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ISSN 1820-8665

Vol. 20 · No1 · MARCH 2019

Serbian Journal of Experimental and Clinical Research

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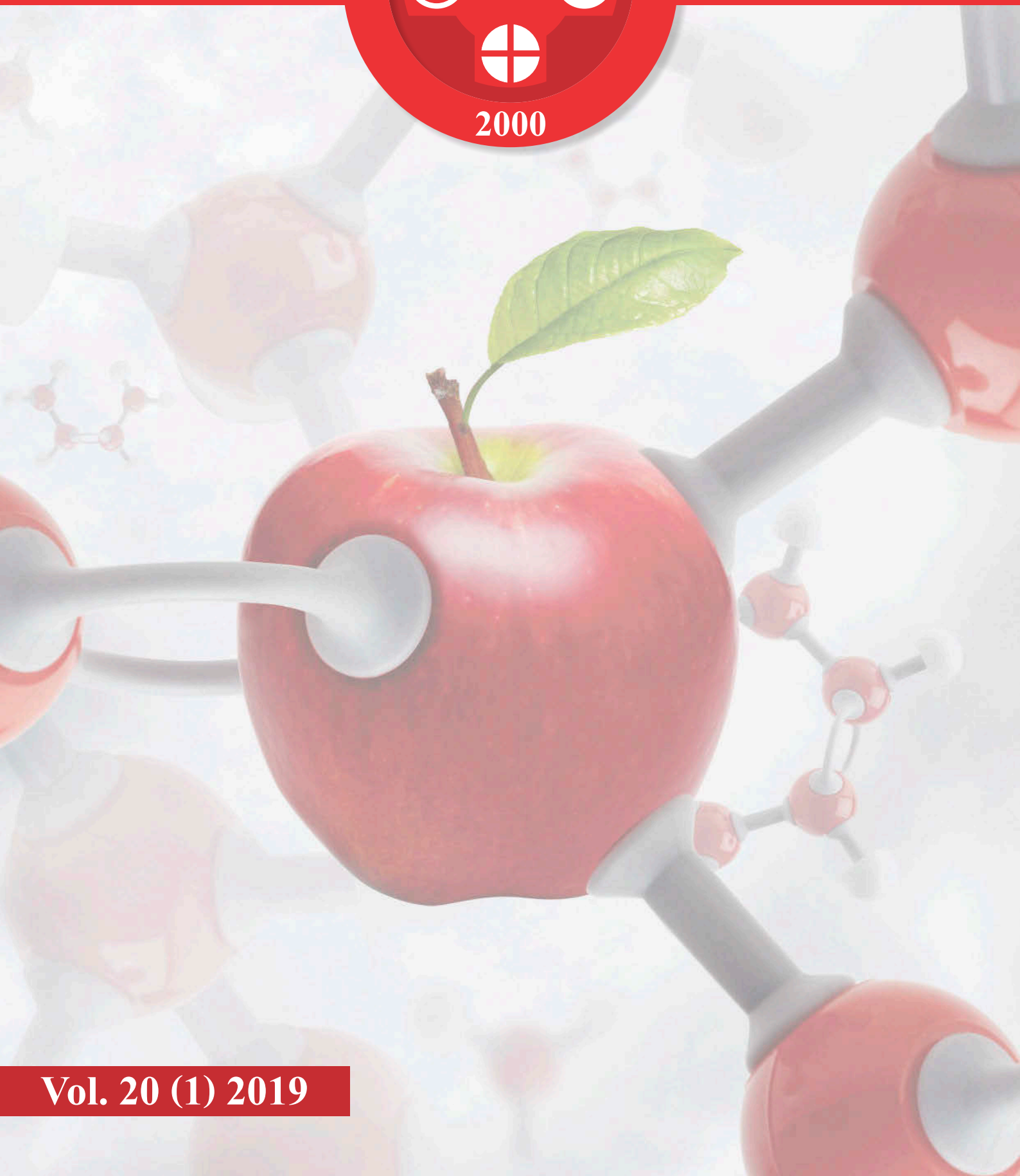

Vol. 20 (1) 2019

Serbian Journal of Experimental and Clinical Research

2000

Print

Faculty of Medical Sciences, University of Kragujevac

Indexed in

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University of Kragujevac, Svetozara Markovica 69, 34000 Kragujevac, PO Box 124
Serbia

https:/medf.kg.ac.rs/sjecr

SJECR is a member of WAME and COPE. SJECR is published four times circulation 250 issues

Serbian Journal of Experimental and Clinical Research is categorized as a scientific journal of M51 category by the Ministry of Education, Science and Technological Development

ISSN 1820 – 8665

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MOGUĆA ULOGA N-METIL-D-ASPARTATNIH RECEPTORA U FIZIOLOGIJI I PATOFIZIOLOGIJI KARDIOVASKULARNOG SISTEMA

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Received / Primljen: 27. 03. 2017. Accepted / Prihvaćen: 28. 03. 2017.

ABSTRACT

N-methyl-D-aspartate (NMDA) receptors belong to ionotropic glutamate receptor family, together with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, kainite receptors and δ-receptors. All of these receptors are tetramers composed of four subunits. NMDA receptors have several unique features in relation to other ionotropic glutamate receptors: requirement for simultaneous action of two coagonists, glutamate and glycine; dual control of receptor activation, ligand-dependent (by glutamate and glycine) and voltage-dependent (Mg^{2+} block) control; and influx of considerable amounts of Ca^{2+} following receptor activation. Increasing number of researches deals with physiological and pathophysiological roles of NMDA receptors outside of nerve tissues, especially in the cardiovascular system. NMDA receptors are found in all cell types represented in cardiovascular system, and their overstimulation in pathological conditions, such as hyperhomocysteinemia, is related to a range of cardiovascular disorders. On the other hand we demonstrated that blockade of NMDA receptors depresses heart function. There is a need for the intensive study of NMDA receptor in cardiovascular system as potential theraputical target both in prevention and treatment of cardiovascular disorders.

Keywords: *NMDA receptors, glutamate receptors, cardiovascular system, homocysteine*

SAŽETAK

N-metil-D-aspartatni (NMDA) receptori pripadaju porodici glutamatnih jonotropnih receptora, zajedno sa AMPA (α-amino-3-hidroksi-5-metil-4-izoksazolpropionska kiselina), kainatnim i δ-receptorima. Svi navedeni receptori su transmembranski proteini sastavljeni od četiri subjedinice. NMDA receptori imaju nekoliko jedinstvenih osobina u odnosu na ostale jonotropne glutamatne receptore: neophodnost istovremenog vezivanja dva koagonista, glutamata i glicina, za aktivaciju receptora; dvojna kontrola aktivacije receptora, ligand-zavisna (vezivanje glutamata i glicina) i voltaž-zavisna (blokada jonom magnezijuma); i ulazak znatne količine jona kalcijuma nakon aktivacije receptora. Sve više istraživanja se bavi fiziološkim i patofiziološkim ulogama NMDA receptora van nervnih tkiva, pre svega u kardiovaskularnom sistemu. NMDA receptori postoje u svim tipovima ćelija koje se nalaze u kardiovaskularnom sistemu, i njihova prekomerna stimulacija u patološkim stanjima, kao što je hiperhomocisteinemija, se dovodi u vezu sa velikim brojem poremećaja kardiovaskularnog sistema. Sa druge strane mi smo ukazali na činjenicu da blokada NMDA receptora izaziva slabljenje srčane funkcije. Nameće se potreba za svobuhvatnim istraživanjem NMDA receptora u kardiovaskularnom sistemu kao mogućeg terapijskog oruđa, kako u prevenciji, tako i u lečenju poremećaja kardiovaskularnog sistema.

Ključne reči: *NMDA receptori, glutamatni receptori, kardiovaskularni sistem, homocistein*

ABBREVIATIONS

ATD - amino-terminal domain
AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CBS - cystathione β-synthase
CNS - central nervous system
CVS - cardiovascular system
CTD - carboxyl-terminal domain
DNA - deoxyribonucleic acid
EAAT - Excitatory Amino Acid Transporters
eNOS - endothelial nitric oxide synthase
Hcy - homocysteine
Hcy TL - homocysteine thiolactone
Hhcy - hyperhomocysteinemia
LBD - ligand-binding domain
MMP - matrix metalloproteinase
NMDA - N-methyl-D-aspartate
NO - nitric oxide
RNA - ribonucleic acid
ROS - reactive oxygen species
TMD - transmembrane domain
VSMC - vascular smooth muscle cells

sciendo
UDK: 612.1.015
Ser J Exp Clin Res 2019; 20 (1): 3-13
DOI: 10.1515/SJECR-2017-0010

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INTRODUCTION

N-methyl-D-aspartate (NMDA) receptors belong to ionotropic glutamate receptor family, together with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, kainite receptors and δ-receptors. All of these receptors are integral membrane proteins, composed of four subunits configured so as to form transmembrane ion channel. Each of these subunits contain four domains: two extracellular domains, 1) the amino-terminal domain (ATD) and 2) the ligand-binding domain (LBD), 3) the transmembrane domain (TMD), and an 4) intracellular carboxyl-terminal domain (CTD). There are several types of subunits that can form the ionotropic glutamate receptor, but each glutamate receptor is made only by subunits within the same receptor group (1). NMDA receptors are made of three types of subunits, usually denoted as GluN1, GluN2 and GluN3. GluN1 and GluN3 subunits bind glycine, and GluN2 subunits bind glutamate. From the fact that NMDA receptors are composed form two obligatory glycine binding GluN1 subunits and two glutamate binding GluN2 subunits (or one GluN2 subunit and one glycine binding GluN3 subunit) derives their unique feature among other glutamate receptors – requirement of both coagonists, glutamate and glycine, for activation (2). Another peculiarity of NMDA receptors is related to magnesium (Mg^{2+}) blockade of ion channel of NMDA receptors. Namely, during resting membrane potential most of subtypes of NMDA receptors are blocked by Mg^{2+} from extracelular space, which greatly hinders the flow of ions through the channel. Due to depolarization of cell membrane, voltage-dependent Mg^{2+} block is removed, allowing the flow of ions through the pore of NMDA receptors. The resulting entry of Ca^{2+} initiates a series of intracellular signaling cascades, which alter the functioning of the cells by activation of different kinases and phosphatases (3).

Beside the known facts about the significance and function of NMDA receptors in central nervous system (CNS), as well as the relationship between dysfunction of NMDA receptors and certain diseases of nervous system (Alzheimer disease), the research data in past years indicated the presence of NMDA receptors in many other organs and tissues (4-7). Regarding the cardiovascular system (CVS), the NMDA receptors were first discovered in a rat cardiomyocytes (8). The NMDA receptors are also found in endothelial cells and vascular smooth muscle cells (VSMC) (9, 10). Bearing in mind the pathophysiological significance of effects of homocysteine (Hcy) in developing of atherosclerosis and consequent disorders, in the limelight comes the possibility that Hcy exerts its negative effects through NMDA receptors (11).

Concerning the crucial role of Ca^{2+} in heart function, as well as in function of vascular smooth muscle cells and endothelial cells, the NMDA receptors could have great importance in maintaining of homeostasis in CVS. In that sense, this review addresses on the recent insights in functions of NMDA receptors in CVS, as well as possibility of targeting of NMDA receptors as potential therapeutic option in various cardiovascular disorders.

Table 1. The names of NMDA receptor subunits in accordance with the NC-IUPHAR nomenclature

	NC-IUPHAR name of subunit	Name of human gene	Location on the human chromosomes
NMDA	GluN1	*GRIN1*	9q34.3
	GluN2A	*GRIN2A*	16p13.2
	GluN2B	*GRIN2B*	12p12
	GluN2C	*GRIN2C*	17q25
	GluN2D	*GRIN2D*	19q13.1
	GluN3A	*GRIN3A*	9q31.1
	GluN3B	*GRIN3B*	19p13.3

NC-IUPHAR - International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification

STRUCTURE OF NMDA RECEPTORS

As already mentioned above, NMDA receptors are composed of three different types of subunits, GluN1, GluN2 and GluN3 (Figure 1A). GluN1 subunit is encoded by single gene, but due to posttranslational processing of RNA arise eight different splice variants, while GluN2 subunit is encoded by four (A – D), and GluN3 (A and B) subunit by two different genes (Table 1) (12-14). By combining of different GluN1 and GluN2 subunits are formed NMDA receptors with different functional characteristics. Different splice variants of GluN1 subunit determine characteristics of NMDA receptors such as modulation by zinc, polyamines, and a protein kinase C, as well as binding to the intracellular proteins (calmodulin, calmodulin-dependent protein kinase II, α-actinin-2) (15, 16). Type of GluN2 subunit determines the biophysical properties of a channel such as the conductivity of a channel, the average time of opening, sensitivity to voltage-dependent Mg^{2+} block (16, 17). If, in addition to GluN1 and GluN2 subunits NMDA receptor contains GluN3A subunit also, it causes a decrease in the conductivity and permeability channels for Ca^{2+}, which was confirmed by registering the ionic currents resulting from activation of the NMDA receptor in neurons in which these receptors do not include subunit GluN3A (18).

Three models have been proposed for the conclusion of NMDA receptors. According to the first model, subunits form stable homodimers, GluN1-GluN1 and GluN2-GluN2, which can then connect and form a tetrameric receptor (19). Tetrameric receptor structure, on the basis of the second proposed model, is created by adding of two GluN2 monomers to GluN1-GluN1 homodimer (20). In accordance with the third model, initially are created GluN1-GluN2 heterodimers, which then tetramerize (21) (Figure 1B).

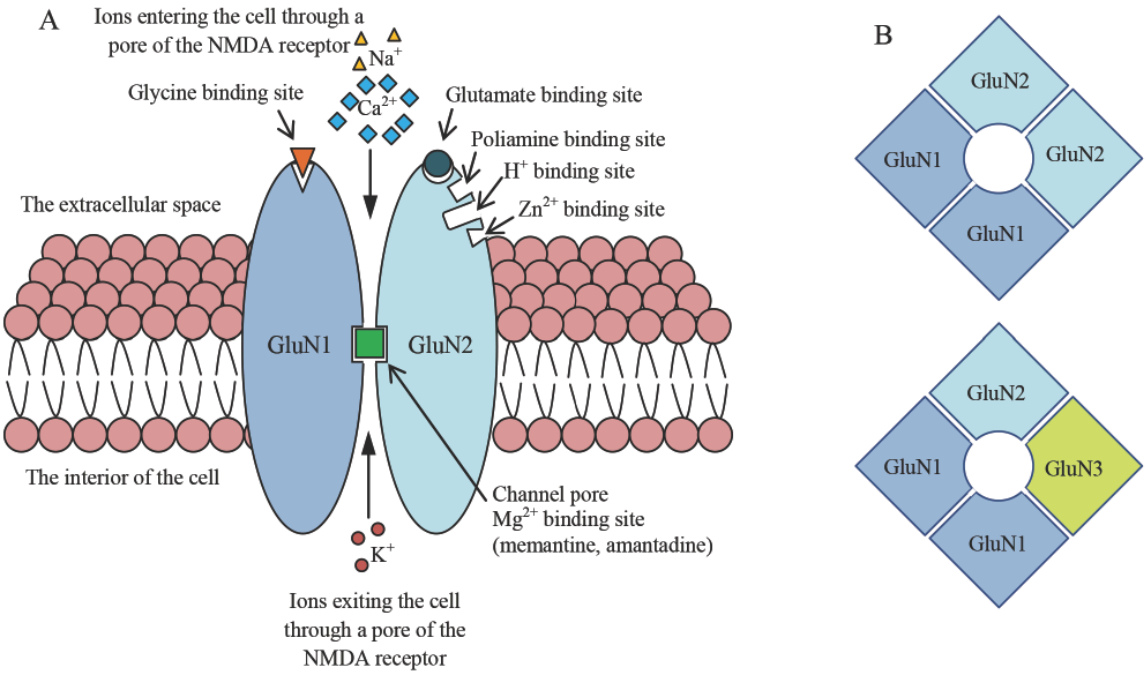

Figure 1. Schematic representation of NMDA receptor (A) and spatial arrangement of subunits that constitute the NMDA receptor (B)

Like other ionothorpic glutamate receptor subunits, all subunits of NMDA receptors are composed of four domains: amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TMD) and carboxy-terminal domain (CTD) (Figure 2). The extracellular ligand-binding domain (LBD) form two extracellular string of amino acids, which are labeled as S1 and S2 (22). All LBD are clamshell-shaped, wherein the polypeptide segment S1, which is connected to the M1 helix of a transmembrane domain, forms a major part of one side of the „clamshell" (D1), while the polypeptide segment S2, which is located between M3 and M4 helices of transmembrane domain, forms a major part of the other side of the „shell" (D2). In this way it is formed a pocked or gap in which is located the agonist binding site. The matching agonists, glutamate, and glycine in the case of NMDA receptors,

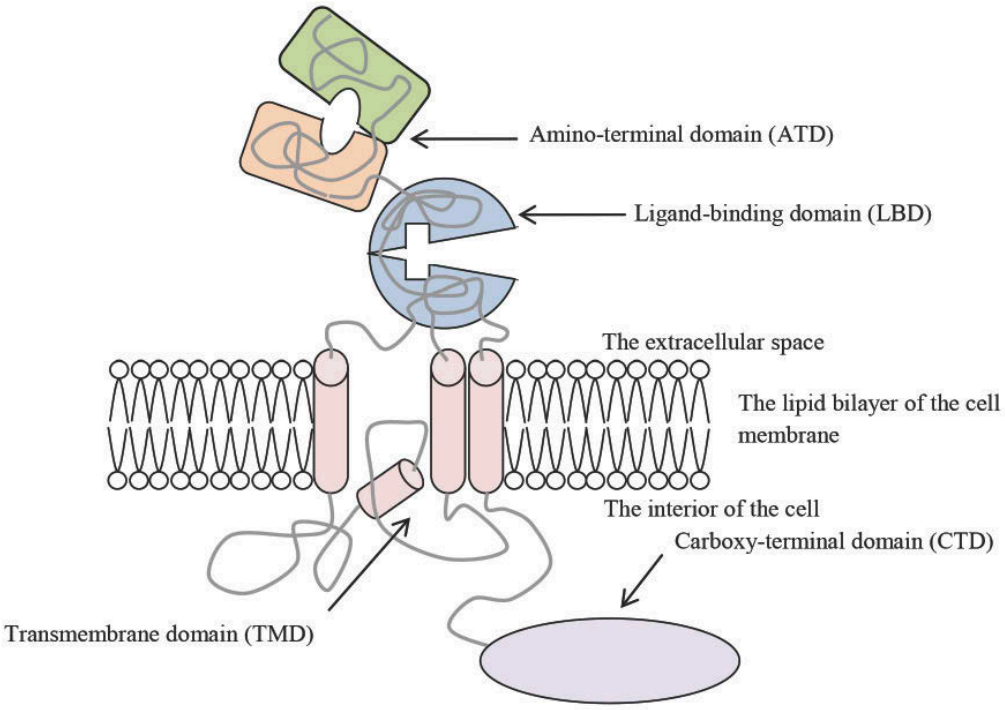

Figure 2. Schematic representation of structure of NMDA receptor subunits

contain groups that bind to the α-amino and α-carboxyl group of LBD. Parts of LBD that form atomic bonds with ligands are highly similar in all glutamate receptors and built mainly by the amino acid residues that belong to the D1 section of LBD. Agonist binding to a LBD causes a conformational change, and it was found that binding of agonist leads to convergence of D1 and D2 and taking more closed conformation form, while in the free form D1 and D2 are separated and take up a more open conformation.

The extracellular amino-terminal domain (ATD) is made up of approximately 350 amino acid residues and, similarly to LBD, forms a clamshell-like structure which reacts with a number of allosteric modulators and thus affects the activity of NMDA receptor. ATD has a role in the regulation of receptor function, and it is not essential for connection of the subunits and construction of the NMDA receptor. It has been proven that ATD of GluN2 subunit control pharmacological and kinetic properties of NMDA receptor, such as: agonist binding activity, deactivation time, the opening probability, the median duration of opening and closing (23). ATD consists of two parts, the R1 and R2, which form a cleft inside which are located more binding sites: 1) a hydrophilic binding site built up of polar amino acid residues that form the binding site for Zn^{2+}, located near the outer aperture of the cleft; 2) a hydrophobic binding site for ifenprodil, located deep in the cleft; and 3) binding place for Na^{+} and Cl^{-} ions, whose role is not fully elucidated (24).

Transmembrane domain (TMD) of all glutamate receptors is linked to LB) by three short links. TMD contains three transmembrane helices, which are marked as M1, M3 and M4, and a membrane re-entrant loop, marked as M2 (25). The transmembrane helices, M1, M3 and M4, each of the four subunits of the receptor form a transmembrane channel; M2 loops limit the inner edge, while parts of the M3 helix limit the outer edge of the channel pore. Parts of M3 helices extending at each other, probably forming a gate that prevents the flow of ions when the receptor is inactive. M1 and M3 helices form the core of the channel, and M4 helix of one subunit makes the connection with the M1 and M3 helices of other subunits. Next to that, linking region preceding M1 (pre-M1) forms a short helix that is parallel to the plane of the membrane and creates links to the carboxyl- and amino-terminal ends of the M3 and M4 helices (25, 26). Pre-M1 helices of all four subunits crate a cuff around the outer channel pore aperture, which may have a significant impact on channel controlling.

The carboxy-terminal domain (CTD) exhibits the highest degree of diversity among all glutamate receptors. It is believed that the CTD affect the stabilization of the receptor, posttranslational modification and labeling of the receptor for breakdown. Deleting the CTD in GluN1 and GluN2A subunits does not affect the function of NMDA receptors, but influences the controlling of the NMDA receptor, because CTD contains a phosphorylation sites and binding sites for intracellular proteins involved in the regulation of its functions (27). Several subunits of NMDA receptors via CTD are associated with Ca^{2+}/calmodulin-dependent protein kinase II, enabling the further expansion of the local signal, thereby allowing the spatial and temporal specificity of receptor regulation. Recent studies suggest the possibility of transmitting the signals through NMDA receptors based on the relationship between CTD and related protein kinases, independently of Ca^{2+} influx (28).

MODULATION OF NMDA RECEPTOR ACTIVITY

NMDA receptors are unique in the group of glutamate receptors since it is required simultaneous binding of glycine for GluN1 (or GluN3) subunits and glutamate for GluN2 subunits for receptor activation (29). In certain sources of literature glycine is mentioned as a modulator of NMDA receptors, in order to distinguish it from the L-glutamate, which is a specific agonist of the entire receptor group. However, since their binding sites are structurally similar, they likely have the equal role in the activation of NMDA receptor. Even though, in physiological conditions, the glycine and glutamate still have different roles in the activation of NMDA receptors. While the L-glutamate is released from the nerve endings in the synaptic cleft and represents an „active" neurotransmitter, is believed that a small amounts of glycine, which normally exists in the synaptic cleft, is sufficient for receptor activation (30).

Within the glycine binding site the α-carboxyl group of glycine forms a hydrogen bond with the amino acid arginine at position 522 (Arg522), the threonine at position 518 (Thr518) and serine at position 688 (Ser688). The amino group of glycine reacts with the carbonyl group of proline at position 516 (Pro516), hydroxyl group of threonine at position 518 (Thr518) and the oxygen of the carboxyl group of aspartic acid at position 732 (Asp732) (1, 31).

Besides the glycine, for glycine binding site also can bind D and L-isomers of serine and alanine, and to act as agonists of the GluN1 subunit (32). D-serine is significantly more potent in comparison to L-serine, and probably represents the primary ligand for GluN1 subunit in particular regions of the brain, such as supraoptic nuclei (33).

Glutamate binds to GluN2 subunits. α-carboxylate group of glutamate reacts with arginine at position 518 (Arg518), and the γ-carboxyl group of glutamate binds to the tyrosine at position 730 (Tyr730), whereby between the domains is formed hydrogen bonds between the tyrosine at position 730 (Tyr730) and the glutamic acid at position 413 (Glu413) (34). In the endogenous agonists of GluN2 subunit, next to glutamate, also include D and L-aspartate, homocysteine and cysteine sulphate (35-37).

There are many competitive antagonists of NMDA receptor that are specific for GluN1 subunit, such as 7-chlorokynurenic acid and its analog - 5,7-dichlorokynurenic acid (5,7-DCKA) (29). Furthermore, the anesthetic effects of xenon are independent of the action of the GABA-ergic nerve transmission, but are dependent on the inhibition of the NMDA receptor (38).

The competitive antagonists of NMDA receptor specific for GluN2 subunit such as (R)-2-amino-5-phosphonopentanoate, are used in order to distinguish the effects of NMDA recepotrs and other ionotropic glutamate receptors (primarily AMRA receptors) (39). Great difficulty arises from the inability to synthesize compounds that would selectively inhibit certain subtypes of GluN2 subunit (A – D), which is consequence of high degree of homology between the LBD of GluN2 subunits.

Noncompetitive antagonists block NMDA receptors while they are closed. In this group of antagonists are classified ethanol and dynorphins, whereby ethanol inhibits GluN2B subunit containing NMDA receptors, and dynorphins inhibit GluN2A subunit containing NMDA receptors (40). Unlike to noncompetitive antagonists, for action of uncompetitive antagonists it is essential that NMDA receptor channel is open, in order to reach their binding site. This group of NMDA receptor antagonist includes memantine and MK-801 (dizocilpine).

Allosteric modulators of glutamate receptors attract great attention lately because of the possibility of fine impact on the physiological functioning of the receptor, as well as the possibility to use for therapeutic purposes. The positive and negative allosteric modulators have a number of therapeutic advantages in comparison with agonists and antagonists of glutamate receptors, including greater selectivity for individual subunits that are part of the receptor. It is believed that better tolerance of allosteric modulators in clinical practice is consequence of their actions on the existing level and the form of the receptor activity, in contrast to the agonists and antagonists that induce or complete blockade or excessive stimulation.

NMDA RECEPTORS AND CARDIOVASCULAR SYSTEM

As already mentioned NMDA receptors play a key role in the functioning of the central nervous system (CNS), but a few decades ago some authors have pointed to the possibility of their existence outside of nerve tissues. Results of the experiments based on the cloning of complementary DNA of humane GluN2C subunit, showed 88% of similarity to rat GluN2C subunit, as well as the great representation of this subunit, as in some parts of the nervous tissue, but also in other tissues, especially in heart (41). The researching of the time and spatial tissue distribution of radiolabelled NMDA receptor antagonists ([3H]CGS and [3H]MK-801) showed widespread distribution of these receptors in a number of organs, such as heart, lung, kidney and stomach (42). Based on the results of their research Leung and coauthors suggested the possible existence of homooligomeric NMDA receptors composed of GluN1 subunits in the rat heart (43). Namely, these authors did not find the GluN2 subunit, but only the GluN1. On the other hand, researching the developmental distribution of NMDA receptors subunits, Seeber and colleagues showed presence of GluN2B subunit in rat heart, which was detected in the cardiac tissue of the early development stages until the tenth week of postnatal life (44). Furthermore, these authors did not find the existence of GluN1 subunits at any stage of development. The results of these studies are inconsistent and in some of its parts are contradictory.

It is also confirmed the existence of NMDA receptors in the endothelium of blood vessels in different parts of the body. The application of glutamate and D-serine (which binds to the glycine binding site) causes the activation of NMDA receptors, which activate endothelial nitric oxide synthase (eNOS) causing increased production of nitric oxide (NO) and vasodilatation in brain arteries. In this cascade mediate astrocytes that store glutamate and D-serine and release them depending on the neural activity (45, 46). Studying the adverse effects of homocysteine (Hcy) on CVS, as well as potential mechanisms by which these effects are achieved, Chen and coworkers indicated the existence of GluN1 and GluN2A subunits in the rat carotid arteries, and the expression of NMDA receptor subunits in rat aortic endothelium (47). Within the same research it was shown that Hcy induces an increase in the expression of GluN1 subunit and increase in cell proliferation, while the previous administration of MK-801 precluded above mentioned effects of Hcy.

Glutamate concentration in cerebrospinal fluid of rats is about 11.4 mmol/L, while the plasma concentrations are significantly higher (48, 49). Based on the aforementioned fact, it can be concluded that peripheral NMDA receptors are under tonic, constant activation. However, the application of activators of these receptors in peripheral tissues increases their activity (50-52). One of possible explanations could be that the interstitial concentration of glutamate is not the same as the plasma concentration. In the CNS the glutamate concentration is maintained within a narrow range due to activity of specific glutamate transporter, EAAT (Excitatory Amino Acid Transporters), which remove glutamate from the synaptic cleft, thereby preventing overstimulation of neurons and the consequent neurotoxicity (53). Since the different isoforms of these transporters are discovered in peripheral tissues inter alia, in the CVS, there is a possibility that the concentration of glutamate in the intercellular space of these tissues is regulated by EAAT (54, 55). Further explanation provides the possibility of lower sensitivity of NMDA receptors in the periphery, or a lower affinity for glutamate and glycine compared to NMDA receptors in the CNS. In that sense, Laketić-Ljubojević and colleagues pointed out the much higher value of dissociation constant (K_d) for glutamate that binds to NMDA receptors on osteoblasts compared to NMDA receptors in the nervous tissue (56).

There is increasing information concerning the importance of NMDA receptors in regulation of the electrical activity of the heart (7). Also, the increasing number of studies are focused on the impact of overstimulation of these receptors in the heart and CVS, where chronic activation of these receptors by agonists cause considerable electrophysiological disorders and increases the probability of ventricular arrhythmias (57).

D'Amico and coauthors showed that inhibition of NMDA receptors during reperfusion decreases risk of arrhythmia occurrence, as well as Ca^{2+} accumulation in mitochondria (58). Similar conclusions were performed by Sun and coworkers based on results of their research (59). Namely, preconditioning with MK-801 and gabapentin (a glutamate release inhibitor), before myocardial infarction induced *in vivo* by ligation of the left anterior descending coronary artery for 30 minutes, significantly mitigated ventricular arrhythmias, improved SERCA2a expression and activity of Ca^{2+}-ATPase in sarcoplasmic reticulum, and consequently reduced Ca^{2+} accumulated in mitochondria. On the other hand preconditioning with dihydrokainate (a glutamate transporter inhibitor) had quite the opposite effect. Furthermore, in this study it was shown that myocardial infarction induces increase in serum glutamate concentration. The ensuing conclusion from the results of this study is that glutamate certainly has a role in the pathogenesis of reperfusion induced arrhythmias, and probable mechanism may be associated with Ca^{2+} overload via the NMDA receptor. Hereupon, reperfusion arrhythmias could be prevented by applying the glutamate release inhibitors or NMDA receptor antagonists.

Increase in Ca^{2+} concentration due to NMDA receptor activation causes imbalance in production and elimination of free radicals and oxidative stress. These effects induced by overstimulation of NMDA receptors could be diminished by MK-801, or by scavengers of reactive oxygen species (ROS) such as glutathione and N-acetylcystein, as it is demonstrated in the study by Gao and coauthors (60). Next to that activation of NMDA receptors in cultured neonatal rat cardiomyocytes, in the context of the results of this research, also increased levels of cytosolic cytochrome c and 17-kDa caspase-3, and depolarized mitochondrial membrane potential, leading to cardiomyocyte apoptosis. These findings suggest that overstimulation of NMDA receptors in the cardiomyocytes could induce apoptosis via a Ca^{2+}, ROS, and caspase-3 mediated pathway, and also point out the importance of NMDA receptors in pathogenesis of myocardial disorders. Furthermore, deletion of GluN1 subunit in cardiomyocytes led to reduced production of ROS induced by Hcy, as well as decreased concentration of NO and matrix metalloproteinase 9 (MMP9) in mitochondria of the heart (61, 62). Meneghini and coauthors also have pointed out to protective effects of the blockade of the NMDA receptors by memantine (63). Memantine prevented the nuclear size reduction in cardiomyocytes in rats exposed to cold stress.

Results of experiments conducted by our research group also indicated the role of NMDA receptors in regulation of heart function. Application of glutamate or glicine did not induce any change in heart function, coronary flow or oxidative stress in retrogradely perfused rat hearts according to Langendorff technique, while on the other hand their combined use caused decrease in observed cardiodynamic parameters, coronary flow and increase in oxidative stress biomarkers (64). In the case of the combined use of glutamate and/or glycine with verapamil (blocker of L type Ca^{2+} channels) in the same experimental model, the slightest changes were observed in the group where verapamil was administered along with glutamate and glycine, which suggests that the activation of the NMDA receptors allows the influx of certain amounts of Ca^{2+}, insufficient to nullifies the effects of verapamil, but sufficient enough to significantly increase observed cardiodynamic parameters compared to other experimental groups in this study (65).

All mentioned facts indicate the importance of NMDA receptors in the regulation of physiological activities, as well as the mechanisms of pathological processes, in the CVS.

NMDA RECEPTORS AND HOMOCYSTEINE IN CARDIOVASCULAR SYSTEM

Homocysteine (Hcy) is non-protein amino acid which occurs as an intermediate product during the metabolism of amino acids methionine and cysteine. In methionine cycle Hcy represents the product in the metabolism of methionine, but also represents a substrate for the synthesis of this amino acid (Figure 3).

On the significance of Hcy in the pathophysiology of atherosclerosis and consequent disorders of the CVS first noted Kilmer McCully almost half a century ago. Namely, Kilmer McCully described a case of advanced atherosclerosis in the medium and small arteries of large number of organs and tissues in child with high concentrations of homocysteine, cystathionine and homocysteine disulfide in the plasma and urine, and also low concentrations of methionine (66). This case report became the basis for homocysteine theory of atherosclerosis, and since then, many researchers deal with the effects of Hcy on the CVS, as well as the molecular mechanisms that mediate in these adverse effects of Hcy.

The significance of the methionine is reflected in large number transmethylation reactions, in which the methyl group of methionine is transferred to other molecules (DNA, RNA, proteins, lipids), as well as in the synthesis of other sulfur-containing compounds (cystathionine, taurine). In a methionine cycle occurs Hcy (Figure 3), which can serve as a substrate for the enzyme methionine synthase in the remethylation pathway, which takes place in the in case of methionine deficiency. This metabolic pathway requires the vitamin B_{12}, which acts as cofactor for enzyme methionine synthase, as well as folate (vitamin B_9), which donates a methyl group to Hcy during remethylation. Otherwise, Hcy enters into the series of reactions which together form a transsulfuration metabolic pathway, where the key role has enzyme cystathione β-synthase (CBS), for whose activity is essential vitamin B_6 (67, 68). Any disruption of described metabolic cascade causes the accumulation of Hcy in the cells which is consequently converted to a considerably more toxic form, homocysteine thiolactone (Hcy TL), by action of the enzyme methi-

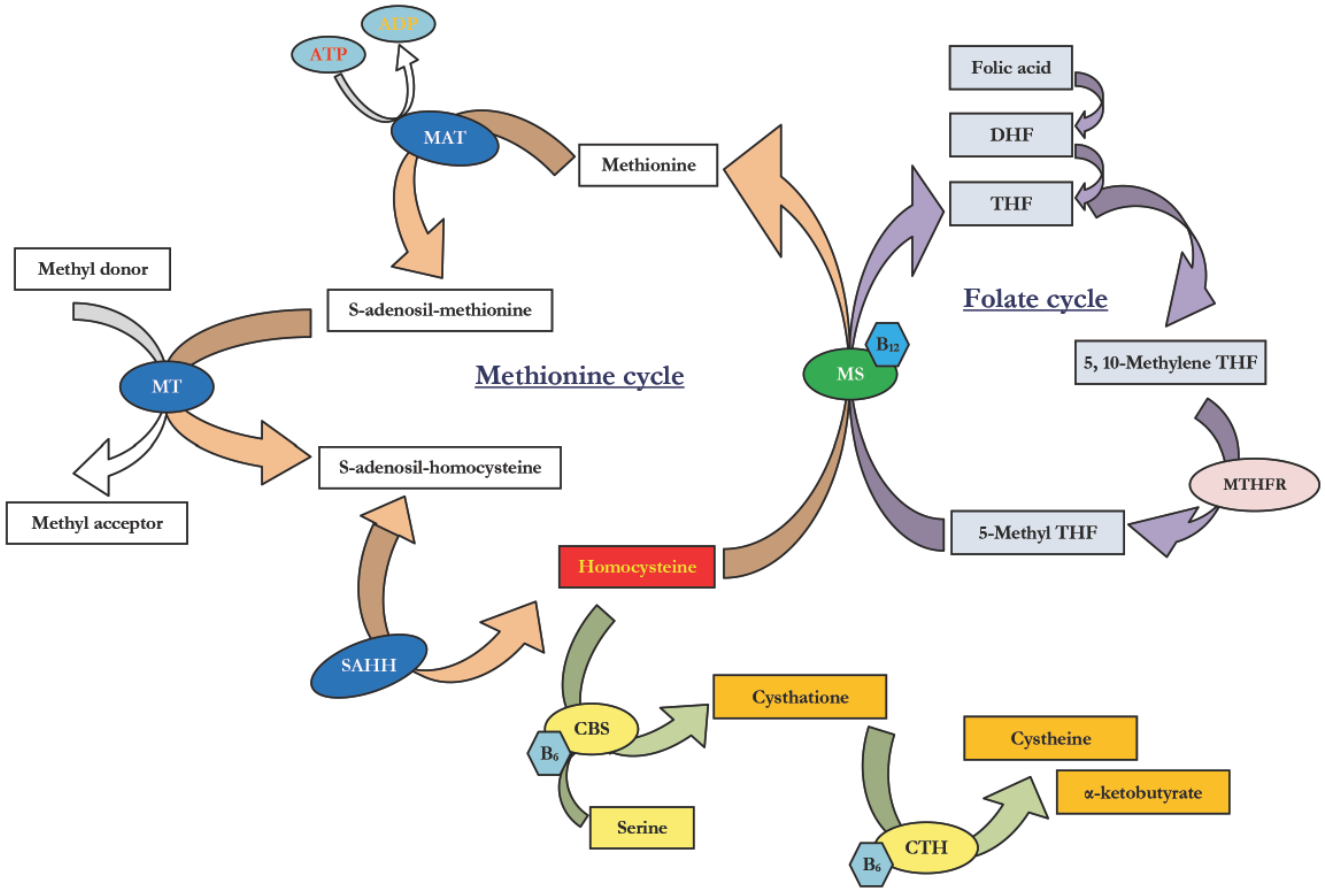

Figure 3. Methionine cycle
MAT, methionine adenosyltransferase; MT, methyltransferase; SAHH, S-adenosylhomocysteine hydrolase; MS, methionine synthase; DHF, dihydrofolate; THF, tetrahydrofolate; MTHFR, 5,10-methylenetetrahydrofolate reductase; CBS, cystathione β-synthetase; CTH, γ-cystathionase.

onyl-tRNA synthetase. Hcy TL reacts readily with amino groups of the large number of proteins, causing homocysteinylation of the protein, which dramatically change the protein activity (69).

