EABR
Experimental and Applied Biomedical Research
ISSN 2956-0454
eISSN 2335-075X
VOL 24 No 1
March, 2023.

REVIEW PAPER

CASE REPORT

ORIGINAL SCIENTIFIC ARTICLE

sciendo

ISSN 2956-0454

Print

Faculty of Medical Sciences, University of Kragujevac

Indexed in

EMBASE/Excerpta Medica, Index Copernicus, BioMedWorld, KoBSON, SCIndeks, Chemical Abstracts Service, Cabell's Directory, Celdes, CNKI Scholar (China National Knowledge Infrastructure), CNPIEC, EBSCO Discovery Service, Elsevier - SCOPUS, Google Scholar, J-Gate, Naviga (Softweco), Primo Central (ExLibris), ReadCube, SCImago (SJR), Summon (Serials Solutions/ProQuest), TDOne (TDNet), WorldCat (OCLC)

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https:/medf.kg.ac.rs/eabr
https://sciendo.com/journal/SJECR

EABR is published four times annually

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

61

EABR : Experimental and Applied Biomedical Research / editor in chief Vladimir Zivkovic. - Vol. 24, no. 1 (mar. 2022)- . - Kragujevac : Faculty of Medical Sciences, University of Kragujevac, 2022- (Kragujevac : Faculty of Medical Sciences, University of Kragujevac). - 30 cm

Tromesečno. - Je nastavak: Serbian Journal of Experimental and Clinical Research = ISSN 1820-8665
ISSN 2956-0454 = EABR. Experimental and Applied Biomedical Research
COBISS.SR-ID 81208329

TABLE OF CONTENTS

THE ROLE OF HEALTH SYSTEMS IN EMERGENCY RESPONSE PLANNING

Vladimir Dobricanin[1], Nebojsa Djokic[2] and Sanja Dobricanin[2]

[1] *Clinical Centre of Montenegro, Medical Faculty University of Montenegro, Podgorica, Montenegro*
[2] *University of Pristina, Faculty of Economy, Kosovska Mitrovica, Serbia*

Received: 05.02.2018.
Accepted: 22.04.2018.

Corresponding author:

Vladimir Dobricanin

Miladina Popovica 9, 81000 Podgorica, Montenegro

Phone: +382 69 385770

E-mail: vladimir.dobricanin@kccg.me

sciendo

UDK: 614.2:616-083.98
Eabr 2023; 24(1):3-6
DOI:10.2478/sjecr-2018-0011

ABSTRACT

The history of mankind is pervaded by many events that have had an incredible influence on the further development of civilization. Unfortunately, many historical milestones have also been marked by unimaginable disasters that have sometimes threatened the very existence of the human race. One of the most important links in the chain of readiness in emergencies and disasters is the national health system with all its institutions at the primary and secondary levels of healthcare. Their capacities and capabilities for adequate response can be significantly enhanced by the development and implementation of appropriate Emergency Response Plans (ERPs). The necessity of ERPs is considered for several important elements: to protect lives, property and the environment; to mitigate the consequences caused by emergencies and disasters and ensure the con-tinuity of general health services; to create a system and network to respond to and recover from the consequences of emergencies and disasters; to use of available resources optimally; and to provide additional resources if needed and improve the cooperation between sectors and agencies. The overall quality of a healthcare system is also measured by its readiness to respond to mass casualty events, emergencies and disasters. The lack of an ERP, a poor ERP, or a nonunderstandable or inaccessible plans leads to partial preparedness that can cause unimaginable consequences to overall health and loss of life of the population.

Keywords: *Emergency, disaster, planning, role, health system.*

INTRODUCTION

A disaster, also called a "calamity" or "catastrophe", is a sudden, devastating event that causes serious disruption of the functioning of a community or society with widespread human, material, economic and/or environmental losses that exceed the ability of the affected community or society to cope using its own level of resources (1).

Modern history, archaeology, palaeontology, medicine, and overall science on a daily basis reveal many pieces of evidence of events that have changed the world at one point of time in history. Today, we are aware that entire civilizations have disappeared in front of the fury of nature.

Over a historical timeline, we have recorded thousands of disasters. Some of them are of local character, whereas others overcome all boundaries and their influence is not only global in a geographical sense but their consequences have been felt by many generations.

The Great Flood of central China in 1931 took ~2.5 million lives (2). For the Great Flood of the Yellow River (Huang He/Hwang Ho) in China in 1887, the number of victims was ~2 million (3). An earthquake in Shaanxi province had an influence on the further development of civilization. Unfortunately, many other historical milestones have also taken place in China, such as in 1556, which resulted in 830,000 victims (4). An earthquake in the Tangshan province of China in 1976 resulted in ~665,000 victims (5). An earthquake in Antioch (Turkey) resulted in 300,000 dead (6). On December 26, 2004, following a strong earthquake in the Indian Ocean, the resulting tsunami took ~230,000 lives in Indonesia. Just a few years later, in January 2010 in Haiti, ~230,000 people were affected by an earthquake (7). Natural disasters are not the only cause of loss of life. Hunger, as one of the four horsemen of the apocalypse, follows the footsteps of humanity even today. From 1958 to 1961, ~43 million people died of starvation in China (8). More than 8.5 million people died of starvation in Russia (USSR) from 1932 to 1933 (Holodomor) (9).

Infectious diseases have taken their time in the overall history of human suffering. From 1300 to 1720, more than 100 million people died from the plague (Black Death) in Europe, Asia and Africa. The measles have taken more than 200 million lives in the last 150 years (10). The smallpox epidemic from 1900 to May 8, 1980, when eradication was proclaimed, claimed ~300 million victims (11).

Malaria in the 20th century accounts for ~250 million victims. In two years (1918-1920), the Spanish fever killed more than 100 million people worldwide (12-14). TBC in the 20th century has taken ~100 million human lives, and AIDS has taken 25,250,000. Even the seasonal flu causes ~250,000 human deaths each year.

In World War I, ~20 million people were killed. In World War II ~60 million people were lost. The An Shi rebellion in China (755-763) resulted in ~36 million deaths. The Mongolian conquest (1207-1727) took ~60 million lives. The conquest of North and South America (14921900) resulted in ~50 million victims (15). The European colonization of Africa and Asia (1758-1970) accounted for some 60 million lives (16). Slavery from the 16th until the 19th century resulted in 2.4 million victims (15).

By paying attention to the information in the last few paragraphs, we can form an impression of the enormous suffering of humanity throughout history, not only from natural disasters but also from hunger, diseases and local and global wars. Being informed by these experiences, we can conclude that there is a necessity to create an Emergency Response Plan (ERP) that can be used during such emergencies and disasters.

One of the most important links in the chain of readiness in emergencies and disasters is the national health system, with all its institutions at the primary and secondary levels of healthcare. Their capacities and capabilities for adequate responses can be significantly enhanced by the development and implementation of an appropriate ERP.

THE HEALTH SYSTEM AND EMERGENCY RESPONSE PLAN

An ERP is an agreed upon set of guidelines for responding to and recovering from disasters and emergencies. Why it is necessary to develop such a plan is considered in several important elements:

- Protect lives, property and the environment;
- Mitigate the consequences caused by emergencies and disasters as well as ensure the continuity of general health services;
- Create a system and network to respond to and recover from the consequences of emergencies and disasters;
- Optimal use of available resources;
- Provide additional resources, if needed;
- Improve the cooperation between sectors and agencies (The Cluster Approach) (17).

The overall quality of a healthcare system is measured also by its readiness to respond to mass casualty events, emergencies and disasters. The lack of an ERP, a poor ERP, or a no understandable or inaccessible plan leads to partial preparedness that can cause unimaginable consequences to overall health and loss of life of the population. A health sector ERP must deal with all aspects of public and environmental health, which includes:

- Casualty management (first aid, triage, transport, prehospital care, and inpatient and outpatient care)
- Communicable disease control (surveillance, tracking, treatment, prophylaxis, isolation and quarantine)
- Continuity of delivery of critical services for all emergency patients

- Management of the dead and missing (disposal, identification and specimen collection, and tagging)
- Management of information (public information, support activities, and health information system)
- Mental health
- Environmental health
- Reproductive health
- Public healthcare programmes (continuity of essential programmes) (17).

