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Kontakt adresa: Časopis Acta Medica Medianae, Medicinski fakultet, Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: acta@medfak.ni.ac.rs
Tel+381-18-4533001 lok. 122 fax. +381-18-4534336
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CONTEMPORARY APPROACHES IN PREVENTION OF SUDDEN CARDIAC DEATH

Tomislav Kostić[1,2], Marina Deljanin-Ilić[2,3], Dragana Stanojević[1], Dragan Milić[4,5], Goran Koraćević[1,2], Svetlana Apostolović[1,2], Zoran Perišić[1,2], Sonja Šalinger-Martinović[1,2], Miodrag Djordjević[5,7], Mladjan Golubović[4,5], Milan Lazarević[4,6]

Sudden cardiac arrest and sudden cardiac death (SCD) are terms that are often used in medicine as synonyms. Sudden cardiac death is defined as "natural, unexpected cardiac death that occurs within one hour of the onset of acute symptoms and is accompanied by a sudden loss of consciousness." The presence of heart disease may be known from before, but the timing and manner of death are unexpected. Coronary artery disease (CAD) is responsible for 75-80% of all SCD. While atherosclerosis is a primary disease in middle-aged and elderly people, in children and young adults (< 35 years), malignant arrhythmias that occur in the cardiac ventricles - monomorphic or polymorphic ventricular tachycardia, and ventricular fibrillation are the most common cause of SCD. The etiology of heart rhythm disorders may be associated with underlying heart disease, but it is most commonly idiopathic in young people. A number of studies have confirmed that malignant arrhythmias are the immediate cause of death. The etiology of cardiac arrhythmias may be related to underlying heart disease, but it may also be idiopathic. Secondary prevention of sudden cardiac death involves the treatment of those who have been fortunate to survive sudden cardiac arrest or have documented hemodynamically unstable ventricular arrhythmias, and the primary prevention is the treatment of those who are at increased risk of sudden cardiac death but without documented prior cardiac arrest or malignant ventricular arrhythmias. Given that the most common arrhythmia preceding cardiac arrest is ventricular tachycardia (VT) that degenerates into ventricular fibrillation (VF), prevention of sudden cardiac death involves effective interruption of ventricular tachycardia (VT). Based on the results of studies and clinical data, it can be concluded that implantation of ICD significantly reduces mortality in both primary and secondary prevention compared to patients who received medication alone. Also, patients who have a pacemaker system in addition to defibrillator therapy and resynchronization therapy have significantly better quality of life, increased left ventricle ejection fraction, and better echocardiographic parameters. Also, the administration of drug therapy in patients with implanted pacemakers reduces the frequency of DC shock delivery, thereby preserving myocardial function and reducing the damage that occurs when current passes through the heart muscle. Implantable cardioverter defibrillators have brought a new chapter in the treatment of the very high risk cardiovascular patients.

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Key words: *sudden cardiac death, primary and secondary prevention, implantable cardioverter defibrillator*

[1]Clinic of Cardiovascular Diseases, Clinical Center Niš, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Department of Internal Medicine, Niš, Serbia
[3]Institute for Rehabilitation and Prevention of Cardiovascular Diseases "Niška Banja", Serbia
[4]Clinic of Cardiothoracic Surgery, Clinical Center Niš, Serbia
[5]University of Niš, Faculty of Medicine, Department of Surgery, Anesthesiology and Reanimatology
[6]University of Niš, Faculty of Medicine, Department of Microbiology and Immunology, Niš, Serbia
[7]Clinic of General Surgery, Clinical Center Niš, Niš, Serbia

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

Introduction

Sudden cardiac arrest and sudden cardiac death are terms that are very often used in medicine as synonyms. However, the definitions of these two terms are fundamentally different.

Sudden cardiac arrest is defined as "a sudden cessation of cardiac activity due to which the patient becomes unresponsive to external stimuli, normal respiratory function ceases and signs of circulation such as pulse activity are absent." If resuscitation measures are not taken quickly, this condition progresses to sudden cardiac death.

Sudden cardiac death is defined as "natural, unexpected death due to cardiac causes that occurs within one hour of the onset of acute symptoms and is accompanied by sudden loss of consciousness." The presence of heart disease may be known from before, but the time and manner of death are unexpected.

Unexplained sudden cardiac death that occurs in individuals aged > 1 year is known as "unexplained sudden cardiac death syndrome", while in individuals aged < 1 year this phenomenon is referred to as "sudden, unexplained death in childhood" (1).

Sudden cardiac death accompanied by negative pathological and toxicological findings is referred to as "sudden cardiac death syndrome due to arrhythmia".

The etiology of sudden cardiac death (SCD) can be:

- ischemic,
- nonischemic and
- non-cardiac.

Coronary heart disease (CHD) is responsible for 75–80% of all SCD cases. While atherosclerosis is the primary disorder in middle-aged and elderly people, children and young people (< 35 years), the cause of SCD is most often malignant arrhythmias that occur in the heart chambers - monomorphic or polymorphic ventricular tachycardia and ventricular fibrillation. The etiology of heart rhythm disorders may be related to the underlying heart disease, but in young people it is most often idiopathic. Also, in addition to malignant arrhythmias, the cause of SCD in young people can be anomalies of the coronary arteries, especially the anomalous origin of the left coronary artery from the pulmonary artery or from the right coronary sinus. In addition to the above causes, it is interesting to note the fact that recent studies have proven a link between air pollution (fine particles with an aerodynamic diameter < 2.5 μm and ozone) and outpatient cardiac arrest (1, 2).

Possible causes of SCD

◇ Myocardial infarction (including myocardial infarction without ST elevation)

◇ Coronary artery abnormalities

◇ Coronary spasm (especially in male smokers without previous KB or with minimal KB)

◇ Prolonged QT syndrome - can be inherited (usually caused by congenital ion channel defects) or acquired (caused by drugs that block the sodium channel known as hERG)

◇ Shortened QT interval syndrome

◇ Brugada syndrome

◇ Early repolarization syndrome

◇ Catecholamine polymorphic ventricular tachycardia

◇ Cardiomyopathies, heart failure, valvular disease, congenital heart defects

◇ Risk factors for SCD largely coincide with risk factors for coronary heart disease, which especially refers to:

- age,
- male gender,
- positive family history of coronary heart disease,
- elevated LDL cholesterol levels,
- hypertension,
- smoking and
- diabetes.

There are also studies that indicate specific risk factors such as:

- Increased heart rate - an independent risk factor for SCD regardless of whether there is heart disease or not. The basis of this connection is not completely clear, but a decreased parasympathetic activity is possible.• excessive alcohol consumption
- electrocardiographic changes in the ST segment and T wave (depression and inversion of the T wave) - may be a risk factor for SCD in patients in whom angina pectoris has not been verified. The corrected QT time > 420 msec in the longer period can be a predictor of SCD, while in the shorter period the predictors of SCD occurrence can manifest as a dispersion of the QT interval, as well as higher amplitudes in the change of T waves amplitudes.
- genetic and hereditary diseases (Brugada syndrome, QT anomaly, adrenergic polymorphic VT) - indicate the importance of family history and genetic testing in the prevention of SCD.
- condition after myocardial infarction - in high-risk patients after myocardial infarction according to various studies (EMIAT, CAMIAT, TRACE, SWORD, DIAMOND-MI) the cumulative incidence of arrhythmic mortality reaches 5% after one year and 9% at the end of the second year, while the incidence of nonarrhythmic cardiac deaths were 4% and 7%, respectively, in the same follow-up period. It is interesting that with the introduction of thrombolytic therapy, the relationship between arrhythmic and non-arrhythmic death did not change significantly.
- Left ventricle ejection fraction (LVEF) - is the most important prognostic parameter in the implementation of primary and secondary prevention of SCD. The value of LVEF ≤ 40% is the most important predictor of SCD. In cases where the LVEF is 10-15%, sudden death is not the most common and is then associated with bardiarrhythmias or electromechanical dissociation rather than ventricular tachyarrhythmias.
- clinically registered VT as well as patients who have already been successfully resuscitated (3, 4).

The joined mechanism of SCD developement, which may be caused by various etiological factors, includes marked activation of the sympathetic nervous system and massive release of catecholamines during the stress response. One of the effects of prolonged catecholamine increase involves the activation of α- and β-adrenoceptors. Activation of β-adrenoreceptors is thought to play a crucial role in the development of SCD. Activation of the stimulatory regulatory G protein (Gs protein - one of the five main components involved in the cyclic AMP (cAMP) signaling pathway) associated with β-adrenoreceptors leads to the activation of adenylyl

cyclase which catalyzes the formation of cAMP from ATP. The formation of cAMP leads to the activation of protein kinase A, which subsequently induces the phosphorylation of voltage-gated L-type calcium channels. Phosphorylation of these channels leads to a significant increase in the influx of Ca_2^+ into cardiomyocytes during cardiac action potential, which further triggers the release of Ca_2^+ from the sarcoplasmic reticulum via the rianodin receptor. This calcium alteration is known to induce ventricular arrhythmias that are often associated with SCD. In addition to the harmful cascade that affects β-adrenoreceptors, it is thought that α-adrenoreceptors may play a significant role in vascular remodeling, proliferation, and hypertrophy during stress responses. Stimulation of α-adrenoreceptors with increased concentration of catecholamines also causes negative effects on coronary arteries.

Prolonged exposure to high levels of circulating catecholamines results in coronary spasm causing myocardial ischemia, which may contribute to the occurrence of etiological factors leading to SCD. Ischemia associated with coronary spasm can also cause aortic pressure reduction and dilatation of arterio-venous shunts leading to multidimensional hypoxia. The detrimental effects of elevated catecholamine levels and their role in the pathogenesis of SCD are further potentiated when these molecules are susceptible to autooxidation. Catecholamine oxidation products lead to the generation of aminochromes that have been shown to have a detrimental effect on cardiomyocytes. The formation of these end products of catecholamine oxidation directly correlates with the increase of superoxide anions and subsequently formed hydrogen peroxide, which is known to cause DNA degradation, protein denaturation and lipid damage, thus increasing the risk of SCD at the molecular level. During the mentioned response to stress, an increased incidence of platelet aggregation and atherosclerotic plaque formation was also noted, which further increases the risk for SCD. Elevated levels of circulating catecholamines have been shown to increase platelet aggregation, resulting in an increased risk of developing coronary artery occlusion leading to myocardial infarction. These conclusions led to the formation of a unique theory for stress-induced SCD that brings together several different etiologies into a single molecular phenomenon (1, 4, 6).

It should also be noted that increased oxidative stress is often associated with vascular endothelial damage. It is known that the endothelium is intimately associated with the regulation of vascular tone, platelet activity, leukocyte adhesion and thrombosis. Damage to this inner lining of the vascular bed can cause serious consequences of various etiologies. In particular, a prolonged response to stress may result in excessive release of endothelin-1 from the endothelium, which may lead to an increased risk for SCD. Endothelin-1 is normally responsible for vasoconstriction and is thought to be in complex equilibrium with vasodilators such as nitrogen monoxide (NO). When this balance is disturbed by excess endothelin-1, patients often suffer from vasospasm, which leads to tissue ischemia and possible necrosis. This imbalance is further complicated by the detrimental effects of oxidative stress leading to endothelial damage and impaired NO release. This disorder may be directly related to the increased risk of SCD through the development of coronary artery disease (3-5).

Diagnosis and screening of patients at risk of SCD

Regarding the diagnosis or screening patients at increased risk for SCD, an ECG, as one of the basic, non-invasive diagnostic procedures, can be helpful. Since many diseases have, as an accompanying manifestation, the disturbance of the electrical conductivity of the heart, this diagnostic method can enable a quick insight into the existing abnormalities and its' progression. It has been noted that 95% of patients who have a high risk of SCD exhibit abnormalities on the ECG. However, ECG findings are very often not specific for SCD and require further diagnosis in order to confirm the existence of a risk factor.

ECG testing can provide insight into the etiology that potentially classifies a patient into a group of people at risk for developing SCD. Arrhythmogenic dyspalsa of the right ventricle is classically manifested by inverted T waves in the middle precordial leads, dilated or fragmented QRS complexes, as well as abnormal complexes on the S wave in the right leads. Patients with evidence of arrhythmogenic right ventricular dysplasia are further examined to assess the possible presence of comorbidities and the potential risk for SCD. In contrast to right ventricular arrhythmogenic dysplasia, congenital coronary artery disease does not normally present with any specific changes on the ECG. In addition, any arrhythmias that occur as a secondary manifestation of the underlying disease may provide information of the potential risk for SCD, but require further investigation to be of clinical significance.

Another important non-invasive imaging technique for detecting the risk of developing SCD in patients is echocardiography. The use of echocardiography may be particularly useful in the detection of valvular abnormalities, aortic root dilatation, left ventricular dysfunction, and left ventricular EF assessment. This diagnostic method is ideal for use in patients with hypertrophic and dilated cardiomyopathy, but cannot be used for the purpose of diagnosing conditions such as congenital coronary anomalies. In certain cases, echocardiography may be used to establish the suspicion of an abnormal origin of the coronary arteries, but it is usually often necessary to conduct further screening to obtain an accurate diagnosis. The parameters obtained on echocardiographic examination have low sensitivity in predicting the risk of developing SCD. The presence of scarring and heterogeneity of myocardial tissue have been shown to be directly related to the developement of cardiac arrhythmias, which is a direct cause of SCD.

Cardiac magnetic resonance imaging is also one of the non-invasive imaging techniques that quantifies scarring and is considered to have predictive significance in patients at risk for SCD. This diagnostic method enables the visualization of the myocardial scar using gadolinium amplification, which has been proven to have an extremely strong correlation with the histopathological finding. Magnetic resonance imaging is also characterized by high spatial resolution, which enables the differentiation of different scar patterns and the detection of interstitial fibrosis and edema. In addition, the topographic distribution of late gadolinium enhancement allows the differentiation of different types of cardiomyopathies, which is of great benefit in stratifying the risk of SCD in patients.

The application of invasive diagnostic procedures, such as cardiac catheterization, is of great importance for determining the etiological factor that caused the changes in the coronary blood vessels. The main benefits of cardiac catheterization include evaluating the presence of coronary artery disease, valvular disease, and/or aortic disease. This invasive procedure can also detect the presence of congenital anomalies of the coronary arteries, which can place the patient in a group at risk for developing of SCD. Cardiac catheterization is most often used when there is a clinical suspicion of coronary heart disease after non-invasive diagnostic procedures (1, 5).

Primary and secondary prevention of sudden cardiac death

Sudden cardiac death is one of the greatest health problems of modern man, and its prevention is one of the greatest challenges of modern cardiology. Sudden cardiac death is an extremely big problem in middle and highly developed countries, because its expression is often the first and last at the same time. Four methods are available for primary and secondary prevention of sudden cardiac death:

1. Drug therapy (antiarrhythmics)
2. Surgical methods to reduce left ventricular arrhythmic focus
3. Catheter ablation of arrhythmic focus
4. Implantation of an implantable cardioverter defibrillator (ICD)

Cardioverter defibrillator (ICD) implantation provides the best prevention of sudden cardiac death, both secondary and primary, and there is no alternative in any of the drugs known today, in selected high-risk patients. It is significant that a recent indication for its implantation is a primary prevention of sudden cardiac death. Of special significance nowadays is the development and use of resynchronization therapy and implantable cardioverter defibrillator in the treatment of heart failure (7).

A number of studies have confirmed that malignant arrhythmias are the immediate cause of death. The etiology of heart rhythm disorders may be related to the underlying heart disease, but it can also be idiopathic. Secondary prevention of sudden cardiac death involves treating those who are lucky enough to survive sudden cardiac arrest or have documented hemodynamically unstable ventricular arrhythmias, and primary prevention involves treating those who are at increased risk for sudden cardiac death but without documented previous cardiac arrest or malignant ventricular arrhythmias. Since the most common arrhythmia that precedes cardiac arrest is ventricular tachycardia (VT), which degenerates into ventricular fibrillation (VF), prevention of sudden cardiac death involves effective cessation of ventricular tachycardia (VT). Large multicenter studies in the 1990s demonstrated the ineffectiveness of antiarrhythmics, including amiodarone in the prevention of sudden cardiac death in those patients in whom VT/VF could not be prevented by treatment of underlying heart disease, and showed significantly better survival in patients treated with implantable CD cardioverter defibrillators (8, 9).

Ventricular fibrillation (VF) and ventricular tachycardia (VT) are the most common causes of sudden cardiac death in the first hours after AIM. They usually occur during the first hour or the first 24 hours. In the last 40 years, with the organization of coronary units, hospital mortality has decreased from 30% to 10-15%, primarily due to the prevention and treatment of arrhythmias and conduction disorders. Ventricular tachycardia may present as nonsustained (lasting 30s) or sustained VT (lasting > 30s). They can often be accompanied by syncope, which depends primarily on the hemodynamic situation in the AIM. Ventricular fibrillation (VF) is most common in the first hour after the infarction, and then the frequency decreases during the first 24-48 hours. Up to 80% of all VF in AIM occur in the first 4 hours. Epidemiological data show that the frequency of primary fibrillation is significantly reduced, most likely due to the correction of electrolyte disorders, due to therapeutic measures that reduce the size of the infarction, as well as due to the early use of B blockers. Unlike VF caused by myocardial ischemia, which is most common in the first hours, VF caused by major necrosis, severe heart failure, left ventricular aneurysm, and other severe AIM complications occurs later, after 48 h (secondary fibrillation) and has a poor prognosis (5).

Implantable cardioverter defibrillators (ICDs) are devices that are designed primarily to conduct therapy for treatment of heart rhythm disorders. In 1980, in cooperation with engineers, Mirowski began the realization of the project of creating an implantable device whose task was to recognize and stop heart rhythm disorders leading to sudden cardiac death. 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 such device was implanted by Prof. Dr. Milan Bane Djordjevic in 1986 at the Clinical Center of Serbia. Today, modern ICD devices are similar in appearance and function to standard bradycardia pacemakers. They use a lithium-vanadium battery due to the reliability of the energy source

and the need to transfer a larger amount of energy in a short period of time (10, 11).

Earlier defibrillators had epicardial patch electrodes, which made necessary to perform the operation with a thoracotomy approach, until today when the placement of the electrode is performed endovenously, practically the same as with standard antibradycardial pacemakers. Detection of heart rhythm disorders is a specific and basic function of the ICD. This detection is based on heart rate, frequency, but requires individual programming, practically for each patient. Detection criteria in ICD have evolved at least as much as therapeutic ones. Initially, the only detection criterion was the number of detected R-R intervals, and to date, detection algorithms have been perfected to prevent maldetection of VT, VF, false detection of VT/VF instead of atrial arrhythmias or sinus tachycardia. Different manufacturers offer different discriminatory criteria, but all have retained the R-R interval stability criterion. Another discriminatory criterion used by all companies is the analysis of the beginning of fast R-R intervals (onset) which avoids false detection of VT in the case of sinus tachycardia. Additional criteria have been introduced with the aim of even better discrimination of VT/VF, such as the criterion of QRS complex width, intrinsic QRS pattern (template) and automatic analysis of P-QRS ratio (PR logic). The greatest advancement in the technological sense was the introduction of gradual tiered therapy, which means that the detected VT is treated with the least aggressive therapy, antitachycardia burst stimulation of different duration of V-V stimulus. After a series of more aggressive ATP options, synchronous cardioversion with a lower current is applied, and finally defibrillation with a maximum current (30-40J). In the case of VF, the maximum strength of the DC shock is applied immediately, with the possibility of changing the polarity (15, 16).

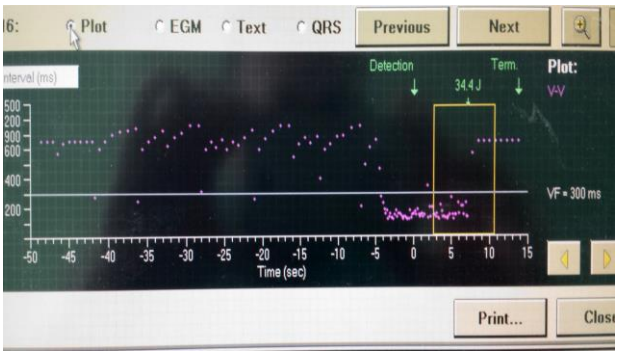

Figure 1. Intracardiac ECG of the ICD, with HF detection and successful therapy

Indications for implantation and therapy with implantable cardioverter defibrillator

Class I

- In patients who have survived cardiac arrest due to VF or haemodynamically threatening VT, and that it is possible to exclude causes resulting from reversible conditions (A)
- In patients with structural heart disease and spontaneous VT (B)
- In patients with obscure syncope with electrophysiologically induced VT or VF (B)
- In patients with EF < 35%, which is a consequence of AIM and from which at least 40 days have passed, and the patient is in functional NYHA class II or III (A)
- In patients with non-ischemic dilated cardiomyopathy and EF ≤ 35%, NYHA class II or III (B)
- In patients with non-repetitive VT after AIM, EF < 40%, and with the possibility of causing VF or VT in electrophysiological examination (B).

ICD installation can be applied:

Class IIa (C)

- In patients with repetitive VT with normal or approximately normal ventricular function
- In patients with hypertrophic cardiomyopathy, arrhythmogenic right ventricular dysplasia, sarcoidosis of the heart, Chagas' disease, in the syndrome of prolonged QT interval who had syncope or VT
- In patients with Brugada syndrome who had syncope or VT (11, 12).

Primary prevention in patients with structural heart disease and damaged left ventricle and/or symptoms of heart failure is currently one of the greatest challenges in cardiology. Primary prevention of sudden cardiac death refers to patients with structural myocardial disease and damaged left ventricle with decreased EF. Several studies have shown the benefit of an implanted ICD over drug therapy. Namely, with a decrease in EF below 35%, the frequency of malignant arrhythmias is not linear

but exponential, so that the occurrence of these life-threatening arrhythmias is significantly higher below this EF limit. The MADIT study showed a 54% reduction in overall mortality in patients with EF < 35% and implanted ICD due to ischemic heart disease. MUSTT study in patients with reduced EF < 35% showed that in the group of patients with ICD the reduction in mortality due to arrhythmias was 75% and the reduction in overall mortality by 60%. These two studies were the first for primary prevention in high-risk patients. The SCD-HEFT study was designed to show if amiodarone or ICD reduce overall mortality in patients with coronary heart disease or nonischemic cardiomyopathy classified as NYHA class II or III and an EF of less than 35%. Patients were randomized into 3 cohorts:

- 847 with placebo,
- 845 with amiodarone, and
- 829 with ICD.

The main conclusion of the study was that patients with congestive heart failure in class II and III, EF less than 35%, with good optimal drug therapy had mortality in the controlled placebo group of 7.2% per year for 5 years. A simple ICD, programmed only for a detection zone of 188/min, and therapeutically only for maximum DC shock reduces mortality by 23%. Amiodarone when taken for primary prevention does not increase survival and has the same effect on primary prevention as placebo. The MADIT II study examined the prophylactic benefit of ICD in patients with coronary heart disease, EF < 30%, who had at least one myocardial infarction. The study showed an absolute reduction in mortality in the group of patients with ICD compared to the group with conventional therapy.

Patients with ICD had a 31% reduction in mortality compared to the group of patients with conventional therapy. Both groups of patients had equivalent and necessary doses of B blockers, ACE inhibitors, diuretics, digitalis and aspirin. The findings of the MADIT II study showed that in patients with myocardial infarction and left ventricular dysfunction, prophylactic administration of ICD significantly increased survival. The inducibility of VT by NEPS, performed during ICD implantation, was associated with more frequent use of VT therapy and reduced use of VF therapy. In one of the recent studies, Moss et al. have shown that the cumulative probability of appropriate VT/VF ICD therapy was40% in the period of 4 years after implantation, which confirms the benefit of ICD in a longer follow-up period (11, 12).

Special groups of patients prone to developing malignant arrhythmias that particularly benefit from ICD implantation are those with: congenital heart disease, hypertrophic cardiomyopathy, Brugada syndrome, idiopathic VT/VF, left ventricular noncompaction, long and short QT interval syndromes, right ventricular arrhythmogenic dysplasia, infiltrative cardiomyopathies (13, 14).

Secondary prevention is indicated in patients who have survived cardiac arrest or sustained VT. Numerous randomized studies have shown that the use of ICD is associated with a reduction in mortality compared with patients treated with any type of drug therapy.

Since the middle of the last decade of the last century, many studies of secondary prevention of sudden cardiac death have been launched to compare the effects of antiarrhythmics, most commonly amiodarone, with ICD in patients with documented, haemodynamically unstable, recurrent ventricular arrhythmias in survivors. Secondary prevention of sudden cardiac death refers to the prevention in those groups of patients who had life-threatening arrhythmias (VT or VF). The most notable of these are the AVID, CIDS, and CASH studies. In each of the studies, ICDs showed a significant and undoubted improvement in survival compared with antiarrhythmics, from 20% in the CIDS study, more than 38% and 39% in the CASH and AVID study to as much as 73% in the Dutch DUTCH CES study. The AVID study was one of the most significant studies of secondary prevention of sudden cardiac death. The study included 1,016 patients and the results showed that the reduction in mortality in the group of patients with ICD compared to drug therapy was 38% in the first year after implantation compared with the group treated with amiodarone or sotalol. In the second year of follow-up, a reduction in mortality of 26% was found, and in the third 30% in relation to patients treated with antiarrhythmics. After this study, for the first time, it was accepted that ICD significantly increased survival in patients with malignant ventricular arrhythmias, and the attitude about ICD implantation, only if antiarrhythmics proved ineffective, was changed. The CIDS and CASH studies also showed a significant reduction in mortality compared to drug therapy (7, 9, 11).

Conclusion

Based on the results of studies and data from clinical practice, it can be concluded that ICD implantation significantly reduces mortality in both primary and secondary prevention compared to patients who received only drug therapy. Namely, patients with implanted resynchronization therapy pacemaker system, in addition to a defibrillator, have significantly better quality of life, increased LVEF, as well as echocardiographic parameters. Also, the use of drug therapy in patients with implanted pacemakers reduces the frequency of DC shock delivery, which preserves myocardial function and reduces the damage that occurs when current passes through the heart muscle. Implantable cardioverter defibrillators have opened a new chapter in the treatment of the cardiovascular patients at the highest risk.

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SAVREMENA PREVENCIJA NAPRASNE SRČANE SMRTI

Tomislav Kostić[1,2], Marina Deljanin-Ilić[2,3], Dragana Stanojević[1], Dragan Milić[4,5], Goran Koraćević[1,2], Svetlana Apostolović[1,2], Zoran Perišić[1,2], Sonja Šalinger-Martinović[1,2], Miodrag Đorđević[5,7], Mlađan Golubović[4,5], Milan Lazarević[4,6]

[1]Klinika za kardiovaskularne bolesti, Klinički centar Niš, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Katedra za internu medicinu, Niš, Srbija
[3]Institut za rehabilitaciju i prevenciju kardiovaskularnih bolesti „Niška Banja", Niška Banja, Srbija
[4]Klinika za kardiohirurgiju, Klinički centar Niš, Niš, Srbija
[5]Univerzitet u Nišu, Medicinski fakultet, Katedra Hirurgija sa anesteziologijom i reanimatologijom, Niš, Srbija
[6]Univerzitet u Nišu, Medicinski fakultet, Katedra Mikrobiologija sa imunologijom, Niš, Srbija
[7]Klinika za hirurgiju, Klinički centar Niš, Niš, Srbija

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

Naprasni srčani zastoj i naprasna srčana smrt su termini, koji se u medicini vrlo često koriste kao sinonimi. Naprasna srčana smrt (NSS) definiše se kao "prirodna, neočekivana smrt usled srčanih uzroka, koja nastaje unutar jednog sata od početka akutnih simptoma i praćena je naglim gubitkom svesti". Prisustvo srčanog oboljenja može biti poznato od ranije, ali su vreme i način smrti neočekivani. Koronarna bolest (KB) odgovorna je za 75% – 80% svih slučajeva NSS. Dok je ateroskleroza primarni poremećaj kod ljudi srednje i starije životne dobi, kod dece i mladih osoba (< 35 godina) uzrok NSS najčešće predstavljaju maligne aritmije, koje se javljaju u srčanim komorama – monomorfna ili polimorfna komorska tahikardija i ventrikularna fibrilacija. Etiologija poremećaja srčanog ritma može biti povezana sa osnovnim srčanim oboljenjem, ali je kod mladih osoba najčešće idiopatska. Veći broj studija potvrdio je to da su maligne aritmije neposredni uzrok smrti. Etiologija poremećaja srčanog ritma može biti vezana za osnovno srčano oboljenje, ali može biti i idiopatska. Sekundarna prevencija naprasne srčane smrti podrazumeva lečenje onih koji su imali sreće da prežive iznenadni srčani zastoj ili imaju dokumentovane hemodinamski nestabilne komorske aritmije, a primarna prevencija podrazumeva lečenje onih koji imaju povišen rizik od nastanka naprasne srčane smrti, ali bez dokumentovanog prethodnog srčanog zastoja ili malignih komorskih aritmija. S obzirom na to da je najčešća aritmija, koja prethodi srčanom zastoju, komorska tahikardija (VT), koja degeneriše u komorsku fibrilaciju (VF), prevencija naprasne srčane smrti podrazumeva efikasno prekidanje komorske tahikardije (VT). Na osnovu rezultata studija i podataka iz kliničke prakse, može se zaključiti da implantacija implantabilnog kardioverter defibrilatora (ICD) značajno smanjuje smrtnost i u primarnoj i u sekundarnoj prevenciji, u odnosu na bolesnike koji su primenjivali samo medikamentnu terapiju. Sem toga, bolesnici kod kojih je ugradnjom pejsmejker sistema, pored defibrilatorske ostvarena i resinhronizaciona terapija, imaju značajno bolji kvalitet života, povećanje EF, kao i ehokardiografske parametre. Takođe, primena medikamentozne terapije kod bolesnika sa ugrađenim pejsmejkerom smanjuje učestalost isporučivanja DC šoka čime se postiže očuvanje miokardne funkcije i smanjuju se oštećenja koja nastaju prilikom prolaska struje kroz srčani mišić. Implantabilni kardioverter defibrilatori doneli su novo poglavlje u lečenju najrizičnijih kardiovaskularnih bolesnika.

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Ključne reči: *naprasna srčana smrt, primarna i sekundarna prevencija, implantabilni kardioverter defibrilator*

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THE TERMINOLOGY USED TO DESCRIBE TEAMWORK IN THE HEALTH CARE SYSTEM: A LITERATURE REVIEW

Stefan Jovanović[1,2], Maja Stanković[2], Tatjana Kilibarda[3], Sanja Trgovčević[3], Sunčica Ivanović[3]

Teamwork in the healthcare system is a necessity because of the complexity of health problems and the emancipation of users. Teamwork characterized by working together, goal, purpose, ethics, problem-solving ideas, good communication, shared responsibility and synergy is a gold standard in the healthcare of patients where each member has specific task and function while respecting the others in the team.

The aims were to describe characteristics and benefits of teamwork in healthcare, to describe the types of teams in health care, and to identify the main limiting factors for a team's success in healthcare.

The effects of teamwork in healthcare are: reducing the length of stay in hospital, reducing costs of healthcare, increasing the satisfaction of users and care givers, improving the quality of health services, efficient use of resources in healthcare, improvement the quality of communication. Unlike the traditional disease focused approach, nowadays, the teams are focused on holistic approach to patient and health-disease phenomenon.

When performing complex tasks that involve quick decision-making, rapid interventions and quick solutions, especially in emergencies in healthcare and in treatment of chronic disease, teamwork is invaluable. The main limiting factors for the success of the team in healthcare are: lack of staff in healthcare, insufficiency in competency of staff, limited financial resources, inability of team members to work in team (lack of experience and education), lack of leadership in the healthcare institution for encourage and promote teamwork.

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Key words: *nursing, team in nursing health care, teamwork, health care system*

[1]Clinical Center of Serbia, Clinic for Burns, Plastic and Reconstructive Surgery, Belgrade, Serbia
[2]Faculty of Medicine Novi Sad, postgraduate student, Novi Sad, Serbia
[3]Academy of Educational and Medical Vocational Studies Kruševac, Department Ćuprija, Ćuprija, Serbia

Contact: Sunčica Ivanović
25. May 69, 18000 Niš, Serbia
E-mail: suncica.ivanovic@yahoo.com

Introduction

In recent years, the emphasis has been placed on the importance of team and team work, as it achieves much better results than those achieved through individual work. A team is a group of people who have common goals that are solved by mutual cooperation. It is not easy to form and maintain a team because team consists of members who have different professions and characters, but it is of great importance that adequate communication exists within the team as well as striving for a common goal (1, 2).

Teamwork in the healthcare system is a necessity because the health care giver as an individual is not in a possibility to meet all of health care user's demands. Numerous studies have proven that effective healthcare teams reduce the length of hospitalization and healthcare costs and increase custom and team member satisfaction.

The two most common terms used in the description of healthcare teams are the multidisciplinary and the interdisciplinary team (3).

A multidisciplinary team consists of individuals who have same or different medical professions who strive to achieve a common goal. Members of an interdisciplinary team have same or different profile and work continuously together, as opposed to a multidisciplinary team where individual members can be involved if needed. A common characteristic of both teams is the provision of the highest quality of patient care that is actually the highest possible quality of the provided health services.

Although teamwork is not a novelty in many sectors, including the healthcare system, there is still a need to explain the purpose of its existence as well as the need for further research.

Defining a team as a term

A team is defined as a series of interpersonal interactions that lead to the achievement of a set goal, and teamwork represents the join efforts of two or more members, as well as the individual achievements of each individual member, in order to solve tasks. The name "Team" is described as: T (together), E (everyone), A (achieve), M (more). A very important characteristic of a team is its success, which should be greater than the work of each member individually. Teams, according to their achievements, are divided into: unsuccessful, average and successful teams in their job. Classical teams use only a part of knowledge and expertise, while synergistic teams use the knowledge and abilities of all members, and they are known as supersumed teams (1, 4).

Before forming a team, it is very important to determine its purpose and tasks. It is important to know that any team has a life cycle that begins with the formation and construction of a team, and continuous with work which aim is to achieve greater team success. The team is made up of people of different beliefs, attitudes, characters, opinions, so it is generally difficult to maintain a balance in teamwork, especially in emergencies (2, 5).

When forming a team, it is very important to answer some questions, such us:

- Will the team be permanent or temporary?
- What is expected of the team?
- How will the team's success be measured?
- What pressures will the team be exposed to?

The team consists of a small number of people with specific skills who are committed to the common approach and realization of goals for which they are responsible. The biggest challenges in forming and operating a team are limited resources and rapid change. Organizing teams and teamwork involves fundamental changes in the way of work and manage (3).

Defining teams in the healthcare system

The healthcare team is defined as a set of many different healthcare professions characterized by teamwork, purpose, ethics and shared responsibility and synergy, and each member is expected to be maximally engaged in accordance with his/her competencies. Due to complexity of the challenges in medicine, and in healthcare in general, teamwork is a necessary factor in organizing quality professional work (6, 7).

Teamwork is the gold standard in the care of patient where each member has a specific task while respecting the tasks and roles of the other members. Teamwork is of utmost importance for the quality of health services, the functioning of the health care system and the organization of health care (9). Some of the benefits of teamwork in healthcare are:

- Reduction of costs
- Shorter length of hospitalization
- Efficient use of health resources
- Better communication
- Better quality of health services

Health care teams are in constant function to meet the needs and complex and multiple health problems of their users. A professional and coordinated team made up of healthcare professionals of different profiles enables healthcare delivery with high quality (8).

The model of teamwork in healthcare was created in 1920 in Great Britain. Unlike the traditional, disease-focused approach, today focus of team is a holistic approach to the patient and the health-disease phenomenon (9, 10). However, teamwork is still largely based on the traditional model that the patient is a passive recipient of health care services, and a team member is an active participant, while to a lesser but positive trend there is an approach to team work that respects the patient's active participation in the healing process (11).

In healthcare, teamwork has been represented since ancient times, as a multidisciplinary team, an interdisciplinary team, and a transdisciplinary team based on the interchange of roles of members within the team. The multidisciplinary team is based on different disciplines and the division of work task accordingly, and the interprofessional team is based on the mutual, collaborative cooperation of all members (12).

Today, healthcare teams are faced with comorbidities and chronic illnesses almost daily, so comprehensive health assessment and healthcare of such patients is required, which requires the collaboration of different professions (13).

The aims

- To describe characteristics and benefits of teamwork in healthcare,
- To describe the types of teams in health care, and
- To identify the main limiting factors for a team's success in healthcare.

Results and discussion

Teamwork in the healthcare system

The team represents a certain form of formal organization of joint work of a smaller or larger number of people, with common tasks and goals, plans, motives and interests. The achievement of one team member directly or indirectly affects everyone else and improves the final results of all work. The key differences between the team and the group are in the organization and some of the differences are shown in Table 1.

Teamwork has its advantages and disadvantages. Some of the benefits are: greater team

member motivation, better performance, more effective decision-making, a willingness to cooperate, enhancing each member's competences and work experiences. Some of the disadvantages are: susceptibility to stress, chronic frustration, or tiredness with teamwork (4).

Table 1. Differences between the team and the group by specific characteristics

Group	Characteristic	Team
Individual	← *Responsibility* →	Individual and group
Low	← *Motivation* →	High
Individual	← *Aims* →	Common
Voting	← *Decision making* →	Consensus
Exist	← *Hierarchy* →	Minimal
Inconsistent	← *Skills* →	Consistent
Little or no	← *Autonomy* →	High degree
Irresistible	← *Organizational changes* →	Resistant

Each is basically a working group, and it grows into a team when its members are well organized and united to carry out their tasks. Teamwork is a form of coordinated activity based on the collaboration and competence of different experts, and its effectiveness is reflected in the evaluation of all that has been achieved, as well as the satisfaction of team members (5). According to WHO, teamwork involves the action of more professionals towards a common goal. The ideal number of members is 3-12 people (6).

Teamwork in healthcare enables coping with complex, risky situations and problems, reducing the use of time, money and other resources, while increasing efficiency. Teamwork is a means by which average people achieve above-average results (7), and a new, modern and flexible way of working for people who need to solve a particular complex problem based on their own judgment, or to suggest the most appropriate way to solve certain issues and problems. Individuals, namely members must constantly be affirmed and motivated in order to maintain mutual trust (3). However, the main limiting factors for team success in health care are: lack of staff, insufficiently competent staff, limited financial resources, inability of team members to work in a team (lack of experience and education), lack of leadership in a healthcare facility to encourage teamwork.

Effectiveness of teamwork in the healthcare system

In addition to professional knowledge, it is important that the team members have the ability to listen to each other and to appreciate others' opinions, namely, the team must have good communication skills such as conflict resolution, counseling, assertiveness, etc. It is proven that a well-functioning team reports only a few professional mistakes and stress. A multidisciplinary team, such as health care teams, most often must possess the aforementioned abilities and skills, because they consist of members of different professions, in order to maximize results and increase patient satisfaction with the services provided (6). Communication in medicine and in healthcare in general is the basis for successful collaboration between patients and their families and between healthcare professionals. According to modern understanding, the four most important components of good clinical practice are, in addition to knowledge and physical examination, communication skills and problem-solving abilities. Any healthcare provider, regardless of position and specialty, is an indispensable part of a whole that can only be maximized through joint collaboration, and numerous studies have shown that collegiality between team members contributes to a positive treatment outcome (8).

Effective healthcare teams are more flexible, take more risks, have new ideas, and make decisions faster. According to Belbin, it is necessary that different, well-coordinated and compatible members exist in order to make team which is successful, because if all team members have similar behavioral styles or similar roles, the team becomes imbalanced. For the proper functioning of the team, it is very important that communication is regular, open and honest and that information is accessible to all team members (7).