Hyperhomocysteinemia is a condition characterized by an increase in the plasma value of total homocysteine concentration above 15 μmol/L. Depending on the value of plasma Hcy, HHcy is classified as moderate (16-30 μmol/L), intermediate (31-100 μmol/L) and severe (higher than 100 μmol/L). The causes of HHcy may be classified into four major categories: 1) genetic disorders of enzymes involved in homocysteine metabolism, 2) inadequate intake of folate, vitamin B_{12} and B_6, 3) increased intake of methionine, and 4) reduction in kidney function (Figure 4), (70). HHcy can also be induced iatrogenically.

HHcy induces interstitial myocardial fibrosis, resulting in developing systolic and diastolic heart dysfunction and chronic heart failure (71). Presumed mechanism of mentioned pathological changes involves the activation of NMDA receptors by Hcy, and this assumption is based on the fact that NMDA receptor blockers reduce the oxidative stress caused by Hcy. As a consequence of stimulation of NMDA receptors in the cardiomyocytes matrix metalloproteinases (MMP - zinc-containing endopeptidases) are activated, and these enzymes change the composition of extracellular matrix in the myocardium. The activation of MMP due to HHsy reduces elastin/collagen ratio, increases collagen deposition in interstitial tissue (fibrosis) between endothelial cells and myocytes, which has the proarrhythmogenic effect, while the use of an NMDA receptor antagonists prevents the activation of MMPs (62, 72).

HHcy induces a cascade of reactions which cause endothelial dysfunction and increased accumulation of ex-

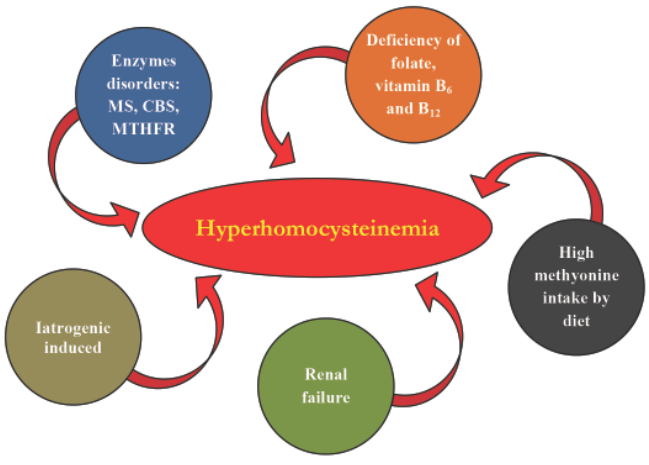

Figure 4. Possible causes of hyperhomocysteinemia

tracellular matrix, and the mechanisms that mediate these disorders include increased production of reactive oxygen and nitrogen species (73-77).

Based on the results of our experimental group it can be concluded that both overstimulation and depression of NMDA receptor activity in heart decrease cardiac function (78). Namely, acute administration of Hcy TL, as well as MK-801, in isolated rat hearts decreased heart function and coronary flow, but the effects of these substances were the opposite in the case of co-administration.

EXPERT OPINION

It is definitely clear, not only that NMDA receptors exist in the CVS, but also to play an important role in the regulation of a physiological process, as well as in pathogenesis of cardiovascular disorders. Bearing in mind the medical and social importance of disorders of CVS in which NMDA receptors could mediate, there is a need for their studying as a potential therapeutic target. NMDA receptors exhibit a considerable complexity regarding the diversity of subunits that compose them and which greatly affect the properties of the receptor, as well as necessity for extremely precise regulation of their action. Namely, it has been shown that blockade of NMDA receptors protect the heart from reperfusion injury (59), but our results showed that blockade of NMDA receptors have adverse effects on heart function (78). It is necessary to focus attention on the synthesis of pharmaceuticals that can specifically act on the peripheral NMDA receptors, and which are capable to finely correct NMDA receptor activity.

ACKNOWLEDGMENTS

This project was supported by Grant no. 175043 from the Ministry of Science and Technical Development of the Republic of Serbia, and the Junior Project 04/2011, Faculty of Medical Sciences, University of Kragujevac, Serbia.

CONFLICT OF INTERESTS

None of the authors of the present study has any actual or potential conflicts of interest to disclose, including financial, personal, or other relationships with specific persons or organisations.

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COST ANALYSIS OF IMAGING DIAGNOSTIC TESTS USED IN THE MANAGEMENT OF PERIPHERAL ARTERIAL DISEASE

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ANALIZA TROŠKOVA VIZUELNIH DIJAGNOSTIČKIH TESTOVA U TRETMANU PERIFERNE ARTERIJSKE BOLESTI

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Received/Primljen: 12.03.2019. Accepted/Prihvaćen: 24.03.2019.

ABSTRACT

The purpose of this paper was to compare the costs of three noninvasive diagnostic investigations as the initial tests in peripheral artery disease management in Romania.

A cost analysis of three imaging diagnostic tests was performed at the university hospital in Timişoara, Romania. The tests were as follows: arterial Duplex Ultrasound Scanning, Computed Tomography Angiography, and Contrast-enhanced Magnetic Resonance Angiography. The evaluation of the diagnostic test performance was performed together with the calculation of the real costs of each investigation. Finally, an economic evaluation of different diagnostic tests was done.

A number of 46 patients (36 male and 10 female) were included in the study. The selected patients have been subjected to a total number of 61 diagnostic tests prior to the therapeutic decision. Both in terms of sensitivity and specificity, Duplex Ultrasound Scanning and Computed Tomography Angiography showed little difference in our study. The cost analysis results showed a net economic advantage if Duplex Ultrasound Scanning is applied as a diagnostic method under conditions of obtaining a similar effect.

In conclusion, Duplex Ultrasound Scanning is accurate, safe, and cost-effective in designing the final therapeutic plan in peripheral artery disease (PAD), especially in the femoropopliteal segment.

Keywords: *peripheral arterial disease, Doppler duplex ultrasonography, computed tomography angiography, cost analysis*

SAŽETAK

Cilj ovog rada je upoređivanje troškova tri neinvazivna dijagnostička ispitivanja kao početnih testova u tretmanu i otkrivanju perifernih arterijskih bolesti u Rumuniji.

U Univerzitetskoj bolnici u Temišvaru u Rumuniji izvršena je analiza troškova tri vizuelna dijagnostička testa. Testovi su bili sledeći: arterijski dupleks ultrazvuk, kompjuterska tomografska angiografija i kontrastno-poboljšana magnetna rezonantna angiografija. Procena učinka dijagnostičkog testa je izvršena zajedno sa izračunavanjem stvarnih troškova svakog ispitivanja. Na kraju je izvršena ekonomska evaluacija različitih dijagnostičkih testova.

U studiju je bilo uključeno 46 pacijenata (36 muškaraca i 10 žena). 46 odabranih pacijenata bilo je podvrgnuto ukupno 61-om dijagnostičkom testu pre terapijske odluke. I u smislu osetljivosti i specifičnosti, Dupleks ultrazvučno skeniranje i kompjuterska angiografija pokazali su malu razliku u našoj studiji. Rezultati analize troškova pokazali su neto ekonomsku prednost ako se Dupleks ultrazvučno skeniranje primeni kao dijagnostička metoda u uslovima dobijanja sličnog efekta.

U zaključku, Dupleks ultrazvučno skeniranje je tačno, sigurno i ekonomično u dizajniranju konačnog terapeutskog plana u PAB u, posebno u femoro-poplitealnom segmentu.

Ključne reči: *periferna arterijska bolest, Doppler dupleks ultrasonografija, kompjuterizovana tomografska angiografija, analiza troškova*

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UDK: 616.13/.14-073:33(498)
Ser J Exp Clin Res 2019; 20 (1): 15-24
DOI: 10.2478/sjecr-2019-0011

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ABBREVIATIONS

CA - Conventional Angiography
CE-MRA - Contrast enhancement Magnetic Resonance Angiography
CTA - Computed Tomography angiography
CMA - Cost-minimization analysis
DUS - Duplex Ultrasound Scanning
PAD - Peripheral Artery Disease

INRODUCTION

Cardiovascular disease was mentioned as the first cause of morbidity, with an average rate of 2557 per 10000 people. Nearly half of this rate was ischemic arterial pathology with a different location than coronary or cerebral arteries (1). According to a survey conducted by the National Agency for Health and Nutrition Examination (NHANES) in the United States in 1999-2000, there were approximately 8-10 million people with PAD. According to the same report, the PAD prevalence increases substantially with age for both genders, at a rate of 1.5-2 times per year. It has been estimated that incipient signs of PAD can be detected in 4% of the population over 40. This prevalence increases to 14.5% for people over 70 years of age (2). People with PAD have 3 times higher risk for all causes of mortality compared to the general population (3). Patients with symptomatic PAD have a 30% risk of death within 5 years of diagnosis and almost 50% after 10 years (4). The risk of death increases in patients with more severe disease requiring surgery. Regarding the costs of treating this pathology, Medicare (the US Health Insurance System) recorded an expenditure of $ 4.37 billion that has been steadily increasing over the time (5).

A patient presenting to the general practitioner with intermittent claudication has a 50% chance of getting better, 25% chances for the disease to be stationary and 25% for the disease to evolve unfavorably. Of those with unfavorable evolution, 20% will need interventions and 8-20% will undergo major amputation (6).

A preliminary diagnosis of intermittent claudication is usually done using the Edinburgh claudication questionnaire. Clinical examination in patients with PAD usually reveals a weak or absent pulse. The next step is represented by an ankle/brachial pressure index (ABPI) measurement. The patient is further investigated only if an invasive intervention is planned (7).

There are several imaging techniques that can be used to asses lower limb vasculature prior to surgery. The rapid development of new imaging techniques makes choosing one of them for preoperative assessment more difficult. Nowadays, the classical gold standard investigation represented by digital subtraction angiography has been largely replaced by less invasive techniques such as contrast-enhanced magnetic resonance angiography (CE-MRA), multirow computed tomography angiography (CTA) and color-coded duplex ultrasound scanning of the artery (DUS). In DUS stenosis is graded by the ratio between the peak systolic velocity of the targeted/stenosed vessel and adjacent or contralateral non-stenosed vessels. This index is called the peak systolic velocity ratio (PSVR). Despite the large-scale adoption of DUS in vascular disease diagnosis, its use as a single investigation before revascularization of the inferior limb remains controversial.

Nowadays, PAD management demands vascular specialists to choose both the most appropriate diagnostic tests for a clinical situation and the most cost-effectiveones.

The aim of this paper was to compare the costs of three noninvasive diagnostic investigations used in PAD management. In order to do this, we followed a three steps strategy. The first step was to assess a selected group of patients with PAD and to analyze it in terms of diagnostic tests, patients' outcome and particularities related to the management of PAD. The second step consisted of the evaluation of the diagnostic performance of the above-mentioned techniques. Finally, the third step was the economic evaluation of the diagnostic tests.

PATIENTS AND METHODS

Study group

We conducted a retrospective clinical research study on patients with suspected PAD admitted between January 2008 and July 2010 at the Clinic of Surgery belonging to the University hospital in Timișoara, Romania. The study was carried out with the agreement of the Ethics Committee of the University hospital. Out of the total number of patients with symptoms raising suspicion of ischemic arterial disease on admission (489), a total of 46 patients who met the inclusion criteria were selected.

The inclusion criteria were represented by: complete information regarding the patient and the pathology in the

study (collected from the medical observation sheet), complete and detailed information about the diagnostic test that determined the therapeutic decision (DUS, CTA and CE-MRA), and details about patient's follow-up. For surgical patients with revascularization techniques, the reference standard was the intraoperative findings. For the remaining patients, the reference standard considered was the patients' vascular status both during hospitalization and follow-up. The minimum period of follow-up was set at six months. Cases requiring readmission, less than 30 days after discharge for treating the same lesion, were reported as cases of unfavorable evolution.

The exclusion criteria were represented by: ischemic artery disease with a different location than lower limbs, the impossibility of following patient's status for a minimum period of six months and, for non-surgical patients, the impossibility to set the reference standard. There was a single exception from these exclusion criteria represented by a patient who died eight days after the vascular intervention.

The diagnostic criterion was represented by the critical stenosis or total occlusion detected in any segment of the entire lower limb. DUS was performed with a 5 MHz linear array transducer. Diagnostic lesion when using DUS was considered, either an increase in PSVR of 2.5 which means a 70% reduction in the luminal diameter or absence of flow on color and power Doppler implying arterial occlusion. All CTA exams were performed on a 16 slice scanner. CE-MRA was performed on a 1.5-T imager. The decision on the appropriate treatment (revascularization procedures, amputation or conservative treatment) was made by a senior surgeon based on the reports and images of one or more (when required) of the following investigations: DUS, CTA, and CE-MRA. Revascularization procedure was chosen as the first option in the presence of no adequate outflow. When the outflow condition was poor, the option of either conservative treatment or amputation was selected according to the clinical features.

Evaluation of the Diagnostic Tests Performance

In order to assess the accuracy of the tests chosen to diagnose PAD, two reference standards were used in order to calculate the sensitivity, specificity, positive and negative predictive values, accuracy and classification error rate. The 2x2 tables of test performance/contingency (Table 1) were used in order to calculate the following indicators:

Table1.Contingency table assessing test performance.

		The evaluated test		
		T+	T-	
Reference standard demonstrating the state of the disease	D+	True positive (TP)	False negative (FN)	No D+
	D-	False positive (FP)	True negative (TN)	No D-
		No T+	No T-	Total No

Sensitivity – proportion of people with affection who have a positive test

=> *SN* = TP/(TP+FN) = TP/No B+

Specificity – proportion of people without affection who have a negative test

=> ***SP*** = TN/(TN+FP) = TN/No D-

Positive prediction value - proportion of people testing positive that have the condition

=> ***PPV***= TP/(TP+FP) = TP/No T+

Negative prediction value - proportion of people testing negative that do not have the condition

=> ***NPV*** = TN/(FN+TN) = TN/No T-

Accuracy => ***AC*** = (TP+TN)/Total No

Classification error rate => ***ER*** = (FN+FP)/Total No

As convention recommended by Moses et al, for all cells with a value of zero, when calculating the indicators, a value of 0.5 was added (8).

Economic Evaluation of Three Diagnostic Tests Used in Deciding PAD Management

The choice of diagnostic test goes far beyond a simple assessment of the performances of an individual assay. In order to be able to choose the best diagnostic test, other aspects, such as patients' disease outcome and cost analysis should be considered.

In this respect, it is necessary to calculate the costs of the investigation and the general formulas for computing the real cost/investigation, regardless of the type of diagnostic test, are presented in Table 2.

Table 2. The general formulas for calculating the real cost/investigations

Operational costs/month = Direct medical expenses + Indirect medical expenses + Non-medical expenses (when appropriate)
Direct medical expenses = Expenditure on wages + Expenditure on materials + Capital costs (damping + wear/depreciation + maintenance service of the investigation equipment)
Indirect medical expenses = Overhead/General costs (facility space cost, utilities, energy, etc) + Ancillary expenses (with staff not directly involved in the execution of the service – cleaning, porters, medical records, accounting service, office supplies)
Real cost/investigation = Operational costs per month/number of working days per month/number of working hours per day/number of investigations per hour

Directly assignable costs included personnel costs, material costs (contrast agents, syringes, etc) and equipment costs. Personnel costs were calculated based on the estimated time spent performing a certain type of diagnostic test per month and the mean wages for each involved personnel category (including 43% tax charges). Costs of materials were calculated based on cost prices and summed for each test. The capital/equipment costs consisted of annual costs of imaging equipment and the annual costs of equipment maintenance. The costs for radiology equipment were computed using the annuitization method with a 5% annual discount/interest (9).

Depreciation of medical devices was made in accounting for a period of 5 years. The costs of maintenance of the equipment were 5%/year. The costs of radiology equipment as well as the costs of equipment maintenance were divided by the proportion of total available room time (80% of a 160-hour work month) (10). Overhead/general costs have been calculated by estimating the costs of utilities, energy, special location conditions. Ancillary costs were not included because they were approximately equal in all three radiology tests studied.

Regarding the economic aspects involved in choosing the preferred diagnostic test, a *cost-minimization analysis (CMA)* was performed.

CMA measures and compares input costs when the outcomes of two or more options are assumed to be equivalent. This type of economic evaluation is often seen as being "Cinderella" of the Cost-effectiveness analysis. Many analysts consider this method a simple cost analysis. The difference between the two is represented by the fact that, when running a CMA, all possible consequences have to be highlighted and compared, and only if the differences among them are considered insignificant, the analysis can be performed (11).

The cost side of a CMA equation is equivalent to that of the other methods used in Cost-effectiveness analysis. In CMA, the least expensive option is preferred. The results of the analyzed options can be compared in the matrix presented in Table 3 (9).

Table 3. Matrix for CMA comparison (9)

		Incremental effectiveness of option compared to control		
		More	Same	Less
Incremental cost of the option compared to control	More	7	4	2
	Same	3	9	5
	Less	1	6	8

Strong dominance for decision	Weak domination for decision	Non-dominance: no obvious decisions
1 = accept option **2** = reject option	**3** = accept option **4** = reject option **5** = reject option **6** = accept option	**7** = Does added effect deserve added cost to adopt the option? **8** = Is the reduced effect acceptable, given reduced cost to adopt the option? **9** = Neutral on costs and effects, other reasons to adopt the option?

RESULTS

Patients' Characteristics

A number of 46 patients (36 male and 10 female) were included in the present study and the general characteristics (distribution by gender and age) of the patients are presented in Figure 1.

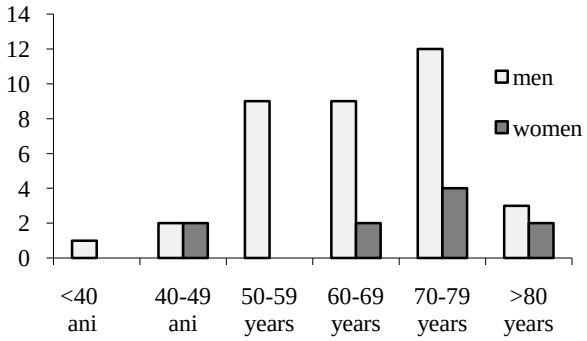

Figure 1.

The diagnostic tests studied in our research were represented by arterial DUS for all the years of the study, CTA mainly for 2009 and 2010, and CE-MRA only for 2008. The type of diagnostic lesion was considered critical stenosis (>70% stenosis) or total occlusion detected in any segment of the lower limbs. The 46 patients selected have been subjected to a total number of 61 diagnostic tests prior to the therapeutic decision. During the hospitalization, the surgical patients were subjected to at least three evaluation exams while the medical ones have received at least one DUS evaluation. Regarding the follow-up period, the patients were examined at least twice with arterial DUS. The distribution of patients with suspected PAD lesions with respect to the diagnostic investigations was as follows: 32 patients were diagnosed with only one type of examination, while in 14 cases the diagnosis was based on information obtained by combining two or all three investigations (Figure 2).

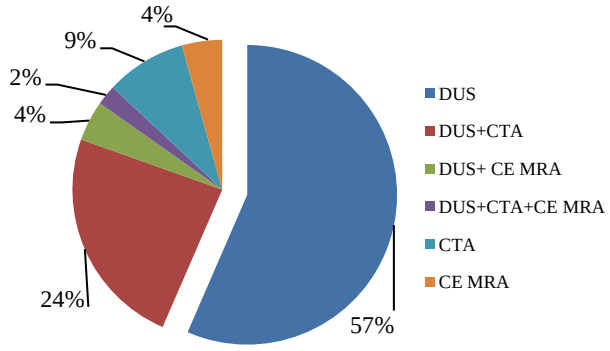

Figure 2.

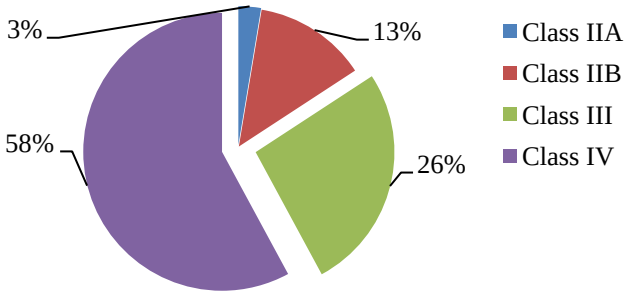

Figure 3.

Based on the results from diagnostic tests, 38 patients were diagnosed with critical stenosis or total occlusion in one or more segments of the lower limb. According to the Leriche-Fontaine classification, our study group was classified as shown in Figure 3 (12).

As we have already mentioned, the patients were differentiated not only on the basis of the diagnostic examination, but also on the type of therapeutic intervention (Figure 4).

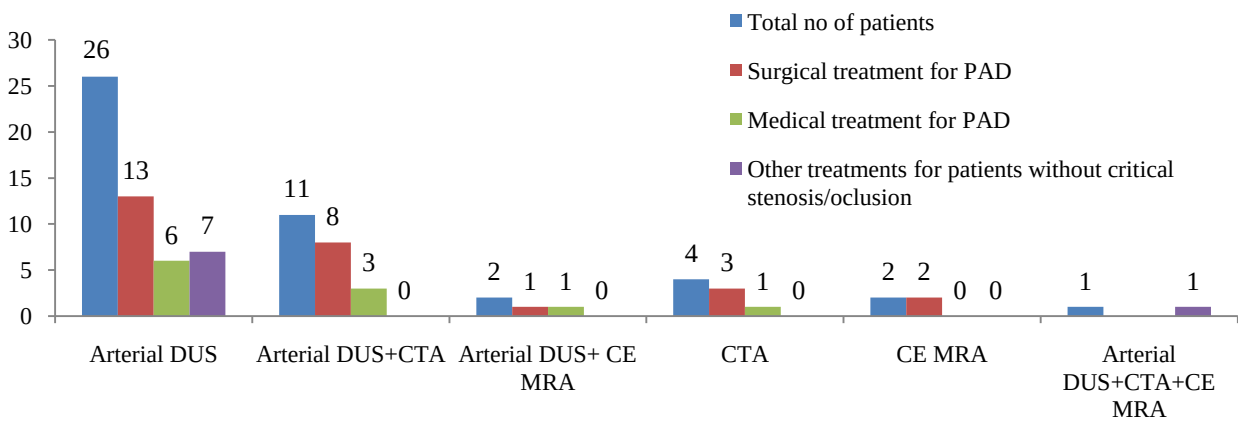

Figure 4.

The lower percentage of surgical cases in the group examined with DUS was determined by the fact that in 7 cases DUS, in fact, excluded the presence of a critical stenosis/occlusion. Out of the 38 patients diagnosed with critical stenosis/occlusion of a lower limb artery, 10 patients (29%) underwent medical treatment and the 28 patients underwent the following types of arterial surgery: 47% revascularization procedures, 16% primary amputations, 8% sympathectomies.

Based on the information presented in Table 4, we can draw conclusions about patient outcomes. As it can be seen, the percentage of surgical patients in the total number of PAD patients analyzed varied from 50% to 100% and appeared to be more related to the severity of the lesions than to the type of diagnostic test. The highest percentage of primary major amputations was recorded in the group investigated exclusively by CTA (33.3%). Cases requiring major amputations after the initial revascularization intervention were recorded in the DUS+CTA group (2 cases), DUS+CE-MRA group (1 case), and DUS group (2 cases). The highest percentage was recorded in the DUS+CTA group (22.2%). The percentage representing both primary and secondary major amputations was approximately 30%. Adverse developments recorded in the same hospitalization or the need for readmission to solve the same lesion was recorded both in the DUS group and in the DUS+CTA and DUS+CE-MRA. These paradoxically negative evolutions appeared to be the consequence of the PAD evolution rather than the applied diagnostic method.

Table 4. Patient outcomes

Intervention/Investigation	DUS	CTA	CE MRA	DUS+CTA	DUS+CE MRA
Surgical patients	(13/19) 68%	(3/4) 75%	(2/2) 100%	(8/11) 72.72%	(1/2) 50%
Revascularization techniques	(8/13) 61.5%	(2/3) 66.6%	(2/2) 100%	(5/8) 62.5%	(1/1) 100%
Lumbar+/-Periarterial Sympathectomy	(1/13) 7.6%	0	0	(2/8) 25%	0
Primary minor amputation	(1/13) 7.6%	0	0	0	0
Primary major amputation	(3/13) 23%	(1/3) 33.3%	0	(1/8) 12.5%	0
Major amputation (secondary to revascularization)	(1/13) 7.6%			(2/8) 25%	(1/2) 50%
Major amputation (primary+secondary)	30.7%	33.3%		37.5%	50
Stage IV Leriche-Fontaine	(13/19) 68%	(3/4) 75%		(5/11) 45.45%	(1/2) 50%
Average no days/hospitalization surgical patients	23.69	26.66	20	37.77	8
Average no days/hospitalization medical patients	10.5	4		17.66	20

The number of days of hospitalization was constantly higher for patients who underwent surgery (average no days/hospitalization=27.35) than those who received drug therapy (average no days/hospitalization=12.72).

Assessment of Diagnostic Accuracy in the Critical Stenosis/Occlusion of Lower Limb Arteries

In order to establish the investigations accuracy in diagnosing PAD, we compared the results for the above-mentioned tests. For all three selected tests, the contingency tables are presented in Tables 5, 6 and 7.

Table 5. Contingency table assessing DUS performance

		Arterial DUS		
		T+	T-	
Reference standard for disease	D+	29	2	31
	D-	0 (0.5)	9	9
		29	11	40

Table 6. Contingency table assessing CTA performance

		CTA		
		T+	T-	
Reference standard for disease	D+	14	1	15
	D-	0 (0.5)	1	1
		14	2	16

Table 7. Contingency table assessing CE MRA performance

		CE MRA		
		T+	T-	
Reference standard for disease	D+	4	0 (0.5)	4
	D-	0 (0.5)	1	1
		4	1	5

After computing the quality indicators for each diagnostic test, a comparison was performed to assess the accuracy (Table 8). Only the indicators for CE-MRA could not be considered reliable view the low number (< 10) of assay performed (13).

Regarding the sensitivity, no significant difference was found between arterial DUS and CTA in the present study (93.5% vs. 93.3%). Similarly, no differences were found with respect to specificity for all three diagnostic tests.

Economic Evaluation of the Diagnostic Tests Used in PAD Management

The prices of diagnostic tests show rather large variation between the state system (i.e., the amount paid by the National Health Insurance system) and the private one, respectively. As neither of these prices are reflecting the real costs of the services, the real cost of the diagnostic tests used was computed. The cost assessment was performed from a health care provider perspective using the 2009 unit costs data in Euros (€), using the RON currency conversion when needed.
The applied exchange rate was €/ RON = 1/4.21 (14).
No discount rate was applied due to the fact that the study was conducted for a period of less than five years.

The real costs calculated for DUS, CTA and CE-MRA are presented in Table 9 whereas a detailed explanation of these costs and prices is presented in Table 10 as follows: the real cost/investigation calculated in this paper and the other two prices, the one settled by the National Health Insurance (15) and the other settled by private practices (16) showing clear differences in prices among the analyzed imaging assays. There is a neat economic advantage if DUS is applied as a diagnostic method. Regarding the effect/consequences, we analyzed in this paper both performances of the tests and patients' outcomes.

Table 8. The quality indicators

Indicator	**DUS**	**CTA**	**MRI angiography**
Sensitivity/SN	93.5%	93.3%	80%
Specificity/SP	100%	100%	100%
Positive prediction value/PPV	1	1	1
Negative prediction value/NP	0.81	0.5	1
Accuracy/AC	95%	93.7%	100%
Classification error rate/ER	6.2%	9.3%	30%

Table 9. Real costs of DUS, CTA and CE-MRA.

Real cost/investigation = Operational costs per month/20 working days per month/6 working hours per day/1 investigation per hour
⇨ (1664 + 30 + 837 + 1000)/20 x 6 x 1 = 3531/120 ⇨ **29.43 €**
Real cost/investigation = Operational costs per month/20 working days per month/6 working hours per day/2 investigations per hour
⇨ (2683 + 5890 + 6088 + 5000)/20 x 6 x 2 = 19661/240 ⇨ **81.92 €**
Real cost/investigation = Operational costs per month/20 working days per month/6 working hours per day/1.5 investigations per hour
⇨ (2683 + 4625 + 17561 + 4000)/20 x 6 x 1,5 = 28869/210 ⇨ **160.38 €**

Table 10. Detailed costs and prices for DUS, CTA and CE MRA

	DUS	CTA	CEMRA
Expenditure on labor/personnel/month	1664 €	2638 €	2638 €
Expenditure on materials/month	30 €	5890 €	4625 €
Expenditure on investigation equipment/month	837 €	6088 €	17561 €
Expenditure on utilities, rent, etc.	1000 €	5000 €	4000 €
Real cost/investigation = Operational costs per month/20 working days per month/6 working hours per day/1 (DUS), 2(CTA), 1.5 (CE MRA) investigations per hour	**= 29.43 €** **~ 30 €**	**= 81.92 €** **~ 82 €**	**= 160.38 €** **~ 161 €**
Prices for arterial DUS, CTA, CE MRA in private system (15)	**~ 43 €**	**~ 131 €**	**~ 162 €**
Maximum rate paid by the Romanian Health Insurance House per investigation (16)	**~ 4.7 €**	**~ 60 €**	**~ 95 €**

The final step consisted of combining the conclusions on similar patient outcomes when applying different diagnostic test with the results of similar sensibility and specificity for DUS and CTA (~93%, 100%) and, finally, with the calculated real cost/investigation (30 €, 82 €) in a CMA matrix.

The outcomes of our approach can be classified in a CMA matrix as "The same incremental effectiveness of option compared to control and a less incremental cost of the option compared to control". This means "Accept option with weak domination for the decision" (9).

DISCUSSION

When choosing a diagnostic test, the cost, availability, acceptability, and usefulness of the diagnostic test must be taken into account in addition to the test performance. Knottnerus et al. proposed a strategy based on diagnostic performance, availability and acceptability, and, last but not least, its cost (17). Our study followed this strategy.

Firstly, we assessed the performance/accuracy of DUS compared to CTA and CE MRA. Regarding DUS and CTA, the values obtained in our study (Table 11) are consistent with those reported within a systematic review by Collins et al. that analyzed and compared the diagnostic accuracy for the assessment of critical limb stenosis/occlusion by means of DUS (20 studies), CTA (11 studies), and CE-MRA (17 studies), respectively (6).

Table 11. Performance indicators for DUS, CTA and CE-MRA

Diagnosis test	DUS	CTA	CE MRA
Sensitivity/SN (literature) Median (interval)	90% (74% la 94%)	97% (89% la 100%)	94% (85% la 100%)
Specificity/SP (literature) Median (interval)	99% (96% la 100%)	99,6% (99% la 100%)	99,2% (97% la 99,8%)
Included studies	20	11	17
Sensitivity/SN in our study	93,5%	93,3%	100%
Specificity/SP in our study	100%	100%	100%

According to their and our results, both DUS and CTA represent a good alternative to conventional angiography (CA). This conclusion was made only on the bases of the test performance. As for the discrepancies observed in the CE-MRA analysis, these are the effect of the small sample size, only 5 assays being performed.

Diagnostic performance/accuracy studies are neither able to compare tests on their final impact on patient outcomes nor to provide cost-effectiveness information.

Because high diagnostic accuracy does not always reflect in the clinical usefulness, the DUS ability to define treatment plans must be proven. There are several studies that have demonstrated the reliability of DUS in establishing the final treatment strategy in PAD (6, 17-22).

Data available regarding the confidence and reproducibility of DUS in the PAD management are controversial. A number of studies indicated a low confidence score obtained by DUS as compared to CE-MRA or CTA (23, 24). Concerning the reproducibility of DUS results and the achievement of a higher inter-observer agreement, the literature is stating that a consensus is required with respect to the hemodynamic relevance of stenosis versus the degree of stenosis (20, 6).

Availability, acceptability and costs are all important issues that need to be considered when choosing a diagnostic test. Our study was conducted at the University hospital where all the above mentioned diagnostic tests (DUS, CTA, CE-RMA, CA) were available. In terms of patient acceptability, DUS is particularly preferred view its non-invasive nature.

The real cost of the investigation for each of the diagnostic test examined (wages, material and contrast medium, capital costs, overheads) was calculated. Our findings are consistent with other studies that have used decision analysis models (25). Moreover, when applying CMA, a similar conclusion was reported by Collins et al (6).

Limitations of the study

The small sample of only 46 patients led to discrepancies with respect to the results regarding the diagnostic accuracy for CE-MRA versus data reported in the literature.

The cost analysis focused on the investigation costs without adding costs for applied surgical procedures or hospitalization. The only way to calculate the cost of hospitalization with surgical/medical treatment (hospital stay, surgical procedure, anesthesiology, intensive care, and clinical laboratory) was based on diagnosis-related group (DRG) prices that the National Health Insurance system paid for each case resolved. This cost was the same for all surgical/medical patients, without any difference related to the type of intervention suffered, the number of days of hospitalization or other differences between cases. Adding this cost would not help our intention to make a difference between the cases investigated by one or the other diagnostic test.

Also, no summary benefit measure was done; only individual clinical outcomes were reported.

CONCLUSIONS

DUS is accurate, safe and cost-effective in designing the final therapeutic plan for PAD, especially in the femoropopliteal segment provided that it is performed by an experienced specialist in a clinic with solid expertise in this respect. The present study will be used as a reference to future address the reliability in time of DUS in terms of cost- effectiveness in our hospital.

ACKNOWLEDGMENTS

None

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THE IMPACT OF SOCIOECONOMIC CHARACTERISTICS AND LIFESTYLES ON VITAMIN D DEFICIT IN MENTALLY ILL PATIENTS

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UTICAJ SOCIOEKONOMSKIH KARAKTERISTIKA I NAČINA ŽIVOTA NA DEFICIT VITAMINA D KOD MENTALNO OBOLELIH PACIJENATA

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Received / Primljen: 16. 12. 2016. Accepted / Prihvaćen: 19. 04. 2017.