BASIC PRINCIPLES OF EMERGENCY RESPONSE PLANNING

The development of an ERP is a *continuous process*. This process is time consuming and cannot be completed in a short period of time. Actually, an adequate ERP is a *never ending story* because it requires constant upgrading and development so it can stay up to date regarding all of its contents. All Emergency Response Plans are 80% generic to all disasters, 15% specific to the hazard and 5% unique to the event. One of the main principles is that the ERP is developed to *attempt to reduce the unknown*. The risk is defined as the probability and consequences of exposure to a hazard. If any potential threat to public health and safety-hazard is well-defined, then we can approximately calculate the risk. By using modern technologies, we can build mathematical models of potential disasters and emergencies and, according to the results, develop an ERP. This is the way by which we can *focus on what is likely to happen* and what will *evoke appropriate actions,* which can lead to possible prevention and mitigate the potential consequences to the population and environment. The ERP also *defines the starting point for response and recovery,* which are the two most delicate actions during emergencies and disasters. They are also the biggest budget and time-consuming processes that require many highly specialized, well-equipped and trained manpower. The development of an ERP is actually a *dynamic learning process* that requires constant learning and gruelling trainings so that responders can give an expected response (18).

KEY COMPONENTS OF EMERGENCY RESPONSE PLANNING

Establishing the line of authority and responsibility of all stakeholders is one of the most key components of an ERP. This will provide an adequate chain of command that is essentially required during emergencies and disasters. Defining the management structure is also necessary so that the authorities can identify, establish and implement the command and coordination mechanisms. These key components are also provisions for the management of logistics and resources, establishing communication and information systems, designating infrastructure and equipment, defining reporting and accounting processes and implementing training and exercises (19).

THE ROLE OF MINISTRY OF HEALTH (MOH) IN EMERGENCY RESPONSE PLANNING

Developing a risk assessment and risk management framework is the first key role of the MoH with subsequent development of integrative planning strategies and national policies. The MoH is responsible for the coordination and cooperation between relevant national and international agencies and coordination of international cooperation and assistance. Deployment of resources and allocation of funds to support expansion of surge capacities in a healthcare system is the responsibility of the MoH. The evaluation of national and community emergency preparedness by the MoH leads towards efficient vulnerability reduction and hazard mitigation; thus, the MoH is responsible for overall medical emergency preparedness.

There are different responsibilities of the MoH regarding the national and provincial/community level. On the national level, the responsibilities of the MoH are to develop policy, guidelines, and standards; mobilize reinforcement of resources; coordinate national and international assistance and evaluate emergency preparedness plans. On the provincial/community level, the responsibilities are to develop emergency preparedness plans; enhance local surge capacity; train and exercise medical teams; and manage information, communication and logistics systems (17).

CONCLUSIONS

We are witnessing global weather changes that are causing excessive loss of life worldwide. Natural disasters are one of the most threatening hazards for humankind. Despite these facts, there have been over 100 significant military conflicts since WWII. Old and newly discovered diseases demand our constant attention and action. These are the main reasons why we ultimately need an Emergency Response Plan so that we can increase public safety and health through prevention, mitigation, efficient response and excessive recovery from emergencies and disasters.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None.

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EFFECTS OF SULFUR AMINO ACIDS ON CARDIODYNAMIC PARAMETERS OF ISOLATED RAT HEART

Nikola Sobot[1], Tanja Sobot[2], Katarina Radonjic[3], Tamara Nikolic Turnic[3], Anica Petkovic[3], Jovana Jeremic[3], Nenad Ponorac[2], Tatjana Lazarevic[4], Sergey Bolevich[5], Vladimir Jakovljevic[5,6] and Dragan Djuric[7]

[1]*Clinic for Cardiac Surgery, University Clinical Centre of the Republic of Srpska, Bosnia and Herzegovina*
[2]*University of Banja Luka, Faculty of Medicine, Department of Physiology, Bosnia and Herzegovina*
[3]*University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Serbia*
[4]*University of Kragujevac, Faculty of Medical Sciences, Department of Internal Medicine, Serbia*
[5] *I.M. Sechenov First Moscow State Medical University (Sechenov University), Department of Human Patholog, Russia*
[6]*University of Kragujevac, Faculty of Medical Sciences, Department of Physiology, Serbia*
[7]*University of Belgrade, Faculty of Medicine, Institute of Medical Physiology „Richard Burian“, Serbia*

Received: 30.06.2020.
Accepted: 01.07.2020.

Corresponding author:

Dragan Djuric, MD, PhD

University of Belgrade, Faculty of Medicine,
Institute of Medical Physiology „Richard Burian“,
Serbia

E-mail: dr_djuric@yahoo.com

UDK: 612.173:577.112.386
Eabr 2023; 24(1):7-15
DOI:10.2478/sjecr-2020-0025

ABSTRACT

Sulfur-containing amino acids are integral part of molecular mechanisms which underlie many aspects of cellular function and homeostasis, facilitated by reversible changes in oxidation states of sulfur atoms. Dysregulation of these pathways is associated with diverse pathologies, notably of the cardiovascular system, which are typically characterized by inappropriate plasma levels of sulfur-containing amino acids. The aim of this study was to assess the acute, direct effects of sulfur-containing amino acids and inorganic NaHS, as H_2S donor, on cardiodynamic parameters in homocysteine treated rats. Moderate hyperhomocysteinemia did not cause significant decrease in myocardial contractility, but our findings suggest that NaHS and L-methionine cause negative effects on cardiac function in hearts of the rats treated with homocysteine, even in a single administration. Further investigations need to be carried out with purpose of better understanding and highlightening the impact of Hcy and sulphur amino acids on cardiac function.

Keywords: *Sulfur amino acids, L-methionine, L-cysteine, N-acetylcysteine, hiperhomocysteinemia, heart function.*

INTRODUCTION

Sulfur-containing amino acids are integral part of molecular mechanisms which underlie many aspects of cellular function and homeostasis, facilitated by reversible changes in oxidation states of sulfur atoms. Sulfur-containing amino acids are metabolically linked by interacting pathways that affect the one-carbon metabolic cycle and generation of methyl groups, the folate cycle, and maintenance of the major intracellular redox buffer, glutathione. Dysregulation of these pathways is associated with diverse pathologies, notably of the cardiovascular (CV) system, which are typically characterized by inappropriate plasma levels of sulfur-containing amino acids (1). Among the four common sulfur amino acids (SAAs) - methionine (Met), cysteine (Cys), homocysteine (HCy), and taurine - only Met and Cys are proteinogenic. In addition to their roles in protein synthesis, Met and Cys also play a variety of essential roles in cells and tissues. Of the two, only Met is considered an essential amino acid, as it cannot be synthesized de novo in the body, whereas Cys can be produced from Met and serine in the liver and other tissues. While dietary sources are required only for Met, both Met and Cys are common constituents in most dietary proteins (2).

The two other sulfur-containing amino acids, Hcy and taurine, are intermediates formed from methionine and cysteine metabolism respectively, that have very different structural and hence functional properties. Hcy can be considered a homologue of cysteine, differing only by anadditional methylene (-CH_2-) group. Although not incorporated into proteins, both Hcy and taurine impact upon cellular functions and have significant effects upon CV health. Indeed, high levels of plasma Hcy have been shown to be an independent risk factor in the development of many CV disorders (CVDs) (3).

Met is an essential sulfur-containing amino acid. It has a crucial role in one-carbon metabolism cycle by providing methyl groups for the synthesis of biomolecules such as DNA, lipids, and proteins (4). Met metabolism is the only source for Hcy in mammals (5). Excess of Hcy is eliminated through remethylation to methionine and transsulfurylation pathway. Given that these processes require B6, B12, and folate as cofactors, lack of these vitamins leads to hyperhomocysteinemia (HHcy) (6).

Although HHcy is recogmized as CVD risk factor due to its correlation with endothelial dysfunction and oxidative stress, it still remains unclear in which content and by what mechanisms HHcy induces cardiovascular disorders and how thay could be prevented (7). It is usually assumed that reduced Hcy is the atherogenic form of Hcy in circulation. Due to the high reactivity of its thiol group, it undergoes oxidation, producing hydrogen peroxide, superoxide anion radical, and other reactive oxygen species, as an injurious agents. Only about 1% of Hcy is available as reduced form (8).

