cells treated only with Man; 0.3 μMCur-cells treated with Man and Cur (0.3 μM); 1 μMCur-cells treated with Man and Cur (1 μM); 3 μMCur-cells treated with Man and Cur (3 μM); #-$p < 0.05$; ##-$p < 0.01$ vs. control cells; *-$p < 0.05$ vs. Man treated cells.



Graph 4. Effect of Mancozeb (Man) and curcumin (Cur) on cytochrome C oxidase (A) catalase activity (B) and ROS production (C) in rat thymocytes

Graph 4. Effect of Mancozeb (Man) and curcumin (Cur) on cytochrome C oxidase (A) catalase activity (B) and ROS production (C) in rat thymocytes. Cells were treated with Man (0.01 μg/ml) without or with increasing Cur (0.3, 1 and 3 μM), for 24 hours. Data were expressed (mean ± SD) as the absorbance ratio of control for further comparison. Man-cells treated only with Man; 0.3 μMCur-cells treated with Man and Cur (0.3 μM); 1 μMCur-cells treated with Man and Cur (1 μM); 3 μMCur-cells treated with Man and Cur (3 μM); ##-$p < 0.01$ vs. control cells; *-$p < 0.05$ vs. Man treated cells.

Since different studies revealed that activity of cytochrome C oxidase activity is closely related to the cell death (30, 31), we next evaluated whether Mancozeb and curcumin treatment had any effect on cytochrome C oxidase activity in rat thymocytes. The results showed that cells exposure to Mancozeb resulted in significantly decreased ($p < 0.01$) cytochrome C oxidase activity, after 24 of incubation (Graph 4A). Simultaneously, co-treatment with curcumin (1, 3 μM) significantly restored ($p < 0.05$) cytochrome C activity in rat thymocytes, as evaluated by colorimetric assay (Graph 4A).

Based on the previous results and because catalase activity may protect rat thymocytes from oxidative injury and apoptosis (17), in next experiments we examined the effect of Mancozeb and curcumin on catalase (CAT) activity in rat thymocytes. As shown in Graph 4B, Mancozeb application to cell culture markedly reduced ($p < 0.01$) catalase activity in rat thymocytes. Also, colorimetric assay revealed that treatment with curcumin (1, 3 μM) significantly restored ($p < 0.05$) altered catalase activity in rat thymocytes, induced by Mancozeb (Graph 4B). Moreover, the analysis of ROS production showed that Mancozeb treatment induced significantly ($p < 0.01$) increased ROS production while application of curcumin (1, 3 μM) reduced ($p < 0.05$) ROS production in rat thymocytes (Graph 4C).

Discussion

Curcumin has been used in traditional Indian and Chinese medicine for centuries due to its various therapeutic properties. Extensive *in vivo* and *in vitro* studies showed that curcumin has a number of biological activities (32, 33), including the increasing of T cell proliferation and inhibition of T cell apoptosis (34).

The current study results demonstrate that Mancozeb application in cell culture decreased viability and increased caspase-3 and caspase-9 activity in rat thymocytes, as evaluated by colorimetric assay. The obtained results correspond with our inhibition experiments which showed that Z-VAD-FMK (a pan-inhibitor of caspases) almost restored viability of the cells while Z-LEHD-FMK (specific caspase-9 inhibitor) strongly suppressed caspase-3 activity in rat thymocytes, indicating that the caspase cascade is involved in Mancozeb-induced cytotoxicity. These observations are in accordance with our previous results (4, 5) demonstrating the pro-apoptotic potential of Mancozeb in rat thymocytes, as well as with other studies in human immune cells (1, 3). Moreover, our results indicate that rat thymocytes exposure to Mancozeb resulted with decreased cytochrome C oxidase and catalase activity, after 24h of incubation. Cytochrome C oxidase represents the terminal enzyme of mitochondrial respiratory chain. It couples electron transfer from cytochrome c to oxygen to form water with transport of protons from matrix to cytosol thereby maintaining mitochondrial (MMP) membrane potential. Furthermore, since this enzyme induces proton transfer and electron exchange takes place within the enzyme, reactive oxygen species (ROS) generation is inherently prohibited (35). It is well documented that Mancozeb possess ability to reduce MMP and induce ROS generation in immune cells (1, 4, 5), showing that mitochondrial dysfunction and alterations in antioxidant defense systems represent major components of Mancozeb-induced toxicity (36). During mitochondrial dysfunction, several key factors of apoptosis (procaspase, cytochrome C, apoptosis protease-activating factor 1-APAF-1) are released into cytosol. The complex formed of cytochrome C, APAF-1 and caspase-9 leads up to a chain activation of other caspases and results in apoptosis (5, 37). These findings correlate with increased caspase-3 and caspase-9 activity in rat thymocytes, after Mancozeb treatment. On the other hand, our results indicated that CAT activity was markedly decreased in rat thymocytes treated with Mancozeb. Given observations are supported by the earlier reports, which indicated that overexpression of CAT protect thymocytes against oxidative injury and apoptosis (17). The decreased activity of CAT in the rat thymocytes indicated the altered CAT activity to degrade hydrogen peroxide. Increased hydrogen peroxide could be converted to toxic hydroxyl radicals that may contribute to oxidative stress and apoptosis (38). Taken together with our results, it seems that rat thymocytes exposure to Mancozeb resulted in altered activities of antioxidant defense system and mitochondrial dysfunction which may lead to caspase cascade activation and cytotoxicity.

p53, as a tumor suppressor gene, plays a prominent role in the regulation of cell apoptosis (27). Moreover, p53 has been linked to evoking apoptosis by transcriptional activation of pro-apoptotic proteins (Bax) and transcriptional repression of anti-apoptotic (Bcl-2) proteins (39). The present study showed that co-treatment with Mancozeb and PFT-α inhibited caspase-3 activity in cells, suggesting the potential role of p53 in Mancozeb-induced toxicity in rat thymocytes. Activated p53 is able to induce the expression of Bax, MMP damage and activation of caspases that lead to apoptosis (40). These observations are in agreement with our recent report which documented that Mancozeb-induced apoptosis through mitochondrial pathway, by disturbing the Bcl-2/Bax protein ratio in rat thymocytes (5). On the other hand, since the rat thymocytes were not completely rescued after PFT-α treatment, it suggests the involvement of another pathway which can be triggered by Mancozeb. Having in mind our previous results, it appears that Mancozeb-induced toxicity in rat thymocytes may be partially associated with p53 signaling activation, with potential secondary immunological consequences.

Taking into account the obtained results, we next tested the possibility that curcumin may modulate Mancozeb-induced toxicity in rat thymocytes. Present study results showed that curcumin (1, 3 μM) markedly inhibited cytotoxicity, caspase-3 and 9 activity, ROS production and restored cytochrome C oxidase and CAT activity in rat thymocytes after Mancozeb treatment. Curcumin (0.3 μM) failure to suppress Mancozeb-induced cytotoxicity is supported by previous findings which proposed that curcumin protective effect is mainly mediated by micromolar concentrations (23). The obtained findings are

consistent with our recently published results, indicating the protective role of curcumin through mitochondrial pathway in Mancozeb-induced rat thymocytes toxicity (5). The decline in mitochondrial respiratory activity (reduced cytochrome C oxidase activity) results in an increased susceptibility to oxidative stress, indicating the unique crosstalk between cytochrome C oxidase activity and cell death machinery (41). These findings correspond with inhibitory effect of curcumin on rOS production. In line with these observations, we showed that CAT activity was suppressed, suggesting that there may be an imbalance between pro-oxidant and anti-oxidant system after Mancozeb treatment. The preventive effect of curcumin on cytochrome C oxidase activity has been shown earlier (42) and here we demonstrated in rat thymocytes, after Mancozeb treatment. In support of this possibility, the protective effect of curcumin may also involve the promotion of mitochondrial respiratory function due to its anti-oxidative properties (42). Together with our results, we can speculate that protective role of curcumin in Mancozeb induced toxicity in rat thymocytes may be partially attributed to p53 inhibition, but this thesis needs additional studies.

In summary, the current study results demonstrate that Mancozeb exerts toxic effects in rat thymocytes, including caspase activation, cytochrome C oxidase and catalase inhibition. Moreover, Mancozeb-induced cell toxicity may be partially mediated through p53 signaling pathway and restored by certain curcumin concentrations. These findings could potentially provide the basis of curcumin as a potential therapeutic strategy for individuals exposed to pesticides which may suppress immunomodulation and secondary immunological consequence development.

References

1. Srivastava AK, Ali W, Singh R, Bhui K, Tyagi S, Al-Khedhairy AA,et al. Mancozeb-induced genotoxicity and apoptosis in cultured human lymphocytes. Life Sci 2012;90 (21-22):815-24.[PubMed][CrossRef]
2. Zahm SH, Blair A. Cancer among migrant and seasonal farmworkers: an epidemiologic review and research agenda. Am J Ind Med 1993;24(6):753-66. [PubMed][CrossRef]
3. Calviello G, Piccioni E, Boninsegna A, Tedesco B, Maggiano N, Serini S,et al. DNA damage and apoptosis induction by the pesticide mancozeb in rat cells: involvement of the oxidative mechanism. ToxicolAppl Pharmacol 2006;211(2):87-96.[PubMed][CrossRef]
4. Pavlovic V, Cekic S, Kamenov B, Ciric M, Krtinic D. The effect of ascorbic acid on Mancozeb-induced toxicity in rat thymocytes. Folia Biol 2015;61(3):116-23. [PubMed]
5. Pavlovic V, Cekic S, Ciric M, Krtinic D, Jovanovic J. Curcumin attenuates Mancozeb-induced toxicity in rat thymocytes through mitochondrial pathway. Food ChemToxicol 2016;88:105-11.[PubMed][CrossRef]
6. Mandarapu R, Ajumeera R, Venkatesan V, Prakhya BM. Proliferation and TH1/TH2 Cytokine Production in Human Peripheral Blood Mononuclear Cells after Treatment with Cypermethrin and MancozebIn Vitro. J Toxicol 2014;2014:308286. [PubMed][CrossRef]
7. Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK,et al. Effects on the immune system associated with living near a pesticide dump site. Environ Health Perspect 2000;108 (12):1113-24. [PubMed][CrossRef]
8. Colosio C, Barcellini W, Maroni M, Alcini D, Bersani M, Cavallo D, et al. Immunomodulatory effects of occupational exposure to mancozeb. Arch Environ Health 1996;51(6):445-51. [PubMed][CrossRef]
9. Domico LM, Zeevalk GD, Bernard LP, Cooper KR. Acute neurotoxic effects of mancozeb and Maneb in mesencephalic neuronal cultures are associated with mitochondrial dysfunction. Neurotoxicology 2006;27 (5):816-25. [PubMed][CrossRef]
10. Guan F, Ding Y, Zhang Y, Zhou Y, Li M, Wang C. Curcumin Suppresses Proliferation and Migration of MDA-MB-231 Breast Cancer Cells through Autophagy-Dependent Akt Degradation. PLoS One 2016;11 (1): e0146553. [PubMed][CrossRef]
11. Demir EA, Oz M, Alp MI, Gergerlioglu HS, Nurullahoglu KE, Yerlikaya FH. Co-administration of cisplatin and curcumin does not altermood-associated behaviors. BratislLekListy2016; 117(2):106-11. [PubMed][CrossRef]
12. Liao YF, Hung HC, Hour TC, Hsu PC, Kao MC, Tsay GJ. Curcumin induces apoptosis through an ornithine decarboxylase-dependent pathway in human promyelocytic leukemia HL-60 cells. Life Sci 2008;82(7-8): 367-75. [PubMed][CrossRef]
13. Mackenzie GG, Queisser N, Wolfson ML, Fraga CG, Adamo AM, Oteiza PI. Curcumin induces cell-arrest and apoptosis in association with the inhibition of constitutively active NF-kappaB and STAT3 pathways in Hodgkin's lymphoma cells. Int J Cancer 2008; 123 (1):56-65. [PubMed][CrossRef]
14. Chen J, Xu T, Chen C. The critical roles of miR-21 in anti-cancer effects of curcumin. Ann Transl Med 2015; 3(21):330. [PubMed]
15. Banerjee A, Kunwar A, Mishra B, Priyadarsini KI. Concentration dependent antioxidant/prooxidant activity of curcumin studies from AAPH induced hemolysis of RBCs. ChemBiol Interact 2008;174(2):134-9. [PubMed][CrossRef]