ABSTRACT

Mental illnesses put patients at high risk for vitamin D deficit. The aim of the research is to examine the impacts of socioeconomic characteristics and lifestyles on vitamin D deficiency in mentally diseased patients. In this cross-sectional study we used blood samples from patients who were treated for mental disorders at Specialist-consultative unit of the Health Center and Clinical Center Kragujevac from May-June 2014. The study used a questionnaire for the assessment of vitamin D status. The study included 220 subjects with different types of mental disorders. Normal values of vitamin D were detected in 16% of patients whereas 64% of patients had vitamin D deficiency. The patients with vitamin D deficit were in average 3 years older than that but the difference is not statistically significant (p>0.05). The patients with vitamin D deficiency were primarily female (p=0.003), people with high-school education from urban environment who lived in bad life conditions (p>0.05). Between patients with and without vitamin D deficiency there is no difference in cigarette consumption, in the number of cigarettes per day, in alcohol usage, in coffee consumption and in nutrition. However, the patients without vitamin D deficiency spent more time outside; during the past year were more exposed to sun and during the past seven days spent more than 30 minutes a day exposed to sunlight (p<0.01). These facts indicate that there is a current need for further research in this area.

Keywords: *vitamin D, vitamin D defi ciency, mental disorders, lifestyle, socioeconomic characteristics*

SAŽETAK

Mentalne bolesti su bitan faktor rizika za nastanak deficita vitamina D. Cilj ovog istraživanja je ispitivanje uticaja sociodemografskih karakteristika i načina života na deficit vitamina D kod mentalno obolelih pacijenata. Istraživanje predstavlja studiju preseka u kojoj su ispitivani uzorci krvi pacijenata koji su, zbog mentalnih poremećaja, lečeni u specijalističko-konsultativnoj jedinici Zdravstvenog centra Kragujevac i Kliničkom centru Kragujevac tokom maja i juna 2014. godine. U istraživanju je korišćen upitnik za ispitivanje nivoa vitamina D. Istraživanje je obuhvatilo 220 ispitanika sa dijagnozom različitih mentalnih poremećaja. Fiziološke vrednosti vitamina D su izmerene kod 16% pacijenata, dok je njih 64% imalo deficit vitamina D. Pacijenti koji su imali deficit vitamina D su u proseku 3 godine stariji, ali ta razlika nije statistički značajna (p>0.05). Među pacijentima sa nedostatkom vitamina D preovlađuju osobe ženskog pola (p=0.003), osobe sa srednjim stepenom obrazovanja iz urbanog okruženja koje žive u lošim uslovima (p>0.05). U poređenju pacijenata sa i bez deficita vitamina D nije bilo značajnih razlika u konzumiranju duvana, broju popušenih cigareta na dnevnom nivou, konzumiranju alkohola i kafe, i načinu ishrane. Međutim, pacijenti koji nisu imali nedostatak vitamina D su provodili više vremena napolju, tokom prethodne godine su se više izlagali sunčevom zračenju i tokom prethodnih sedam dana su provodili više od 30 minuta napolju na sučevoj svetlosti (p<0.01). Navedene činjenice ukazuju na potrebu za daljim istraživanjima u ovoj oblasti.

Ključne reči: *vitamin D, deficit vitamina D, mentalne bolesti, način života, socioekomoske karakteristike*

sciendo
UDK: 615.356:577.161.2; 616.89-008.44(497.11)"2014"
Ser J Exp Clin Res 2019; 20 (1): 25-31
DOI: 10.1515/SJECR-2017-0026

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INTRODUCTION

According to the World Health Organization, at the beginning of the new millennium, there were 450 million people suffering from a mental illness or behavioral disorder in the world. About 33% of their lifespan they lived with disability due to their neuropsychiatric disorder. Only the depression bears more than 10% of YLD (Years Lived with Disability) (1).

In our country intense acute and chronic stress and accumulated trauma, which have affected all our society in the past two decades, due to wars and transition, had significant consequences on mental health. According to the Institute of Public Health of Serbia "Dr Milan Jovanović Batut", the number of mental and behavioral disorders has increased by 13.5% last ten years and their total morbidity and mortality are on the rise. Thus, these disorders are now the second biggest health problem of the population in Serbia after cardiovascular diseases (2).

Vitamin D is synthesized in the body from its precursor, 7-dehydrocholesterol, 80% of which is produced in skin under the influence of UV rays and 20% is taken orally through food and/or supplements. Vitamin D has pleiotropic effects and does not work like other vitamins only on biochemical processes. It regulates the transcription of a large number of genes and the synthesis of cellular proteins (3-5). Vitamin D deficiency causes rachitis and osteomalacia and increases the chances of developing osteoporosis, allergic and autoimmune diseases, hypertension, malignant diseases and mental disorders (6-11). Vitamin D receptors are found in neurons and glial cells which are mapped in areas of the brain that are responsible for the development of depression. This indicates the role of vitamin D in psychosomatic disorders (12-14). The antidepressant effect of vitamin D is achieved due to the impact it has on the hypothalamic-pituitary-adrenal interface which consequently regulates the production of adrenaline, noradrenaline and dopamine (15). Without any doubt, it is shown that its deficit causes cognitive disorders and depressive moods (12), but the results of the previous studies different (13).

THE AIM OF WORK

The aim of the research is to examine the impacts of sociodemographic characteristics and lifestyles on vitamin D deficiency in mentally diseased patients.

MATERIAL AND METHODS

Study description

This study was designed as a cross-sectional study. The study used blood samples of patients who were treated for a newly-diagnosed illness or in a stage of exacerbations (a relapse) of a chronic mental disease. All patients gave their consent to be included in the study. Patients were treated at Specialist-consultative unit of the Department of Neuropsychiatry of Health Centre Kragujevac and at the Clinic of Psychiatry, Clinical Centre Kragujevac from May – June 2014. Both outpatient and inpatient cases were included. The participants had the following characteristics: adults of both sexes aged from 19 to over 81 years. Their psychiatric diagnoses include the following mental disorders: organic and symptomatic mental disorders, mental and behavioural disorders caused by psychoactive substances, schizophrenia, schizotypal and delusional disorders, mood disorders, neurotic stress-related and somatoform disorders, syndromes of disturbed behaviour associated with physiological disturbances and physical factors, personality disorders and behavioural disorders, mental retardation, developmental disorders of the psyche (F00-F89), epilepsy (G80) and poisoning suicide attempts (T42) with antiepileptic, sedative-hypnotic and anti-parkinsonian drugs. The study was approved by the Ethics Committees of the Clinical Centre Kragujevac and the Health Centre Kragujevac.

The patients with the following characteristics were excluded: patients who were younger than 18 years, patients with diseases of a liver and kidneys, patients with tumour-induced osteomalacia, patients with hyperthyroidism, hyperparathyroidism, granulomatous disorders, sarcoidosis or tuberculosis; those who refused to participate in the study or were prevented from participating by any other circumstance were also not included.

The assessment of a mental status of patients was obtained by examining the medical (psychiatric) documentation that was previously made by a competent psychiatrist. The study used a questionnaire for the assessment of vitamin D status (16) which was adapted to the needs of this research. It consists of segments related to: demographic characteristics of the respondents, history of a present illness, personal medical history, psychiatric status, habits of respondents in terms of diet, sun exposure, physical activity, bad habits (smoking and alcohol) and personal and family history.

In order to eliminate geographical and seasonal differences, i.e. the different degrees of sunshine, all respondents came from a single, narrow geographic area (territory of Kragujevac and the surrounding area – northern latitude of the city of Kragujevac is 44 ° 22'). Also, the blood samples were collected during the same season.

The dependent variable was the concentration of vitamin D in the form of 25(OH) within the serum. The defined cut-off for vitamin D deficiency is <12 ng/ml, the inadequacy ranges from 12 – 20 ng/ml, and normal values are above 20 ng/ml (17). The study population was divided into two groups: the first with the serum 25(OH)D < 12 ng/ml and the other with 25(OH)D > 12 ng/ml.

Statistical analysis

For the harmonization of sampling distribution with a normal distribution, we used Normal Q-Q Plot and Histogram charts and Kolmogorov-Smirnov and Shapiro-Wilk tests. In order to describe the parameters of significance

Table 1. Deficiency of vitamin D and demographic characteristics of the subjects

Demographic characteristics	Total	25 (OH)D status		Test
		Deficiency <12 ng/ml	Without deficiency >12 ng/ml	
Sex				
Male	72 (32.7%)	36 (25.71%)	36 (60.0%)	**p=0.003**
Female	148 (67.3%)	104 (74.29%)	44 (40.0%)	
Age				
Mean (±SD)	48.68 (±13.3)	49.66(±13.63)	46.98 (±12.59)	p=0.370
Median (Range)	49 (19 – 81)	49 (21 – 81)	49 (19 – 71)	
Education				
No education	2 (0.91%)	2 (1.43%)	0 (0%)	p=0.781
Incomplete primary	11 (5.00%)	6 (4.29%)	5(6.25%)	
Primary education	45 (20.45%)	30 (21.43%)	15 (18.75%)	
Secondary education	140 (63.64%)	86 (61.43%)	54 (67.5%)	
High education	9 (4.09%)	6 (4.29%)	3 (3.75%)	
University education	13 (5.91%)	10 (7.14%)	3 (3.75%)	
Residence				
Urban	175 (79.55%)	114 (81.43%)	61 (76.25%)	p=0.305
Rural	44 (20.00%)	25 (17.86%)	19 (23.75%)	
No data available	1 (0.45%)	1 (0.71%)	0 (0%)	
Living conditions				
Good	61 (27.73%)	34(24.29%)	27 (33.75%)	p=0.322
Medium	75 (34.09%)	50 (35.71%)	25 (31.25%)	
Bad	81 (36.82%)	54 (38.57%)	27 (33.75%)	
No data	3 (1.36%)	2 (1.43%)	1 (1.25%)	
Total	220 (100%)	140 (100%)	80 (100%)	

depending on their nature we used the measures of descriptive statistics: frequency, percentage, mean (average), median, ± standard deviation (SD) and scope (range). For the level of statistical significance we chose the value of α=0.05. In testing the difference between independent groups depending on the nature of the investigated parameters we used: Pearson χ^2 test, Fisher exact test and Mann-Whitney test. χ^2 agreement test was used to examine the concordance of the results obtained through this study with the referent results. The data analysis was performed in the statistical program SPSS version 19.0.

RESULTS

The study included 220 subjects with different types of mental disorders. 135 (61%) patients were treated at the Psychiatric Clinic of Clinical Centre Kragujevac and 85 (39%) at Psychiatric Department of Health Centre Kragujevac. More than a half, namely 135 patients (61%) were treated at the Psychiatric Clinic of Clinical Center Kragujevac and 85 (39%) at Psychiatric Department of Health Center Kragujevac. Moreover, 88 patients (40%) were included in the inpatient service and 132 (60%) in outpatient service. Normal values of vitamin D level were detected in 16% of the patients and 64% had vitamin D deficiency.

Three quarters of the total number of respondents were women so the distribution in terms of gender differs significantly from what was expected (χ^2_1=8.60, p=0.003). The mean age was 49 years. The youngest subject was at the beginning of the adult age while the oldest belonged to the category of very old people. People with vitamin D deficiency were on average about three years older but the difference is not statistically significant (p=0.370). More than a half of respondents completed secondary school, a quarter of them had primary or incomplete primary education while there was the lowest number of subjects with higher or high education. Most of the respondents lived in an urban environment and the largest number, regardless of the place of residence, lived in bad living conditions. The number of respondents who lived in poor conditions is higher in the group with vitamin D deficiency (p=0.322) (Table 1).

Smokers made 55% of the total number of subjects. In average, they smoked one pack a day. Most subjects did not use alcohol (85%), and only 8% did not drink coffee. Most subjects who drank coffee intensively (more than two cups a day) were in the group with no vitamin D deficit (p=0.248) (Table 2).

During the day, 68% of the subjects were physically active and 77.5% of them had no vitamin D deficit. The subjects were active for about 5 hours a day in average – the group without the deficit for 5.75 hours and the group with a deficit for 4.42 hours (p=0.005). 6% of the subjects did exercises and all of them had no vitamin D deficit (p=0.007). Subjects exercised 3.8 times per week in average; most commonly those without the deficit (4.3 times a week) but the difference is not statistically significant (p=0.077).

Table 2. Vitamin D deficiency and bad habits (smoking, alcohol and coffee consumptions)

Bad habits	Total	25 (OH)D status		Test
		Deficiency <12 ng/ml	Without deficiency >12 ng/ml	
Smoking				
Yes	122 (55.45%)	72 (51.43%)	50 (62. 5%)	p=0.128
No	87 (39.55%)	62 (44.29%)	25 (31.25%)	
Earlier	10 (4.55%)	5 (3.57%)	5 (6.25%)	
No data	1 (0.71%)	0 (0%)	1 (0.45%)	
Number of cigarettes a day				
Mean (±SD)	21.49 (±15.05)	22.08 (±17.04)	20.75 (±12.25)	p=0.994
Median (Range)	20 (1-80)	20 (2-80)	20 (1-50)	
Alcohol				
No	186 (84.55%)	122 (87.14%)	64 (80%)	p=0.096
Yes, not every day	19 (8.64%)	8 (5.71%)	11(13.75%)	
Yes, two glasses a day	1 (0.45%)	0 (0%)	1 (1.25%)	
Yes, more than two glasses a day	13 (5.91%)	9 (6.43%)	4 (5%)	
No data	1 (0.45%)	1 (0.71%)	0 (0%)	
Coffee				
Intensively	75 (34.09%)	43 (30.71%)	32 (40%)	p=0.248
Moderate	126 (57.27%)	82 (58.57%)	44 (55%)	
No	18 (8.18%)	14 (10%)	4 (5%)	
No data	1 (0.45%)	1 (0.71%)	0 (0%)	
Total	220 (100%)	140 (100%)	80 (100%)	

Table 3. Vitamin D deficiency and sun exposure

Sun exposure	Total	25 (OH)D status		Test
		Deficiency <12 ng/ml	Without deficiency >12 ng/ml	
Being outdoors long				
Yes	75 (34.09%)	37 (26.43%)	38 (47. 5%)	**p=0.001**
No	144 (65.45%)	102 (72.86%)	42 (52. 5%)	
No data	1 (0.45%)	1 (0.71%)	0 (0%)	
Sun exposure during the last year				
Yes	128 (58.18%)	71 (50.71%)	57 (71.25%)	**p=0.002**
No	92 (41.81%)	69 (49.28%)	23 (28.75%)	
Sun exposure during the last week				
<5 minutes a day	76 (34.55%)	55 (39.29%)	21 (26.25%)	**p=0.002**
5 – 15 minutes a day	6 (2.73%)	4 (2.86%)	2 (2.5%)	
15 – 30 minutes a day	44 (20%)	34 (24.29%)	10 (12.5%)	
>30 minutes a day	94 (42.73%)	44 (33.57%)	47 (58.75%)	

Less respondents (34%) spent time outside (exposed to sun) and most of them belonged to the group without the deficit of the vitamin D (p=0.001). During the previous year, more than a half subjects had exposed themselves to sun with higher percentage in the group without the deficit (p=0.002). Most subjects who had spent less than five minutes during the previous week belonged to the group with the deficit. Most subjects had spent more than 30 minutes outside during the past week and most of them belonged to the group without the deficit (p=0.002) (Table 3).

The question about regular diet (3 meals, 2 fruit snacks and consumption of fruit and vegetables every day) was answered affirmatively by 16%, negatively by 64%, while 20% of the subjects stated that they eat regularly at times. There is not statistically significant difference between groups with and without deficiency in terms of regular nutrition (p=0.145).

DISCUSSION

New epidemiological data show that about billion people worldwide, including North America and Europe, have vitamin D deficiency (18,19). Such results are most commonly explained by the fact that nutrition is poor with vitamins and that exposure to sun is low (20,21). Mental illnesses put patients at high risk for vitamin D deficiency. It is very likely that a mental illness disrupts the socio-economic conditions that are required for optimal vitamin D levels, especially in terms of nutrition and healthy lifestyles like physical activity and sun exposure. New studies indicate that certain meidcines used in the treatment of mental illnesses deepen the deficit of the vitamin D (22). A systematic review of 14 epidemiological studies shows that the prevalence of low vitamin D values is about 30%

higher in people with depression, who during the course of the disease are more than twice more likely to develop D hypovitaminosis than people in the general population (23). The results of the most recent meta-analysis indicate that two-thirds of patients with schizophrenia have values of vitamin D in the serum which can be classified as clinically significant deficit and that such people are about two times more likely to develop schizophrenia (24). Our study confirms the findings of earlier studies that the presence of mental disorders (mostly depression and schizophrenia) is associated with clinically significant deficiency of vitamin D, but with a much higher prevalence (25).

Two main factors responsible for the occurrence of vitamin D deficiency are insufficient exposure to sunlight and/or inadequate nutritional intake of vitamin D. In addition, it has been proven that there are numerou factors that contribute to this phenomenon: age, skin type, body mass index (BMI), geographic areas of residence, gender, and the usage of creams with SPF (26-28). Although the sex is often insignificant variable for the occurrence of vitamin D deficiency, low levels of vitamin D in women are explained by higher fat content in women which affects the formation of a depot of vitamin D in the body (29). In women during pregnancy and immediately after giving birth, who had depression, low levels of vitamin D were significantly associated with the severity of depressive symptoms (30). Low levels of vitamin D during pregnancy are actually identified as a risk factor for the development of postpartum depression (31). In our research, most patients with vitamin D deficiency are women and the difference between genders is statistically significant. The average age indicates that the majority of our respondents were at the end of the fifth decade of life. Older people have vitamin D deficiency more frequently (32,33). The study, which involved over 1,500 people aged 60 and more, found that each fifth person had moderate or severe deficiency of vitamin D, close to half of them had depressive symptoms and their vitamin D levels were significantly lower than in other subjects (34). Low levels of vitamin D represent a marker of poor health status in this population and vitamin D supplementation in the elderly can reduce the overall mortality rate (35).

Three quarters of our subjects lived in urban areas in bad or satisfying conditions and most had secondary education. Large multicentric study conducted in several countries indicated that inferior material situation, higher costs for housing, lower levels of education, female gender and divorce are important factors associated with the presence of depression (36). In our study, the highest percentage of respondents who live in rural areas had no deficit of vitamin D which is in line with data from the literature that children and adults who are exposed to sun and who live in rural areas have better vitamin D status, especially in the summer months (37).

It is well known that smoking is associated with lower vitamin D levels. The level of vitamin D was significantly lower in smokers than in nonsmokers (38). In our study, a little over half of the respondents were smokers. The largest number of them consumed about one pack of cigarettes a day, and they started smoking in twenty-first year of life in average. These data are in accordance with the well-known fact that smoking is significantly present in people with mental illnesses. Accordingly, the surprising fact is that in our study the group without vitamin D deficit had more smokers.

Almost all respondents regularly consumed coffee, mostly one cup a day. A high intake of caffeine, 4 or more cups a day, is a significant risk factor for insufficiency of vitamin D (39,40).

Data from the literature suggests that lower levels of vitamin D were measured in patients being treated for alcohol dependence (41). This research shows that 85% of the subjects never consumed alcohol which was expected due to the fact that patients used psychotropic drugs. The amount of vitamin D that is generated in the skin depends on the level of melanin which is excellent absorber of UVB radiation (42). As latitude increases, the amount of UVB radiation decreases and there are more seasonal variations in UVB radiation (43). In our region from May to October it is sufficient to spend 5 to 15 minutes exposed to sun (1000 – 2000 IU is being created) (27). However, the analysis shows that there is a statistically significant difference in the time spent outdoors being exposed to sun during past year and week between patients with and without a deficiency of vitamin D.

Vitamin D deficiency in patients with somatic and mental illnesses is actually the result of the underlying disease (44). Physical exercise acts as a mild protective factor for the development of depressive disorders (45,46). In our study, more than a half of the respondents said that they had certain physical activity, but only a small number of them did exercises. Some of the factors that contribute to such inactivity are certainly working disengagement and antipsychotic medications with extrapyramidal side effects which can be very severe (47).

Poor diet and the lack of sun exposure are common in patients with psychiatric disorders. Both factors play a significant role in the development of vitamin D deficiency (48). Analyzing consumer habits in terms of diet, we have found that more than half of the respondents in our study population did not care about proper nutrition. In recent years, recommendations for vitamin D intake increased significantly so it is difficult to meet such demands them only through diet (49). In our study population respondents practically did not use supplements of vitamin D.

CONCLUSION

Based on the presented results we may say that this study has contributed to better understanding of the role of vitamin D in patients suffering from mental disorders. Lack of vitamin D could be potentially solved through public-health measures relating to changes in the general lifestyle, a higher exposure to the sun and better nutrition. They all

belong to the field of preventive medicine. Mental health care must be a necessary and important aspect of overall health care and public health systems. Mental disorders affect the functioning of individuals, causing emotional distress, impairment of life quality, alienation, stigma and discrimination. Their influence extends to the entire community and represents an enormous social and economic burden. In general, the question of the true nature of the relations between vitamin D and mental illnesses is still not sufficiently clarified. It is not clear whether the existence of mental illness primarily defines vitamin D deficiency (eg, disorders of nutrition, physical activity, stay in the open air, etc.) or, conversely, a low concentration of vitamin triggers or contributes to pathophysiological mechanisms of development of a psychiatric illness, and if the answer is affirmative, to what extent. Also, the question remains whether and to what extent therapeutic interventions in psychiatry, especially the application of relevant psychotropic medications, affect the disorders of homeostasis of vitamin D (22). These facts point to a current need for further research in this area, which should focus on identifying the causes of lack of vitamin D and its role in improving overall health of persons with mental disorders (23).

ACKNOWLEDGMENTS

This work was supported by grant from the Faculty of Medical Sciences, University of Kragujevac (project JP 13-14).

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TUMOR NECROSIS FACTOR-ALPHA AS DIFFERENTIAL DIAGNOSTIC MARKER FOR PATIENTS WITH FEVER OF UNKNOWN ORIGIN

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FAKTOR NEKROZE TUMORA-ALFA KAO DIFERENCIJALNO DIJAGNOSTIČKI MARKER U EVALUACIJI FEBRILNIH STANJA NEPOZNATOG POREKLA

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Received / Primljen: 04. 04. 2017. Accepted / Prihvaćen: 18. 05. 2017.

ABSTRACT

Febrile conditions of unidentified origin are still unknown in modern medicine despite the development of diagnostic procedures. There are various agents of long-term temperature encompassing numerous infectious or non-infectious diseases.

The aim of this study was to determine if there was a statistically significant difference in the values of proinflammatory cytokines (IL-1, TNFα, IL-6) in patients who meet the criteria for febrile conditions of unidentified origin, between the group of infectious, malignant, rheumatic, "other" diseases and undiagnosed patients.

The study was conducted in the Immunology laboratory of the Center for Molecular Medicine and Stem Cells Research of the Faculty of Medical Sciences in Kragujevac. Blood samples were taken from patients tested at the Clinic for Infectious Diseases, of the Clinical Center of Kragujevac, in the period from 2014 to 2016. The study included 70 patients.

The measured values of the level of TNFα showed significantly higher values in a group of malignant diseases than in the group of infectious diseases, while the values of IL-1 and IL-6 did not show statistical significance.

TNFα can improve diagnosing in case of patients with an unknown febrile condition, which can shorten the length of the hospital stay and reduce the volume of performance of diagnostic procedures.

Keywords: *temperature, temperature of an unknown cause, IL- 1, TNFα, IL-6*

SAŽETAK

Febrilna stanja nepoznatog porekla i dalje predstavljaju nepoznanicu u savremenoj medicini i pored razvoja dijagnostičkih procedura. Uzročnici dugotrajne temperature mogu biti različiti, obuhvatajući brojne infektivne ali i neinfektivne bolesti.

Cilj istraživanja je bio da utvrdimo da li postoji statistički značajna razlika u vrednostima proinflamatornih citokina (IL- 1, TNFα, IL-6) kod bolesnika koji ispunjavaju kriterijume za febrilna stanja nepoznatog porekla, između grupe infektivnih, malignih, reumatoloških, „drugih" bolesti i nedijagnostikovanih bolesnika.

Istraživanje je rađeno u Imunološkoj laboratoriji Centra za molekulsku medicinu i istraživanja matičnih ćelija Fakulteta medicinskih nauka u Kragujevcu. Krv za analizu uzeta je od bolesnika ispitivanih na Klinici za infektivne bolesti, Kliničkog Centra Kragujevac, u periodu od 2014. godine do 2016. godine. U ispitivanju je učestvovalo 70 bolesnika.

Izmerene vrednosti nivoa TNFα su pokazale statistički značajno više vrednosti u grupi malignih bolesti u odnosu na grupu infektivnih bolesti, dok vrednosti IL-1i IL-6 nisu pokazale statističku značajnost.

TNFα može unapredi postavljanje dijagnoze kod bolesnika sa nejasnim febrilnim stanjem, čime se može skratiti trajanje hospitalizacije i smanjiti obim izvođenja dijagnostičkih procedura.

Ključne reči: *temperatura, temperatura nepoznatog uzroka, IL- 1, TNFα, IL-6*

ABBREVIATIONS

ELISA - engl. *enzyme-linked* immunosorbent assay
IL - interleukin (engl. *interleukin*)
FUO - febrilna stanja nepoznatog uzorka (engl. *Fever of unknown origin*)
^{18}F-FDG - *2-deoxy-2-(^{18}F) fluoro-D-glucose*
PET - pozitronska emisiona tomografija (engl. *Positron emission tomographu*)
PGE2 - prostaglandin E2
serr - standardna greška (engl. *standard error*)
SD - standardna devijacija (engl. *standard deviation*)
TNF-α - faktor nekroze tumora-α (engl. *tumor necrosis factor-α*)
TRAIL - (eng. *TNF related apoptosis-inducing ligand-TRAIL)*

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UDK: 616.8-008.24-02
Ser J Exp Clin Res 2019; 20 (1): 33-38
DOI: 10.1515/SJECR-2017-0023

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INTRODUCTION

In the pathogenesis of fever, proinflammatory cytokines IL-1, IL-6, and TNF have an important role which, by circulating from sites of infection or inflammation, go to the blood brain barrier in the hypothalamus (1, 2, 3). On endothelial cells of the preoptic region of the hypothalamus they are band to their receptors and they induce the formation of prostaglandin E2 (PGE2), which is the main pyrogenic mediator in the genesis of elevated temperature (4, 5).

Tumor necrosis factor (TNF) is a proinflammatory cytokine that shares many biological similarities with IL-1, which is a result of the activation of the same transcription factors, despite the existence of structural and receptor differences (6). It represents a mediator of acute inflammatory response and is responsible for many systemic complications during the infection (7, 8). TNF in the case of a large production may have a number of system effects, and thus causes the loss of myocardial contractility and the inhibition of the tone of blood vessels, which has a role in the development of shock (9). It stimulates endothelial cells to express the activators of coagulation and to inhibit the expression of thrombomodulin coagulation inhibitor, which consequently leads to the formation of intravascular thromboses (10, 11, 12). It is believed that TNF α mediates in oncogenesis, by activating the transcription of the pro-inflammatory transcription factor NF-κB, which allows expression of genes associated with tumor survival, proliferation, invasion, angiogenesis and metastasis (13).

Febrile condition of unidentified origin represents a special condition that refers to an elevated temperature above 38.3 °, which is registered on several occasions, and lasts longer than three weeks without any diagnoses despite an adequate testing during three visits to the doctor that is after a three-day stay at the hospital. (14). There are various agents of unclear febrile conditions and they include a large number of clinical entities. Petersdorf and Besson described four groups of diseases and divided them according to the etiological agents (15). The division has been valid nowadays as well, and it comprises: infectious, malignant, and other rheumatic diseases involving granulomatous diseases, subacute thyroiditis, inflammatory bowel diseases, drug-induced temperature and artificially induced temperature (16-18).

It has been discovered that some of the proinflammatory cytokines have a role of endogenous pyrogens, while on the other hand, certain cytokines can have antipyretic effect. (19) Some of these circulating proteins are mediating intracellular communication between the immune and nervous systems, which is the reason why the cytokine profile is under examination in this study (20).

PATIENTS AND METHOD

Testing of the cytokine profile was done in Immunological laboratory of the Center for Molecular Medicine and Stem Cells Research of the Faculty of Medical Sciences in Kragujevac. Blood samples taken from patients were tested at the Clinic for Infectious Diseases, of the Clinical Center of Kragujevac, in the period from 2014 to 2016. 70 patients participated in the study. FUO group of patients, involved 38 patients who met the criteria for febrile conditions of unknown etiology (22) which comprises: a) a repeated appearance of the temperature over 38.3 ° C within three weeks; b) the origin of temperature remains unresolved after three visits to the doctor of the ambulance service that is three days of hospital tests. The control group included hospitalized patients diagnosed with any of the preexisting acute infectious diseases, of a viral or bacterial etiology. The study included 32 patients.

The aim of this study was to determine whether there is a statistically significant difference in the values of proinflammatory cytokines (IL-1, TNFα, IL-6) between the group of infectious, malignant, rheumatic, "other" diseases and undiagnosed patients.

a) Determining the concentration of the cytokine in the serum

Blood samples, with the amount of 10 ml, were taken before treatment. The blood sample was centrifuged at 2000 rpm for 5 minutes. After the centrifugation serum was separated and distributed in 3-5 tubes. The tubes were labeled and serum sample was stored at -20 ° C. For determining serum level of cytokines, IL-1β, IL-6, TNFα, commercial ELISA kits (R&D Systems, Minneapolis, MN, USA) have been used.

For performing the ELISA test manufacturer's manual was used. Optical density was read on a microplate reader (ZENYTH 3100 MyLTi-Mode Detector, Anthos, Austria) at a wavelength of 450nm.

Measured values of absorbance have, before the analysis, been decreased for the value of the absorbance of blind probe (deionized water). The reading values of the standard were used to construct a standard curve, and then equation was determined according to which concentration of the tested cytokines was calculated.

b) Statistical data processing

Data were analyzed using the statistical program SPSS version 22. For describing the linear relationship between two variables Pearson linear correlation coefficient r was used as well as Spearman's rank correlation coefficient rho. Results of the study and experiments are expressed as an average value +/- standard error (SE) or standard deviation (SD). For a statistically significant difference in the values obtained between groups, we used the two levels of statistical significance: statistically significant difference $p<0.05$ and highly statistically significant difference $p<0.01$. The obtained results are presented in tables and graphs.

RESULTS

The study included 70 patients who were examined and treated at the Clinic for Infectious Diseases, of the Clinical Center of Kragujevac. Criteria for unclear febrile conditions were met by 38 patients. After the diagnosis, FUO patients were classified according to the etiology into infectious and non-infectious group of diseases. 11 patients (29%) were diagnosed with some of the infectious diseases, whereas 24 of FUO patients (63%) were diagnosed with non-infectious disease. In 3 patients (8%) after exhausting all diagnostic procedures the cause of febrile condition was not discovered, and they belonged to a group of undiagnosed patients. 10 of rheumatic patients (26%), 9 who were diagnosed with malignancy (23%), and 5 (13%) patients who belong to the group of "other diseases" belonged to the group of non- infective agents of FUO (Figure 1).

The control group consisted of 32 patients who were diagnosed with some of the acute infectious diseases.

The results showed that there was no statistically significant difference in the levels of IL-1ß serum between two groups. Values of IL-1ß in the group of FUO patients were approximately equal to values which were measured in patients with acute infectious diseases, and in the study they represented the control group. The average value of IL-1ß in the group of rheumatic diseases was higher than the control group, but there was not a statistically significant difference (Figure 2).

Tumor necrosis factor is a proinflammatory cytokine that shares many biological similarities with IL-1. In the group of FUO patients there was a trend of increasing serum levels of TNFα as opposed to patients diagnosed with acute infectious diseases, but not any statistical significance was achieved.

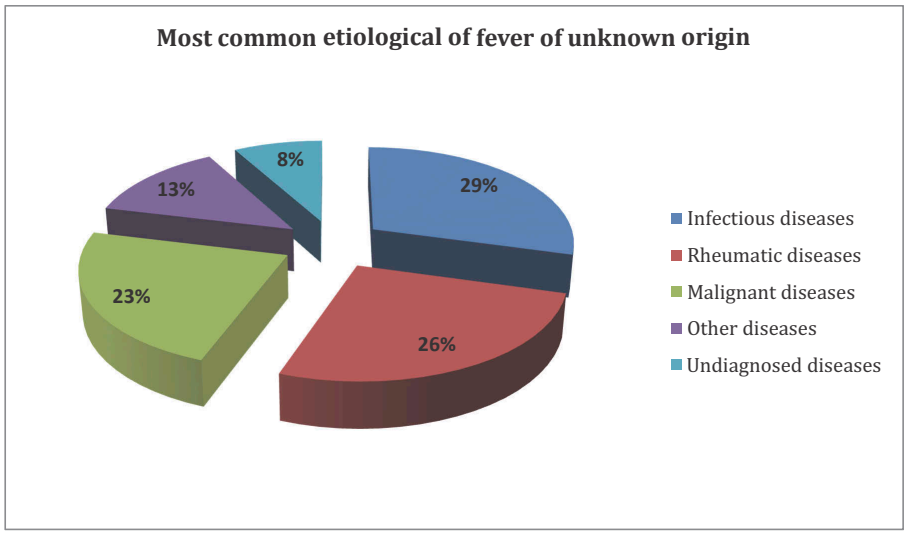

Figure 1. TNFα values in sera of FUO patients vs. Control group.
Results are presented as average ± SD, *p<0.05, **p<0.01

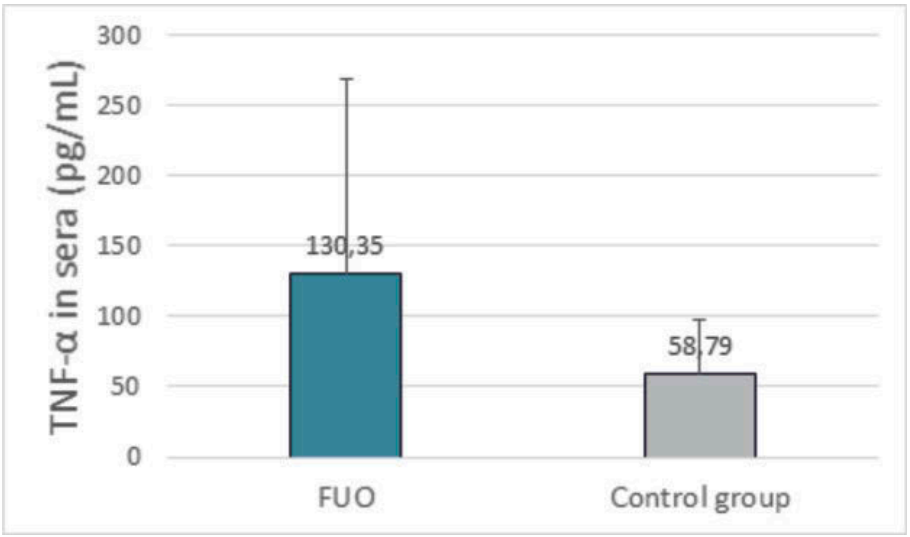

Figure 2. TNFα in sera among different groups of FUO patients.
Results are presented as average ± SD, *p<0.05, **p<0.01

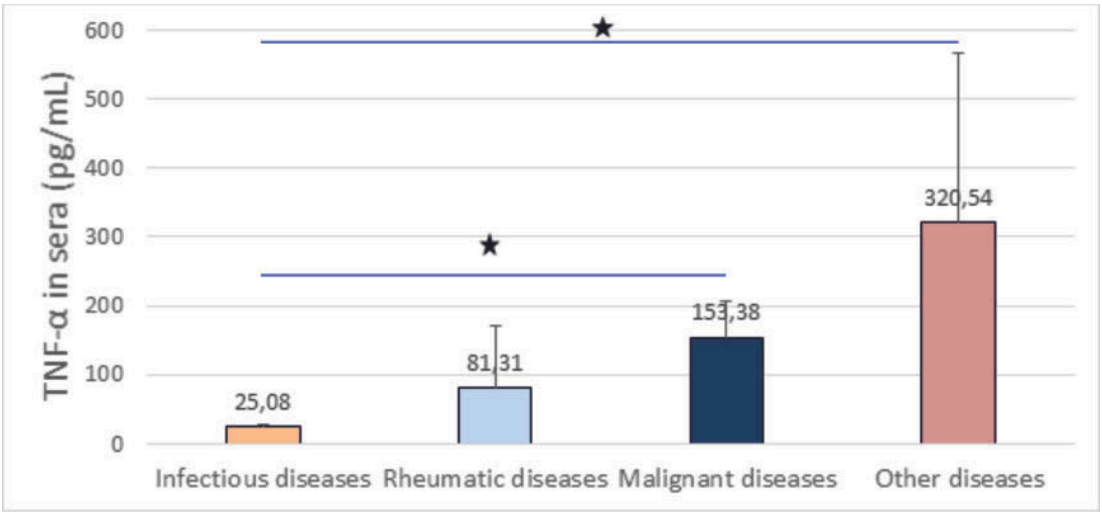

Figure 3. TNFα in sera among different groups of FUO patients.
Results are presented as average ± SD, *p<0.05, **p<0.01

Serum levels of TNFα in the systemic circulation were significantly lower in the group of infectious diseases, as compared to the group of patients with malignant diseases and other diseases. (Figure 3).