Dilemmas of naming the teams in the healthcare system

The four most common terms used to describe health care teams are: multidisciplinary, interdisciplinary, multiprofessional and interprofessional (14). The prefix "multi" implies the diversity of the employees that make up the team, while "inter" denotes joint and mutual tasks, and interprofessional and interdisciplinary teams are teams with formal structure (15). According to the way they func-

tion, health teams are interdisciplinary or multidisciplinary teams (7). Although these terms are most represented in the literature, they are not always clearly defined. Multidisciplinary healthcare teams are the most represented, especially when it comes to chronic diseases, and interprofessional teams differ from multidisciplinary teams in that the main team member is the patient.

The development of healthcare teams was initially non-interdisciplinary, i.e., the team consisted of, for example, nurses working in the same department (16). Although there are different names for describing healthcare teams, none of them should affect communication within the team or quality of team work (17).

The term "interprofessional" refers to the education of health professionals from different disciplines, and the term "multidisciplinary team" most commonly describes healthcare teams composed of different disciplines (10). In describing teams on the basis of their structure or cooperation, some authors use the name multiprofessional, but use the name interdisciplinary when referring to the interventions performed by the team, while the term interprofessional means both. In describing healthcare teams, there is no one universal name which can cause some problems or ambiguities. Haggerty et al. in 2007, in his study defined a multidisciplinary team as a joint collaboration of professionals from different medical disciplines while providing healthcare (18).

In some studies, the term "interprofessional team" is defined as work between two or more clinical professions, while the term "interdisciplinary team" refers to the work of physicians of different specialties, all with the aim of improving mutual cooperation and the quality of services provided (19).

Numerous studies consider that the healthcare teams consist not only of health professionals of different profiles, but also non-medical staff such as administrative staff and others, while Mills et al. in 2010, in their study included staff in management position in healthcare facilities from other cities or countries as members of the healthcare teams (20).

A multidisciplinary and interdisciplinary team

The multidisciplinary team is made up of members of different medical profiles who, through their work and effort, contribute to the improvement and promotion of health, while the interdisciplinary team consists of health professionals who, through mutual cooperation, decide on the well-being of patients. Teamwork has positive effects because it contributes to improved results, efficiency and safety (21).

The term "team" is universally used in all working groups of health and social care. Despite the development of teams, interdisciplinary teamwork is not clear enough for practitioners. In order to achieve efficiency and reliability in an interdisciplinary team, it is essential that all members have specific knowledge, skills and attitudes in order to monitor each other's performances and maintain a positive climate and collaboration within the team (22).

An interdisciplinary team consists of members of a particular specialty, support staff, and administrative staff who work together toward a common goal. The Australian study promoted interdisciplinary collaboration as a priority in health care delivery with mandatory feedback. Evaluation of teamwork can improve team cohesiveness, team focus, as well as professional identity (20). Clinical knowledge and skills of interdisciplinary healthcare teams have been improved in recent years. There is also a need for increasing research on interdisciplinary teams, specifically their achievements. According to some studies, the interdisciplinary team is also called the participatory team (23, 24).

A holistic approach to the patient and the principle of working together towards the set goal is a characteristic of interdisciplinary teamwork. According to some authors, the interdisciplinary teamwork is considered to be highly effective, which contributes to socioeconomic advantage in the healthcare system. According to the literature, members from interdisciplinary teams show higher levels of job satisfaction (9).

Interprofessional and multiprofessional team

Interprofessional teams are characterized by teamwork and knowledge sharing due to the diversity of the professions that make them, but the multiprofessional team is determined by the structure of the team (10). Although there are numerous dilemmas when describing healthcare teams, they are still a key base of clinical practice. According to some definitions, interprofessional work involves interaction within team members, while the work of multiprofessional and multidisciplinary teams is based on the presence of different professions, but does not imply mandatory mutual communication of all members, which is one of the main problems for effective team work. Interprofessional work involves the mutual learning of collaboration and dialogue. However, there is insufficient data about terminology used to describe healthcare teams.

In their studies, some authors refer to healthcare teams as multifunctional teams, meaning all of the above terms. The team members are individuals with different skills, experiences and traits, and because of that conflict can easily occur, so the operation of such team in practice is a challenge. Especially such are the interprofessional teams. The work of the interprofessional team is based on interprofessional education, which involves the mutual exchange of knowledge and experience between different professions in order to achieve high quality health care. An interprofessional team is defined as an association of multiple health professions, working together to achieve greater quality in problem solving (21). Failure to follow the principles of teamwork can be the cause of many problems. Some members of the team may be in the shadow of other members, and according to some studies, it has been noted that nurses have been most often denied to make some suggestions and opinions even though they are an equal and obligatory member of the health team (22). Unlike the interprofessional and multiprofessional approach, the multidisciplinary

approach to patient care has proven to be the most appropriate when it comes to chronic diseases (23, 24).

Multiprofessional teams consist of members with different or the same professions that work together to take care of the patient's condition and meet his or her needs. The multiprofessional team can be changed in order to meet the demands of patients. The approach of the multiprofessional team has shown high-level results in palliative care (25).

According to some studies, there are also teams that collaborate remotely, using modern technologies whose progress is also being noticed in the healthcare system. These are the so-called virtual teams and they are working in various branches of telemedicine, especially when it comes to data sharing. A virtual team is a group of individuals who share common goals, and who operate using communication technologies regardless of time and space (26).

Ambiguities in the terminology of healthcare teams

Standardized definitions of healthcare teams are not reliable when it comes to understanding and using different terms on a daily basis. The consistency of applying a standardized term in clinical practice, policy, education can enhance understanding, collaboration and communication between sectors (10). According to the latest research, the most important factors of any healthcare team is the communication and holding meetings with the aim of solving the current problem, as well as adequate education of its members (27).

When describing teams in healthcare, different terms are used that relate to the place, job description, etc. The most represented descriptors are interdisciplinary and interprofessional, as well as multidisciplinary and multiprofessional teams, however, due to the inconsistency of using only one fixed term and numerous ambiguities, further research on this topic is needed (10).

Some authors find that difficulties in defining and understanding the context of teams are due to the use of different terms. For these reasons, there is a need to adopt a general term to describe healthcare teams that will maintain consistency in education and clinical practice. A characteristic of a professional team is education of its members, but, according to some studies, there is a small number of theoretical education programs and practical training in teamwork (9).

Conclusion

When performing complex tasks involving quick decision-making, rapid intervention, and rapid finding of solutions, especially in emergencies and treatment of chronic diseases, teamwork is invaluable.

Different terms are used in describing healthcare teams, and the most represented are: multidisciplinary and interdisciplinary team. Also, it is necessary to reduce the need to describe certain types of health teams by job titles or job descriptions, but it is desirable to define a standardized name that will be understandable to the general population, because the essence of teamwork is important.

There are certain ambiguities regarding the use of these terms, but the essence of all teams is cooperation, honesty, good communication, motivation and realization of goals that have been set.

What healthcare professionals lack are experience in working in a team and teamwork training. The main limiting factors for the success of the team in health care are: lack of staff, insufficiently competent staff, limited financial resources, inability of team members to work with the team (lack of experience and education), lack of leadership in the healthcare institutions to encourage and promote team work.

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TERMINOLOGIJA KOJA SE KORISTI ZA OPISIVANJE TIMSKOG RADA U ZDRAVSTVENOM SISTEMU

Stefan Jovanović[1,2], Maja Stanković[2], Tatjana Kilibarda[3], Sanja Trgovčević[3], Sunčica Ivanović[3]

[1]Klinički centar Srbije, Klinika za opekotine, plastičnu i rekonstrukovnu hirurgiju, Beograd, Srbija
[2]Medicinski fakultet Novi Sad, student postdiplomskih studija, Novi Sad, Srbija
[3]Akademija vaspitačko-medicinskih strukovnih studija Kruševac, Odsek Ćuprija, Ćuprija, Srbija

Kontakt: Sunčica Ivanović
25. maja 69, 18000 Niš, Srbija
E-mail: suncica.ivanovic@yahoo.com

Timski rad u zdravstvenom sistemu predstavlja neophodnost zbog kompleksnosti zdravstvenih problema i emancipacije korisnika. Timski rad je zlatni standard u zbrinjavanju bolesnika, gde svaki član ima svoju određenu ulogu, uz uvažavanje uloga drugih članova i koji karakterišu zajednički rad i cilj, zajedničke svrha, etika i razmena ideja usmerena ka rešavanju problema, dobra komunikacija, zajednička odgovornost i sinergija.

Ciljevi rada bili su opisati karakteristike i prednosti timskog rada u zdravstvenoj zaštiti, opisati vrste timova u zdravstvenoj zaštiti i prepoznati osnovne limitirajuće faktore za uspeh tima u zdravstvenoj zaštiti. Odabrana metoda rada bila je pregled literature.

Efekti timskog rada u zdravstvu su: smanjenje dužine hospitalizacije, smanjenje troškova i povećanje zadovoljstva korisnika i članova tima, unapređenje kvaliteta zdravstvenih usluga, efikasno korišćenje zdravstvenih resursa i bolja komunikacija. Za razliku od tradicionalnog pristupa, koji je bio usmeren isključivo na bolest, danas se timovi fokusiraju na holistički pristup bolesniku i fenomenu zdravlje–bolest.

Pri obavljanju složenih zadataka, koji podrazumevaju brzo donošenje odluka, brzu intervenciju i brzo pronalaženje rešenja, pogotovo u hitnim situacijama i tretmanu hroničnih bolesti, timski rad je od neprocenjivog značaja. Osnovni limitirajući faktori za uspeh tima u zdravstvenoj zaštiti su: nedostatak kadra, nedovoljno kompetentan kadar, ograničeni finansijski resursi, nekompetentnost članova tima za rad u timu (nedostatak iskustva i edukacije), nedostatak vođstva u zdravstvenoj ustanovi, nedostatak ohrabrivanja i promocije timskog rada.

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Ključne reči: *zdravstvena nega, zdravstveni tim, timski rad, zdravstveni sistem*

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IS ULNAR NERVE DECOMPRESSION AND ANTERIOR TRANSPOSITION EFFECTIVE ON ELBOW RANGE OF MOTION IN THE TREATMENT OF CUBITAL TUNNEL SYNDROME?

Özgür Korkmaz[1], Yildiray Genç[2]

The aim of this study was to evaluate the clinical results and elbow range of motion in patients with cubital tunnel syndrome who underwent decompression and anterior ulnar nerve transposition.

There were 11 patients, 7 male and 4 female. Mean age of the patients was 45.45 ± 16.22 years. The mean follow-up period of the patients was 14.81 ± 6.98 months. Decompression and anterior transposition of the ulnar nerve were performed in all patients. Patients were classified according to McGowan-Goldberg classification system before surgery and in the last control after surgery. VAS scores, flexion and extension range of motion were evaluated before surgery and in the last control after surgery.

Eight patients had stage 2A according to McGowan-Goldberg classification system and 3 patients had stage 2B preoperatively. Postoperatively, 10 patients were stage 0 and one patient was stage 1. Preoperative mean VAS score was 8.45 ± 0.93 and postoperative mean VAS score was 1.45 ± 2.29. Preoperative mean elbow flexion-extension range of motion was 118.64° ± 11.42° and postoperative mean elbow flexion-extension range of motion was 128.63° ± 7.77°. We found statistically significant difference between preoperative and postoperative VAS values and elbow flexion extension range of motion.

Anterior subcutaneous ulnar nerve transposition is an effective surgical treatment method with increasing elbow range of motion in patients with cubital tunnel syndrome.

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Key words: *cubital tunel, ulnar nerve, decompression*

[1]Orthopaedic and Traumatology Department, Bahçeşehir University Medical School, VM Medicalpark Pendik Hospital, İstanbul, Turkey
[2]Orthopaedic and Traumatology Department, İstanbul Cerrahi Hospital, İstanbul, Turkey

Contact: Özgür Korkmaz
Fevzi Çakmak Mah. D100 Cemal Gürsel caddesi no: 9, 34899 İstanbul, Turkey
E-mail: ozkorkmaz00@yahoo.com

Introduction

Cubital tunnel syndrome is the second mostly seen compression neuropathy of the upper extremity after carpal tunnel syndrome (1). There are dynamic and static factors in the etiology. Static factors include osteoarthrosis of the elbow, cubitis valgus, ganglion cysts around the nerve, trauma and the masses causing compression. Increased pressure on the cubital tunnel with elbow flexion, recurrent minor micro-traumas and subluxation of the ulnar nerve constitute dynamic factors (2-4).

The ulnar nerve can be compressed at 5 anatomical points around the elbow. These are arcade of Struthers, the medial intermuscular septum, the epicondylar groove, the Osborne ligament, and flexor pronator aponeurosis. The most common compression is seen in the Osborne ligament and epicondylar groove (5).

The loss of sensation and numbness in the sensory area of the ulnar nerve, loss of grip strength and clawing of the fingers are symptom of cubital tunnel syndrome. Ulnar nerve dysfunction is divided into 3 phases by McGowan.

Stage 1: sensory changes,
Stage 2: reduction in muscle strength,
Stage 3: paralysis and muscle atrophy (6).

Surgical treatment procedures are applied if conservative treatment does not make any improvement in clinical signs and symptoms. Surgical approaches are divided into two groups as superficial and deep. In situ decompression and anterior subcutaneous transposition of the ulnar nerve are superficial approaches, medial epicondylectomy or anterior sub or intramuscular transposition are deep surgical approaches (7-9).

The aim

The aim of this study was to evaluate the clinical results and elbow range of motion in patients who underwent decompression and anterior ulnar nerve transposition without any response to conservative treatment.

Materials and methods

This retrospective study was approved by the Ethics Board of our institution (approval number 2019-17/01) and conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all patients included in the study.

In this study, 11 patients who were diagnosed with cubital tunnel syndrome and confirmed with electromyography (EMG) were included in the study. There were 7 male and 4 female patients. Decompression and anterior transposition of the ulnar nerve were performed in all patients.

The inclusion criteria comprised patients between 18-75 years of age, who had no neurological disease and did not respond to 6-week conservative treatment. Patients with cervical pathologies were excluded from the study. Patients under 18 years of age and over 75 years of age and patients who had undergone surgical treatment for cubital tunnel syndrome were excluded from the study.

All patients had positive Tinel's sign and elbow flexion tests with complaints of cubital tunnel syndrome. The diagnosis of cubital tunnel syndrome was confirmed by EMG and nerve conduction velocity. All patients with cubital tunnel syndrome were included in a 6-week physical therapy program. Patients who did not benefit from physical therapy and rehabilitation program were treated surgically.

Patients were classified according to McGowan-Goldberg classification system before surgery and in the last control after surgery (6, 10). VAS scores, flexion and extension range of motion were evaluated before surgery and in the last control after surgery.

Surgical technique

All surgeries were performed under general anesthesia and no tourniquet was used. The patients underwent surgery in the supine position. Incision was about 7-8 cm and started approximately 2 cm proximal to the medial epicondyle to the medial side of the flexor carpi ulnaris parallel to the ulnar nerve trace. Release and decompression were performed for all soft tissues which left the ulnar nerve under pressure in the cubital tunnel. Release of the ulnar nerve was performed from the distal side to the proximal side (Figure 1). The ulnar nerve was transposed into the subcutaneous area and the subcutaneous tissue was sutured into the deep fascia to cover the ulnar nerve. A new channel was formed for the ulnar nerve. After surgery, the operated extremities were not immobilized and were immediately allowed to range of motion.

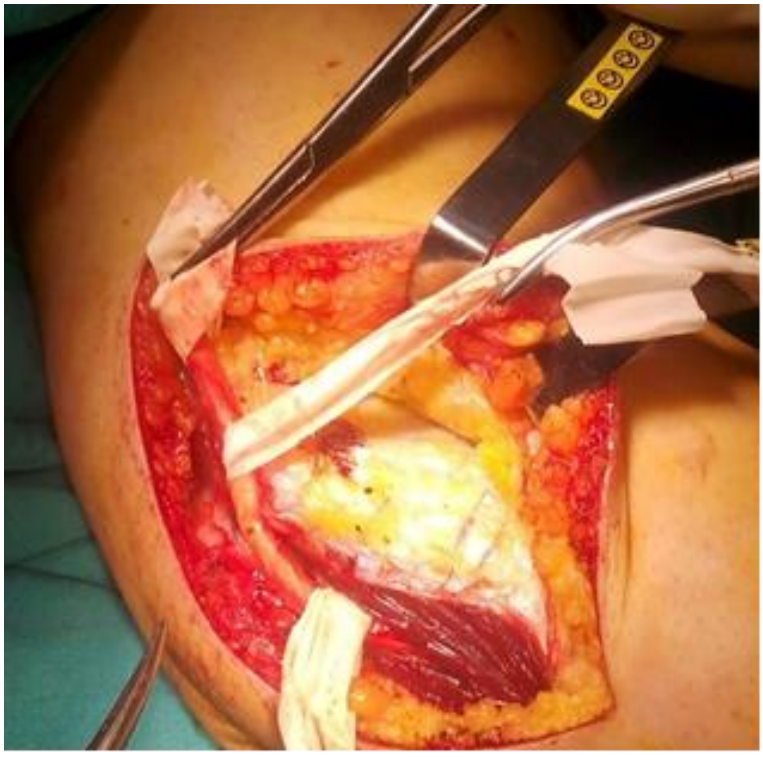

Figure 1. Release and decompression of the ulnar nerve from the distal side to the proximal side

Statistical analysis

The normal distribution of the data was tested and the Wilcoxon test was used as the non-parametric version of the matched test since they were not distributed normally. A value of $p < 0.05$ was considered statistically significant in the 95% confidence interval.

Results

Of 11 patients, in 8 patients surgery was performed on the right side and in 3 patients on the left side. When the etiologies of 11 patients were evaluated, it was found that all of the patients did not have cubital tunnel syndrome secondary to trauma and all were idiopathic. Mean age of the patients was 45.45 ± 16.22 years. The mean follow-up period of the patients was 14.81 ± 6.98 months. Out of 11 patients, 10 patients reported that they were satisfied after surgery. Eight patients had stage 2A according to McGowan-Goldberg classification system and 3 patients had stage 2B preoperatively. Postoperatively, 10 patients were stage 0 and one patient was stage 1 (Table 1). Preoperative mean VAS score was 8.45 ± 0.93 and mean postoperative VAS score was 1.45 ± 2.29. Preoperative mean elbow flexion extension range of motion was 118.64° ± 11.42° and postoperative mean elbow flexion extension range of motion was 128.63° ± 7.77° (Table 2). We found statistically significant difference between preoperative and postoperative VAS values and elbow flexion extension range of motion ($p \leq 0.05$). No infection was detected related to surgery during the follow up period. We did not see any wound problems like hematoma due to bleeding problems.

McGowan-Goldberg classification system	Stage 2A	Stage 2B	Stage 1	Stage 0
Preoperative	8	3	0	0
Postoperative	0	0	1	10

Table 1. Preoperative and postoperative distribution of the patients according to McGowan-Goldberg classification system

	Mean VAS score	Mean range of elbow motion
Preoperative	8.45 ± 0.93	118.64° ± 11.42°
Postoperative	1.45 ± 2.29	128.63° ± 7.77°

Table 2. Preoperative and postoperative VAS scores and elbow range of motion values

Discussion

The oval form of the cubital tunnel becomes elliptical with elbow flexion. A 55% reduction in cubital tunnel volume and increased pressure around the ulnar nerve was detected with elbow flexion (11-14). The elbow flexion leads to an elongation of 4-7 mm in ulnar nerve due to the distance of the ulnar nerve from the elbow center of rotation and increase compression on ulnar nerve during elbow flexion due to the effect of traction (15). Anterior transposition of the ulnar nerve decreases the tension on ulnar nerve with elbow flexion and elbow extension does not create additional tension on ulnar nerve in elbow extension (16). The main idea of ulnar nerve anterior transfer is to reduce the intrinsic pressure on the nerve during elbow flexion (17). Ulnar nerve tension has been found to increase by 29% during elbow flexion (18). Simple decompression has been shown not to reduce traction forces on the ulnar nerve during elbow flexion (19).

The results of the meta-analysis comparing the patients who underwent simple decompression and ulnar nerve transposition in patients with idiopathic cubital tunnel syndrome showed no difference between the clinical results and nerve conduction velocities of both groups. Simple decompression was proposed due to easier surgical technique and faster recovery period (20).

Seyfettinoglu et al. compared anterior transposition and simple decompression in relation to blood flow and functional results. They found no statistically significant difference between both groups

about functional results but no arterial blood flow of ulnar nerve was found in five of seven patients who underwent anterior transposition and 2 patients had blood flow decrease of 20 cm/s. Mean 10 cm/s blood flow decrease was detected in the simple decompression group. There was a significant difference between the two groups in relation to blood flow. As a result of the study, simple decompression may be considered as the first option (21).

Macadam et al. compared clinical outcomes of the patients in whom they performed anterior ulnar nerve transposition and simple decompression. Their results showed that patients who underwent anterior transposition had better clinical outcomes than patients who underwent simple decompression (22). Our clinical results showed the improvement in pain and range of motion but the lack of a comparison group restricts our study.

Keith and Wollstein decided to perform anterior transposition according to the stability of the ulnar nerve during surgery and found improvement in upper extremity functions in 90% of patients (23). Stability of the ulnar nerve can be an important criterion for transfer to the anterior but we did not control the stability of the ulnar nerve during surgery, however, we detected a statistically significant difference in terms of elbow range of motion before and after surgery.

Black et al. found that subcutaneous ulnar nerve transposition was easier surgical technique than submuscular transposition with less bleeding, and shorter surgical time (24). Subcutaneous transfer of the ulnar nerve causes less pain and allows early motion after surgery (25, 26). Allowing elbow motion in the early postoperative period allows the ulnar nerve to slide during movement and prevent the formation of perineural fibrosis (27). We detected increased elbow range of motion after surgery. We think that there are two main reason for improved range of motion: the first one is allowing elbow movements in the early postoperative period, the second one is making the ulnar nerve trace get closer to motion center of elbow joint by anterior transfer thereby reducing traction on the ulnar nerve.

Infection rates are less in patients who underwent subcutaneous ulnar nerve transfer. Bartels et al. showed that infection rates were higher in patients who underwent submuscular ulnar nerve transposition (28). We detected no infection during the follow up period. Our results are in accordance with the literature.

Conclusion

Anterior subcutaneous ulnar nerve transposition is an effective surgical treatment method with increasing elbow range of motion in patients with cubital tunnel syndrome.

Conflict of interest

None of the authors of this manuscript received funding, grants, or in-kind support in support of this research or the preparation of this manuscript. The authors have no financial relationships with any company. Each author certifies that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/ licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article. We, the authors, declare that we have all participated in the design, data collection, statistical analysis, data interpretation, literature search and manuscript preparation of the paper, and that we have approved the final version.

Ethical Review Committee Statement

The study was approved by the Ethics Committee of Bahçeşehir University Medical School (number: 2019-17/01).

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DA LI DEKOMPRESIJA I ANTEARIORNA TRANSPOZICIJA ULNARNOG NERVA POZITIVNO UTIČU NA OPSEG POKRETA U LAKTU U LEČENJU SINDROMA KUBITALNOG TUNELA?

Özgür Korkmaz[1], Yildiray Genç[2]

[1]Odeljenje za ortopediju i traumatologiju, Medicinska škola Univerziteta Bahcesehir, Bolnica VM Medicalpark Pendik, Istanbul, Turska
[2]Odeljenje za ortopediju i traumatologiju, Bolnica Istanbul Cerrahi, Istanbul, Turska

Kontakt: Özgür Korkmaz
Fevzi Čakmak Mah. D100 Cemal Gursel 9, 34899 Istanbul, Turska
E-mail: ozkorkmaz00@yahoo.com

Cilj ove studije bila je procena kliničkih rezultata i opsega pokreta lakta kod bolesnika sa sindromom kubitalnog tunela, kod kojih su urađeni dekompresija i transpozicija anteriornog ulnarnog nerva.

Ukupno je bilo 11 bolesnika, sedmoro muškaraca i četiri žene. Prosečna starost bolesnika iznosila je 45,45 godina ± 16,22 godine. Prosečno trajanje praćenja bolesnika bilo je 14,81 mesec ± 6,98 meseci.

Dekompresija i anteriorna transpozicija ulnarnog nerva urađeni su kod svih bolesnika. Bolesnici su bili klasifikovani prema McGowan-Goldberg klasifikaciji pre operacije, kao i na poslednjem kontrolnom pregledu, nakon operacije. VAS skor, opseg pokreta u fleksiji i eksteziji, takođe su procenjivani pre operacije, ali i na poslednjem kontrolnom pregledu nakon operacije.

Prema McGowan-Goldberg klasifikacionom sistemu, osmoro bolesnika je na skali ocenjeno sa 2A, dok je troje bolesnika ocenjeno sa 2B, pre hirurške intervencije. Postoperativno, desetoro bolesnika je na skali dobilo 0, dok je jedan bolesnik na skali dobio 1. Preoprativno, srednja vrednost VAS skora iznosila je 8,45 ± 0,93, dok je njegova vrednost u postoperativnom periodu bila 1,45 ± 2,29. Preoprativno, srednja vrednost opsega fleksije i ekstenzije iznosila je 118,64° ± 11,42°, dok je postoperativno vrednost ovog opsega bila 128,63° ± 7,77°. Utvrđena je statistički značajna razlika u vrednostima VAS skora i opsega pokreta pri fleksiji i ekstenziji pre i posle operacije.

Anteriorna subkutana transpozicija ulnarnog nerva efikasna je hirurška metoda lečenja, koja povećava opseg pokreta u laktu kod bolesnika sa sindromom kubitalnog tunela.

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Ključne reči: *kubitalni tunel, ulnarni nerv, dekompresija*

Original article

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CLINICAL SIGNIFICANCE OF CD44 EXPRESSION IN SEROUS OVARIAN CANCER

Irena Conić[1,2], Slavica Stojnev[2,3], Aleksandra Dimitrijević[1], Ljubinka Janković-Veličković[2,3], Biljana Djordjević[2,3], Ivana Djordjević[3], Ivica Pejčić[1,2], Svetislav Vrbić[1,2]

Ovarian cancer is a devastating disease causing more than 180.000 deaths a year, with often insidious course, delayed clinical diagnosis, and limited response to therapy. The CD44 cell surface glycoprotein is involved in metastatic spread and progression in various types of cancer, including ovarian serous cancer. This study aimed to investigate the profile of CD44 immunohistochemical expression in ovarian serous cancer, and to determine its potential significance in prognosis of the disease. A total of 124 primary serous ovarian cancers were analyzed for the expression of CD44 by immunohistochemical method and assessed for possible relation with clinical and pathological parameters, as well as with patients' survival. High CD44 expression was observed in 67.7% of the investigated tumors. A positive family history of malignancies was associated with low expression CD44 in cancer cells ($p = 0.004$). Low expression of CD44 was more frequent in FIGO stage IV tumors than in other stages, as well as in high grade cancer compared to low grade, however these differences were not statistically significant. Mean survival was significantly longer in patients with high CD44 expression compared to those with absent or low expression ($p = 0.009$). The fatal outcome during the follow-up period occurred in 65% of patients with low CD44 expression, and in 42.86% of patients with high CD44 expression, with statistically significant difference between the groups ($p = 0.035$). In conclusion, the adverse clinical course of serous ovarian cancer was associated with the absence or low expression of CD44.

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Key words: *ovarian cancer, serous cancer , CD44*

[1]Clinic of Oncology, Clinical Center Niš, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia
[3]Center for Pathology, Clinical Center Niš, Niš, Serbia

Contact: Irena Conić
48 Dr. Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: irenaconic@yahoo.com

Introduction

Ovarian cancer is a devastating disease causing more than 180.000 deaths a year (1), with often insidious course, delayed clinical diagnosis, and limited response to therapy. Most ovarian cancers start in the cells covering the ovaries and are called epithelial ovarian cancer. High-grade serous ovarian carcinoma (HGSOC) is the most frequent type of ovarian cancer and has a poor outcome (2).

There are different types of cells in the tumor environment, such as vascular cells, fibroblasts, cells of the immune system, extracellular matrix (ECM) components, as well as growth factors and cytokines. The tumor cells receive paracrine signals from the local microenvironment. In this way they often alter the cellular and molecular composition and lead to tumor progression. The tumor environment is important for the occurrence of the metastatic phenotype of cancer cells and it has been the subject of extensive research (3, 4). The disruption of the ECM results in abnormal intercellular and/or intracellular signaling, so that dysregulation of cell proliferation, growth, and cytoskeletal reorganization can occur (5, 6).

The CD44 cell surface glycoprotein is involved in the interaction between cells, cell adhesion and migration. It is considered to be a cell surface marker of metastasis and progression in various types of cancer, including ovarian cancer. CD44 is a receptor for hyaluronic acid (7). Hyaluronic acid is a major component of the ECM in most mammalian tissues and it accumulates at the site of cell division and rapid matrix remodeling, which occurs during

embryonic morphogenesis, inflammation, and tumorigenesis. Hyaluronic acid is the basis of glycosaminoglycans and it is found in the extracellular matrix. It is the main component of the peritoneum where ovarian cancer metastases mostly occur. Hyaluronic acid (HA) induces signals upon binding to CD44, and in turn, CD44 can react with other molecules, including collagen, fibronectin, osteopontin, growth factors and matrix metalloproteinase (MMPs) (8-9).

CD44 interacts with hyaluronic acid and activates Nanog-Stat3 and signaling pathways into which ankyrin is included. The activation of these signaling pathways is thought to be responsible for the specific behavior of tumor stem cells because it has an effect on transcriptional activation, on the growth of tumor cells and drug resistance in ovarian and breast cancers. The interaction of CD44 with the tumor stroma and the tumor environment is closely related to the metastatic growth of cancer (10-11).

Based on the previous findings, the question arises whether the suppression of the CD44 protein can improve chemotherapy efficacy and prevent the occurrence of metastases. Such suppression can potentially be achieved by using siRNAs generated by targeting CD44 with mRNA (7). This study aimed to investigate the profile of CD44 immunohistochemical expression in ovarian serous epithelial cancer, and to determine its potential clinical significance in this devastating disease.

Material and methodology

This study included female patients with serous epithelial ovarian cancer diagnosed between 2005 and 2011 in the region of Southern Serbia. Their survival was monitored until October 2013, with median follow-up of 60 months. A total of 124 primary ovarian cancers were analyzed including the following: ovarian cancer limited to the ovaries (FIGO stage I), ovarian cancer extended to the pelvic organs (FIGO stage II), ovarian cancer with peritoneal metastases (FIGO stage III) and ovarian cancers with distant metastases (FIGO stage IV).

The clinical parameters of the patients with ovarian cancer were monitored, as well as their pathological characteristics. The clinical parameters analyzed were: age, FIGO stage, the presence and size of residual tumor, the type of therapy, and the response to the therapy. The pathological characteristics analyzed were: histologic tumor type, histologic grade, and nuclear grade.

Case histories of the female patients with ovarian cancer who were treated at the Oncology Clinic of the Clinical Center Niš were used as the source of the relevant clinical data.

Pathohistological analysis and immunohistochemical reaction scoring

The pathological and immunohistochemical analysis of the ovarian cancer samples was performed at the Institute of Pathology of the Faculty of Medicine in Niš.

The pathohistological analysis was performed on the biopsy specimens of ovarian cancers that were fixed in 10% buffered formalin, processed in an automatic tissue processing machine, embedded in paraffin and cut in the microtome at 5 μm thickness. The monoclonal antibody to CD44 (ab157107, Abcam, 1:100 dilution) was used for the immunohistochemical analysis. Briefly, the sections were deparaffinized, rehydrated and heat mediated antigen retrieval procedure was performed. After incubation with primary antibody at 4 °C overnight, the slides were further treated with standard immunoperoxidase detection system, and diaminobenzidine (DAB) was used as a chromogen for the visualization of reaction. Slides were counterstained with Mayer's hematoxylin, dehydrated, and mounted with DPX.

The analysis of the immunohistochemically stained microscopic ovarian cancer preparations was performed using a Leica DM 1000 light microscope. The positive finding was a brownish cytoplasmic or membranous staining. The immunohistochemical reaction of the expression of CD44 was based on the evaluation of the positive response in the tumor cells, and was characterized as low expression if less than 25% of the cells showed immunoreactivity, or high expression, if ≥ 25% of cancer cells showed staining with moderate to strong intensity, i.e. marked or intense brown precipitate within the tumor cells (7).

The results obtained were systematized and grouped, and the statistical significance was tested by appropriate statistical tests according to the examined parameters and the size of the samples.

Statistical methods

Microsoft Office 2007 Excel was used to write, rank, group, tabulate and graph the data, while SPSS 26.0 Software was used for quantitative statistical analysis. Comparison of mean values of numerical features between two groups of subjects was performed by Student's t test. The comparison of the frequency of individual categories of attribute characteristics between some groups of the subjects was performed by Chi square test or Fisher exact test in cases where some of the expected frequencies were less than 5. As a threshold for statistical significance, a permissible error rate of less than 5% ($p < 0.05$) was used in the inference.

Results

High CD44 expression was observed in 67.7% of the investigated tumors (Figure 1). The mean age of the patients with high CD44 expression was 58.75 ± 13.69 years, and they were not significantly older than the subjects with low expression (Figure 2) whose mean age was 55.13 ± 12.1 years ($p > 0,05$) (Table 1).

A statistically significant difference was observed in term of a positive family history for malignancies between the two groups; more often family history was positive for cancer in the group of patients with low CD44 expression compared to

those with high CD44 (30% vs. 9.52%; p = 0.004) (Table 1).

The highest ratio of the tumors with low expression of CD44 was recorded in FIGO stage IC (low CD44 found in 66.6% of the tumors), and in FIGO stage IV, where half of the tumors (50%) had low CD44 expression, in contrast to other FIGO stages, where tumors with high CD44 expression predominated. However, these differences were not statistically significant ($p \geq 0.05$) (Table 2).

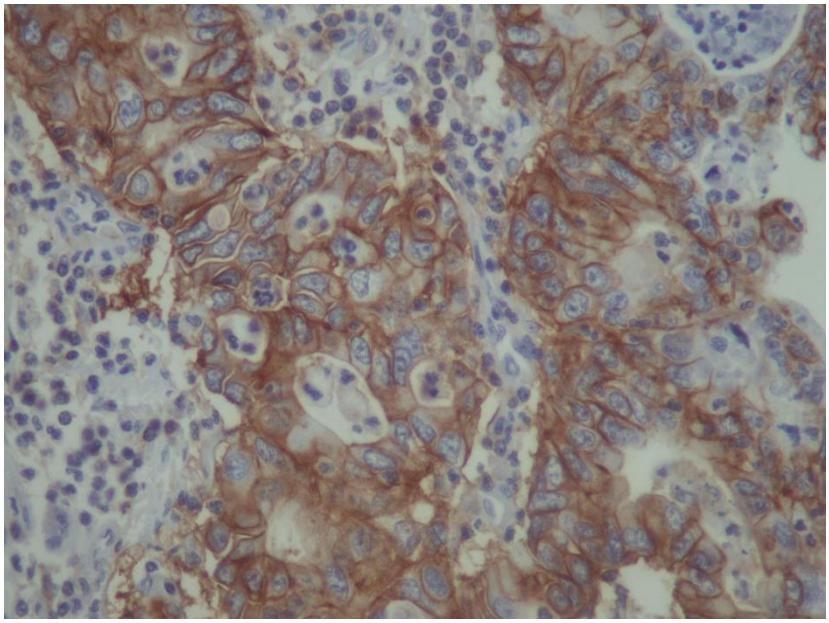

Figure 1. Strong diffuse membranous expression of CD44 in poorly differentiated high grade serous ovarian carcinoma (x400)

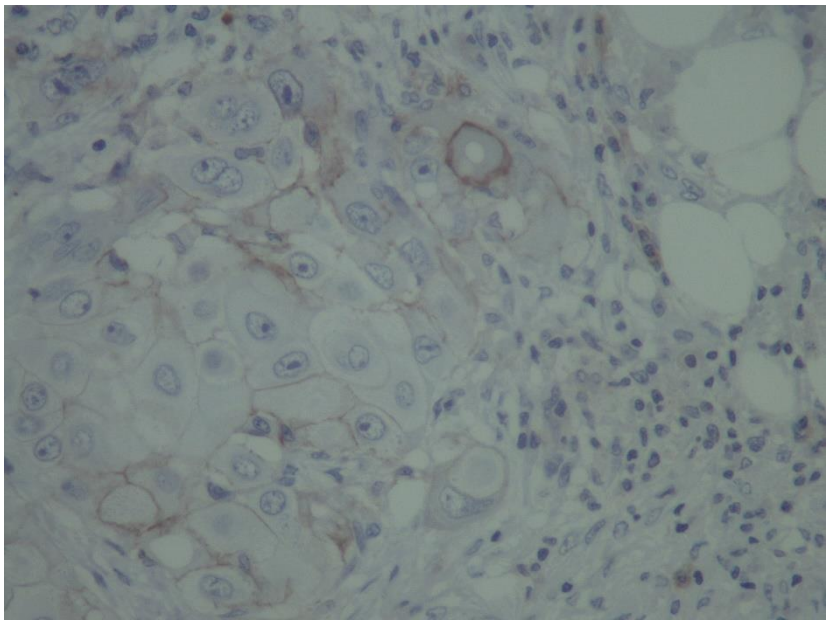

Figure 2. Loss of immunoreactivity of CD44 in metastatic serous ovarian carcinoma (x400)

Table 1. The characteristics of the subjects and tumor type in relation to CD44 expression

Characteristics	Expression of CD44		Comparison
	Low expression (n = 40)	High expression (n = 84)	
Age (years)	58.75 ± 13.69	55.13 ± 12.1	p = 0.069
Menarhe (years)	13.55 ± 1.55	13.53 ± 1.59	p = 0.481
Menopause (years)	39.2 ± 19.93	34.1 ± 22.07	p = 0.108
First birth (years)	19.57 ± 6.12	20.75 ± 6.57	p = 0.172
Number of children	1.82 ± 0.64	1.8 ± 0.79	p = 0.424
Number of abortions	1.6 ± 3.04	1.37 ± 2.01	p = 0.312
Positive family history	12 (30%)	8 (9.52%)	p = 0.004
Time to diagnosis(months)	3.55 ± 3.21	4.5 ± 4.03	p = 0.096

Chi square test was performed. A p-value of ≤ 0.05 was considered significant.

Table2. CD44 immunohistochemical expression in relation to FIGO stage

FIGO stage	Expression CD44		Comparison
	Low expression (n = 40)	High expression (n = 84)	
borderline	4 (10%)	8 (9.5%)	p = 0.933
IA	1 (2.5%)	3 (3.6%)	p = 1.000
IB	0 (0%)	0 (0%)	/
IC	4 (10%)	2 (2.4%)	p = 0.085
IIA	0 (0%)	2 (2.4%)	p = 1.000
IIB	0 (0%)	5 (5.9%)	p = 0.174
IIC	1 (2.5%)	3 (3.6%)	p = 1.000
IIIA	5 (12.5%)	11 (13.1%)	p = 0.926
IIIB	9 (22.5%)	18 (21.4%)	p = 0.977
IIIC	8 (20%)	24 (28.6%)	p = 0.308
IV	8 (20%)	8 (9.5%)	p = 0.103

Chi square test was performed. A p-value of ≤ 0.05 was considered significant

According to the FIGO grading system, each patient group was divided into two categories: high grade and low grade. In both patient groups, the majority of tumors belonged to a more aggressive histologic grade (high grade). In the group of patients with low CD44 expression high grade tumors comprised 80%, while in the group of patients with high CD44 expression 75% of the tumors, with no significant difference in the distribution between the groups (p = 0.318) (Table 3).

Mean survival was significantly longer in patients with high CD44 expression compared to those with absent or low expression (43.81 ± 21.9: 32.81 ± 28 months; p = 0.009) (Table 4).