16. Guo LD, Chen XJ, Hu YH, Yu ZJ, Wang D, Liu JZ. Curcumin inhibits proliferation and induces apoptosis of human colorectal cancer cells by activating the mitochondria apoptotic pathway. Phytother Res 2013; 27(3):422-30. [PubMed][CrossRef]
17. Pavlovic V, Cekic S, Sokolovic D, Djindjic B. Modulatory effect of monosodium glutamate on rat thymocuyes proliferation and apoptosis. BratislLekListy 2006: 107(5):185-91. [PubMed]
18. Meslin F, Thiery J, Richon C, Jalil A, Chouaib S. Granzyme B-induced Cell Death Involves Induction of p53 Tumor Suppressor Gene and Its Activation in Tumor Target Cells. J BiolChem 2007;282(45):32991-9. [PubMed][CrossRef]
19. Nishimura Y, Oyama TB, SakanashiY, Oyama TM, Matsui H, Okano Y. Some characteristics of quercetin-induced cytotoxicity on rat thymocytes under in vitro condition. ToxicolIn Vitro 2008;22(4):1002-7. [PubMed][CrossRef]
20. Park GB, Choi Y, Kim YS, Lee HK, Kim D, Hur DY. ROS and ERK1/2-mediated caspase-9 activation increases XAF1 expression in dexamethasone-induced apoptosis of EBV-transformed B cells. Int J Oncol 2013;43 (1): 29-38.[PubMed][CrossRef]
21. Rohm. Dithane fungicide. Material Safety Data Sheetpp. Philadelphia: X; 1995. p. 1–11.
22. Vaccari A, Saba P, Mocci I, Ruiu S. Dithiocarbamate pesticides affect glutamate transport in brain synaptic vesicles. J PharmacolExpTher 1999;288(1):1-5. [PubMed]
23. Koizumi K, Kawanai T, Hashimoto E, Kanbara Y, Masuda T, Kanemaru K, et al. Cytometric analysis on cytotoxicity of curcumin on rat thymocytes: Proapoptotic and antiapoptotic actions of curcumin. ToxicolIn Vitro 2011;25(4):985-90.[PubMed][CrossRef]
24. Hori R, Kashiba M, Toma T, Yachie A, Goda N, Makino N, et al. Gene transfection of H25A mutant heme oxygenase-1 protects cells against hydroperoxide-induced cytotoxicity. J BiolChem 2002;277(12): 10712-8. [PubMed][CrossRef]
25. Al-Gayyar MM, Al Youssef A, Sherif IO, Shams ME, Abbas A. Protective effects of arjunolic acid against cardiac toxicity induced by oral sodium nitrite: Effects on cytokine balance and apoptosis. Life Sci 2014;111 (1-2):18-26. [PubMed][CrossRef]
26. Choi EY, Shin KC, Lee J, Kwon TK, Kim S, Park JW. Treatment with a Small Synthetic Compound, KMU-193, induces Apoptosis in A549 Human Lung Carcinoma Cells through p53 Up-Regulation. Asian Pac J Cancer Prev 2015;16(14):5883-7. [PubMed][CrossRef]
27. Slovackova J, Smarda J, Smardova J. Roscovitine-induced apoptosis of h1299 cells depends on functional status of p53. Neoplasma 2012;59(6):606-12. [PubMed][CrossRef]
28. Dastjerdi MN, Valiana A, Mardani M, Ra MZ. Adenosine A1 receptor modifies P53 expression and apoptosis in breast cancer cell line Mcf-7. BratislLekListy 2016; 117(4):242-6. [PubMed][CrossRef]
29. Amaral JD, Xavier JM, Steer CJ, Rodrigues CM. The role of p53 in apoptosis. Discov Med 2010;9(45):145-52. [PubMed]
30. Srinivasan S, Avadhani NG. Cytochrome c oxidase dysfunction in oxidative stress. Free RadicBiol Med 2012;53(6):1252-63. [PubMed][CrossRef]
31. Leadsham JE, Sanders G, Giannaki S, Bastow EL, Hutton R, Naeimi WR, et al. Loss of cytochrome c oxidase promotes RAS-dependent ROS production from the ER resident NADPH oxidase, Yno1p, in yeast. Cell Metab 2013;18(2):279-86. [PubMed][CrossRef]
32. Srinivasan A, Menon VP, Periaswamy V, Rajasekaran KN. Protection of pancreatic beta-cell by the potential antioxidant bis-o-hydroxycinnamoyl methane, analogue of natural curcuminoid in experimental diabetes. J Pharm PharmSci 2003;6(3):327-33. [PubMed]
33. Tu CT, Han B, Liu HC, Zhang SC. Curcumin protects mice against concanavalin A induced hepatitis by inhibiting intrahepatic intercellular adhesion molecule-1 (ICAM-1) and CXCL10 expression. Mol Cell Biochem 2011;358(1-2):53-60. [PubMed][CrossRef]
34. Li X, Liu X. Effect of curcumin on immune function of mice. J HuazhongUnivSciTechnolog Med Sci 2005;25 (2):137-40. [PubMed][CrossRef]
35. Tripathy MK, Mitra D. Differential modulation of mitochondrial OXPHOS system during HIV-1 induced T-cell apoptosis: up regulation of Complex-IV subunit COX-II and its possible implications. Apoptosis 2010;15 (1):28-40. [PubMed][CrossRef]
36. Medjdoub A, Merzouk S, Merzouk H, Chiali F, Narce F. Effects of mancozeb and metribuzin on in vitro proliferative responses and oxidative stress of human and rat spleen lymphocytes stimulated by mitogens. PesticBiochemPhysiol 2011;101(1):27-33. [PubMed][CrossRef]
37. Badr H, Hashemi M, Javadi G, Movafagh A, Mahdian R. Assessment of global ischemic/reperfusion and Tacrolimus administration on CA1 region of hippocampus: gene expression profi les of BAX and BCL2 genes. BratislLekListy 2016; 117(6):358-62. [PubMed][CrossRef]
38. Kaushik S, Kaur J. Chronic cold exposure affects the antioxidant defense system in various rat tissues. ClinChimActa 2003;333(1):69-77.[PubMed][CrossRef]
39. Brady CA, Attardi LD. p53 at a glance. J Cell Sci 2010;123:2527-32. [PubMed][CrossRef]
40. Kotipatruni RP, Ren X, Thotala D, Jaboin JJ. NDRG4 is a novel oncogenic protein and p53 associated regulator of apoptosis in malignant meningioma cells. Oncotarget 2015;6(19):17594-604. [PubMed][CrossRef]
41. Schüll S, Günther SD, Brodesser S, Seeger JM, Tosetti B, Wiegmann K, et al. Cytochrome c oxidase deficiency accelerates mitochondrial apoptosis by activating ceramide synthase 6. Cell Death Dis 2015;6: e1691. [PubMed][CrossRef]
42. Kaur H, Bal A, Sandhir R. Curcumin supplementation improves mitochondrial and behavioral deficits in experimental model of chronic epilepsy. Pharmacol BiochemBehav 2014;125:55-64. [PubMed][CrossRef]

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EFEKAT KURKUMINA I P53 SIGNALNOG PUTA U TOKSIČNOSTI PACOVSKIH TIMOCITA IZAZVANE MANKOZEBOM

Voja Pavlović[1], Snežana Cekić[1], Maja Petrović[2]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za fiziologiju, Niš, Srbija
[2]Klinika za očne bolesti, Klinički centar u Nišu, Niš, Srbija

Kontakt: Pavlović Voja
Bulevar dr Zoran Đinić 81, 18000 Niš, Srbija
E-mail: vojapav@yahoo.com

Mankozeb, kao ditiokarbamatni fungicid, nalazi se u širokoj upotrebi u poljoprivredi, prvenstveno zbog svoje male akutne toksičnosti i kratkog poluživota u spoljašnjoj sredini. U našem radu ispitivali smo preventivnu upotrebu kurkumina na mankozebom indukovanu toksičnost pacovskih timocita, kao i potencijalne mehanizme uključene u ovaj proces. Timociti pacova bili su izloženi delovanju mankozeba (0,01 μg/ml) i/ili kurkuminu u rastućim koncentracijama (0.3, 1 3 μM). Varijabilnost ćelija, aktivnost kaspaze 3, aktivnost kaspaze 9, aktivnost citohrom C oksidaze, katalazna aktivnost, produkcija reaktivnih kiseoničkih radikala (ROS) i aktivnost p53 signalnog puta ispitivani su nakon inkubacije od 24 sata. Ćelije tretirane mankozebom pokazale su povećanu toksičnost, aktivnost kaspaze 3 i kaspaze 9 i produkciju ROS-a, zajedno sa sniženom aktivnošću citohroma C oksidaze i sniženom katalaznom aktivnošću. Inhibicija aktivnosti kaspaze 3 i kaspaze 9 dovela je do smanjene toksičnosti timocita pacova, dok je inhibicija p53 signalnog puta suprimirala aktivnost kaspaze 3 u ćelijama. Kotretman kurkuminom (1, 3 μM) pokazao je značajnu redukciju toksičnosti, aktivnosti kaspaze 3 i 9 i produkcije ROS-a, zajedno sa povećanom aktivnošću citohroma C i povećanom katalaznom aktivnošću u ćelijama. Dobijeni rezultati pokazuju da je mankozebom indukovana apoptoza u timocitima pacova zavisna od kaspaza, kao i da se parcijalno odigrava preko p53 signalnog puta. Odgovarajuće koncentracije kurkumina mogu modelirati mankozebom indukovanu toksičnost pacovskih timocita, prvenstveno preko svog antioksidativnog efekta, što može predstavljati potencijalno mesto terapijske strategije u imunomodulaciji koja je indukovana mankozebom.