The highest levels of TNFα in a total sample of FUO patients have been observed in a patient suffering from Castleman's disease, assigned to a group of other diseases, in which maximum values of this pro-inflammatory mediator were registered.

By the analysis of level IL-6 in the serum, high values in patients with acute infectious diseases were registered compared to FUO patients but without reaching any statistical significance. Average values of five etiological patient groups were approximately equal.

DISCUSSION

In recent decades there have been numerous developments of both laboratory and visual diagnostic procedures, but the number of undiagnosed FUO patients is still approximately 10-30% (21, 22). Such a high number of undiagnosed patients may be the cause of a fatal outcome, especially for those patients who hide malignancy.

Our results show that in patients with prolonged febrile condition non-infectious diseases are dominant. Most of the patients were diagnosed with some of the rheumatic or malignant diseases.

By analyzing previous studies it was observed that the distribution of etiological agents since 1961 until today has constantly been changing. In 1961 Petersdorf and Beeson announced that the largest number of patients with unknown febrile conditions has an infection or infectious diseases (36%) (14). After infectious diseases, the leading position was occupied by malignant diseases that nowadays many authors consider as an important cause of long-term fever. More recent investigations show that a great importance in the identification of the etiology of FUO has a positron emission tomography with fluoro-deoxy-glucose (FDG-PET), especially in the case of occult infections and tumor (23, 24).

The published results of the authors of unclear febrile conditions indicate that over the last decade a trend of increasing the number of patients with rheumatic diseases has been recognized, as shown by our results as well (25).

Although in recent decades the development of modern diagnostic procedures has occurred, febrile conditions of unidentified origin still remain a diagnostic challenge, which is why there is a constant need for a new research.

In order to evaluate FUO patients, serum concentration levels of TNFα with FUO patients have been measured as well as in patients with acute infectious diseases, which in the study presented a control group. Statistically speaking, significantly higher values have been achieved in the group of malignant diseases than in the group of infectious diseases.

Tumor necrosis factor is an important pro-inflammatory cytokine, which may be a link between inflammation and carcinogenic process (26). The role of TNFα in carcinogenesis, is reflected in the activation of the transcription factor NF-kB (27). NF-kB is responsible for the enhanced expression of genes that have a role in the survival, invasion, proliferation, angiogenesis and metastasis of tumor cells (28). In our study, in patients belonging to the group of malignant diseases breast cancer, ovarian cancer, colon cancer, etc. were diagnosed, and it is known that their tumor cells can constitutively express TNFα. Most of the cells with these characteristics are responsible for the permanent activation of NF-kB (29).

Unlike TNFα, other members of the TNF superfamily may have a suppressive role in tumor immunity. It was found that TRAIL can induce apoptosis in tumor or altered cells but not in case of healthy cells, causing the conducted clinical trials for the treatment of tumors (30).

The highest levels of TNFα in a total sample of FUO patients have been observed in a patient suffering from Castleman's disease, assigned to a group of other diseases, in which the maximum values of this pro-inflammatory mediator are registered. Castleman's disease, a rare lymphoproliferative disorder that is defined as a localized hyperplasia of the lymph follicles with or without germinative centers, with the existence of endothelial hyperplasia (31). It is believed that in the pathogenesis of Castleman's disease a cytokine dysfunction of pro-inflammatory cytokines plays an important part, with an involvement of hypersecretion, IL-6 and TNFα, as was the case in this study (32).

CONCLUSION

Febrile conditions of unidentified origin remain a diagnostic challenge to clinicians because it encompasses a heterogeneous group of diseases which belong to infectious illnesses and infections as well as non-infectious diseases. In view of the achieved results, determining cytokine profiles in FUO patients, can improve the diagnosis, which can shorten the duration of hospitalization, and reduce the volume of the performance of diagnostic procedures.

ACKNOWLEDGMENTS

This work was supported by grant from the Faculty of Medical Sciences, University of Kragujevac (project JP 12-14).

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HISTOPATHOLOGY OF HEPATIC SINUSOIDAL OBSTRUCTION SYNDROME AFTER NEOADJUVANT OXALIPLATIN-BASED CHEMOTHERAPY

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HISTOPATOLOGIJA SINDROMA SINUSOIDALNE OPSTRUKCIJE JETRE NAKON NEOADJUVANTNE HEMOTERAPIJE NA BAZI OKSALIPLATINA

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Received / Primljen: 27. 03. 2017.

Accepted / Prihvaćen: 21. 05. 2017.

ABSTRACT

Sinusoidal obstruction syndrome („blue liver syndrome“) has been frequently associated with oxaliplatin-based neoadjuvant chemotherapy in patients with colorectal liver metastasis. Hepatotoxic vascular lesions in the nontumourous liver parenchyma result in hypoperfusion and tissue hypoxia leading to lower tumour response to oncologic treatment and to increase the risk of liver metastasectomies. Furthermore, hepatic parenchyma injuries could be aggravated by hepatic resection itself. Contrary to standard surgical techniques, radiofrequency assisted liver resection significantly reduce harmful intraoperative blood loss and perfusion-reperfusion effects. We compared histological alterations in 59 specimens of bloodless radiofrequency-assisted liver recetions made for colorectal metastases to those in 38 specimens of standard liver resections. In general, the main histologic alterations in both examined groups related to oxaliplatin include SOS lesions (69.35%), fibrosis (50.95%) and steatosis (38%). After scoring of histopathological parameters based on modified criteria according to Rubbia-Brandt et al., they were statistically insignificant between both groups for portal and/or porto-portal fibrosis (59.3% vs 47.4%, respectively) and moderate/severe macrovacuolar steatosis (10.2% vs 26.3%). Similar distribution between groups was shown for surgical hepatitis with „borderline“ statistical significance (23,7% vs 42,1%, p= 0.05). However, there were significant differencies in vascular lesions, particularly for hemorrhagic centrilobular necrosis (10,2% vs 31,5%, p=0.01) and peliosis (15,2% vs 36,8%, p= 0.04), but were not significant for sinusoidal dilatation and congestion as well as surgical necrosis. High-grade vascular lesions such as hemorrhagic centrilobular necrosis and peliosis are less frequent in cases of radiofrequency-assisted liver recetions and might be associated with better clinical outcome in these patients.

Keywords: *sinusoidal obstruction syndrome, hepatic vascular lesions, oxaliplatin, hepatotoxicity, colorectal liver metastases, liver resection*

SAŽETAK

Sindrom sinusoidalne opstrukcije (“sindrom plave jetre”) je često povezan s primenom neoadjuvantne hemoterapije na bazi oksaliplatina kod pacijenata sa kolorektalnim metastazama u jetri. Hepatotoksične vaskularne lezije u netumorskom parenhimu jetre rezultiraju hipoperfuzijom i hipoksijom tkiva i slabijim odgovorom tumora na onkološki tretman i povećavanjem rizika za resekcije jetrenih metastaza. Osim toga, povrede jetrenog parenhima mogu biti otežane samom hepatalnom resekcijom. Za razliku od standardnih resekcija jetre, radiofrekventne hirurške tehnike mogu značajno smanjiti štetne efekte intraoperativnog gubitka krvi i fenomena perfuzije-reperfuzije. Uporedili smo histološke promene u 59 slučajeva beskrvne radiofrekventne resekcije jetre učinjenih zbog kolorektalnih metastaza s onima u 38 slučajeva standardnih resekcije jetre. Generalno, glavne histološke promene jetre nakon upotrebe oksaliplatina u obe ispitivane grupe se odnose na SOS lezije (69.35%), fibrozu (50.95%) i steatozu (38%). Nakon bodovanja histopatoloških parametara na osnovu modifikovanih kriterijuma Rubia-Brandt i saradnika, nije bilo statistički značajne razlike između obe grupe za portnu i/ili porto-portnu fibrozu (59,3% prema 47,4%) i za umerenu/tešku makrovakuolarnu steatozu (10.2% u odnosu na 26,3%). Slična distribucija između ispitivanih grupa je pokazana za hirurški hepatitis sa “graničnim” statističkim značajem (23,7% prema 42,1%, p = 0,05). Međutim, utvrđena je značajna razlika za vaskularne lezije, naročito za hemoragijsku centrilobularnu nekrozu (10,2% prema 31,5%, p = 0.01) i peliozu (15,2% u odnosu na 36,8%, p = 0,04) , ali nije bilo statistički značajne razlike za sinusoidalnu dilataciju i kongestiju kao i za hiruršku nekrozu. Visoko rizične vaskularne lezije, kao što su hemoragijska centrilobularna nekroza i pelioza ređe su u slučajevima radiofrekventne resekcije jetre i mogu biti povezane sa boljim kliničkim ishodom kod ovih bolesnika.

Ključne reči: *sindrom sinusoidalne opstrukcije, vaskularne lezije jetre, oksaliplatin, hepatotoksičnost, kolorekralna jetrena metastaza, resekcija jetre*

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UDK: 616.36-091.8
Ser J Exp Clin Res 2019; 20 (1): 39-46
DOI: 10.1515/SJECR-2017-0025

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ABBREVIATIONS

RALR – radiofrequency-assisted liver resection
CASH – chemotherapy associated steatohepatitis
SOS – sinusoidal obstruction syndrome

INTRODUCTION

Sinusoidal obstruction syndrome (SOS) is distinctive hepatotoxic and rapidly developing vascular lesion with compromised hepatic microcirculation. SOS has a strong potential to lethal liver failure and portal hypertension-related complications (1). It is also known as "blue liver syndrome" because of its characteristic intraoperative subcapsular livid appearance and similar macroscopic „marble" bluish-red discoloration on cut surface. SOS is caused by toxic injury to hepatic sinusoidal endothelial cells producing their swelling and leading to the loss of sinusoidal wall integrity, impaired sinusoidal blood flow and consequent sinusoidal congestive obstruction (2). In some cases it can result in further obstructive fibrovascular alterations on hepatic veins and is often associated with other histopathologic findings in the liver parenchyma. Therefore, this syndrome was originally described as hepatic venoocclusive disease, but since DeLeve et al. coined the term SOS, previous term is no longer recommended primarily because the major lesion is located at the level of hepatic sinusoids, not necessarily involving centrilobular vein (3). The classical clinical triad of SOS includes ascites, hepatomegaly and increased bilirubin levels (>2 mg/dl), but one can often find other signs of portal hypertension, weight gain, pleural effusion, lower limbs edema, splenomegaly and rarely upper gastrointestinal bleeding. The frequent recognition of SOS has resulted from numerous complications after the use of chemotherapy before hemopoietic stem cell transplantation, immunosuppressive agents in solid organ transplantation and inflammatory bowel diseases, after irradiation as well as long list of hepatotoxic drugs and herbal remedies, including frequent oncologic treatment of colorectal liver metastases by oxaliplatin (4). Contrary to the West, in the Far East SOS is usually caused by herbal medicine containing pyrrolizidine alkaloids (5).

The liver is most common site of colorectal carcinoma metastases with initial rate of metastatic disease estimated to 15-20% of all patients (6). Oxaliplatin is frequently used as a part of neoadjuvant or systemic chemotherapy prior to hepatic resection of colorectal cancer liver metastases in patients who were estimated as initially inoperable (7). The results of combined medical oncology and modern surgery increase the number of curative liver resections from 15% to 30% and 5-year survival rates are reported to be 30-35% (8,9). However, oxaliplatin-based chemotherapy provokes sinusoidal obstruction syndrome in association with other histologic alterations of liver tissue not involved by tumor (10). Hepatic steatosis was initially regarded as the most significant pathological alteration in these settings and all other changes, including vascular changes, were attributed to steatohepatitis or secondary effects (11). At present, more scientific reports favour vascular lesions to be more closely associated with prehepatectomy oxaliplatin-containing regimens over steatosis and chemotherapy associated steatohepatitis (CASH) in the non-tumour liver parenchyma. As a consequence, SOS after neoadjuvant oxaliplatin-based chemotherapy might significantly increase morbidity, reduces the effect of oxaliplatin, bevacizumab or other chemotherapeutic agents in colorectal metastases (lower tumour response to oncologic treatment) and increases the risk of liver resection metastasectomies.

Liver resection itself can contribute further histologic changes since intraoperative blood loss and perfusion-reperfusion effects are often associated with postoperative morbidity, mortality and shorter long-term survival. The introduction of advanced surgical techniques have increased the percentage of curative liver resections, such as the novel technique of radiofrequency-assisted liver resection (RALR), which is thought to be transfusion-free, easy and safe (12). It broadens therapeutic options for patients with initially unresectable colorectal liver metastases and convert them to resectability.

The aim of this study was to assess the frequency and severity of SOS and associated histologic alterations in the non-tumor bearing liver parenchyma in the resected specimens after oxaliplatin-based chemotherapy in patients with colorectal liver metastases. However, little is known about possible differences between liver resection-related histologic alterations in standard resections and RALR. Therefore, SOS and its histologic variables and other hepatic alterations were correlated to main liver resection types.

MATERIAL AND METHODS

In a retrospective study a total of 97 liver resection specimens were examined from patients with colorectal liver metastases from November 2001 to December 2005 in a single institution (First Surgical Clinic, Clinical Centre of Serbia, Belgrade) who had been previously operated for colorectal cancer, underwent preoperative neoadjuvant oxaliplatin-based chemotherapy and first partial hepatec-

Table 1. Indications for radiofrequency-assisted liver resection (RALR) with minimal blood loss and facilitated tissue sparing, potentially reducing the risk of the development of SOS

Indications for resection	Patients	n %
Colorectal metastases	59	65.7
Primary liver cancer	17	18.9
Giant liver hemangioma	3	3.3
Lung cancer metastases	2	2.2
Ovary cancer metastases	1	1.1
Gallbladder cancer	1	1.1
Liver hydatid cyst	2	2.2
Liver cystadenoma	1	1.1
Recurrent liver abscess	1	1.1
Liver actinomycosis	1	1.1
Solitary necrotic liver nodule	1	1.1
Metastasis – undetectable primary tumor	1	1.1
Total	**90**	**100.0**

tomy. The majority of patients (59/90) were among cases with strong indications for RALR as a new surgical technique in a series of 81 patients with malignant tumors and 9 patients with benign diseases (Table 1). The rest of 38 patients were underwent standard surgical procedures for metastasectomies which all needed blood transfusion after significant blood loss (>1 unit of packed red blood cells transfusion). Selection of cases followed this exclusion criteria: (a) less than 6 cycles regimen of preoperatively chemotherapeutic treatment with fluorouracil plus oxaliplatin; (b) extrahepatic spread of the disease; (c) no sufficient amount of remnant non-tumour bearing liver parenchyma; (d) other known vascular disorders or focal lesions, including hemangioma; (e) simultaneous operative procedures on other organs and (f) less than 6 months period of successful postoperative follow-up.

Selected tissue samples were taken from non-tumour bearing liver parenchyma at minimum distance of 20 mm from the tumour, avoiding subcapsular region and from peritumoural zone. Standard 4% buffered formalin fixed and paraffin embedded samples were cut and 4-μm sections routinely stained for hematoxylin-eosin, reticulin and Masson trichrome staining. Histomorphological analysis and scoring of histopathological parameters were based on the criteria according to Rubbia-Brandt et al. (table 2) (13). In concordance with the study of Aloia et al. we focused mainly to categories of vascular lesions, fibrosis and macrovesicular steatosis (14). In addition to these morphological variables, we examined the presence of necrotic lesions induced by operative manipulation of the liver, so-called surgical hepatitis (surgical necrosis) defined by hepatocyte

Table 2. Histological features of hepatic sinusoidal obstructive syndrome (SOS) evaluated according to Rubbia-Brandt L. et al. (13)

Parenchymal lesions
Sinusoidal dilation - Grades:
0, absent
1, mild (centrilobular involvement limited to one-third of the lobular area)
2, moderate (centrilobular involvement extending in two-thirds of the lobular area)
3, severe (complete lobular involvement or centrilobular involvement extending to adjacent lobules with bridging congestion)
Perisinusoidal haemorrhage
Peliosis
Nodularity (including nodular regenerative hyperplasia) – Grades:
0, absent
1, mild (focal occasionally distinct nodular hyperplasia on reticulin staining but indistinct on H&E staining)
2, moderate (focal distinct nodular hyperplasia apparent on H&E staining, clearly highlighted on reticulin staining)
3, severe [diffuse nodular hyperplasia, distinct in most areas on H&E staining and highlighted on reticulin staining, corresponding to nodular regenerative hyperplasia (NRH)]
Steatosis – Grades:
0, absent
1, mild (steatosis 10–30% of the hepatocytes)
2, moderate (steatosis in 30–60% of the hepatocytes)
3, severe (steatosis in >60% of the hepatocytes)
Steatohepatitis
Hepatocellular damage (necrosis, apoptosis, atrophy)
Venular lesions
Centrilobular vein or portal vein lesions (endothelial cell rounding) – Grades: 0, 1, 2
Fibrosis
Perisinusoidal fibrosis (grade 0, 1, 2)
Centrilobular vein fibrosis (grade 0, 1, 2)
Portal vein fibrosis (grade 0, 1, 2)
Fibrosis grades:
0, absent;
1, mild (<50% of veins and sinusoids evaluated on 20 fields at ×200 magnification);
2, moderate (>50% of veins and sinusoids evaluated on 20 fields at ×200 magnification).
The area of a single high-power field is 0.315 mm^2.

Table 3. Liver histology changes in non-tumour bearing tissue in patients after neoadjuvant oxaliplatin-based chemotherapy operated for colorectal liver metastasis with radiofrequency-assisted liver resection (RALR) and with standard surgical procedure (non-RALR)

Histopathologic parameters examined	RALR (N=59)		Non-RALR (N=38)		
	N	%	N	%	*p*
Steatosis	**20**	**33.9**	**16**	**42.1**	**0.18**
Mild	14	23.7	6	15.8	0.12
Moderate	5	8.4	9	23.7	0.12
Severe	1	1.2	1	2.6	0.5
Fibrosis	**35**	**59.3**	**20**	**52.6**	**0.35**
Portal	35	59.3	18	47.4	0.69
Septal	0		0		ns
Cirrhosis	0		2	5.3	0.43
Vascular lesions	**26**	**44**	**36**	**94.7**	**0.21**
Sinusoidal dilatation and congestion	14	23.7	6	15.8	0.86
Peliosis	9	15.2	14	36.8	0.04
Haemorrhagic centrolobular necrosis	3	5.1	13	34.2	0.01
Regenerative nodular hyperplasia	0		3	7.9	0.01
Surgical necrosis	**14**	**23.7**	**16**	**42.1**	**0.05**

necrosis associated with neutrophils that is disseminated throughout the periportal or centrilobular areas.

Detailed histological examination of 59 specimens was performed on samples of liver specimens of transfusion-free RALR and was compared to histological changes on samples of non-tumorous liver tissue from 38 specimens of standard liver resections. Furthermore, we analyzed samples distal and proximal to the treated tumour, i.e. samples taken from non-tumour bearing liver parenchyma at minimum distance of 20 mm from the tumour and compared to those from peritumoural zone.

Statistical analysis were performed using SPSS version 15.0 statistical software (SPSS Inc., Chicago, IL, USA). The chi-square test was used to correlate the different groups of patients according to the liver resection types versus the presence of lesions associated with oxaliplatin based chemotherapy. *P*-values were considered statistically significant if <0.05.

RESULTS

The study included 97 patients previously operated for colorectal cancer and underwent preoperative neoadjuvant oxaliplatin-based chemotherapy for colorectal liver metastases. There were 50 men and 47 women, ranging from 18 to 78 years old (mean age 57.71 years). According to subsequent liver resection type they were divided in two groups: 59 patients underwent bloodless radiofrequency-assisted liver resection (RALR), and 38 patients were treated by standard surgical procedures for colorectal metastasectomies (non-RALR).

Detailed histomorphological examination on oxaliplatin-related histological alterations comprises 59 specimens of non-tumorous liver tissue (RALR group) and 38 specimens of cases with standard liver resections (non-RALR group) including both samples taken from non-tumour bearing liver parenchyma at minimum distance of 20 mm from the tumour and those from peritumoural zone. After careful examination and scoring of numerous histomorphological parameters we systematized them in ascending order of severity, mostly grouping them to low-grade and high-grade lesions. SOS lesions were divided according to grading systems in two groups: low-grade lesions included grades 0 (absent) and 1 (mild) and high-grade lesions included grades 2 (moderate) and 3 (severe). For histological analysis of SOS parameters in this study we finally reported only high-grade lesions (moderate or severe grades).

We found most frequent and most important four histologic entities: (a) fibrosis, futher categorized as portal fibrosis, porto-portal fibrosis, septal fibrosis, and cirrhosis; (b) vascular lesions, specified as sinusoidal vasodilatation and congestion, peliosis, hemorrhagic centrilobular necrosis and regenerative nodular hyperplasia; (c) macrovacuolar steatosis, and (d) surgical hepatitis (surgical necrosis). The most important histopathological alterations in both examined groups are briefly presented (Table 3).

In general, the main histologic alterations in both examined groups related to oxaliplatin include SOS lesions (69.35%), fibrosis (50.95%) and steatosis (38%). After scoring of histopathological parameters based on modified criteria according to Rubbia-Brandt et al., they were statistically insignificant between both groups for portal and/or porto-portal fibrosis (59.3% vs 47.4%, respectively)

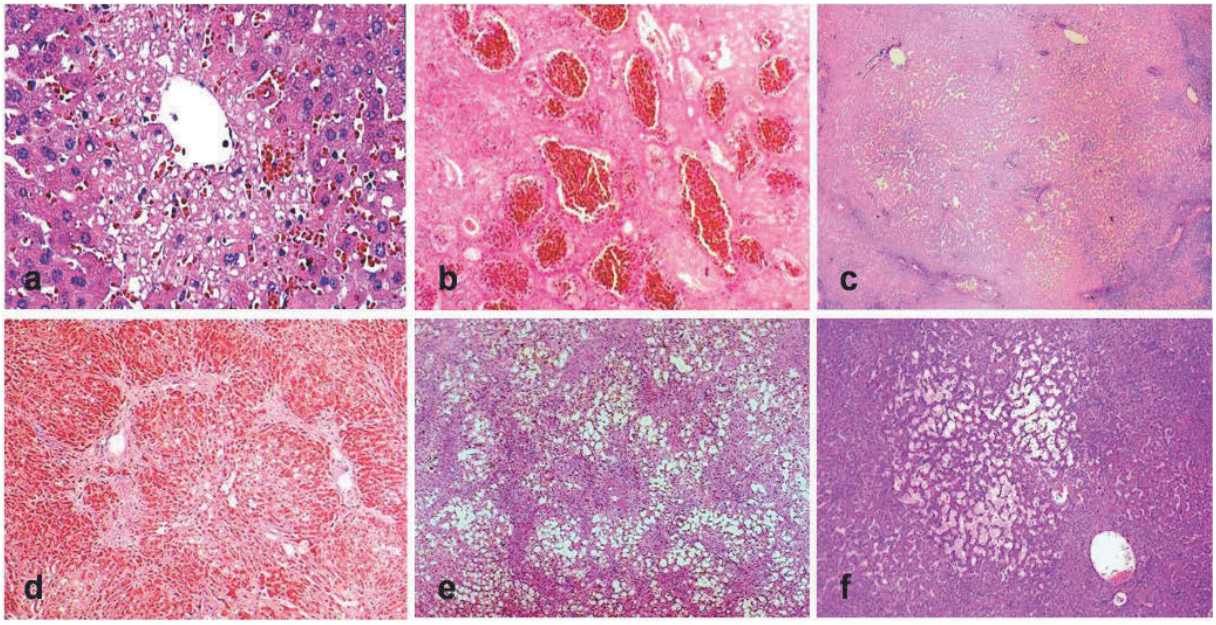

Figure 1. High-grade SOS lesions were seen more often in non-RALR group of patients and may be related to worse clinical outcome in this group of patients such as: hemorrgahic centrilobular necrosis (a) and peliosis, representing large haemorrhagic cystic-like pattern of sinusoidal dilation, (b) as well as regenerative nodular hyperplasia (c). The most consistent findings in non-tumoural liver tissue related to effects of neoadjuvant oxaliplatin-based chemotherapy were: portal fibrosis (d), macrovacuolar steatosis (e) and sinusoidal dilatation (f)

and moderate/severe macrovacuolar steatosis (10.2% vs 26.3%), some of the with elements of CASH. We have not seen isolated centrilobular vein thrombosis and fibrosis which is quite interesting and unusual result. Similar distribution between groups was shown for surgical hepatitis with „borderline" statistical significance (23.7% vs 42.1%, $p = 0.05$). However, there were significant differencies in „high-grade" SOS vascular abnormalities, particularly in the presence of hemorrhagic centrilobular necrosis (10.2% vs 31.5%, $p = 0.01$) and peliosis (15.2% vs 36.8%, $p = 0.04$), but were not significant in the presence of sinusoidal dilataton and congestion as well as surgical necrosis. Two cases of micronodular cirrhosis were noted only in RALR group and three cases of regenerative nodular hyperplasia and two cases of hepatic atrophy only in non-RALR group. In our series of cases liver parenchymal and fibrotic lesions were dominant (Figure 1).

We have also compared the main features of SOS lesions and other associated alterations in samples distal and proximal to the treated tumour. Although there is a lesser degree of hemorrhagic and necrotic lesions in samples taken from non-tumour bearing liver parenchyma (at minimum distance of 20 mm from the tumour) compared to those from peritumoural zone, we observed evidently narrower (although variable) zone of necrotic and vascular lesions with slightly dilated sinusoidal bed (figure 2). There were visible differences for

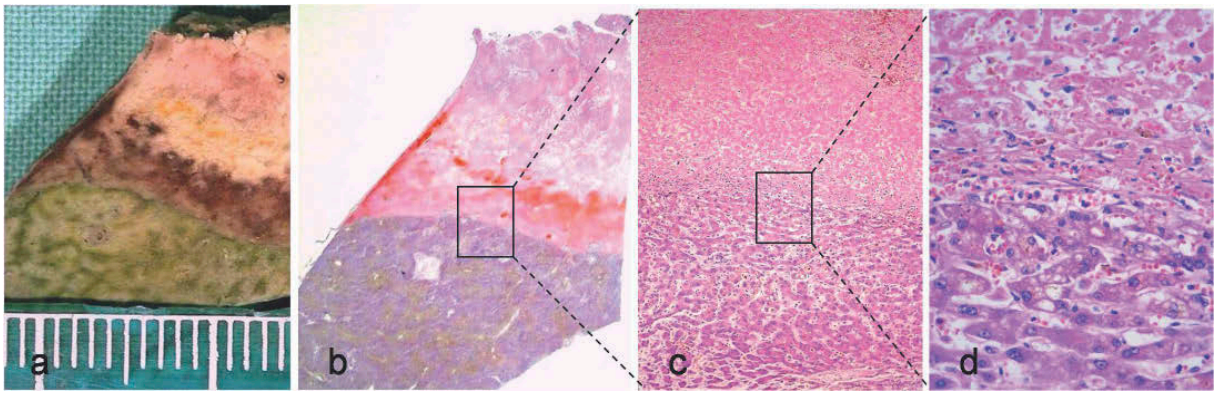

Figure 2. Macroscopic aspect of SOS shows liver congestion with irregular nodularity of hepatic parenchyma and congestive discoloration below necrohemorrhagic zone of treated metastatic colorectal carcinoma (a); the same area presented by whole mount section with boundary zone between necrotic tumour and liver parenchyma in the rectancle (b); closer view depicts RALR-related sharp delineation of necrotic to viable liver tissue showing sinusoidal dilatation and irregular trabeculae of hepatocytes with the same intensity in peritumoural and peripheral zones (c); the loss of endothelial cells and hepatocyte degeneration is less pronounced and restricted to narrow peritumoural zone in cases treated with RALR (d)

histologic lesions in peritumoural zones between both RALR and non-RALR groups but were not significant: hemorrhagic centrilobular necrosis (23.2% vs 39.9%, p=0.81), surgical necrosis or surgical hepatitis (31.2% vs 44,7%, p=0.76) and sinusoidal dilataton and congestion (45.7% vs 76.8%, p=0.64).

DISCUSSION

Histopathology of SOS and associted histopathologic alterations significantly broadens the role of pathologist in evalutation the surgical and oncological treatment for colorectal liver metastases. In addition to verification of histological type and grade as well as residual status by cheking the margins of the resection, the up-to-date pathological report includes further information on other major determinants in management and outcome: the regression grade, i.e. the degree of histological response of the CRLM to preoperative chemotherapy and possible histological alterations in non-tumour bearing liver parenchyma with special attention to the distinctive toxic hepatic lesions associated with chemotherapy (15).

The rationale of neoadjuvant preoperative use of oxaliplatin-based chemotherapy in selected patients with colorectal liver metastases relates to downsizing the tumors that may increase the rate of curative resections, to conversion of unresectable metastases to those that may become eligible for hepatic resection, to identification of good responders among patients and via the regression grade (the degree of response to chemotherapy) to evaluate biologic aggressiveness of tumors as those who progress under chemotherapy may not benefit from resection. However, oxaliplatin-based treatment is accompanied by serious side effects. Several studies has established the prevalence of complex vascular lesions of SOS in oxaliplatin-related liver damage in patients treated for colorectal liver metastases. There are also detailed pathological descriptions and grading of these changes, because its occurrence was associated with high mortality rates. According to current data, one can expect sinusoidal lesions to be found in aproximately two thirds of patients receiving oxaliplatin-based chemotherapy (ranging 38-74%) dependent on the heterogeneity of applied regimens and type of administration as well as on the level of clinical and histopathological recognition of SOS. Our result with prevalence of 69.35% of SOS lesions with more than a half with low-grade lesions is consistent with most of european studies (4, 14). SOS is in these settings almost always variably associated with various patterns of fibrosis, steatosis and/or steatohepatitis and to lesser degree regenerative nodular hyperplasia. Although the severity and the outcome of SOS is hardly predictable, potentially strong hepatotoxicity can be observed in patients receiving intrahepatic arterial administration of high doses of oxaliplatin and little is known whether these lesions are eventually reversible. Less hepatotoxicity is reported for irinotecan and 5-fluorouracil-based chemotherapy (25-40%) but coadministration of bevacizumab has been shown to reduce the incidence and severity of sinusoidal changes (4).

On the other hand, pathological lesions of non-neoplastic liver treated by surgery alone showed the prevalence of steatosis present in 37% (but other types of lesions were not found) and that was not significantly different from that of cases treated by neoadjuvant chemotherapy (39.8%) (13). Furthermore, development of CASH, i.e. severe steatohepatitis can occur in patients who have received certain types of chemotherapy for colorectal liver metastases such as preoperative administration of oxaliplatin and/or irinotecan, especially in the obese. CASH can limit the ability to perform an extensive hepatectomy and can contribute to postoperative patient morbidity and mortality (11). However, all further studies revealed that most of questions about the interpretation of the histologic lesions has led to the conclusion that SOS develops in the context of steatohepatitis rather than aggravate steatohepatitis itself. This issue is important in order pathogenesis to be better understood and prevention strategies developed (16).

Liver resection type can influence the severity of SOS. In the context of standard liver surgerical procedures for colorectal liver metastases, oxaliplatin-related SOS could increase the risk of intraoperative bleeding and postoperative liver insufficiency. In patients undergoing a major resection combined with radiofrequency procedures, oxaliplatin-associated liver injury was not associated with an increased risk of perioperative (90-day) morbidity or mortality. In contrast, CASH steatohepatitis was associated with irinotecan and with an increased perioperative risk of death, especially (17). This is also consistent with our results showing "high-grade" SOS lesions specially hemorrhagic centrilobular necrosis and peliosis in non-tumour bearing liver parenchyma less frequent in patients underwent RALR. It was shown that most severe vascular lesions from the spectrum of SOS that are found in standard liver metastasectomies are associated with increased intraoperative transfusions due to significant blood loss and perfusion-reperfusion effects in the liver sinusoidal bed (14). Bloodless liver resection type such as RALR may represent at least partial explanation for lower rates of surgical hepatitis (surgical necrosis) as well as known better clinical outcome in these patients. Therefore, the extent of liver resection and type and duration of applied chemotherapy regimen should be carefully considered and individualized, including a consideration for body mass index and related comorbid factors.

Etiopathogenesis of SOS is still not well elucidated but has similar and quite consistent histomorphologic features regardless of its cause. In general, it is hypothesized as multifactorial activation of the hepatic sinusoidal endothelial cells and the subsequent toxic destruction of endothelial cells with sloughing and eventually downstream occlusion of terminal hepatic venules (18). Contributing factors include glutathione and nitric oxide depletion of sinusoidal endothelial cells, increased intrahepatic expression of matrix metalloproteinases and vascular endothelial growth factor, and activation of coagulation cascade. However, these mechanisms are described predominantly

in SOS observed after hematopoietic stem cell transplantation or pyrrolizidine alkaloid exposure. It is still not known whether SOS observed in oxaliplatin based chemotherapy are identical to them, or are mostly related to pro-inflammatory mechanisms. Unlike the previous two, endothelial destruction and hepatocyte necrosis are inconspicuous in oxaliplatin-related SOS both in clinical and in experimental studies and an activation of VEGF and IL6 pathway mediators can result only in sinusoidal dilatation and hepatocyte atrophy (19, 20). There is also evidence that an activation of pro-inflammatory pathways can be induced in the tumor itself by chemotherapeutic agents (21).

The role of biopsy with respect to raising the awareness of possible development of SOS is unclear. Some authors have recommended preoperative liver biopsy to evaluate for SOS, steatosis and/or steatohepatitis. However, given the problems associated with unknown or unclear clinical context, sampling error as well as intra-and interobserver variation in the evaluation of histologic alterations, we do not stand for this approach. Rather, laparoscopy before laparotomy is advocated in patients with preoperative imaging suggested steatosis to directly evaluate the liver. Systematic review by Wang et al. disclosed that nearly half of patients with SOS underwent liver biopsy to diagnose the syndrome (210 patients with SOS caused by herbal medicine Tusanqi) in addition to the currently well-established diagnostic criteria for SOS include original or modified Seattle criteria and Baltimore criteria with four major components: bilirubin, hepatomegaly, ascites and weight gain (22). There is no data on diagnostic accuracy of hepatic biopsies as the diagnosis is currently based on sinusoidal lesions, independently of hepatic venous lesions. It depends on the size and type of biopsy samples because sinusoidal and endothelial lesions are discrete: dilated and empty sinusoids could be easily overlooked as well as subintimal edema and fibrin deposits in sinusoids and/or terminal hepatic venules. Peliosis and nodular regenerative hyperplasia could hardly be recognized on routine hematoxylin-eosin staining of needle biopsies but more clearly seen in reticulin preparations and until detailed clinical data and clinical suspicion are presented in the request. Fine needle biopsies are almost useless in diffuse vascular lesions. Acute hepatic congestion should be considered if perivenular sinusoids are dilated and associated with necrosis, little inflammation and brown-ceroid pigment laden Kupffer cells. Chronic venous congestion with fibrotic veins is difficult to demonstrate within the fibrous tissue without some of trichrome or collagen stainings. Rubia-Brandt stated that in pathologic studies, occlusion of the centrilobular veins occurs in 50-75% of patients who develop SOS after hematopoietic stem cell transplantation, but only in approximately 50% of patient with SOS after oxaliplatin-based chemotherapy (2).