The fatal outcome (death) during the follow-up period occurred in 65% of patients with low CD44 expression, and in 42.86% of patients with high CD44 expression, with statistically significant difference between the groups (p = 0.035).

Table 3. CD44 immunohistochemical expression in relation to FIGO grade

FIGO grade	Expression CD44		Comparison
	Low expression (n = 40)	High expression (n = 84)	
High grade	32 (80%)	73 (75%)	p = 0.318
Low grade	8 (20%)	11 (15%)	

Table 4. Disease outcome in relation to CD44 expression

Disease outcome	Expression CD44		Comparison
	Low expression (n = 40)	High expression (n = 84)	
Survival (months)	32.81 ± 28	43.81 ± 21.9	p = 0.009
Fatal outcome	26 (65%)	36 (42.86%)	p = 0.035

Discussion

The results of our study indicated that the patients with high CD44 expression in cancer cells had significantly longer mean survival compared to those with decreased or absent CD44 (43.81 ± 21.9 vs. 32.81 ± 28 months; p = 0.009). According to Sillanpaa et al. (12), the decreased expression of CD44 is associated with the advanced stage of ovarian epithelial cancer and it is an independent prediction factor of shorter survival time, which is in agreement with our results. On the other hand, we failed to recognize the significant association between CD44 expression and FIGO stage. Although tumors of advanced stage demonstrated more frequent loss of immunohistochemical expression of CD44, this difference was not significant (p > 0.05).

The general prognostic role of CD44 in human cancers is controversial. The reduced expression of CD44 and its variants (CD44v and CD44s) is associated with poor disease outcome in other malignant tumors as well, such as melanoma (13), prostate cancer (14), and colorectal cancer (15). Conversely, the increased expression of CD44 is a predictor of poor prognosis and survival in kidney cancer, non-small cell lung cancer breast cancer in stage II and III and cervical cancer in FIGO stage IB (16-19).

The studies regarding the prognostic role of CD44 in ovarian cancer have yielded inconsistent results so far. Several previous studies were congruent that high CD44 expression was associated with a relatively good prognosis for ovarian cancer, but these studies included relatively small number of patients. No association between CD44 expression and disease prognosis has been found in three studies, while one study has shown that the poor prognosis of a disease is associated with high CD44 expression, which contradicts our results. Regardless of the sufficient number of samples, the reasons for the discrepancy in the results may lay in the type of the used antibodies. In addition, this study revealed an interesting finding about the possible association of hereditary background and CD44 expression in ovarian tumors. Namely, patients with positive family anamnesis for malignant disease had significantly lower expression of CD44 in cancer tissue. The significance of this association is yet to be elucidated (20-24).

Epithelial ovarian cancer is spread by the implantation of tumor cells into the peritoneal cavity. *In vitro* studies have shown that CD44 on the surface of ovarian cancer cells binds the hyaluronic sheath of mesothelial cells and may contribute to the formation of peritoneal metastases (25, 26). The monoclonal antibodies whose target is CD44 significantly inhibit the adhesion of ovarian cancer cells to mesothelial cells and their peritoneal implantation in mice (27, 28). This indicates that CD44 in tumor cells may contribute to the spread of ovarian cancer in humans. However, our results do not support the idea that high expression of CD44 is associated with high grade histology of serous epithelial carcinoma or aggressive clinical behavior. On the contrary, a high percentage of CD44 positive ovarian cancer cells occur in well-differentiated tumors and less aggressive histological subtypes in the presented material. In addition, high CD44 expression in tumor tissue is associated with a favorable disease prognosis, and longer overall survival. The other molecular mechanisms, such as integrins (28) and proteoglycans (29), may play a role in the implantation of ovarian cancer cells.

Previous studies additionally suggested that the expression of CD44 in cancer tissue did not alter during the metastatic process (29, 30), while some authors observed a downregulation of CD44 during

tumor progression in mice (31) and tumor cells from ascitic fluid in humans. It must be taken into account that changes in CD44 other than total expression levels may also contribute to malignant growth (32-33). The regulation of receptor's ability to bind hyaluronan may modify the roles and significance of CD44 in various cancer types (34). In epithelial ovarian cancer (34), a positive correlation present in primary tumors between cells bound by hyaluronan and CD44 was lost in metastases, possibly reflecting the changes in the function of the hyaluronan receptor for CD44. However, there is no straightforward direct relationship between the hyaluronan accumulation in tumor stroma and CD44 expression in tumor cells. CD44 expression does not necessarily mean tumor cell adherence to hyaluronan and, on the other hand, the levels of CD44 do not always correlate with affinity for hyaluronan. A previous study suggests that hyaluronan accumulation in the stroma represents independent prognostic factor in ovarian cancer, as well as the loss of CD44 positivity in primary tumors, indicating that both molecules have independent, separate influence to the prognosis of ovarian cancer (10). The results of our study are in accordance with these conclusions, and suggest that the decrease of CD44 expression is linked with poor clinical outcome. The fatal outcome after follow-up period occurred in 65% of patients with low CD44 expression, and in 42.86% of patients with high CD44 expression, with statistically significant difference between the groups ($p = 0.035$).

Conclusion

1. A positive family history of ovarian cancer malignancies is associated with low expression of CD44 in cancer cells.
2. No significant association was found between the immunohistochemical expression of CD44 and FIGO stage and grade of serous epithelial ovarian carcinoma.
3. The adverse clinical course of ovarian cancer was associated with the absence or low expression of CD44.

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KLINIČKI ZNAČAJ EKSPRESIJE CD44 KOD SEROZNOG KARCINOMA JAJNIKA

Irena Conić[1,2], Slavica Stojnev[2,3], Aleksandra Dimitrijević[1], Ljubinka Janković-Veličković[2,3], Biljana Đorđević[2,3], Ivana Đorđević[3], Ivica Pejčić[1,2], Svetislav Vrbić[1,2]

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

Kontakt: Irena Conić
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: irenaconic@yahoo.com

Karcinom jajnika je bolest koja uzrokuje više od 180.000 smrtnih slučajeva godišnje, sa često podmuklim tokom, kasnim simptomima i kliničkom dijagnozom i ograničenim odgovorom na terapiju. Površinski glikoprotein CD44 uključen je u širenje i pojavu metastaza kod različitih vrsta karcinoma, uključujući i serozni karcinom jajnika. Ovo istraživanje imalo je za cilj da analizira profil imunohistohemijske ekspresije CD44 kod seroznog karcinoma jajnika i da utvrdi njegov potencijalni značaj u prognozi bolesti. Na ekspresiju CD44 analizirano je ukupno 124 primarnih seroznih karcinoma jajnika imunohistohemijskim metodama i procenjena je moguća povezanost sa kliničkim i patološkim parametrima, kao i sa preživljavanjem bolesnika. Primećena je visoka ekspresija CD44 kod 67,7% ispitivanih tumora. Pozitivna porodična anamneza maligniteta bila je povezana sa niskom ekspresijom CD44 u ćelijama karcinoma ($p = 0,004$). Niska ekspresija CD44 bila je češća kod tumora u FIGO stadijumu IV, nego u ostalim stadijumima, kao i kod karcinoma visokog gradusa, u poređenju sa karcinomima niskog gradusa. Međutim, ove razlike nisu bile statistički značajne. Prosečno preživljavanje bilo je značajno duže kod bolesnika sa visokom ekspresijom CD44, u poređenju sa onima kod kojih je bila odsutna ili slaba ekspresija CD 44 ($p = 0,009$). Smrtni ishod tokom perioda praćenja dogodio se kod 65% bolesnika sa niskom ekspresijom CD44 i kod 42,86% bolesnika sa visokom ekspresijom CD44, sa statistički značajnom razlikom između grupa ($p = 0,035$). Istraživanje je pokazalo to da je nepovoljni klinički tok seroznog karcinoma jajnika bio povezan sa odsutvom ekspresije ili slabom ekspresijom CD44.

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Ključne reči: *karcinom jajnika, serozni karcinom jajnika, CD44*

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HEALTH SELF-EVALUATION OF COMPLEMENTARY AND ALTERNATIVE MEDICINE USERS IN SERBIA: CROSS-SECTIONAL NATIONAL STUDY

Marina Luketina-Šunjka[1], Nemanja Rančić[2], Nataša Mihailović[3], Svetlana Radević[4], Saša Dragović[5], Mihajlo Jakovljević[6]

The aim of the research was to examine the influence of the respondents' health status on the use of complementary and alternative medicine methods. This was a population-based, cross-sectional study. The sample consisted of 550 interviewed persons, from the third National health survey of the residents of Serbia in 2013, who had used complementary and alternative medicine (CAM) services in the preceding 12 months. Just over 2/3 of CAM users had chronic health disorders ($p < 0.01$), the most commonly diagnosed chronic health disorder among CAM users was hypertension (36.7%). Every seventh and partially every fourth CAM user had been seriously restricted as regards undertaking normal daily activities for the previous 6 months or longer. CAM users were more satisfied with the services they received in private practice ($p < 0.01$). The two out of three users of CAM services rated their health as good and/or average. CAM users were more satisfied with the services they received in private practice, the highest percentage of them rated their health as good. The analysis of the impact of respondents' health status, the analysis of the correlation between the respondents' self-health assessment and the use of alternative medicine methods, analysis of the impact of socio-demographic characteristics on the use of CAM, along with a comparative analysis of the use of health care services – would significantly contribute to better recognition of CAM by the Ministry of Health.

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Key words: *health self-assessment; health status; complementary and alternative medicine; chronic diseases*

[1]European Center for Peace and Development, University for Peace established by the United Nations, Belgrade, Serbia
[2]Centre for Clinical Pharmacology, Military Medical Academy; Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia
[3]Department of Biostatistics and Informatics, Institute of Public Health Kragujevac, Kragujevac, Serbia
[4]Department of Social Medicine, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[5]Clinic for General Surgery, Military Medical Academy, Belgrade, Serbia
[6]Department of Global Health Economics and Policy, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

Contact: Nemanja Rančić
Crnotravska 17, 11000 Belgrade, Serbia
E-mail: nece84@hotmail.com

Introduction

The terms complementary medicine and alternative medicine (CAM), in practice, are often used interchangeably and are considered synonymous, but there are, nevertheless, some differences between them (1, 2). If those methods are used to replace conventional medicine methods, then they are considered to represent alternative medicine, i.e., alternative medicine is completely independent while performing diagnostics and treatments, while complementary medicine means activities that supplement conventional medical treatment and are used jointly with the methods of conventional medicine (1, 2). The following terms may also be found in the literature: non-allopathic, unorthodox (unrecognized), unconventional, traditional, mental and physical medicine, mental and physical medicine and natural medicine (3, 4). Holistic and integrative are very commonly used terms (4). The World Health Organization defines traditional medicine as "health practice, approach, knowledge and beliefs related to herbal, animal or mineral preparations, spiritual therapy, as well as manual techniques and exercises applied individually or in combination for the purpose of diagnosis, treatment and preventing disease, that is, for health reasons" (5).

CAM include a number of different diagnostic and therapeutic procedures (methods), as well as the use of various natural products for the purpose

of healing or healing of patients which do not belong to conventional medicine (allopathic, modern, western medicine, i.e. evidence-based medicine using methods whose safety and efficacy have been demonstrated in well-designed randomized controlled clinical trials) (1–3). There is a growing interest for CAM around the world in both developed and underdeveloped countries (6). The reasons for the increased popularity of CAM are complex and have not yet been fully explored. Some studies indicate that in addition to various motivational factors in the individual sphere, socio-political factors play an important role, as well as the inability of modern medicine to solve all the problems of ill citizens (1).

If we were to compare the prevalence of CAM use across studies, we would soon realize the shortcomings of such processes (7). And the flaws lie in the following:

- there are often differences in how CAM is defined
- what methods and procedures were considered when prevalence was determined (7)
- the implementation procedures are different, not clearly defined
- the surveys were carried out at different time intervals
- consumption is expressed in different currencies and there is no systematization (8).

The analyses of the database of the International Social Survey Programme for the period 2011-2013 (module health and health care), in which data from respondents from 32 countries and regions were available with a final study sample of 52,801 respondents for whom available data on the use of CAM, showed that the average 12-month prevalence of users of CAM practitioner services was on average 26.4% (9). This study found that there were significant differences in prevalence among different countries: 34.7% in Australia, below 10% in Europe in Bulgaria, Poland and Slovenia, up to 35.4% in France, in Asia from 16.7% in Russia, up to over 50% in China, the Philippines, the Republic of Korea and over 20% in the US, Chile and South Africa (9).

Analysis of data from the 2014 European social survey showed that during the last 12 months, 25.9% of the respondents used CAM, and that among users of CAM, 69.4% had used only one kind of CAM modality, whereas 19.9% had used two kinds of CAM modalities (11). About 8% of CAM users have used only CAM without any visits to health care providers in the previous 12 months (alternative use) (7). Alternative use rates were highest for spiritualism (14.9%) and acupressure (12.1%), and they were lowest for osteopathy (4.1%), homeopathy (5.6%) and acupuncture (6.3%) (11).

A systematic review of 89 studies addressing the prevalence of CAM use in the United Kingdom showed that on average, the one-year prevalence of CAM use was 41.1% (range 9.2-100%) (12). The use of herbal medicines was the most popular type of CAM in the 24 included studies, followed by homeopathy in 8 studies and aromatherapy in 6 studies (12).

The prevalence of use of any CAM method in Australia ranged from 68.9% in 2005 (13) to 66% in a study published in 2017 (14).

In Canada, the prevalence of CAM use ranged between 50% in 1997 (15) and 54% in 2006 (16). In 1997, the most commonly used CAM method was prayer/spiritualism (18%) (15), and in 2006 – massage (19%) (16).

A systematic review of the literature published between 1998 and 2009 (148 publications, 152 surveys) showed that the prevalence of current CAM use in cancer patients ranged from 9-88%, with the pooled prevalence of 40% showing significant heterogeneity (17). A significant difference between countries was also noted in this literature review, with the highest pooled prevalence in the USA (50%) and the lowest in Italy and the Netherlands (22%) (17). The systematic review which included 61 research articles published between 2009 and 2018 found that, on average, 51% of cancer patients used CAM, and the range was from 16.5-93.4% (18).

As regards a systematic literature review (42 studies), it has found that the prevalence of CAM use ranged from 8% to 90% (median = 30%) in patients with prostate cancer (19). In a population of patients with colorectal cancer, a systematic review of 4 studies showed that up to 75% of them used at least one CAM method (20). The most commonly used methods of CAM were biologically based therapies: herbal remedies (48.7%), homeopathy (20.5%), vitamins/minerals (17.9%), medicinal teas (15.4%), and body-mind-spirit medicine procedures and techniques (15.4%) and relaxation techniques (12.8%) (20).

According to the results of a systematic literature review that included 27 studies examining the use of CAM in the cardiovascular population, the prevalence of CAM use ranged from 22-68%, herbal medicines used from 2-46%, vitamins, minerals and other dietary supplements were used between 3-54% (most vitamin B/B12 or vitamin B complexes, vitamin C, vitamin E, glucosamine/chondroitin, coenzyme Q10, calcium and magnesium), and mind-body medicine from 2-57% (21).

The prevalence of CAM use in patients with low back pain ranged from 6-76.4% (mean 34.2%) according to the results of a systematic review that included 30 studies, and the most commonly used modalities of CAM were acupuncture (from 6-60.2%, average: 27.4%), chiropractic (from 16.1-74%, average 39.3%), osteopathy (from 4.1-48.4%, average: 17.3%) and massage (7-41.4%, average: 25.9%) (22).

In the Republic of Serbia, the Acupuncture Section was first established within the Serbian Medical Society in 1980, then the Homeopathy Section in 2002 and the Quantum medicine board in 2006 within the Acupuncture section (23). The Section for Traditional Medicine of the Serbian Medical Society was established in 2012 and at its regular monthly meetings lectures are organized

and given by experts, practitioners and theorists of traditional medicine methods (23).

A study conducted in eight Serbian cities among physicians, dentists and pharmacists employed by public and private healthcare institutions, as well as medical, dental and pharmacy students from two state universities found that dental students were better informed about CAM than medical students, pharmacists better than university professors, while healthcare professionals working at the primary health care level were more familiar with CAM than pharmacists in public pharmacies (24). This research shows weaknesses in the attitudes of current and future healthcare professionals in Serbia towards CAM (24, 25).

The aim of the study was to examine the influence of the respondents' health condition on the use of Alternative medicine methods, as well as to examine the correlation between the health self-assessment of the respondents and the use of Alternative medicine methods, and to examine the health self-assessment of CAM users in relation to the possibility of performing daily activities and the presence of long-term health disorder.

Methods

Study design and sampling

This was a population-based, cross-sectional study. The analyzed data were used from the latest National Health Survey of the Republic of Serbia administered during 2013 and sponsored by the Ministry of Health of the Republic of Serbia. It was based on the general population of citizens of the Republic of Serbia aged 15 and over who lived in private households. The survey was conducted in accordance with the methodology and instruments of the European Health Survey - Second Wave (European Health Interview Survey - EHIS wave 2) (26).

The sample included all households listed in all enumeration areas in the census conducted in 2011. A stratified two-stage sample was selected to provide a reliable assessment of a large number of factors which indicate the population health at the national level, as well as at the level of four geographic areas and the level of different settlement types. The units of the first stage of sampling were 670 census enumeration areas defined in the 2011 population Census, while the units of the second stage of sampling were randomly selected households. The study included 6500 randomly selected households with 3909 household from urban and 2591 from other areas, with 19,079 respondents aged 15 and over. The number of persons interviewed who had used alternative medicine services in the previous 12 months was 550.

The survey was approved by the Ethical Board of the National Institute of Public Health of the Republic of Serbia and the Ministry of Health. The principles of ICH Good Clinical Practice were strictly followed and the approval from the Ethics Committee of Republic of Serbia was obtained. Ethical Standards at Healthcare Research are aligned with the International Medical Association Declaration of Helsinki and legislative specific to our country's laws. Aiming to align with General Data Protection Regulation (GDPR) policies to preserve the discretion of gathered respondents' data all steps stipulated by the Law on protection of personal data (Official Gazette of the Republic of Serbia No. 97/08, 104/09), the Official Statistics Law (Official Gazette of the Republic of Serbia No. 104/09) and Directive 95/46/EC of the European Parliament on the protection of individuals with regard to the processing of personal data and on the free movement of such data (27).

Instruments

A standardized face-to-face self-reported questionnaire was used for data collection. The data collection was performed by specially trained teams of interviewers. The participation in the study was voluntary and all participants signed a written consent.

Study variables

The main independent variable was related to whether the respondent personally used Alternative Medicine services in the previous 12 months. Alternative medicine services included the following services: acupuncture, homeopathy, phytotherapy, chiropractic applied in a state health institution and/or with a private individual, which included private practice and folk healers.

The auxiliary dependent variables used in the analysis were the following: health self-assessment, health self-assessment of CAM users in relation to the ability to perform daily activities and the presence of long-term health disorders, health status of respondents, presence of chronic diseases and satisfaction with health care, in the previous 12 months.

Statistical analysis

All the data of interest were presented and analyzed by adequate statistical methods appropriate for the data type. Categorical variables were presented as frequency and percentage; n(%). The Chi-square test was used to compare proportions between groups. All statistical calculations were performed using commercial, standard software package SPSS Inc., version 18.0, Chicago, IL.

Results

Health status of respondents

Just over 2/3 of complementary and alternative medicine users had chronic health disorders ($p < 0.01$).

The most commonly diagnosed chronic health disorders among CAM users were the following: hypertension (36.7%), followed by back problems (28%) followed by hypercholesterolemia (23.1%),

allergy, without asthma (20.9%), and the cervical spine problems (18.9%). One in ten users had kidney problems, i.e., ischemic heart disease, or arthrosis, or depression. Four point four percent of CAM users were diagnosed with malignancy (Table 1).

Table 1. Chronic health disorder of complementary and alternative medicine (CAM) users

Disorder	Yes (n)	%	No (n)	%
Asthma bronchiale	33	6.0	517	94.0
Bronchitis chronica, COPD	41	7.5	507	92.5
Myocardial infarction	23	4.2	526	95.8
Ischemic heart disease	59	10.7	487	89.3
Hypertension	202	36.7	343	63.3
Brain stroke	18	3.3	530	96.7
Arthrosis	59	10.7	489	89.3
Back pain	154	28.0	395	82.0
Neck pain	104	18.9	445	80.1
Diabetes	52	9.5	495	90.5
Allergy with no asthma	115	20.9	433	79.1
Cirrhosis of the liver	5	0.9	543	99.1
Urinary incontinence	32	5.8	516	94.2
Kidney problems	60	10.9	489	89.1
Depression	56	10.2	491	89.8
Malignancy	24	4.4	525	95.6
Hypercholesterolemia	127	23.1	408	76.9

COPD- Chronic obstructive pulmonary disease

Every other user of CAM (54.9%) had a long-term illness/disorder. Every seventh and partially every fourth CAM user had been seriously restricted as regards performing their daily activities for the previous 6 months or longer. There was no statistically significant difference in the use of CAM services in relation to the presence of long-term health disorders ($p > 0.05$).

Health Self-Evaluation

Two out of three users of CAM services rated their health as good and/or average. Respondents who reported very poor health status (in their own estimation) were the least frequent users of complementary and alternative medicine services (Graph 1).

There was a significant difference in the self-assessment of the health of CAM users in relation to the presence of long-term illness ($p < 0.01$) and the ability to perform normal daily activities ($p < 0.01$). Namely, users of CAM who had a long-term illness, as well as those who had difficulty in performing daily activities, had a lower rating of their health compared to users of CAM who did not have it (Graph 2).

In the previous month, 61.8% of CAM users felt physical pain. The pain was usually moderate in intensity. There was a moderate positive association between pain intensity and the ability to perform daily activities of CAM users ($ro = 0.630$, $p < 0.01$).

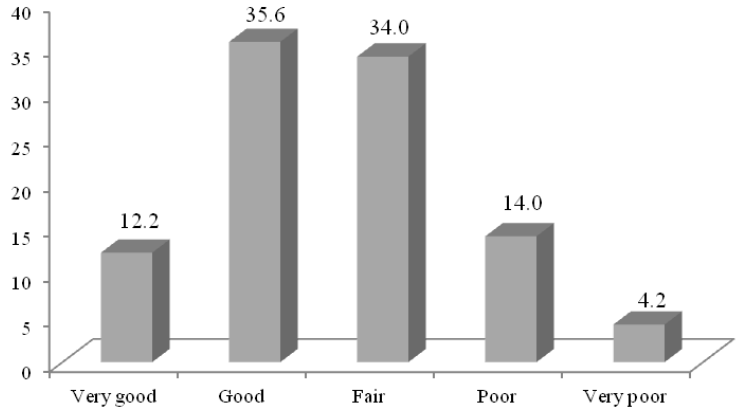
A quarter of Complementary and Alternative medicine service users were already diagnosed with hypertension, and every 10th of those were diagnosed with heart and blood vessel diseases. Almost all CAM users believed that their behavior did not put them at risk for liver cirrhosis, sexually transmitted diseases and/or injuries. At the same time, one in five, or six users of CAM considered themselves to be at risk of becoming obese or developing diabetes (Table 2).

Satisfaction with healthcare and unmet needs for health care

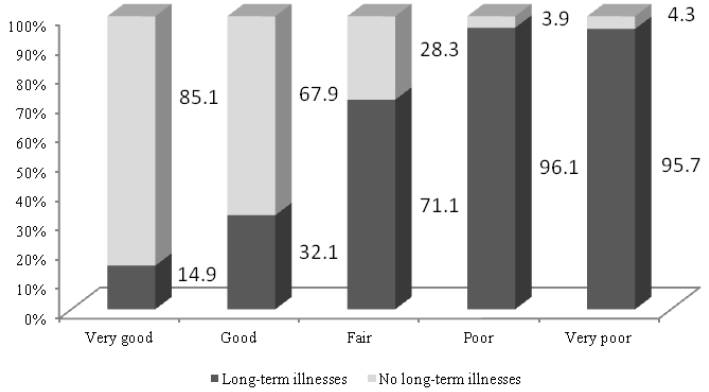
CAM users were more satisfied with the services they received in private practice ($p < 0.01$). Specifically, almost half of the respondents who were dissatisfied with the services provided in government institutions were satisfied with the services provided in the private sector. At the same time, persons who were very dissatisfied with state institutions were either satisfied (41.1%) or neither satisfied nor dissatisfied (23.2%) with private health care institutions. CAM users who were very satisfied with the services provided in the state health services, at the same time in 82.1% of cases were very satisfied with the services provided in private offices (Graph 3).

Waiting lists and financial constraints along were considered to be the main reasons for the lack of the necessary form of healthcare for CAM users (Graph 4). The lack of money for every fifth user of

CAM caused the lack of the necessary form of medical healthcare, and for every sixth – the lack of necessary dental health care, i.e., the inability to purchase the prescribed medications.



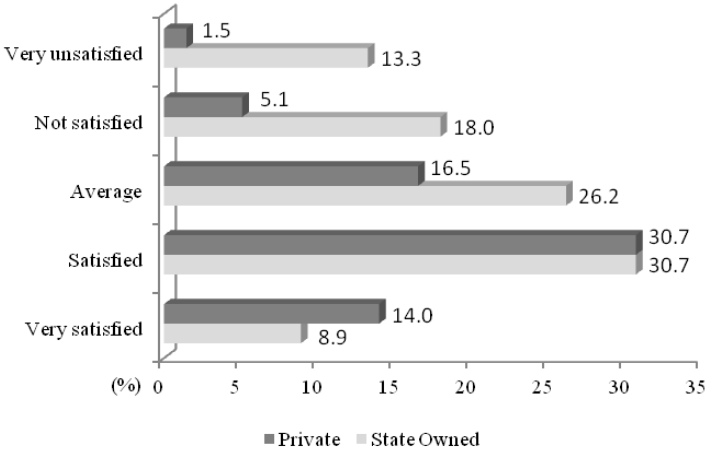
Graph 1. Health Self-Evaluation of complementary and alternative medicine (CAM) users



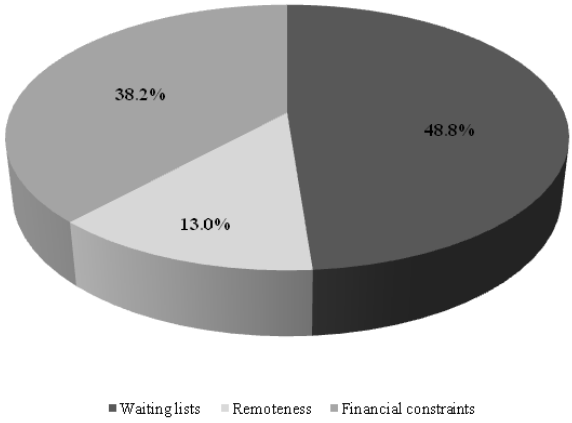
Graph 2. Health Self-Evaluation of complementary and alternative medicine (CAM) users compared to their ability of performing daily activities and the presence of long-term illnesses

Table 2. Risk of illness of complementary and alternative medicine (CAM) users due to risky behavior

Health Disorder; n(%)	Yes	No	Confirmed illness	I don't know
Obesity	126 (22.9)	391 (71.1)	27 (4.9)	6 (1.1)
Hypertension	101 (18.4)	287 (52.2)	148 (26.9)	14 (2.5)
Diabetes	81 (14.7)	414 (75.3)	39 (7.1)	16 (2.9)
Diseases of the heart and blood vessels	145 (26.4)	331 (60.2)	57 (10.4)	17 (3.1)
Pulmonary diseases	68 (12.4)	447 (81.3)	19 (3.5)	16 (2.9)
Malignancy	50 (9.1)	447 (81.3)	21 (3.8)	32 (5.8)
Cirrhosis of the liver	10 (1.8)	527 (95.8)	3 (0.5)	10 (1.9)
Sexually Transmitted Diseases	5 (0.9)	537 (97.6)	/	8 (1.5)
Injuries	37 (6.7)	492 (89.5)	5 (0.9)	16 (2.9)



Graph 3. Healthcare Satisfaction, Private/Government Practice



Graph 4. Reasons for lack of the required form of healthcare

Discussion

Health status and health perceptions are among the significant factors affecting the use of CAM, primarily poor health or inaccurate perceptions of one's own health (9, 11, 28, 29) in patients with chronic illnesses or disabilities (9) and more severe forms of disease (19, 30, 31), longer disease duration and complications present (32), often due to the lack of effects of conventional medical treatments or the unavailability of general practitioners (GPs) (33). Some studies have shown an association between the use of CAM and good health (28).

Additionally, it was observed that CAM use was more prevalent among patients who had already been hospitalized (11), who used multiple drugs, or who had been treated surgically (34).

A systematic review of the literature covering the cancer patient population showed that significant predictors of CAM use were younger age, female gender, higher education, higher income, and previous CAM use, whereas the most common reasons for its use were related to the fact that patients thus wished they could affect not only the cancer but their general health as well, as well as treat existing complications of cancer or therapy (18). In prostate cancer patients, the use of CAM in most studies was significantly higher in patients with higher education/income and in patients with more severe forms of disease (19).

In patients with low back pain, the results based on recent US studies indicate that the use of CAM is significantly associated with younger age, female gender, non-Hispanic background, and at least high school graduation. The most commonly cited reasons for using CAM were the presence of frequent, incapacitating and chronic low back pain, dissatisfaction with the availability of GPs, length of waiting times for GP's appointments, or lack of efficacy of conventional medical treatments (22).

The scientific research conducted in Serbia showed that just over 2/3 of the users of Complementary and Alternative medicine services had chronic health disorders. The most commonly diagnosed chronic health disorders among CAM users were the following: hypertension (36.7%), followed by back

problems (28%), followed by hypercholesterolemia (23.1%), allergy, without asthma (20.9%), and the cervical spine problem (18.9%). Four point four percent of CAM users were diagnosed with malignancy.

The beneficiaries of CAM services cite financial difficulties (28.5%), remoteness (6.8%) and waiting lists (26.8%) – as the main reasons for the lack of the necessary form of healthcare.

The two out of three users of CAM services rated their health as good and/or average. Respondents who reported poor health status (in their own estimation) were the rarest users of complementary and alternative medicine services.

The study conducted at the Institute for Oncology of Vojvodina, by interviewing patients diagnosed with gastroenterological malignancy, showed that 48 (24.9%) patients did not use any of the alternative medicine methods given, while 145 (75.1%) patients used at least one form of alternative therapy (35). Approximately 64% of patients used herbal preparations, most commonly beet juice (about 57%) (34). Special diets were used by 19.2% of patients, mind-body therapies were used by 16.6% of patients, while spiritual therapy was used by 18.1% of patients (35). Patients were most often informed of alternative therapy by other patients, relatives and neighbors (70.5% of patients) (35). As regards the reasons for using alternative medicine, 75.1% of patients indicated that they wanted to increase the chance of healing in combination with standard oncology therapy, whereas 47.7% of patients used alternative medicine for the purpose of improving their immunity, 27.5% believed they could prolong their life in this manner, and 18.6% believed that it would result in achieving a complete cure for malignant diseases (35).

Although worldwide research shows an accelerated upward trend in the use of CAM, in the Republic of Serbia there is only scarce evidence related to the extent of CAM use (25).

Conclusion

The analysis of the impact of respondents' health status, the analysis of the correlation between the respondents' health self-assessment and the use of alternative medicine methods, analysis of the impact of socio-demographic characteristics on the use of CAM, along with a comparative analysis of the use of health care services – would significantly contribute to better recognition of CAM by the Ministry of Health of the Republic of Serbia.

By making such a comparison, one could potentially work on how to further enhance defining patient treatment strategies.

Statement of Ethics

The Serbian National Health Survey 2013 was approved by the Ethical Board of the National Institute of Public Health of Serbia "Dr Milan Jovanović Batut" and the Ministry of Health.

Disclosure Statement

The authors declare that no conflicts of interest exist.

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SAMOPROCENA ZDRAVLJA KORISNIKA KOMPLEMENTARNE I ALTERNATIVNE MEDICINE U SRBIJI: NACIONALNA STUDIJA PRESEKA

Marina Luketina-Šunjka[1], Nemanja Rančić[2], Nataša Mihailović[3], Svetlana Radević[4], Saša Dragović[5], Mihajlo Jakovljević[6]

[1]Evropski centar za mir i razvoj, Univezitet pod pokroviteljstvom Ujedinjenih Nacija, Beograd, Srbija
[2]Univerzitet odbrane, Medicinski fakultet Vojnomedicinske akademije, Centar za kliničku farmakologiju, Vojnomedicinska akademija, Beograd, Srbija
[3]Centar za informatiku i biostatistiku u zdravstvu, Institut za javno zdravlje Kragujevac, Kragujevac, Srbija
[4]Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Katedra za socijalnu medicinu, Kragujevac, Srbija
[5]Klinika za opštu hirurgiju, Vojnomedicinska akademija, Beograd, Srbija
[6]Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Katedra za globalno zdravlje, zdravstvenu ekonomiju i politiku, Kragujevac, Srbija

Kontakt: Nemanja Rančić
Crnotravska 17, 11002 Beograd, Srbija
E-mail: nece84@hotmail.com

Cilj istraživanja bilo je ispitivanje uticaja zdravstvenog stanja ispitanika na upotrebu metoda komplementarne i alternativne medicine (KAM). Ovo je bila populaciona studija preseka. Uzorak je činilo 550 ispitanika, evidentiranih u trećem nacionalnom istraživanju zdravstvenog stanja stanovnika Srbije iz 2013. godine, koji su u predhodnih 12 meseci koristile usluge KAM. Nešto više od 2/3 korisnika usluga KAM ima hronični poremećaj zdravlja ($p < 0,01$). Najčešći dijagnostikovani hronični poremećaj zdravlja bila je hipertenzija (36,7%). Ozbiljno ograničen u obavljanju dnevnih aktivnosti u poslednjih 6 meseci i duže bio je svaki sedmi ispitanik, a delimično svaki četvrti korisnik KAM. Korisnici KAM zadovoljniji su uslugama koje dobijaju u privatnoj praksi ($p < 0,01$). Dvoje od troje korisnika usluga KAM sopstveno zdravlje ocenjuje kao dobro i/ili prosečno. Korisnici KAM zadovoljniji su uslugama koje dobijaju u privatnoj praksi i u najvećem procentu svoje zdravlje ocenili su kao dobro. Analiza uticaja zdravstvenog stanja ispitanika, povezanosti samoprocene zdravlja ispitanika i upotrebe metoda alternativne medicine, analiza uticaja sociodemografskih karakteristika na upotrebu KAM, uz uporednu analizu korišćenja usluga zdravstvene zašite, doprineli bi boljem pre-poznavanju KAM od strane Ministarstva zdravnja Republike Srbije.

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Ključne reči: *samoprocena zdravlja, zdravstveno stanje, komplementarna i alternativna medicina, hronične bolesti*

Case report

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MULTIPLE MODALITIES OF ARACHNOID CYST TREATMENT AND POSSIBLE COMPLICATIONS

Predrag Milošević[1], Aleksandar Kostić[1,2], Boban Jelenković[1], Luka Berilažić[1], Slavko Živković[1], Bojan Stanojević[1]

Arachnoid cysts are benign, congenital lesions of the brain that grow inside the leaves of the arachnoid. They represent 1% of all intracranial *mass* lesions and are most frequently detected during childhood and adolescence. In most cases, they do not cause any symptoms. However, they can grow to a large size and cause life-threatening symptoms, especially if they grow in a confined space such as a posterior cranial fossa.

Our case is a symptomatic arachnoid cyst of the posterior cranial fossa in a 22-year old man. The patient had symptoms and signs of progressive intracranial hypertension. CT of the brain showed the presence of an arachnoid cyst in the posterior cranial fossa that compresses the surrounding structures. The cyst was surgically treated with suboccipital craniectomy, excision of the cyst wall and drainage.

In our study, we examined the simultaneous use of different methods of arachnoid cyst drainage and their complications.

The goal was to show that the simultaneous use of multiple alternative methods of surgical treatment of these cysts have high efficiency in treatment because of the low possibility of recurrence.

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Key words: *arachnoid cyst, treatment modalities, intracranial hypertension*

[1]Clinical center Niš, Clinic for Neurosurgery, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Predrag Milošević
10 Jovana Skerlića St., 18000 Niš, Serbia
E-mail: pedja@doctor.com

Introduction

Arachnoid cysts are congenital lesions of the brain that arise from the separation of arachnoid layers. They are filled with cerebrospinal fluid (CSF) and do not interact with the ventricular system or subarachnoid space. Arachnoid cysts represent about 1% of all intracranial mass lesions (1) and their incidence, looking at the incidence of all arachnoid brain cysts, is about 20% in the posterior cranial fossa (2). Most of these cysts do not cause any symptoms. Those that are symptomatic generally manifest themselves as early as childhood in the form of convulsions, headaches, hemiparesis, craniomegaly, developmental disorders and premature puberty. Arachnoid cysts of the posterior cranial fossa can compress the fourth brain ventricle and cause hydrocephalus by pressing aqueduct of Sylvius.

In clinical practice, if they do not cause symptoms or cause a mass effect, these cysts are not treated, regardless of location and size.

The goal of surgical treatment of arachnoid cysts is not only their evacuation but also the prevention of re-accumulation of cerebrospinal fluid and recurrence. For this reason, the most recommended definitive treatment for these cysts is shunting into the peritoneum (3) or the vascular system.

Case report

Our case is a symptomatic arachnoid cyst of the posterior cranial fossa in a 22 year old man. The patient was admitted to the Clinic for Neurosurgery of the Clinical Center Niš due to severe headache, vomiting and blurred vision. The headache has been present for the last month and has been treated medically. A brain CT indicated a cystic formation in the cerebellum, which compresses the surrounding structures (Figure 1). An examination of the ocular fundus indicated pupillary stasis of 2 diopters.

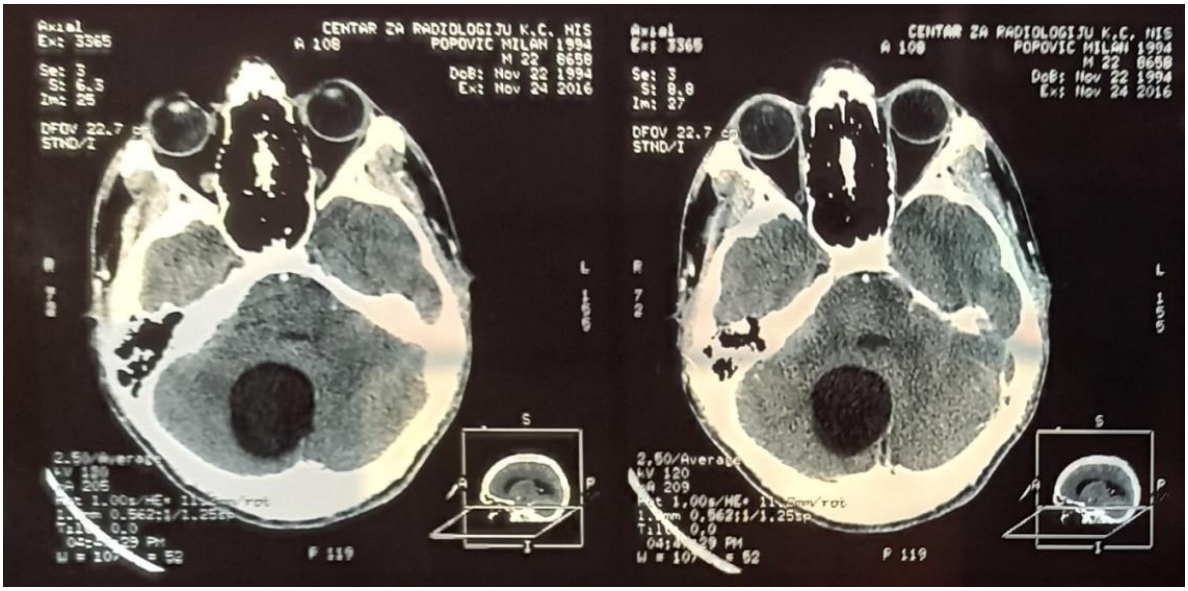

Figure 1. Cystic formation in the cerebellum that compresses the surrounding brain structures

The patient was conscious on admission – Glasgow Coma Score (GCS) was 15, vital parameters stable. The neurological examination showed no pathological findings. As the preparation for the surgery had begun, which included routine laboratory analysis and the coagulation status (INR 1.4, TR 349x10 * 9-l), the patient deteriorated to GCS 12.