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Ključne reči: *mankozeb, kurkumin, p53, timociti toksičnost*

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THE IMPACT OF DEPRESSION ON THERAPY OF ACUTE CORONARY SYNDROME

Snežana Ćirić-Zdravković[1,2], Olivera Žikić[1,3]

Epidemiological studies suggest that besides classic factors for the development and prognosis of acute coronary syndromes, depression and anxiety are important as well. Depression and coronary heart disease occur simultaneously in the same person due to a common pathophysiological mechanism, possible genetic dysfunction of serotonin receptors. The aim of the paper was to examine the incidence and severity of the depression and anxiety in acute coronary syndrome patients and to examine whether the presence of depression had an impact on the decision for invasive or non-invasive approach.

We included 38 patients (23 males and 15 females, aged 63.5 ± 10years) hospitalized in the Clinic for cardiology Clinical Centre Niš, with the acute coronary syndrome (ACS). The patients were divided according to the type of ACS therapy: the invasive group (28 patients) with percutaneous coronary intervention and stenting and the second group of patients who underwent angiography without indication for stenting (non- invasive group of 10 patients). The anamnestic and clinical data, biomarkers of cardiac necrosis, standard laboratory, lipid profiles, and markers of inflammation were done. To examine the levels of depression and anxiety we used different questionnaires: The general questionnaire for socio-demographic data and the data about the disease progression, Beck Depression Inventory – questionnaire to measure the intensity of the depressive symptoms and State and Trait Anxiety Inventory (STAI) questionnaire to measure the intensity of actual anxiety (state anxiety), and anxiety as a personal characteristic (trait anxiety), Health Locus of Control – the questionnaire which measures where a patient puts the centre of the control of the disease. According to the level of depression, we formed four groups - 19 patients without depression (50 %), mild: 10 patients (26.3 %), moderate: 8 patients (21 %), and with severe depression: 1 patient (2.7 %).

The most common risk factors were: hypertension with 81.6 %, lipid disorders 68.4% and family history of cardiovascular disease in 52.6 %. Cardiovascular risk factors did not differ significantly between genders. Previous coronary artery disease (CAD) had 42.1 % hospitalised patients with ACS with a similar proportion of patients of both genders. The STEMI was the most common clinical presentation of ACS in 47.4 % of patients. The stenting procedures were performed in 28 (73.6 %) patients and medicamentous therapy in 10(26.4 %). The patients in non- invasive group were significantly older, more commonly obese, hyperlipidemic, with positive family history for CAD, with anamnestic data about previous AP and heart failure, and with higher heart rate. The presence of depression, especially a moderate level of depression was more common in non -invasive group (90.0 % and 50.0 % prospective) than in invasively treated group (35.7 % and 10.7 %) ($p < 0.01$ and $p < 0.05$). The correlation was found between the duration of hospitalization and the degree of hyperglycemia and depression and anxiety that exacerbate the progression of CAD.

Increased depression leads to increased anxiety and higher blood glucose levels - both additional risk factors for the progression of CAD. Patients with depression more likely had actual anxiety and will be treated by non–invasive medicamentous therapy for ACS. Increase in anxiety in patients diagnosed with the CAD increases the risk of MI, lethal outcome of coronary disease.

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Key words: *acute coronary syndrome, depression*

[1]University of Niš, Faculty of medicine, Niš, Serbia
[2]Clinic for cardiovascular diseases, Clinical Centre Niš, Serbia
[3]Clinic for psychiatry, Clinical Centre Niš, Serbia

Contact: Snežana Ćirić-Živković
Bulevard dr Zoran Djindjić 48, 18000 Niš, Serbia
E-mail: sczdravkovic@yahoo.com

Introduction

According to studies, it has been suggested that depressive symptomes occur in 15 to 30 % of patients with coronary artery disease (CAD). Risk ratios for the first and recurrent cardiac events related to depression are comparable to well-established CAD risk factors and range from 2 to 7. During the

last decades, there is increasing evidence that psychological factors, including depression, lack of social support, anger, job- related stresses could have an effect on prognosis in cardiovascular patients. Recently a number of studies pointed out that depression is a big psychosocial problem (1, 2) .It is important to note that a certain amount of negative emotional reaction should be expected in acute coronary syndrome. A physician should expect everyday anxiety from the patient, irritability, and sadness. A physician should respond to those reactions with support, warmth and to give hope for recovery.

These basic human emotions have a positive influence on the patients' recovery and progress. For example, the fear of the heart attack in the future could stimulate the acceptation of the proposed therapy. However, for some patients the fear is disproportional in comparison to the treatment and it is not adaptive such as the case with the intensive fear which leads to insomnia, atypical chest pain and avoiding of low-risk activities (3). Unlike the sudden fear which is a short-term reaction, the behaviour caused by serious illness is not seen immediately. Some patients are better at dealing with the changes in their lifestyles, including the stopping of some activities, while some others have difficulties with acceptance of their state and the emotional healing process is slow like curing of the infected wound. This unresolved grief could be the first sign of depression. Long stay in an intensive care unit or numerous re-hospitalizations could also trigger the depression. Epidemiological data suggest that on the medical ward cardiologist would probably see 16 % of the patients with major depression and 20 % a with minor depression form. Numbers are similar for hospitalized patients with myocardial infarction, unstable angina pectoris, and coronary bypass, angioplasty or heart failure (4, 5).

In the last 10 years, evidence has suggested that various psychological factors, including depression, lack of social support, anger, stress at work, can affect the prognosis of cardiac patients. More recently, the increasing number of well-designed studies published in medical journals and cardiology, has drawn attention to the great depression as a psychosocial risk.

Thrombus formation is a key factor in the rapid progression of CAD and in the occurrence of acute MI and unstable angina. The hypothesis of an association between depression and increased platelet activation, in particular, appears to explain the link between depression and mortality from heart diseases. Although serotonin itself is a weak agonist for platelet aggregation, serotonin can potentiate the effect of other agonists in the induction of platelet aggregation. Serotonin may further encourage the formation of thrombus by induction of coronary vasoconstriction of damaged vessels. During many years, platelets were used in psychiatric researches as a model of serotonin pre-and post-synaptic function in the brain (1, 2).

Aims

The aim of the study was to determine the incidence and severity of the depression and anxiety in acute coronary syndrome patients, as well as to examine its influence on the type of in-hospital therapy.

Methods

We included in the study 38 patients who were hospitalized in the Clinic for the cardiology Clinical Centre Niš with the acute coronary syndrome (unstable angina pectoris – UAP, myocardial infarction without ST - segment elevation – NSTEMI and myocardial infarction with ST - segment elevation – STEMI). The patients were divided into two groups according to tthe type of ACS therapy: the first group (invasive group 28 patients) has a percutaneous coronary intervention with stenting and second group has angiography without indication for stenting (non -invasive group 10 patients).

In all patients besides anamnestic data, routine laboratory examinations were done in Central laboratory Clinical Centre Niš. From the markers of necrosis, we followed troponin I (TnI), creatinine kinase isoform MB (CK-MB), from inflammation markers high sensistive C reactive protein (CRP) and white blood cells count were followed and lipid profile – triglycerides, cholesterol, LDL and HDL fractions. Standard 3 channel ECG, echocardiography and invasive examinations were done in all patients.

The patients with chronic non -cardiovascular diseases, chronic or acute inflammatory states, rheumatologic and systemic diseases were excluded from the analysis. The patients with an indication for further cardio-surgical revascularisation were also excluded from the study.

To obtain additional data about socio-demographic characteristics and the level of depression and anxiety we used different questionnaires:

1. The general questionnaire to obtain socio-demographic data and the data about the disease progression the (length of illness, number of hospitalizations), hereditary diseases, the association with psychological or stress disorders.

2. Beck Depression Inventory – the questionnaire to measure the intensity of the depressive symptoms. It has 21 questions and offered answers are 4-scaled (0-3). The final score is the sum of all the answers. According to the score, we could divide depression into 4 groups – without, mild, moderate and severe depression.

3. State and Trait Anxiety Inventory (STAI) – the questionnaire has 40 questions which measure the intensity of two anxiety forms – actual anxiety (state anxiety) and anxiety as personal characteristics (trait anxiety). According to the score, both types of anxiety could be divided into mild, moderate and intensive. With special tables, we could transform primary scores into Z- scores (0-100) which allow comparing those two forms of anxiety.

4. Health Locus of Control – questionnaire which measure where a patient puts centre of the control of the disease – internal (he thinks that he is dominantly responsible for his health and disease progression) and external (he thinks that the external factors determine if he will be sick or he will be healed as fate, by a physician and other people). With processing the results we get the intensity scores for (a) internal control centre, (b) control centre associated with the fate/coincidence, (c) doctors, and (d) other persons.

The data were processed in SPSS 16 program, including T test, Chi-square test, ANOVA and correlation analysis. The significance level was set to < 0.05.

Results

In the examined group we followed 38 patients, 23 men (60.5 %) and 15 women (39.5 %) aged 63.5 ± 10.0 years. The average age of male subjects was 65.2 ± 10.0 years and 61.1 ± 10.14 years for female, the difference was not statistically signi-ficant ($t = 1.2$, $p > 0.05$) (Table 1).