An accurate and noninvasive tool to predict SOS lesions in the subset of patients receiving oxaliplatin preoperatively is missing, although some risk factors of SOS have been identified. The increase of spleen volume could be helpful in the early diagnosis and pathogenesis of SOS. In a recent animal study of vascular endothelium toxicity caused by herbal medicine Tusanqi, various platelet disorders and other hematologic effects were observed to be associated with splenomegaly (22). It seems more likely that splenomegaly is caused by the change in endothelin in the intersection of liver-spleen axis, but not necessarily linked to chronic portal hypertension (23). Imai et al. also found that the splenomegaly should be an independent predictor of the development of SOS in patients with colorectal metastases who received oxaliplatin-based chemotherapy with or without bevacizumab (24). Previously, Soubrane et al. reported a low preoperative platelet count and high aspartate aminotransferase to platelet ratio index (APRI) might represent the most reliable indicators to predict SOS severity (25). Among determinants for the development of SOS after chemotherapy genetic polymorphism in drug metabolizing enzymes, such as glutathione S-transferase may be important (19).

This study had several limitations. First, all patients with 6 or more cycles regimen of preoperatively chemotherapeutic treatment with fluorouracil plus oxaliplatin were included. However, variable duration of neoadjuvant chemotherapy and dose-dependent oxaliplatin-related effects could be relevant for the severity of SOS and other histological abnormalities. Second, there was no groupings of patients who underwent major and minor liver resection, although no statistical difference was found between the patients with regard to the frequency of blood transfusion (12). Third, only a short-term (6-24 months) surgical follow-up result could be provided in this study, mostly with relatively good outcome. Most of death events and other major complications occurred during a relatively short follow-up period. The most common cause of death might be acute liver failure, rather than portal hypertension-related complications. However, the long-term follow-up was lacking.

CONCLUSION

Our results confirmed recent reports that sinusoidal obstruction syndrome is a major feature of hepatic lesions associated with oxaliplatin neoadjuvant chemotherapy for liver colorectal metastases. Although the impact od oxaliplatin-related SOS on clinical outcome is unclear and in many cases minimal, it can be associated with increased postoperative morbidity after major liver resections and with a decreased tumor response. We found severe vascular abnormalities in non-tumour bearing liver parenchyma such as hemorrhagic centrilobular necrosis and peliosis to be less frequent in patients underwent RALR for colorectal liver metastases and they might be associated with better clinical outcome in these patients.

These preliminary findings might be important for clinicians as well as pathologists to recognize SOS with respect to possible complications and the impact on overall therapeutic results. Further studies should be necessary to explore the long-term follow-up results and refine diagnostic possibilities and the treatment strategy.

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PREDICTIVE PARAMETERS FUNCTIONING ARTERIOVENOUS FISTULA FOR HEMODIALYSIS IN THE ELDERLY

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PREDIKTIVNI PARAMETRI FUNKCIONISANJA ARTERIOVENSKE FISTULE ZA HEMODIJALIZU KOD STARIJIH OSOBA

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Received / Primljen: 02. 03. 2017. Accepted / Prihvaćen: 12. 05. 2017.

ABSTRACT

Elderly patients with end stage kidney disease represent a challenge for surgeons to create a vascular access.

Determine predictive parameters functionality of the arteriovenous fistulas for hemodialysis in the elderly.

The study was organized as a retrospective study at the Center for Dialysis, Clinic for Urology and Nephrology, Clinical Center Kragujevac. The study included patients older than 65 years with arteriovenous fistula thrombosis, in the period of four years, in which there is information on the length of the functioning fistula. The study included 48 patients, mean age 71.3±5.2 years, 29 (60%) men and 19 (40%) women. The data were analyzed according to gender and demographic structure, type of anastomosis, positioning, length of functioning fistulas, and the lumen diameter of the arteries and veins that are used to create a fistula.

The median length of functioning arteriovenous fistula, based on Kaplan-Meier model, is 16 months (95% CI 6.9-25.1). Median functioning for proximaly located fistulas was 24 months (range, 1-259), while median functioning in patient with distally located fistulas was 8 months (range, 1-96). The difference in relation to the positioning of the fistula was statistically significant (p=0.006). In univariate Cox regression model, a statistically significant predictor of the functioning of arteriovenous fistulae is fistula positioning (B=0.700; p=0.022).

The predictive parameter of survival of arteriovenous fistulas in elderly is proximally located fistula.

Keywords: *old patients, hemodialysis, arteriovenous fistula, duration, functioning*

SAŽETAK

Stariji bolesnici sa terminalnom bubrežnom insuficijencijom predstavljaju izazov za hirurge koji kreiraju vaskularne pristupe. Cilj rada bio je da se utvrde prediktivni parametri funkcionalnosti arteriovenske fistule za hemodijalizu kod starijih osoba.

Istraživanje je organizovano kao retrospektivna studija u Centru za dijalizu Klinike za urologiju i nefrologiju, Kliničkog centra Kragujevac. Analizirani su bolesnici stariji od 65 godine, sa trombozom arteriovenskom fistulom, u periodu od četiri godine, kod kojih postoji podatak o dužini funkcionisanja fistule. U istraživanje je uključeno 48 bolesnika, prosečne starosti 71,3±5,2 godine, 29 (60%) muškaraca i 19 (40%) žena. Analizirana je polna i demografska struktura, tip anastomoze, pozicioniranje, dužina funkcionisanja, kao i dijametar lumena arterije i vene koji su korišćeni za kreiranje fistule.

Medijana dužine funkcionisanja arteriovenske fistule, na osnovu Kaplan-Meireovog modela iznosi 16 meseci (95% Cl 6,9-25,1). Medijana funkcionisanja proksimalno lociranih arteriovenskih fistula iznosila je 24 meseca (opseg, 1-259), dok je kod ispitanika sa distalno lociranim fistulama 8 meseci (opseg, 1-96) a razlika u odnosu na pozicioniranje fistule je statistički značajna (p=0,006). U univarijantnom Cox-regresionom modelu statistički značajan prediktor dužine funkcionisanja arteriovenske fistule je pozicioniranje fistule (B=0,700; p=0,022).

Prediktivni parametar dužine preživljavanja arteriovenske fistule kod starijih osoba je proksimalno locirane fistule.

Ključne reči: *stari bolesnici, hemodijaliza, arteriovenska fistula, dužina funkcionisanja*

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UDK: 616.13/.14-089.86; 616.61-78-053.9
Ser J Exp Clin Res 2019; 20 (1): 47-51
DOI: 10.1515/SJECR-2017-0021

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INTRODUCTION

Functional arteriovenous fistula is one of the main factors of survival in hemodialysis patients. There are many parameters that affect the functionality of arteriovenous fistula. A significant increase in the number of elderly patients with end stage kidney disease, points to serious problems in the creation of vascular access. Complications of arteriovenous fistula were significantly more frequent in elderly patients. Important parameters such as life expectancy, price, number of required audits, as well as the duration of hemodialysis should be analyzed in future studies to clarify the best options for a dramatic increase in the elderly population (1).

In the case of the failure arteriovenous fistula, in elderly patients, a better option is to create a new fistula, than to attempt of rescue current fistula. In the current guides there are no specific guidelines for the creation of fistula in the elderly. The relationship between unnecessary and necessary operations in order to create a vascular access has always been higher in the elderly compared to younger patients. Old hemodialysis patients have a shorter life expectancy, 50% of patients aged 75 years dies in the first year of hemodialysis treatment. Establishing an optimal vascular access in the elderly patients, is a major challenge. Recent studies have shown that two-thirds of older patients, which had fistula created, die before it's use. In patients with glomerular filtration rate less than 15ml/min or when the maintenance hemodialysis onset is expected in the next six months, the most rational solution is creation of fistula (2, 3).

The procedure of creating vascular access, and expected complications are an important cause of morbidity and mortality in the population of hemodialysis patients. Therefore, the assessment of the older patient, and his or her suitability for the formation of vascular access is of fundamental value, as it represents the key component of the planning process. Fatigue, exhaustion, malnutrition and general frailty is a common feature of older patients. In patients with end stage kidney disease are recorded bath skin and a greater possibility of forming a hematoma. Likewise, the use of anticoagulants can potentially increase the risk of bleeding. Older patients have a higher incidence of peripheral vascular disease, atherosclerosis, hypertension and arterial calcification (4, 5).

Help and support in making joint decisions regarding the creation of vascular access will be focused on improving communication, knowledge transfer between patients and doctors, in order to generate information that will allow patients and physicians realistic expectations about the different treatment options.

The purpose of this study was to determine predictive parameters for functionality of the arteriovenous fistulas for hemodialysis in the elderly.

MATERIALS AND METHODS

The study was designed as retrospective, conducted at the Center for Dialysis, Clinic of Urology and Nephrology, Clinical Center Kragujevac. We analyzed all patients on hemodialysis, who were older than 65 years, with thrombosis of arteriovenous fistula, where there is information on the length of functioning fistula.

The study included 48 patients, mean age 71.3±5.2 years, 29 (60%) men and 19 (40%) women.

Clinical and demographic parameters

The data were analyzed according to gender and demographic structure, type of anastomosis, positioning and length of functioning fistulas, as well as the diameter of the arteries and veins that are used to create a fistula.

The study was retrospective, conducted at the Center for Dialysis, Clinic of Urology and Nephrology, Clinical Center Kragujevac. We analyzed all patients on hemodialysis, who were older than 65 years, with thrombosis of arteriovenous fistula, where there is information on the length of functioning fistula.

The study included 48 patients, mean age 71.3±5.2 years, 29 (60%) men and 19 (40%) women.

The study was approved by the Ethics committee of the Clinical Center Kragujevac and was performed in accordance with the Helsinki declaration for medical research.

Statistical analysis

For the analysis of primary data we used descriptive statistical methods, for testing statistical hypotheses and methods for the analysis of time to the occurrence of events of interest. From the descriptive statistical method we used measures of central tendency, measures of variability and relative numbers. For testing statistical hypothesis we used Mann-Whitney test. For an analysis of the duration of the arteriovenous fistula was used Kaplan-Meier method, to find independent predictors of length of the Cox regression model with 95% confidence interval. The statistical hypotheses were tested for statistical significance level of 0.05.

RESULTS

In our study, of the 48 respondents in total, 29 (60%) patients were men and 19 (40%) women. Eight patients (17%) had end-to-end anastomosis, while 40 (83%) had termino-lateral anastomosis. In relation to the positioning of the fistula, 29 (60%) patients had proximal fistula, whereas 19 (40%) patients had a distal fistula. In relation to the placement of central venous catheter 30 (62.5%) of the respondents had a catheter, as a temporary vascular access. The mean value of the vein diameter, in our patients, was 2.5±0.6 mm, while mean of the artery diameter was 2.7± 0.6 mm (Table 1).

Evaluation of the median duration of arteriovenous fistula, based on Kaplan-Meire model is 16 months (95% CI 6.9 to 25.1), (Figure 1).

Table 1. Clinical characteristics of patients included in the study

Variables	n = 48
Age (years), mean ± sd	71.3 ± 5.2
Gender, n(%)	
men	29 (60%)
women	19 (40%)
Type of anastomosis, n(%)	
termino-terminal	8 (16.7%)
termino-lateral	40 (83.3%)
Location arteriovensous fistula, n(%)	
proximal fistula	29 (60%)
distal fistula	19 (40%)
Central-venous catheter, n(%)	30 (62.5%)
Diameter of the vein (mm), mean ± sd	2.5 ± 0.6
Artery diameter (mm), mean ± sd	2.7 ± 0.6

Table 2. Correlation lengths functioning of the arteriovenous fistula in relation to the examined clinical variables

Variables	Mediana (range)	p-value
Gender, n(%)		
men	18 (1-95)	0.916
women	16 (1-259)	
Type anastomosis, n(%)		
termino-terminal	15.5 (2-56)	0.430
termino- lateral	17 (1-259)	
Central-venous catheter, n(%)		
yes	21 (1-259)	0.749
no	15 (1-96)	

Table 3. Univariate Cox regression models

Predictors	Hazard ratio (96% CI)	p-value
Age (years)	1.02 (0.97-1.08)	0.387
Gender	0.73 (0.39-1.36)	0.315
Type anastomosis	0.62 (0.28-1.36)	0.233
Location of the arteriovenous fistula	2.01 (1.10-3.67)	0.022*
Central-venous catheter	1.02 (0.56-1.86)	0.944
Diameter of the vein	1.46 (0.81-2.64)	0.213
Artery diameter	1.28 (0.72-2.28)	0.411

* Statistically significant difference

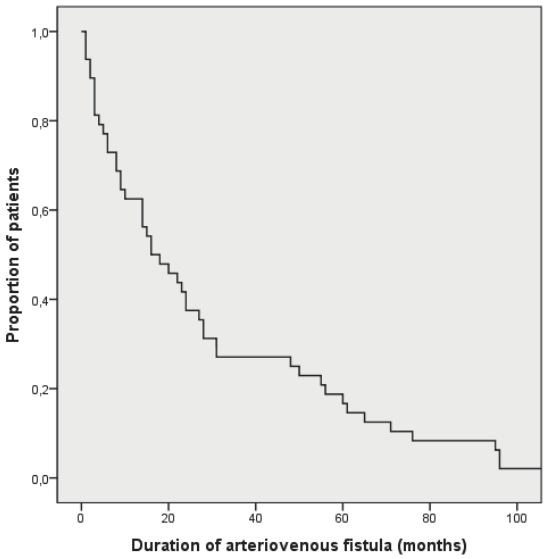

Figure 1. Kaplan-Meire curve proportion of respondents with regard to the functioning of arteriovenous fistula

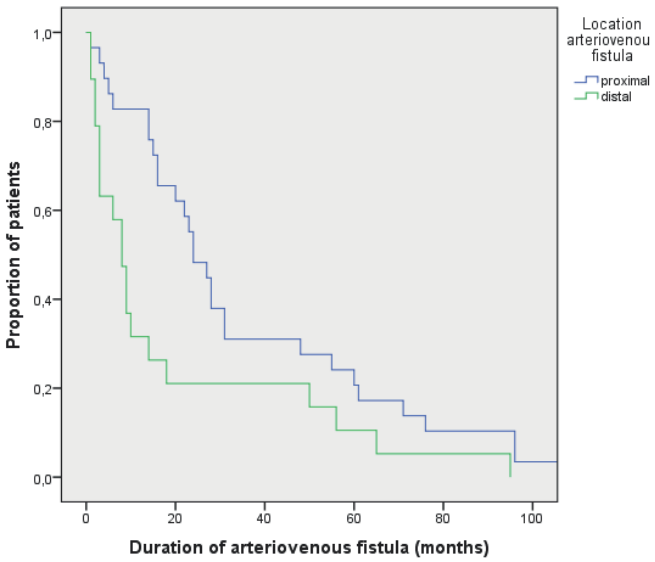

Figure 2. The correlation of the duration in relation to the location of the arteriovenous fistula

Median functioning of the arteriovenous fistula in men was 18 months (range, 1-95), while in female respondents it was 16 months (range, 1-259), which is not a statistically significant difference (p=0.916). In relation to the type of the arterio-venous anastomosis, there was no statistically significant difference, compared to the length of the functioning fistula (p=0.430). There was no statistically significant difference shown in the length of functioning arteriovenous fistula between the patients with and without a central-venous catheter (p = 0.749), (Table 2).

Median functioning for proximally located fistulas was 24 months (range, 1-259), while median functioning in patient with distal located fistulas was 8 months (range, 1-96). The difference in relation to the positioning of the fistula was statistically significant (p= 0.006). Median functioning arteriovenous fistula in patients with proximal location is 24 months (range, 1-259), while in patients with distal location 8 months (range, 1-95). A statistically significant difference was found in relation to the location of the arteriovenous fistula (p=0.006). Subjects with proximally located fistula had statistically significant longer duration od arteriovenous fistula. Figure 2.

Univariate Cox regression models found that a statistically significant predictor duration is positioning of arteriovenous fistula (B = 0.700; p = 0.022), Table 3.

DISCUSION

Not so long ago, a contraindication for the initiation of hemodialysis, was patients age over 45 years. Today, however, the number of elderly patients on hemodialysis reaches 25-30% of the total number of patients with end stage kidney disease (1, 2). Number of elderly patients over 75 years seems almost a third of all patients on chronic hemodialysis with a tendency of further growth (6). The mean age of the participants was 71.3 years. Having an insight into its own database (unpublished data), trend can not be established of continuous increasing in elderly, who requested the creation artriovenous fistulas for hemodialysis.

The latest research establishes doubt about previous strategies, when it comes to the type of vascular access in the elderly and advocate for greater flexibility in the choice of the vascular access for hemodialysis. Restriction policy, aimed at optimizing vascular access for hemodialysis has certain omissions, because it does not consider complexity and challenges of the disease, as well as the different experiences and characteristics of individual patients with chronic kidney disease (3). Experience of our center showed that the optimal vascular access is arteriovenous fistula, regardless of age, unless there are significant contraindications.

In accordance with current guide, radiocephalic fistula is a primary choice of the vascular access for hemodialysis. However, this strategy is more frequently contested, especially when it comes to older populations. Due to the significant comorbidity in older patients, such as uremic or ischemic cardiomyopathy, peripheral vascular disease, diabetes mellitus, there is greater doubt in the successful functioning of distal arteriovenous fistula. Preserving the proximal blood vessels, for the purpose of creating a vascular access in the elderly, is of no crucial importance, especially with the position of patients with limited life expectancy. Cubital arteriovenous fistula in elderly hemodialysis patients have greater functionality for the year, compared with radiocephalic fistulas. Such policy can reduce the rate of initial afunction and the need to use temporary central-venous catheters (4). In two-year follow of duration of functioning arteriovenous fistula in elderly patients in our study is 16 months (95 CI 6.9 to 25.1), with no statistical differences in relation to gender, nor in relation to the placement of central-venous catheters. Median functioning of the proximal arteriovenous fistula is 24 months, a distal 8 months. We found that subjects with proximal fistulas have longer duration. Nearly two-thirds of our respondents have proximal location of arteriovenous anastomoses, which is in line with current data (6, 7).

On the other hand, permanent central venous catheter hemodialysis patients in the United States is used in over 80% of patients. It is interesting to notice the results of avaliable studies that, when looking at period less than two years, there is no difference in mortality between patients with permanent central-venous catheters and arteriovenous fistulas in the elderly (8). The results of our study showed that 62.5% of respondents had a placed central venous catheter, but only as temporary vascular access.

Central venous catheter is used more frequently in elderly hemodialysis patients in Europe, Australia, North America, but rarely in Japan (2). On the other hand, analysis of hospitalizations between 1995 and 2006 in the United States showed that the lowest number of hospitalized older patients on hemodialysis was conducted because of infection of the vascular access (9).

When it comes to vascular accesses, there are not appropriate recommendations which regards the elderly. Existing guidelines suggest that the choice of distal arteriovenous fistula in elderly is associated with frequent primary afunction. Benefits of radiocephalic fistula, in the event of failure, is that there is a possibility of repeated, proximal attempts. However, only 12% of elderly patients have a adequate radio-cephalic fistula (4). In our study, 19% of respondents have a distal fistula, probably because of the policy of our Center that is primarily focused on the creation of native arteriovenous fistula and, wherever possible, distally, regardless of their age.

Stratified studies suggest discordance between clinical practice guidelines for vascular access, and measures of clinical performance, suggesting the need of individual approach in many aspects of care of patients with end stage kidney disease. This could be in conflict with the current clinical practice guidelines. In fact, strict application of these recommendations may be inappropriate for certain patients (3, 10-12).

The absence of guidelines related to vascular access for older people, is result of the exclusion of older people from the process of achieving certain guidelines. There is almost declarative consensus that brachiocephalic fistula have significantly higher rate of the duration, compared to radiocephalic fistula (2, 13). The results of our studies have found that a statistically significant predictor of the length of the functioning of arteriovenous fistulae in older patients, is proximal positioning of arterio-venous anastomosis (B= 0.700; p=0.022).

Data on the successful creation of functional fistula, in elderly patients, are contradictory, primarily due to non-compliance definitions of age (5). REDUCE-FTM I study (14), in one-year period, showed that elderly patients have 70% greater chance for a non-functional fistula. In the elderly patients, with end stage kidney disease, due to lower lumen of blood vessels, the higher rate of the initial dysfunction, shorter life expectancy, and a larger number of comorbidities, arteriovenous fistula should not be a universal option for vascular access. It is interesting that today, according to some titles, it seems more and more that the problem of vascular access creation goes beyond the reality of clinical nephrology, and that theology and religion is included in the phenomenology of creating arteriovenous fistula (15).

Elderly patients, during their lives undergo numerous diagnostic and medical procedures, which often require

numerous venipuncture, which is a proven risk factor for stenosis and consequential dysfunction of arteriovenous fistula (1, 16). The current data identifies risk factors (age, diabetes mellitus, smoking, peripheral vascular disease, predialysis hypotension, characteristics of blood vessels, obesity, use of adjuvant therapy) (17), but there is no data on the impact of gender and type of anastomosis, as a predictive parameter functioning arteriovenous fistula, except in terms of increased incidence of steal syndrome in older people with termino-terminal anastomosis (18).

CONCLUSION

Predictive parameter for duration of arteriovenous fistulas for hemodialysis in patients of older age, in our study, is proximal location of the arteriovenous anastomoses.

ACKNOWLEDGEMENTS

This research is partly supported by the Ministry of Education and Science of Serbia, Grants III41010.

CONFLICT OF INTEREST

The authors declare no financial or other conflict of interests regarding this paper.

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CIRCULATING IL-10 LEVELS IN CAROTID ARTERY DISEASE

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CIRKULIŠUĆI IL-10 NIVOI U BOLESTI ARTERIJE KAROTIDA

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Received / Primljen: 03. 07. 2017. Accepted / Prihvaćen: 16. 07. 2017.

ABSTRACT

Carotid atherosclerosis may be associated with neurosymptoms including cerebral infarction. IL-10 exerts atheroprotective effects, but its role in carotid disease is not fully defined. We aimed to investigate serum IL-10 levels in patients undergoing endarterectomy and their relation to the degree of carotid stenosis, plaque types and neurosymptoms.

Two hundred consecutive patients with atherosclerotic carotid stenosis and 29 healthy controls were enrolled in this study. Plaque types were classified according to AHA criteria. Serum IL-10 levels were determined by ELISA.

Patients undergoing endarterectomy had significantly higher circulating IL-10 levels (18.7 ± 3.2 pg/ml) in comparison with healthy controls (7.2 ± 1.8pg/ml; P=0.0001) and IL-10 has good discriminatory efficacy between these two groups (ROC curve, AUC = 0.723, P=0.0001). Patients with < 70% and those with > 70% of carotid stenosis did not differ in terms of age, sex, cardiovascular risk factors except hypertension, neurosymptoms and AHA plaque types. Circulating IL-10 levels differed significantly among patients with different carotid plaque types (P = 0.002). Patients with uncomplicated plaques had significantly higher serum levels of IL-10 (23.0 ± 6.1 pg/ml) compared to those with complicated plaques (13.0 ±1.4 pg/ml, P=0.035) and IL-10 can differentiate patients between these two groups (ROC curve, AUC = 0.413, P= 0.035).

Our findings reveal an important role for IL-10 in carotid atherosclerosis. IL-10 might be a potential biomarker in discriminating patients with carotid disease from healthy controls. Decreased serum levels of IL-10 are related to complicated carotid plaques.

Keywords: *carotid disease, atherosclerotic plaque, cytokines, IL-10*

SAŽETAK

Karotidna bolest se manifestuje aterosklerotskim suženjem karotidnih arterija i može biti udružena sa neurosimptomima. IL-10 ispoljava atero-protektivna dejstva, ali njegova uloga u karotidnoj bolesti nije u potpunosti ispitana. Cilj studije je bio ispitivanje nivoa IL-10 u serumu bolesnika podvrgnutih endarterektomiji sa različitim tipovima karotidnih plakova, stepenima karotidne stenoze i neurosimptomima.

U studiju je bilo uključeno 200 bolesnika sa aterosklerotskom stenozom karotidne arterije i 29 zdravih osoba. Aterosklerotski plakovi su klasifikovani prema AHA kriterijumima. Serumske koncentracije IL-10 su određivane ELISA metodom.

Bolesnici podvrgnuti endarterektomiji su imali značajno više serumske vrednosti IL-10 (18.7 ± 3.2 pg/ml) u poređenju sa zdravim kontrolama (7.2 ± 1.8pg/ml; P=0.0001) i serumski IL-10 može da diskriminiše zdrave osobe i bolesnike sa karotidnom bolesti (ROC kriva, AUC = 0.723, P=0.0001). Bolesnici sa stepenom stenoze karotidne arterije <70% i >70% se nisu razlikovali u odnosu na pol, starost, kardiovaskularne rizikofaktore izuzev hipertenzije, neurosimptome i tip plaka. Serumski IL-10 se značajno razlikuje između bolesnika sa različitim tipovima aterosklerotskog plaka (P=0.002). Bolesnici sa nekomplikovanim plakom su imali značajno više vrednosti serumskog IL-10 (23.0 ±6.1 pg/ml) u odnosu na bolesnike sa komplikovanim plakom (13.0 ±1.4 pg/ml, P=0.035). Cirkulišući IL-10 može da diferencira bolesnike sa komplikovanim plakovima od onih sa nekomplikovanim plakom (ROC kriva; AUC = 0.413, P=0.035).

Dobijeni rezultati pokazuju značajnu ulogu IL-10 u aterosklerotskoj karotidnoj bolesti. Serumski IL-10 može biti potencijalni biomarker za razlikovanje bolesnika sa karotidnom bolešću od zdravih osoba. Niži nivoi IL-10 su povezani sa prisustvom komplikovanih plakova.

Ključne reči: *karotidna bolest, aterosklerotski plak, citokini, IL-10*

sciendo
UDK: 616.133-004.6:577.12
Ser J Exp Clin Res 2019; 20 (1): 53-63
DOI: 10.1515/SJECR-2017-0040

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ABBREVIATIONS

AHA – American Heart Association
CAD – coronary artery disease
CEA – carotid endarterectomy
ICA – internal carotid artery
IL – Interleukin
IFN-γ – Interferon - gamma
MDCT - multi-detector computerized tomography
PAD – peripheral artery disease
ROC – Receiver's operating characteristic curve
TIA – transient ischemic attacks
TNF-α – tumor necrosis factor-alpha

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease of arterial blood vessels leading to complications including stroke and myocardial infarction (1). Atherosclerosis is systemic inflammatory disease that is initiated and regulated by cells of the immune system. Plaques are formed when lipoproteins are deposited at specific sites of the arterial vessel walls through intimal inflammation, necrosis, fibrosis, and calcification (2). Carotid plaques may cause narrowing of blood vessel lumen or they may rupture causing thrombosis and life threatening clinical disease. Carotid disease is typically manifested as atherosclerotic stenosis and may be associated with neurological complications including amaurosis fugax, transient ischemic attacks (TIAs) and cerebral infarction. Plaques prone to rupture have thin fibrous cap and are classified as unstable or vulnerable plaques, while those with thick fibrous cap, less inflammation and small lipid and necrotic core are stable plaques. Complicated plaques are defined when they have features including intraplaque calcification, hemorrhage, thrombi or plaque erosion.

The precise molecular and cellular mechanisms underlying carotid plaque formation are incompletely defined. Intraplaque expression of cytokines or their circulating levels are associated with vascular events and the stage of atherosclerotic disease (3). Circulating levels of inflammatory biomarkers, including high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6, tumor necrosis factor-α (TNF-α) or interleukin (IL)-18 are predictive for vascular events (4-7), carotid plaque vulnerability and higher intima-media thickness (8, 9). In contrast, elevated serum levels of the anti-inflammatory cytokine interleukin (IL)-10 and Th2 cytokine interleukin (IL)-5 are associated with a favorable prognosis of atherosclerotic disease (10, 11).

IL-10, a type II cytokine, was initially described by Mosmann, Fiorentino and Bond in 1989, as a cytokine that inhibits synthesis and excretion of proinflammatory cytokines by Th1 lymphocytes (12). Its main biological action is inhibition of presentation of antigens by dendritic cells and macrophages to Th1 cells, through inhibition of MHC class II expression, as well as costimulatory molecules expression (13-16). In this way antigen presenting cells are deprived of stimuli to synthesize and excrete Th1 associated cytokines (IL-2, IFN-γ). IL-10 also inhibits synthesis of proinflammatory cytokines by macrophages and dendritic cells including IL-1, IL-6, IL-12, and TNFα. Furthermore, it inhibits inflammation by increasing the release of IL-1 receptor antagonist by macrophages (17-19). However, not all effects of IL-10 are immunosuppressive as IL-10 costimulates B-cell activation, influences immunoglobulin class-type switching, and prolongs survival of B-cells (20).

Most experimental data confirms atheroprotective role of IL-10 in animal models of atherosclerosis (21-25). The results obtained in experimental animal models, where atherosclerosis is result of certain experimental conditions may be opposing to the studies emerging from patients with atherosclerosis that can be influenced by various genetic and environmental factors.

We investigated serum levels of IL-10 in 199 patients submitted to elective carotid endarterectomy. Patients were grouped according to the degree of carotid stenosis, determined by color duplex scan of carotid arteries, those with carotid stenosis less or equal to 70%, and those with atherosclerotic plaques that narrowed carotid arteries more than 70%. We analyzed the association of circulating IL-10 levels with the grade of carotid stenosis, histologic features of carotid plaques and neurosymptoms.

PATIENTS AND METHODS

Study population

We enrolled 200 consecutive surgical patients with atherosclerotic carotid stenosis, admitted to IKVB Dedinje for carotid endarterectomy. Color Doppler scan and MDCT (multi-detector computerized tomography) were performed on each patient, to obtain information on a degree of carotid stenosis. We used North American Symptomatic Carotid Endarterectomy Trial criteria to define the level of stenosis of carotid artery. The patients selected for this operation were either symptomatic, or asymptomatic. Symptomatic patients, 99 of entire group, had in their previous medical history some form of neurological incident, weather as transient ischemic attack (TIA), amaurosis fugax (AFX) or stroke, regardless of the degree of stenosis at the time of surgery.

Blood samples were obtained from 200 patients who underwent surgery, prior to the surgery. We also collected blood from 29 healthy volunteers.

Blood samples were collected from patients by a single needle stick. Sera were separated, aliquoted and stored at –20°C until assayed.

Histopathologic analysis of carotid atherosclerotic plaques

Atherosclerotic plaques removed from patient's carot - id artery were immediately frozen, and then submitted for further histological examination. The frozen samples were cut into sections of 5 μm thicknesses and stained using a hematoxylin eosin staining by standard procedure (26). After the staining all sections were examined using the Olympus BX41 microscope. The grade of atherosclerosis in observed plaques was established according to the classification of the American Heart Association Committee on Vascular Lesions of the Council of Atherosclerosis (27).

The evaluation of plaques stability was performed according to the criteria as previously described (28, 29). Stable plaques (fibrous plaques and fibroatheroma) had low lipid content, thick fibrous cap, low level of inflammation and absence of plaque complications. Briefly, all

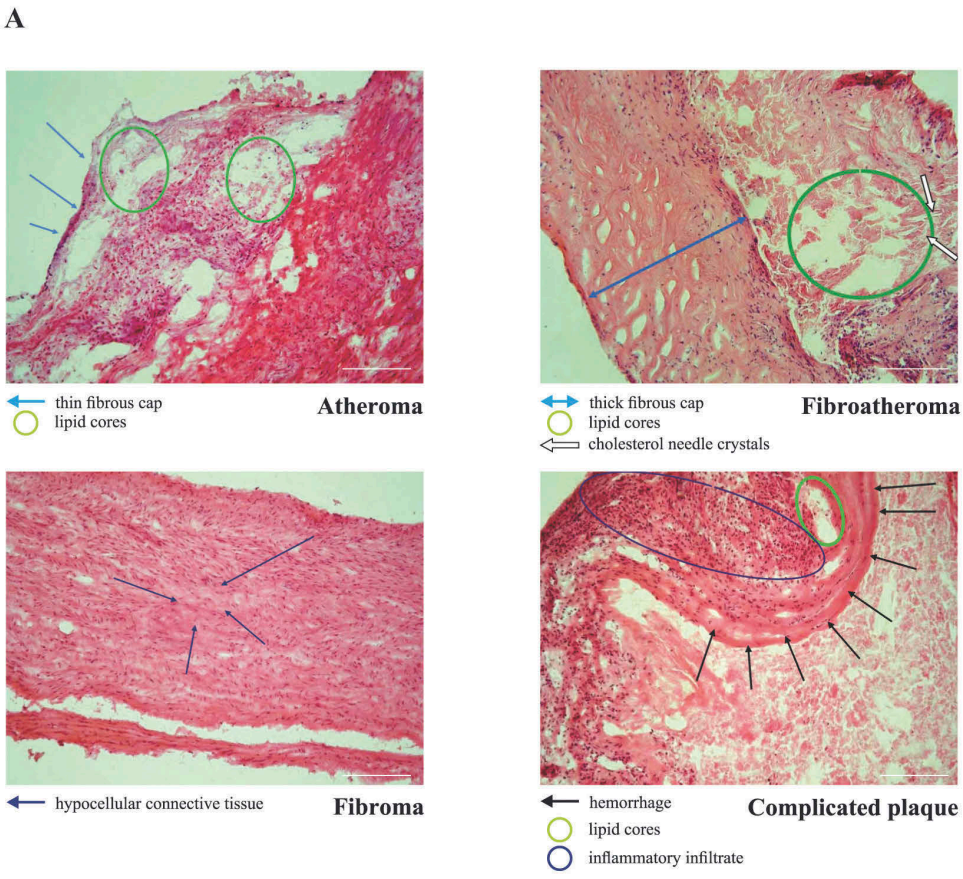


Figure 1. AHA classification of carotid plaques in studied population

A. Representative images of carotid plaque types
B. Proportions of patients with carotid disease with different types of plaques according to AHA criteria

Table 1. Demographic, clinical and histological characteristics of patients undergoing endarterectomy

Cardiovascular risk factors and plaque histology[a]	*Carotid stenosis ≤70% n=18 (9.3%)*[c]	*Carotid stenosis >70% n=176 (90.7 %)*[c]	*P*
Age	66.3 ± 6.8	66.4 ± 8.0	0.994
Gender			
Male	12 (11.2)	95 (88.8)	0.303
Female	6 (6.9)	81 (93.1)	
Diabetes mellitus			
Yes	5 (7.8)	59 (92.2)	0.566
No	13 (10.4)	112 (89.6)	
Smoking			
Yes	15 (10.6)	127 (89.4)	0.419
No	3 (6.2)	45 (93.8)	
Hypertension			
Yes	14 (7.7)	168 (92.3)	0.003[b]
No	4 (50.0)	4 (50.0)	
Neurosymptoms			
Yes	109 (98.2)	2 (1.8)	0.097
No	85 (96.6)	3 (3.4)	
Triglycerides			
mmol/L, (SD)	1.42 ± 0.46	1.54 ± 0.55	0.400
Creatinine			
mmol/L, (SD)	113.63 ± 87.95	83.85 ± 18.57	0.273
PAD			
Present	4 (16.0)	21 (84.0)	0.259
Absent	14 (8.3)	155 (91.7)	
CAD			
Present	5 (6.2)	76 (93.8)	0.207
Absent	13 (11.5)	100 (88.5)	
Intraplaque inflammatory infiltration			
Present	1 (14.3)	6 (85.7)	1.000
Absent	17 (9.1)	170 (90.9)	
Intraplaque calcification			
Present	7 (9.7)	65 (90.3)	0.870
Absent	11 (9.0)	111 (91.0)	
Intraplaque hemorrhage			
Present	2 (12.5)	14 (87.5)	0.648
Absent	16 (9.0)	162 (91.0)	
Rupture of plaque			
Present	4 (7.1)	52 (92.9)	0.514
Absent	14 (10.1)	124 (89.9)	
AHA classification of carotid plaques			
IV	0 (0.0)	13 (100.0)	
Va	3 (15)	17 (85)	
Vb	3 (10.3)	26 (89.7)	0.737
Vc	7 (10.9)	57 (89.1)	
VIa	3 (5.9)	48 (94.1)	
VIb	2 (13.3)	13 (86.7)	
VIc	0 (0.0)	2 (100.0)	

PAD, peripheral artery disease; CAD, coronary artery disease

[a] Values are expressed as mean ± SE or absolute numbers with calculated percentage

[b] Statistically significant (Chi-Square, Independent-Samples T, Mann-Whitney U and Kruskal-Wallis H tests; $P < 0.05$)

[c] Data for six patients on the degree of stenosis were not available

other plaques contained large, lipid-rich necrotic core with a thin and inflamed cap (< 65 μm of thickness) and other features such as expansive remodeling, plaque hemorrhage, neovascularization, adventitial inflammation, and "spotty" calcifications. The evaluation of plaque stability was done by two independent investigators in a blinded fashion (S.M., I.T.).