Cys also plays numerous important roles as a key extracellular reducing agent and rate-limiting precursor for glutathione and taurine synthesis (9). Contrarily, it exerts cytotoxicity in vitro, exhibits autooxidation properties, and generates free radicals (10). Few studies demonstrated an independent association between cysteinemia, CVD, and atherosclerotic lesions in hyperlipidemic patients (11). Cys is positively related to increased cholesterol, diastolic blood pressure, and BMI (12). In coronary heart disease, high concentrations are noticed, depending on the associated levels of Hcy. Certain authors showed that the concentration of Hcy-Cys mixed disulfide after Met load was slightly higher in coronary heart disease patients (13). In addition to protein synthesis, Cys is also utilized in the production of a number of important cellular agents, including GSH, hydrogen sulfide (H2S), taurine, and sulfate (14-16).

On the other hand, N-acetyl-l-cysteine (NAC) is widely recognized as a powerfull antioxidant which has an anti-inflammatory effects in different tissues (17, 18). During the last decade, numerous in vitro and in vivo studies have suggested that NAC has beneficial medicinal properties, including the inhibition of carcinogenesis, tumorigenesis, and mutagenesis, as well as the inhibition of tumor growth and metastasis. Although NAC is an excellent scavenger of free radicals and chelator of heavy metal, it remains unclear whether this compound is effective protector in induced oxidative stress (19).

Inorganic sodium hydrogen sulphide (NaHS) is an exogenous hydrogen sulphide (H_2S) donor. H_2S has been identified as a gas signalling molecule in the body, and has previously been shown to have vasorelaxant properties (20). H_2S has been reported to function as an antioxidant and is able to improve cardiac function, limit leukocyte adhesion and to enhance angiogenesis in a number of animal models (21). Similarly, hydrogen sulfide has also been shown to attenuate hyperhomocysteinemia-induced cardiomyocytic endoplasmic reticulum stress in rats (22). Endogenous H_2S can protect cardiomyocytes via varied mechanisms, such as protection against oxidative stress and reduction of mitochondrial damage (23). A previous study has revealed the role of H_2S in cardioprotective signaling pathways that converge toward the mitochondria (24, 25). NaHS is an important exogenous donator of H_2S.

Therefore, the main objective of this study was to assess the acute, direct effects of sulfur-containing amino acids (L-meth, L-cys, NAC) and inorganic NaHS on the cardiodynamic parameters in hyperhomocysteinemic rats.

MATERIALS AND METHODS

The present study was carried out using 80 adult male *Wistar* albino rats (16-week-old body mass 300-400 g). Animals were housed under controlled environmental

conditions, with a temperature of 25°C and a 12-h light/dark cycle and they had ad libitum access to food and water (they were obtained from the Military Medical Academy, Belgrade, Serbia).

The animals were divided into two groups: control and experimental group with induced HHcy. HHcy in rats was induced by subcutaneous injection of DL-homocysteine, in dose of 0.45 μmol/g of body weight, twice a day (every day at the same time), for a period of 2 weeks. After that, HHcy was confirmed by the high-performance liquid chromatography analysis of the blood (HPLC method). The blood for the determination of Hcy (3 ml) was collected into the tubes without the addition of anticoagulants, during the sacrifice of the animals, after which it was centrifuged to separate the serum. After centrifugation and separation, they were frozen at -20 °C within one hour of serum extraction. Hcy was measured on the ACL Elite Pro apparatus, (HPLC) according to the manufacturer's instructions.

Each group was divided into five subgroups (8 animals in each subgroup), depending on the acute treatment with sulfur-containing compounds (in same single dose of 0.5 mmol/l):

1. Control subgroup (acute administration of distilled water)
2. L-Meth subgroup (acute administration of L-meth)
3. L-Cys subgroup (acute administration of L-cys)
4. NAC subgroup (acute administration of NAC)
5. NaHS subgroup (acute administration of NaHS)
6. Control+DL-Hcy subgroup (acute administration of distilled water)
7. L-Meth+DL-Hcy subgroup (acute administration of L-meth)
8. L-Cys +DL-Hcy subgroup (acute administration of L-cys)
9. NAC +DL-Hcy subgroup (acute administration of NAC)
10. NaHS+DL-Hcy subgroup (acute administration of NaHS)

Isolated rat heart preparation and experimental protocol

The hearts of rats were excised on the 15th day from the beginning of research and perfused using a Langendorff apparatus (Experimetria Ltd, 1062 Budapest, Hungary). After a short ketamine/xylazine-induced narcosis the rats were sacrificed by cervical dislocation, where blood samples were collected. Following a quick thoracotomy and rapid cardiac arres by superfusion with ice-cold isotonic saline, the hearts were promptly excised and attached to the Langendorff apparatus via aortic cannulation. The hearts were retrogradely perfused under a constant perfusion pressure (CPP) of 70 cm H_2O with complex Krebs-Henseleit solution composed of the following (in mmol/L): NaCl 118, KCl 4.7, $CaCl_2 \times 2H_2O$ 2.5, $MgSO_4 \times 7H_2O$ 1.7, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, pyruvate 2, equilibrated with 95 % O_2 plus 5 % CO_2 at 37ºC (pH 7.4). Immediately after the establishment of automatic operation by opening the left atrium of the heart and dissecting the mitral valve, the sensor was inserted (*transducer BS4 73-0184, Experimetria Ltd, Budapest, Hungary*) into the left ventricle for continuous registration of myocardial function.

The hearts from all study groups were undergone to a 25 minutes stabilization period. Coronary flow (CF) was determined flowmetrically. When the CF was stabilised (three repeated measurements of the same value), samples of coronary effluent were collected (control value - C), and the experimental protocol was initiated. In order to assess the effects of acute administration of L-meth, L-cys, NAC and NaHS, the hearts were perfused individually with each of the mentioned substances, in same single dose of 0.5 mmol/l, during the 5 minutes. During the last minute of acute treatment, a sample of coronary venous effluent was collected (effect value). After acute treatment, followed the wash-out period of heart. At the end of this period, a sample of coronary venous effluent was collected (wash-out value). Using the sensor within the left ventricle, the following parameters of myocardial function were determined:

1. The maximum rate of pressure development in the left ventricle (dp/dt max)
2. The minimum rate of pressure development in the left ventricle (dp/dt min)
3. The systolic left ventricular pressure (SLVP)
4. The diastolic left ventricular pressure (DLVP)
5. The heart rate (HR)

In the experimental work were respected the provisions prescribed acts (EU Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes 86/609/EEC) and the principles of ethics. The experimental protocol was approved by the Ethics Committee for the welfare of experimental animals, the Faculty of Medical Sciences of the University of Kragujevac.

Drugs

All drugs used in this experimental protocol were provided by Sigma-Aldrich, Germany.

Statistical analysis

Statistical analysis of experimental data included the following basic descriptive statistics: the mean value (x), standard deviation (SD) and standard error mean (SEM). For testing the normality of the distribution parameters, the Kolmogorov-Smirnov test was used. To test the statistical significance of the results and to confirm the hypothesis, the following statistical tests were used: Student's t test (parametric test), for dependent and independent variables. A database analysis of the results was performed using software package SPSS 20 (SPSS Inc., Chicago, IL, USA). A p value ≤ 0.05 was considered statistically significant.

RESULTS

Concentrations of homocysteine in the control groups of animals (untreated with homocysteine) were within the reference values, with an average value of 8.11 μM/L. In Hcy-treated group average concentration of homocysteine was 5,66 μM/L.

3.1.The effects of sulphur amino acids on the cardiodynamic parameters and coronary flow in isolated rat heart in control groups

3.1.1. The effects of acute perfusion of L-methionine

Values of dp/dt max, dp/dt min, SLVP, HR and CF maintained almost constant during the experiment (control, effect, wash-out period), except values of DLVP which were significantly increased in wash-out period (Figure 1).

3.1.2. The effects of L-cysteine

Similarly, during the whole acute experiment, values of all parameters of cardiac function, except DLVP insignificantly differ. Use of single dose of this amino acid induced increase in values of all parameters compared to L-methionine group (Figure 1).