Table 1. Characteristics of the study population by age and gender

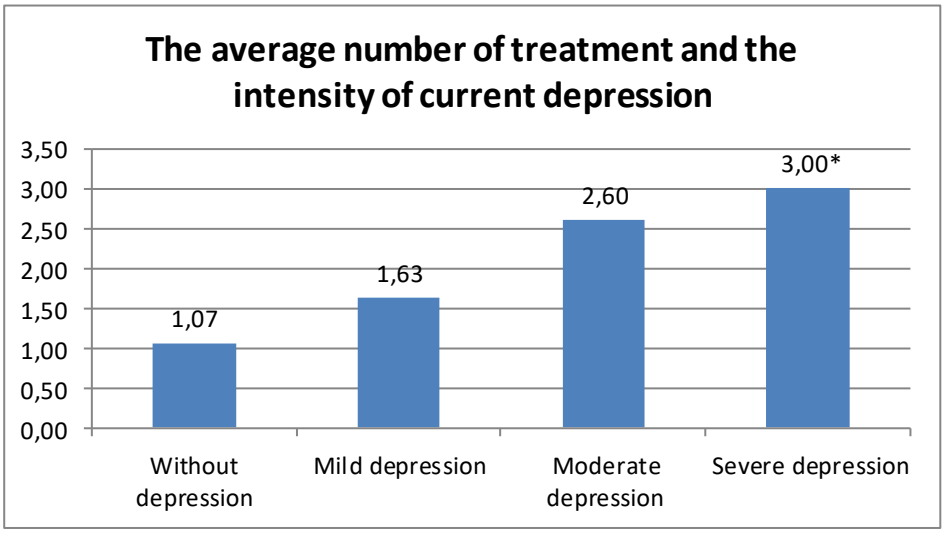
	Male	Female	Total
n/%	23 / 60.5	15 / 39.5	38 / 100.0
Age mean±SD (y)	65.2±10.0	61.1±9.8	63.5±10.0
Age min - max (y)	40-79	41-76	40 - 79
Cardiovascular risc factors			
Hypertension	20 / 87.0	11 / 73.3	31 / 81.6
Diabetes melitus	8 / 34.8	4 / 26.7	12 / 31.6
Smoking	5 / 21.7	3 / 20.0	8 / 21.1
Lipid disorders	17 / 73.9	9 / 60.0	26 / 68.4
Obesity	5 / 21.7	8 / 53.3*	13 / 34.2
Family history of CVD	11 / 47.8	9 / 60.0	20 / 52.6
Hystory of IM	3 / 13.0	3 / 20.0	6 / 15.8
Hystory of AP	5 / 21.7	2 / 13.3	7 / 18.4
Hystory of heart failure	2 / 8.1	1 / 6.7	3 / 7.9
Hystory of CABG	2 / 8.6	1 / 6.6	3 / 7.9
Cardiovascular parameters			
Heart rate	76.2±15.1	84.1±17.6*	79.3±18.3
Systolic pressure	147.1±22.8	140.7±32.8	144.5±30.0
Diastolic pressure	87.1±19.4	82.3±17.8	85.2±19.9
Laboratory findings			
Total cholesterol	6.2±1.8	6.4±1.8	6.3±1.7
LDL cholesterol	4.1±2.1	4.9±2.8	4.4±2.7
HDL cholesterol	1.1±0.4	1.2±0.4	1.1±0.4
Tryglicerides	2.5±1.1	2.8±0.9	2.6±1.2
Clinical presentations of ACS			
UAP	1 / 4.3	3 / 20	4 / 10.5
NSTEMI	9 / 39.1	7 / 46.7	16 / 42.1
STEMI	13 / 56.6	5 / 33.3	18 / 47.4
Depresion			
with depression	8 / 34.8	11 / 73.3*	19 / 50.0
mild depression	4 / 17.4	6 / 40.0	10 / 26.3
moderate depression	4 / 17.4	4 / 26.7	8 / 21.0
severe depression	0 / 0.0	1 / 6.6	1 / 2.7

Data are presented as mean±SD or n / group %; CVD- cardiovascular disease, IM-infarctus myocardi, AP-angina pectoris; UAP-unstable angina pectoris, NSTEMI-myocardial infarction without ST segment elevation, STEMI-myocardial infarction with ST segment elevation; *$p < 0.05$ vs. male

Table 2. Characteristics of the examined groups

	Invasive	Non invasive
n/%	28 / 73.6	10 / 26.4
Age mean±SD (y)	60.1±9.5	67.8±9.8*
Cardiovascular risc factors		
Hypertension	21 / 75.0	10 / 100.0
Diabetes melitus	8 / 28.6	4 / 40.0
Smoking	7 / 25.0	1 / 10.0
Lipid disorders	17 / 60.7	9 / 90.0*
Obesity	4 /14.3	9 / 90.0**
Family history of CVD	12 / 42.9	8 / 80.0*
Hystory of IM	4 /14.3	2 / 20
Hystory of AP	2 / 7.1	5 / 50.0**
Hystory of heart failure	0 / 0.0	3 / 30.0*
Hystory of CABG	1 / 3.5	2 / 20.0
Cardiovascular parameters		
Heart rate	75.1±14.2	88.8±19.6*
Systolic pressure	140.3±20.7	148.1±31.8
Diastolic pressure	86.1±18.3	88.3±16.8
Clinical presentations of ACS		
UAP	0 / 0.0	4 / 40.0
NSTEMI	10 / 35.7	6 / 60.0
STEMI	18 / 64.3	0 / 0.0
Depresion		
with depression	10 / 35.7	9 / 90.0**
mild depression	7 / 25.0	3 / 30.0
moderate depression	3 / 10.7	5 / 50.0*
severe depression	0 / 0.0	1 / 10.0

Data are presented as mean±SD or n/%; CVD- cardiovascular disease, IM-infarctus myocardi, AP-angina pectoris; UAP-unstable angina pectoris, NSTEMI-myocardial infarction without ST segment elevation, STEMI-myocardial infarction with ST segment elevation; *p<0.05 vs. invasive, **p < 0.01 vs. invasive group



*p < 0.05 vs. without and mild depression, Students t test

Graph 1. The average number of treatment and the intensity of the current depression

The most common risk factors were: hypertension with 81.6 %, lipid disorders 68.4 % and family history of cardiovascular disease in 52.6 %. Cardiovascular risk factors did not differ significantly between genders. Previous coronary artery disease had 34.2 % hospitalised patients with ACS with similar proportion of patients of both genders. The STEMI was the most common clinical presentation of ACS. The patients with NSTEMI and UAP were somewhat more frequent women while STEMI were frequently men, but without significant difference between genders.

Depression was presented in 50 % of patients, significantly more in females 73.3 % than males 34.8 % ($p < 0.05$) with similar distribution of depression grades among gender (Table 1).

The stenting procedures were performed in 28 (73.6 %) patients and medicamentous therapy in 10(26.4 %) (Table 2). The patients in the non–invasive group were significantly older, more commonly obese, hyperlipidemic, with positive family history for CAD, with anamnestic data about previous AP and heart failure, and with higher heart rate.

T5he presence of depression, especially moderate level of depression was more common in the non-invasive group (90.0 % and 50.0 % prospective) than in the invasively treated group (35.7 % and 10.7 %) ($p < 0.01$ and $p < 0.05$). Characteristics of invasively and noninvasively treated groups were presented in Table 2.

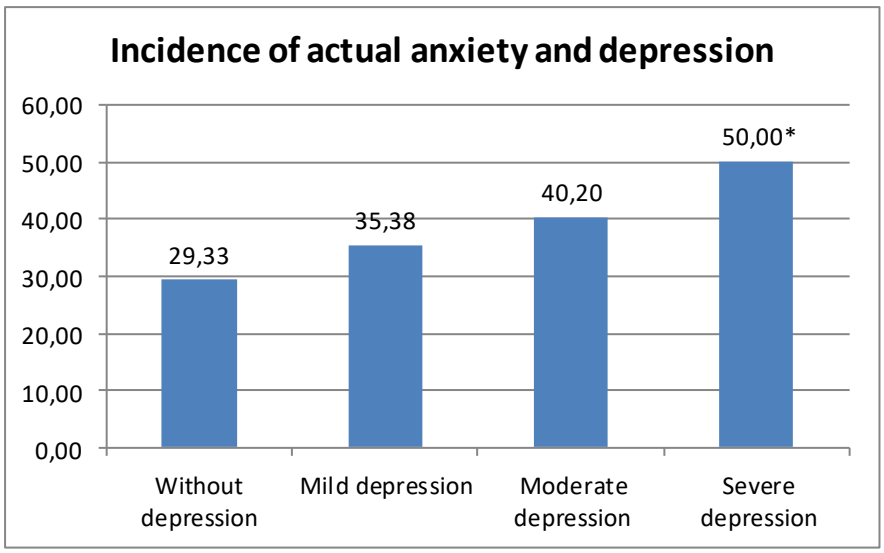
In the study population, with the number of hospitalizations, the severity of depression increased, and an average number of hospitalisations was significantly higher in severe depression compared to mild and no depression patients ($p < 0.05$) (Graph 1).

Actual anxiety was a very common finding in the observed coronary heart disease patients and strongly associated with level of depression. Its incidence was the lowest in patients without depression and the highest in patients with severe depression. Half of the examined patients (50 %) with severe depression had actual anxiety state (Graph 2).

Patients who had severe anxiety traits had the lowest values of internal locus of control, and differed from the others in a statistically significant level ($F = 5379$, $P = 0.011$) (Graph 3).

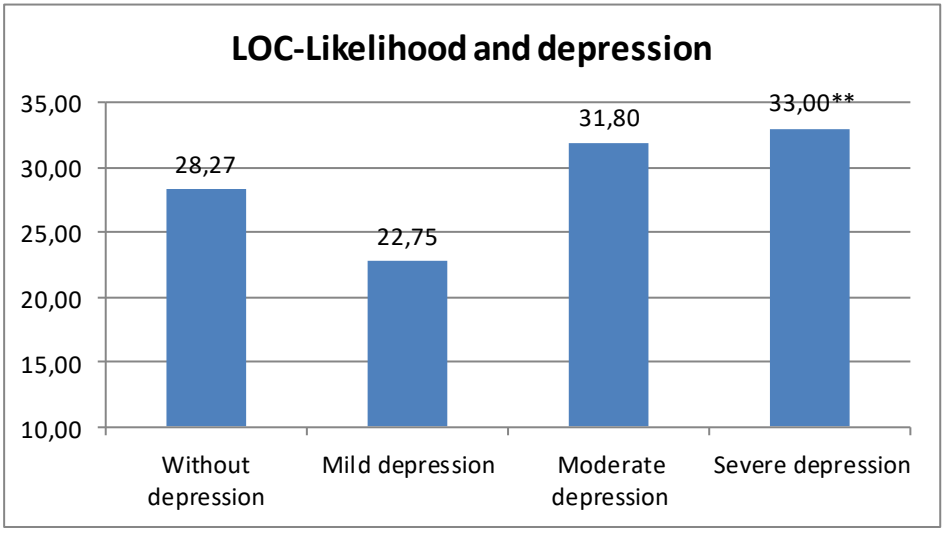
The Annalys of severity of depression according to the severity of current anxiety is shown in Graph 4. There is a significantly higer proprtion of patients with mild anxiety among patients without depression (66.5 %) compared to severe depression, where mild anxiety was not presented, only moderate in 35.6 %. Correlation analysis revealed a statistically significant positive correlation of severity of depression and severity of current anxiety ($C = 0.48$, $p < 0.05$) (Figure 4).

Correlation analysis revealed a significant po-sitive correlation among anxiety and fasting glicemia with strenght of depresion ($C = 0.59$ and $C = 0.45$, prospective), while this correlation was inverse with cholesterol values, Table 3.