Carotid plaques were classified as complicated (fibroatheroma with calcifications Vb, ruptured atheroma with calcifications VIa, fibroatheroma with hemorrhage VIb, ruptured atheroma with thrombus VIc) or uncomplicated plaques (atheroma IV, fibroatheroma Va and fibrotic plaques Vc).

Quantification of IL-10 in sera

Cytokine levels were measured using highly sensitive enzyme-linked immunosorbent assay (ELISA) kits (R&D

Systems Minneapolis, MN) specific for the human IL-10 according to the manufacturer's instruction. The standard stock was serially diluted in Reagent Diluent to generate seven points for the standard curves. After incubation with diluted sera and standards, 100 µl of the Detection Antibody was added to each well and incubated for 2 hours at room temperature followed by Streptavidin-HRP (100 µl). The incubation was terminated after 20 min at room temperature and 100 µl of Substrate Solution was added. Then, 50 µl of Stop Solution was added to each well, and the optical density of each well was immediately determined using a microplate reader. Absorbance was measured on ZENYTH 3100 apparatus, at 590nm, and obtained data was then processed by Software for Anthos Multimode Detectors. The results were expressed in pg/ml. (30).

Statistical analysis

Results are expressed as median and ranges (25 and 75 percentiles) or mean ± SE. Clinical and plaque histological features were compared between groups of patients with >70% or <70% of carotid artery stenosis by $\chi 2$ test. The Kruskal-Wallis and Mann–Whitney non-parametric tests or independent Student t-test were used to investigate the significance of differences between groups depending on the normality of the data.

Receiver's operating characteristic (ROC) curve was constructed to determine the discriminating efficacy of the circulating IL-10 between healthy individuals and patients with carotid disease and between patients with complicated and uncomplicated plaques.

A P value < 0.05 was considered statistically significant. All statistical calculations were performed with the IBM SPSS statistics version 20.

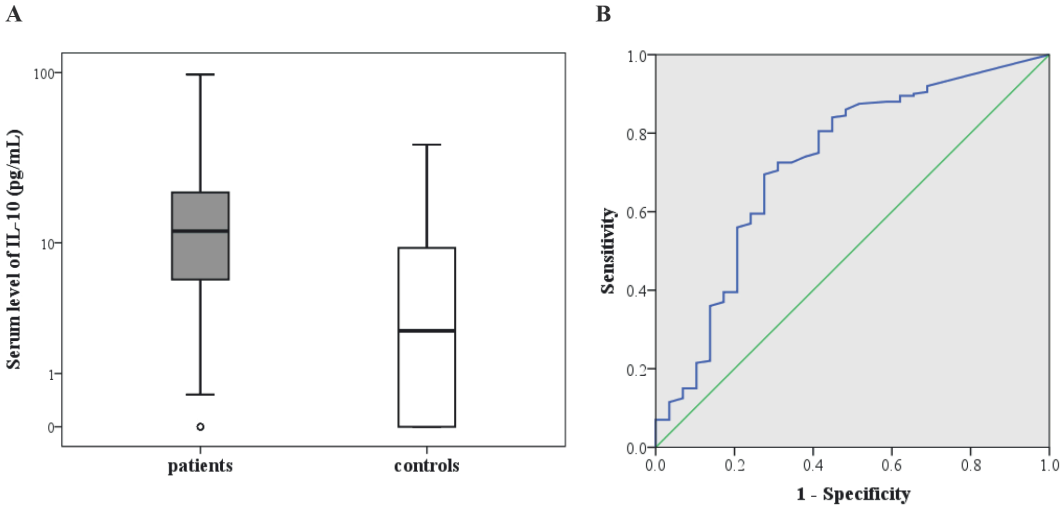
RESULTS

Demographic, clinical and histologic plaque characteristics of patients with carotid atherosclerosis. Serum levels of IL-10 are increased in patients undergoing endarterectomy

This study of the 200 patients that underwent carotid endarterectomy was composed of 110 males (55%) and 90 females (45%), ranging from 43 to 83 years old (mean 66.5 ± 7.9). Our data show that average serum IL-10 level in healthy subjects was 7.2 ± 1.8 pg/ml. There was a significantly higher serum level of IL-10 in patients with carotid atherosclerosis compared with healthy subjects (Fig 1A). Patients undergoing endarterectomy had substantially higher average level of IL-10 (18.7 ± 3.2 pg/ml; P=0.001) in comparison to healthy subjects. The ROC curve showed that serum IL-10 levels exhibited a good discriminatory efficacy between healthy individuals and patients with carotid disease (AUC = 0.723, P = 0.0001) (Fig 1B).

The patients were stratified into two groups based on the degree of carotid stenosis: those with less or equal to 70% or greater than 70% of stenosis as shown in Table 1. There were no significant differences between the two groups of patients in terms of age, sex, cardiovascular risk factors: dyslipidemia, smoking status, and diabetes, presence of peripheral artery disease (PAD), coronary artery disease (CAD) or neurosymptoms, but there was a significantly higher number of patients with hypertension with carotid stenosis greater than 70%.

Based on carotid plaque histological characteristics, patients were classified into groups IV, V and VI according to AHA criteria. Out of 200 analyzed carotid plaques, 13 were atherosclerotic plaques in the stage of atheroma (type IV lesion), 20 were at the stage of fibroatheroma (type Va), 30



Area Under the Curve

Area	Std. Errora	Asymptotic Sig.b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.723	.055	**.000**	.615	.831

Figure 2. Serum levels of IL-10 in patients with carotid disease
A. Serum levels of IL-10 are increased in patients undergoing endarterectomy
B. ROC curve for IL-10 in healthy individuals versus patients with carotid atherosclerosis

Table 2. Serum levels of Interleukin-10: Comparison between patients with different clinical features

Variable [a]	*Serum level of IL-10 (pg/ml) n (%)*	*P*
Age		
≤ 65	12.4 (7.2 – 23.2)	
	92 (47.4)	.627
> 65	10.8 (4.6 – 16.6)	
	102 (52.6)	
Gender		
Male	12.3 (7.3–22.2)	
	109 (54.8)	.520
Female	11.0 (3.9 – 17.1)	
	90 (45.2)	
Smoking		
Yes	11.3 (5.6 – 20.19)	
	146 (74.9)	.701
No	13.2 (6.3 – 20.4)	
	49 (25.1)	
Diabetes Mellitus		
Yes	11.0 (4.0 – 22.8)	
	66 (34.0)	.551
No	12.0 (6.4 – 19.8)	
	128 (66.0)	
Hypertension		
Yes	11.6 (5.7 – 20.2)	
	187 (95.9)	.658
No	12.5 (8.5 – 29.7)	
	8 (4.1)	
Degree of carotid stenosis		
≤ 70%	9.9 (6.2 – 18.6)	
	18 (9.2)	.839
> 70%	12.3 (5.7 – 21.4)	
	176 (90.8)	
CAD		
Present	15.7 (7.9 – 25.6)	
	85 (42.7)	.031[b]
Absent	9.4 (4.8 – 15.5)	
	114 (57.3)	
PAD		
Present	9.8 (4.2 – 16.7)	
	26 (13.1)	.524
Absent	12.2 (6.0 – 16.7)	
	173 (86.9)	
Neurosymptoms		
Yes	11.0 (5.4 – 17.5)	
	111 (55.8)	.918
No	13.4 (6.1 – 23.7)	
	88 (44.2)	

PAD, peripheral artery disease; CAD, coronary artery disease;
[a] Values are presented as median and range/25th – 75th percentiles or absolute numbers with calculated percentage
[b] Statistically significant (Mann-Whitney U test; P < 0.05).

were at the stage of fibroatheroma with calcifications (Vb lesion), 67 were fibrous plaques (type Vc lesion), 51 were at the stage of ruptured atheroma with calcifications (type VIa) and 16 plaques were atheroma complicated by previous rupture, thrombosis or hemorrhage (type VIb lesion) and 3 plaques were VIc (Fig 2B). Table 1 shows morphological plaque features and analyzes revealed no significant differences between the two groups in terms of plaque inflammatory infiltration, intraplaque calcification, hemorrhage and rupture and carotid plaque types. Representative images of carotid plaque types are shown in Fig 2A.

Serum levels of Interleukin-10 in patients with different clinical features

There were no significant differences in serum levels of IL-10 in patients in terms of age, sex, cardiovascular risk factors: hypertension, dyslipidemia, smoking status and diabetes, and the degree of carotid stenosis or neurosymptoms. Patients with CAD had significantly higher serum levels of IL-10 in comparison to patients without CAD (25.8 ± 7.1 pg/ml vs. 12.1 ±1.2, *P* = .031), while patients with PAD has similar serum levels of IL-10 compared to those without PAD. These results are summarized in Table 2.

Serum levels of IL-10 are related to histological features of carotid plaques

There were no significant differences in serum levels of IL-10 in patients in terms of the presence of intraplaque inflammation, hemorrhage, rupture or calcification (Table 3). There was a significant difference in serum levels of IL-10 among patients with different types of carotid plaques according to AHA criteria as shown in Fig 3. When serum IL-10 level was analyzed in relation to histological features of the carotid plaques the highest level of IL-10 was found in the group of patients with fibrotic plaques (26.8 ± 9.0 pg/ml), followed by those with fibroatheromatous plaques (18.0 ± 3.5 pg/ml). Patients with fibroatheroma complicated with hemorrhage had serum level of IL-10 of 15.1 ± 2.4 pg/ml; patients with ruptured atheroma with calcifications 13.1 ± 1.6 pg/ml, patients with fibroatheroma with calcifications 11.5 ± 3.3 pg/ml and the lowest serum level of IL-10 was detected in the group of patients with atheroma complicated by previous thrombosis (10.3 ± 2.3 pg/ml) and

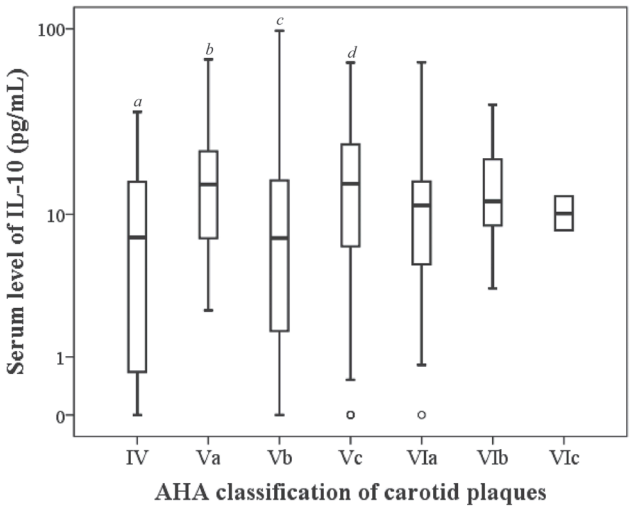


Figure 3. Circulating IL-10 levels depend on carotid plaque type
A. Serum IL-10 significantly differ among patients with different types of carotid plaques (AHA classification)); [a] type IV vs. Vc *P* = .081; [b] type Va vs. Vb *P* = .014; [c] type Vb vs. Vc *P* = .004; [c] type Vb vs. VIb *P* = .035; [d] type Vc vs. VIa *P* = .068

atheroma (10.9 ± 3.2 pg/ml). The results of the circulating IL-10 levels between patients with different types of carotid plaques are summarized in Table 3.

Next, we analyzed the difference in serum IL-10 levels in patients with stable and unstable carotid plaques. Patients were divided according to the presence of stable carotid plaques (fibrous plaques and fibroatheroma) (87 patients; 43.5%) and those with unstable carotid plaques (atheroma, atheroma and fibroatheroma with complications including calcification, hemorrhage, rupture or thrombosis (113 patients; 56.5%)). Serum level of IL-10 was significantly higher in patients with stable plaques compared to those with signs of plaque instability (24.8 ± 7.0 vs. 14.0 ± 1.8 pg/ml; $P = 0.005$).

One of the clinical criteria for performing elective carotid artery surgery is stenosis of internal carotid artery (ICA) greater than 70%. The group of patients with <70% stenosis had mean IL-10 serum level 16.2 ± 5.07 pg/ml, while patients with >70% stenosis had mean IL-10 level 18.30 ± 3.59 pg/ml, without statistically significant difference between groups (P > 0.05).

Patients undergoing endarterectomy were grouped in relation to the presence or absence of neurological symptomatology prior to surgery. Patients with neurosymptoms had mean serum IL-10 level of 18.30± 5.50 pg/ml, while patients without previous neurological symptomatology had mean serum IL-10 level of 17.62 ±1.77 pg/ml. Again, no statistically significant difference was observed (P > 0.05).

Circulating IL-10 differentiate patients with complicated plaques from patients with uncomplicated plaques

Patients were divided in two groups based on the histological characteristics of plaques. Group of patients with any form of complication in the plaque itself, like the rupture of the plaque, hemorrhage in the plaque, recanalization of the plaque or the thrombosis of the plaque, and also presence of large lipid core with thin fibrous cap or calcification and patients whose plaques were without aforementioned features.

Serum IL-10 concentrations were significantly lower in patients with complicated plaques as compared to the patients with uncomplicated plaques (13.03 ±1.36 pg/ml vs. 23.04 ±6.15 pg/ml; $P = .035$) (Fig 4A). The ROC curve for serum IL-10 levels showed a significant discriminatory efficacy between patients with complicated plaques from patients with uncomplicated plaques (AUC = 0.413, $P = 0.035$) (Fig 4B).

Table 3. Serum levels of Interleukin-10: Comparison between patients with different histological features of carotid plaques

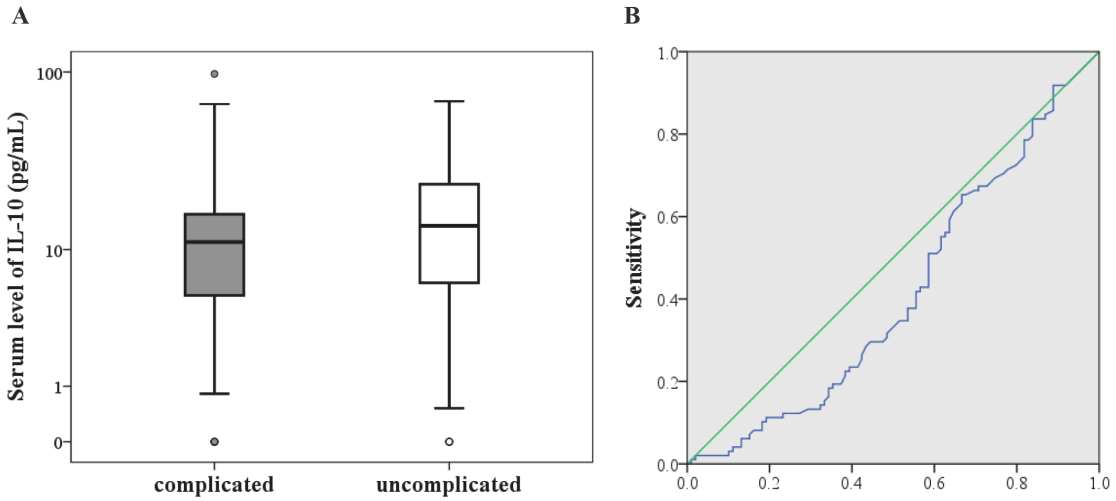
Carotid plaque histology[a]	*Serum level of IL-10 (pg/ml) n (%)*	*P*
Intraplaque inflammatory infiltration		
Present	21.4 (13.8 – 26.8)	
	7 (3.9)	.640
Absent	(8.0 – 70.2)	
	192 (96.1)	
Intraplaque calcification		
Present	11.1 (3.8 – 15.7)	
	73 (36.7)	.189
Absent	13.0 (6.6 – 23.4)	
	126 (63.3)	
Intraplaque hemorrhage		
Present	13.0 (8.6 – 21.8)	
	17 (8.5)	.808
Absent	11.6 (5.4 – 20.0)	
	182 (91.5)	
Rupture of plaque		
Present	11.1 (6.5 – 15.5)	
	56 (28.1)	.351
Absent	12.5 (5.7 – 23.0)	
	143 (71.9)	
AHA classification of carotid plaques		
IV	7.3 (0.3 – 18.0)	
	13 (6.5)	
Va	14.7 (7.3 – 23.00)	
	20 (10.0)	
Vb	7.3 (1.3 – 15.6)	
	30 (15)	
Vc	14.9 (6.4 – 24.6)	.041[b]
	67 (33.7)	
VIa	11.3 (4.8 – 15.7)	
	51 (25.5)	
VIb	11.9 (8.5 – 21.9)	
	16 (8.0)	
VIc	10.4 (8.1 – 12.6)	
	3 (1.5)	

[a] Values are presented as median and range/25th – 75th percentiles or absolute numbers with calculated percentage

[b] Statistically significant (Mann-Whitney U and Kruskal-Wallis H tests; P < 0.05).

DISCUSSION

The results obtained in this study imply that IL-10 may have substantial influence on the evolution of atherosclerotic process in carotid arteries. That is, our work shows higher levels of circulating IL-10 in the group of patients with carotid disease in comparison to healthy subjects. Recent papers show association between reduced serum IL-10 levels and the risk of cerebral infarction (31). Study of Ambrosius W. et al (32) show lower levels of IL-10 in patients which had higher values of carotid intima-media thickness (IMT), which is an ultrasound marker of the level of atherosclerosis. Our results are not in agreement with these studies. In our study patients who underwent surgical repair of carotid artery had significantly higher serum levels of IL-10 when compared to control subjects. Our findings are in line with recent evidence of higher levels of IL-6 and IL-10 in patients with progressive carotid sclerosis (33). Moreover, in this study IL-6 and IL-10 were found to be independent variables for the unfavorable dynamics of changes in the morphology of atherosclerotic carotid plaques. Atherosclerotic plaques may be influenced by alterations in systemic levels of circulating cytokines including IL-10. We can conclude that, according to our data,



Area Under the Curve

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.413	.041	**.035**	.333	.493

Figure 4. Serum levels of IL-10 in patients with complicated and uncomplicated plaques

A. Serum levels of IL-10 are increased in patients with uncomplicated plaques

B. ROC curve for serum IL-10 in patients with complicated and uncomplicated plaques

higher circulating levels of IL-10 do not reflect atheroprotective action in carotid artery disease.

Our results show that stage of carotid artery disease and structure of plaque does depend on the circulating level of IL-10. Our paper shows that patients with stable plaques enriched with fibrous tissue, like fibroma and fibroatheroma (type Va and Vc lesions) had significantly higher levels of circulating IL-10 than patients who had unstable plaques. This may be the reflection of the propensity of IL-10 to induce Th2 immune response in local milieu, and to drive tissue macrophages towards type 2 inflammatory response, and stimulation of tissue repair and activation of fibroblasts. This would potentially lead to beneficial outcome of atherosclerotic process since these types of plaques are less prone to rupture, then vulnerable plaques. They, however, do cause narrowing of blood vessel diameter, and in that way compromise inflow of blood to the brain. In the work of Pinderski et al, (22), decrease in accumulation of lipids in experimental model of atherosclerosis was observed, regardless of circulating levels of IL-10. Possible mechanisms might be imbalance between pro-inflammatory and anti-inflammatory cytokines.

Halvorsen et al (35) show, however, that IL-10 stimulates lipid accumulation in oxLDL treated macrophages and inhibits their apoptosis. When apoptosis is inhibited, there are less free cholesterol crystals and fatty acids which could potentially induce inflammatory response in local environment. When apoptosis is abundant, as shown in some recent papers, atherosclerotic plaque becomes more susceptible to rupture (36-38). In this way, as authors explain, IL-10 diminishes the size of necrotic core of atherosclerotic plaque. This may stabilize the plaque itself, making it less prone to rupture. It has been shown that IL-10 modulates lipid metabolism in macrophages, both by stimulating cholesterol uptake and efflux from cells (39). There are reports showing that efficient efflux of cholesterol is mostly confined to M2 macrophages, induced in Th2 immune response (40, 41). Our work also concurs with these findings, since we found higher levels of IL-10 in patients with uncomplicated plaques. We now may conclude that higher levels of IL-10 are related to plaque structure less prone to rupture, and, in that way, this cytokine may exert atheroprotective activity.

As mentioned above, numerous experimental data show atheroprotective role of IL-10 in highly controlled environment and in knock-out animals lacking IL-10, where it exerts unquestionable favorable influence on atherosclerotic process both through inhibition of inflammation, and by stimulation of restorative action of fibroblasts (21, 23-25). However, there are recent papers showing no benefit of IL-10 in slowing down atherosclerotic process (42) in different animal models, the findings that reflect multifactorial background in atherogenesis. Also, experimental results cannot be simply translated to human pathology. This is shown in our findings that levels of IL-10 are significantly higher in patients with coronary artery disease, but not in patients with peripheral artery disease. It is well known that myocardial infarction and angina pectoris are accompanied by various alterations in levels of pro- and anti-inflammatory cytokines (43, 44). This may be due to the fact that myocardial disease may provoke systemic inflammatory response, as seen in severe bacterial infections, only without microbiological stimuli (45, 46). This type of systemic inflammation may sometimes occur in severe cerebral infarction, but never occurs during the development of atherosclerotic lesion.

Moreover, it is well established that prognosis of atherosclerosis outcome relates more to the structure of atherosclerotic lesion, its cellular content and level of inflammation within the plaque (47). These characteristics determine whether undesirable event may occur, with possible fatal consequences, disabilities, prolonged hospitalization, and need for various medical interventions. Degree of artery stenosis remains one of stable indications for medical interventions, since narrowing of blood vessel occurs independently from plaque structure, and different types of plaques may cause the same blood flow impairment if there is similar degree of stenosis.

All these facts imply that process of atherosclerosis may be influenced by altering immune reactions which could possibly lead to preferred outcome, stabilization of atherosclerotic lesion and reduction of its size. There are pioneer projects towards this aim, and results need to be evaluated only after longtime follow-up (48).

This work clearly shows that differences in IL-10 serum levels reflect differences in types of atherosclerotic lesions, rather than size of the lesion. We have also shown that different clinical parameters do not relate to the degree of stenosis or type of atherosclerotic lesion. According to our findings, IL-10 could be employed in atheroprotective manner, as the highest serum IL-10 levels were found in patients with uncomplicated plaques.

Atherosclerotic process results from numerous environmental and intrinsic factors, and represents the dynamical and complex process which pathogenesis is only partly defined. Investigating the roles of cytokines in the development, growth and destabilization of atherosclerotic lesions is necessary in search of drugs that by affecting the concentration of certain cytokines may be important for inhibition of atherosclerosis progression.

ACKNOWLEDGEMENT

The authors thank Irena Tanaskovic (Faculty of medical sciences, University of Kragujevac) for help in histopathologic analysis of carotid plaques. The authors also thank Nikola Jankovic (Faculty of medical sciences, University of Kragujevac) for help in statistical analyses and Aleksandar Ilic (Faculty of medical sciences, University of Kragujevac) for technical assistance. We thank Vera Maravic-Stojkovic for help in collecting the samples. We thank Miljan Nedeljkovic for excellent technical assistance.

This work was supported by grants from the Serbian Ministry of Science and Technological Development (ON175103 and ON175069), Serbia and Faculty of Medical Sciences, University of Kragujevac, Serbia (Grant no. JP 07-14).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SHORT TITLE:

IL-10 in carotid atherosclerosis

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THE EVALUATION OF THE EFFECTS OF N-ACETYLCYSTEINE ON CISPLATIN-INDUCED ALTERATIONS IN EXPLORATORY ACTIVITY IN ELEVATED PLUS MAZE TEST IN RATS

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ISPITIVANJE EFEKATA N-ACETILCISTEINA NA PROMENE EKSPLORATIVNE AKTIVNOSTI U TESTU UZDIGNUTOG KRSTASTOG LAVIRINTA IZAZVANE CISPLATINOM KOD PACOVA

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Received / Primljen: 13. 10. 2017. Accepted / Prihvaćen: 16. 10. 2017.

ABSTRACT

The aim of this study was to evaluate the potential beneficial effect of N-acetylcysteine (NAC) on cisplatin-induced alterations in anxiety levels in rats, by means of parameters of the exploratory activity obtained in the elevated plus maze (EPM) test. Animals were divided into four groups: control group, cisplatin group (7.5 mg/kg/weekly of cisplatin), N-acetylcysteine group (500 mg/kg/weekly of NAC), and cisplatin plus N-acetylcysteine group (7.5 mg/kg/weekly of cisplatin, and 500 mg/kg/weekly of NAC). After two weeks of treatment, exploratory activity (estimated by means of the number of rearings, head-dippings and the number of total exploratory activity episodes) was significantly reduced in cisplatin group comparing to control values. Although NAC induced no alterations in exploratory activity when applied alone, simultaneous administration with cisplatin resulted in significant attenuation of cisplatin-induced decline in exploratory activity. The exploratory activity gradually decreased in time-dependent manner during five minutes of EPM test in all groups. The results of this study confirmed clear beneficial effect of NAC supplementation against cisplatin-induced neurotoxicity in rats. Antioxidative properties of NAC were manifested through restoration of exploratory activity, confirming that NAC administration can attenuate anxiogenic effect of cisplatin therapy. Those results could recommend NAC supplementation as a potential protection against cisplatin-induced neurotoxicity.

Keywords: *exploratory activity, cisplatin, N-acetylcysteine, neurotoxicity, rat*

SAŽETAK

Cilj ovog istraživanja je bio ispitivanje potencijalno korisnog efekta N-acetilcisteina (NAC) na promene nivoa anksioznosti izazvane primenom cisplatine kod pacova, kroz parametre eksplorativne aktivnosti u uzdignutom krstastom lavirintu. Životinje su bile podeljene u četiri grupe: kontrolna grupa, cisplatina grupa (7.5 mg/kg/nedeljno cisplatine), N-acetilcistein grupa (500 mg/kg/ nedeljno NAC-a) i kombinovana grupa (7.5 mg/kg/nedeljno of cisplatin i 500 mg/kg/ nedeljno NAC-a). Nakon dvonedeljnog tretmana, eksplorativna aktivnost (ispitivana kroz broj uspravljanja, naginjanja i broj epizoda ukupne eksplorativne aktivnosti) u cisplatina grupi je bila značajno smanjena u poređenju sa kontrolnim vrednostima. Iako samostalna primena NAC-a nije izazvala promene eksplorativne aktivnosti, simultana primena sa cisplatinom je uzrokovala značajno ublažavanje smanjenja eksplorativne aktivnosti izazvanog cisplatinom. Eksplorativna aktivnost se postepeno smanjivala u funkciji vremena u svim grupama tokom petominutnog izvođenja testa uzdignutog krstastog lavirinta. Rezultati ove studiji jasno potvrđuju da suplementacija NAC-om može biti korisna kod neurotoksičnosti izazvane cisplatinom kod pacova. Antioksidantna svojstva NAC-a su se ispoljavala kao poboljšanje eksplorativne aktivnosti, što je potvrdilo da primena NAC-a može umanjiti anksiogeni efekat terapije cisplatinom. Ovi rezultati ukazuju da suplementacija NAC-om može biti potencijalna zaštita od neurotoksičnosti izazvane cisplatinom.

Ključne reči: *ekplorativna aktivnost, cisplatina, N-acetilcistein, neurotoksičnost, pacov*

ABBREVIATIONS

NAC - N-acetylcysteine
EPM - elevated plus maze
TEA - total exploratory activity
ROS - reactive oxygen species

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UDK: 615.277.065; 547.466
Ser J Exp Clin Res 2019; 20 (1): 65-72
DOI: 10.1515SJECR-2017-0053

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INTRODUCTION

Cisplatin is anticancer drug which is widely used in the therapy of various types of malignancies. More than 40 years used as primary drug in the treatment of ovarian and testicular cancer, cisplatin also has been shown very effective in the treatment of other tumors, such as sarcoma, microcellular lung cancer, lymphoma, urinary bladder cancer, and cervical cancer. Great antitumor potential of cisplatin is based on its ability to form complexes with water molecules, replacing chlorine with water (aqua complexes), incorporating in DNA molecule and activating apoptosis (1).

Although it is considered as highly effective in treating tumors, cisplatin therapy is usually associated with various adverse effects such as: nephrotoxicity (2), hepatotoxicity (3), cardiotoxicity (4), hematotoxicity (5), nausea and vomiting (6), as well as neurotoxicity (7). In humans, neurotoxicity is usually manifested by peripheral nerves dysfunction and sensory disorders, seizures, reduced cognitive abilities and various mood disorders (8, 9). Studies performed on animal experimental models confirmed cisplatin-induced neurotoxicity by means of motor impairment (10), decline of cognitive functions (11), mood disorders (12) and alterations in nociception (13).

Adverse effects of cisplatin therapy are confirmed to be associated with increased oxidative damage (14). It has been reported that cisplatin treatment leads to enhanced free radicals production (15), reduced activity of antioxidant enzymes (16) and declined concentration of tissue glutathione (17) in various tissues. Since many adverse effects of cisplatin therapy seem to be accompanied with alterations in redox status, numerous antioxidants have been applied as supplementation during cisplatin therapy in order to diminish its toxicities. Among others, it has been reported that administration of vitamin C (18), vitamin E (19), selenium (20), ginger (21), and/or ellagic acid (22) can decrease the severity of cisplatin adverse effects. Although neurotoxicity is not considered as one of the most frequent side effect of cisplatin, antioxidant supplementation showed beneficial effects by means of reduction in severity of cisplatin-induced neuronal damage. It has been reported that cisplatin-induced neurotoxicity may be attenuated by numerous antioxidants, such as: ethoxyguin (23), Echinophora cinerea (24), and flavaglines (25).

Numerous papers reported increased anxiety levels obtained in an adequate behavioral tests in various species following cisplatin treatment (26, 27). Since exploratory activity is considered as reliable indicator for evaluation of the anxiogenic action (28), the aim of this study was to evaluate the potential beneficial effect of N-acetylcysteine (NAC, sulfur containing amino acid with antioxidant properties) on cisplatin-induced alterations in anxiety levels in rats, by means of parameters of the exploratory activity obtained in the elevated plus maze (EPM) test.

MATERIAL AND METHODS

Three months old male Wistar albino rats (weighting between 250-300 g, n=32) were used in this study. Animals were housed in groups of 3-4 per (polycarbonate) cage, under controlled environmental conditions of temperature (23±1 ℃) and light (12/12h light/dark cycle). Rats had *ad libitum* access to food and water. Animals were divided into four groups (8 animals in each group): control group, cisplatin group, N-acetylcysteine (NAC) group, cisplatin plus N-acetylcysteine group (cisplatin+NAC, i.e. combined group).

Cisplatin group received cisplatin (Bristol-Myers Squibb Co., USA, 7.5 mg/kg/weekly, intraperitoneally), while the rats from NAC group received NAC (Sigma–Aldrich, Germany, 500 mg/kg/weekly, intraperitoneally). Combined group was administered with cisplatin (7.5 mg/kg/weekly, intraperitoneally) and NAC (500 mg/kg/weekly, intraperitoneally). Control group received saline (intraperitoneally, approximately in the same volume as experimental groups). All protocols lasted for two weeks.

Two days after the protocols were finished, the rats were placed in a testing room for 1-2 h to accommodate before behavioral testing in EPM. During the behavioral testing, the maze was cleaned following the trial for each animal in order to remove possible interfering scents.

Apparatus used for testing was made of black wood, and consisted of two opposite open (50 x 20 cm) and two opposite enclosed arms (50 x 20 x 30 cm), extended horizontally at 90° from the central area (20 x 20 cm). EPM was elevated 100 cm from the floor. Each animal was placed in the centre of the EPM facing one of the open arms. The tests lasted five minutes. The testing was recorded by digital video camera, placed approximately 2.5 m above the elevated plus maze. Video files were analyzed and the following parameters were estimated: the number of rearings, the number of head-dippings and then the total exploratory activity (TEA) was calculated according to previously described procedure (29). Also, we evaluated the exploratory activity in EPM test in time–dependent manner i.e. by analyzing the parameters mentioned above in successive periods during the whole test (5 minute), and expressed the values for applied parameters in each consecutive (one minute) sequences.

Statistical analysis

The data were expressed as the means ± S.E.M. Parameters obtained in EPM test were initially submitted to Levene's test for homogeneity of variance and to Shapiro-Wilk test of normality. Comparisons between groups were performed using One-way ANOVA, followed by Fisher's least significant difference (LSD) multiple comparisons test. Comparisons within the groups (for TEA) were performed using unpaired Student t-test. Significance was determined at $p<0.05$. Statistically analysis was performed with SPSS version 20.0 statistical package (IBM SPSS Statistics 20).

All research procedures were carried out in accordance with European Directive for welfare of laboratory animals N° 86/609/EEC and principles of Good Laboratory Practice (GLP), approved by Ethical Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia.

RESULTS

As shown in Figure 1, applied protocols significantly altered the number of rearings in EPM test (df=3, F=4.135, 6.936, 16.880, 4.143 and 0.768 for five consecutive minutes, respectively). Cisplatin administration significantly decreased the number of rearings compared to the control group (statistically significant during the first four minutes of the test, $p<0.01$). Independent application of NAC did not alter the number of rearings comparing to the control group maintaining the values significantly above cisplatin treated animals (also, statistically significant during the first four minutes of the test, $p<0.01$). On the other hand, simultaneous administration of NAC during cisplatin treatment resulted in increased number of rearings comparing to cisplatin group (statistically significant in the 2nd and 3rd minutes, $p<0.05$).

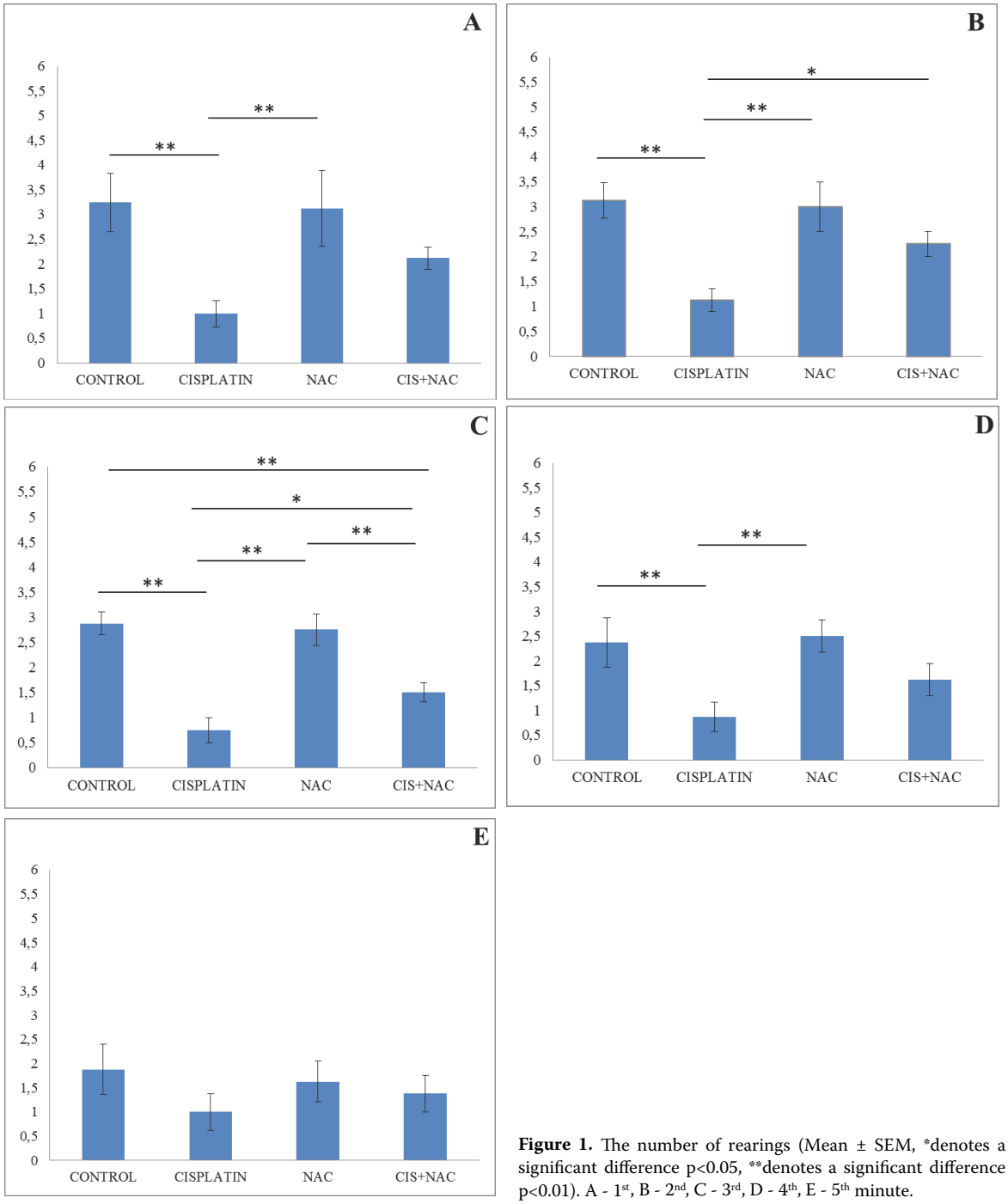

Figure 1. The number of rearings (Mean ± SEM, *denotes a significant difference $p<0.05$, **denotes a significant difference $p<0.01$). A - 1st, B - 2nd, C - 3rd, D - 4th, E - 5th minute.