3.1.3. The effects of NAC

NAC administration induced insignificantly change of almost all parameters during the experiment, except for dp/dt max and dp/dt min which significantly decreased during acute perfusion. During wash-out period dp/dt max and dp/dt min reached values similar to control period (Figure 1).

3.1.4. The effects of NAHS

Acute administration of NaHS significantly depressed dp/dt max and SLVP in rats compared to the effects of control group. Our results showed that coronary flow was significantly increased during perfusion period compared to application of other amino acids (Figure 1a, 1c, 1f).

3.2. The effects of sulphur amino acids on the cardiodynamic parameters and coronary flow in isolated rat heart in Hcy-treated group

3.2.1. The effects of DL-Hcy + L-methionine

L-methionine in HHcy rats induced almost similar values of dp/dt max and CF during whole experiment found in control group. We also noticed increased values of systolic and diastolic blood pressure compared to untreated rats. Met caused depression of heart rate in comparison with control group (Figure 2).

3.2.2. The effects of DL-Hcy + L-cysteine

In rats subchronically treated with homocysteine we noticed statistically decreased values of dp/dt max, dp/dt min, SLVP compared to untreated animals. Heart rate and CF were preserved, with similar values found in control group (Figure 2a, 2b, 2c, 2e, 2f).

3.2.3. The effects of DL-Hcy + NAC

Administration of NAC induced elevated values of dp/dt max, dp/dt min, SLVP and CF compared with untreated rats, during the whole experiment. In contrast, DLVP values were decreased while HR maintained almost constant values with control group (Figure 2).

3.2.4. The effects of DL-Hcy + NaHS

Values of all cardiodynamic parameters except HR and CF were decreased during the period of experiment with high statistical significance. HR and CF were depressed during application of amino acid compared with untreated rats, while values of coronary flow were similar like those in control group (Figure 2).

DISCUSSION

Methionine is essential, sulfur-containing amino-acid that has an important role in one-carbon metabolism. Methionine is converted to its active form, S-adenosylmethionine (SAM), the major donor of methyl-group in the numerous intracellular transmethylation reactions. SAM is converted to S-adenosylhomocysteine (SAH), which is intermediate of all transmethylation reactions. SAH is hydrolyzed by a reversible enzyme SAH-hydrolase to Hcy and adenosine (26). Homocysteine is sulphurated, non proteinogenic amino acid exclusively derived from ingested methionine in the reaction of demethylation. Homocysteine-methionine cycle plays a crucial role in maintaining the biochemical balance by methylation reactions within the whole body (27). Hcy is normally metabolized via two biochemical pathways-remethylation, which converts Hcy back to methionine and transsulfuration that result in the conversion of Hcy to cysteine and taurine. Under normal conditions, approximately 50% of Hcy is remethylated to form Met. There are two distinct routes of remethylation of Hcy back to Met to complete the methyl cycle. Under normal conditions, Hcy levels are maintained in a narrow range (5-15 μM) as a result of balance between remethylation and transsulfuration processes (28). In our study, rats were subchronically treated with homocysteine in order to induce hyperhomocysteinemia. Our results showed that concentrations of homocysteine in the control groups of animals (untreated with homocysteine) were within the reference values, as well as in Hcy-treated group. Concentrations in our study are close to the lower limit, but still not enough to classify it as HHcy. Nevertheless, some changes observed in cardiac function might be due to these levels of Hcy. There was no statistically significant change after Met

load, which is in line with findings of Deutz and coworkers (29).

Cysteine, the limiting amino acid for GSH synthesis, is a sulfur-containing amino acid that plays an important role as an extracellular reducing agent. Cys is the rate-limiting precursor of intracellular GSH synthesis. Cys is also an independent risk factor for occlusive vascular disease. Autoxidation of Cys in vitro promotes several processes considered to be involved in atherogenesis and thrombogenesis (30). Cys supported superoxide-mediated modification of LDL, which might facilitate foam cell formation. A significant relationship between plasma total Cys and cardiovascular disease after adjustment for tHcy, creatinine, and other cardiovascular disease risk factors has been observed, suggesting that Cys may increase the risk of cardiovascular disease (31). Although Cys and NAC have the similar structure and both are precursors of GSH, many data from literature describe their opposite effects on cardiovascular system. However, it is known that sulfur amino acids (Hcy, Cys, NAC) and GSH can act as a antioxidant and pro-oxidant by scavenging hydroxyl radicals or by generating thyol radicals, respectively (32). The type of reaction depends on the composition of the medium in which the reactions take place and on the levels of each component. Excessive Hcy has pro-oxidant potential and physiological levels of Cys antagonize the oxidative damage (33). Our results showed that acute perfusion of isolated rat heart with L-cysteine obtained from untreated rats did not induce significant changes of almost all cardiodynamic parameters, except diastolic left ventricle pressure (Figure 1). On the other hand, in Hcy-treated group we noticed that acute application of Cys induce depression of cardiac contractility and systolic pressure, while frequency and coronary circulation obtained unchanged compared to control group (Figure 2).

Antioxidant treatment (N-acetylcysteine) has been shown to reduce dysfunction and damage to the myocardium caused by induced oxidative stress due to hyperomocysteinemia (34). On the other hand, a number of pharmacologically active substances from several pharmacological groups affect the level of homocysteinemia by different mechanisms. Three pharmacologically active sulfhydryl compounds reduce the level of homocysteinemia. These are: dimethylcysteine (D-penicillamine), metallic exchange agent for use in the treatment of rheumatoid arthritis, N-acetylcysteine, mucolytic agent and 2-mercampoietane sulfonate, chemotherapeutic protector. All compounds of this type have a free sulfhydryl group and are capable of forming disulfides in plasma, and in the chemical interaction of this type also enter homocysteine, lowering the concentration of the same (35).

Supplementation with sulfuric amino acids is gaining importance as an important adjuvant therapy in the prevention of diabetes and cardiovascular disease. Numerous studies report damaging the amino acid metabolism in diabetes and the beneficial effects of these amino acids that are positively correlated with the rise in levels of these amino acids in plasma. In addition, it is known that oxidative stress plays a major role in the pathophysiology of cardiovascular diseases, and it is exactly the addition of sulfuric amino acids to be mentioned as a potential solution in reducing the level of oxidative stress induced by pathological disorders. Experimental and clinical studies mention the modulatory effects of cysteine, N-acetyl cysteine and cysteine residues in the reduction of oxidative stress and damage due to this (36). We found that administration of NAC in HHcy rats induced elevated values of dp/dt max, dp/dt min, SLVP and CF compared with untreated rats, during the whole experiment (Figure 2). Several studies that assessed the effects of NAC in heart demonstrated antioxidant effects and attenuation of cardiomyocytes damage during heart failure (37). This is a possible explanation of improved cardiac function in this study after administration of NAC.

Sodium hydrogen sulphide (NaHS) is a compound formed by the coupling of hydrogen sulfide (H_2S) and an alkali metal base. It is especially important as a donor of hydrogen sulphide, a gaseous transmmiter with potential abilities to lower the level of oxidative stress but also as a promising potential therapeutic agent in the treatment of many vascular diseases (38). Hydrogen sulphide is also the metabolite of homocysteine, so studies show that exogenous sodium hydrogen sulphide as a donor of endogenous hydrogen sulfide significantly affects the level of homocysteine in the blood, on the vascular tone, and on the reduction of damage to the blood vessel wall (39, 40). Treatment with sodium hydrogen sulphide in experimental studies in an isolated heart affects the functional characteristics of the myocardium, reducing the heart rate and DLVP (41). We obtained similar findings in our research, with significantly decreased values of all cardiodynamic parameters during the period of experiment (Figures 1 and 2).