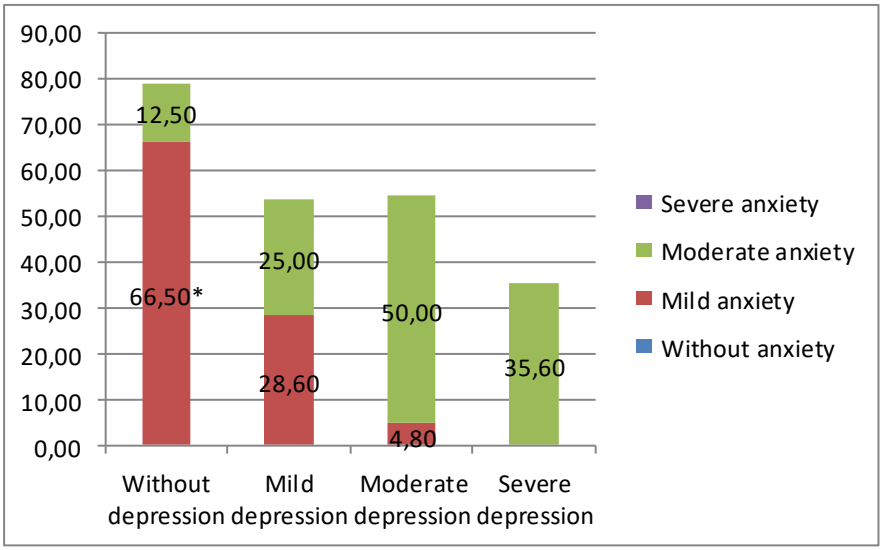
*$p < 0.05$ vs. without and mild depression, Hi square test

Graph 2. Incidence of actual anxiety and depression



**p < 0.01 vs. others

Graph 3. Average score of internal health locus of control and anxious personality traits



*p < 0.05 vs. moderate and severe depression, Hi square test

Graph 4. The current anxiety and depression

Table 3. Correlations of anxiety level and bichemical analyisis with depression

	Pearson correlation coefficient	P value
Actual anxiety	0.592	0.001
Cholesterol	-0.382	0.045
Glycemia	0.452	0.014

Discussion

To understand the occurrence of depression in patients with coronary artery disease it is required to understand that depression is neither the cause nor the result of coronary artery disease, but rather that it exists together in the same patient due to the patient having the same pathophysiological mechanism. It is possible that genetic dysfunction of serotonin receptors induces depression development and in platelets it could induce the risk of thrombotic events (1, 4, 5).

The relation between depression and coronary artery disease may be viewed through three different types of relationships. Firstly, depression may directly cause cardiac mortality through biological or behavioural mechanisms, as well as the type of ACS in-hospital and home therapy. Secondly, depression may be a consequence of the systemic complications of cardiovascular disease or its treatment. Finally, both depression and heart disease may share the common genetic and pathophysiological cause and have no causal correlation between each other (6).

Two other common pathophysiological mechanisms are low - intensity chronic inflammation and low intake of omega-3 fatty acids. Low - intensity chronic inflammation is an integral part of coronary artery disease. In patients with coronary artery disease markers of inflammation as CRP, IL6 or soluble intracellular adhesion molecules are high and are associated with a worse prognosis. They predict the development of CAD in a healthy population.

There is an interesting relation between depression and regulation of the immune function and inflammation. Meta-analyses of studies on patients without CAD concluded that major depression is associated with high leucocytes count, high CD4/CD8 ratio, and an increase of haptoglobin, prostaglandin E2, IL 6, lower natural killer cell cytotoxicity and low response on mitogens. Authors concluded that there is evidence that major depression is associated with one immune activation of reminiscent acute phase in inflammation response (5, 6).

Parallels can be seen between clinical data on inflammation and data in experimental studies. Animal experimental studies suggest that immune activation can induce depression similar behaviour and, conversely, that chronic stress induces the release of pro-inflammatory cytokines in the brain with consequent systemic responses. Pro-inflammatory cytokine-1 and tumor necrosis factor- alpha produced by activated immune cells promote the production of interleukin-6 from leukocytes, adipocytes or endothelial cells. Interleukin-6 induces broad systemic effects, including liver production of acute phase proteins (such as C-reactive protein) inhibition lipoprotein lipase activity, and increased platelet aggregation in response to ADP and epinephrine. Interestingly, systemic pro-inflammatory cytokines circulating in the blood also induce interleukin-1 activity in the hippocampus and hypothalamus, which acts as a messenger and SRES stimulate serotonin and norepinephrine neurotransmission and the release of corticotropin-releasing factor.

A conclusion can be drawn that the prognostic impact and prevalence of depression in patients with coronary artery disease may be attributed to the immune activation associated with progression of coronary artery disease score inducing depressive episodes in susceptible patients. The psychological concept of "vital exhaustion", defined as a combination of excess fatigue, irritability, and low morale may be particularly relevant for understanding the relationship between immune activation and symptoms of depression. Big epidemiological study of the elderly without coronary disease was found, although the C-reactive protein was associated with symptoms of depression and fatigue symptoms, the relationship remains independent of cardiovascular covariates and measures of physical weakness for symptoms of fatigue. It happens that the patient communicating vital exhaustion symptoms may be more likely to have concomitant immune activation than more common symptoms of depression. Finally, there is a statement about the normalization of inflammatory markers after antidepressant treatment (6).

In the discussion, we expect that higher level of depression and anxiety lead to higher blood glucose levels - both additional risk factors for the progression of coronary artery disease with an increase in the anxiety in already diagnosed patients which increases the risk for myocardial infarction, lethal outcome, and sudden coronary death. In addition to the direct effects on myocardial vulnerability, anxiety can lead to diabetes, hypertension, and hyperlipidemia. Chronically increased catecholamine levels were shown to increase the level of lipoprotein lipase induced hyperglycemia and increase blood pressure.

Considerable epidemiological evidence supports a link between chronic emotional stress and coronary heart disease /CHD/. Emotional factors related to atherosclerosis and adverse cardiac events include primarily disorders such as depression, anxiety, anger, and hostility. It is now well established that depression is associated not only with the incidence of CHD but also with the prognosis of patients with the disease. In meta-analysis relating to the role of depression in the development of coronary artery disease, Regulies found that individuals with clinical depression have > 2.5 fold increased risk of myocardial infarction or coronary death than the general population. In the patients with established coronary heart disease, major depression is not only a significant predictor of mortality after acute myocardial infarction, but also the level of depressive symptoms has a dose-dependent relationship with cardiac mortality over several years of monitoring. There is no evidence that could recommend systematic screening for depression in CAD patients. Self-reported questionnaire similar to BDI is quick and easy but it is too sensitive, and it gives many false positive diagnoses. However, systematic screening can overcome already scarce mental health resources available for a patient seeking psychiatric help. Instead, we need cardiologists to ask a few questions about mental health problems during regular visits. It can also encourage the patient to accept the referral to a psychiatrist when necessary. Studies have demonstrated that patients with CAD experience depression at a higher rate than the general population. Because of this connection, it is

critical to recognize depression and manage it effectively for people with CAD. Studies have also provided evidence that identifying and treating depression in patients early after myocardial infarction improve clinical outcomes. In addition, a number of studies have discussed the negative effects that can occur from untreated depression in those patients. The cited negative effects include mortality, recurrent myocardial events, and a worse quality of life (7). Some simple questions can be used to open a chapter of emotional distress without using the word depression, which often leads to rejection (8). Based on all of this evidence, it is important to diagnose and manage depression in patients with coronary artery disease. As such, depression screenings in cardiovascular disease care should be performed more often, because currently, they are under-performed due to the issue of optimal screening tolls cut-off (9).

Conclusion

In our study, increased depression leads to increased anxiety and higher blood glucose levels - both additional risk factors for the progression of CAD. Patients with depression more likely had actual anxiety and will be treated by non- invasive medicamentous therapy for ACS. Increase in anxiety in patients diagnosed with the CAD increases the risk of MI, lethal outcome of coronary disease and poor further outcome. Depression is associated with an increased incidence of cardiac death and re-hospitalization, as well as continues with chronic depression.

To prevent the adverse impact of depression on the prognosis of acute coronary syndrome there should be promptly implemented psychiatric and therapeutic support.

References

1. Lespereance F, Frasure-Smith N. What Cardiologists Need to Know on Depresion in Acute Coronary Syndromes. In: Pierre Theroux, editor. Acute Coronary Syndromes: A Companion to Braunwald's Heart Disease. 2nd ed. Philadelphia: Sauders; 2003. p. 131-43.
2. Singh PJ, Muller EJ. Triggers to Acute Coronary Sindromes. In: Pierre Theroux, editor. Acute Coronary Syndromes: A Companion to Braunwald's Heart Disease. 2nd ed. Philadelphia: Sauders; 2003. p. 108-18.
3. Smith C, Sidney Jr. Acute Coronary Syndromes: National and International Dimensions of the Problem. In: Pierre Theroux, editor. Acute Coronary Syndromes: A Companion to Braunwald's Heart Disease. 2nd ed. Philadelphia: Sauders; 2003. p. 10-3.
4. Hemingway H, Marmot M. Evidence based cardiology: Psychosocial Factors in the aetiology and prognosis of coronary heart disease: systemic review of prospective cohort. BMJ 1999; 318(7196):1460-7. [PubMed] [CrossRef]
5. Albert CM, Chae UC, Rexrode KM, Manson JAE, Kawachi I. Phobic Anxiety and Risk of Coronary Heart Disease and Sudden Cardiac Death Among Women. Circulation 2005; 111(4):480-7. [PubMed] [CrossRef]
6. Lett SH, Blumenthal JA, Babyak MA, Sherwood A, Strauman T, Robinsom C, et al. Depression as a Risc Factor for Coronary Artery Disease: Evidence, Mechanisms and Tretment. Psychosom Med 2004; 66(3): 305-15. [PubMed] [CrossRef]
7. DiSante JL, Bires AM, Cline TW, Waterstram-Rich K. An Analysis of the Prevelance of Depression Post-Myocardial Infraction. Crit Care Nurs Q 2017; 40(2):124-36. [PubMed] [CrossRef]
8. Wittsteuin IS. Depresion, anxiety, and platelet reactivity in patients with coronary heart disease. Eur Heart J 2010; 31(13):1548-50. [PubMed] [CrossRef]
9. Forsdahl SH, Torvik T, Schirmer H. Changes in symptoms of anxiety and depression following diagnostic angiography: a prospective cohort study. Eur Heart J Qual Care Clin Outcomes 2018; 4(2):106-12. [CrossRef]

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UTICAJ DEPRESIJE NA TERAPIJU AKUTNOG KORONARNOG SINDROMA

Snežana Ćirić-Zdravković[1,2], Olivera Žikić[1,3]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Klinika za kardiovaskularne bolesti, Klinički centar Niš, Niš, Srbija
[3]Klinika za psihijatriju, Klinički centar Niš, Niš, Srbija

Kontakt: Snežana Ćirić-Zdravković
Bulevar dr Zoran Đinđić 48, 18000 Niš, Srbija
E-mail: sczdravkovic@yahoo.com