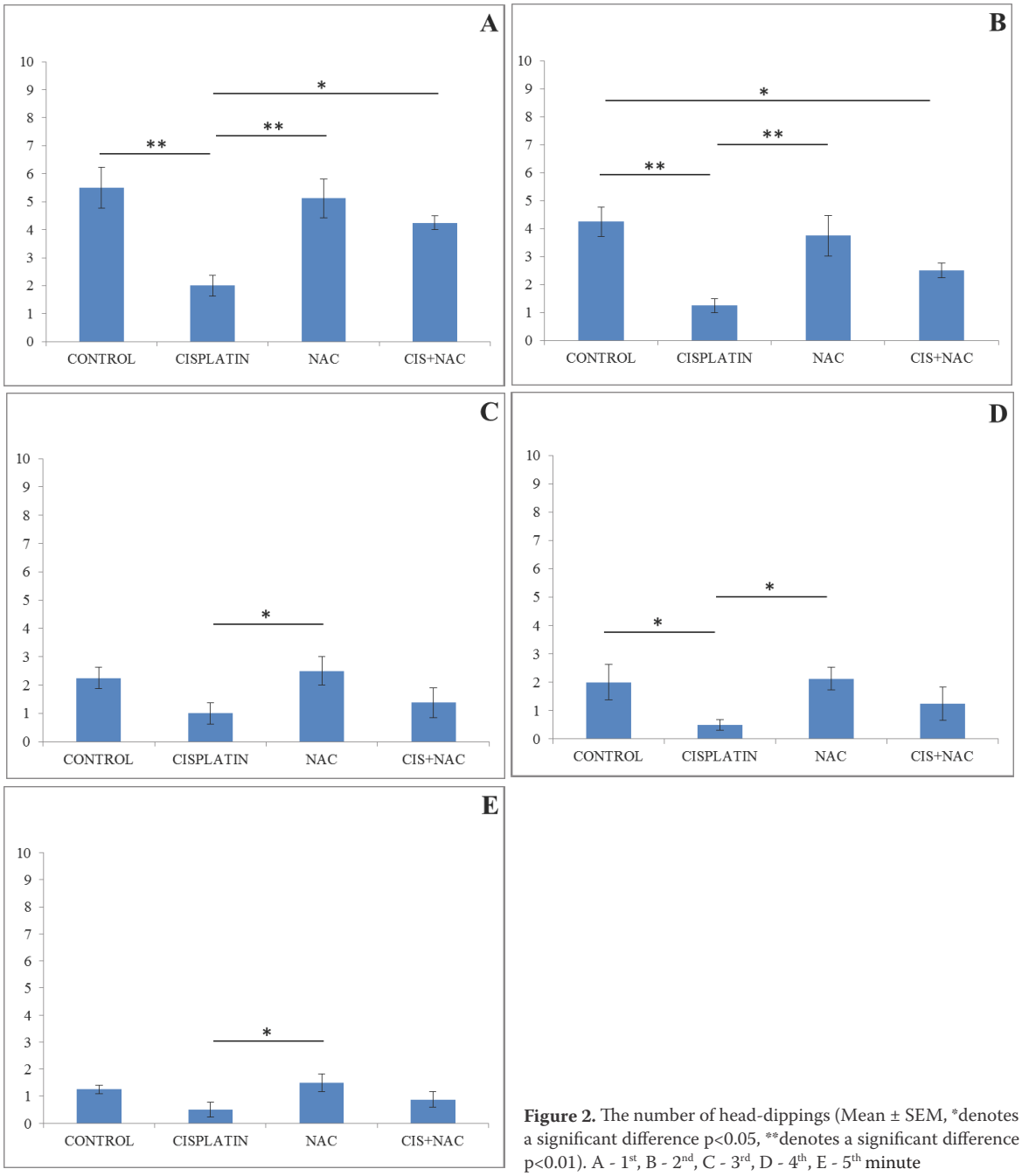

Figure 2. The number of head-dippings (Mean ± SEM, *denotes a significant difference p<0.05, **denotes a significant difference p<0.01). A - 1st, B - 2nd, C - 3rd, D - 4th, E - 5th minute

Analyzing the exploratory activity in open arms of EPM, significant alterations in the number of head-dippings was induced by different protocols (df=3, F=8.066, 7.711, 2.488, 2.422 and 2.618 for five consecutive minutes, respectively). It was noticed that cisplatin treatment resulted in significant decrease in the number of head-dippings (statistically significant in the 1st and 2nd minute, as well as in the 4th minute, p<0.01 and p<0.05, respectively). As for the number of rearings, when applied alone, NAC treatment did not affected the number of head-dippings compared to control group, and the values were also significantly higher when compared to cisplatin group for the whole duration of the test (first two minutes, p<0.01, 3rd to 5th minute p<0.05). The number of head-dippings observed in the combined group (Figure 2) was higher comparing to the cisplatin group (significantly only for the 1st minute, p<0.05), maintaining lower values when compared to control group (significantly only for the 2nd minute, p<0.05).

Applied protocols significantly altered TEA observed in EPM test (df=3, F=17.495, 21.608, 9.399, 8.675 and 1.829 for five consecutive minutes, respectively). As shown in Figure 3, the total exploratory activity was diminished in cisplatin group (statistically significant during the first four minutes of the test, p<0.01) comparing to control. When applied alone, NAC did not produce significant alterations in TEA comparing to the control group, and the values were also above the observed in cisplatin group (statistically significant in 1st, 2nd and 4th minute, p<0.01). Simul-

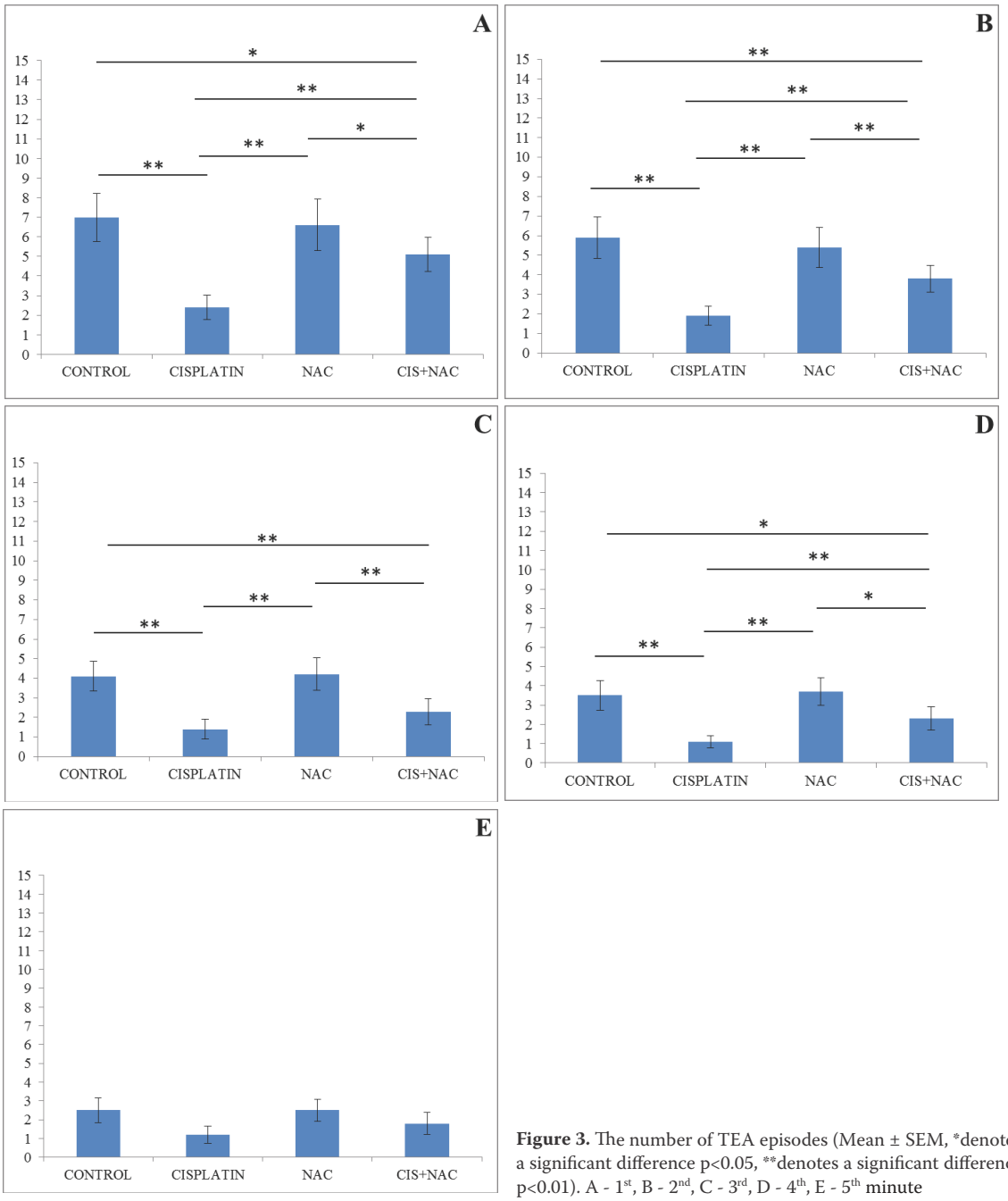

Figure 3. The number of TEA episodes (Mean ± SEM, *denotes a significant difference p<0.05, **denotes a significant difference p<0.01). A - 1st, B - 2nd, C - 3rd, D - 4th, E - 5th minute

taneous administration NAC and cisplatin resulted in significant increase in TEA when compared to cisplatin group (statistically significant in 1st, 2nd and 4th minute, p<0.01), but the values remained significantly lower comparing to the control (statistically significant during the first four minutes of the test, p<0.01).

Analyzing the time distribution of exploratory activity observed in EPM test (Figure 4), it is obvious that TEA progressively decline during the test. The stepwise attenuation of exploratory activity was observed in all tested groups. Since TEA gradually decreased, significant decline between two consecutive minutes was rarely observed: between 2nd and 3rd minute in control group (p<0.01), between 4th and 5th minute in NAC group (p<0.05), as well between 1st and 2nd (p<0.01), and 2nd and 3rd minute in combined group (p<0.05). Although TEA also progressively decreased following cisplatin administration, there was no significant decrease in time-dependent manner in cisplatin group.

DISCUSSION

Rising incidence of malignant diseases resulted in increased use of chemotherapeutic drugs, including cisplatin. Such therapeutic approach to malignancies often interferes with the course of treatment, so it is important to evaluate adverse effects of chemotherapy. Neurotoxicity is one of the many adverse effects of cisplatin therapy. In order to reduce

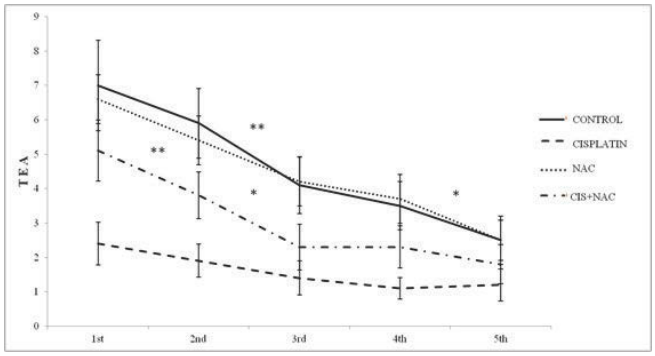

Figure 4. The time distribution of exploratory activity (Mean ± SEM, *denotes a significant difference p<0.05, **denotes a significant difference p<0.01)

the effects of cisplatin-induced neurotoxicity, it seems reasonable to investigate the possible underlying mechanisms of that specific side effect of cisplatin. It is well known that numerous metals of high atomic number (30), including platinum (31), lead to increased oxidative damage in various organic systems (32). Therefore, potential attenuation of oxidative damage that can improve clinical appearance during cisplatin treatment may be of substantial clinical relevance. According to the literature data, the dose of cisplatin applied in this study was sufficient to increase the platinum levels in serum and various tissues in clinical trials, as well as in animal experimental models. Consequently, this dose may also be considered as an adequate for investigation of cisplatin-induced neurotoxicity (33).

Exploratory activity has been reported as a good parameter for evaluation of anxiety state level, in such a manner that decreased exploratory activity may be considered as indicator of increased anxiety (34). Cisplatin treatment resulted in significant reduction in exploratory activity in the EPM test by means of all estimated parameters. This finding is consistent with the results of another study (35) which also observed the development of anxiety-like behavior in cisplatin-treated rats in the EPM test. The results of our study (as shown in Figure 4) also showed that under control conditions animals demonstrate the highest level of exploratory activity at the very beginning of the test. The exploratory activity decreases during the testing, but without statistical significance. The observed reduction in exploratory activity may be explained by the fact that the animals adapt to the new environment over time. Such regularity was observed in all groups of animals. Gradual decline in exploratory activity was observed throughout the whole test, except for cisplatin group, by the end of the test (4^{th} and 5^{th} minute). Although NAC, as shown in the Figure1-3, did not lead to changes in exploratory activity compared to the control group when applied alone, combined application of NAC and cisplatin resulted in significant mitigation of cisplatin-induced reduction in exploratory activity. The results of this study showed the beneficial effect of NAC by means of reduced oxidative damage that consequently attenuated anxiogenic effect of cisplatin and restored exploratory activity almost to control values. Observed neuroprotective effect of NAC may be achieved as the result of reduced free radicals production (36), increased activity of antioxidant enzymes (37) and/or tissue glutathione (38). A similar beneficial effect of an antioxidant supplementation on cisplatin-induced neurotoxicity (manifested by means of behavioral manifestetions) was observed for various nutritiens, such as walnuts. Walnuts contain potentially neuroprotective substances with anti-inflammatory and antioxidant properties (vitamin E, folate, melatonin, flavonoids and numerous polyphenolics) that were able to diminish cisplatin-induced neurotoxicity in rats (39). It is worth to notice that NAC administration also showed the beneficial effects by means of amelioration of cisplatin-induced cardiotoxicity in rats (33). The observed cardioprotective effect of NAC supplementation during cisplatin administration was accompanied with attenuation of oxidative damage induced by cisplatin administration. Antioxidant role of NAC in cisplatin-induced cardiotoxicity was achieved by means of decreased ROS production, with no alterations in tissue glutathione levels (33).

In conclusion, the results of this study confirmed clear beneficial effect of NAC supplementation against cisplatin-induced neurotoxicity in rats. Antioxidative properties of NAC were manifested through restoration of exploratory activity, confirming that NAC administration can attenuate anxiogenic effect of cisplatin therapy. Those results could recommend NAC supplementation as a potential protection against cisplatin-induced neurotoxicity.

ACKNOWLEDGEMENTS

This work was supported by Faculty of Medical Sciences (JP 01/13), University of Kragujevac, Serbia.

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ORGANISATIONAL AND ECONOMIC ACTIVITIES FOR PROVISION OF MEDICAL REHABILITATION SERVICES ON AN OUTPATIENT BASIS TO PATIENTS THAT HAVE SUFFERED AN ACUTE CEREBROVASCULAR ACCIDENT

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ORGANIZACIONE I EKONOMSKE AKTIVNOSTI ZA SPROVOĐENJE MEDICINSKE REHABILITACIJE U AMBULATNIM USLOVIMA BOLESNIKA KOJI SU DOŽIVELI AKUTNI CEREBROVASKULARNI DOGAĐAJ

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Received/Primljen: 07.11.2018. Accepted/Prihvaćen: 21.12.2018.

ABSTRACT

The aim of this study was to develop legal, organizational and economic activities for providing medical rehabilitation on an outpatient basis to patients that have suffered an acute cerebrovascular accident (CVA). The study included patients who had suffered a CVA and were undergoing medical rehabilitation on an outpatient basis (400 individuals participated in a retrospective study and 400 individuals took part in a sociological survey) and medical rehabilitation specialists providing care on an outpatient basis to patients who had suffered a CVA (n = 50). All included patients received medical rehabilitation in accordance with the Guidelines of the Ministry of Health of the Russian Federation (No: 70). It should be noted that patients who suffered a CVA occupy a central place in the system of medical rehabilitation performed on an outpatient basis. Medical rehabilitation is important for their medical and social characteristics, motivation, environment, adherence to treatment and a healthy lifestyle. In accordance with the above mentioned, the following activities should be planned and implemented: a) work with doctors, b) work with patients; c) work with patients' relatives; d) organizational aspects, and e) economic aspects. When organizing medical rehabilitation on an outpatient basis, it should be considered as a system of interaction between all participants in the rehabilitation process, in the center of which the patient is located. The main organizational activity for conducting medical rehabilitation in an outpatient setting is the implementation of a comprehensive interaction of all participants in the rehabilitation process.

Keywords: *stroke, medical rehabilitation, organization of health care system.*

SAŽETAK

Cilj ove studije je bio razvijanje pravnih, organizacionih i ekonomskih aktivnosti za sprovođenje medicinske rehabilitacije u ambulantnim uslovima pacijenata koji su doživeli akutni cerebrovaskularni događaj (CVI). Studija je obuhvatala pacijente koji su u istoriji bolesti imali CVI i bili su medicinski rehabilitovani u ambulantnim uslovima (400 ljudi je učestvovalo u retrospektivnoj studiji, 400 ljudi u sociološkom istraživanju) i specijaliste koji su obavljali medicinsku rehabilitaciju za pacijente sa CVI (n = 50). Svi pacijenti koji su bili uključeni dobili su medicinsku rehabilitaciju u skladu sa članom 70 Ministarstva zdravlja Ruske Federacije. Treba napomenuti da pacijent koji je imao CVI ima visok značaj i zaokuplja pažnju u sistemu medicinske rehabilitacije. Medicinska rehabilitacija u ambulatnim uslovima je veoma važna u pogledu njegovih medicinskih i socijalnih karakteristika, motivacije, njegovog okruženja, pridržavanja tretmana i zdravog načina života. U skladu sa tim, plan je sprovesti sledeće aktivnosti: a) rad sa doktorima; b) rad sa pacijentima; c) rad sa okruženjem; d) organizacioni aspekti i e) ekonomski aspekti. Prilikom organizovanja medicinske rehabilitacije u ambulantnim uslovima, treba imati u vidu sistem interakcija između učesnika u procesu rehabilitacije, u čijem centru se nalazi pacijent. Glavna organizaciona aktivnost za sprovođenje medicinske rehabilitacije u ambulantnim uslovima je sprovođenje sveobuhvatne interakcije svih učesnika u procesu rehabilitacije.

Ključne reči: *moždani udar, medicinska rehabilitacija, organizacija sistema zdravstvene zaštite.*

sciendo
UDK: 616.831-005.1:614.2
Ser J Exp Clin Res 2019; 20 (1): 73-79
DOI: 10.2478/sjecr-2018-0082

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INRODUCTION

At the present stage of development of health care systems in the economically developed countries of the world, one of the priority problems of strengthening and preserving health of the population is reducing the mortality rate due to the circulatory system diseases. In the structure of the causes of mortality in Russia, these diseases have occupied the first place, from the middle of the twentieth century to the present days. In 2016 and 2017, they accounted for more than 50 % of deaths among the country's population (1, 2).

A special role in resolving this problem should be given to activities aiming to improve prevention and organization of medical care and rehabilitation for patients with acute cerebrovascular accident (CVA). Almost half of the patients who suffered a CVA die in the first year after their stroke, and only about 20% of the sick return to work (2, 3).

There are different options for managing patients who have had a CVA, after completing their treatment in an intensive care unit. One of the options is the recommendation that most patients after discharge from an intensive care unit should be in a rehabilitation hospital. Other options are that some patients should undergo treatment in a hospital, and others who have a mild neurological deficit should receive rehabilitative treatment on an outpatient basis. The organization of targeted quality rehabilitation care at the outpatient stage is one of the directions in the reform of medical care (3, 4).

Monitoring of the treatment and observation of long-term treatment outcomes are some of the main duties of doctors (neurologists, surgeons, orthopedists, physiotherapists, and rehabilitation specialists), and this group of specialists has a responsibility to follow up with their patients for a long time (4,5).

Medical rehabilitation of patients with CVA is a task of supreme importance, as the number of patients grows each year, and the number of severe forms of this pathology accompanied by profound neurological deficits simultaneously increases. This clinical picture leads to a permanent loss of ability to work, most often due to late treatment of patients in an outpatient care unit. The timing of admission to the outpatient unit of the health system is often missed, often due to the lack of recommendations on the timing of admission of patients that have had a CVA on an outpatient basis (5-7).

In modern conditions of healthcare development, it is necessary to improve the continuity of therapeutic, diagnostic, rehabilitation and health-improving activity at all levels of care. First of all, it concerns the activities that are carried out at the stage of outpatient rehabilitation (8). A timely rehabilitation will prevent disability of patients and facilitate a return of the able-bodied population to work, thereby reducing the economic burden on the society and the state (9).

This applies not only to the choice of medical support to patients, but also to the organization of the third stage of rehabilitation as a system where the patient reaches the maximum possible effect for the degree of his or her neurological deficit and one of the main criteria for successful rehabilitation is a return to work. According to that, the aim of the study was to develop legal, organizational and economic activities for provision of medical rehabilitation services on an outpatient basis to patients who have suffered an acute cerebrovascular accident.

MATERIALS AND METHODS

Study protocol

The study was conducted at the Polyclinic centers of the Moscow City Health Department, Moscow, Russia, on the patients with diseases of the central and peripheral nervous system and who used medical rehabilitation on an outpatient basis. Patients are sent to the polyclinic from hospitals, outpatient centers, city polyclinics and outpatient clinics of Moscow and the Moscow region. In the period from 2011 to 2015, patients were admitted to treatment from the city polyclinics, hospitals, and their request. The study was implemented in the period from 2015 to 2018 (the analysis of data from the primary medical documentation for the period from 2011 to 2015 was carried out retrospectively).

Study population

The subjects of the study were patients who had suffered a CVA and were undergoing medical rehabilitation on an outpatient basis (400 individuals participated in a retrospective study and 400 individuals took part in a sociological survey) and specialists providing medical rehabilitation on an outpatient basis for patients who had suffered a CVA (n = 50). All included patients received medical rehabilitation in accordance with Article 70 of the Decree of Ministry of Health of the Russian Federation dated 29 December, 2012 No. 1705n "On the order of organization of medical rehabilitation". Randomization was carried out by mechanical selection.

The main inclusion criteria were: written informed consent of a patient to participate in the research; age above 18 years; diagnosis of "cerebral infarction" (code I 63 according to ICD-10) or "transient ischemic attack" (code G 45 according to ICD-10); conducting rehabilitation activities in an outpatient-polyclinic medical organization.

The main exclusion criteria were: age less than 18 years; patient’s refusal to further participate in the research; focal lesions of the brain, which developed as a result of other causes not related to CVA; lack of data about the implementation of rehabilitation activity at the third stage; patient's failure to further participate in the research.

Data collection

During the study, the primary medical documentation was selected, the volume of the sample was calculated, the data was copied from the primary medical documentation (form 025/y "Medical card of the outpatient"), and the received information was entered in the appropriate database. The data mining map included the following sections:

I Passport part (socio-demographic characteristics of the patient);

II Information about the CVA;

III Information about the medical therapy and use of non-drug rehabilitation methods;

IV Information about the consultations provided by specialists of different profiles;

V Conducted sanatorium-and-spa treatment.

The sociological research of patients and specialists was carried out on the basis of analysis of questionnaires that were specially developed for the corresponding group of respondents. The complex methodology of scientific research included the following methods: analytical, organizational, sociological, economic analysis, economic modeling, comparative and statistical analysis, content analysis, and intellectual analysis of data.

RESULTS AND DISCUSSION

Basic socio-demographic characteristics of the study population

The average age of a patient undergoing medical rehabilitation on an outpatient basis was 54.07 years, and 50.2% of the able-bodied age group. The structure of patients was dominated by individuals whose occupation was associated primarily with intellectual work (71.1%) in the area of higher education (64.25%). The relationship between the level of education and a good prognosis of medical rehabilitation, in persons with higher education, was the following among women ($r = 0.83$, $p < 0.001$) and among men ($r = 0.93$, $p < 0.001$).

Clinical characteristics of the study population

The overwhelming majority of patients had no disability ($80.6 \pm 1.9\%$). More than half ($68.7 \pm 2.5\%$) of the patients had suffered a CVA for the first time. The clinical picture was characterized mainly by vestibule-cerebellar disorders ($65.9 \pm 1.1\%$), motor reflex ($14.8 \pm 2.2\%$), and disorders of cranial nerves ($11.8 \pm 2.2\%$ %).

The average duration of rehabilitation activity performed on an outpatient basis was 28.3 days. Patients who had suffered a CVA and were undergoing rehabilitation were consulted by doctors - neurologists, physicians, physiotherapists, ophthalmologists, and cardiologists.

An important characteristic of a stroke patient is a restriction of his or her vital activity. Restriction in labor activity was experienced by $77.8\% \pm 3.2\%$ of the patients and restriction in self-service was experienced by $55.3\% \pm 3.5\%$ of the patients. There was also a violation of orientation in space ($81.2\% \pm 2.8\%$), a restriction in movement ($11.5\% \pm 3.8\%$), training ($5.7 \pm 1.5\%$), and communication ($4.1 \pm 1.3\%$). Moreover, $47.8 \pm 2.4\%$ of the respondents showed a significant degree of dependence in the household activity and the evaluation was carried out using the Barthel-index.

Satisfaction of patients with the health care system

Satisfaction with the services of medical rehabilitation in outpatient conditions on a 5-point scale was 3.46 ± 0.9 points, depending on a number of factors. High evaluation of the provided services was given by individuals aged 61 to 70 years, and taking into account a work history, those were working patients with higher education. Patient's satisfactio n is also determined by the state of their health. It was revealed that the lower the patient's subjective assessment of his or her health, the lower the satisfaction rating ($r = + 0.86$, $p = 0.010$). At the same time, low satisfaction ratings were noted among those who did not consider it necessary to monitor their lifestyle and adjust it. Low ratings of satisfaction with medical rehabilitation on an outpatient basis were associated with a shortage of staff and diagnostic equipment in the polyclinic, and an inadequate set of diagnostic and therapeutic activities.

Activity implementation plan

It should be noted that patients who have suffered a CVA occupy a central place in the system of medical rehabilitation performed on an outpatient basis. It is important for their medical and social characteristics, motivation, environment, adherence to treatment and a healthy lifestyle. In accordance with this, the following activities should be planned and implemented (Figure 1): a) Work with doctors; b) Work with patients; c) Work with relatives; d) Organizational aspects, and e) Economic aspects. Therefore, medical rehabilitation on an outpatient basis should be organized as a system of interaction between participants in the rehabilitation process, in the center of which the patient is located.

Work with doctors

Doctors, being the main sources of information about a patient's condition, should be fully involved in the rehabilitation of patients on an outpatient basis. As the results of the study showed, the best prognosis (according to the subjective estimates of the patient) is observed when the medical assistance was provided by an experienced specialist trained in medical rehabilitation. It is extremely important to maintain continuity and interdisciplinarity, which implies the involve-

ment of subspecialists in preparation of an individual program for the rehabilitation of patients; however, 12.0±4.5% of the respondents did not take part in this. The implementation of this approach when working with doctors provides a basic principle for realization of medical rehabilitation for patients who suffered an acute cerebrovascular accident - complexity. At the same time, the basic factors influencing the positive outcome of medical rehabilitation are not only the complex work of all specialists (as 64.0±3.3% of specialists believe), but also the right therapy, and a strong-willed patient. This indicates the need for continuous, dynamic and comprehensive support for patients throughout the course of medical rehabilitation with the involvement of specialists such as a psychologist and psychotherapist.

The use of information materials and monitoring of the results should also be considered as some of the main organizational activities necessary to be implemented in everyday practice. At the same time, the obtained results of the study demonstrate a lack of understanding on the part of specialists what are the goals, objectives and mechanisms for monitoring rehabilitation process. More than half of the surveyed respondents do not monitor, since they believe that it is not their responsibility, and specialists do not know the goals, objectives and mechanisms for its implementation. Thus, it is necessary to upgrade the skills of specialists with a view to their preparation for the implementation of full-fledged and high-quality medical rehabilitation.

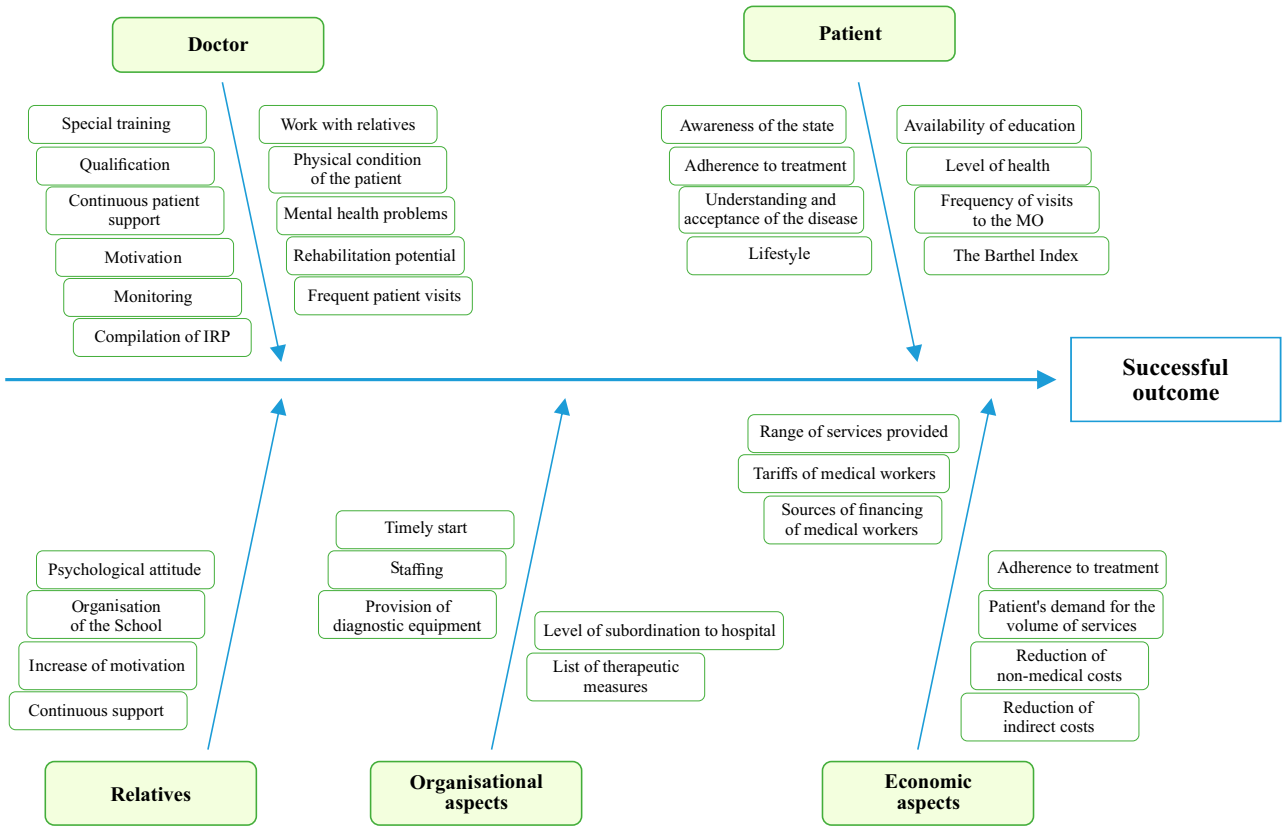

Figure 1. Organisational and management measures that promote successful outcome of medical rehabilitation on an outpatient basis

CONCLUSIONS

The emotional-volitional factor of specialists performing medical rehabilitation has a special significance. Our study shows that 68% of the specialists, against a background of moderately high degree of maturity, lack motivation and, as a result, there is a decrease in their responsibility and awareness of their involvement in the task. The importance of this factor is determined by its direct influence on adherence to treatment of a particular patient and, as a consequence, on the outcome of the rehabilitation. When interacting with patients, it is important to take into account the frequency of their visits to the medical organization and the patient's rehabilitation potential. Specialists should pay attention to the patient's psychological state and physical condition. The results of the research show the need for specialists to interact with the patient's relatives for the purpose of their education and the correct approach to rehabilitation activity that can be carried out at home.

Work with patients

The next direction, which determines the success of rehabilitation, is working with patients. Namely, a positive outcome is influenced by the patient's satisfaction with the rehabilitation process. As a rule, highly educated individuals who also work at the time of the disease are more satisfied with rehabilitation activity.

An important aspect is the patient's state of health. The study demonstrated low interest of patients in maintaining a healthy life style; therefore, organizational activities should be aimed at establishing a "School of Health" and quality educational activities among patients. Patients, as immediate participants in the rehabilitation process, should be informed about their condition in order to understand and accept their illness. Clarification of these aspects is the task of medical staff. The success of rehabilitation directly depends on patient adherence to treatment, during which the patient needs a long and continuous support and, possibly, control during the patient’s regular visits in the period of medical rehabilitation in a medical organization.

Of particular importance in forecasting a favorable outcome of medical rehabilitation is the magnitude of the Barthel-index. Obviously, the higher the index of daily activity, the better the rehabilitation forecast. However, patients with a low Barthel-index often enter rehabilitation on an outpatient basis. In this case, the goal of medical rehabilitation is to plan the maximum possible increase in the index of their daily activities.

Work with relatives and friends

Work with relatives and friends can qualitatively improve and accelerate the process of recovery. As the results of the study showed, both specialists and patients assigned an important place to this interaction in the medical organization where rehabilitation is carried out.

A significant number of patients with difficulties in daily self-care live with relatives and close people, thus being a burden on them. Virtually all patients with a Barthel index of less than 20 do not live alone, which means that a significant share of the costs falls not only in the form of direct costs for treatment, but also indirectly in the form of disability of the patients themselves and their relatives who are responsible to take care of them.

Relatives understanding of the goals and objectives of medical rehabilitation, as well as the availability of their capabilities and desire to provide all possible assistance in the implementation of an individual rehabilitation program are necessary for the full and correct implementation of the doctor's recommendations. In this regard, it would be convenient to organize special schools for relatives and friends of the patient in order to teach them how to take proper care of the patient and to explain the necessity of accompanying the patient throughout the course of medical rehabilitation.

Organizational aspects

Organizational aspects are essential for the success of medical rehabilitation. The favourable outcome of rehabilitation necessitates a resolution of a number of organizational problems, such as: lack of medical and nursing staff in a polyclinic at the place of residence and a shortage of diagnostic equipment.

A good prognosis is also influenced by appropriate medical activities. Some patients indicate a link between the prognosis and the level of medical organization, believing that federal medical organization provide better quality health care. With all the subjectivity of this opinion, as the research shows, it can affect the patient's psycho-emotional state and, as a result, the outcome of rehabilitation.