Figure 1. Effects of sulfur amino acids (L-methionine, L-cysteine, NAC) and NaHS on cardiodynamic parameters and coronary flow in healthy rats. Data are expressed as mean ± SD (Standard Deviation). The values were measured in three period times (C - control, E - effect, W - wash-out)

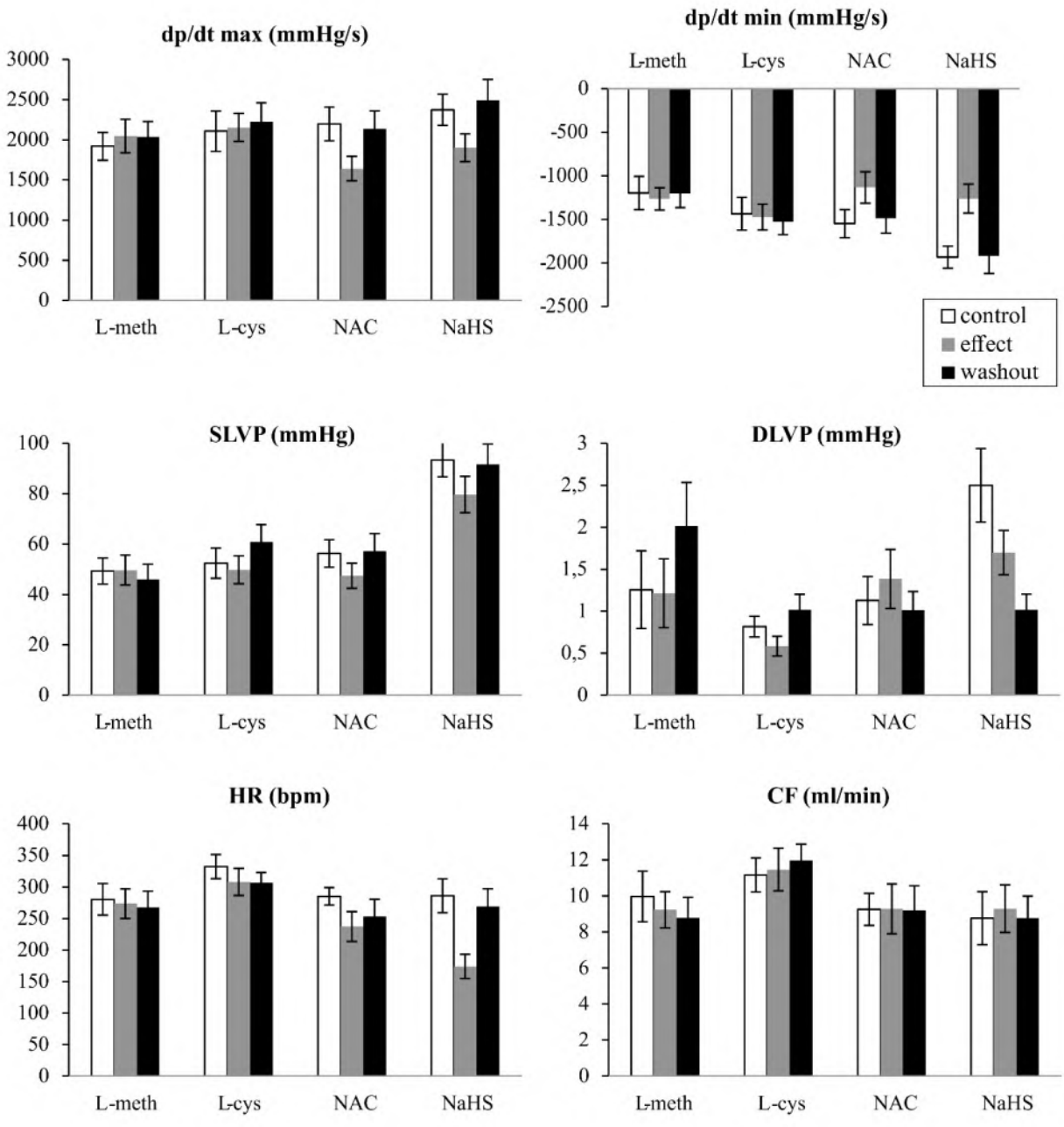
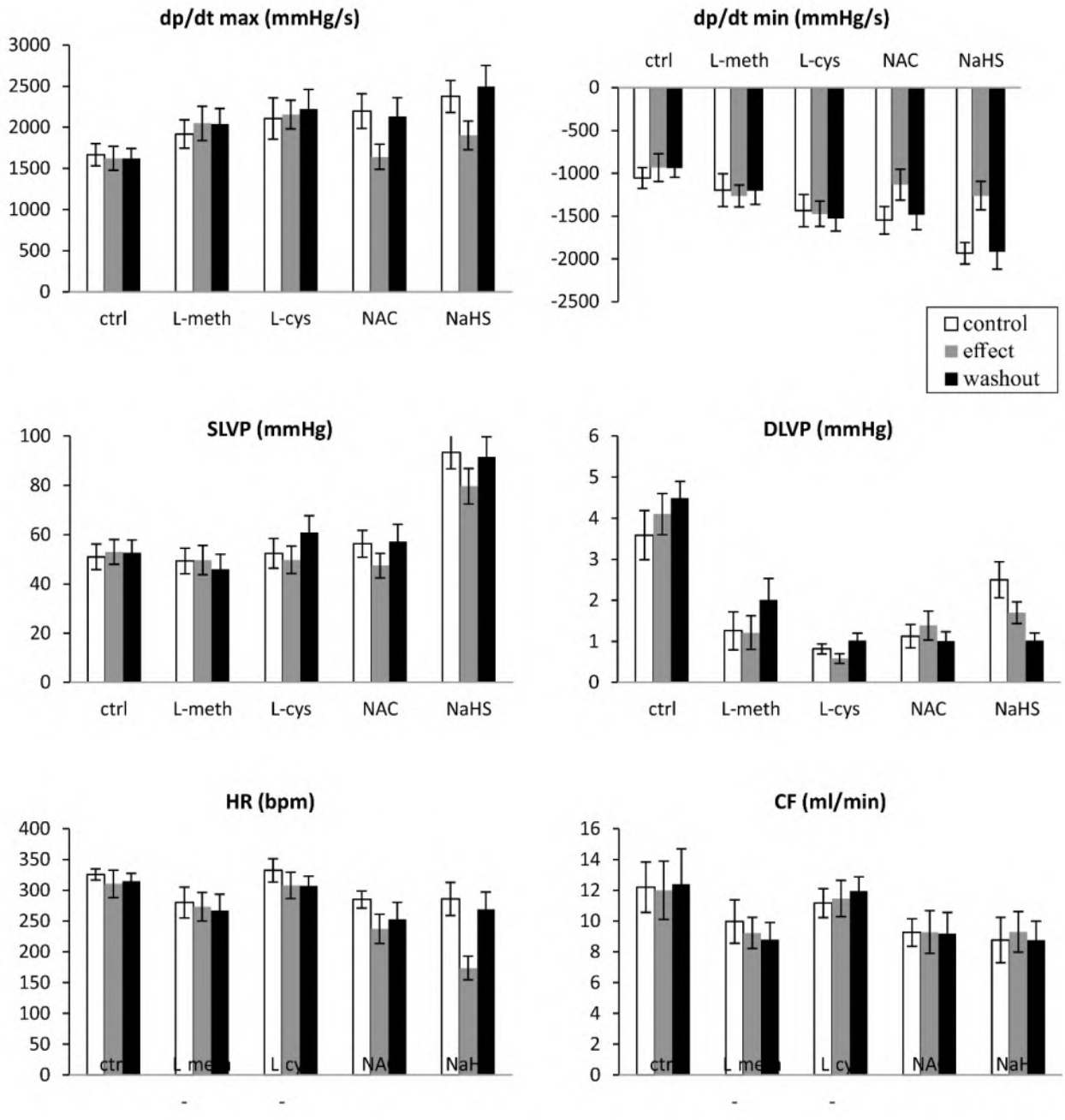


Figure 2. Effects of sulfur amino acids (L-methionine, L-cysteine, NAC) and NaHS on cardiodynamic parameters and coronary flow in Hcy treated rats. Data are expressed as mean ± SD (Standard Deviation). The values were measured in three period times (C - control, E - effect, W - wash-out)



CONCLUSION

Moderate hyperchomocysteinaemia did not cause a decrease in myocardial contractility, whereas severe hyperhomocysteinemia exhibited a negative effect on myocardial function and caused diastolic myocardial dysfunction. Our results suggesting that the NaHS and L-meth causes negative effects on cardiac function in HHcy hearts even in a single administration. Further investigations need to done with purpose of better understanding the impact of Hcy on cardiac function.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 175043), and the Faculty of Medical Sciences, University of Kragujevac (Junior Project 09/2011).