Epidemiološke studije ukazuju da su pored uobičajenih faktora za razvoj i prognozu akut-nog koronarnog sindroma, depresija i anksioznost bitni faktori. Depresija i koronarna arte-rijska bolest pojavljuju se simultano kod bolesnika zbog zajedničkog patofiziološkog mehanizma, odnosno mogućih genetskih disfunkcija seratoninskih receptora. Cilj ovog rada je ispitati incidenciju i ozbiljnost depresije i anksioznosti kod bolesnika sa akutnim koronarnim sindromom i dati uvid u to da li prisustvo depresije ima uticaj na odluku o tome da li treba pri-meniti invazivni ili neinvazivni pristup. Ispitivano je 38 bolesnika (23 muškaraca i 15 žena, starosti 63,5 ± 10 godina) lečenih na Klinici za kardiologiju Kliničkog centra Niš, sa akutnim koronarnim sindromom (ACS). Bolesnici su bili odvojeni po tipu ACS terapije: invazivna grupa (28 bolesnika) sa perkutanom koronarnom intervencijom i stentiranjem i druga grupa koja je bila podvrgnuta angiografiji bez indikacija za stent (neinvazivna grupa, 10 bolesnika). Odra-đeni su anamnestički klinički podaci, biomarkeri miokardne nekroze, standardna laboratorija, profil lipida, marker zapaljenja. Da bi se ispitao nivo depresije i anksioznosti koristili smo razli-čite upitnike: generalni upitnik o socio-demografskim podacima i podacima o razvoju bolesti, Beck Depression Inventory – upitnik koji meri intenzitet depresivnih simptoma i State and Trait Anxiety Inventory (STAI)-upitnik koji meri intenzitet aktuelne anksioznosti (state an-xiety) i anksioznost kao karakternu crtu (trait anxiety). Health Locus of Control – upitnik koji meri gde bolesnik postavlja centar kontrole bolesti. Po ozbiljnosti depresije formirali smo četiri grupe – 19 bolesnika bez depresije (50 %), 10 sa blagom depresijom (26,3 %), 8 sa srednjom depresijom (21 %) i jedan bolesnik sa teškom depresijom (2,7 %).

Najčešći faktor rizika bili su hipertenzija (81,6 %), poremećaj lipida (68,4 %) i poro-dična istorija kardiovaskularnih bolesti (52,6 %). Kardiovaskularni faktori rizika nisu se bitno razlikovali između polova. Prethodnu koronarnu bolest (CAD) imalo je 42,1 % hospitalizovanih bolesnika sa ACS, sa proporcionalnim brojem bolesnika oba pola. STEMI je bio najčešća kli-nička prezentacija ACS u 47,4 % bolesnika. Implantacija stenta urađena je kod 28 (73.6 %) bolesnika i medikamentozna terapija kod 10 (26.4 %) bolesnika. Pacijenti u neinvazivnoj grupi značajno su stariji, češće gojazni, sa hiperlipoproteinemijom, sa porodičnom istorijom CAD, sa anamnestičkim podacima prethodne AP i srčane insuficijencije i sa većim brojem otkucaja srca. Prisustvo depresije, posebno srednjih nivoa depresije, češći su u neinvazivnoj grupi (90,0 % i 50,0 % prospektivno) nego kod invazivne grupe (35,7 % i 10,7 %) ($p < 0,01$ i $p < 0,05$). Korelacija je pronađena između dužine trajanja hospitalizacije i nivoa povišenog šećera i depresije i anksioznosti koji ubrzavaju progresiju CAD.

Teža depresija vodi ka težoj anksioznosti i višim vrednostima šećera u krvi – od kojih su oba faktora rizika za progresiju CAD. Pacijenti sa depresijom češće su imali aktuelnu an-ksioznost i bili su lečeni neinvazivnom medikamentnom terapijom za ACS. Porast anksiozno-sti kod pacijenata sa dijagnozom CAD povećava rizik za MI, smrtni ishod.

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MUSCLE STRENGTH TEST PERFORMANCE CHANGES OVER TIME IN SERBIAN CHILDREN

Jadranka Kocić[1], Darko Stojanović[2], Sladjana Stanković[3], Lana Petrović[1], Aleksandar Ignjatović[3], Zoran Savić[1], Vesko Milenković[1], Tijana Stojanović[4], Zoran Momčilović[5]

The aim of this study was to identify the changes in motor performance test over an interval of four decades in eleven-year-old children in Serbia. The secular trend of body height and weight showed constant increase over time, but positive influence on the motor performance was lacking. Most studies found some decline in various motor skills over different time periods. However, none of them investigated the standing long jump results over the period of four decades. Data were collected from three separate cross-sectional samples examined in 1971, 2014 and 2018. Measurements were conducted by a team of qualified testers, coordinated by the Faculty of Physical Culture from Belgrade in 1971, Serbian Institute of Sport and Sport Medicine in 2014, and authors of this study in 2018. Motor performance test that was evaluated including the standing long jump. Despite the average increase in longitudinal skeleton dimensions, an average distance of the long jump showed a significant decrease. Decreased values after four decades ranged between 10 % and 18 % depending on the sample. The results of this study raise serious concerns about the contemporary way of life of children and trends in their motor skills. Without changes in lifestyle and healthy nutritional habits, and most importantly, greater participation in organized physical activities that target at deficits in muscular fitness and motor skill performance early in childhood, these contemporary trends of motor test performance will most likely continue to decrease in the future.

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[1]University of Priština, Kosovska Mitrovica, Faculty of Sport and Physical Education, Leposavić, Serbia
[2]University of Union - Nikola Tesla, Faculty of Sport, Belgrade, Serbia
[3]University of Kragujevac, Faculty of Education, Jagodina, Serbia
[4]Club for synchronized swimming "Niš", Niš, Serbia
[5]University of Niš, Pedagogical Faculty in Vranje, Vranje, Serbia

Contact: Jadranka Kocić
Ratka Vukićevića 1/10, 18000 Niš, Serbia
E-mail: jadranka.kocic@pr.ac.rs

Introduction

Insufficient level of physical activity has been identified to be one of the leading risk factor for global mortality worldwide (1). The rising levels of childhood obesity are likely to have major public health consequences since being overweight during childhood will most likely continue into adulthood. Low scoring in physical fitness tests are important risk factor for many diseases like diabetes, cancer, high blood pressure, osteoporosis, lipid disorders, depression, anxiety etc (1).

Positive secular trends in height and weight have occurred in all socioeconomic groups in the first half of the twentieth century (2). The changes recorded from the 1960's through the 2000's are variable among countries (2). Secular changes were remarkably similar for boys and girls, and there is no doubt that children are becoming fatter (1, 3), so it's questionable if we could call this trend the "positive" one. With this trend, it would be surprising if performance on jumping tests were not declining (4). The trend of a decrease in acceleration has also been confirmed in some other studies on secular height trend conducted in Serbia (5, 6).

Unfavorable trends in children's weight status have been documented in many countries. Overweight prevalence remained high in most developed countries in the world (7). An increase was observed for both boys and girls (3, 7). However, in recent years, a stabilization in overweight prevalence has been suggested by researches from several different countries (8, 9). Some researchers suggest that this plateau in overweight prevalence may mask an increase in certain vulnerable groups of children (10).

In the period from 2002 to 2010, overweight prevalence increased predominantly in Eastern Europe compared to the rest of the Europe and US (3). Recent data showed that in 2016, approximately 50 million girls and approximately 74 million boys worldwide, were obese. That estimated pooled population-based data regarding trends from 1975 to 2016 in mean BMI in Eastern Europe showed an increase of 1.00 kg/m^2 per decade; for boys, there was a non-significant increase of 0.09 kg/m^2 per decade (3).

An important health marker in youth is muscle strength. For adequate and balanced children development, some form of resistance training is highly recommended as a part of regular daily activities (11). In addition to increased muscular strength, regular engagement in resistance exercises has the potential to influence several other aspects of health. It may result in improvement of body composition, increased bone mineral density, increased cardio-respiratory fitness, enhanced mental health and well-being and a more positive attitude towards lifetime physical activity (11).

Several studies and epidemiological research (12, 13) reported a decline in physical fitness in children and adolescents over time, suggesting that the present-day children and adolescents are not as active as their peers few decades ago. Evidence-based research (14, 15) showed a constant decrease over the past decades in children's participation in physical activity and organized community sport. An evident decreasing trend in youth muscular capabilities are observed in English, Spanish, Lithuania and Serbian primary school children and adolescents (13, 16-18).

However, studies on different muscle performance capabilities have shown divergent trends: for example, despite the decline in upper body strength, the leg muscle power increased (16), which could partially be explained by the constant bearing of larger body weight. Similarly increased body weight could lead to increased handgrip strength but it is not necessarily accompanied by the increase in lower body strength (19). Changes in fitness may depend on the population studied and tests applied, so it is necessary to use the same standardized procedures in the same population (20).

Moreover, muscular fitness in adolescents has been shown to be associated with cardiovascular problems and cardiovascular mortality later in life, independent of the aerobic capacity (21). The aerobic performance of children has declined over the last few decades in most of the countries. Sedentary lifestyle contributed to easy availability of energy-rich but low-micronutrient-content foods and declines in community-based physical activity have been implicated (22).

The aim

The present study was aimed at investigating how secular changes in body height and weight in Serbian children aged 11 have been changing since 1971, and how this changes influence the motor test performance that requires muscle strength, being also depended on anthropometric characteristics such as body height and weight.

Materials and methods

Data Sources

An extensive review of the domestic literature was undertaken to locate studies that have published data on motor test performance or reported motor test performance of normal children and adolescents aged 11. Briefly, studies were selected via the online search of bibliographic databases and the University library catalogue and through a manual search of all hard copy monographs and PhD thesis at the University of Niš.

Inclusion/exclusion criteria

Data were collected from three separate cross-sectional samples examined in 1971 and 2014. Measurements were conducted by a team of qualified testers, coordinated by Faculty of Physical Culture from Belgrade in 1971 (23) and Serbian Institute of Sport and Sport Medicine in 2014 (24).

Data were collected in 2018 from elementary school children in Jagodina. The testing was conducted in the Faculty of Education exercise hall. Height was measured by a stadiometer. The subjects did not wear shoes, and height was converted to the nearest centimeter. Weight was measured using a digital scale (Tefal, France, accuracy of 0.1 kg). Body mass index was calculated by dividing weight (kilograms) by height (centimeters) squared and then multiplying the result by 10,000, in accordance with international standards.

The standing long jump test, also known as the standing broad jump, is the test used for the assessment of explosive muscle strength of lower extremities. It was a part of test batteries in both assessments. The participant stood behind the starting line and was instructed to push off explosively and jump as far as possible. The children had to land with the feet together and stay upright. The test was repeated twice, and the best score was retained to the nearest 0.1 cm.

Statistical analysis

All descriptive data were extracted into Excel (Microsoft Office) using a standardized data extraction table. The following descriptive data were extracted by one author and checked by another for accuracy: year of testing, sex, and age, because only data on children aged 11 years were included in further analysis.