Economic aspects

Adherence to treatment by patients is significantly influenced by the source of funding for medical rehabilitation. The patient's demand for the volume of medical services depends on the source of funding. At the same time, the source of funding has an impact on the choice of specialists in the range of services assigned to patients for medical rehabilitation, which in turn also affects the outcome of treatment. Taking into account the incidence of CVA in Moscow, as well as the number of patients who can provide medical rehabilitation on an outpatient basis, the costs were very high, about 2 billion rubles. However, it should be noted that the state's payments in connection with indirect medical and indirect costs are made annually. Life expectancy in Russia in 2017 was 74 years; therefore, if we take into account that the average age of patients that have suffered a CVA is 54 years, the indirect medical expenses for this group of patients, taking also into account annual inflation, are 17,601,143,566 rubles. Thus, there is the possibility of preventing state expenses related to

this category of patients, provided that patients are timely admitted to outpatient facilities. Based on the foregoing, it can be concluded that the costs of medical rehabilitation with a subsequent return to work are much lower than the costs borne by the state and society in case of failure of providing medical rehabilitation on an outpatient basis.

Thus, organizational, managerial, legal and economic activities for the implementation of medical rehabilitation on an outpatient basis should be aimed at the integrated interaction of all participants of the rehabilitation process. Specialists who provide medical rehabilitation should have the necessary qualifications and training and at the same time be in constant interaction with patients and their relatives for dynamic and continuous monitoring and monitoring of the results.

The patient, as the central link of the rehabilitation process, must have complete and reliable information about his state of health and understand the features of the current disease and its risks. At the same time, the patient is responsible for adjusting the lifestyle, with the aim of leveling the risk factors for the occurrence of repeated acute conditions.

Among legal tasks, an important aspect is the revision of territorial tariffs, and as a result, the expansion of the range of services provided to a patient served by the CHI tariff, and at the same time increasing the patient's demand for services and adherence to treatment. The sources of law regulating medical rehabilitation do not pay enough attention to the programs of medical rehabilitation and the continuous, long-term strategy for monitoring patients who have suffered a CVA, proceeding from individual characteristics of patients. As the economic analysis shows, non-medical and indirect costs faced by individuals in need of the third stage of rehabilitation can be reduced through their timely admission to this stage of rehabilitation.

DISCUSSION

Medical rehabilitation of patients who suffered a CVA is carried out in three stages. The first stage is carried out in the acute period of the course of the disease or trauma in intensive care units. The second stage should be carried out in the early recovery period of the disease or trauma, and the late rehabilitation period, as the period of residual phenomena of the course of the disease, should be the chronic course of the disease without exacerbation under hospital conditions of medical organizations (rehabilitation centers, rehabilitation departments). The third stage is carried out in the early and late rehabilitation periods by specialists who provide medical care on an outpatient basis, through doctors visiting patients at home and in the presence of the prospect of restoration of functions (rehabilitation potential) confirmed by the results of the survey.

The need to develop organizational and management activity for medical rehabilitation of patients who have suffered a CVA is caused by a significant increase in morbidity, mortality and a decrease of life quality among people of working age. Up to the third stage of medical rehabilitation, no more than 60% of patients survived after they had suffered a CVA. Despite the high importance of this problem, complex, continuous, and multidisciplinary medical rehabilitation with a return to work is foreseen only in a number of countries (9-11).

An analysis of regulatory documentation has shown that the Russian Federation does not have a legal basis needed for implementation of a medical rehabilitation program and there are no long-term strategies for continuous and integrated support for patients who have suffered a CVA. Professional communities are working on programming and organizing rehabilitation activities; however, the routing of such patients and their timely admission to the third stage of rehabilitation are not fully understood.

This situation requires international cooperation in the implementation of clinical guidelines, intersectional interaction of social and medical services, improving the understanding of financial departments of the need for investment in medical rehabilitation, as well as interdisciplinary cooperation to ensure better continuity of patients with CVA at different stages. It should be noted that the important organizational and managerial aspects are the complex interaction of the patient, the doctor, relatives, and also the satisfactory organizational and economic models for the organization of medical rehabilitation.

The following necessary activities should be organizational, legal and economic aspects of medical rehabilitation of CVA patients on an outpatient basis: participation of all specialists involved in patient rehabilitation programs in the preparation of individual rehabilitation programs before counseling or other medical assistance. Correctly chosen therapy and emotional-volitional state of mind of the patient should become another task for the doctor. Continuous, dynamic and comprehensive support of patients throughout the course of medical rehabilitation with the involvement of specialists such as a psychologist and psychotherapist can solve the task. In addition, increasing the number of patients who ongoing monitoring results by activity of specialists who provide care to patients (12).

There is an emotional-volitional factor of a specialist that influences adherence of patients to treatment and, as a result, a decrease in responsibility and awareness of their involvement in the task performed can result in an undesirable outcome of rehabilitation. One of the important organizational activities that can help to improve rehabilitation outcomes is patient satisfaction and interest in adherence to a recommendation and adherence to a healthy lifestyle. This task can be achieved through establishment of special "Schools of Health".

Patient’s relatives and people close to the patient have a significant impact on the recovery process (13). It is necessary

to conduct educational work among them for the rapid achievement of the goals and objectives of rehabilitation of the patient who has suffered a CVA. The lack of staff in a polyclinic at the place of residence and the shortage of diagnostic equipment are significant limitations in achieving a good outcome of rehabilitation (7, 13).

Patients who have suffered a CVA are a significant burden on society in cases of late or no admission to an outpatient facility, which proves the high economic efficiency of timely medical rehabilitation and continuous patient support.

Application of the obtained results in medical practice will allow improving the measures of the state policy, for people who have suffered a CVA, to predict long-term results of restoring damaged or compensated lost functions and determine the appropriateness of improving the legislative and material-technical base, forming the personnel potential of the industry, conducting activities in the direction of developing measures for disability prevention.

CONCLUSION

Specialists who carry out medical rehabilitation in outpatient settings note the need for a comprehensive individual rehabilitation plan and are engaged in drawing up an individual plan with other specialists or individually. When assessing organizational activities, specialists noted the need for continuous comprehensive support for patients throughout the course of medical rehabilitation. The main organizational activity during medical rehabilitation in an outpatient setting is a comprehensive interaction of all participants in the rehabilitation process. The patient, as the central link of the rehabilitation process, must have full and reliable information about his or her state of health and understand the features of the current disease and its risks. Thus, the interaction doctor-patient-relatives, registration of organizational activities aimed at providing staff at equipment, and timely admission to an outpatient unit for rehabilitation will reduce and prevent non-medical and indirect costs of CVA patients, enabling their return to work.

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NEUROPHYSIOLOGICAL ASSESSMENT OF COGNITIVE DYSFUNCTION IN PATIENTS WITH MULTIPLE SCLEROSIS

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NEUROFIZIOLOŠKO MERENJE KOGNITIVNIH DISFUNKCIJA KOD BOLESNIKA SA MULTIPLOM SKLEROZOM

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Received / Primljen: 28. 06. 2016. Accepted / Prihvaćen: 14. 07. 2016.

ABSTRACT

Cognitive impairment occurs in a high percentage in all forms of multiple sclerosis, regardless of physical disability. Slowing the speed of information processing is one of the most difficult and the most frequently mentioned, but impairment of memory, attention, executive functions are included also. Long latency event related potentials (ERP) are much more objective means of cognitive functioning evaluation. Different types of immunomodulatory therapies which are used for relapsing- remitting forms of multiple sclerosis may affect the results of ERP. ERP can evaluate subclinical changes and provide important information on the evolution of cognitive changes in patients with MS.

Keywords: *multiple sclerosis, cognitive function, cognitive evoked potentials*

SAŽETAK

Oštećenje kognitivnih funkcija se javlja u visokom procentu u svim formama multiple skleroze nezavisno od fizičke onesposobljenosti. Usporavanje brzine procesuiranja informacija je jedna od najtežih i najčešće pominjanih, ali tu spadaju i oštećenje pamćenja, pažnje i egzekutivnih funkcija. Kognitivni potencijali kasnih latenci (ERP) su mnogo objektivnije sredstvo za evaluisanje kognitivnog funkcionisanja. Različite vrste imunomodultaornih terapija koje se koriste kod relapsno-remitetne forme multiple skleroze mogu imati uticaj na rezultate ERP. ERP nalazi mogu otkriti subkliničke promene i obezbediti važne informacije o kognitivnim oštećenjima u bolesnika sa multiplom sklerozom.

Ključne reči: *multipla skleroza, kognitivne funkcije, kognitivni evocirani potencijali*

ABBREVIATIONS

MS – Multiple Sclerosis **ERP** – Event related potential

INTRODUCTION

Cognitive impairment is a common feature in patients with multiple sclerosis, documented the high frequency up to 65% (1). Cognitive impairment occurs independently of physical disability and duration of the disease and occurs in all forms of MS (2). Slowing of the speed of information processing is one of the most difficult and the most frequently mentioned dysfunction, but there are damage of working memory, attention, executive function as well. Some patients early show neurobehavioral changes during the disease, while others never. The degree of cognitive deficit is highly variable.

Cognitive dysfunction can be evaluated by a battery of different neuropsychological tests, procedures that last about 3 to 5 hours and depend on the cooperation of patients. The main limitation in the application of these tests in patients with MS is a physical disability, reduced visual activity and motoric limit. On the other hand, it was shown that quick Mini Mental test (MMSE) is very insensitive for mild cognitive dysfunction. PASAT (Paced Auditory Serial Addition Test) proved to be a test which score is affected by education, training, anxiety and physical ability that is often impaired in MS (3).

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UDK: 616.832-004.2:159.943
Ser J Exp Clin Res 2019; 20 (1): 81-83
DOI: 10.1515/SJECR-2016-0056

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Long latency event related potentials (ERP) are much more objective means of cognitive functioning evaluation (4). Typical ERP waves elicited by the "oddball" paradigm with auditory and visual stimulation, in which occasional target stimuli have to be detected in a train of frequent irrelevant standard stimuli evokes a number of ERP components. In the performing of this test, the response to the rare tone is more complex, consisting P300 negative deflection. P300 component (P3) is known as an objective measure of individual cognitive functioning. P300 is a neurophysiological correlate of cognition and can be considered an internal memory model. It occurs 300 ms after stimulation and the maximum amplitude is recorded with the parietal scalp. This method of assessment of cognitive functioning in MS patients is very suitable because it is relatively independence from their visual and motor impairment, that are often in these patients.

Still the generator source of these waves is not reliably confirmed, but a lot of data suggest that the medial part of the temporal lobes and the temporal-parietal associative cortex can play a major role in the generation of these waves. It is assumed that the peak latency of this wave is the indicator of information processing speed since it is completely independent of motor activity and it is the process of making reaction choice. Prolonged latency of P300 represents an extension of time information processing while reducing the amplitude and abnormal topographic distribution reflects either weaker activation of the generator (frontal, parietal cortex, the thalamus and the temporal-medial cortex) or time dispersion of information processing. There is not enough data that the prolonged latency P300 in diseases is associated with general cognitive decline especially if it is characterized by slowing of the intellectual process.

Previous electrophysiological studies suggest that patients with MS have a significantly increased latency and decreased amplitude waves P300 with the auditory ERP-compared to healthy (4, 5). Whelan presented a significant difference in amplitude and latency between MS patients andthe healthy (6). The same author finds that were no significant differences between RRMS (relapsing-remitting multiple sclerosis) and SPMS (secondary progressive multiple sclerosis) patients on any ERP component. Longitudinal study presented that the decreased in amplitudes over 12-month period was greater in MS patients relative to controls and change in P3 amplitudes correlate with change in PASAT score in MSpatients over 12-month period (7). Although there are conflicting opinions that there is no difference in P3b latency and amplitude between MS patients and healthy (8). It is shown that latency of P300 poorly correlated with the duration of the disease (4). Nevertheless, recently published work indicates that cognitive capacity at MS patients is better expressed withlower amplitude and longer response time (9).

For now, the relationship between the lesions and neuropsychological cognitive function is not clearly defined in the MS. The application of MRI technique is the attempt to explain the mechanisms responsible for the development of cognitive deficits in patients with MS. Available data suggest that focal lesions in the white matter of the brain crucial areas play an important role in the development of cognitive deficit (2,10). Irreversible tissue loss, brain atrophy, is associated with cognitive deficits. And other components of MS pathology such as diffuse damage, the phenomenon of the normal-appearing white matter (NAWM) and damage the gray matter (GM) play the role in determining the cognitive profile (10). It has been shown that delayed P300 latency correlated with the existence of extensive lesions of the white matter of the brain seen at magnetic resonance. This can lead to the assumption that white matter lesions may be responsible for cognitive deficits, abnormalities of ERP, which is explained by blocking functional fibers that connect different cortical regions. Some authors associate P300 latency extension with white matter lesions around the frontal horn and brain stem (5).

Study with functional magnetic resonance imaging (fMRI) indicate that performance of cognitive task in RRMS patients is associated with activation of higher cortical area in bilateral prefrontal and lower parietal cortex compared with control (11). Different patterns of cortical activations demonstrated an adaptive role in some stages of the disease.

Different types of, immunomodulatory therapies: Interferon beta and Natalizumab, which are applied in these patients may have an impact on the result of ERP (12). The authors give examples of the administration of high doses of corticosteroids, which are used in these patients relapses therapy may significantly reduce latency of P300 (13). It not only affects the level of motor and sensory functions but also at the high level of cognitive functioning. The same authors believe that the latency of P300 waves can be used to detect the changes caused by therapy and thus monitor the effect of treatment.

Dysfunction in speed of information processing is the prominent feature of cognitive impairment in MS and it is therefore important to identify cognitive deficits and often to control it. It is shown that training and motivation improve performance and significant impact on P300 amplitude (14).

By measuring the fluctuations in cognition may be useful as an additional measure for monitoring disease activity, monitoring therapy in MS. ERP can evaluate subclinical changes and provide important information on the evolution of cognitive changes in patients with MS.

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TRUE ANEURYSM OF TEMPORAL SUPERFICIAL ARTERY ARISE SPONTANEOUSLY. CASE REPORT

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SPONTANO NASTALA ANEURIZMA TEMPORALNE SUPERFICIJALNE ARTERIJE. PRIKAZ SLUČAJA

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Received / Primljen: 26. 04. 2017. Accepted / Prihvaćen: 07. 07. 2017.

ABSTRACT

Aneurysms of the temporal superficial artery (TSA) are very rare clinical entity. From 1861 to the present day, is described less than 200 cases. The most common cause of these aneurysms is so called blunt head trauma but there are described many cases of iatrogenic aneurysms, very rarely, aneurysms arise spontaneously. We report a case of 17-year-old patient with spontaneously formatting aneurysm of TSA. Three months prior to admission, he noticed the existence of tumefaction localized frontotemporal on the right side. MSCT angiography of blood vessels of the scalp showed an aneurysm on the frontal branch of TSA diameter of 15 mm. The aneurysm was resected with uneventful postoperative course. PH findings pointed to a true aneurysm. Treatment protocol for the aneurysms of the TSA include clinical monitoring, compression of the aneurysm, the injection of thrombin, endovascular treatment and surgical resection. Surgical resection has proven to be a safe and effective treatment modality and still is the method of choice.

Keywords: *aneurysm, temporal superficial artery*

SAŽETAK

Aneurizme temporalne superficijalne arterije (TSA) su vrlo retke. Od 1861. godine do danas je opisano manje od 200 slučajeva. Najčešći uzrok nastanka ovih aneurizmi su tupe povrede glave, ali je opisan i veliki broj aneurizmi jatrogene etiologije, dok su spontano nastale aneurizme još ređe. Opisali smo slučaj pacijenta muškog pola, starog 17 godina, kod kojeg je dijagnostikovana sponatno nastala aneurizma TSA. Tri meseca pre hospitalizacije, pacijent je primetio postojanje tumefakcije u čeonoslepoočnoj regiji sa desne strane. MSCT angiografija krvnih sudova poglavine je ukazala na aneurizmu čeone grane TSA dijametra 15mm. Aneurizma je hirurški eksicidirana, postoperativni tok je uredno protekao. Patohistološka analiza je pokazala da se radi o pravoj aneurizmi. Protokol za zbrinjavanje TSA aneurizmi podrazumeva kliničko praćenje, kompresiju aneurizme, injekciju trombina, endovaskularni tretman i hiruršku resekciju. Hirurška resekcija se pokazala kao siguran i efikasan modalitet lečenja i još uvek je metoda izbora.

Ključne reči: *aneurizma, temporalna superficijalna arterija*

ABBREVIATIONS

MRI - Magnetic resonance imaging
MSCT - Multislice computed tomography
PH - Patohistology
TSA - Temporal superficial artery

INTRODUCTION

Temporal superficial artery (TSA) is the terminal branch of the external carotid artery from that usually separates behind the angle of the mandible. It has two terminal branches, frontal and parietal. Aneurysms of the TSA are rare clinical entity. Thomas Bartholin has published the case of a young patient who had blunt head trauma and who has aneurysms on the TSA in 1740 (1).

From 1861 to the present day is described less than 200 cases (2, 3). Indications for treatment of aneurysms of the

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UDK: 616.134-007.64
Ser J Exp Clin Res 2019; 20 (1): 85-88
DOI: 10.1515/SJECR-2017-0037

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TSA are headache, aesthetic reasons, and most importantly the prevention of aneurysm rupture or erosion of bone.

The most common cause of these aneurysms is so called blunt head trauma but there are described many cases of iatrogenic aneurysms, very rarely, aneurysms arise spontaneously.

We report a case of 17-year-old patient with spontaneous aneurysm of TSA.

CASE REPORT

Male patient 17 years old noticed the existence of tumefaction localized on the right side of forehead three months prior to admission. He thought that the change is an atheroma and has not been to a medical examination. After two and a half months, the patient noticed that the change is increased in size and it became painfully sensitive to touch. Then he reported to our clinic.

By physical examination, we found pulsating palpatory painfully sensitive tumefaction measuring 1x2 cm. Flank mass was mobile in relation to the bone. MSCT angiography of blood vessels of the scalp showed an aneurysm on the frontal branch of TSA (Figure 1).

After preoperative evaluation, the patient was operated. The aneurysm was resected and the postoperative course was uneventful. The patient was discharged home the next day. Control MSCT angiography of blood vessels of the scalp showed absence of vascular anomalies. Sutures are removed 7 days after the surgery. PH findings pointed to a true aneurysm. In the period of fourth months of follow up, there were no signs of recurrence.

DISCUSSION

Aneurysms of TSA is rarely seen. So far is described less than 200 cases of these aneurysms which are solitary in most cases, but sometimes can be multiple (4, 5). In 89% percent of reported cases were pseudo aneurysms, while the remaining 11% were true aneurysms of TSA (1). Pseudo aneurysms usually occur after blunt head trauma in 75% of cases (6, 7). The most commonly occur on the front branch of TSA because of its anatomical position. The frontal branch of TSA pass over upper temporal line where is the junction between frontal and temporal muscle and arteries passing through the hole between the two muscles and remains unprotected (8). Apart from blunt trauma to the head, there are also described cases of aneurysm formation after firearms injuries (9, 10).

Also, a certain percentage of pseudo aneurysms occurs iatrogenic after bypass surgery (11) after craniotomy (12), after a hair transplantation (13) and even as a complication of Botox injections (14). True aneurysms occur mainly spontaneously (15). Causes of it still is not completely understood. It is assumed that atherosclerotic changes may have important influence on the spontaneous occurrence of true aneurysms (16). In addition, given that the true aneurysms occur in younger patients, it must be assumed that there are other causes. According to some authors congenital defect of the vessel wall also has a role in the development of true aneurysms, as well as segmental arteriopathic amyloidosis (17) or non-specific temporal arteritis (5). Differentiation whether it is a true or pseudo aneurysms is only possible by histopathological analysis.

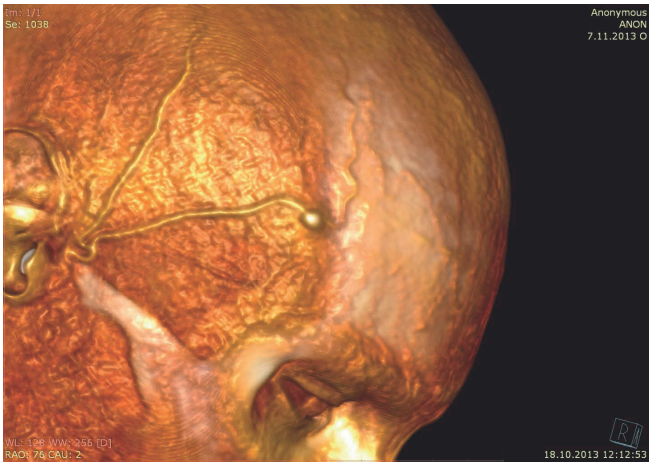

Figure 1. MSCT angiography of scalp blood vessels

Aneurysms of TSA is usually first suspected after physical examination. It is usually presented as a subcutaneous non adhesive pulsating mass whose pulsations coincide with the pulsations of blood pressure. The use of duplex ultrasound can help in the diagnosis (18). Native CT scan and MRI exam can be used in diferential diagnosis purposes to preclude the existence of fistula of medial meningeal artery (19). According to the review of the literature, some authors have used the DS angiography of blood vessels. By their opinion that accurate preoperative identification of afferent vessels accelerates and facilitates the identification of the aneurysm and its surgical excision (20). From our point of view, and according to other authors (1) the DSA is an invasive procedure and carries a minimum, but still existing, risk of stroke, dissection of intima of the vessels and bleeding, and as such is unusefull for this type of pathology. It is much safer and yet extremely illustrative 3D MSCT angiography (21) that we used in this case.

Unlike degenerative aneurysms of TSA, which are increased gradually over the time, traumatic aneurysms are usually increased rapidly over a period of 1 to 6 weeks after the trauma (22). The natural course of the disease is in most cases the rupture, rarely thrombosis.

In the treatment of aneurysms of TSA can apply conservative and radical methods. Treatment protocol for the aneurysms of the TSA include clinical monitoring, compression of the aneurysm, the injection of thrombin, endovascular treatment and surgical resection. Edwards M. R. published the first conservative treatment method for the aneurysm of TSA in 1861. The patient was student who was pressing the aneurysm and caused spontaneous thrombosis (4). Most of the patients, according to the

literature, underwent surgical resection of the aneurysm (23 - 25), which proved to be a safe and effective treatment. Still, there were described complications of surgery in the form of postoperative paresis n. VII (13, 26) and n. XII (most likely mobilization of the upper portion of the extenal carotid artery to be correlated to stretching of the nerve during the surgery) (23). Also, some authors for the treatment of aneurysms of the TSA suggest direct puncture and injection of thrombin with ultrasound navigation (27, 28). The disadvantage of this method is that it is difficult to control the dispersion of thrombin, which can lead to necrosis of the flap (29).

Standard endovascular intervention of coiling aneurysms (30, 31) may be an alternative to surgical resection in some cases and that in the case of aneurysms in the facial region. Still the potential embolic complications represent a significant limitation of this method. Some authors have used direct percutaneous coiling embolization of aneurysms guided by ultrasound (32).

CONCLUSION

Aneurysms of the TSA are rare clinical entity. Indications for treatment of aneurysms of the TSA are headache, aesthetic reasons, and most importantly the prevention of aneurysm rupture or erosion of bone. Protocol treatment of aneurysms of TSA include clinical monitoring, compression of the aneurysm, the injection of thrombin, endovascular treatment and surgical resection. Surgical resection has proven to be a safe and effective treatment modality and it is the method of choice. In some cases, need to think about injections of thrombin or endovascular treatment.

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THE SURGICAL MANAGEMENT OF GARDNER SYNDROME MANIFESTATION IN THE MAXILLOFACIAL REGION: A CASE REPORT

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HIRURŠKA INTERVENCIJA GARDNEROVOG SINDROMA MANIFESTOVANOG U MAKSILOFACIJALNOJ REGIJI: PRIKAZ SLUČAJA

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Received / Primljen: 20. 06. 2017. Accepted / Prihvaćen: 13. 07. 2017.

APSTRAKT

Gardner syndrome is a rare autosomal-dominant genetic disorder, considered a variant of familial adenomatous polyposis. It is characterized by intestinal polyposis and various bone and soft-tissue tumors, including osteomas, epidermal and dermal cysts, lipomas and fibromas. Intestinal polyps have high potential to become malignant, and the screening of intestinal lesions is mandatory. Maxillofacial manifestations, such as jaw osteomas, odontomas, dental abnormalities and soft tissue tumors frequently precede the intestinal manifestations. Thus, maxillofacial surgeons and dental practitioners may have important role in early detection of Gardner syndrome. In this case report we present a 22 year old male patient who was reffered to maxillofacial surgeon due to osteoma of the mandible. After obtaining clinical and radiological examinations, as well as data from family history, Gardner syndrome was suspected, which was later confirmed after gastroenterological examination.

Keywords: *gardner syndrome, extraintestinal manifestation, mandible osteoma, treatment*

SAŽETAK

Gardnerov sindrom je redak autozomno dominantni genetski poremećaj, varijanta familijarne adenomatozne polipoze. Karakteriše ga polipoza creva i različiti tumori kostiju i mekih tkiva, uključujući osteome, epidermalne i dermalne ciste, lipome i fibromatoze. Crevni polipi imaju visoki maligni potencijal, pa je redovna kontrola ovih lezija od strane gastroenterologa obavezna. Manifestacije u maksilofacijalnoj regiji, kao što su osteomi vilica, odontomi, zubne abnormalnosti i tumori mekog tkiva često prethode crevnim manifestacijama. Dakle, maksilofacijalni hirurzi i stomatolozi mogu imati važnu ulogu u ranom otkrivanju Gardnerovog sindroma. U ovom slučaju prikazujemo 22-godišnjeg muškog pacijenta koji je upućen na pregled maksilofacijalnog hirurga zbog osteoma donje vilice. Na osnovu kliničkog i radiološkog pregleda, kao i na osnovu anamnestičkih podaataka, dijagnostikovan je Gardnerov sindrom, što je potvđeno nakon pregleda gastroenterologa

Ključne reči: *gardnerov sindrom, ekstraintestinalna manifestacija, mandibularni osteom, lečenje*

INTRODUCTION

Gardner syndrome (GS) is a rare autosomal-dominant genetic disorder characterized by multiple colorectal polyps and various types of tumors of the skin, bones and glands (1-4). People affected by GS have a high risk of developing colorectal cancer at an early age as well as other familial adenomatous polyps related cancers: small bowel, stomach, pancreas, thyroid, central nervous system, liver, bile ducts, and adrenal gland. Extraintestinal symptoms of GS include dental abnormalities, odontogenic tumors, osteomas, various skin abnormalities such as epidermoid cysts, fibromas, lipomas and desmoid tumors (4).

The diagnosis of GS is in most cases based on clinical findings. The significance of GS to the oral and maxillofacial surgeons and dental practitioners is that the jaw lesions and dental abnormalities may be apparent before those in the bowel and could be of diagnostic importance (3, 4). Early detection of multiple jaw osteomas, odontogenic tumors, supernumerary teeth and multiple cutaneos cysts may lead to appropriate early detection of colon polyps. Since the syndrome is genetically inherited, molecular genetic testing is available and the diagnosis has implications for family members (5).

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UDK: 616-056.7:616.31
Ser J Exp Clin Res 2019; 20 (1): 89-91
DOI: 10.1515/SJECR-2017-0038

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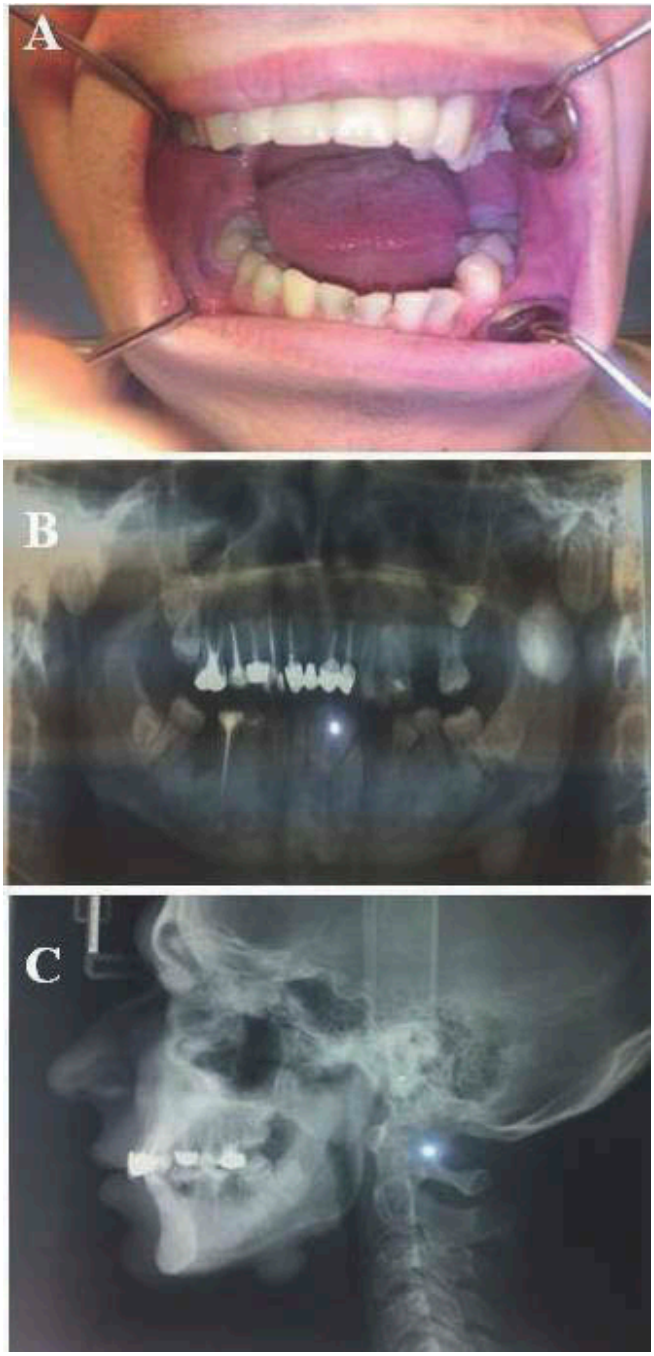

Figure 1. A. An intraoral appearance of patient; B. Panoramic radiograph showing multiple osteomas, impacted teeth, odontomas and diffuse sclerosis throughout the mandibular body; C. Profile view of the skull

Although there is no cure for GS, management options are available to reduce the risk of cancer. Thus colectomy is advised when more than 20 polyps are detected (6). Dental abnormalities may be treated, osteomas and epidermoid/dermoid tumors may be removed when causing cosmetic or functional disabilities (1, 2).

The aim of this case report is to present patient with GS and to discuss management of maxillofacial manifestations and need for screening intestinal lesions.

REPORT OF A CASE

A 22-year-old male was referred to the Department of Maxillofacial Surgery at Military Academy, Belgrade, Serbia, for the management of mandible osteomas and odontomas as well as dermoid cysts on the neck. In the patient's family history we found out that the patient mother died due to the colon carcinoma and that his sister has similar symptoms.

A panoramic radiograph showed the presence of multiple round radiopaque lesions in the mandible (corpus, ramus and condyle on the left side), impacted teeth and multiple odontomas in both maxilla and the mandible. There was a diffuse bone sclerosis throughout the mandibular body (Fig 1).

The patient main complaint was of cosmetic nature and the visually irregularity of the face. An initial clinical examination showed a hard palpable nodular formation along the left mandibular angle and in the temporomandibular joint region, each measuring approximately 1,5-2 cm in diameter. On palpation, the lesions were adherent to the bone, hard, well limited, and nonadherent to the skin. There were no disfunction of the temporomandibular joints. The mouth opening was normal. Intraoral examination revealed partial edentulous maxilla and mandible, rotated and inclinated teeth as well as multiple caries lesions. Examination of the neck revealed an dermoid cyst in the left supraclavicular region, measuring approximately 2 cm in diameter, well limited on palpation, nonadherent to deeper tissues and movable.

The excision of the osteomas of the left mandible condyle was performed via intraoral approach. Each osteoma measured approximately 1,5 cm. Histopathologic examination revealed a dense, acelular compact lamellar bone with minimal marrow spaces and rare irregular Haversian canals. The postoperative course was uneventful.

The patient was instructed that he and his family members need an examination for possible intestinal lesions and genetical testing. Because the suspicion the patient had GS, the gastroenterologist indicated colonoscopy which revealed multiple polyps in the colon and colectomy was indicated.

DISCUSSION

GS is considered a variant of familial adenomatous polyposis (FAP), in which various extracolonic manifestations develop. GS is caused by mutations in a portion of the APC gene (codons 1403 and 1578) that differs from classic FAP (codons 169-1600) (7). In all untreated patients, cancer of the large intestine develops before the age of 40 (8). This has implication for all family members if the diagnosis is positive. Although there is no cure for GS, people affected must undergo screening for the polyps and associated intestinal tumors to permit early diagnosis and treatment. Standard screening regimens include:

- Sigmoidoscopy or colonoscopy every one to two years, beginning at age ten to 12 years. Once polyps are detected, colonoscopy is recommended annually until colectomy;
- Eesophagogastroduodenoscopy beginning by age 25 and repeated every one to three years;
- Thyroid evaluation beginning in the late teenage years;
- Screening for desmoid tumors and hepatoblastoma (8-11).

Genetic testing is the most efficient mode of identifying gene mutations in family members and should be assessed before the initiation of regular endoscopic screening (12). Screening colonoscopy should begin at age 10-12 years for patients with proven APC mutations (13). A colectomy is usually recommended when multiple advanced polyps are identified.

The prognosis for people with GS depends on the presence of malignant alteration of colonic polyps and the age of diagnosis (14). By the fourth decade of life more than 95% of affected people have polyps (10). The polyps rapidly increase in number and without colectomy the colon cancer is inevitable (8). Thus, early diagnosis and management are mandatory. Osteomas in the facial bones and cranium are found in about 25% of patients affected by GS and frequently precede the diagnosis of colon polyps, which is important for early detection (15, 16). The most common localizations of osteomas are cranial vault and mandible (4, 17). Osteomas may be removed for cosmetic reasons, usually do not cause medical problems and do not become malignant.

The oral and maxillofacial manifestations of GS can show up before intestinal lesion polyposis which potentiate the role of maxillofacial surgeons in early diagnosis of GS (4). Various orofacial manifestations may be treated if there are cosmetic of functional issues. Dental abnormalities such as impacted teeth, malpositioned teeth, orthodontic anomalies may be treated surgically and orthodonticaly. Surgical treatment of dental abnormalities usually consists of surgical removal of supernumerary and impacted teeth and extirpation of odontogenic tumors. Surgeons should have in mind that there may be increased difficulty of teeth extraction in GS patients because of the dense nature of the alveolar bone and hypercementosis (2). The skin abnormalities associated with Gardner syndrome are mainly of cosmetic concern and do not become malignant (1).

To conclude, maxillofacial surgeons and dental practitioners may have important role in early detection of Gardner syndrome since osteomatous and various dental lesions frequently precede intestinal manifestations of this rare syndrome. Furthermore, these patients as well as their family members should be instructed for genetical testing and screening for intestinal lesions due to the fact that all affected patients develop intestinal polyps which become malignant in high percentage.

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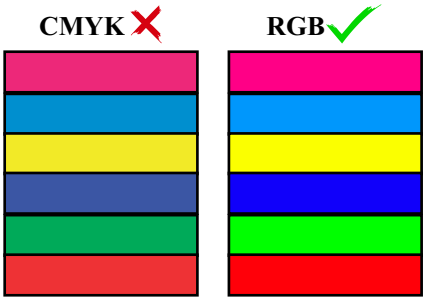

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SERBIAN Journal of Experimental and Clinical Research
editor-in-chief Vladimir Jakovljević.
- Vol. 9, N° 1 (April 2008) -
- Kragujevac (Svetozara Markovića 69) :
Medical Faculty, 2008 - (Kragujevac : Medical Faculty). - 29 cm

Je nastavak: Medicus (Kragujevac) = ISSN 1450-7994
ISSN 1820-8665 = Serbian Journal of
Experimental and Clinical Research
COBISS.SR-ID 149695244

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Serbian Journal of Experimental and Clinical Research

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