Emergency surgery was performed, where external ventricular drainage was inserted after minor occipital craniectomy. The catheter tip was placed in the right occipital horn of the lateral cerebral ventricle. The resulting CSF does not come out under great pressure. Suboccipital craniectomy was then performed paramedially on the right side. Upon opening the dura, a cyst was found. The cyst wall was excised (4) and the liquid resembling CSF spontaneously drained under great pressure. The cyst wall was taken for PH analysis and the liquid resembling CSF was taken for cytological analysis. After hemostasis, a Torkildsen shunt was placed, where the cyst lumen was connected by a drain to the cisterna magna (5). Another drain was placed, connecting the cyst lumen with the subgaleatic space (Figure 2), as an alternative drainage, in case of necessity to place a cysto-peritoneal shunt, which is the definitive and according to the literature data the best way to resolve arachnoid cysts (2). The subgaleatic end of the drain was closed with a silver clip and additional ligatures. The dura was then sewn and plasticized with muscle and fibrin glue. The epidural drainage was placed suboccipital on the right and the soft tissues sewn by layers.

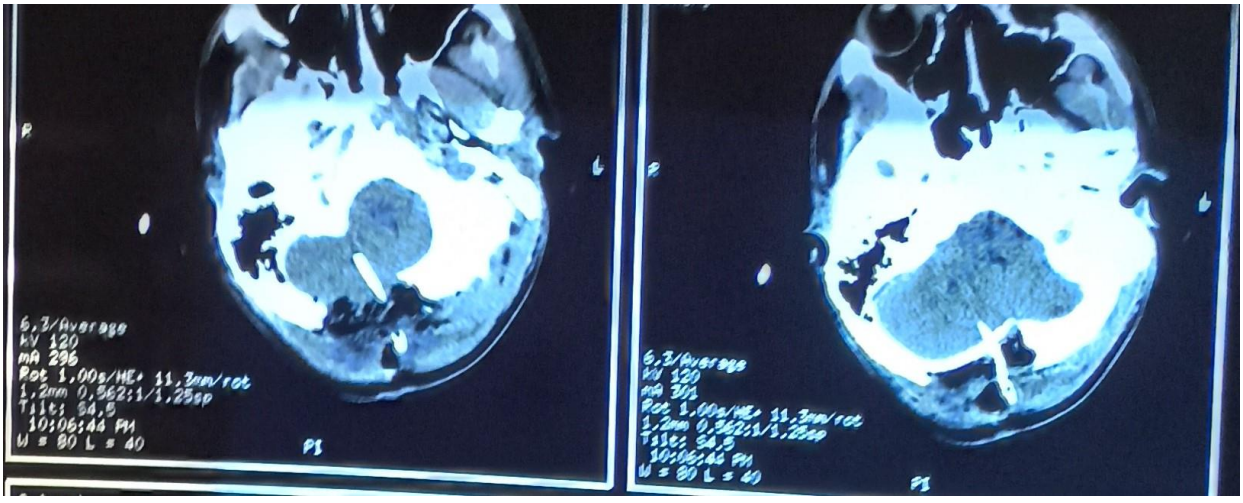

Figure 2. Drainage catheter connecting the cyst lumen to the subgaleatic space

Postoperatively, the patient was confused with evidence of left-sided hemiparesis in neurological status. A brain CT scan indicated the right sided acute parietal epidural hematoma (Figure 3). An emergency reoperation was performed in the form of parietal craniotomy with the evacuation of the epidural hematoma, placement of dural suspensions and epidural drainage. After this operation, the patient was conscious and without any neurological deficit. Control brain CT was without rests of epidural hematoma (Figure 4).

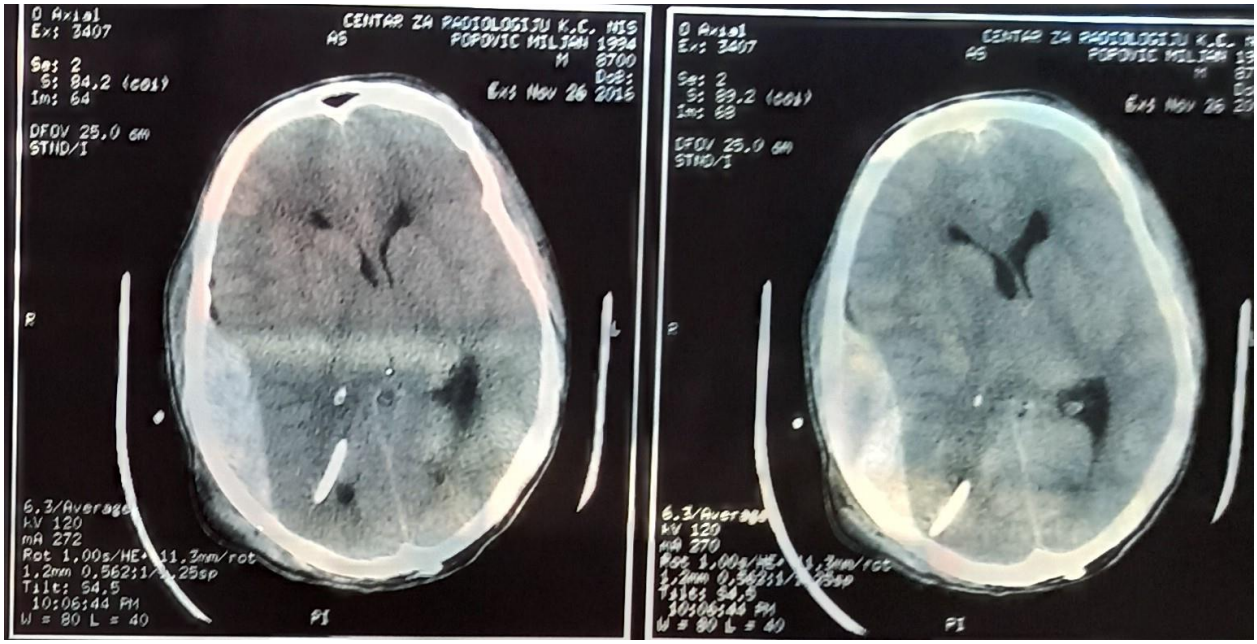

Figure 3. The right sided acute parietal epidural hematoma

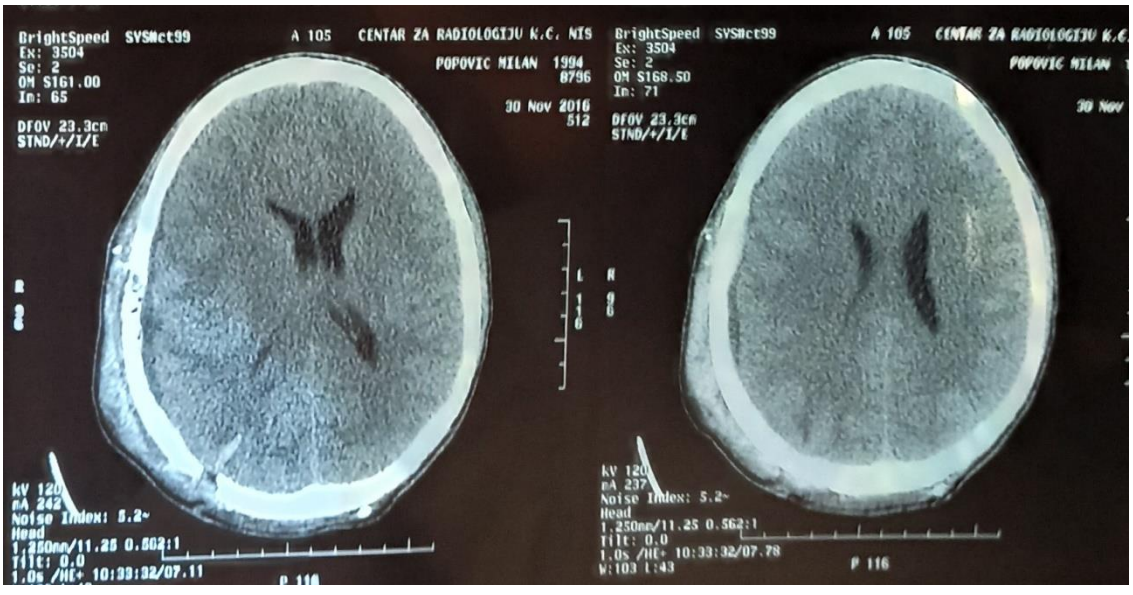

Figure 4. Control CT of the brain after evacuation of epidural hematoma

Postoperatively, the patient's condition was complicated with the development of subcutaneous cerebrospinal fluid collection in the suboccipital region and with febrile periods. The subcutaneous cerebrospinal fluid collection was resolved by compressive occipital circular bandages (6), and with the needle assisted aspiration of the subcutaneous collection. CSF sampled three times did not indicate

bacterial infection. Laboratory findings indicated neutrophil predominance in leukocytosis. Conventional empirical antibiotic therapy led to a normalization of body temperature and laboratory parameters.

The patient was discharged after 3 weeks with fully healed wounds, Glasgow Coma Score was 15, vital parameters stable, without any neurological deficit.

Discussion

Arachnoid cysts are congenital lesions that become subject to treatment only when they cause disturbances in patients in the form of convulsions, psychomotor disturbances or symptoms and signs of intracranial hypertension (7), which was the clinical course in our patient. The most common types of surgical treatment for these cysts are excision and evacuation of the cyst after craniotomy, needle aspiration, endoscopic cyst fenestration, and shunting of the cyst into the peritoneum or vascular bed (2). One of the most common complication of operations is a CSF leak of 5-17% (8). According to the literature (2), the CSF leak can be minimized or completely eliminated by the prophylactic use of external ventricular drainage. Therefore, prior to cyst evacuation surgery, we placed external ventricular drainage. It is a well-known fact (7) that arachnoid cysts have a recurrence rate of 10%, so after we evacuated the cyst, we placed the Tokildsen system of cysto-cisternal drainage and an additional cysto-subgaleatic shunt as an alternative precaution in case of inefficiency of the Tokildsen system. We closed the cysto-subgaleatic shunt with a silver clip and ligatures in the subcutaneous tissue, thus allowing us to open the shunt rapidly and convert it into a cysto-peritoneal shunt, if necessary.

Postoperatively, an acute epidural hematoma appeared in the region where the primary surgery was not performed. The patient had no coagulation disorders (INR 1.4, TR 349x10 * 9-l). External ventricular drainage was placed occipital on the right side, the burr hole was in the immediate vicinity of the site where the epidural hematoma was developed. The incidence of postoperative epidural hematomas is 1% (9). The Mayfield head holder was used, because the primary surgery was performed in a sitting position, whose pins (10) could cause a skull fracture if too much pressed into the skull, and thus potentially cause an epidural hematoma outside the operating region. However, postoperative brain CT did not verify non-operative skull trauma. Preoperatively, the patient was given medical therapy against brain swelling, and during the operation three methods were performed that could reduce the intracranial volume - external ventricular drainage, cysto-cisternal shunt and cysto-subgaleatic shunt. Cyst evacuation surgery itself also reduces intracranial volume. All of these methods, collectively or individually, can cause abrupt intracranial decompression and provoke the formation of an epidural hematoma due to dural separation off of the skull. External ventricular drainage can induce spontaneous epidural hematoma due to sudden excessive drainage of the CSF (11). A cysto-cisternal shunt can cause excessive drainage, especially if there is communication of the cyst with the ventricular system of the brain. The subgaleatic shunt was closed in our case, but the reliability of this was questionable because of the fact that the patient had a persistent subcutaneous collection of CSF at the point where the subgaleatic part of this drain was placed.

Conclusion

Using multiple alternative methods for surgical treating of arachnoid cysts in the same act, have the advantage in bigger chance for preventing of the cyst recurrence. Standard procedure in treatment of posterior fossa lesions usually is intra or preoperative ventricular drainage. In our case, the complication was a delayed epidural hematoma (12), caused by a minor craniectomy that was used for external ventricular drainage and not by alternative drainage methods of arachnoid cyst. For this reason, it is necessary to do adequate neurological monitoring of the patient after surgery and perform early CT diagnostics in case of the patient's deterioration. In our case, those measures led to early detection of the epidural hematoma as a complication.

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MULTIPLI MODALITETI LEČENJA ARAHNOIDALNE CISTE I MOGUĆE KOMPLIKACIJE

Predrag Milošević[1], Aleksandar Kostić[1,2], Boban Jelenković[1], Luka Berilažić[1], Slavko Živković[1], Bojan Stanojević[1]

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

Kontakt: Predrag Milošević
Jovana Skerlića 10, 18000 Niš, Srbija
E-mail: pedja@doctor.com

Arahnoidalne ciste su benigne, kongenitalne lezije mozga, koje rastu unutar listova arahnoideje. Predstavljaju 1% svih intrakranijalnih *mass* lezija i najčešće se otkrivaju u detinjstvu i adolescenciji. U većini slučajeva, one ne izazivaju nikakve simptome. Međutim, mogu narasti do velikih dimenzija i prouzrokovati po život opasne simptome, posebno ako rastu u skučenom prostoru, kao što je zadnja lobanjska jama.

U ovom radu predstavljamo simptomatsku arahnoidalnu cistu zadnje lobanjske jame kod dvadesetdvogodišnjeg muškarca. Bolesnik je imao simptome i znakove progresivne intrakranijalne hipertenzije. CT mozga pokazala je prisustvo arahnoidalne ciste u zadnjoj lobanjskoj jami, koja pritiska okolne strukture. Cista je hirurški tretirana subokcipitalnom kraniektomijom, ekscizijom zida ciste i drenažom.

U našem istraživanju, ispitali smo istovremeno korišćenje različitih metoda drenaže arahnoidalne ciste i njihove komplikacije.

Cilj je bio pokazati da istovremena primena više alternativnih metoda hirurškog lečenja ovih cisti ima prednost u odnosu na korišćenje jedne metode lečenja, zbog manje mogućnosti recidiva.

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Ključne reči: *arahnoidna cista, modeliteti lečenja, intrakranijalna hipertenzija*

Original article

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IMMUNOHISTOCHEMICAL AND MORPHOMETRIC STUDY OF ADENOHYPOPHYSEAL GONADOTROPIC CELLS IN MALE CADAVERS OF DIFFERENT AGES

Jovana Čukuranović-Kokoris[1], Ivan Jovanović[1], Sladjana Ugrenović[1], Vesna Stojanović[1], Miljana Pavlović[1], Braca Kundalić[1], Vladimir Živković[1], Milorad Antić[1], Rade Čukuranović[1]

The literature data suggest that with advancing age there occurs a functional decline in the gonadotropic cells, while the data concerning structural changes in these cells are rather scarce. The aim of this paper was to detect and quantify the changes in human gonadotropic cells of the anterior pituitary in male cadavers of different ages using immunohistochemical and morphometric methods. The material in this study consisted of adenohypophyseal tissue from 14 male cadavers of different ages, starting from the fourth decade of life. Adenohypophyseal tissue sections were routinely histologically processed and stained with immunohistochemical monoclonal anti-LH antibody to detect gonadotropic LH cells. Digital images of the visual fields of immunohistochemically processed adenohypophyseal sections were then morphometrically analyzed using the Image J system. Statistical analysis was performed using the SPSS statistical software package. The results of the morphometrical analysis showed that volume density of LH cells did not change significantly with advancing age, while their area, perimeter and Feret's diameter increased statistically significantly. Nuclear morphometric parameters did not change significantly, while the nuclear-cytoplasmic ratio of LH cells decreased with ageing, with a statistically significant decline observed in cases aged over 70 years. Based on the obtained results the conclusion may be drawn that the density of LH cells does not change significantly with ageing, but that they undergo hypertrophy in order to maintain normal hormonal secretion. Long-lasting hypertrophy of these cells ultimately leads to their functional decline, which reaches statistical significance after 70 years of age.

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Key words: *luteinizing gonadotropic cells, adenohypophysis, immunohistochemistry, morphometry, ageing*

[1]University of Niš, Faculty of Medicine, Department of Anatomy, Niš, Serbia

Contact: Jovana Čukuranović-Kokoris
81 Dr. Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: jovana.cukuranovic.kokoris@medfak.ni.ac.rs

Introduction

Gonadotropic cells, i.e., LH and FSH cells, represent about ten percent of the overall cellular population of the anterior lobe of the pituitary. They are evenly distributed in the distal portion of adenohypophysis and histologically appear basophilic. Gonadotropes are oval cells with round nuclei and well developed organelles. Their cytoplasm contains electron-dense secretory granules which measure 200-250 nm in diameter, and are filled with hormones (1).

The main secretory products of gonadotropes are luteinizing (LH) and follicle stimulating hormone (FSH). Gonadotropin-releasing hormone (GnRH) stimulates the production and secretion of these hormones, while gonadal steroids, inhibin, follistatin and activin, by way of negative feedback loop, inhibit their production and secretion.

In women, LH is involved in ovulation and follicular luteinization, while in men it stimulates Leydig cells to secrete testosterone. FSH in women stimulates follicle maturation, while in men, it stimulates Sertoli cells to secrete androgen-binding protein (2, 3), which binds specifically to testosterone and transports it through the germinative epithelium into the lumen of the tubules. Hereby, it enhances spermatogenesis in the seminiferous tubules of the testis.

LH and FSH represent low molecular weight glycoproteins of about 30 kDa. They exert their effects on target tissues activating the cyclic adenosine monophosphate secondary messenger system,

which further activates specific enzyme systems in appropriate target cells (4).

From the point of view of endocrinology, ageing is predominantly characterized by a decline of function of the systems which support tissue anabolism. In males, it mostly relates to two very important components of the neuroendocrine system: gonadotropic and somatotropic axes. The term gonadopause generally describes a decline in biological availability of testosterone (T), while the term somatopause denotes a decline in biological availability of growth hormone (GH) and/or IGF-1. Nevertheless, these processes may markedly vary between different individuals, and their intensity is influenced as well by environmental factors and life habits.

In men, adenohypophysis begins to secrete gonadotropic hormones with the onset of puberty, and continues with the secretion throughout life following a gradually decreasing pattern. However, in most men, gradually decreasing sexual function becomes evident in late 40s or 50s, and is correlated with decreased testosterone secretion. Testosterone secretion usually drops significantly after 50 years of life, and in 80-year olds it ranges between 20% and 50% of the testosterone amount at the time of its maximal secretion. Despite this, testicular morphology, spermatogenesis and fertility demonstrate only minor changes in older men (4, 5).

Gonadopause (or late hypogonadism) is characterized by a progressive dysregulation of the hypothalamic-pituitary-gonadal axis, most probably under the influence of age-related decline in maximal hypothalamic secretion of GnRH, decline of the maximal and average LH pulse amplitude (with preserved basal frequency of LH pulse secretion), decline of LH-stimulated testosterone secretion, and reduction of testosterone-mediated negative feedback loop (6). The consequence of all of the above is *biochemical* late hypogonadism, with lower serum concentrations of total testosterone, bioavailable testosterone and free testosterone, then with a moderate rise of serum LH and FSH and ultimately with elevated serum levels of globulins which bind sex hormones (7). Late hypogonadism *clinically* manifests with the symptoms similar to those in hypogonadism which affects younger males, including reduced bone and muscle mass, abdominal obesity, loss of pubic hair and beard hair. Moreover, hypogonadism results in a number of non-specific symptoms, including nervousness, irritability, psychological depression, memory loss, weakness, insomnia, hot flashes, periodical sweating, and loss of sexual drive (7).

In their paper, Schwartz et al. (8) have reported that the function of adenohypophysis probably represents the result of integration of multiple input signals, including hypothalamic, peripheral and intrapituitary ones. Intrapituitary factors may exert stimulatory or inhibitory effects on hormone production by the adenohypophysis. The production and secretion of LH is also controlled by a variety of locally produced signalling molecules, which form a complex network and are involved in autocrine/ paracrine control of the function of gonadotropes (9). According to Denef (10), gonadotropes are involved in interactions with other hormonal (mostly lactotropic, somatotropic and corticotropic) and non-hormonal cells, such as folliculostellate (FS) cells.

Considering the fact that hypogonadism which occur with ageing may be the consequence of structural changes at all levels of the gonadotropic axis and that age-related histomorphologic changes, when this regulatory system is concerned, are thoroughly studied only in testis, our aim in this study was to detect and quantify the changes in human gonadotropic cells of the adenohypophysis, as a component part of the gonadotropic axis, in cases of different age, using immunohistochemical and morphometric methods (measurements of volume density, area, perimetar, Feret's diameterand calculation of the nuclear-cytoplasmic ratio).

Materials and methods

The study took place at the Institute of Anatomy, University of Niš Faculty of Medicine, Center for Forensic Medicine in Niš, and Center for Pathology, Clinical Center Niš, all of which are the teaching bases of this Faculty. Immunohistochemical processing of histological sections of adenohypophysis was done at the *Siniša Stanković* Institute for Biological Research in Belgrade.

Material

The study material consisted of hypophyseal tissue taken from 14 male cadavers, aged 41 to 87 years. The tissue samples were taken during routine autopsies performed at the Center for Forensic Medicine in Niš, after a post-mortem period which did not exceed 24 hours, abiding by the ethical norms regulating the use of cadaveric material for biomedical research purposes (decision by the Ethics Committee of the University of Niš Faculty of Medicine, № 12-2307-2/8 of March 10, 2016). The cadavers used in this study were without any diagnosed neurological, psychiatric or endocrine disorders during life. There was no visible damage to the brain or hypophysis on autopsy in any of the cases. Further, histopathological evaluation of the brain and hypophysis excluded the presence of a possible hidden or misdiagnosed disease. Cadavers were classified into three age groups: first (I), with cases aged 30-49 years; second (II), with cases aged 50-69 years; and third (III), with cases aged 70 years and older.

The methodology of the study involved an adequate dissection procedure for sampling of the hypophysis, followed by histological preparation of the samples and their morphometrical and statistical analysis.

Dissection procedure

The sellar diaphragm (*diaphragma sellae*) was the first to be removed by dissection. After that, the pituitary stalk (*infundibulum*) was sectioned, and the hypophysis was then carefully detached from the

surrounding osseous structures of the sella turcica and removed *en bloc*.

Histological preparation

Our histological analysis, in the sense of identification of possible changes in the structure of gonadotropic cells of the adenohypophysis with ageing, was based on the light microscopy evaluation of their properties.

The removed hypophyses were fixed for 24 hours in 10% buffered formalin and then embedded in paraffin. The resulting paraffin molds were used to obtain up to 5 μm thick hypophyseal tissue sections using the Leica 2235 microtome, which were then routinely stained with hematoxylin-eosin (H&E) and immunohistochemically processed. The presence of cells with a positive reaction to the applied immunohistochemical marker was established by immunohistochemical analysis.

Immunohistochemical staining of LH gonadotropic cells of the adenohypophysis

The peroxidase-antiperoxidase (PAP) method was used for immunohistochemical staining of LH gonadotropic cells (11). Immunocytochemical methods, in particular the specific reaction between the primary antibody and antigen enables differential staining of cells carrying the specific antigen, in this case a hormone. After deparaffinization and rehydration of the tissue sections, first the activity of endogenous peroxidase was blocked by incubation of tissue sections in 0.3% H_2O_2 in methanol for 15 minutes. The sections were then washed in 0.01M phosphate buffer (Phosphate Buffer Saline PBS; pH 7.6; 2 x 5 min.), and the reduction of non-specific staining was accomplished by incubation of the sections in normal swine serum (Normal swine serum, Dako Dakopatts, Denmark; dilution 1:10 in PBS) for 1 hour.

Primary anti-LH (1:100) anti-rat antibodies (a donation from Dr Parlow; NIH, Bethesda, Md., USA) diluted in PBS were used to detect LH gonadotropic cells (12). The incubation lasted for 24 hours at room temperature. After the incubation with the primary antibody, the sections were washed in PBS (2 x 5 minutes) and then incubated for 1 hour with secondary conjugated antibody (Policlonal swine anti rabbit IgG (HRP), Dako Dakopatts, Denmark; dilution 1:200 in PBS). After washing in PBS, visualization was made possible by using DAB (Dako). Contrast enhancement was performed using Mayer's hematoxylin solution (Merck-Alkaloid, Alkaloid, Skopje, Republic of North Macedonia), dehydration with increasing ethanol series, and mounting by using DPX.

Morphometric analysis

Morphometric analysis was performed using the digital image taken with 1.3 megapixel digital camera. Twenty visual fields were obtained from each of the adenohypophyseal lateral wings and 20 from the middle portion in each analyzed case, with 60 visual fields in total per each analyzed case. Image analysis was accomplished using the ImageJ system (https://imagej.nih.gov/ij/).

Astereological analysis

Our astereological analysis of gonadotropic LH cells included the measurement of their area (ALH), perimeter (BLH) and Feret's diameter (DfLH). Additionally, for each of the selected somatotropic LH cells, we measured the parameters of area (ANLH), perimeter (BNLH) and Feret's diameter (DfnLH) of the nucleus. The nuclear-cytoplasmic ratio, as the parameter of metabolic activity and functional status of somatotropic LH cells ((N/C)LH), was calculated as the ratio of nuclear area and cytoplasmic area, with the cytoplasmic area obtained as the difference of the area of these cells and area of their nuclei. The measurement of 60 gonadotropic LH cells was performed for each of the analyzed cases.

Stereological analysis

Stereological analysis was performed using the multipurpose test system M168 (d = 17.88 μm, a = 15.49 μm^2, AT = 2601.54 μm^2, LT = 1501.92 μm), placed over the analyzed digital image of histological sections. Volume density of gonadotropic LH cells (VVLH) was obtained as the ratio of the number of dots in the test system which hit immunopositive cells (PF) and the total number of dots in the system (PT = 168) (13), per each analyzed field.

The values of area, perimeter and Feret's diameter, then of nuclear area, perimeter and Feret's diameter, nuclear-cytoplasmic ratio and volume density of LH cells per each analyzed case were obtained as the average of values for all measured visual fields.

Statistical analysis

Statistical analysis was performed using the SPSS statistical software package (version 16). The correlation of age with the measured morphometric parameters was evaluated by the calculation of linear correlation and linear regression.

A more precise dynamics of the values of morphometric parameters for the age groups was analyzed using One Way ANOVA and Tukey-Kramer *post hoc* test.

The t-test was used to establish statistical significance of the differences between two dependent samples.

Results

Histological analysis

Histological analysis of adenohypophyseal gonadotropic LH cells during ageing involved the analysis of immunohistochemically stained tissue sections of the gland.

In younger cases, gonadotropic LH cells were dispersed in the lateral wings of adenohypophysis, while in the mucoid, wedge-shaped portion of the gland these cells were localized within the acinar structures, the number of which varied between cases. Gonadotropic LH cells were in such cases oval or polygonal and with centrally or (more often) eccentrically positioned immunonegative blue-stained euchromatic nucleus. Their cytoplasm was immunopositive, brown-stained, with prominent granular appearance due to the presence of numerous secretory granules (Figure 1).

In older cases, except for the interstitial fibrosis of variable degree and consequential reduced presence of blood vessels, the distribution and presence of LH cells did not differ significantly from that in younger cases. Gonadotropic cells were larger, more often round, and with eccentrically positioned, smaller, hyperchromatic, immunonegative nuclei compared to younger cases. Immunopositivity reaction was similar to that of the same gonadotropes in younger cases (Figure 2).

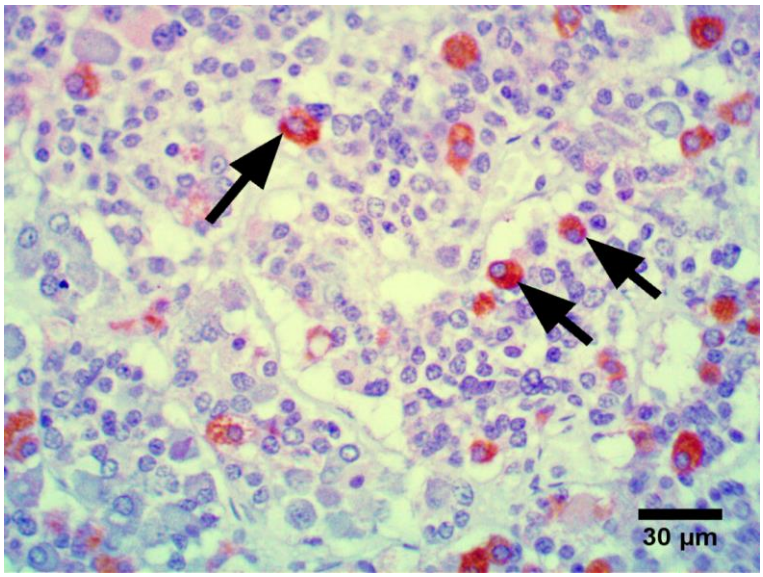


Figure 1. Tissue section from a 41-year old man; immunopositive LH cells with eccentric or centrally positioned euchromatic nucleus (arrows); anti-LH antibody; PAP, x40

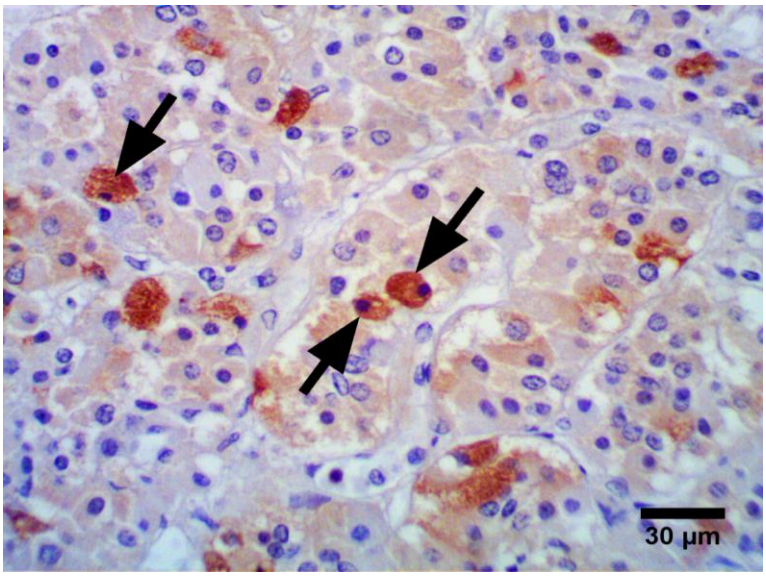


Figure 2. Tissue section of an 87-year old man; immunopositive LH cells of the adenohypophysis; large immunopositive LH cells with small, eccentrically positioned hyperchromatic nuclei (arrows); anti-LH antibody; PAP, x40

Morphometric analysis

The results of morphometric analysis of adenohypophyseal LH immunoreactive cells are presented in Table 1.

The case number 1 was excluded from further morphometric analysis due to a significant age difference compared to the other group 1 cases.

Correlation analysis indicated the presence of a statistically significant correlation between age and astereological parameters (average area, perimeter and Feret's diameter) and nuclear-cytoplasmic ratio of LH immunoreactive cells of the adenohypophysis in analyzed cases (Table 2).

Table 1. Results of morphometric analysis of adenohypophyseal LH immunoreactive cells

Case	Age	Group	A_{LH} (μm^2)	B_{LH} (μm)	D_{FLH} (μm)	A_{NLH} (μm^2)	B_{NLH} (μm)	D_{FNLH} (μm)	$(N/C)_{LH}$	V_{VLH} (%)	V_H (cm^3)
1	22	I	123.26	41.16	14.94	20.35	16.62	5.97	0.20	2.90	0.7
2	41	I	107.60	38.57	13.84	22.16	17.56	6.29	0.26	3.12	0.5
3	45	I	136.72	43.83	15.63	24.37	17.99	6.32	0.22	3.31	0.4
4	48	I	109.52	38.92	13.83	20.60	16.82	6.09	0.23	3.45	0.7
5	48	I	142.16	44.87	16.18	28.55	19.67	7.17	0.25	3.61	0.7
6	57	II	131.29	42.64	15.50	23.54	17.89	6.45	0.22	4.13	0.25
7	61	II	121.01	40.54	14.57	24.18	17.92	6.28	0.25	8.04	0.3
8	65	II	147.83	45.66	16.37	27.37	19.28	6.96	0.23	4.35	0.4
9	65	II	138.10	43.29	15.50	20.58	16.68	5.94	0.18	3.00	0.4
10	66	II	156.08	45.90	16.55	30.12	20.10	7.23	0.24	8.23	0.5
11	76	III	165.11	50.00	18.51	26.25	19.12	6.93	0.19	7.70	0.8
12	76	III	123.46	42.41	15.82	19.97	16.73	6.21	0.19	2.54	0.4
13	77	III	161.76	48.14	17.76	27.55	19.64	7.26	0.21	5.54	0.6
14	78	III	129.99	43.13	15.65	23.46	17.82	6.42	0.22	1.92	0.4
15	87	III	168.33	49.03	17.73	21.55	17.41	6.32	0.15	4.82	0.4

Table 2. Correlation matrix of age and values of morphometric parameters of LH immunoreactive cells of the adenohypophysis in analyzed cases

Parameter		A_{LH}	B_{LH}	D_{FLH}	A_{NLH}	B_{NLH}	D_{FNLH}	$(N/C)_{LH}$	V_{VLH}
Age	R	0.92	0.92	0.92	0.20	0.27	0.35	-0.86	0.25
	p	< 0.001	< 0.001	< 0.001	0.49	0.36	0.22	< 0.001	0.38
	N	14	14	14	14	14	14	14	14

Linear regression analysis was aimed at evaluation of the association between astereological parameters (average area, perimeter and Feret's diameter) of adenohypophyseal gonadotropic cells and age of the analyzed cases (Table 3). The results demonstrated that the age of analyzed cases was a statistically significant predictor of area ($(F(1,12) = 68.59$, $p < 0.001$), perimeter ($F(1,12) = 63.37$, $p < 0.001$) and Feret's diameter ($F(1,12) = 62.67$, $p < 0.001$), which can be represented by the following three models: ALH = 28.83 + Age x 1.79, BLH = 24.03 + Age x 0.32 and DFLH = 8.04 + Age x 0.13. The average area, perimeter and Feret's diameter of gonadotropic cells of the adenohypophysis statistically significantly increased during the process of ageing, and age could be held accountable for 85% of total variance of area ($R^2 = 0.85$) (Graph 1), 84% of total variance of perimeter ($R^2 = 0.84$) and 84% of variance of Feret's diameter ($R^2 = 0.84$) (Graph 2), and in all three cases represents a large magnitude effect.

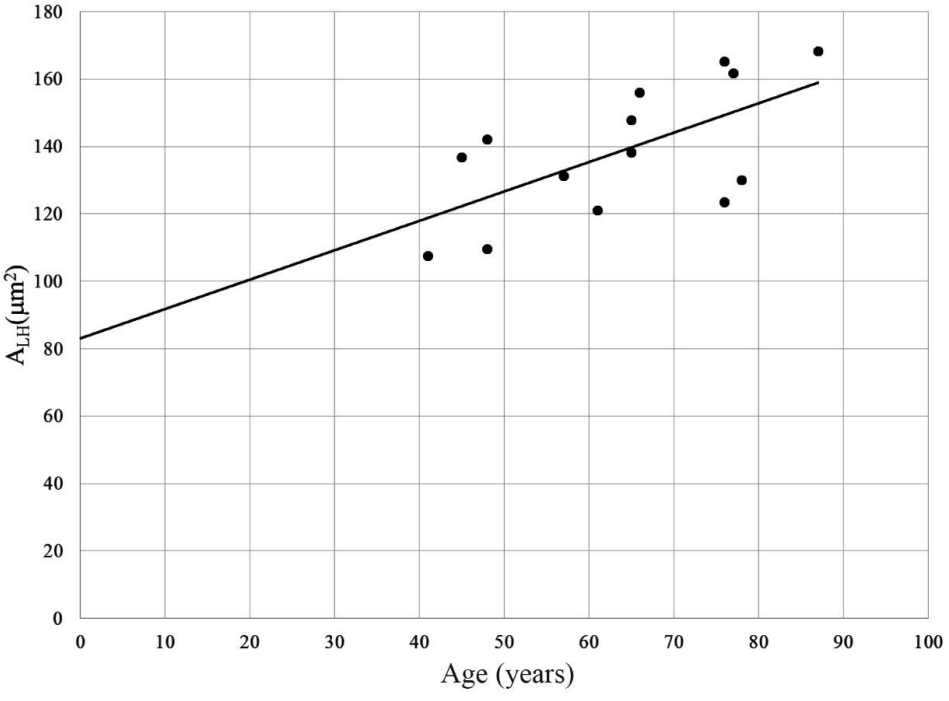
In contrast to the above, age was not a statistically significant predictor when astereological parameters of the nuclei of gonadotropic adenohypophyseal cells were concerned (area, perimeter and Feret's diameter) in the examined cases ($p > 0.05$) (Table 2).

Age represented a statistically significant predictor of average nuclear-cytoplasmic ratio of adenohypophyseal gonadotropic cells ($F(1,12) = 32.53$, $p < 0.001$) (Table 3). This ratio could be identified using the following model: $(N/C)LH = 0.384 - Age \times 0.003$, which meant that the values of the nuclear-cytoplasmic ratio statistically significantly declined during ageing (Graph 3). Age was accountable for 73% of total variance of this parameter ($R^2 = 0.73$), which was a large magnitude effect.

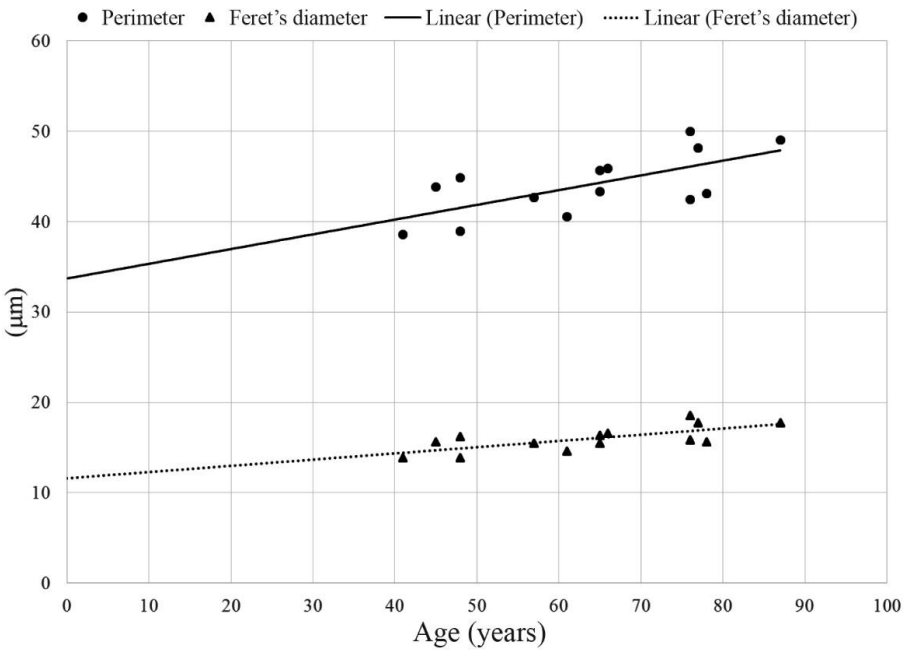
Age did not represent a statistically significant predictor of volume density of adenohypophyseal gonadotropic cells in the same cases ($p > 0.05$) (Table 2).