The data are presented as mean ± SD unless otherwise stated. Mean differences of the secular trends in leg strength between measurement points were analyzed. We calculated the effect size statistics as Cohen's d (standardized mean differences) and 95% confidence interval. The values of Cohen's

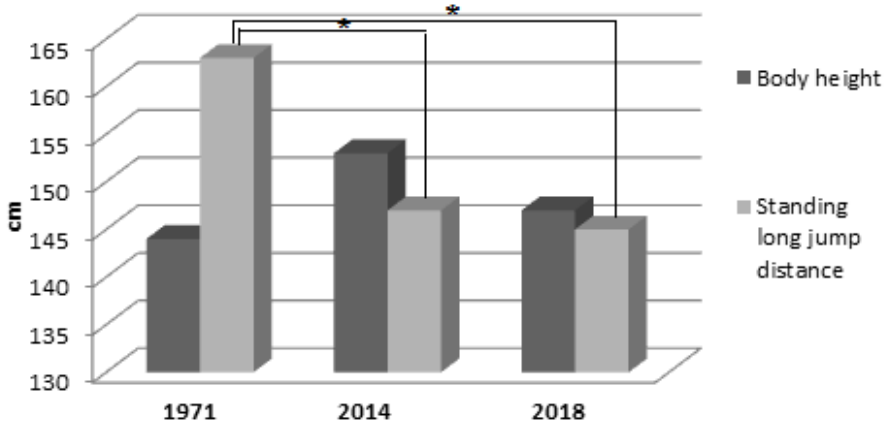
d~0.2, ~0.5 and ~0.8 are considered as small, medium and large effect sizes, respectively.

Results

The investigation included results from three different periods – the years 1971, 2014 and 2018. Mean (M) and standard deviation (SD) are presented in Table 1. The average value of standing long jump height in 1971 was 163.2 cm for boys and 156.2 for girls in the sample of 848 eleven- year-old children, 431 boys and 417 girls. These values have been decreased in a recent measurement. In 2014, an average standing long jump for boys and girls was 147.5 cm and 134.9 cm, respectively. In 2018, an average standing long jump was 145.3 cm and 128.2 cm, respectively.

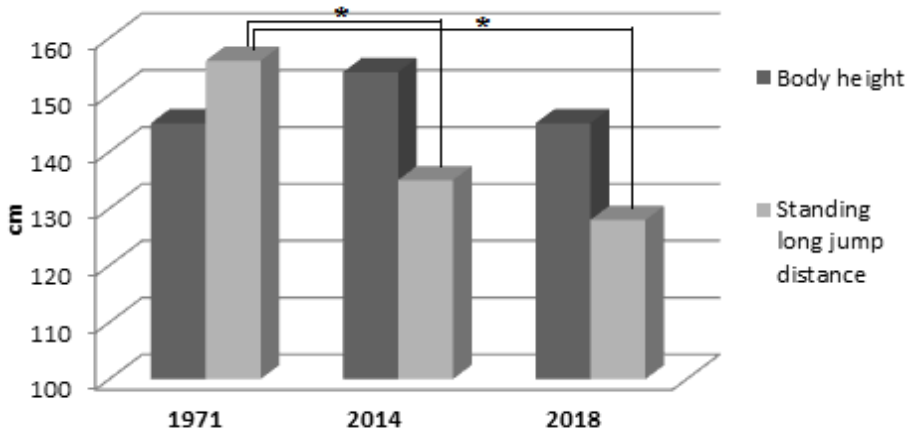
Table 1. Means and stadard deviations from three different data point

Year of intervention	1971		2014		2018	
	Boys	Girls	Boys	Girls	Boys	Girls
Sample number (n)	431	417	50	57	48	44
Body height (cm)	144.02 (6.83)	144.99 (7.42)	153.07 (7.11)	154.04 (7.65)	147.44 (7.33)	145.36 (7.45)
Body weight (kg)	36.07 (6.80)	36.89 (7.15)	45.87 (11.84)	45.60 (10.29)	40.5 (9.20)	41.5 (8.80)
Standing long jump (cm)	163.21 (15.32)	156.21 (16.34)	147.52 (22.53)	134.93 (21.92)	145.3 (18.87)	128.2 (16.54)



* - Significant difference between measurements Cohen D, large differences

Graph 1. Body height and long distance jump in eleven years old boys



* - Significant difference between measurements Cohen D, large differences

Graph 2. Body height and long distance jump in eleven years old girls

The results showed significant differences in the standing long jump distance between the two periods (1971 and 2014). Cohen effect size are considered large as values for 11-year-old boys were d = 0.84 and for girls Cohen's d = 1.09. Mean difference between 1971 and 2018 also showed large Cohen's d = 1.05 and 1.75, for boys and girls, respectively. Cohen effect size between two samples from 2014 and 2018 was considered small, 0.1 and 0.3, for boys and girls, respectively.

Similar results in body height and weight between 1971 and 2014 showed significant effect size of d = 1.28; d = 1.19 for body height, and d = 1.02; d = 1.04 for body weight, for boys and girls, respectively. Most medium differences on basic anthropometric data were found between 1971 and 2018 for body height d = 0.43; d = 0.14 and for body weight d = 0.5; d = 0.62 for boys and girls, respectively. Between 2014 and 2018 there were large differences in mean difference between samples, d = 0.85; d = 1.28 on body height and medium differences d = 0.37; d = 0.39 on body weight, for boys and girls, respectively.

Discussion

In this study, we examined the secular trend of lower body muscle strength test performance in the last four decades. This is the first study to have quantified muscle strength performance changes in eleven-year-old children in Serbia in the longer period. Using data from previous studies from 1971 and those from 2009 and 2014, and comparing them with this study data, it can be observed that the standing long jump performances have declined remarkably over the past four decades (Graph 1 and 2).

The results of Cohen effects size showed large effects in all measurements between 1971 and two more intervention periods (2014 and 2018). Children in 1971 had lower height and body mass than their peers several decades after (Table 1). The largest difference was noted between measurements conducted in 1971 in children measured in different schools across the Republic of Serbia (23) and children measured only in Belgrade in 2014 (24). Difference in body height of eleven-year-old boys were increased in favor of recent measurements by 10 cm. A sample from 2018 from central Serbia showed 3 cm increases compared to the measurement conducted in 1971. Body weight showed similar increases of 10 kg and 4.5 kg, respectively. The previous study (6) that considered changes over the period 1971–2001 in 11-year-old children from the city of Novi Sad showed also a growing tendency of body weight. However, body weight increases during three decades were 4.3 kg in eleven-year-old girls, while in boys, weight increased by 3.4 kg over the period 1971-1991 and this trend failed to continue until 2001, most probably due to the economic situation in the country (6) or a small sample used in investigation. On the other hand, the previously mentioned investigation (6) found relatively small positive changes (less than 2 cm) in secular trends of body height during that period. Additionally, over the last decade, the secular trend of height has slowed down both in the world and Serbia (5).

An increase in height leads to proportional increase of longitudinal dimensions such as upper leg height which should logically positively affect the standing long jump performance. However, despite the average increase in longitudinal skeleton dimensions, an average distance of the long jump showed significant decrease over the same period. Results in the standing long jump (Table 1.) showed a decrease after four decades between 16 to 28 cm depen-

ding on sample and sex. Presented in percentages, there is a decrease in the standing long jump distances of 10 % to 18 % depending on the sample, which raises serious concerns about children's way of life today and the observed trends in their motor skills.

In the last decade, positive trends in body weight were identified for children and adolescents living in different parts of the world (2). On the other hand, most studies (13, 16-18) found some decline in various motor skills over different time periods. However, none of them compared the standing long jump results over the period of four decades.

A study (16) compared the standing long jump results in Spanish youths aged 12 - 17 years. They found a decrease of 12 cm after five years (2001/02-2006/07). Cohen ES was 0.5. A similar study on Estonians and Lithuanians children (18) found no significant change for Estonians in the standing long jump over the period of 10 years (1992-2002), while this decline was observed in Lithuanians.

Finally, there are limitations to this investigation as well. Muscular fitness was measured by only one field tests. Compared with testing in the laboratory, this test may result in errors due to the level of test performance and previous experience. Additionally, small sample size in recent sample groups (2014 and 2018) may contribute to research bias. A larger research sample and more motor performance test with same protocol are required to obtain more credible data regarding this important topic.

Conclusion

The results of our investigation have demonstrated that motor test performance in children aged 11 have been decreased substantially over the last four decades. Without changes in P.E. curriculum, healthy nutritional habits, and most importantly, greater participation in organized physical activities that target at deficits in muscular fitness and motor skill performance early in childhood, these contemporary trends of motor test performance will most likely continue to decrease in the future.

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References

1. World Health Organization. Global Recommendations on Physical Activity for Health. Geneva; 2010. A from http://www.who.int/dietphysicalactivity/publications/9789241599979/en/.
2. Malina RM. Secular trends in growth, maturation and physical performance: A review. Anthropological Review 2004; 67: 3-31.
3. Abarca-Gómez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. The Lancet 2017; 390: 2627-42. [PubMed]
4. Tomkinson GR, Olds TS, Kang SJ, Kim DY. Secular Trends in the Aerobic Fitness Test Performance and Body Mass Index of Korean Children and Adolescents (1968–2000). International Journal of Sports Medicine 2007; 28: 314–20. [CrossRef][PubMed]
5. Pavlica TM, Rakić RS, Popović BK, Puškaš VP. Secular trend in growth and nutritional status in a sample of girls aged 7-9 years from Serbia. HOMO – Journal of Comparative Human Biology 2018; 69: 280-6. [CrossRef][PubMed]
6. Božić-Krstić V, Pavlica T, Rakić R. Body height and weight of children in Novi Sad. Annals of Human Biology 2004; 31: 356-63. [CrossRef][PubMed]
7. Ahluwalia N, Dalmasso P, Rasmussen M, Lipsky L, Currie C, Haug E, et al. Trends in overweight prevalence among 11- 13- and 15-year-olds in 25 countries in Europe, Canada and USA from 2002 to 2010. European Journal of Public Health 2015; 25: 28-32. [CrossRef][PubMed]
8. Olds T, Maher C, Zumin S, Peneau S, Lioret S, Castetbon K, et al. Evidence that the prevalence of childhood overweight is plateauing: data from nine countryes. International Journal of Pediatric Obesity 2011; 6: 342-60. [CrossRef][PubMed]