ETHICS APPROVAL

All research procedures were carried out in strict accordance with the European Union Directive for the welfare of laboratory animals (No. 2010/63/EU).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None.

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PERIPHERAL NERVE BLOCK VERSUS SPINAL ANESTHESIA FOR TOTAL KNEE REPLACEMENT IN ELDERLY PATIENTS

Milovan Vukotic[1], Aleksandra Vukotic[2], Zoran Bascarevic[3,4] and Nebojsa Videnovic[5]

[1]*Department of Anesthesia, Reanimatology and Intensive Care, Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia*
[2]*Clinic for Anesthesiology and Critical Care, University Hospital Center „Dr Dragisa Misovic-Dedinje", Belgrade, Serbia*
[3]*Department of Orthopedic Surgery, Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia*
[4]*University of Belgrade, Medical Faculty, Belgrade, Serbia*
[5]*University of Pristina in Kosovska Mitrovica, Medical Faculty, Kosovska Mitrovica, Serbia*

Received: 13.11.2019.
Accepted: 11.01.2020.

Corresponding author:

Milovan Vukotic

Department of Anesthesia, Reanimatology and Intensive Care, Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia

Phone: +381 116 660 466

E-mail: vukotic.milovan@gmail.com

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UDK: 617.3-089.5-053.9
Eabr 2023; 24(1):17-26
DOI:10.2478/sjecr-2020-0002

ABSTRACT

Spinal anesthesia and peripheral nerve block anesthesia are used in total knee replacement. The aim of the study was to examine whether peripheral nerve block anesthesia would provide a more stable hemodynamic profile and analgesic effect in elderly patients undergoing total knee replacement, as compared to spinal anesthesia. This is a single-center case-control trial, with patients from our prospectively followed registry. The patients were divided into two groups, those with peripheral nerve block anesthesia and spinal anesthesia. Propensity score analysis was performed in 1:1 ratio. The primary outcome was analgesia with total analgesic effect and the secondary outcome was intraoperative hemodynamic status. The patients in peripheral nerve block anesthesia group had a longer length of analgesia (606.19±219.35 vs 359.48±106.82, P<0.01) and pain scores during 24h and 48h after the surgery were lower in the same group of patients (3.21±1.74 vs 5.02±2.23, P=0.037; 3.03±1.57 vs 5.67±2.51, P=0.028). Spinal anesthesia group had a larger number of patients with significant hypotension (3.84% vs 15.38%, P=0.01), as well as a larger number of patients who received vasopressors (0% vs 9.61%, P<0.01). Both anesthesia methods demonstrated sufficient analgesic efficacy in total knee replacement, although there was less pain severity and longer analgesic effect of peripheral nerve block anesthesia in patients who were 60 years old or older. Spinal anesthesia showed a significantly higher degree of hypotension than in those patients receivingperipheral nerve block anesthesia.

Keywords: *Total knee replacement, spinal anesthesia, peripheral nerve block anesthesia.*

INTRODUCTION

Total knee replacement (TKR) remains the most effective treatment of the end-stage osteoarthrosis. It is most frequently performed among older patients, where sensitivity to the different type of drugs applied during anesthesia is higher, thus resulting in more frequent hemodynamic instability.

TKR may be performed under both general and regional anesthesia. The ability to provide superior postoperative analgesia, rapid postoperative rehabilitation and reduced cost of medical care may have resulted from thoughtfully implemented regional anesthetic and analgesic techniques. Also, recent studies have shown that in patients undergoing regional anesthesia, there was a significantly lower level of stress hormones and more rarely postoperative cognitive dysfunction in comparison to general anesthesia (GA) (1,2). Because of this, the use of regional anesthesia, including spinal, epidural, and peripheral block has increased.

Peripheral nerve block (PNB) carries potential advantages such as hemodynamic stability and better postoperative pain control (3,4). PNB is reported to provide effective analgesia, facilitate physical therapy, and reduce length of hospital stay compared to other regional anesthetic techniques (5,6). In our institution, TKR surgery is commonly done under spinal anesthesia (SA) or PNB. Therefore, the main aim of the study was to examine whether PNB would provide a more stable hemodynamic profile and postoperative pain scores in elderly patients undergoing TKR surgery, as compared to SA.

MATERIALS AND METHODS

Study design and patient population

The study was designed as single-center case-control trial, with patients from our prospectively followed registry. Between January 1st 2013 and January 1st 2015, the total number of consecutive 895 TKRs was done at the Institute for Orthopedic Surgery „Banjica“. Out of these, 104 (11.62%) were done in PNB, 52 (5.81%) in GA and 739 (82.56%) in SA. Those operated in PNB and SA were recruited for this study (Figure 1). The study protocol was approved by the Ethics Committee of the institution and was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all patients.

The inclusion criteria were the patients: older than 60 years, undergoing elective single TKR surgery; who had met the American Society of Anesthesiologists physical (ASA) status I -III and that had no allergy to local anesthetics.

The exclusion criteria were: contraindications for PNB and SA (coagulation defects, infection at the puncture site), bone degenerative disease and posttraumatic condition of the spine, current severe psychiatric disease, alcoholism or drug dependence, no dementia (Mini-Mental Score Examination > 23) and severe visual or auditory disorder.

The data were collected about demographics (age, body mass index and sex), baseline comorbidities (smoking status, presence of hypertension, hyperlipidemia and diabetes mellitus), ASA score, the intraoperative data (time of anesthesia induction, length of surgery, leg/side being operated, total anesthesia time - defined as the time from anesthesia induction until the end of surgery, the total amount of fluid expressed in milliliters, the administration of either coloid solutions or blood transfusion, blood loss, the use of vasopressors and opioids and the episode of significant hypotension defined as a drop of more than 30% of systolic blood pressure during the operation compared to the baseline values. Also, the intraoperative data reflecting the hemodynamic status (systolic - SBP and mean blood pressure - MAP, as well as the heart rate - HR) were collected every 5 minutes during the first 30 minutes, and then every 10 minutes until the end of surgery. Postoperative characteristics (length of analgesia defined as the time from anesthesia induction untilthe first reappearance of pain which necessitated the administration of opioids and length of hospital stay) as well as the average numerical pain score (NPS) rating (numeric score 0-10; 0 without pain, 1-3 mild, 4-6 moderate and >7 strong pain) for the first three postoperative days were collected.

The patients were divided into two groups, those with PNB anesthesia and those with SA. All patients who underwent PNB met the inclusion criteria. The propensity score analysis was performed by matching PNB group of patients to SA patients controlling the demographics, baseline comorbidities and ASA vaules in 1:1 ratio (Figure 1).

Regional anesthesia techniques

Since all patients underwent TKR at the same hospital, regional anesthesia techniques were completely standardized. All patients received 500 ml of intravenous cristaloids prior to anesthesia. For sedation, the patients received Midazolam (0.07-0.1 mg/kg) intramuscularly. Standardized monitoring, such as non-invasive blood pressure (BP), heart rate (HR), electrocardiography and pulse oximetry, was conducted. To measure BP, a BP cuff was fitted to the patient's upper arm and BP was measured in the patient in the supine position.

The patients in PNB group had lumbar plexus, sciatic and femoral nerve blocks done with a nerve stimulator (STIMUPLEX®S,B BRAUN,Germany). After the sterile preparation and draping, the nerve blocks were administered using a 21 gauge needle (Stimuplex®A, Insulated Needle, 21G × 4“,0.80 ×100mm, B BRAUN, Germany). Posterior approach to lumbar plexus block (LPB) was performed in patients in the lateral decubitus position. The puncture site was at the level of intercristal line 3 cm caudal and 5 cm lateral midline to the spinous processes. Sagittal insertion direction

was used. The puncture depth was typically 6 -10 cm. After the insertion of the needle in the psoas space, a sign of the lumbosacral plexus identification muscle activity (with the nerve stimulator settings at 2 Hz frequency and current at 0.3 -0.5 mA) from the quadriceps muscle was noted. After the negative aspiration test, 10-15 ml of 1,3% lidocaine and 10-15 ml of 0,25% bupivacaine were injected. The aspiration test was repeated on every 3-4 ml of a given anesthetic.