Table 3. Results of bivariate linear regression analysis of age as an independent, and analyzed morphometric parameters of adenohypophyseal LH immunoreactive cells as the dependent variables

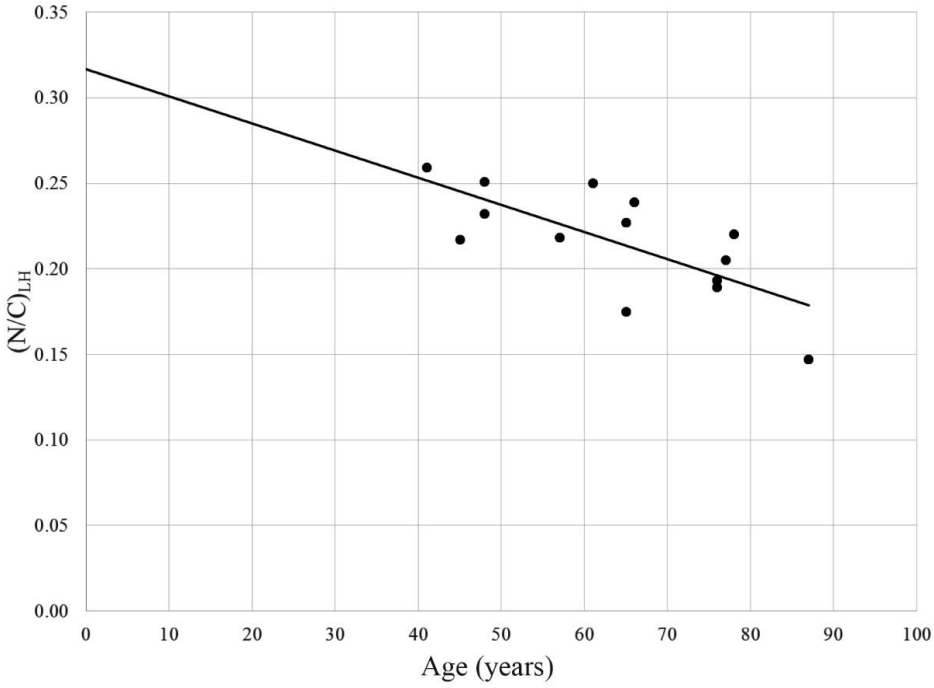
A_{LH}					
Variable	B	SEB	β	t	p
Constant	28.83	14.03		2.05	0.062
Age	1.79	0.22	0.92	8.28	< 0.001
$R^2 = 0.85$; $F(1,12) = 68.59$, $p < 0.001$; Model: $A_{LH} = 28.83 + Age \times 1.79$					
B_{LH}					
Variable	B	SEB	β	t	p
Constant	24.03	2.64		9.09	< 0.001
Age	0.32	0.04	0.92	7.96	< 0.001
$R^2 = 0.84$; $F(1,12) = 63.37$, $p < 0.001$; Model: $B_{LH} = 24.03 + Age \times 0.32$					
D_{FLH}					
Variable	B	SEB	β	t	p
Constant	8.04	1.05		7.67	< 0.001
Age	0.13	0.02	0.92	7.92	< 0.001
$R^2 = 0.84$; $F(1,12) = 62.67$, $p < 0.001$; Model: $D_{FLH} = 8.04 + Age \times 0.13$					
$(N/C)_{LH}$					
Variable	B	SEB	β	t	p
Constant	0.384	0.031		12.511	< 0.001
Age	-0.003	0.0005	-0.8547	-5.7036	< 0.001
$R^2 = 0.73$; $F(1,12) = 32.53$, $p < 0.001$; Model: $(N/C)_{LH} = 0.384 - Age \times 0.003$					



Graph 1. Correlation between age and area of adenohypophyseal LH immunoreactive cells in the examined cases



Graph 2. Correlation between age and perimeter, i.e. Feret's diameter, of adenohypophyseal LH immunoreactive cells in the examined cases



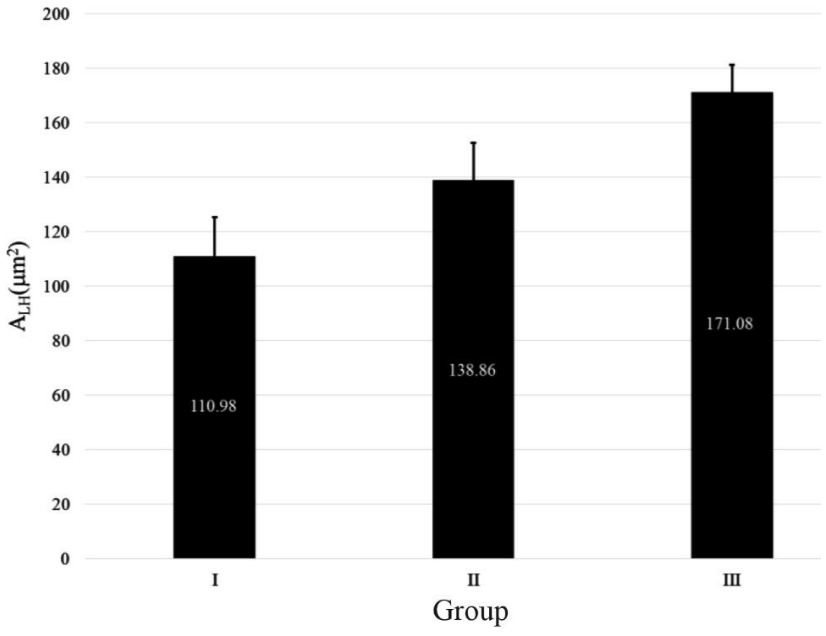
Graph 3. Correlation between age and nuclear-cytoplasmic ratio of adenohypophyseal LH immunoreactive cells in the examined cases

A more detailed dynamics of age-related changes of the average values of morphometric parameters of adenohypophyseal gonadotropic cells was evaluated using the One Way ANOVA test (Table 4).

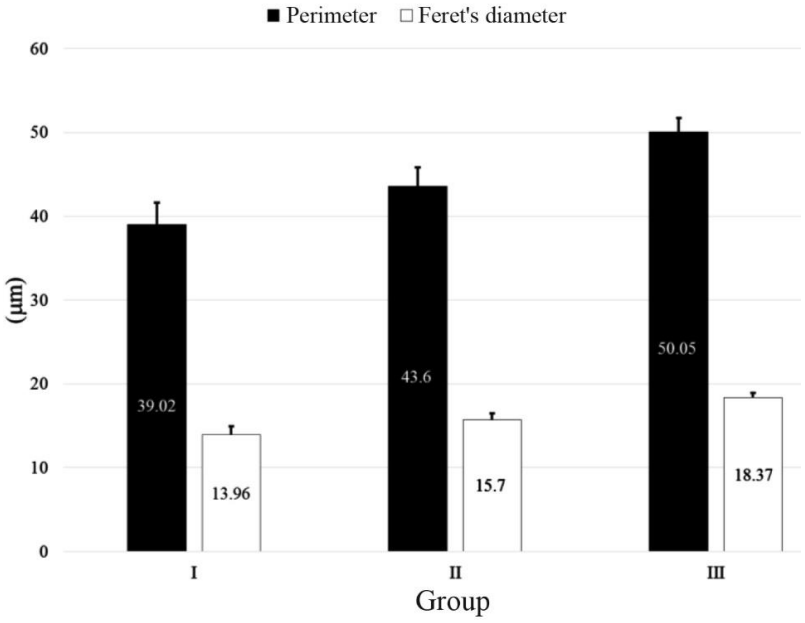
Average area ($F(2,11) = 24.88$, $p < 0.001$), then average perimeter ($F(2,11) = 29.82$, $p < 0.001$) and average Feret's diameter ($F(2,11) = 37.61$, $p < 0.001$) of adenohypophyseal gonadotropic cells statistically significantly increased during the process of ageing (Graphs 4 and 5).

Table 4. Results of univariate ANOVA test involving the average values of morphometric parameters of adenohypophyseal LH immunoreactive cells in the analyzed age groups

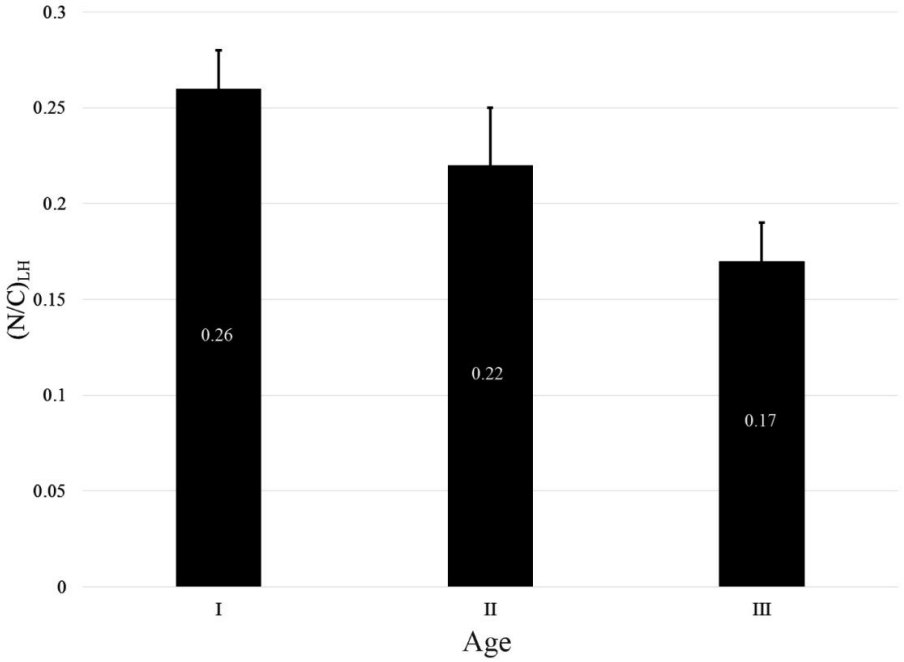
Parameter	Group	N	Average	SD	SE	95% CI		Tukey post hoc test
						LB	UB	
A_{LH} (μm²)	I	4	110.98	14.41	7.20	88.06	133.9	a, b
	II	5	138.86	13.72	6.14	121.82	155.9	a, c
	III	5	171.08	10.25	4.58	158.36	183.81	b, c
ANOVA	F (2,11) =24.88, p < 0.001							
B_{LH} (μm)	I	4	39.02	2.62	1.31	34.85	43.19	a, b
	II	5	43.60	2.23	1.00	40.83	46.38	a, c
	III	5	50.05	1.64	0.73	48.01	52.08	b, c
ANOVA	F (2,11) = 29.82, p < 0.001							
D_{FLH} (μm)	I	4	13.96	0.95	0.48	12.45	15.48	a, b
	II	5	15.70	0.80	0.36	14.71	16.69	a, c
	III	5	18.37	0.57	0.25	17.66	19.08	b, c
ANOVA	F (2,11) = 37.61, p < 0.001							
A_{NLH} (μm²)	I	4	22.87	3.67	1.83	17.04	28.71	/
	II	5	25.16	3.68	1.64	20.60	29.72	/
	III	5	24.37	2.89	1.29	20.78	27.96	/
ANOVA	F (2,11) = 0.51, p = 0.62							
B_{NLH} (μm)	I	4	17.50	1.22	0.61	15.55	19.45	/
	II	5	18.37	1.33	0.60	16.72	20.03	/
	III	5	18.27	1.27	0.57	16.69	19.84	/
ANOVA	F (2,11) = 0.60, p = 0.57							
D_{FNLH} (μm)	I	4	6.25	0.45	0.22	5.54	6.96	/
	II	5	6.57	0.52	0.23	5.93	7.22	/
	III	5	6.65	0.50	0.23	6.02	7.27	/
ANOVA	F (2,11) = 0.78, p = 0.48							
$(N/C)_{LH}$	I	4	0.26	0.02	0.01	0.23	0.29	b
	II	5	0.22	0.03	0.01	0.19	0.26	c
	III	5	0.17	0.02	0.01	0.14	0.20	b, c
ANOVA	F (2,11) = 16.26, p = 0.001							
V_{VLH} (%)	I	4	3.45	0.23	0.12	3.07	3.82	/
	II	5	5.55	2.42	1.08	2.55	8.55	/
	III	5	4.73	2.06	0.92	2.17	7.29	/
ANOVA	F (2,11) = 1.33, p = 0.30							
a - I : II, p < 0.05; b - I : III, p < 0.05; c - II : III, p < 0.05								



Graph 4. Average area of adenohypophyseal LH immunoreactive cells in the analyzed age groups



Graph 5. Average perimeter and average Feret's diameter of adenohypophyseal LH immunoreactive cells in the analyzed age groups



Graph 6. Average nuclear-cytoplasmic ratio of adenohypophyseal LH immunoreactive cells in the analyzed age groups

The *post hoc* Tukey-Kramer test indicated that the above parameters showed an identical trend during ageing, with the values in the III age group being statistically significantly greater compared to the II and I age group ($p < 0.05$). Average values of these parameters in the II age group were also statistically significantly greater compared to the I age group ($p < 0.05$) (Table 4).

Average nuclear-cytoplasmic ratio of adenohypophyseal gonadotropic cells was statistically significantly different between I and III and between II and III analyzed age group, while the difference between groups I and II did not reach statistical significance ($F(2,11) = 16.26$, $p = 0.001$). Nuclear-cytoplasmic ratio decreased from I to III age group, but the decrease was statistically significant in age

group III, suggesting a functional decline of gonadotropic cells with ageing, which was most conspicuous after 70 years of life (Table 4, Graph 6).

Discussion

In recent decades, more and more authors have considered the endocrine, i.e. neuroendocrine system, responsible for numerous changes occurring in the body with growing age. In particular, in the endocrine, similar to other tissues in the body, some structural changes occur with ageing, which have as a consequence respective functional abnormalities. These are reflected in a disturbed synthesis and secretion of hormones created in particular glands. The pattern of hormonal secretion at different levels (hypothalamus and peripheral gland tissues) varies considerably. With ageing, the levels of some hormones increase, the levels of others decrease, while the levels of some of them do not change significantly.

Ageing of the hypophysis, from the functional point of view, manifests with a decline of its secretory activity, above all by decreasing levels of growth hormone, gonadotropic hormones, prolactin and thyroid-stimulating hormone in the blood. These changes lead to so called ageing diseases, which predominantly affect the target-organs of these hormones (14-16). The above adenophyseal changes are most commonly only a link in the chain of changes which involve the hypothalamic-adenohypophyseal axes (hypothalamic-somatotropic (HSO or GH), hypothalamic-pituitary-gonadal (HPG) axis, hypothalamic-pituitary-adrenal (HPA) axis, and hypothalamic-pituitary-thyroid axis). These functional changes stem from the structural changes at different levels of these axes.

Although adenohypophysis has an important role in the maintenance of overall homeostasis in the organism and is characterized by progressive functional decline with ageing, the knowledge of the accompanying structural changes is not sufficient, especially regarding the quantity and dynamics of these changes.

In the available literature on the subject older studies tend to prevail; these have been performed mostly on experimental animals and utilizing semi-quantitative methodologies (17-20).

The performed quantitative immunohistochemical and electron-microscopy studies used as their material mostly the hypophyses obtained from experimental animals, especially rats (21-26).

Danilova et al. (27) have analyzed ultrastructural changes in all types of adenohypophyseal endocrine cells in rats during ageing and identified changes in almost all cellular organelles: nuclei, Golgi apparatus, endoplasmatic reticulum, mitochondria and secretory granules, reporting also the presence of numerous lysosomal bodies and fat vacuoles. Older animals had also a greater number of atrophic cells, which indicated their progressive degeneration with growing age. In apparently morphologically normal cells of these animals the signs of ultrastructural changes were seen, which indicated their increased functional activity. These changes were aimed at preservation of the levels of hormonal secretion, but in the long term they resulted in a depletion of the functional reserves of adenohypophyseal endocrine cells in older animals. It was clear that these changes in an ageing hypophysis could be viewed as an attempt of compensatory structural reorganization which would preserve normal gland functioning and help in the maintenance of basic homeostatic mechanisms in the ageing organism.

Analyzing rat hypophyses with quantitative immunohistochemistry methods, Console et al. (28) reported a significant decrease in the number, volume and surface density, area and perimeter of somatotropic cells with ageing. They also noticed a reduced number of other adenohypophyseal cell types, such as gonadotropic cells (FSH and LH), but the reduction was not associated with a corresponding decline of LH and FSH levels.

In general, morphometric studies about the cellular composition of adenohypophysis are relatively rare, especially those dealing with human adenohypophysis in the process of ageing.

Morphometric studies of age-related changes of gonadotropes are also rare. In contrast to other hormonal cells of the adenohypophysis which store a single hormone within special cell types, numerous gonadotropes show immunoreactivity to both LH and FSH. This additionally complicates their quantification in the process of ageing, since in theses cells, FSH and LH expression may vary significantly in different phases of postnatal life.

Meeran et al. (29) evaluated the changes in gonadotrope subtypes in puberty and adolescence of rhesus monkeys and concluded that the number of gonadotropes increased in adults compared to juvenile rhesus monkeys, mostly due to increased numbers of LH and bihormonal cells.

Console et al. (22) performed a morphometric analysis of gonadotropes from young, old and very old rats and established a progressive age-associated reduction of cellular density, volume density and surface density of LH cells. On the other hand, area and perimeter of gonadotropes increased in both young and old animals, but were drastically reduced in oldest animals. Basal levels of serum LH and FSH showed a tendency identical to that established for surface and perimeter of gonadotropes.

Kurosumi et al. (30) classified rat gonadotropes according to the size of their secretory granules into two types: type I, which contained both small and large secretory granules and expressed both FSH and LH; and type II, which contained only small granules, immunopositive to LH. They found that in young adult rats type I gonadotropes are more common compared to type II ones. In middle-aged rats, type I gonadotropes were predominant, but the expression of FSH in most of them was rather weak. In older rats, the ratio of type I and II was reversed compared to young and middle-aged rats, i.e. type II gonadotropes were more common. Kurosumi et al. finally concluded that with advancing age LH cells became predominant in the hypophysis of male rats, which was associated with a considerable decline in FSH and a slow depletion of LH contents.

In our study, the density of LH cells did not change significantly with ageing. However, in contrast to the findings of Console et al. (22), the area of LH cells increased significantly even after 70 years of age. In contrast to the factor of area, nuclear-cytoplasmic ratio gradually decreased with advancing age, and the decrease became significant after 70 years of life. Therefore, our findings of relatively stable density of LH cells and their larger size with advancing age, together with age-related increased production of LH reported by some authors (31), led us to the conclusion that in men these cells probably developed hypertrophy (32) with ageing. This may have indicated their exposure to increased functional stress with time, probably due to disturbed testosterone feedback loop or excessive stimulation by some extra- or intrapituitary factors. Gradual decline of nuclear-cytoplasmic ratio of LH cells represents the sign of their functional decline, which becomes significant after 70 years of life, most probably due to their exhaustion caused by long-lasting hypertrophy. This agrees with the results obtained by Kurosumi et al. (30), who noticed gradual depletion of LH contents with ageing.

However, with the exception of a greater irregularity and reduced amplitude of the LH pulse, as we have described, the prevailing opinion in the scientific community is that LH secretion does not only change insignificantly, but that in men it even slightly increases with advancing age (33-35). This is inconsistent with the findings of reduced GnRH secretion and the assumption that disturbed testosterone feedback loop at the level of hypothesis and hypothalamus releases LH secretion during ageing (36).

Conclusion

Based on the investigations performed so far, a conclusion may be drawn that there is a relatively stable density and increased size of adenohypophyseal gonadotropic LH cells during ageing in men, which probably reflects their hypertrophy in order to maintain normal hormonal secretion, with the consequence of their functional decline.

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IMUNOHISTOHEMIJSKO I MORFOMETRIJSKO PROUČAVANJE GONADOTROPNIH ĆELIJA ADENOHIPOFIZE KADAVERA MUŠKOG POLA RAZLIČITE STAROSTI

Jovana Čukuranović-Kokoris[1], Ivan Jovanović[1], Slađana Ugrenović[1], Vesna Stojanović[1], Miljana Pavlović[1], Braca Kundalić[1], Vladimir Živković[1], Milorad Antić[1], Rade Čukuranović[1]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra Anatomija, Niš, Srbija

Kontakt: Jovana Čukuranović-Kokoris
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: jovana.cukuranovic.kokoris@medfak.ni.ac.rs

Podaci iz literature ukazuju na opadanje funkcije gonadotropnih ćelija sa starenjem, dok su podaci koji se odnose na strukturne promene istih oskudni. Cilj ovog rada bio je da se primenom imunohistohemijske i morfometrijske metode, detektuju i kvantifikuju promene kod humanih gonadotropnih ćelija adenohipofize kadavera muškog pola različitog uzrasta. Materijal je predstavljalo tkivo adenohipofize 14 kadavera muškog pola različite životne dobi, počev od četvrte decenije. Tkivni preseci adenohipofize standardno su histološki obrađivani i bojeni imunohistohemijski monoklonalnim anti–LH antitelom za detekciju gonadotropnih LH ćelija. Digitalne slike vidnih polja imunohistohemijski obrađenih preseka adenohipofize, zatim su morfometrijski analizirane pomoću Image J sistema. Statistička analiza vršena je pomoću SPSS statističkog paketa. Rezultati morfometrijske analize pokazali su to da se zapreminska gustina LH ćelija nije značajno menjala sa godinama, dok su njihova area, perimetar i Feretov dijametar statistički značajno rasli. Nuklearni morfometrijski parametri nisu se značajno menjali, a nukleocitoplazmatski odnos LH ćelija opadao je sa starenjem, pri čemu je taj pad bio statistički značajan kod slučajeva starijih od 70 godina. Na osnovu dobijenih rezultata, može se zaključiti da se tokom starenja gustina LH ćelija ne menja značajno, već da one hipertrofiraju, u cilju održavanja normalne sekrecije hormona. Dugotrajna hipertrofija ovih ćelija na kraju dovodi do njihovog funkcionalnog pada, koji postaje značajan nakon 70. godine života.

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Ključne reči: *luteinizirajuće gonadotropne ćelije, adenohipofiza, imunohistohemija, morfometrija, starenje*

Case report

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PREOPERATIVE RADIOTHERAPY IN ESOPHAGEAL CANCER: A CASE REPORT

Milica Radić[1,2], Dušan Mitić[1], Milica Ćosić[3], Nataša Simonović[1]

Carcinoma of the esophagus is a relatively rare tumor, accounting for 1.5% of overall cancer morbidity. Throughout the world, 480,000 people are affected by the disease each year and men twice as often as women, causing about 400,000 deaths a year.

Most cases are reported in underdeveloped environments, among the middle-aged or elderly. Smoking, excessive alcohol consumption, deficiencies of some dietary microelements and vitamins are among the principal risk factors for the disease. The main symptoms of the disease are progressive dysphagia and dyspepsia, while pain, hoarseness, and cough are common in more advanced disease stages. The diagnosis is established on the basis of histopathology confirmation of the disease from the biopsied material sampled by way of esophagogastroscopy or from the material obtained by way of endoscopic resection. The therapy used in the treatment of esophagus cancers is multidisciplinary. The most common is surgery, radiotherapy, and chemotherapy. Often, these three basic forms of therapy are combined. In this case report, the neoadjuvant therapy consisting of chemotherapy according to the PF protocol and transcutaneous radiotherapy gave an extremely good therapeutic response, and the disease from an unresponsive state was converted into a state of radical operability.

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Key words: *esophageal cancer, multimodality treatment, radiotherapy, preoperative therapy*

[1]Clinic for Oncology, Department of Radiotherapy, Clinical Center Niš, Niš, Serbia
[2]University of Niš, Faculty of Medicine, PhD student, Niš, Serbia
[3]Clinic for Infection Diseases, Clinical Center Niš, Serbia

Contact: Milica Radić
48 Dr. Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: milica91nis@ymail.com

Introduction

Carcinoma of the esophagus is a relatively rare tumor, accounting for 1.5% of overall cancer morbidity. Throughout the world, 480,000 people are affected by the disease each year and men twice as often as women, causing about 400,000 deaths a year (1). Most cases are reported in underdeveloped environments, among the middle-aged or elderly (average age at onset, 59.5 years). Smoking, excessive alcohol consumption, deficiencies of some dietary microelements and vitamins (selenium, vitamin E, beta-carotene) are among the principal risk factors for the disease. Individuals with certain diseases and conditions also have an increased risk for the disease, such as *tylosis palmaris*, severe dysplasia in Barrett's esophagus, untreated achalasia and celiac disease (2, 3). Radiation to the mediastinum (in breast or lung cancer, for instance) also carries an increased risk, similar to the strictures occurring after caustic injuries, and association of esophageal cancer with human papillomavirus has also been reported (1, 4).

Two most common histopathological forms of esophageal carcinoma are planocellular and adenocarcinoma forms (5). Starting from the upper to the lower esophageal portions, the incidence of planocellular type is decreasing, with adenocarcinoma being more and more common (originating from the superficial and deep esophageal glands, embryonic remnants of the glandular epithelium and metaplastic glandular epithelium) (5, 6).

The main symptoms of the disease are progressive dysphagia (in 80-90%) and dyspepsia, isolated or associated with difficulty swallowing. Pain, hoarseness, and dry cough are common in more advanced disease stages, as well as bleeding when other adjacent organs become infiltrated (7). The diagnosis is made based on histopathology confirmation of the disease from the biopsied material sampled by way of esophagogastroscopy or from the material obtained by way of endoscopic resection. Biopsy of multiple sites (usually 6-8) in the changed portion of the esophagus is usually recom-

mended (8). Histopathological classification of the World Health Organization (2010) is used (9). Endoscopic ultrasound, chest x-ray, chest and upper abdomen CT, and PET CT scanning are useful in the assessment of disease spread.

The initial treatment plan for these patients should be formulated at a multidisciplinary tumor board, basing the decision not only upon scientific evidence, but also the aspects present in each individual patient, taking into account his general health condition and existing comorbidities. The tumor board should involve a gastroenterologist, pathologist, radiologist, thoracic surgeon, oncologist, radiation oncologist and, as required, nutritionist and psychiatrist.

Case report

We report a male 68-year-old patient in whom the complaints appeared in January 2018 with difficult swallowing of solid foods. These difficulties progressively worsened in the following few months, with the patient losing 9 kg in body weight (until June 2018).

He consulted a doctor for the first time in April 2018, when esophagogastroduodenoscopy (EGDS) was performed at the Clinic of Gastroenterology and the visualized change was biopsied. The definitive diagnosis was made at the Clinical Center Niš Center for Pathology, and the change was identified as carcinoma squamocellulare partim kerathodes invasivum oesophagei HG II, NG II-III. At the MSCT finding from May 2018, the distal esophagus with an infiltrative tumor change was seen, measuring up to 36 mm in axial diameter and up to 48 mm in craniocaudal diameter, with scarce post-contrast enhancement. In the same month, bronchoscopy was done as well, with no endoscopic abnormalities detected. In that period, the patient was referred to the Clinical Center of Serbia (in Belgrade), Department for Esophageal Malignancies, for a consultancy examination, where the tumor board decision was made, after thorough clinical and laboratory evaluations (with x-ray, ultrasound and MSCT), that the patient should receive neoadjuvant chemoradiotherapy in a regional center. During this hospitalization, a control MSCT scanning of the thorax and abdomen was done, visualizing two nodular lesions in the lung parenchyma, measuring in diameter 5 mm (at the interface of the lateral and medial segment of the middle lobe, on the right) and 2 mm (anterobasal, on the right), which were not highly suspicious for secondary deposits, so that only surveillance of these was then recommended. The thoracic esophagus extended from the level of the ostium of the inferior vena cava caudally for 6.7 cm of tumorously enlarged wall, with the largest axial diameter of 20x25 mm and largest infiltration thickness of about 11 mm, without any signs of infiltration of adjacent structures on CT scanning. It seemed that cardia was not involved with tumor. In the fifth group of mediastinal lymph nodes, several lymph nodes measuring in diameter up to 7 mm could be seen. There were no signs of lymphadenopathy. A solitary focal lesion involving the corpus of Th12 was detected as well, which could not be seen on a repeated control examination. The findings in other structures, as visualized by CT scanning, were without any abnormalities. The decision concerning neoadjuvant chemoradiation was confirmed after the tumor board at the Clinic of Gastroenterology, Clinical Center Niš, in June 2018. The patient received one chemotherapy cycle according to the PF protocol (cisplatin plus 5-fluorouracil) and two cycles of chemotherapy by the PF protocol with radiation therapy of 45 Gy TD in 25 fractions (chemopotentiation).

The patient tolerated well his neoadjuvant chemoradiation treatment, and a month after the completion of planned therapy, a control MSCT scanning of the thorax and abdomen was done at the Clinic of Oncology, Clinical Center Niš, Department of Radiation Therapy, in order to assess the treatment response. At MSCT scanning, a slower passage of peroral contrast medium could be seen, with a stenosis of irregular luminal contours of the distal part of the thoracic esophageal portion, measuring 38 mm in craniocaudal diameter and 27 mm in the axial slice. The findings in other structures, as visualized by CT, were without abnormalities, which suggested disease remission. The patient was then referred to the Clinical Center of Serbia, Department for Esophageal Malignancies, so that possible surgical treatment could be considered. After an adequate preoperative preparation, the patient was surgically treated two months after the completion of his oncological treatment. A "minimally invasive Ivor Lewis" procedure was done (oesophagectomia subtotalis, lymphadenectomia mediastini posterioris, medialis et inferioris et oesophagogastroplasticam per thoracoscopiam et laparoscopiam; explorative thoracoscopy; laparoscopy). Histopathological verification was done at the Department of Pathology, Clinical Center of Serbia - Oesophagitis fibrosa segmentalis chronica postiradiatione sine tumoris, T0, N0 (0/19) Mx L0 V0 PN0. Complete regression (TR 1/5, RCRG 1/3).

Postoperative MSCT findings two months later confirmed the observed postoperative status, with a massive pleural effusion on the left, with compressive atelectasis of the lower lobe on the left. However, tumor cells were not found in the pleural puncture fluid; instead, the presence of lymphocytes and lipoprotein masses were seen (December, 2018; Center for Pathology, Niš). The patient was then presented at the tumor board for oncology in Niš, where the decision was made, on the basis of a satisfactory response to chemoradiation, that specific oncological treatment was not required; regular control visits to his surgeon were recommended.

The patient visited his surgeon in January 2019, two and a half months after the above mentioned interventions, feeling subjectively well, gaining 2 kg in body weight. The next control visit was scheduled for April 2019, and at that visit thoracic and abdominal CT showed pulmonary parenchyma without secondary deposits, with the presence of free fluid basally on the left (up to 6 HU, indicating a transudate), esophageal stump with preserved

lumen and without any apparent recurrence. The anastomosis and most part of the stomach presented mediastinally without any apparent recurrence. Lymph nodes could be seen para-aortally, measuring up to 7 mm. Osseous parts of the thorax were without any visible infiltrations. Abdominal findings were without any abnormality. Laboratory and biochemical parameters were within reference ranges, and tumor marker values were as follows: CEA: 3.8 ug/L; CA 19-9: 5.5 ku/L; and CA 72-4: 1.1 U/mL, which, in addition to other evaluation tests and methods, indicated a complete disease remission. The patient denied having any pains or problems with digestion, and had regular stools and urination, stating that he had gained 10 kg in body weight since the operation.

Discussion

According to the TNM classification, our patient was classified as T3 N0 M0, and as a potentially resectable case received neoadjuvant therapy: one cycle of chemotherapy by PF protocol, and was further treated with chemoradiation, with chemotherapy potentiated by radiation (chemopotentiation). The degree of tumor response to therapy was excellent in our patient, since viable tumor cells could not be demonstrated in the operative material. The degree of tumor response to neoadjuvant therapy, according to the College of American Pathologist classification, was estimated as a complete tumor response and thus belonged to stage 0 (10).

Although carcinoma of the esophagus belongs to the group of chemoresistant tumors, the success of therapy was significantly influenced by concomitant chemo- and radiation therapy. Chemotherapy preceding the combined approach was the same as that used in combination with radiation. Cisplatin and 5-fluorouracil (PF protocol) are the agents commonly used. The first cycle is administered four weeks before the beginning of radiation. Cisplatin is given on the first day of the cycle at a dose of 100 mg/m^2, while 5-fluorouracil is given as a 24-hour infusion at a dose of 1000 mg/m^2, from days 1 to 5 of the chemotherapy cycle. After the first cycle, radiation therapy is introduced. Transcutaneous therapy of esophageal cancer is administered on the linear accelerator "Electa", using high-energy x-rays (10 MeV or more) and with three radiation portals. As a standard, the patient is irradiated in supination, with hands raised above the head, using the appropriate radiotherapeutic accessories. The dose of 45 Gy in 25 fractions is administered to the tumor tissue, using 3D conformal radiation technique, taking into account radiosensitivity of high-risk anatomical structures (spinal cord, healthy lung, heart, pericardium) (Figure 1, 2). ICRU 50 and supplement ICRU 62 were used in the contouring of target volumes. During the radiation therapy, for the purpose of reproducibility, radiotherapy portal precision check-ups were done each week. During the treatment, there were no significant adverse effects.

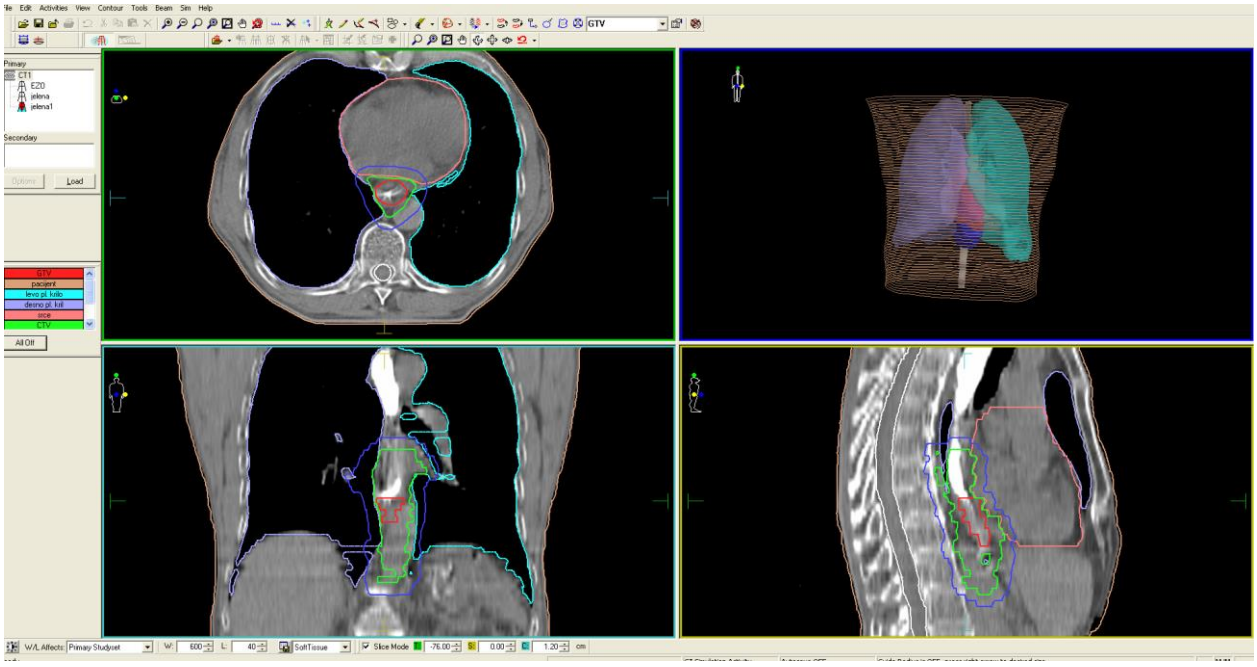

Figure 1. Presentation of delination of target volumens and organs of risk (3DCRT), neoadjuvant (chemo) radiotherapy for oesophageal cancer

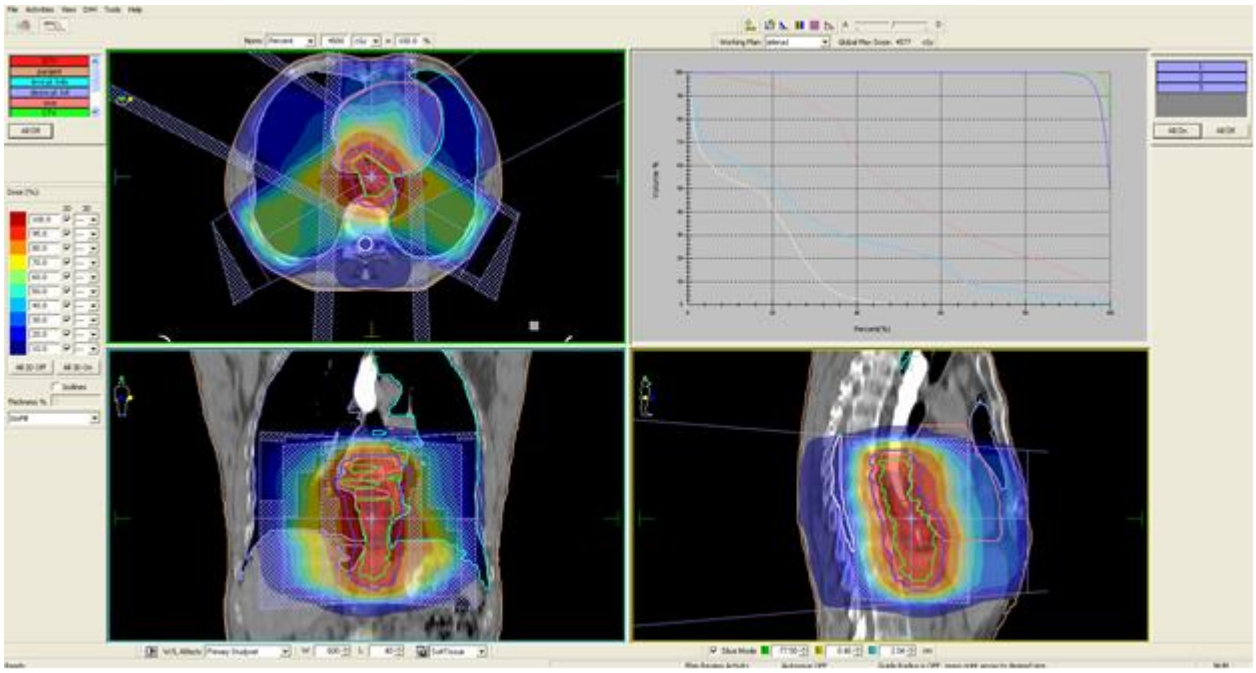

Figure 2. Dose distribution and field arrangement in 3D conformal preoperative radiotherapy for oesophageal cancer ("mercedes" technique)

Thanks to an excellent therapeutic response to chemoradiation, the treatment was continued in the Clinical Center of Serbia, where the surgical procedure was performed. Surgical therapy is the therapy of choice for patients with limited disease, i.e. those with lower disease stages (0, I, IIA, IIB). Although the disease is operable in these stages, a significantly prolonged survival period has been observed in patients with *in situ* disease (11). Neoadjuvant chemoradiation followed by surgical resection is the treatment option for both histopathological tumor forms in locally advanced stage of esophageal cancer, as is the case with the patient we are presenting (12). Postoperative chemotherapy has yielded therapeutic effects in patients with esophageal adenocarcinoma localized in the lower third of the esophagus, while for the disease involving other portions of the esophagus and in planocellular carcinoma cases these effects have not been observed (13). According to the results published so far in the literature, the use of postoperative radiotherapy in cases with positive resection margins results in better local disease control rates, but it affects overall survival rates as well (14). In patients with advanced disease, the only treatment modality is palliative radiation therapy, aiming to improve the act of swallowing and to alleviate pain (15, 16).