9. Rokholm B, Baker JL, Sorensen TI. The levelling off of the obesity epidemic since the year 1999 - a review of evidence and perspectives. Obesity Reviews 2010; 11: 835-46. [CrossRef][PubMed]
10. Sjoberg A, Lissner L, Albertsson-Wikland K, Marild S. Recent anthropometric trends among Swedish school children: evidence for decreasing prevalence of overweight in girls. Acta Paediatrica 2008; 97: 118-23. [CrossRef][PubMed]
11. Lloyd RS, Cronin JB, Faigenbaum AD, Haff GG, Howard R, Kraemer WJ, et al. National Strength and Conditioning Association Position Statement on Long-Term Athletic Development. Journal of Strength and Conditioning Research 2016; 30: 1491-509. [CrossRef][PubMed]
12. Tudor-Locke C, Johnson W, Katzmarzyk PT. Accelerometer-determined steps per day in US children and adolescents. Medicine and Science in Sports and Exercise 2010; 42: 2244-2250. [CrossRef][PubMed]
13. Ignjatović A, Gudmir-Ninković G, Stevanović N. Hand grip strength in 12 years old children in Serbia – changes over 50 years. Sport and business 2017; 3: 77-80.
14. Dollman J, Norton K, Norton L. Evidence for secular trends in children's physical activity behaviour. British Journal of Sports Medicine 2005; 39: 892-7. [CrossRef][PubMed]
15. Donnelly JE, Lambourne K. Classroom-based physical activity, cognition, and academic achievement. Preventive Medicine 2011; 52: S36-S42. [CrossRef][PubMed]
16. Cohen DD, Voss C, Taylor MJ, Delextrat A, Ogunleye AA, Sandercock GR. Ten-year secular changes in muscular fitness in English children. Acta Paediatrica 2011; 100: 175-7. [CrossRef][PubMed]
17. Moliner-Urdiales D, Ruiz JR, Ortega FB, Jiménez-Pavón D, Rey-López JP, Martinez-Gómez D, et al. Secular trends in health-related physical fitness in Spanish adolescents: the AVENA and HELENA studies. Journal of Science and Medicine in Sport 2010; 13: 584-8. [CrossRef][PubMed]
18. Jürimäe T, Volbekiene V, Jürimäe J, Tomkinson GR. Changes in Eurofit test performance of Estonian and Lithuanian children and adolescents (1992-2002). Medicine and Sport Science 2007; 50: 129-42. [CrossRef][PubMed]
19. Dos Santos FK, Prista A, Gomes TN, Daca T, Madeira A, Katzmarzyk PT, et al. Secular trends in physical fitness of Mozambican school-aged children and adolescents. American Journal of Human Biology, 2015; 27: 201-6. [CrossRef][PubMed]
20. Venckunas T, Emeljanovas A, Mieziene B, Volbekiene V. Secular trends in physical fitness and body size in Lithuanian children and adolescents between 1992 and 2012. Journal of Epidemiology and Community Health 2017; 71: 181-7. [CrossRef][PubMed]
21. Timpka S, Petersson IF, Zhou C, Englund M. Muscle strength in adolescent men and risk of cardiovascular disease events and mortality in middle age: a prospective cohort study. BMC Medicine 2014; 12: 62. [CrossRef][PubMed]
22. French SA, Story M, Jeffery RW. Environmental influences on eating and physical activity. Annual Review of Public Health 2001; 29: 309-35. [CrossRef][PubMed]
23. Kurelić N, Momirović K, Stojanović J, Šturm J, Radojević Đ, Viskić-Štalec N. Struktura i razvoj morfoloških i motoričkih dimenzija omladine. Beograd: Institut za naučna istraživanja Fakulteta za fizičku kulturu; 1975.
24. Ivanović J, Gajević A. Razlike u antropološkom status dece osnovnoškolskog uzrasta. Beograd, Srbija: Republički zavod za sport; 2015.

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PROMENE U TESTU ZA PROCENU MIŠIĆNE SNAGE KOD DECE U SRBIJI

Jadranka Kocić[1], Darko Stojanović[2], Slađana Stanković[3], Lana Petrović[1], Aleksandar Ignjatović[3], Zoran Savić[1], Vesko Milenković[1], Tijana Stojanović[4], Zoran Momčilović[5]

[1]Univerzitet u Prištini, Kosovska Mitrovica, Fakultet za sport i fizičko vaspitanje, Leposavić, Srbija
[2]Univerzitet "Union - Nikola Tesla", Fakultet za sport, Beograd, Srbija
[3]Univerzitet u Kragujevcu, Fakultet pedagoških nauka u Jagodini, Srbija
[4]Klub za sinhrono plivanje "Niš", Niš, Srbija
[5]Univerzitet u Nišu, Pedagoški fakultet u Vranju, Vranje, Srbija

Kontakt: Jadranka Kocić
Ratka Vukićevića 1/10, 18000 Niš, Srbija
E-mail: jadranka.kocic@pr.ac.rs

Cilj ove studije bio je ispitivanje promena u uspešnosti izvođenja motoričkog testa kod jedanaestogodišnjaka u Srbiji. Za praćenje u intervalu od četiri decenije izabran je motorički test za koji je potrebno ispoljavanje mišićne snage nogu. Sekularni trend telesne visine i težine pokazao je konstantno povećanje u odnosu na prošlost, ali je pozitivan uticaj na uspešnost u izvođenju motoričkog zadatka izostao. Većina prethodnih istraživanja utvrdila je određeni pad raznih motoričkih veština tokom različitih vremenskih perioda. Međutim, nijedno od njih nije se bavilo uspešnošću u skoku udalj u periodu dužem od četiri decenije. Podaci su prikupljeni sa tri odvojena uzorka ispitanika iz 1971, 2014. i 2018. godine. Merenja su sprovedena od strane kvalifikovanih merioca sa Fakulteta fizičke kulture iz Beograda 1971. godine, Instituta za sport i sportsku medicinu Srbije 2014. godine i autora ove studije 2018. godine. Testom skok udalj iz mesta ispitivana je motorička sposobnost. Uprkos prosečnom povećanju longitudinalne dimenzionalnosti skeleta, prosečna dužina skoka udalj imala je trend opadanja. Smanjenje posle četiri decenije bilo je između 10 % i 18 % u zavisnosti od uzorka i pola. Rezultati ove studije izazivaju ozbiljnu zabrinutost o načinu života dece i trendovima u motoričkim sposobnostima. Bez promena u načinu života, stvaranja zdravih navika u ishrani, i što je najvažnije, većeg učešća u organizovanim fizičkim aktivnostima, pomenuti trendovi opadanja nivoa motoričkih sposobnosti najverovatnije će se nastaviti i u budućnosti.

Acta Medica Medianae 2019;58(2):154-160.

Ključne reči: *mišićne promene, motoričke sposobnosti, snaga nogu, deca*

JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

Ideja o postavljanju jedinstvenih kriterijuma za objavljivanje radova u časopisima za biomedicinske nauke iskristalisana je 1978. godine u Vankuveru. Ovi kriterijumi za rukopise, uključujući pravila za pisanje bibliografije, prvi put su objavljeni 1979. godine. Vankuverska grupa je vremenom prerasla u Međunarodni komitet urednika medicinskih časopisa — International Committee of Medical Journal Editors (ICMJE). Trenutno je na snazi peta revizija kriterijuma za objavljivanje radova u biomedicinskim časopisima, doneta 1997. godine.

Kriterijumi za citiranje i navođenje referenci

Reference se obeležavaju arapskim brojevima u zagradama, pri čemu se reference obele-žavaju brojevima onim redosledom kojim se pojavljuju u tekstu. Reference citirane jedino u tabelama ili legen-di moraju se obeležiti brojem u skladu sa redosledom pojavljivanja u tekstu.

Naslove medicinskih časopisa treba pisati u skraćenom obliku onako kako su navedeni u poglavlju **List of Journals Indexed in Index Medicus**. Lista skraćenih naziva medicinskih časopisa objavljuje se svake godine u januarskom broju ***Index Medicusa***. Ova lista se takođe može naći na adresi **www.nlm.nih.gov**

Izbegavati upotrebu apstrakata kao referenci, već koristiti samo izvorne tekstove (*in extenso* članci). Reference koje se odnose na radove koji su prihvaćeni, ali još nisu odštampani, treba označiti sa "u štampi", pri čemu autor mora imati pismeno odobrenje da citira takve radove i da priloži pismeni dokaz da je citirani rad prihvaćen za štampu. Informacije iz rukopisa koji nisu prihvaćeni za štampanje mogu se citirati u tekstu kao "neobjavljeni rezultati", ali sa pismenom dozvolom autora.

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Vega KJ, Pina I, Krevsky B. Heart transplantation is associated with an increased risk for pancreatobiliary disease. Ann Intern Med 1996; 124(11):980-3.

Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood–leukaemia in Europe after Chernobyl: 5 year follow-up. Br J Cancer 1996;73:1006-12.

2. Organizacija kao autor

The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. Med J Aust 1996;164:282-4.

3. Članak bez poznatih autora

Cancer in South Africa (editorial). S Afr Med J 1994;84:15.

4. Volumen sa suplementom

Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.

5. Broj sa suplementom

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1 Suppl 2):89-97.

6. Volumen sa više delova

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem 1995;32(Pt 3):303-6.

7. Broj sa više delova

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. N Z Med J 1994;107(986 Pt 1):377-8.

8. Časopisi sa brojem bez volumena

Turan I, Wredmark T, Fellander-Tsai L. Arthroscopic ankle arthrodesis in rheumatoid arthritis. Clin Orthop 1995;(320):110-4.

9. Časopisi bez volumena i broja

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. Curr Opin Gen Surg 1993:325-33.

10. Reference u obliku apstrakta ili prethodnih saopštenja

Enzensberger W, Fischer PA. Metronome in Parkinson's disease (letter) Lancet 1996;347:1337.

Clement J, De Bock R. Hematological complica–tions of hantavirus nephropathy (HVN) (abstract). Kidney Int 1992; 42:1285.

Udžbenici i monografije

11. Monografija

Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

12. Autori kao urednici

Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

13. Organizacija kao autor i izdavač

Institute of Medicine (US). Looking at the future of the Medicaid program. Washington: The Institute; 1992.

14. Poglavlje u knjizi

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

15. Conference proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

16. Conference paper

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors.

MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-5.

17. Istraživački ili tehnički izveštaji

Službeni izveštaji (Issued by funding / sponsoring agency):

Smith P, Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX): Dept. of Health and Human Services (US), Office of Evaluation and Inspections; 1994 Oct. Report No.: HHSIGOEI69200860.

Sponzorisani izveštaji (Issued by performing agency)

Field MJ, Tranquada RE, Feasley JC, editors. Health services research: work force and educational issues. Washington: National Academy Press; 1995. Contract No.: AHCPR282942008. Sponsored by the Agency for Health Care Policy and Research.

18. Magistarske i doktorske disertacije

Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

Druge vrste publikovanog materijala

Neobjavljeni materijal

19. U štampi (In press)

Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med. In press 1996.

Elektronski zapisi

20. Internet članak u elektronskom formatu

Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar "cited 1996 Jun 5"; 1(1)(24 screens). Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm

21. Monografija u elektronskom formatu

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

22. Kompjuterski podaci

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

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