Sciatic nerve block (SNB) was performed in the same position. The puncture site was at the middle of the line connecting posterior superior illiac spine and greater trochanter and 5cm caudal (Labat line). After the field preparation, the needle was inserted at 6-8 cm depth below the skin puncture, and both plantar and dorsal flexation of the foot was elicited as a reaction from tibial and peroneal part of the ischiadic nerve. Again, after the negative aspiration test, 3-4 ml anesthetic (1,3% lidocaine and 0,25% bupivacaine) was injected intermittently with a constant repetition of the aspiration test, until the total dose of 10 ml 1.3% lidocaine and 10-15 ml 0.25% bupivacaine.

The supine approach was used for femoral nerve block (FNB). The leg position was in a slight abduction. The femoral artery pulse was palpated 1-2 cm below the inguinal ligament. The punction site was 1-1.5 cm laterally from the artery and the femoral nerve was indentified 2-3cm below the skin level. With a similair current, a 10 mL of of 1,3% lidocaine and 10-15 ml of 0,25% bupivacaine were injected.

The puncture site for the patients in SA was at the L3 or L4 level with a 25 gauge spinal needle (Spinocan, 25G, B Braun, Germany) in the patient in the sitting or lateral decubitus position, under the aseptic technique. With perpendicular position of the needle and avoidance of moving the needle, 2 ml of 2% lidocaine and 3 ml of 0,5% bipuvacaine were administered. Following the administration of SA or PNB, the patients were placed in the supine position for a surgery.

The patients who developed hypotension were given intravenous ephedrine in titrated boluses, then resuscitated with intravenous fluids as needed. After the operation, all patients were prescribed intravenous Paracetamol (acetaminofen), nonsteroidal anti-inflamatory drugs or opioids (Tramadol) if needed, when the average NPS was six or higher. The patients were followed up on the wards later in order to determine pain-free duration and pain scores in the first three postoperative days.

Statistical analysis and outcomes

Continuous variables were presented as mean±standard deviation. Group comparisons were performed using the Student t-test or ManneWhitney U test, as appropriate. Categorical data were expressed as percentages and were compared using the chi-square test or Fisher exact test. Differences were considered statistically significant at $p < 0.05$. Differences between curves were tested using the log-rank test. Analyses were done with SPSS software, version 20.0 (SPSS, Chicago, IL, USA).

The primary outcome was analgesia assessed by: duration of analgesia (expressed as the time from anesthesia induction until the reappearance of pain necessitating the administration of opioids) and postoperative numeric pain scores until the fourth postoperative day. The secondary outcomes were intraoperative hemodynamic status, as well as the use of vasopressor drugs and the appearance of signficant hypotension as defined above.

RESULTS

Demographic and baseline characteristics

The demographic data and baseline clinical characteristics are shown in Table 1. There were no significant differences between the groups in terms of these data. The intraoperative variables are shown in Table 2. The anesthetic induction time of PNBs was longer (22.03±9.31 vs 7.89±2.87 minutes, P<0.01), as well as the total anesthesia time (160.17±40.92 vs 145.96±37.71, P<0.01), although it did not change the total operative time (115.02±28.17 vs 113.54±36.46 minutes, P=0.77). The overall administration of fluids during the operation (1834.62±617.43 vs 1040.38±421.85 ml, P<0.01), as well as the total number of patients that received coloid solutions (41.34% vs 3.84%, P<0.01) and opioids (8.65% vs 0%, P<0.01) were higher in SA group.

Hemodynamic variables

The hemodynamic variables are shown in Table 3 and Figures 2, 3, 4, 5. We compared changes in the hemodynamic variables during the surgery in SA and PNB groups. Compared with the patients in PNB group, the patients in SA group had an overall lower SBP (114.38±13.30 vs 130.87±14.83 mmHg; P<0.01) and MAP (84.61±9.47 vs 96.15±10.63, P=0.015). There was no difference in the mean heart rate (78.23±10.07 vs 80.69±12.18, P=0.41) throughout most of the observation period after induction of anesthesia. Compared to PNB group, SA group had a considerably larger number of the patients with significant hypotension (3.84% vs 15.38%, P=0.028), as well as a larger number of the patients who received vasopressors (0% vs 9.61%, P=0.019) (Table 4).

Analgesic efficacy

All patients received opioids postoperatively. The patients in PNB group had a statistically significant longer length of analgesia (606.19±219.35 vs 359.48±106.82, P<0.01). Pain scores during 24h and 48h after the surgery were lower in PNB patients (3.21±1.74 vs 5.02±2.23, P=0.037; 3.03±1.57 vs 5.67±2.51, P=0.028), but there was not a statistically significant difference in the same pain scores during 72h and 96h respectively (2.57±1.05 vs 4.21±1.82, P=0.094; 2.08±0.88 vs 2.83±1.69, P=0.43) (*Table 5,* Figure 5). The patients receiving PNB as an anesthetic technique had a significantly shorter intrahospital stay (7.81±2.68 vs 8.77±3.66, P=0.03) (Table 2).

Table 1. Baseline clinical characteristics

Characteristics	PNB* (n=104)	SA* (n=104)	p value
Demographics			
Age	67.96±6.10	68.98±6.51	0.24
Body mass index	25.64±3.10	26.16±3.15	0.23
Female	74 (71.15%)	68 (65.38%)	0.67
Risk factors			
Smoker	43 (41.34%)	41 (39.42%)	0.88
Hypertension	64 (61.53%)	66 (63.46%)	0.86
Hyperlipidemia	17 (16.34%)	15 (14.42%)	0.84
Diabetes mellitus	8 (7.69%)	7 (6.73%)	1.00
*ASA score**			
I	33 (31.73%)	33 (31.73%)	1.00
II	64 (45.71%)	62 (59.61%)	0.88
III	8 (7.66%)	9 (8.66%)	1.00

ASA score* - American Society of Anaesthesiologists score, PNB* - peripheral nerve block, SA* - spinal anesthesia

Table 2. Intra- and postoperative characteristics

Characteristics	PNB* (n=104)	SA* (n=104)	p value
Intraoperative data			
Anesthetic induction time, minutes	22.03±9.31	7.89±2.87	**<0.01**
Length of surgery	115.02±28.17	113.54±36.46	0.77
Side of surgery, right/left	59/45	64/40	0.57
Anesthesia time, minutes	160.17±40.92	145.96±37.71	**0.01**
Fluid administration, ml	1040.38±421.85	1834.62±617.43	**<0.01**
Coloid solutions	4 (3.84%)	43 (41.34%)	**<0.01**
Blood loss, ml	300.05±716.11	311.54±322.11	0.88
Blood transfusion	4 (3.84%)	6 (5.76%)	0.74
Opioids	0 (0%)	9 (8.65%)	**<0.01**
Postoperative data			
Intrahospital stay, days	7.81±2.68	8.77±3.66	**0.03**

PNB* - peripheral nerve block, SA* - spinal anesthesia

Table 3. Intraoperative hemodynamic status

Hemodynamic variable	Groups	Baseline value	End of surgery	p value
SBP* (mmHg)	PNBA	145.21±10.56	125.58±17.61	**<0.01**
	SA	148.37±25.78	122.45±19.25	
MAP* (mmHg)	PNBA	105.08±8.11	94.59±11.23	**<0.01**
	SA	101.67±7.41	88.19±10.78	
Heart rate (beats/minute)	PNBA	79.56±10.87	81.77±13.04	0.58
	SA	82.15±13.04	78.18±10.95	0.37

PNBA* - peripheral nerve block anesthesia, SA* - spinal anesthesia, SBP* - systolic blood pressure, MAP* - mean arterial pressure

Table 4. Comparison between PNB and SA group with significant hypotension and need for vasopressors

Characteristics	PNBA* (n=104)	SA* (n=104)	p value
Significant hypotension*	4 (3.84%)	16 (15.38%)	**0.028**
Need for vasopressors	0 (0%)	10 (9.61%)	**0.019**

*Significant hypotension is defined as a drop of >30mmHg from baseline systolic blood pressure
PNBA* - peripheral nerve block aneshesia, SA* - spinal anesthesia