Since R0 resection was histologically confirmed, as well as the fibrosis of resected change, the patient was relocated from a potentially incurable condition to the category of potentially curable patients. Taking into account that 5-year survival of patients with esophageal cancer is 17.9% according to most studies, we consider that the treatment of this patient was very successful and that there is a possibility that the patient would enjoy a longer period without disease relapse.

Conclusion

Our patient was treated with neoadjuvant therapy in accordance with the tumor board decision and was relocated from the category of unresectable to the category of resectable disease. Neoadjuvant therapy, involving chemotherapy by the PF protocol and transcutaneous radiation therapy, yielded an excellent therapeutic response. Chemoradiation produced in this case a complete pathological response, so that the patient could be radically treated by surgery. It can be seen from our report that radiation therapy significantly contributed to the success of treatment of this patient. We expect that with further advancements in the domains of equipment and radiation techniques we will be in the position to offer to these patients intraluminal brachytherapy as well.

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PREOPERATIVNA RADIOTERAPIJA KARCINOMA JEDNJAKA: PRIKAZ SLUČAJA

Milica Radić[1,2], Dušan Mitić[1], Milica Ćosić[3], Nataša Simonović[1]

[1]Klinika za onkologiju, Odeljenje radioterapije, Klinički centar Niš, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, student doktorskih studija, Niš, Srbija
[3]Klinika za infektivne bolesti, Klinički centar Niš, Niš, Srbija

Kontakt: Milica Radić
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: milica91nis@ymail.com

Karcinom jednjaka je relativno redak tumor i u ukupnom morbiditetu učestvuje sa 1,5%. Svake godine u svetu oboli oko 480.000 ljudi, među kojima je veći broj muškaraca u odnosu na žene, a oko 400.000 obolelih godišnje ima letalan ishod. Većina slučajeva registruje se u manje razvijenim sredinama, u srednjoj i starijoj životnoj dobi. U osnovne faktore rizika ubrajaju se pušenje, prekomerno konzumiranje alkohola, deficit pojedinih mikroelemenata i vitamina u ishrani. Osnovni simptomi su: progredijentna disfagija, dispepsija, dok se bol, promuklost i nadražajni kašalj, javljaju u odmaklim stadijumima bolesti. Dijagnoza se postavlja na temelju patohistološke potvrde bolesti iz biopsiranog materijala uzetog ezofagogastroskopijom ili iz materijala dobijenog endoskopskom resekcijom. Terapija koja se primenjuje u lečenju karcinoma ezofagusa je multidisciplinarna. Najčešće je reč o hiruškoj, i zračnoj terapiji, kao i hemioterapija. Često se ova tri osnovna vida terapije kombinuju. U ovom prikazu, neoadjuvantna terapija, koja se sastojala iz hemoterapije po PF protokolu i transkutane radioterapije, dala je izuzetno dobar terapijski odgovor, pa je bolest iz neresektabilnog stanja prevedena u stanje radikalne operabilnosti.

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Ključne reči: *karcinom jednjaka, multidisciplinarni tretman, radioterapija, preoperativna terapija*

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NON-INVASIVE PARAMETERS IN TREATED HYPERTENSIVE PATIENTS HAVE BETTER CORRELATION WITH TARGET ORGAN DAMAGE THAN THE POSSIBILITY OF PREDICTING 5-YEAR TREATMENT OUTCOME

Dragan Djordjević[1,2], Ivan Tasić[1,2], Bojana Stamenković[1,2], Svetlana Kostić[1], Milan Lović[1], Dragan Lović[3], Nikola Djordjević[2]

The objective of the research was to investigate which of routine non-invasive parameters could predict the occurrence of target organ damage and treatment outcome during 5-year follow-up in patients with arterial hypertension. The research included 176 patients (average age 66.9 ± 9.3) with arterial hypertension who were previously treated for 144 ± 90 months. Patient monitoring was continued for 63.6 months on average. Average values of 24-hour ABPM were 121.5 ± 14.6 mmHg for systolic (SBP) and 69.1 ± 9.2 mmHg for diastolic (DBP) pressure. The total of 36 (20.4%) patients had diabetes, while 115 (65.4%) patients had lipid disorder. Furthermore, 116 (63.4%) patients had left ventricular hypertrophy (LVH). Left ventricular mass index was, on average, 135.4 ± 30.7 g/m^2. During the follow-up period, we registered 21 (11.9%) new events, 3 (1.7%) of which were stokes, 6 (3.4%) were acute coronary events with accompanying revascularization, two stable angina pectoris (2.6%), two pacemaker insertions (2.6%), one acute thrombosis of leg artery (0.6%), one dementia (0.6%) and 6 (3.4%) new atrial fibrillations. Independent predictor for total new events was the size of left atrium (coefficient beta 0.295; $p < 0.01$). Patients with LVH had higher SBP and DBP values obtained from 24-hour ABPM and home measurement ($p < 0.01$). Independent predictor for the presence of LVH was the length of hypertension treatment (coefficient beta 0.180; $p < 0.03$). Predictors of lower values of creatinine clearance (for model $p < 0.01$) were age (beta 0.187; $p < 0.02$) and glycemic value (coefficient beta 0.232; $p < 0.01$). Routine non-invasive parameters in patients with arterial hypertension cannot predict 5-year treatment outcome during the treatment, but have a good correlation with damage of target organs.

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Key words: *arterial hypertension, treatment, target organs damage, cardiovascular events*

[1]Institute for Treatment and Rehabilitation Niška Banja, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia
[3]Intermedica – dr Lović, Niš, Serbia

Contact: Dragan Djordjević
81 Dr. Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: ddj964@gmail.com

Introduction

Blood pressure which is above the normal range leads to hypertensive damage of target organs and, along with risk factors for the occurrence of cardiovascular disease, leads to vascular, i.e. arteriosclerosis complications and adverse events. European Guidelines for Arterial Hypertension Recommendations state that the normal systolic pressure is below 140 mmHg and normal diastolic pressure is below 90 mmHg (1). Recently published American Guidelines have stated that normal values of systolic and diastolic blood pressure should be below 130 mmHg and 80 mmHg, respectively, and thus have defined the target values of blood pressure (2). Nowadays, there are numerous discussions on the values of normal blood pressure, as well as on target blood pressure in patients treated from high blood pressure. The Systolic Blood Pressure Intervention Trial (SPRINT) has confirmed that optimum value of systolic blood pressure in patients with arterial hypertension without diabetes is < 120 mmHg (3). If compared to the target value of < 140 mmHg, intensive reduction of blood pressure has led to the reduction of mortality by 27%. However, other studies, such as the study Action to Control Cardiovascular Risk in Diabetes Blood Pressure (ACCORD BP) have not confirmed the advantages of intensive reduction of systolic blood pressure below the standard target value (4).

Aside from blood pressure level, the presence of other risk factors was significant for the outcome of hypertensive disease. Joint effects of risk factors were shown in 10-year absolute risk tables in European Recommendations (1). However, reaching the target values defined in the guidelines was not possible in clinical practice. This was clearly shown by the results of EUROASPIRE (European Action on Secondary and Primary Prevention by Intervention to Reduce Events) study which was conducted in 24 European countries and which focused on cardiovascular disease risk factors (5).

Having in mind the above stated, the expected outcome of hypertensive disease will be different depending on our ability to adjust the therapy and bring blood pressure and other relevant risk factors closer to the targeted values. It is important for clinical experts to review the results of their work and explore the possibility of predicting the disease outcome, all based on the success of correcting non-invasive parameters during treatment. The objective of the research was to determine which of routine non-invasive parameters could predict the occurrence of target organ damage and treatment outcome during 5-year follow-up in patients with arterial hypertension.

Materials and methods

The study was based on routine ambulatory medical examinations at Niška Banja Institute. The patients were referred to the institute from primary health protection unit due to high blood pressure. Treatment and monitoring were continued at the Institute. The second medical check-up was conducted after two weeks, the third was after a month, after which the check-ups were conducted each 5-6 months. All patients were subjected to clinical examination which included anthropometric measurements (waist, height and weight), electrocardiography, blood pressure measurement in ambulatory conditions, home conditions and 24-hour ambulatory monitoring, echocardiography and ergometric test. Patients with arterial hypertension and cardiovascular or cerebrovascular complications were not included in the study. The total of 176 patients, 72 males and 104 females, met the criteria for being included in the study. Table 1 shows the basic characteristics of the tested group. Median for risk factors, such as age, gender, smoking habits, diabetes, hypercholesterolemia, obesity, physical inactivity, alcoholism and heredity, was 4 risk factors for cardiovascular diseases. Thirty-six patients (20.4%) had diabetes, while 115 (65.4%) patients had lipid disorder. Ideal weight was recorded in 40 (21.9%) patients, while obesity was recorded in 56 (30.6%) patients. There were 61 (34.7%) smokers in the group, 19 (10.8%) of whom never stopped smoking. Physical inactivity was recorded in 62 (35.2%) patients.

Table 1. Basic features of the tested group

Parameters	Values
Male (%) / female (%)	72 (40.9)/104 (59.1)
Hypertension duration (months)	12.0 ± 7.5
Monitoring duration (years)	5.4 ± 3.0
Systolic blood pressure at outpatient unit (mmHg)	127.1 ± 14.4
Diastolic blood pressure at outpatient unit (mmHg)	77.7 ± 6.3
Body mass index (kg/m^2)	28.7 ± 4.7
Left ventricular mass (g)	255.3 ± 70.5
Left ventricular mass index (g/m^2)	135.3 ± 30.7
Left ventricular ejection fraction (%)	64.0 ± 5.8
Aortic root diameter (mm)	33.7 ± 3.3
Left atrial diameter (mm)	42.2 ± 6.5
Glycaemia (mmol/L)	6.0 ± 1.9
Cholesterol (mmol/L)	5.7 ± 1.2
HDL-cholesterol (mmol/L)	1.4 ± 0.7
LDL-cholesterol (mmol/L)	3.6 ± 1.1
Triglycerides (mmol/L)	1.9 ± 1.5
Creatinine (μmol/L)	87.8 ± 24.9
Number of risk factors	3.7 ± 1.3

In terms of important medications, at the end of the study 144 (81.1%) patients used beta blockers, 142 (80.7%) used ACE inhibitors or blockers of angiotensin 1 receptors , 85 (48.3%) patients used calcium channel blockers, 113 (64.2%) patients used diuretics and 72 (40.9%) patients used statins.

Blood pressure measurement

Blood pressure was initially measured in ambulatory conditions twice in two minutes at both hands. After the change of therapy, blood pressure reached the optimal limits, which was confirmed during the control check-ups. Corrected values of blood pressure were used in the study as average values monitored at control check-ups. The patients were trained to measure blood pressure at home twice a week, in the morning and in the evening for a period of one month, between the second and third check-up. Twenty-fourhour ambulatory blood pressure monitoring was conducted minimum once within the period of six months from the second medical check-up.

Laboratory analysis

We conducted the following standard laboratory analyses: complete blood count, glycaemia, lipid status, transaminase, urea, creatinine, uric acid, electrolytes and urine. The therapy was altered in accordance with the results of laboratory tests. Values of laboratory parameters which were taken minimum two months after taking the regular therapy were taken for the needs of this study. Total cholesterol larger than 5 mmol/L and/or triglycerides larger than 1.7 mmol/L were defined as lipid status disorder. Patients who suffered from diabetes mellitus needed endocrinologist report or evidence on prescribed medications for diabetes. Glomerular filtration rate was calculated by Cockcroft-Gault equation and identified five levels of kidney function (6).

Exercise testing

Exercise test, without beta blockers for minimum 24 hours, was done by standard Bruce protocol. The test was completed after reaching submaximal heart rate (85% of maximum theoretical heart rate) or due to pain in the chest or legs, fatigue or vertigo. Additionally, we respected patient's request to terminate the test if the patient felt bad. Ergometric test was conducted with the aim of excluding the patients due to coronary disease or discovering coronary disease as adverse vascular event during the later tests.

Echocardiography

Echocardiography was conducted by means of standard two-dimensional technique, while the measurement of heart structure was done by M-mode technique. Additionally, the assessment of valve function and testing the flow was done by means of Color Doppler, including pulse and/or Continuous-Wave Doppler. Having in mind that the patients had no segmental contractility disorder, all measurements were conducted in M-mode technique. Echocardiographic parameters which were taken into consideration were aortic root diameter, left ventricular diameter and ejection fraction. Left ventricular mass was calculated based on left ventricular wall thickness at the end of diastole and diastole diameter. Left ventricular mass index was obtained by dividing left ventricular mass by body surface area (7). Male patients with left ventricular mass index larger than 134 g/m^2 and female patients with mass index larger than 110 g/m^2 were considered to suffer from left ventricular hypertrophy. Patients with moderate and severe heart defects were excluded from the study.

Statistical data processing

Numerical parameters were shown as mean value ± standard deviation. Statistical significance between the subgroup was defined by means of Student's t-test. Distribution of attributive marks was shown by absolute values, percentage and median. Testing statistical significance between the groups was conducted by means of χ^2 test. Pearson correlation coefficient was obtained by bivariate correlation method. Multivariate linear regression analysis was used for defining significant predictors of adverse events and target organ damage. The value of p less than 0.05 was considered as statistically significant.

Results

During the follow-up period, we registered 21 (11.9%) new events, 3 (1.7%) of which were strokes, 6 (3.4%) were acute coronary events with revascularization, 2 (2.6%) stable angina pectoris, 2 (2.6%) pacemaker insertions, 1 (0.6%) acute arterial leg thrombosis, 1 (0.6%) dementia and 6 (3.4%) new atrial fibrillations.

Table 2 shows characteristics of patients with adverse event as compared to patients who have never experienced an adverse event. After including the parameters such as gender, age, hypertension duration, systolic and diastolic blood pressure values, factors of risk, body mass index, waist, glycaemia, total cholesterol, creatinine value and echocardiographic parameters, we obtained a model with one prognostic marker ($R = 0.277$; $R^2 = 0.077$; adjusted $R^2 = 0.070$; standard error of the estimate = 0.32600; $p < 0.001$). The only independent predictor of total new events was left atrial size (beta coefficient 0.277; $p < 0.01$). There was no difference in terms of the use of specific medications. After excluding non-vascular events from the analysis, there was not a single parameter that was statistically different between the groups.

Table 2. Most important characteristics of patients with and without adverse events

Parameters	Without adverse event	With adverse event
Age	66.6 ± 9.4	69.8 ± 8.6
Systolic blood pressure (mmHg)	127.2 ± 14.7	125.7 ± 12.8
Diastolic blood pressure (mmHg)	77.9 ± 6.6	75.7 ± 5.4
Ejection fraction (%)	64.0 ± 5.9	63.9 ± 5.5
Left atrial diameter (mm)	41.8 ± 5.9	46.1 ± 9.4*
Left ventricular mass index (g/m^2)	135.6 ± 30.2	134.4 ± 35.5
Glycaemia (mmol/L)	6.0 ± 2.0	5.6 ± 1.0
Total cholesterol (mmol/L)	5.7 ± 1.2	5.1 ± 1.0*
Creatinine (μmol/L)	88.1 ± 26.1	85.2 ± 15.3
Factors of risk	3.7 ± 1.3	3.3 ± 1.3

* - $p<0.05$

Both male and female patients with left ventricular mass index above limit value were older (age 68.4 ± 8.6 vs. 64.1 ± 10.0; $p < 0.01$), had longer history of treating arterial blood pressure (12.8 ± 7.4 vs. 10.3 ± 7.3 months), had higher values of 24-hour systolic (123.9 ± 10.0 vs. 119.2 ± 9.5 mmHg; $p < 0.05$) and diastolic (70.9 ± 10.2 vs. 66.1 ± 4.8 mmHg; $p < 0.05$) blood pressure. Additionally, these patients had higher values of home systolic (124.8 ± 11.2 vs. 118.3 ± 9.1 mmHg; $p < 0.05$) and diastolic blood pressure (71.5 ± 7.9 vs. 67.8 ± 5.3 mmHg; $p < 0.05$). Independent predictor for the presence of left ventricular hypertrophy was hypertension treatment duration (beta coefficient 0.180; $p < 0.03$; for model: $R = 0.180$; $R^2 = 0.032$; adjusted $R^2 = 0.026$; standard error of the estimate = 0.46992 $p < 0.03$). In terms of medication use, we found significant difference in the use of ACE inhibitors or angiotensin II receptor blockers in the group of patients with left ventricular hypertrophy (85.3% vs. 71.6%; $p < 0.05$), as compared to patients who did not suffer from left ventricular hypertrophy.

Predictors of higher creatinine values (for model: $R = 0.289$; $R^2 = 0.033$; adjusted $R^2 = 0.027$; standard error of the estimate = 24.23117 $p < 0.01$) were age (beta coefficient 0.178; $p < 0.02$) and glycaemia values (beta coefficient 0.231; $p < 0.01$). There was no significant correlation between eGFR and other parameters after excluding parameters which were parts of eGFR formula, except the value of systolic blood pressure ($r = 0.200$; $p < 0.05$). In terms of glomerular filtration rate, 53 (30.1%) patients had I-degree kidney function, 82 (46.6%) patients had II-degree kidney function, 38 (21.6%) patients had III-degree kidney function, only 3 (1.7%) patients had IV-degree kidney function, while there were no patients with V-degree of kidney function.

Figure 1 shows direct bivariate correlation between glycaemia and creatinine values. Correlation coefficient was not high, but it was statistically significant ($r = 0.231$; $p < 0.01$).

Ultrasound examination of carotid artery was conducted in 56 (31.8%) patients, 18 of whom had normal results (intima-media complex < 0.9 mm). Patients whose blood pressure could not be regulated despite the therapy (19 patients – 10.8%) were subjected to fundus examination, the result of which were changes of II degree.

Success in achieving the target values of specific parameters was shown in Graph 1.

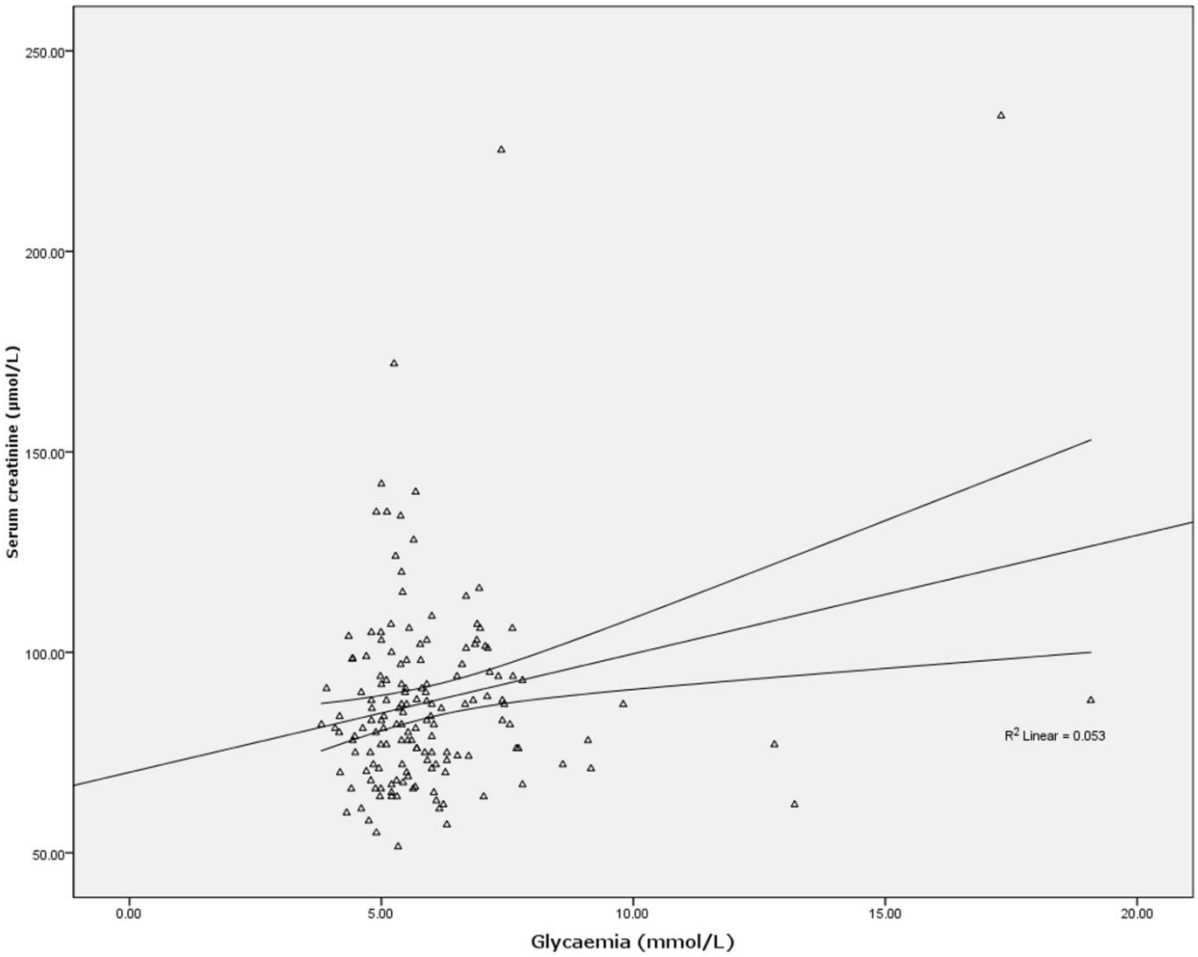
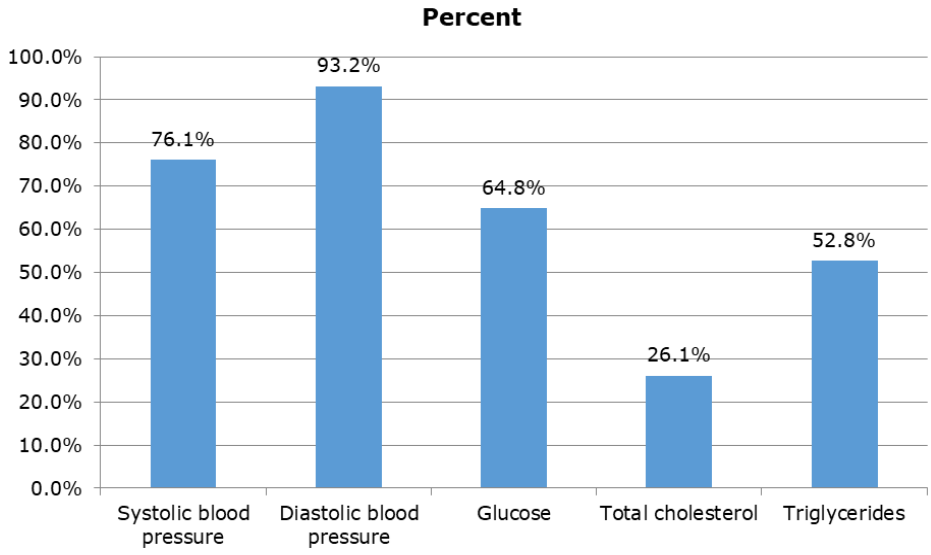


Figure 1. Direct bivariate correlation be-tween glycaemia and creatinine values.



Graph 1. Success in achieving the target values in accordance with European guidelines

Discussion

Vascular events (stroke and coronary disease) had a significant share in the structure of adverse events analyzed in this study. As for non-vascular events, atrial fibrillation was very important as well. This could account for the difference between the group with adverse event and the group without adverse events in terms of left atrial size. This difference became irrelevant if we observed patients with vascular complications only. Vaziri et al. proved that systolic and diastolic pressure level led to the increase of left ventricle, but that this was not the only cause (8). Moreover, age and body mass index played a very important role. According to the research of Dan et al. arterial hypertension was an important factor for the occurrence of composite events in patients with atrial fibrillation, but that it was not an independent prognostic marker in one-year total mortality (9). Higher value of total cholesterol in group of patients without adverse events could be explained by intensive statin therapy, as the difference in the use of statin was not recorded in the two subgroups.

Left ventricular hypertrophy occurred as a result of various factors, such as high arterial blood pressure, numerous neurohumoral factors and genetic factors (10). Patients with left ventricular hypertrophy who participated in this study were older, had longer history of treating arterial hypertension, higher values of systolic and diastolic blood pressure measured at home and during 24-hour ambulatory monitoring, but did not have high blood pressure measured in ambulatory conditions. Monitoring of blood pressure in ambulatory conditions was easier, but pressure was controlled better if home measurement was included in the entire process (11). This was cheaper and easier way of controlling blood pressure, and the studies showed that the results had good correlation with 24-hour ambulatory blood pressure measurement (12, 13). Niiramen et al. pointed out that even a one-day measurement could have prognostic significance. However, blood pressure should be measured at home for at least seven days in order to obtain the proper hypertension diagnosis (14). On the other hand, home measurement of blood pressure could predict morbidity and mortality of patients with arterial hypertension (15, 16). Having in mind literature data, the results of this research were compatible in terms of blood pressure rate and age. However, one could argue the fact that duration of arterial hypertension treatment correlated and acted as independent prognostic marker for the occurrence of left ventricular hypertrophy. It was hard to determine the moment when hypertension occurred and the moment when systematic treatment was initiated. Increased use of ACE inhibitors/angiotensin II receptor blocker was in accordance with the guidelines for treating arterial hypertension, having in mind that most potent medications for hypertrophy regression were the medications from this group (1, 17).

In terms of hypertension, kidney is the second target organ and its damage should be detected by non-invasive methods on regular basis. According to the consensus of the three European associations: the European Association of Cardiovascular Imaging, the European Society of Cardiology Council on Hypertension and the European Society of Hypertension, assessment of glomerular filtration rate, detection of microalbuminuria and definition of albumin-creatinine ratio in urine are recommended for early detection of kidney damage (18). However, due to insufficient availability, we usually measure creatinine and calculate glomerular filtration for all patients, as we have done to all patients who participated in this study. It is important to mention that it takes several years to change glomerular filtration arte (19). After diabetes, arterial hypertension is an important cause of kidney failure. Appropriate regulation of blood pressure reduces the frequency of kidney failure and vice versa, higher blood pressure measured by 24-hour ambulatory monitoring increases the frequency of kidney function deterioration (20, 21). Our study has demonstrated the connection between glomerular filtration rate and systolic blood pressure. Some studies have shown that dyslipidemia increases the risk of kidney failure (22). However, there are studies which have not confirmed the results illustrated in our study (23). Our study has found the correlation between glycaemia and creatinine. However, our study has not confirmed the correlation between glomerular filtration and diabetes, even though it has been confirmed in other studies (22).

Even though the guidelines emphasize the necessity of conducting ultrasound examination of carotid arteries as markers of vascular damage, it is not easy to conduct such examination in clinical conditions, and thus we have managed to conduct ultrasound examination in 31.8% patients (18). Even though they were mentioned in the guidelines, pulse velocity test and ankle–brachial index were not conducted during the study due to their unavailability. Detection of microvascular changes in the brain by means of magnetic resonance were not conducted because majority of patients had no neurological deteriorations, no deterioration of cognitive function, except one patient with verified dementia and changes during magnetic resonance of the brain. Patients with acute cerebrovascular events were subjected to detailed neurological diagnostics and therapy.

Conclusion

Routine non-invasive parameters in patients with arterial hypertension cannot predict five-year treatment outcome during the treatment, but have a good correlation with the degree of target organ damage.

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NEINVAZIVNI PARAMETRI KOD HIPERTENZIVNIH BOLESNIKA, KOJI SE LEČE, BOLJE KORELIRAJU SA OŠTEĆENJEM CILJNIH ORGANA U ODNOSU NA MOGUĆNOST PREDVIĐANJA PETOGODIŠNJEG ISHODA LEČENJA

Dragan Đorđević[1,2], Ivan Tasić[1,2], Bojana Stamenković[1,2], Svetlana Kostić[1], Milan Lović[1], Dragan Lović[3], Nikola Đorđević[2]

[1]Institut za lečenje i rehabilitaciju „Niška Banja", Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[3]Intermedica – dr Lović, Niš, Srbija

Kontakt: Dragan Đorđević
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: ddj964@gmail.com

Cilj istraživanja bio je ispitati koji od rutinskih neinvazivnih parametara može, kod lečenih bolesnika od arterijke hipertenzije, predvideti nastanak oštećenja ciljnih organa i ishod bolesti, tokom pet godina praćenja. U istraživanje je uključeno 176 bolesnika (prosečne starosti 66,9 godina ± 9,3 godine) sa arteijskom hipertenzijom, koji su bili prethodno lečeni, prosečno 144 meseci ± 90 meseci. Praćenje bolesnika nastavljeno je još prosečno 63,6 meseci. Prosečne vrednosti dvadesetčetvoročasovnog ABPM bile su za sistolni (SBP) 121,5 mmHg ± 14,6 mmHg i za dijastolni (DBP) 69,1 mmHg ± 9,2 mmHg. Dijabetes je imalo 36 (20,4%) bolesnika, a poremećaj lipida 115 (65,4%) bolesnika. Hipertrofiju leve komore (LVH) imalo je 116 (63,4%) bolesnika. Indeks mase leve komore bio je prosečno 135,4 g/m^2 ± 30,7 g/m^2. Tokom perioda praćenja registrovan je 21 (11,9%) novi događaj, od toga 3 (1,7%) šloga, 6 (3,4%) akutnih koronarnih događaja sa sledstvenom revaskularizacijom, dve stabilne angine pektoris (2,6%), dve implantacije pejs-mejkera (2,6%), jedna akutna tromboza arterije nogu (0,6%), jedna demencija (0,6%) i 6 (3,4%) novih atrijalnih fibrilacija. Nezavisni prediktor za ukupne nove događaje bila je veličina leve pretkomore (koeficijent beta 0,295; p < 0,01). Bolesnici sa LVH imali su značajno veće SBP i DBP dobijenog iz dvadesetčetvoro-časovnog ABPM i kućnog merenja (p < 0,01). Nezavistan prediktor za prisustvo LVH bila je dužina lečenja hipertenzije (koeficijent beta 0,180; p < 0,03). Prediktori nižih vrednosti klirensa kreatinina (za model p < 0,01) bile su godine starosti (beta 0,187; p < 0,02) i vrednosti glikemije (koeficijent beta 0,232; p < 0,01). Rutinski neinvazivni parametri kod bolesnika sa artrijskom hipertenzijom tokom lečenja, ne mogu predvideti petogodišnji ishod lečenja, ali dobro korelišu sa oštećenjem ciljnih organa.

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Ključne reči: *arterijska hipertenzija, lečenje, oštećenje ciljnih organa, kardiovaskularni događaji*

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TOTAL HIP ARTHROPLASTY USING FITMORE® STEM IN TREATMENT OF HIP OSTEOARTHRITIS – A 3-YEAR FOLLOW-UP

Predrag Stojiljković[1], Marko Mladenović[1], Milan Mitković[1], Ivan Golubović[1], Sonja Stamenić[2], Andrija Krstić[3]

Hip osteoarthritis is a chronic degenerative disease which usually presents after the age of 50. In advanced stages of the disease, the method of choice in treating hip osteoarthritis is total hip arthroplasty. Fitmore® hip stem belongs to short-stems which preserve bone mass in the region of the greater trochanter and the distal part of the femur. In this paper we present the results of total hip arthroplasty using Fitmore® stem in hip osteoarthritis treatment.

We report a series of 10 patients with osteoarthritis of hip that were treated by total cementless hip endoprosthesis with Fitmore® Hip Stem at the Clinic of Orthopedics and Traumatology, Clinical Center Niš. The average age of patients was 56 (48-65) years. There were 5 women and 5 men. The patients were followed for 3 years postoperatively and the functional outcome was assessed according to Harris Hip Score. Radiographic analysis was performed on the radiograph of the hip joint in anterior-posterior projection.

In 7 patients we had excellent (over 90 points) and in 3 patients we had good (over 80 points) functional outcome according to the Harris Hip Score. After 3 years, all patients' radiological findings showed good integration of the Fitmore® stem. Four patients had cortical hypertrophy of the femur. In patients with hypertrophy of cortex, there were no clinical manifestations.

Implantation of total cementless hip joint endoprosthesis with Fitmore® stem in the treatment of hip osteoarthritis is a good choice when treating younger patients with good bone quality.

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Key words: *hip osteoarthritis, total hip arthroplasty, Fitmore® Hip Stem*

[1]Clinic of Orthopaedic Surgery and Traumatology, Clinical Center Niš, Niš, Serbia
[2]Clinic of Anesthesia and Intensive Therapy, Clinical Center Niš, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Predrag Stojiljković
48 Dr. Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: predragssss@gmail.com

Introduction

Hip osteoarthritis (degenerative arthritis of the hip) is a chronic degenerative disease which usually presents after the age of 50. The main symptoms are pain and limitation of movement. Osteoarthritis symptoms often develop slowly and worsen over time.

Total hip arthroplasty improves the quality of life in many patients with severe hip osteoarthritis. The main goal of total hip arthroplasty is to achieve a painless, stable and moving hip (1).

A problem with total hip arthroplasty (THA) that persists in modern orthopedic surgery is the treatment of young patients. With a new model of cementless hip endoprosthesis, good and excellent long-term survival rates have been reported even in young patients (2, 3, 4). The aim of modern cementless hip endoprosthesis is to achieve a longer implant survival. Year after year, we encounter new types of hip endoprosthesis (new materials and designs) which should enable the achievement of this aim.

Bone preservation is necessary during total hip arthroplasty. By removing a small part of bone during a primary operation, the surgeon leaves more options for revision surgery. The Fitmore® Hip's short curved stem save the bone. Three-dimensional shape of the stem and Titan Vacuum Plasma Spray layer for press fit fixation enable good fixation and osteointegration (5).

In this paper, we present the results of total hip arthroplasty using Fitmore® stem in treatment of hip osteoarthritis.

Methods

At the Clinic of Orthopedic Surgery and Traumatology, Clinical Center Niš, ten total hip arthroplasties with Fitmore® hip stem were done in patients with hip osteoarthritis.

The average age of patients was 56 (48-65) years. There were 5 women and 5 men.

In all patients we used preoperative radiological templating. The aim of radiological templating is to set the correct stem family. Following preoperative preparation, hip replacement using cementless total endoprosthesis with Fitmore® Hip Stem was done under spinal anesthesia in all patients. The Fitmore® stem instrumentarium is compatible with all traditional approaches for hip arthroplasty. In one patient, we used posterior approach to the hip and in nine patients we used lateral Watson Johnes approach.

The stem system is comprised of 3 stem families A, B and C (family B with two offsets), to cover different anatomies (5).We used the Fitmore® stem family B in eight patients, and in other two we used the Fitmore® stem family A. In all patients we used Trilogy® Acetabular System. Mean surgery time was 75 minutes.

Operative and early postoperative course were without complications in all the patients. All patients were mobilized using under arm crutches with non-weight bearing on the operated leg. On the fifth postoperative day, the patients were discharged and transferred to physical therapy. Full-weight bearing was allowed after six weeks. All of the patients made a full recovery and they returned to work and everyday life activities 4-6 months after surgery.

Patients were followed for 3 years postoperatively. Functional outcome was assessed according to Harris Hip Score (6).

Radiographic analysis was performed on the radiograph of the hip joint in anterior-posterior projection.

Results

The Harris hip score analyzes pain severity (1 item, 0-44 points), function (7 items, 0-47 points), absence of deformity (1 item, 0-4 points) and range of motion (2 items, 0-5 points). Scores range from 0 (worse disability) to 100 (less disability).Three years after the operation, excellent functional results (over 90 points) according Harris hip score were achieved in 7 patients (70%) and good (over 80 points) in 3 patients (30%). All of the patients returned to work and full everyday life activity after the surgery.

Follow-up radiograph showed good integration of endoprosthesis in all patients (Figure 1, 2, 3).

Cortical hypertrophy of the femur was found in 4 hips (40%) in Gruen (7) zones 3 and 5.

Figure 1. Radiography of the pelvis before left hip arthroplasty

Figure 2. Radiography of the pelvis immediately after left hip arthroplasty with total cementless endoprosthesis with Zimmer Fitmore® Hip Stem

Figure 3. Follow-up radiograph of the pelvis 3 years after left hiparthroplasty. We can see the cortical hypertrophy in Gruen zone 5

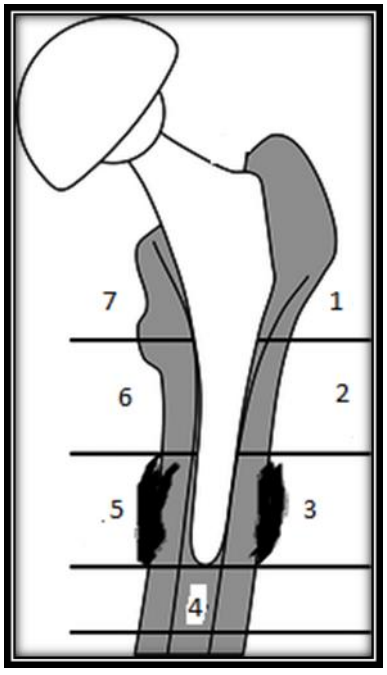

Figure 4. The 2 patients had cortical hypertrophy in Gruen zone 5 and 2 patients in Gruen zone 3

Two patients had medial cortical hypertrophy of the femur in projection of lower part of stem (Gruen zone 5). Two patients had lateral cortical hypertrophy of the femur in projection of lower part of the stem (Gruen zone 3) (Figure 4). In patients with hypertrophy of the cortex, there were no clinical manifestations.

Discussion

Hip osteoarthritis is the most common cause for hip arthroplasty. Incidence of hip osteoarthritis ranges from 3-5% in people older than 55 years. More and more younger patients undergo total hip arthroplasty due to the development of new and better hip endoprosthesis. In the last two decades cementless total hip arthroplasty shows excellent long-term implant survival, with a mean of 94.7% after 16 years (8).

A very important requirement during cementless total hip replacement is a good primary fixation. good press fit (primary fixation) with low micro motions between implant and bone improves bone ingrowth (secondary fixation) (9). For the long life of the hip endoprosthesis, the balance between primary and secondary fixation is very important (decreasing primary and increasing secondary fixation). Bad balance between primary and secondary fixation may result in implant loosening (10).

With time, a hip stem changes the bone structure of the proximal and distal femur (11). Non-physiological stem position and high intrafemoral stress have an effect on the bone remodeling process. A reduced load transfer to the femur may induce a reduction of bone density around the stem (12). Increased load may increase bone density (hypertrophy) (13). Endoprosthesis parameters, such as material or design have an effect on these remodeling processes (14, 15).

Bone conservation is very important during total hip arthroplasty. By removing as little bone as possible during a primary operation, the surgeon leaves more options for future revision surgeries. The total cementless hip joint endoprosthesis with Fitmore® stem in the treatment of hip osteoarthritis is a good choice in the treatment of younger patients with good bone quality. Hip's short curved stem design helps conserve the bone. The triple taper design and proximal Ti-Plasma coating enables secure press-fit, while the trapezoidal cross-section provides rotational stability. The Fitmore® stem's short length and curved design helps preserve the patient's natural bone in the greater trochanter. The Fitmore® rasps and implants allow for a curved atraumatic trajectory and insertion along the calcar arch (5).

Before implantation of cementless total endoprosthesis with Fitmore® Hip Stem it is important to make preoperative planning. The primary objective of templating the femur is to choose the appropriate family and size of the stem. It requires anteroposterior X-ray of the pelvis, which includes the proximal third of the femur (5).

In their biomechanical comparison between Fitmore® stem and classic long stem, Pepkeet all

found that Fitmore® stem can cause remodeling of periprosthetic bone structure. Fitmore® stem has higher rigidity, which is the reason why the remodeling process of the bone is more prominent in this type of prostheses. Further clinical studies are necessary to follow the Fitmore® stem (frequency and severity of cortical hypertrophies, and the clinical long-term outcome of this stem) (16).

In his study, Gustke reports results of 500 Fitmore® stems, with a mean follow-up of 1.3 years. He reported a survival rate of 99.4 %. In this study, more than 60% of the hips showed cortical hypertrophy, but without having a significant effect on the clinical outcome in the early follow-up period (17).

In their retrospective clinical and radiological study, Michael et al. annualized 100 Fitmore® stem implanted in 97 patients (mean age 59 years, 19 to 79 with a 2 year follow-up). They showed cortical hypertrophy in 50 cases (predominantly in Gruen zones 3 and 5). Only in 2 patients with hypertrophy, there was a severe pain in their thigh and in the trochanter region during physical activity. Cortical hypertrophy had no effect on the clinical outcome. Clinical and the radiographic outcome, as well as a good survival rate confirm the excellent results for short, curved cementless stems (18).

In their study, Gasbarro et al. followed osseointegration (with x-ray evaluation and bone densitometry) and functional results (Harris Hip Score) in 33 patients with a Fitmore® stem, 12 months after surgery. They showed good results and predicted a long-term survival rates for this type of stem (19).

Our results also show good functional results and predict a long-term survival rates for Fitmore® stems. Further long-term follow-up is necessary, especially due to the possible clinical manifestation of cortical hypertrophy.

Conclusion

Total hip arthroplasty is the method of choice in the treatment of hip osteoarthritis in advanced stages of the disease. In modern society, there is an increase in the number of young patients undergoing total hip arthroplasty. Better, high-quality materials and innovative designs of modern hip prostheses enable nearly complete and painless movement in the hip with a long-term implants survival. Implantation of total cementless hip joint endoprosthesis with Fitmore® stem in the treatment of hip osteoarthritis is an optimal choice in the treatment of younger patients with good bone quality.

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TOTALNA ARTROPLASTIKA KUKA FITMORE® STEMOM U LEČENJU OSTEOARTROZE KUKA – TRI GODINE PRAĆENJA

Predrag Stojiljković[1], Marko Mladenović[1], Milan Mitković[1], Ivan Golubović[1], Sonja Stamenić[2], Andrija Krstić[3]

[1]Klinika za ortopediju i traumatologiju, Klinički centar Niš, Niš, Srbija
[2]Klinika za anesteziju i intenzivnu terapiju, Klinički centar Niš, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Predrag Stojiljković
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: predragssss@gmail.com

Osteoartroza kuka je hronična degenerativna bolest, koja se uobičajno manifestuje posle 50. godina života. Totalna zamena kuka endoprotezom u odmaklim stadijumima bolesti je metoda izbora lečenja. Fitmore® stem pripada grupi kratkog stema, koji štedi koštanu masu u regionu velikog trohantera i u distalnom delu femura. U radu prikazujemo rezultate lečenja osteoartroze kuka implantacijom bescementne endoproteze kuka Fitmore® stemom.

Prikazujemo seriju od 10 bolesnika sa osteoartrozom kuka kojima je ugrađena bescementna endoproteza kuka Fitmore® stemom na Klinici za ortopediju i traumatologiju, KC Niš. Prosečna starost bolesnika iznoslia je 54,5 (48 – 65) godina. Bilo je 5 žena i 5 muškaraca. Bolesnici su praćeni 3 godine, postoperativno. Funkcionalni rezultat procenjivan je po Harris Hip Score-u. Analizirana je rendgenografija kuka u AP projekciji.

Kod 7 bolesnika imali smo odličan, a kod 3 dobar funkcionalni rezultat po Harris Hip Score-u. Kod svih bolesnika, radiološki nalaz posle 3 godine pokazuje dobro urastanje Fitmore® stema. Kod 4 bolesnika, nakon analize radiološkog nalaza primećuje se hipertrofija korteksa femura. Kod bolesnika sa hipertrofijom korteksa, nije bilo kliničkih manifestacija.

Implantacija totalne bescementne endoproteze zgloba kuka Fitmore® stemom u lečenju osteoartroze kuka, dobar je izbor u lečenju mlađih bolesnika sa dobrim kvalitetom koštanog tkiva.

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Ključne reči: *osteoartroza kuka, totalna artroplastika kuka, Fitmore® stem*

Review article

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THE USING OF SOCIAL NETWORKS AS A HEALTH RISK

Zoran Milošević[1,2], Natalija Premović[4], Miodrag Stojanović[1,2], Aleksandar Višnjić[2,5], Aleksandra Ignjatović[1,2], Marija Apostolović-Andjelković[1,2], Sanja Milošević[3]

Social networks represent the most used type of communication. They are the largest virtual community and an inexhaustible source of information. The essential characteristic of social networks is virtual socializing and communication between people around the world.

Today, it is known that excessive use of social networks for an extended period can cause significant health problems. The most common health problems are highlighted in this paper, and they are addiction, impaired concentration and attention, negative impact on intellectual abilities, increased levels of stress and fatigue, and negative impact on emotions.

It is a fortunate circumstance that it is possible to reduce the negative impact of social networks on health. It is advised to limit the time of using social networks. It is best to connect to social networks from time to time and to look at the content that is offered to you several times during the day. It would be advisable to have access at a time that is convenient and limited for you.

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[1]University of Niš, Faculty of Medicine, Department of Medical Statistics and Informatics, Niš, Serbia
[2]Institute of Public Health Niš, Niš, Serbia
[3]Clinical Center Niš, Niš, Serbia
[4]Health Center Niš, Niš, Serbia
[5]University of Niš, Faculty of Medicine, Department of Social Medicine, Niš, Serbia

Contact: Zoran Milošević
48 Dr. Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: zoran.milosevic@medfak.ni.ac.rs

Introduction

Social networks have occupied our lives. Thanks to the dizzying development of technology, this kind of communication is available to almost everyone on the planet today. The development of telecommunication equipment and the appearance of the smart phone leads a large number of people to spend most of the day with their phone and in online communication with their "friends". We live in the 21st century; everything is different than before; our day begins and ends with some technological device in our hands. By organizing a Wi-Fi network, uninterrupted communication devices are available. Unfortunately, in-ear headphones are mandatory for a large number of people, not just young people. In everyday life, wherever you go, you meet people who use their phones and other communication devices (1-3).

We should all try not to spend our time on the Internet and social networks. There is not even anything social in them. Instead of drinking coffee and talking "face to face", we do it through a chat. If you pay attention, you will see young people in cafes looking at their phone screen non-stop.

Social networks represent the most used type of communication nowadays. They are a large virtual community and an inexhaustible source of information. The primary characteristic of social networks is virtual socializing and communication between people around the world. Besides, people can get to know each other, connect with someone with whom they have lost contact, new jobs can be promoted through social networks; they can be used to exchange information and opinions. Nowadays, current socializing is online in all generations, and unfortunately mostly among young people. The big problem is when young people meet, most of their attention is focused on the status and events on their mobile phone. Nearly half of the world's population (3.48 billion people) uses social networks. The latest research shows that we spend an average of 116 minutes a day on social networks (1, 2).

Social networking sites are a global phenomenon and enjoy an exponential increase in their use (e.g. Facebook, Twitter). Participation on these sites is currently one of the main activities on the Internet, especially for the younger generations (3).

The digital connection of young people is increasing over the years, and social networking sites play a significant role in their personal and professional relationships. According to Statista, the participation of young people on websites and social networks is growing every year (1). More than half of individuals between the ages of 16 and 74 use the Internet for social networks, and the percentage of young people is continuously increasing (1-5).

Today, social networks are indeed an integral part of the modern health care system. The population can be informed and warned daily with the help of social networks about important life issues, among other things and about issues important for health. Social networks significantly helped to raise the level of health culture and positive behaviour in the fight against the virus during the COVID 19 Pandemic.

Most social networks are used to find out what friends we have online are doing, in second place is the reception of information, news and various events, in third place is the use of free time.

The impact of social networks on health

Theoretically, social networks represent an excellent platform for the promotion of healthy lifestyles, consultations with appropriate medical doctors, scheduling examinations, the flow of health information necessary for the diagnosis and treatment of patients, as well as other vital issues important for health. Modern technology has created technical conditions so that you can always be online wherever you are.

Exaggeration in anything inevitably leads to illness. Therefore it is crucial not to overdo the use of social networks. In the world of electronics, social network users are increasingly lonely because they have more friends on social networks compared to real life.

According to a few studies published in The Lancet Child magazine in England, based on interviews with almost 10,000 children aged 13 to 16, it was concluded that depression is related to the use of social networks (3-7). These studies argue that the issue may be more complicated than experts think. Researchers believe that the use of social networks by adolescents disturbs sleep and leads to a decrease in physical activity. Also, the use of social networks leads to greater exposure of young people to various harmful contents. The content mostly causes the problem of sleep disorders and reduced physical activity that young people are inevitably exposed to on social networks. The existence of psychological problems was also recorded.

A lot of surveys showed that Facebook users have symptoms of mental health disorders, unlike young people who do not use this social network (7-9). These researches have proven that the same time spent on the Internet does not cause mental health problems.

There are identical data from the survey of Twitter users' behaviour. A large amount of various information has a lot of content which is harmful and affects mental health disorders. Attitudes and opinions are shared on Twitter; therefore, there is an eager need for followers' confirmation. The correlation between the time spent on Twitter and the increase in the negative mood of users was unequivocally confirmed (3-8).

Young people on social networks have the feeling that they are members of a virtual society and that they are worth more. It's probably the trigger to start ignoring friends in the real world and spending much more time on social media. Inevitably, young people start to compare themselves with others and try to achieve other people's standards, which are often not real.

As the widespread use of social media is associated with depression, anxiety, self-harm, and suicide, there is no consensus (3, 6, 7). Still, most social media impact analyzes appear to stem from unwarranted panic rather than extensive analysis. A study investigating social networks shows that young people today use them the most to alleviate boredom and to connect socially (3, 7, 8).

Biolcati et al. in the study published in the Journal of Clinical Medicine analyzing the use of Facebook, noted that it is becoming more widespread around the world as a communication platform. Young people, in particular, use this social networking site daily to maintain and establish relationships. This study aimed to deepen the under-standing of the relationship between personality traits, social and emotional loneliness, life satisfaction and Facebook addiction. Behaviour and life satisfaction are also discussed as independent variables to explain the difference depending on Facebook (8). Another research showed that conscientiousness, extraversion, neuroticism, and loneliness (social, family, and romantic) are a direct consequence of excessive use of social networks. They conclude that excessive use of this social network significantly affects people's behavior (9).

Social networks are not necessarily the cause of these problems, but the content to which young people are exposed is more likely, as well as their disruption of healthy living habits. There are several negative influences of social networks, and here we will consider only some of them.

Social network's addiction

It has long been known that excessive computer use can develop an addiction, especially when it comes to video games, and the same happens with the use of social networks. Excessive use of social networks creates addiction because it stimulates the pleasure centres in the brain that are activated every time someone leaves you a positive comment or "likes" a photo. Satisfaction is caused by stimulation of the neurotransmitter dopamine. Dopamine is responsible for the feeling of satisfaction, such as the desire for a more significant presence on social networks. It is generally known that the pleasure centre is influenced by communication, and thus also on social networks.

Most social network users have a large number of "friends" who regularly post photos, write statuses and thus consistently communicate. In real life, you will hardly get so many congratulations on various occasions, or positive comments for a new wardrobe, hairstyle or car. Live communication requires much more time than social networks, and people rarely have the opportunity to be in live contact with as many people as it is on social networks. Communication on the Internet is fast and easy, a large number of comments and likes to arrive, which leads to an increase in the amount of dopamine and satisfaction. A significant number of social media users due to the large number of compliments and satisfaction it evokes can create an illusion of their greatness.

The pleasure caused by using social networks, a simple, fun and quick form of communication can very easily lead to addiction.

Apart from the mentioned, when we have nothing to do, or we are just bored, we take a light look at our mobile phone and review what "friends" have posted on one of the social networks. Today, in the era of the smart phone, it is available to almost everyone. In this way, one enters a vicious circle of habit and addiction, and after a while, we cannot imagine life without social networks. And then, more significant problems such as lack of attention and concentration inevitably develop ; you will notice how it is more and more challenging to focus on one thing and how less and fewer things hold your attention.

Previous research suggests that excessive engagement on social networks and the perceived need of their users to be continuously connected to these services is considered problematic (3). Although the use of social networks is a typical behaviour, excessive and compulsive use has recently been proposed as behavioural addiction (3-6).

People around the world are showing inappropriate and problematic use of social networking sites, such as Facebook and Instagram. The symptoms of overuse of social networks are similar to the symptoms of substance use and behavioural disorders similar to an abstinence crisis, such as relapse when trying to stop using drugs (9, 10).

Morioka H, and co-authors conducted a study aimed at elucidating the link between smoking and internet addiction and internet overuse among Japanese adolescents. Responses were received from 100,050 high school students. The results of multiple logistic regression analyses showed that the adjusted odds coefficients for internet addiction and internet overuse were significantly higher in students who smoked (including those who smoked before) than in those who never smoked ($p < 0.01$ for all comparisons). This study found that adolescents who smoked usually or those who smoked more cigarettes a day had a higher risk of problematic Internet use than adolescents who did not (11).

Kumar et al. investigated the prevalence and pattern of problematic use of the Internet among engineering students from various faculties in India. After research, they concluded that students tend to use the Internet in a way that could negatively affect several aspects of their lives. This study is one of the most extensive studies being done in India, which aims to understand the existing pattern of internet use and assess the prevalence of problematic internet use among students (12).

The results obtained in the Salem survey among students in Egypt show that this population is less dependent on social networks. On a sample of 835 Facebook users, findings showed that in most cases, there was a slight Facebook dependence (48.7%) and an average Facebook dependence (46.7%). Very few students had severe symptoms of Facebook addiction (1%), and regular Facebook addiction (5.5%). No differences were found between men and women in their dependence on Facebook (13).

It has been proven that the use of a smartphone can take an addictive form. At the same time, through the literature, evidence of the connection between various psychopathological manifestations and tendencies with the possible addictive use of a smartphone is singled out. A study conducted at the Faculty of Medicine in Belgrade examined the effect of anxiety, depression and impulsiveness concerning addictive phone use (14).

The first signs of addiction are impatience, obsession, exclusion from the environment. These signs are followed by avoidance of obligations and meals, avoidance of personal hygiene and finally, aggression.

Disorder of concentration and attention

Excessive time on social networks can cause a disorder of concentration and attention in healthy people. Decreased concentration may occur continuously, work or study will be neglected to see what is new on social networks. Comments are read quickly and switched from one topic to another, from one place to another. New posts are tracked, messages replied quickly, and comments and likes are sent. For these reasons, attention is more difficult to maintain for a long period of time. This can cause a problem in communicating with real people and can, therefore, cause problems at school or work.

The information that comes through social networks is mostly concise and can also affect the way someone learns new things. This can affect concentration and attention. Excessive time spent on social media can cause a problem when you want to read a printed text, book or textbook; a person can feel quite uncomfortable because they will not receive any feedback. People who have had an attention deficit disorder so far need to know that social media will exacerbate that problem.

Negative impact on intellectual abilities

Activity on social networks, which means watching the posts of other users of social networks and possibly commenting on them, can only lead to

stagnation of intelligence. Viewing and commenting on other people's messages, pictures or other content can in no way be classified as an intellectual activity. No specialized knowledge or education is required to use social networks, reviewing the content of social networks is a movement through the virtual world, and it is certain that it will not lead to more intelligent behavior in real life. On the contrary, it will negatively affect the intellectual abilities of social network users. There is also a great danger that the user of social networks does not have time for real communications, but will be more and more obsessed with communications on social networks.

Increased levels of stress and fatigue

Long-term monitoring of content on social networks can instead of rest, as some believe, lead to increased fatigue. Receiving a large amount of various information will surely result in fatigue and increased stress. Although at the beginning, it seems to users that this process is the opposite. Information arrives every hour, the user is bombarded with it, it is usually short and varied, which on the other hand, certainly requires an effort to follow everything, and if users post and comment, it is an even more significant problem for them. Most of the unnecessary information during the day that just arrives, occupies the user's attention and thus the brain is strained at all times, which certainly makes him or her tired. There is a shortening of sleep over time, and instead of resting, the user of social networks wants to see some more information, and thus he or she is certainly stressed and tired every day. Insufficient rest and focused attention during the day produces stress, which is the trigger for the development of health problems. Physical activity will inevitably be reduced, because walking and staying outdoors will be replaced more and more by activities with a computer screen, lab cannon or smart phone.

The use of social networks has caused profound changes in the way people communicate. Some of these changes in communication can affect certain normal aspects of human behavior and cause psychiatric disorders. Several studies have suggested that excessive use of social networks, such as Facebook, may be associated with signs and symptoms of fatigue, stress, and depression (11-15).

Influence of social networks on emotions

The emotions of social network users when they start sharing or following others are certainly in danger. More and more social media users want to see what others think of their post on the networks or comment on other people's posts.

By following the news and posts on social networks, their user directs his or her attention to other people's emotions. In that way, he or she suppresses his or her own emotions. And with that, one enters a vicious circle, because by suppressing one's own emotions, stress and an emotional problem arise. Day by day, the problems accumulate and it is very difficult to solve them later. It is necessary for each of us to dedicate a lot of time to the analysis of our own feelings, and to deal with problems as little as possible and distract our attention from other people's emotions and problems.

Is it possible to reduce the negative impact of social networks on health?

Social networks, in addition to the negative impact on health, can be useful if you spend moderate time on an electronic device where you have social networks installed and it strongly depends on the content you share on them. With the help of social networks, you can communicate with people you care about, it is possible to get information from all areas of human activity, and thus it is possible to gain some knowledge that will be useful for your health.

The limit where the positive impact ends and the negative impact on your health begins is very small. The basic requirement to avoid negative impact is to limit the time of using social networks. It is best not to be constantly involved than to look at the content that is offered to you several times during the day. It would be advisable to have access at a time that is convenient and limited for you. And choose the time so that it does not disturb your biorhythm and the plans you have for the day. Do not focus your attention on the correspondence and photos of your "friends", but try to read and follow the pages that match your affinities and the work you do. Social networks are full of useful information and content that can be very useful to you. Try not to follow the likes and photos, but follow the content that interests you professionally. This recommendation is especially important for everyone who is in any cycle of their education. Or even better advice and activity is to take a walk in the park or in the woods outside the city when you are tired of your core business. If that doesn't seem feasible to you either, you can physically relax in your bed and take a break from daily activities.

Awareness of what social networks can bring us will come with age, and maybe we will just outgrow them. Until then, if a person really annoys you with his or her posts, delete either his or her or your profile.

The Internet can also be used for other activities

Since the beginning of the Internet, there have been daily discussions about whether it is necessary, whether it is harmful and how much. Most people today need the internet every day. Sure social networks are one of the possibilities, but the Internet offers much wider possibilities. Today, with the help of the Internet, you can find out all the important information, scientific newspapers and many things that people are interested in.

When it comes to content that Internet users pay attention to, they most often watch video content, visit various websites, as well as friends' profiles and edit their own profiles. The dominant social network is still Facebook, and it is similar in

other countries in the region. Approximately 77 percent of the internet population in Serbia visits Facebook more than once a day. In Serbia, 88 percent of them visit Facebook several times during the day, 44% of Internet users post only their pictures, not pictures of their friends (2, 3).

In the coronavirus pandemic, the Internet enabled students to actively participate in teaching, thus saving the second semester of school and the semester of studies.

The Internet provides unlimited opportunities to get informed and learn if you wish. Social networks can serve as a time of relaxation to have fun and relax from gaining new knowledge. It is certainly important to maintain live contacts with your loved ones, so do not let your life be "online".

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DA LI JE KORIŠĆENJE DRUŠTVENIH MREŽA RIZIK PO ZDRAVLJE?

Zoran Milošević[1,2], Natalija Premović[4], Miodrag Stojanović[1,2], Aleksandar Višnjić[2,5], Aleksandra Ignjatović[1,2], Marija Apostolović-Anđelković[1,2], Sanja Milošević[3]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za medicinsku statistiku i informatiku, Niš, Srbija
[2]Zavod za javno zdravlje Niš, Niš, Srbija
[3]Klinički centar Niš, Niš, Srbija
[4]Zdravstveni centar Niš, Niš, Srbija
[5]Univerzitet u Nišu, Medicinski fakultet, Katedra za socijalnu medicinu, Niš, Srbija

Kontakt: Zoran Milošević
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: zoran.milosevic@medfak.ni.ac.rs

Društvene mreže su, u svetu, fenomen današnjice i predstavljaju najkorišćeniji vid komunikacije, najveća su virtuelna zajednica i neiscrpan izvor informacija. Osnovne karakteristike društvenih mreža su virtuelno druženje i komunikacija među ljudima širom sveta.

Danas je poznato to da, na duži vremenski period, prekomerno korišćenje društvenih mreža može izazvati značajne zdravstvene tegobe. Najčešći problemi sa zdravljem istaknuti su u ovom radu, a oni su: zavisnost, poremećaj koncentracije i pažnje, negativni uticaj na intelektualne sposobnosti, povećani nivoi stresa i umora i negativan uticaj na emocije.

Srećna je okolnost to da je moguće smanjiti negativan uticaj društvenih mreža na zdravlje. Predlaže se ograničenje vremena korišćenja društvenih mreža. Najbolje je da se povremeno konektuje na društvene mreže i da se nekoliko puta u toku dana pogledaju sadržaje, koji se nude. Bilo bi preporučljivo da pristup bude u neko zgodno i ograničeno vreme.

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Ključne reči: *društvene mreže, internet, zdravstveno vaspitanje*

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QUANTIFICATION OF CD11C-IMMUNOPOSITIVE CELLS IN DIFFERENT TYPES OF CHRONIC TONSILLITIS

Vladimir Petrović[1], Ivan Nikolić[1], Marko Jović[1], Ivana Graovac[2]

CD11c is a transmembrane protein, belonging to the β2 integrin subfamily. It is generally accepted as a marker of conventional dendritic cells, but can also be found on macrophages, neutrophils, and some B cells. The aim of this paper was to determine numerical areal density of CD11c-immunopositive cells in different morphological compartments of tonsillar tissue in recurrent tonsillitis (RT) and chronic hypertrophic tonsillitis (CHT). As a material we used tonsils which were taken after tonsillectomy, from patients of both sexes, aged 10-29 years: six tonsils with RT and nine tonsils with CHT. The quantification of the CD11c-immunopositive cells was performed on 5 μm thick serial paraffin tissue slices, which were stained immunohistochemically, by using mouse monoclonal anti-CD11c antibody. For quantification we used ImageJ software. Our results showed that CD11c-immunopositive cells were present in all morphological compartments of tonsils with RT and CHT. The higher value for numerical areal density of CD11c-immunopositive cells in RT showed statistically significant difference in crypt epithelium and subepithelial lymphoid tissue compared to CHT. There was not statistically significant difference of CD11c-immunopositive cells in lymphoid follicles and interfollicular regions between the groups. Crypt epithelium and subepithelial lymphoid tissue represent the first site of contact between antigens and tonsillar tissue, and are crucial for the initiation of the immune response. The higher number of CD11c-immunopositive cells in crypt epithelium and subepithelial lymphoid tissue in RT might be connected with more efficient immunological response of this morphological compartment, compared to CHT.

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Key words: *recurrent tonsillitis, chronic hypertrophic tonsillitis, CD11c, dendritic cells*

[1]University of Niš, Faculty of Medicine, Department of Histology and Embryology, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Department of Anatomy, Niš, Serbia

Contact: Vladimir Petrović
81 Dr. Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: vladimir.petrovic@medfak.ni.ac.rs

Introduction

Palatine tonsils, as part of the mucosal immune system of oral cavity, are lymphoepithelial organs that have significant role in local and systemic immunity (1, 2). Due to their anatomical position, on the crossroad of digestive and respiratory system, they come in contact with airborne and alimentary antigens (3). The immune function of palatine tonsils is facilitated by its specific histological structure consisting of four well defined compartments: reticulated crypt epithelium, subepithelial tissue, lymphoid follicles and interfollicular region (1, 4). Interfollicular region is mainly composed of T cells, interdigitating cells (type of dendritic cells) and macrophages (2, 5). Lymphoid follicles are further structurally and functionally divided into mantle zone and germinal center. Mantle zone contains mostly small B memory lymphocytes, while in the germinal center there are large dividing B lymphoblasts (centroblasts) and their differentiated non-dividing forms called centrocytes, both of which have undergone Ig switch recombination (6, 7). Beside them, germinal center contains specific subset of T helper cells, germinal center dendritic cells and follicular dendritic cells that should not be mistaken for conventional or plasmacytoid dendritic cells (2, 6, 8).

Upon the entry through the crypt epithelium, antigens reach the interfollicular region or secondary lymphoid follicles of palatine tonsil where they are being caught and processed by dendritic cells and macrophages, and subsequently presented to $CD4^+$ T helper lymphocytes (2, 9). T helpers lymphocytes then stimulate germinal center B cells that start to proliferate, and develop into antibody-expressing B

memory cells and antibody-producing plasma cells (10).

Dendritic cells (DC) are professional antigen-presenting cells with ability to activate naïve T cells and to initiate, coordinate, and regulate adaptive immune responses (11, 12). These cells are classified into two groups, as conventional DC (cDC) and plasmacytoid DC (pDC), both of which are found to be present in the parenchyma of human palatine tonsils (12). Conventional DC exibit strong expression of CD11c, and can be further classified as cDC type 1 and type 2 (13). cDC_1 present antigens to CD8+ T cells and promote cytotoxic T lymphocytes and Th1 cells, while cDC_2 present antigens via MHC II and promote Th1, Th2 and Th17 immune response (13-15). Plasmacytoid DC do not express CD11c as a surface marker and are included in the response to viral or bacterial infection, by producing high amounts of interferons and by recruiting natural killer T lymphocytes (16). In resting state, pDC are weak antigen-presenting cells, however, when they are activated, they gain the ability to present antigens directly to the T lymphocytes (13).

Bearing in mind that the lymphoid tissue of the palatine tonsil is constantly stimulated by airborne and alimentary antigens, the chronic inflammations of this organ are common pathological conditions. According to Surjan et al., there are two types of the chronic tonsillitis in adults: chronic hypertrophic tonsillitis (CHT) which is characterized by augmented palatine tonsils and hypertrophy and hyperplasia of the lymphoid follicles and recurrent tonsillitis (RT) whose main features are smaller number of lymphoid follicles with active germinal centers, presence of the fibrosis in extrafollicular lymphoid tissue and thin and damaged crypt epithelium (17).

Cd11c is a transmembrane protein, belonging to the β2 integrin subfamily. It is generally accepted as a marker of cDC, but can also be found on macrophages (especially in the digestive and respiratory system), neutrophils, some memory B cells, as well as in acute nonlymphocytic leukemia and some B cell chronic lymphocytic leukemias (18, 19).

In order to determine the possible differences in the number of antigen-presenting cells between CHT and RT, the aim of this paper was to quantify CD11c-immunopositive cells in different morphological compartments of chronically diseased human palatine tonsil.

Material and methods

Material and tissue processing

The material consisted of chronically diseased palatine tonsils, taken after tonsillectomies from patients of both genders: 9 palatine tonsils with CHT obtained from patients aged 18-22 years, and 6 palatine tonsils with RT obtained from patients aged 10-29 years. All palatine tonsils were fixated in 10% buffered formalin and were routinely processed through ascending series of alcohols (75%, 96%, 100%) and chloroform to paraffin blocks. The paraffin blocks were cut on Leica RM2255 microtome (Leica Micro-Systems, Reuil-Malmaison, France). 5 μm thick tissue slices of tonsillar tissue were adhered to microscopic slides and submitted to hematoxylin-eosin and immunohistochemical staining.

Immunohistochemistry

After deparaffination of tissue slices in thermostat at 64 ^{O}C and xylene, the rehydration was done in descending series of alcohols (100%, 96%, 75%) and distilled water. Heat antigen retrieval was performed for 30 minutes in citrate buffer and the endogenous peroxidase was blocked with 3% H_2O_2 for 10 minutes. For detection of CD11c antigen we used mouse monoclonal anti-CD11c antibody (Abcam, ab52632, 1:500) overnight at 4 ^{O}C. The secondary antibody was applied for 45 minutes and tissue slices were then stained with DAB (diaminobenzidine) and counterstained with Mayer hematoxylin. The secondary antibody, DAB and washing buffer, needed for rinses between the steps of immunohistochemical staining, were used from EnVisionFLEX, HighpH visualization system (Agilent, K8000/8002).

Morphometric and statistical analysis

Stained slides were examined using light microscope Olympus BX50 (Olympus, Japan) and images of different morphological compartments of palatine tonsil, under different magnifications, were captured in TIFF format with digital camera Leica DFC295 (Leica Microsystems, Germany). In order to quantify CD11c-immunopositive cells, we determined their numerical areal density (average number of cells in 1 mm^2 of tissue) in all morphological compartments of palatine tonsil:

a) crypt epithelium and subepithelial lymphatic tissue,

b) interfollicular region and

c) lymphoid follicles. As a tool, we used ImageJ software version 1.48v (Wayne Rasband, National Institute of Health, USA). In each group of palatine tonsils, we examined 30 fields (under magnification x40) in each morphological compartment per tonsil. The obtained values for numerical areal density were compared between the groups by using Mann-Whitney rank sum test.

Results

The slices of tonsillar tissue stained with hematoxylin-eosin were used to confirm the clinical diagnosis of RT or CHT.

CD11c-immunopositive cells were found in all morphological compartments of palatine tonsil, both in CHT and RT, and showed the same pattern of distribution in tonsillar parenchyma of both examined groups (Figure 1). They were mostly seen as diffusely dispersed single cells, with centrally located, large euchromatic nucleus and numerous cytoplasmatic processes. Their cytoplasmatic processes were in contact with surrounding lymphocytes, basement

membranes of the postcapillary venules or with the processes of adjacent CD11c-immunopositive cells thus forming a network (Figure 2). In interfollicular regions, CD11c-immunopositive cells were often seen grouped around the lymphoid follicles in a form of a ring. Lymphoid follicles contained CD11c-immunopositive cells predominantly in germinal centers, while they were lower in number in mantle zones.

Rarely, in interfollicular region and lymphoid follicles, CD11c-immunopositive cells exhibited the morphology of large oval cells containing large eu-chromatic centrally located nucleus and without cytoplasmatic processes.

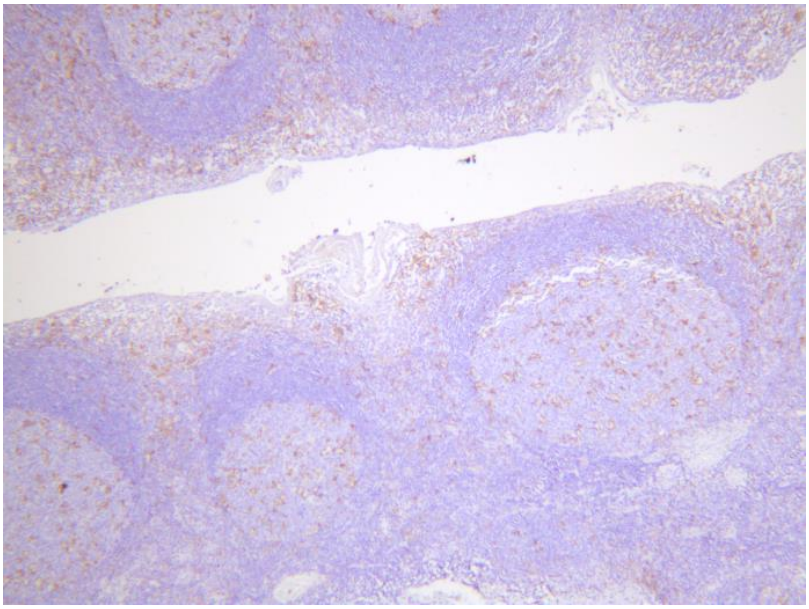

Figure 1. Expression of CD11c antigene in palatine tonsil with CHT. CD11c-immunopositive cells are present in all morphological compartments, x8.

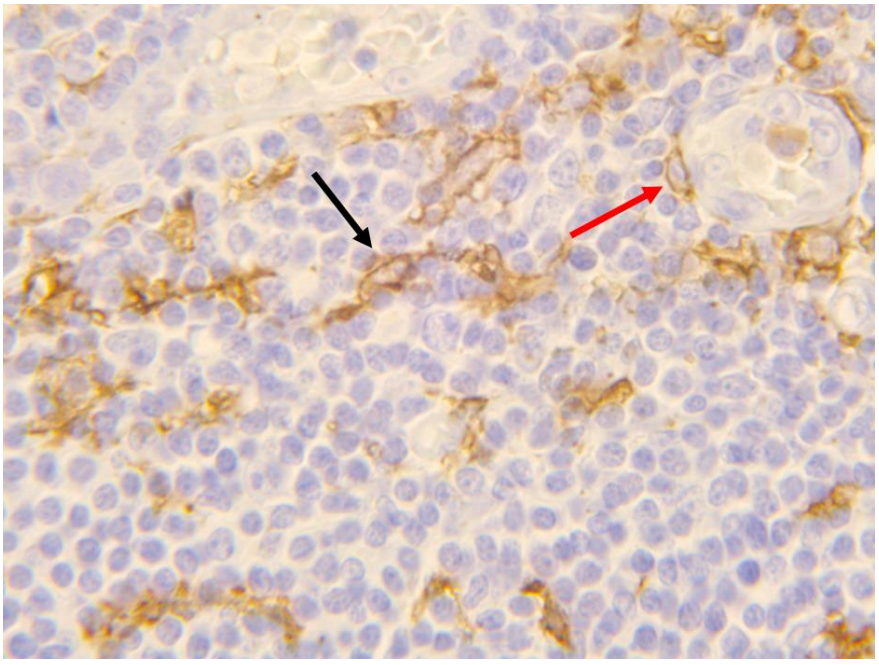

Figure 2. CD11c-immunopositive cells in interfollicular region in tonsil with RT. They are seen as single, dispersed cells between the lymphocytes and macrophages, with long cytoplasmatic processes (black arrow) or around the basement membrane of postcapillary venule (red arrow), x80.

The results for numerical areal density of CD11c-immunopositive cells in CHT and RT are presented in Table 1. The statistically significant difference in the number of CD11c-immunopositive cells was found in crypt epithelium and subepithelial lymphoid tissue, while we did not find statistically significant difference in the number of these cells in interfollicular region and lymphoid follicles between the groups.

Table 1. Value for numerical areal density of CD11c-immunopositive cells in morphological compartments of palatine tonsil with RT and CHT.

Morphological compartments of palatine tonsil	**RT**	**CHT**
	n = 6	n = 9
	X ± SD	X ± SD
Crypt epithelium and subepithelial lymphoid tissue	563.77 ± 65.34*	396.39 ± 104.65*
Interfollicular region	1012.97 ± 151.81	1042.79 ± 103.88
Lymphoid follicles	1137.77 ± 53.75	1161.23 ± 175.84

n – number of palatine tonsils per group, X – average value of numerical areal density, SD – standard deviation, *statistical significance, $p < 0,05$.

Discussion and conclusion

The presence of CD11c-immunopositive cells in human palatine tonsil is well documented in previous studies (12, 20-24). However, to our knowledge, there are no data concerning the morphological distribution and quantification of CD11c-immunopositive cells in human palatine tonsil. The studies of CD11c-immunopositivity were mainly limited to dendritic and some B cell populations, where this marker was used for isolation and identification of these cell types. Both cDC and pDC were found to be present in the tissue of human palatine tonsil, however, pDC are shown to be prevalent dendritic cell type in palatine tonsil (12, 24). Bearing in mind that CD11c is not a surface marker of pDC, it is clear that CD11c does not mark the whole dendritic cell population, but rather the cDC subtype.

The results of our study showed that CD11c-immunopositive cells were distributed in all morphological compartments of chronically diseased human palatine tonsils, with only crypt epithelium and subepithelial lymphoid tissue showing statistically significant difference in the number of these cells, in favor of tonsils with RT. Bearing in mind that almost all cells immunolabeled with CD11c antibody in our tissue slices showed dendritic morphology with the presence of cytoplasmatic processes, we can conclude that these cells correspond to conventional dendritic cells. Follicular dendritic cells in the lymphoid follicles of palatine tonsils, that display dendritic morphology similar to conventional DC, were shown to be CD11c negative in previous studies, and therefore were not taken in consideration (8, 25, 26). Rare, occasional CD11c-immunopositive cells were oval in shape with a centrally located nucleus, and without the presence of cytoplasmatic processes. Taking in consideration their morphology, we assume that these cells most probably correspond to macrophages and memory B cells.

Gorfien et al. examined the presence of dendritic cells in human healthy palatine tonsil, RT and CHT with anti-S100 (marker for cDC), CD1a (marker for cDC1) and RFD1 (marker for interdigitating cells) antibodies by using quantitative approach. Their results, like ours, showed the presence of dendritic cells in all morphological compartments of palatine tonsil. Statistically significant difference was only shown for CD1a immunopositive cells in crypt epithelium in RT/CHT compared to healthy tonsils (27, 28). However, while they found that S100-immunopositive dendritic cells did not show statistically significant difference between the groups, our results show the difference in the number of dendritic cells in crypt epithelium between RT and CHT. This difference might be due to the fact that we observed crypt epithelium and subepithelial lymphoid tissue as one morphological compartment.

The surface epithelium of palatine tonsils makes invaginations in the tonsillar parenchyma in the form of 10-30 tubular, branched crypts that can anastomose among themselves, and which enlarge the tonsillar surface up to 300 cm^2 (29). The crypt epithelium, also referred to as lymphoepithelium, is stratified squamous epithelium that, beside epithelial cells, contains T cells, immunoglobulin-expressing B cells, M-cells and dendritic cells (2). This epithelium represents the first site of contact between antigen and palatine tonsil, and has essential role in the initiation of the immune response (30, 31). Subepithelial tissue contains the cells that migrated from

interfollicular regions. Our results show that the number of CD11c immunopositive cells in crypt epithelium and subepithelial tissue is higher in RT compared to CHT. All CD11c-immunopositive cells in crypt epithelium and subepithelially showed dendritic morphology. However, recent studies of M-cells and Payer patches in the intestine, showed that there is a specific population of CCR6+CD11+ B cells found between the epithelium and lymphoid follicles, that is necessary for differentiation and maturation of M cells (32). Further research is needed to establish whether similar B cell population exists in palatine tonsils.

The studies of dendritic cells in human tonsil reported the presence of three subsets of DC: $CD11c^+$ $CD4^+$ $CD3^-$ cDC and $CD11c^-$ $CD4^+$ $CD3^-$ pDC, both localized in the interfollicular region and a subset of $CD11c^+$ $CD4^+$ $CD3^-$ cDC located within the germinal center, evenly distributed in both the dark and light zone, and in close contact with T cells. These CD11c-immunopositive cells had strong antigen-presenting ability and were also able to directly react with B lymphocytes in vitro (20, 24). $CD11c^+$ $CD4^+$ $CD3^-$ cDC exert their function by secreting chemokine ligand 18 (CCL-18) and chemokine ligand 13 (CXCL-13). CCL18 has the role in attracting naïve T cells ($CD45RA^+$), and CXCL13 is essential for homing of lymphocytes into secondary lymphoid organs and for the development of B-cell follicles (33).

Summers et al. identified five subsets of dendritic cells in human palatine tonsil. Three types expressed CD11c on their surface: $HLA\text{-}DR^{hi}$ $CD11c^+$, $HLA\text{-}DR^{mod}$ $CD11c^+$ $CD13^+$ and $HLA\text{-}DR^{mod}$ $CD11c^+$ $CD13^-$, and all these cells were shown to have strong antigen-presenting potential. Two subtypes were Cd11c immunonegative and were lacking the ability of antigen presentation (23).

In conclusion, CD11c-immunopositive cells are present in all morphological compartments of palatine tonsils with RT and CHT. Statistically significant difference in the number of these cells was observed only in crypt epithelium and subepithelial lymphoid tissue. It was proposed that the chronic infections may structurally alter crypt epithelium and contribute to local immunosuppression (31). The higher number of CD11c-immunopositive cells in crypt epithelium in RT might be explained with preserved immunological function in this morphological compartment, compared to CHT. In order to define the role of these cells in RT and CHT, it is necessary to further research their functional maturation, activation and secretion profile.

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Declarations of interest

None.

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KVANTIFIKACIJA CD11c-IMUNOPOZITIVNIH ĆELIJA U RAZLIČITIM TIPOVIMA HRONIČNOG TONZILITISA

Vladimir Petrović[1], Ivan Nikolić[1], Marko Jović[1], Ivana Graovac[2]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za histologiju i embriologiju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Katedra za anatomiju, Niš, Srbija

Kontakt: Vladimir Petrović
Bulevar dr Zorana Đinđića 81, Niš, Srbija
E-mail: vladimir.petrovic@medfak.ni.ac.rs

CD11c je transmembranski protein koji pripada β2 podfamiliji integrina. Smatra se markerom koncencionalnih dendritičnih ćelija, ali se takođe može eksprimirati na makrofagima, neutrofilima i nekim B limfocitima. Cilj rada bio je da se odredi numerička arealna gustina CD11c imunopozitivnih ćelija u različitim morfološkim odeljcima tonzile sa rekurentnim tonzilitisom (RT) i hroničnim hipertrofičnim tonzilitisom (HHT). Materijal su činile tonzile uzete nakon tonzilektomije bolesnika oba pola, starosti od 10 godina - 29 godina i to: šest tonzila sa RT i devet tonzila sa HHT. Kvantifikacija CD11c-imunopozitivnih ćelija vršena je na serijskim parafinskim presecima debljine 5 μm, koji su bojeni imunohistohemijski, korišćenjem mišijeg monoklonalnog anti-CD11c antitela. Za kvantifikaciju korišćen je program Image J. Naši rezultati pokazuju to da su CD11c-imunopozitivne ćelije prisutne u svim morfološkim odeljcima tonzila sa RT i HHT. Veća vrednost numeričke arealne gustine CD11c-imunopozitivnih ćelija u RT, pokazuje statistički značajnu razliku u odnosu na HHT. Nije pronađena statistički značajna razlika u broju ovih ćelija u limfnim folikulima i interfolikularnim regionima između grupa. Kriptični epitel i subepitelno limfno tkivo predstavljaju prvo mesto kontakta između antigena i tkiva tonzile i imaju ključnu ulogu u započinjanju imunološkog odgovora. Veći broj CD11c-imunopozitivnih ćelija u kriptičnom epitelu i subepitelnom limfnom tkivu tonzila sa RT, ukazuje na efikasniji imunološki odgovor u ovom morfološkom odeljku, u odnosu na HHT.

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Ključne reči: *rekurentni tonzilitis, hronični hipertrofični tonzilitis, CD11c, dendritične ćelije*

Review article

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VENOMOUS SNAKEBITES IN SERBIA THROUGH 125 YEARS: WHAT WE DO (NOT) KNOW IN COMPARISON WITH NEIGHBORING COUNTRIES. A LITERATURE REVIEW

Sonja Nikolić[1,2]

In Serbia, only three autochthonous species of venomous snakes can be found; the bites of two can be harmful or fatal to humans. All three are protected at the national and/or international levels. The author found only four publications depicting small numbers (164 in total) of venomous snakebites in Serbia between 1893 and 2018. On the other hand, only in 2017, 94 persons were hospitalized due to snakebites. Yet, in a ten-year period, only four deaths were reported. Apparently, no attempts were ever made at systematizing the long-term information of this kind for the entire territory of Serbia. For other ex-Yugoslav countries, the author collected the analyses of around 4,000 cases, for approximately the same period. People often confuse non-venomous species for vipers and fear them all. More importantly, from the available sources, it could be concluded that physicians in Serbia are not always well informed about the species of snakes which inhabit the areas they work in. Therefore, transdisciplinary education of both the general public and medical personnel is necessary in this regard. Also, the collecting of information regarding venomous snakebites should become obligatory and centralized, and their analyses could be published periodically. Although envenomation can be a serious health issue, in Serbia there is no reason for panic regarding venomous snakes.

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Key words: *venomous snakebites, education, interdisciplinary cooperation*

[1]University of Belgrade, Faculty of Biology, Institute of Zoology. Belgrade, Serbia
[2]Serbian Herpetological Society "Milutin Radovanović", Belgrade, Serbia

Contact: Sonja Nikolić
16 Studentski trg, 11000 Belgrade, Serbia
E-mail: sonjadj@bio.bg.ac.rs

Introduction

Worldwide, over 100,000 people die and another 400,000 become permanently physically disabled due to venomous snakebites every year, usually in rural, typically poor parts of Africa, Latin America, and Asia (1). The current global situation with snakebites was recently defined as a crisis, not only humanitarian but also one of the data (1). At the time of this manuscript preparation, no systematized data regarding snakebites existed in Serbia (2).

Of the approximately 600 venomous snake species worldwide, 219 were identified as medically important (1). In Serbia, only three species of venomous snakes occur, *Vipera ammodytes* (nose-horned viper with two subspecies), *V. berus* (European adder, subsp. *bosniensis*), and *V. ursinii* (meadow viper, subsp. *macrops*), and all are strictly protected or protected by national and/or international laws (3). In addition to not being capable of inflicting serious damage to humans, *V. ursinii* in Serbia inhabits only a few small, remote and uninhabited areas (3, 4). Nevertheless, because *V. u*. subsp. *rakosiensis* still inhabits southern parts of the Hungarian portion of the Pannonian Plain (5), the experts did not fully exclude the possibility that the meadow viper could have persisted in some remnants of suitable habitats in northern Vojvodina Province (6). The other two species are fragmentally (adder) or widely distributed (nose-horned viper) in our country and often can be found near villages and arable lands, and even in cities (3, personal field observations).

Like in other species of the genus *Vipera*, the venoms of Serbian vipers are complex mixtures of various components with numerous toxic effects, and are extremely variable (7, 8). Although *Vipera* species have highly sophisticated venom injection apparatus, "there is no reason to fear them too much", because the amount of venom they can produce is not enough to kill an adult, otherwise healthy human (9). However, children and the elderly, especially those with some chronic diseases, are at higher risk of fatal outcomes than average healthy adults; the same stands for pregnant women (10-

13). Nevertheless, severe envenomations are not necessarily more frequent in older patients compared to middle-aged (13).

In certain parts of Europe and the Balkans, snakes were systematically exterminated. In 1909, Radić (14) informed about the state-financed killing of snakes in France: "in three counties, in 1889 and 1890 only, 161,313 venomous snakes were killed, while previously in these areas many deaths due to snakebites occurred". He suggested that our country should follow the example of "more developed" states. In Bosnia and Herzegovina, according to official records, 271,685 venomous snakes were killed in 1907 only; in six-year periods, between 790,000 and 863,000 venomous snakes were exterminated (15, 16). Between 1907 and 1911, 40 people died, of the 780 reported snakebites (15): per single human death around 20,000 snakes were slaughtered.

Due to inadequate general education, laypeople usually cannot distinguish between the species of venomous snakes, and often even between the venomous and non-venomous ones. This can become a problem for the physicians because the bitten individuals are not always capable of correctly describing the snake that hurt them.

The aim

What the author presented here is, apparently, the first attempt at collecting the long-term data on venomous snakebites for entire Serbia. The purpose of this article is to inspire other competent researchers to thoroughly search for and adequately analyze and publicize the information regarding this long-neglected health issue and to devise and implement proper educational programs for laypeople and professionals regarding venomous snakes.

Materials and methods

The author searched through the available scientific publications for all kinds of analyses of venomous snakebites in Serbia and other countries of ex-Yugoslavia published until the end of 2018. She wrote to the editors of national medicinal journals and librarians in respective institutions. To obtain concrete data regarding the numbers of fatalities due to snakebites (code T63.0: 44), the author contacted the Statistical Office of the Republic of Serbia (request No. 19956 of December 14, 2018). For the numbers of persons hospitalized due to snakebite envenoming, she communicated with the Institute of Public Health "Milan Jovanović Batut", Center for Informatics and Biostatistics.
Only the "one-sample t-test between percents" was used (www.quirks.com/tools/calculator).

Results

The author found only four papers concerning actual cases of venomous snakebites in Serbia (17–20). Two additional ones were theoretical reviews of venomous snakebites, their effects and treatment (21, 22), and yet another publication described the reactions of a single person to the bite of a non-venomous snake, but this is questionable, because "two punctiform wounds" were reported (23). The retrieved publications cover the period between 1893 and 2007, with a total of 164 cases. Of these, 155 were analyzed in a single paper, from the hospital in Western Serbia (18). For the neighboring ex-Yugoslav countries, on the other hand, a lot of well-systematized information exists, with approximately 4,000 of in-depth analyzed cases found. In all studies, bites of *Vipera ammodytes* prevailed. In Serbia no fatalities were presented, while in other countries 48 deaths were recorded, 40 of these between 1907 and 1911. Regarding the reactions to snake venoms, i.e., clinical pictures in the hospitalized persons, the majority was classified as minor to moderate (Table 1).

The highest numbers of snakebites in Western Serbia were recorded between June and August (127 of 155, 81.9%) although variations in months with most bites were registered among years (18). Other bites in Serbia were recorded between April and July (17, 19, 20). A comparison of data available for Serbia with some of the information from Bosnia and Herzegovina and Croatia (12, 13, 18, 19, 29, 39) showed slight variations in months with maximum numbers of snakebites.

In the only overview available for Serbia (18), among the bitten individuals, "children and pupils" prevailed (45%), followed by "housewives" (29%) and farmers (13.5%). The youngest patient was a 15-month young child, and the oldest had 64 years. Most people were bitten on feet and hands, less on legs and other parts of the body (18, 19).

In many among the analyzed cases, the snakes bit unwary people – farmers working in fields or walking through rough terrain, people picking plants/berries/mushrooms – who did not see the animals and provoked them incidentally (12, 13, 18, 25, 27, 39). Nevertheless, in some cases, the snakes defended from intentional attempts at their capturing and unprofessional handling (19).

According to the Statistical Office of Serbia, there were four cases of death related to snakebites between 2008 and 2017: women fatalities were recorded in 2008, 2013 and 2017 and a man died in 2011.

During 2017, 94 persons were hospitalized in Serbia with the ICD (International Statistical Classification of Diseases and Related Health Problems) code T63.0: Toxic effect of snake venom. One-sample t-test between percents showed that more male (58, 61.7%) than female patients (36, 38.3%) were treated in hospitals: $t_{93,\ 0.05} = 2.334$, $p = 0.022$. According to the national Statistical Office, in 2017, the assessed number of inhabitants of Serbia was 7,020,858, therefore the morbidity and mortality rates due to snakebites (per 100,000 inhabitants) in the given year were 1.34 and 0.01, respectively.

Table 1. Overview of the available data regarding venomous snakebites in most ex-Yugoslav countries. Surveyed periods (and probably some data) in certain areas/hospitals partly overlap.

Region/Municipality	Period	Duration (years)	Total number of cases reported	Clinical signs and symptoms severity: numbers/percentages	Reference(s)
Serbia					
Srem District , Fruška Gora Mt.	1893, 1897	12 years of practice	2 confirmed	moderate	17
Western Serbia, Užice	1960–1968	9	155	many mild and moderate several serious no fatalities	18
Banat province, Vršačke Planine Mts.	1978–1996	18	6	no effect 1 systemic intoxication 5 neurointoxication 3 fatalities 0	19
Southeastern Serbia, Leskovac	2007		single case report	serious (alcohol consumption and walking)	20
Sum for Serbia	***1893–2007***	***114***	***164***	***No fatalities***	***4***
OTHER EX-YUGOSLAV COUNTRIES					
Slovenia					
national Poison Control Centre	1999–2008	10	39	Not provided	24
central part	not specified		single case report	grade 2b (of max 3)	25
Ljubljana	2015	1	3	moderate 2 serious 1	26
Croatia					
Rakovica Municipality	1966–1975	10	24	mild 2 moderate 16 serious 6 fatalities 0	27
Rakovica Municipality	1979	1	single case report	toxic shock: direct bite to blood vessel	28
Split Area	1968–1979	12	397	moderate dominate mild 9 severe present fatalities 0	29
Split	1980–1995	16	391	no complications 78.2% local and general complications 21.8% fatalities 2 (soldiers)	30
Split	1982–2002	21	542	minor 15.1% mild 40.5% moderate 26% severe 18% fatalities 0.4% (2 persons)	13
Zadar	1999–2009	11	93	mild 60.2% intermediate 29% severe 10.8%	31
Zagreb	2000–2012	13	54	4 serious cases no fatalities	32
Split Area	1979–2013	35	160 children < 18 yrs	minor 9.4% mild 35% moderate 30.6% severe 24.4% fatalities 0.6% (1 case, 45-day-old infant)	34, 33
Split Area	2018		2	moderate (woman of 84) severe (boy of 9)	35
Bosnia and Herzegovina					
entire country	1907–1911	5	780 officially reported	40 deaths	15

Trebinje	1971–1980	10	130	mild and moderate effects one fatality: bitten directly on the varicose node	36
Mostar	1972–1983	12	189	provided percents of local and general symptoms, no grades; no fatalities	37
Mostar	1997–2002	6	71	mild 5.6% moderate 90.1% severe 4.2% fatalities 0	38
Mostar	1983–2006	24	341 confirmed	mild 49 (14.4%) moderate 277 (81.2%) severe 16 (4.7%) fatalities 1 (0.3%) 85-yr old woman, rich medical history	12
Konjic Municipality	2010–2014	5	30	mild 63.34% moderate 20% severe 6.66% fatalities 0	39
Montenegro					
entire country	1971–1980	10	971		40
Sum for other ex-Yugoslav countries	***1907–2015***	***108***	***4,219***	***48 fatalities***	***20***

Discussion

Although envenomation resulting from venomous snakebites can be a serious health issue and the author did not intend to underestimate it in any way, in Serbia and the surrounding countries there is no reason for the usual panic regarding venomous snakes: on the population level, stings by hymenopterans and ticks and bites by dogs are more numerous and sometimes more dangerous (22, 27).

To the best of the author's knowledge, the information regarding venomous snakebites in Serbia is being collected, but at the time of the preparation of this manuscript the data were not available (2, 41), i.e., the central register still does not exist. Similar is the case in other parts of the world, including those inhabited by numerous species of highly venomous snakes (1, 42). Being non-infectious, snakebites are not a condition which the physicians are obliged to report to the national public health authority – despite the appeals from several decades ago that they should be better monitored (18, 29).

The highest numbers of venomous snakebites occurred in summer; nevertheless, there were few cases of bites in winter months, when the snakes were disturbed in their hiding places (12, 29). Variations in annual numbers of snakebites were probably related to weather conditions (18, 29).

Incidence/morbidity of snakebites and fatalities resulting from envenomation in Serbia are negligible compared to the neighboring countries and, especially, to strongly affected continents: from 41, i.e., 2.1 (at the beginning of the XX century) in Bosnia and Herzegovina (15), through 2.64/0.027 in Hungary (43) and 5.2, i.e., 1.8% in Croatia (13), up to 653, i.e., 54 in Africa, and Asia and Oceania (42). For comparison, in two towns in Serbia, the incidences of dog bites per 100,000 population were 148.5 and 284.3 (22).

Patients of all ages were treated in hospitals – from babies to persons older than 80 (12, 18, 27, 33, 34). Relatively high numbers of children and women bitten by venomous snakes in previous decades were related to the everyday activities of these two groups: compared to modern generations, they were spending much more time outdoors, keeping livestock, collecting wood or picking berries (29). Later on, an increase was recorded in the numbers of snakebites in elderly people (due to general demographic trends), in tourists and those active in outdoor sports (12, 13, 27).

In the published overviews, the time elapsed from bites to admission in hospitals varied from app. half an hour to several days. The reasons for some of the delays were the self-administered first aid or attempts at applying some traditional cures; importantly, fear was indicated as a significant reason for early visits to general practitioners and subsequent admission to hospitals (12, 17, 19, 38).

Most reactions to venomous snakebites developed into mild to moderate clinical pictures; some of the serious complications occurred because the snakes hit blood vessels (Table 1). The rare fatal outcomes of envenomation were recorded in small children and patients with a history of chronic diseases (12, 34). Serum sickness and anaphylactic shock are also very rare; some cases of anaphylactic shock (in Hungary) resulted from repeated (up to 13, intentional!) snakebites (12, 32, 43). The author reminds that systemic, anaphylactic reaction can quickly develop after the sting of a single bee, wasp

or hornet (22) – the animals that people are much more likely to meet compared to snakes.

In neighboring countries, numerous studies were also performed on the composition, effects and mechanisms of toxicity of venoms of the Balkan *Vipera* species, including the efficacy of various antivenoms (7, 25, 26, 44). For Serbia, the author found only two (45, 46).

During a single year, 94 people were hospitalized in Serbia due to snakebites. This amounts to 57% of the number of all cases described in available scientific publications during the previous 125 years. Annual numbers of snakebites are surely higher: the data regarding the numbers of people who were bitten and treated in (primary) medical facilities are not readily obtainable: one would have to contact individual local medical facilities throughout Serbia and ask for the data. Also, bitten persons sometimes do not seek medical help at all.

Vipers are not aggressive and try to bite only in self-defense, i.e., when they are cornered or handled (47, personal field experience); often they remain motionless, possibly napping, seemingly "hoping" they will stay unnoticed, but that can be dangerous because people do not see them (14, 47).

The media contribute much to the general attitudes towards snakes: most often, the reports of snake occurrences (even regarding non-venomous species) and rare bites in Serbia are unrealistic and dramatized. Unfortunately, thanks to various foreign TV shows, people are usually still better acquainted with exotic species than with local herpetofauna (14, 48). Acknowledging their power, the media could be used in reversing the picture regarding snakes and nature in general.

Traditional treatments of venomous snakebites were known and described even in Serbia 120 years ago (17). In many other parts of the world people still use local traditional remedies/treatments and do not (or are not in the position to) seek the help of official medicine hence still many cases of snakebites remain unreported and the official medical statistics usually underestimate their numbers (1, 15, 16, 27, 41, 49).

Authors have repeatedly concluded that farmers (the most affected category) often work barefoot; in the reviews of bites, many were inflicted on bare skin. In short, as the best prevention of the bites of venomous snakes, simple changes in everyday routine and proper education of both laypeople and medical personnel were suggested, for over a century now (14, 15, 18, 22, 29, 50). Despite the deeply rooted fear, people do react well to appropriate education regarding snakes (51).

In contrast to legitimate bites to local people by autochthonous snakes, many bites are inflicted by exotic, "pet" snakes. In Hungary, for example, 97 snakebites were recorded in 36 years, by 19 exotic (61 of all bites, 62.9%) and two native snake species: bites by the exotics "resulted in severe or life-threatening envenomations" (43). Sometimes, this results from the unavailability of antivenoms. In 2018, a man in Serbia was bitten by his "pet" rattlesnake (52).

Numerous authors emphasized that not all physicians are trained well enough for treating snakebites; also, the inexistence of information regarding this health issue was accentuated numerous times: these can have serious consequences, including the inappropriate treatment of envenomation (14, 18, 12, 39, 43, 53). In previous times some wrong advice (e.g. the sucking of the wound) was available in the literature, even in professional and medical papers (14, 18). This did change recently (22), but apparently still has not reached the wider audience (also 43).

The classification of cases according to their severity varies and changes with new scientific insights, as does the treatment of patients (12, 18, 54). Therefore, continuous education of medical personnel and standardization of procedures is needed in this area, based on the most recent scientific findings (41).

Laypeople often confuse non-venomous species (including *Natrix tessellata*) for vipers and sometimes report the bites erroneously (e.g. adder bites from the regions this species was never recorded in). However, some mistakes are possible: according to the most recent analysis, the ranges of the three *Vipera* species in Serbia do overlap in several places: *V. ammodytes* and *V. berus* were found in sympatry in 22 localities, *V. berus* and *V. ursinii* in two, and *V. ammodytes* and *V. ursinii* in one (3). The three Serbian vipers look alike and usually clearly differ from non-venomous snakes (the exception is *Coronella austriaca* which can resemble adders) hence given due caution mistakes in recognition are highly improbable. Only the melanistic *V. berus* can puzzle inexperienced persons thus presenting a potential threat. Nevertheless, there is no reason whatsoever to intentionally touch/catch/torture any snake: by simply avoiding them, people can be safe. Constant caution is surely necessary while walking, picking fruits/flowers, climbing rocks, hiking, etc., in the areas inhabited by venomous snakes. Fortunately, the viper anti-venom available in Serbia (produced from the *V. ammodytes* venom) is effective in the treatment of both *V. ammodytes* and *V. berus* bites (18, 21, 55).

People use(d) to kill and bring to medical facilities the snakes that bit them, and this was even recommended, not only in Serbia (12, 13, 19, 27, 29, 50). The killing of snakes has to be permanently precluded: all ten snake species in Serbia are (strictly) protected by law, with fines prescribed for their killing and disturbing (56, 57). Populations of *V. ammodytes* declined seriously due to their multidecennial collecting for the venom extraction (58).

Sometimes, mostly in older references, the species lists provided in the available medical publications were incorrect (16, 27, 29, 23, 39). Apparently, the authors made no attempts at communicating with experts in herpetology and faunistics/taxonomy. This can raise a concern: high variability of snake venoms and their effects are long-known facts (8, 18). Several recent publications describe the variability of Vipera sp. venoms at various life stages/seasons and levels (between species, subspecies, populations, among individuals within a

single population, and during a lifetime of an individual) – with consequent concerns regarding the production of antivenins. The effectiveness of available antivenins could be unsatisfying/suboptimal in certain cases (7, 8, 25, 26, 59). In Europe, it is still hard to find comparable studies of Vipera sp. antivenoms and their effects, "and none is licensed with the European Medicines Agency" (60).

Conclusion

In 1909, Radić (14) wrote about field-based education regarding wildlife in North America and recommended that our teachers and priests should be better educated so they could instruct their pupils and flocks. He observed that we are all more interested in the exotic, "overseas" plants and animals – actually completely useless information – convinced that we already know all that should be known about our nature. Instead, he suggested, we should first learn about the species we can see in our country. Recent studies support such an attitude (48, 51).

Medical doctors should attempt to collect, analyze and publicize precise information regarding venomous snakebites, from single hospitals to the national level. Also, they should be constantly educated about the new approaches in the treatment of this health issue.

As noted already by Milićević (18), people from rural areas are well aware of the changes and fluctuations of wildlife in their surroundings (including the numbers of snakes observed in successive years). They should also be consulted in future assessments of both distribution and population sizes and dynamics of snakes in Serbia.

In short, the exchange of information among several disciplines and transdisciplinary education of both the general public and medical personnel is necessary in this regard. Simple brochures and posters could be produced and distributed to health facilities, schools, farmers, etc., with descriptions and distributional details of the three Serbian viper species. This should be accompanied by lectures (and fieldwork: 48, 51) held together by biologists and physicians. Also, collecting and mapping (1, 61, 62) of information regarding venomous snakebites should become obligatory and centralized, and their analyses should be published periodically. Such an approach would benefit all: the incidence of snakebites would be lowered, the treatment of bitten persons improved, the distribution maps of species filled in, and fewer snakes would be intentionally killed.

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UJEDI OTROVNIH ZMIJA U SRBIJI TOKOM 125 GODINA – ŠTA SVE (NE) ZNAMO U POREĐENJU SA SUSEDNIM ZEMLJAMA: PREGLED LITERATURE

Sonja Nikolić[1,2]

[1]Univerzitet u Beogradu, Biološki fakultet, Institut za zoologiju, Beograd, Srbija
[2]Srpsko herpetološko društvo „Milutin Radovanović“, Beograd, Srbija

Kontakt: Sonja Nikolić
Studentski trg 16, 11000 Beograd, Srbija
E-mail: sonjadj@bio.bg.ac.rs

U Srbiji se mogu naći samo tri autohtone vrste otrovnih zmija; ujedi dveju mogu biti opasni ili čak fatalni po ljude. Sve tri vrste otrovnih zmija zaštićene su na nacionalnom i/ili međunarodnom nivou. Autorka je pronašla samo četiri publikacije, u kojima je opisan mali broj (ukupno 164) ujeda otrovnih zmija u Srbiji između 1893. i 2018 godine. Sa druge strane, samo tokom 2017. godine, zbog ujeda zmija, hospitalizovane su 94 osobe. Ipak, u desetogodišnjem periodu, prijavljena su samo četiri smrtna ishoda. Izgleda da do sada nije bilo pokušaja da se sistematizuju dugoročni podaci ovog tipa za celu Srbiju. Što se ostalih republika bivše Jugoslavije tiče, autorka je prikupila analize gotovo 4.000 slučajeva, za približno isti period. Ljudi često mešaju neotrovne zmije sa otrovnicama i plaše se svih. Još značajnije je to što se, iz dostupnih izvora, može zaključiti da ni lekari u Srbiji nisu uvek dovoljno dobro obavešteni o vrstama zmija, koje naseljavaju područja u kojima rade. Stoga je neophodna transdisciplinarna edukacija o ovoj temi, kako šire javnosti tako i medicinskog kadra. Osim toga, poželjno je da prikupljanje informacija o ujedima otrovnih zmija postane obavezno i centralizovano, a njihove analize bi se mogle periodično objavljivati. Iako envenomacija može biti ozbiljan medicinski problem, u Srbiji nema razloga za paniku po pitanju otrovnih zmija.

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Ključne reči: *ujedi otrovnih zmija, edukacija, međudisciplinarna saradnja*

Case report

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NEUROTOXOCARIASIS: CORRELATION OF CLINICAL SYMPTOMS AND RADIOGRAPHIC IMAGING: A CASE REPORT

Stefan Todorović[1,2], Ana Pražić[1,2], Srdjan Ljubisavljević[1,3], Marta Petrović[2]

Human toxocariasis is a parasitic infection caused by the roundworms *Toxocara canis* or *Toxocara cati*. *Toxocara* larvae can cross the blood-brain barrier leading to the neurotoxocariasis. Involvement of the central nervous system is extremely rare. The clinical presentation consists of a wide spectrum of neurological manifestation. Here we present a case of a 63-year-old woman with a rapidly progressive form of neurotoxocariasis. Her initial head computed tomography scan showed multiple hypervascular lesions in her brain and a contrast enhanced magnetic resonance imaging scan showed multiple T1W and T2W enhancing lesions in basal ganglia and cerebellum. Thereafter, in further processing, serological tests showed the presence of higher titer of anti-Toxocara antibody in the serum, as well as the presence of eosinophilia in the serum or cerebrospinal fluid. This case highlights an unusual case of neurotoxocariasis in the nonendemic area, in a patient who was not immunocompromised, who was diagnosed with serological tests and reviews of the relevant radiological findings.

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Key words: *toxocariasis, central nervous system, neurotoxocariasis*

[1]Clinic of Neurology, Clinical Center Niš, Niš, Serbia
[2]University of Niš, Faculty of Medicine, PhD student, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Department of Neurology, Niš, Serbia

Contact: Stefan Todorović
48 Dr. Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: todorovicstefan815@gmail.com

Introduction

Human toxocariasis is a rare parasitic zoonosis with variable clinical picture, caused by the larval ascarids *Toxocara canis* and *Toxocara cati*, that can lead to infection of central nervous system (1). The clinical presentation consists of a wide spectrum of signs and symptoms, but human infection can also be asymptomatic. Clinical presentation depends on immune status, host age, number of ingested larvae, previous contact to larvae, affected tissue. Involvement of the central nervous system is extremely rare. Neurotoxocariasis is a potentially fatal parasitic infection that most commonly occurs in immunocompromised individuals.

The case highlights the difficulties in diagnosing cerebral toxocariasis. Here we present the case of a 63-year-old woman with a rapidly progressive form of the disease, and without other significant diseases. Computed tomography (CT) and magnetic resonance imaging (MRI) scans noted multiple lesions in the right basal ganglia and cerebellum. Clinical presentation and such imaging findings have led the diagnosis in the wrong direction. During the diagnostic processing, numerous diagnostic tests were performed, including a serological blood test for Toxocara antibody, which contributed to the establishment of final diagnostics.

Case report

A 63-year-old woman was receivedto hospital forheadache, forgetfulness, dizziness, instability when standing andwalking and peripheral facial nerve paresis. A week before admission to hospital treatment, the patient became confused, forgetful and complained of dizziness and instability when standing and walking. She was examined by a neurologist (e.g., art. Vertebrobasalis, HTA), after which she was referred to outpatient treatment. The following day, the weakness of the musculature of the right half of the face occurred, with an exacerbation of earlier subjective problems. She returned to the Clinic of Neurology and she was hospitalized.

CT scan showed multiple hypervascular tumoral lesion in the posterior limb of internal capsule, thalamus and mesencephalon. In the further diagnostic processing we also performed magnetic resonance imaging scan which showed multiple T1W and T2W enhancing lesions in basal ganglia (Figure 1) and cerebellum (Figure 2). Such a finding may have

corresponded to multifocal metastasis disease at that moment. For this reason, we have expanded our diagnostic work-up to detect a possible primary tumor process.

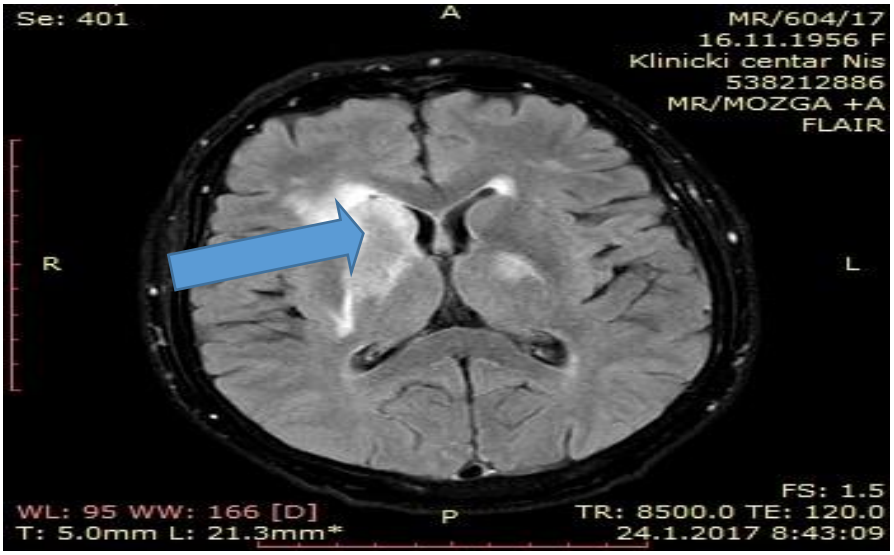

Figure 1. Axial Flair MR image showed multiple hyperintense lesions in the right basal ganglia

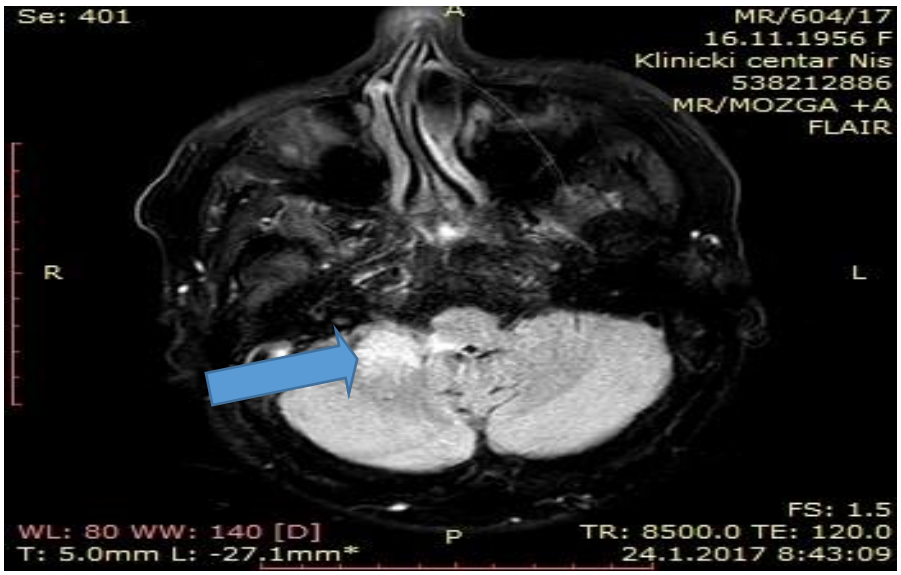

Figure 2. Axial Flair MR tomogram image showed multiple hyperintense lesions in the right cerebellar peduncle

As we did not receive a confirmed primary tumor, we continued with the diagnostic processing. Immunohistochemical stains for cANCA antibodies, HIV, cytomegalovirus (CMV), HSV, varicella zoster virus (VZV), Brucella, *Toxoplasma gondii* were all negative. We have detected the presence of eosinophilia in serum or cerebrospinal fluid, and serological tests showed presence higher titer of anti-Toxocara antibody in the serum. In addition, we confirmed the high level of IgE immunoglobulin. Then we started therapy with antiparasitic drugs (Albendazole) and corticosteroids. Further, after the diagnosis was confirmed, we conducted an epidemiological survey, but did not determine the possible way of infection

(direct contact with an infected animal or ingestion through food).

Her neurological status continued to decline and she died. Her entire hospital stay was 28 days. The family did not consent for autopsy.

Discussion

Human toxocariasis is a zoonosis, which may result in central nervous system injury. Toxocariasis is caused by *Toxocara canis* and *Toxocara cati*, whose definitive host is digestive tract of dogs and foxes for *T. canis* and cats for *T. cati*. The parasite tends to be more prevalent in tropical regions. In our country the frequency of this infection is relatively low (2).

Toxoplasmosis is a parasitosis that most often occurs in people with a weakened immune status. The female *Toxocara* can produce more than hundreds of thousands of eggs per day. Eggs reach to the environment through the dogs or cats feces (3). Human can become infected by direct contact with dogs or cat or by the ingestion of contaminated food. Toxocara eggs hatch in the small intestine and release immature larvae, and then penetrate through the small intestine mucosa, migrate to the portal circulation, lungs and left heart, form where they disseminate via systemic circulation, including the central nervous system (4).

Toxocara canis larve can cross the blood-brain barrier, invading the CNS, leading to neurotoxocariasis. Neurotoxocariasis is rare and occurs mainly in middle aged people. Numerous autopsy studies have proven the presence of *Toxocara larvae* in the leptomeninges, cerebellum, and the spinal cord (5).

Typical clinical presentation of toxocariasis include general clinical symptoms such as hyperthermia, headache, anorexia, nausea, vomiting, body weight loss, cough, dyspnea, possible development of bronchitis or pneumonia, hepatomegaly, cardiac, rheumatological and lymphatic signs and symptoms. Neurotoxocariasis in humans is rare. The clinical presentation of neurotoxocariasis includes a wide range of neurological manifestations such as meningitis, encephalitis, myelitis, cerebral vasculitis. In addition to the central nervous system, the peripheral nervous system may also be affected. Peripheral nervous system manifestation involves radiculitis or inflammation skeletal muscles, but that manifestation is extremely rare. In many scientific literature, the possible association of neurotoxocariasis and some other neuropsychiatric disorders such as schizophrenia, seizure, cognitive deficits, idiopathic Parkinson's disease, and dementia have been discussed. Cognitive and developmental delays have been observed in some infected children. The main clinical manifestations of neurotoxocariasis are vasculitis, obstructive hydrocephalus, encephalitis, meningitis and myelitis (6).

The diagnosis of neurotoxocariasis is a challenge because there is no distinct clinical syndrome. Clinical and radiological picture, laboratory blood test, biochemical, cytological examination of cerebrospinal fluid (CSF), determination of antibodies against *Toxocara* spp. in blood or CSF, are necessary to confirm the diagnosis.

Positive serum IgG antibody by ELISA is required to confirm the diagnosis.

On the other hand, peripheral eosinophilia and CSF pleocytosis are also important for diagnosis. Increased serum IgE levels were almost always positive.

Metastatic disease has the greatest differential diagnostic significance in terms of imaging. Magnetic resonance showing single or multiple subcortical, cortical, or white matter hyperintense lesions on FLAIR and T2WI, with enhancement after contrast. Although such imaging findings are important for diagnosis, they are not specific to neurotoxocariasis. Therefore, postcontrast MRI findings usually indicate multiple enhancing lesions, which often overlap with CNS metastatic disease (7).

Definitive diagnosis is made by histological confirmation, but it is rarely followed.

Treatment is still matter of debate. The drugs used in therapy are anthelmintic drugs and corticosteroids. Albendazole is the drug of first choice in treatment due to a better pharmacological profile than other anthelmintic drugs, with a dosage 800 mg per day (8).

Conclusion

This case emphasizes the difficulties of diagnosing neurotoxocariasis. Clinical diagnosis can be difficult due to atypical clinical presentation that may indicate other neurological diseases. On the other hand, imaging findings may overlap with metastatic disease. Heightened awareness of the occurrence of neurotoxocariasis is needed because clinical and radiological presentation may be unspecific. These can make difficulties to establish an accurate diagnosis. In our case, the final diagnosis was rendered by serological tests, which once again proved to be the gold standard for diagnosis.

Neurotoxocariasis can result in varying and atypical neurological manifestation. It is typical that the infection occurs in immunocompromised patients, but rare cases can be seen in relatively immunocompetent patients. Neurotococariasis is a serious parasitic disease that, if not treated adequately, can have severe consequences, including death.

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Prikaz bolesnika

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NEUROTOKSOKARIAZA: PRIKAZ BOLESNIKA U KORELACIJI SA KLINIČKIM SIMPTOMIMA I RADIOLOŠKIM IMIDŽINGOM

Stefan Todorović[1,2], Ana Pražić[1,2], Srđan Ljubisavljević[1,3], Marta Petrović[2]

[1]Klinika za neurologiju, Klinički centar Niš, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, student doktorskih studija, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakutlet, Katedra Neurologija, Niš, Srbija

Kontakt: Stefan Todorović
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: todorovicstefan815@gmail.com

Ljudska toksokariaza je parazitska infekcija, koju izazivaju okrugli crvi *Toxocara canis* ili *Toxocara cati*. Larve toksokare mogu preći krvno-moždanu barijeru i izazvati neurotoksokariazu. Zahvaćenost centralnog nervnog sistema je izuzetno retka. Klinička prezentacija sastoji se od širokog spektra neuroloških manifestacija. Ovde predstavljamo slučaj šezdesettrogodišnje žene sa brzo progresivnim oblikom neurotoksokariaze. Početno snimanje kompjuterizovanom tomografijom endokranijuma, pokazalo je više hipervaskularnih lezija u mozgu, a kontrastno snimanje magnetnom rezonancom pokazalo je više lezija sa pojačanjem signala T1W i T2W u bazalnim ganglijima i molom mozgu. Nakon toga, u daljoj obradi, serološki testovi pokazali su prisutnost povišenog titra antitoksokara antitela u serumu, prisutnost eozinofilije u serumu i eozinofilne pleocitoze u cerebrospinalnoj tečnosti. Ovo je neobičan slučaj neurotoksokariaze u neendemskom području, kod bolesnika koji nije imunokompromitovan, gde je dijagnoza postavljena serološkim testom, koji pregledava relevantne radiološke nalaze.

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Ključne reči: *toksokariaza, centralni nervni sistem, neurotoksokariaza*

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.

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