Table 5. Pain analysis

Hemodynamic variable	PNBA* (n=104)	SA* (n=104)	p value
Length of analgesia, minutes	606.19±219.35	359.48±106.82	**<0.01**
Pain scores 24h after the surgery	3.21±1.74	5.02±2.23	**0.037**
Pain scores 48h after the surgery	3.03±1.57	5.67±2.51	**0.028**
Pain scores 72h after the surgery	2.57±1.05	4.21±1.82	0.094
Pain scores 96h after the surgery	2.08±0.88	2.83±1.69	0.43

PNBA* - peripheral nerve block anesthesia, SA* - spinal anesthesia

Figure 1. Flow chart diagram showing patient enrollment

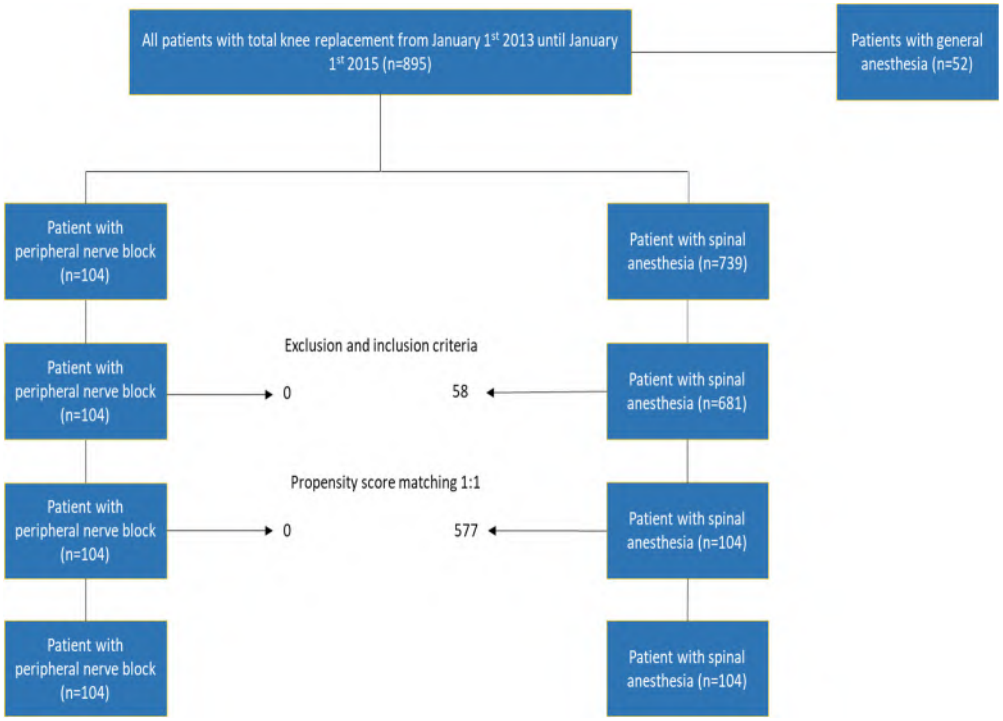


Figure 2. Systolic blood pressure (SBP) for SA group and PNB group every 5 minutes during the first 30 minutes and then every 10 minutes until the end of surgery. Data are presented as the mean ± standard deviation (SD).

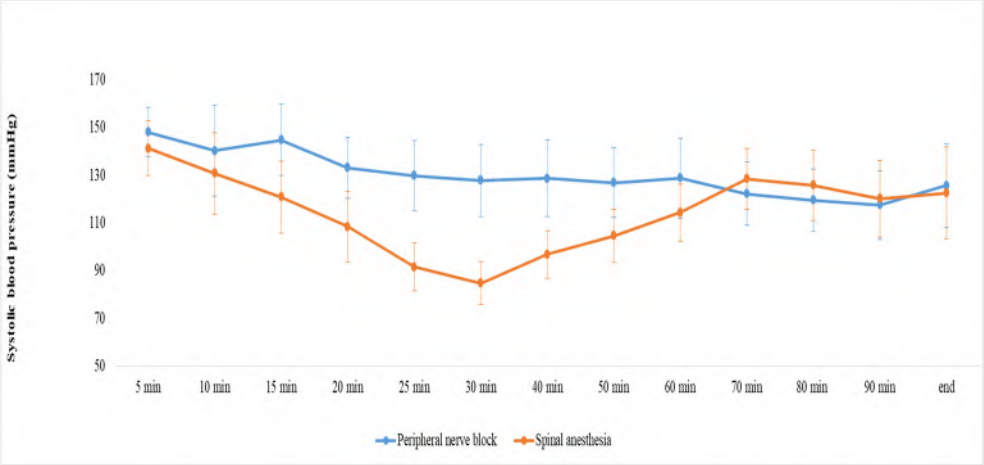


Figure 3. Mean arterial pressure (MAP) for SA group and PNB group every 5 minutes during the first 30 minutes and then every 10 minutes until the end of surgery. Data are presented as the mean ± standard deviation (SD).

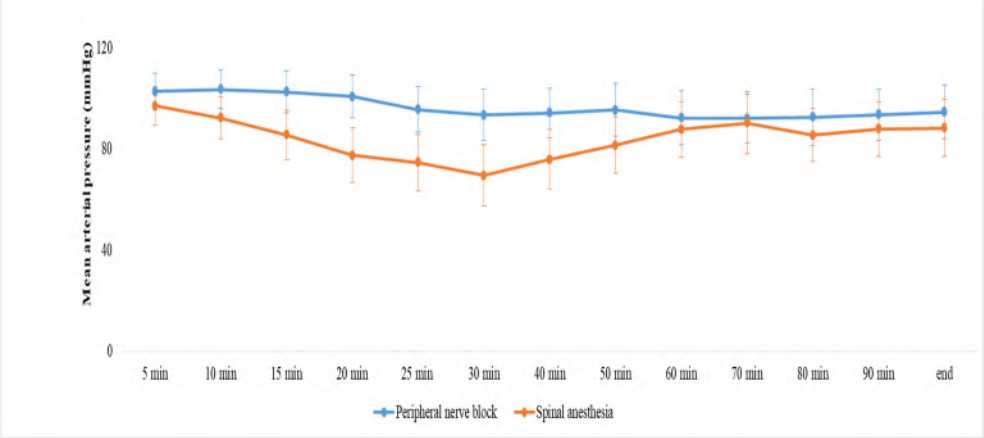


Figure 4. Heart rate (HR) for SA group and PNB group every 5 minutes during the first 30 minutes and then every 10 minutes until the end of surgery. Data are presented as the mean ± standard deviation (SD).

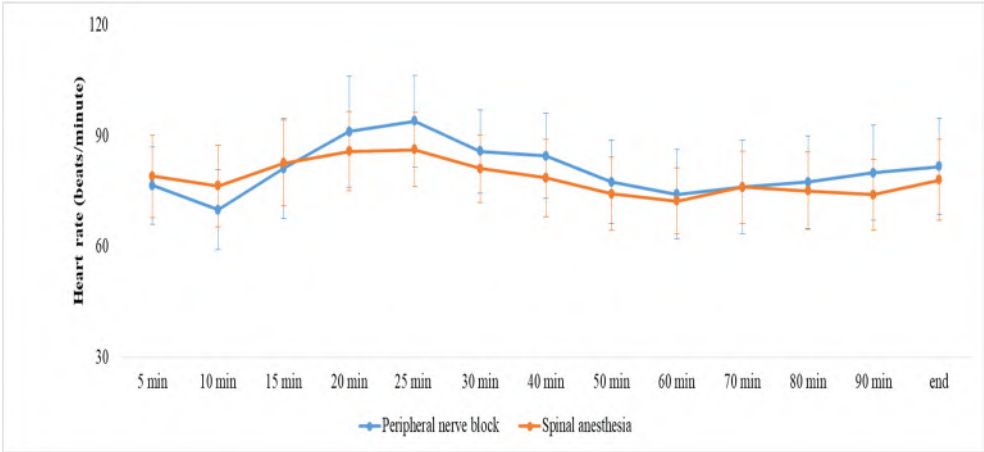
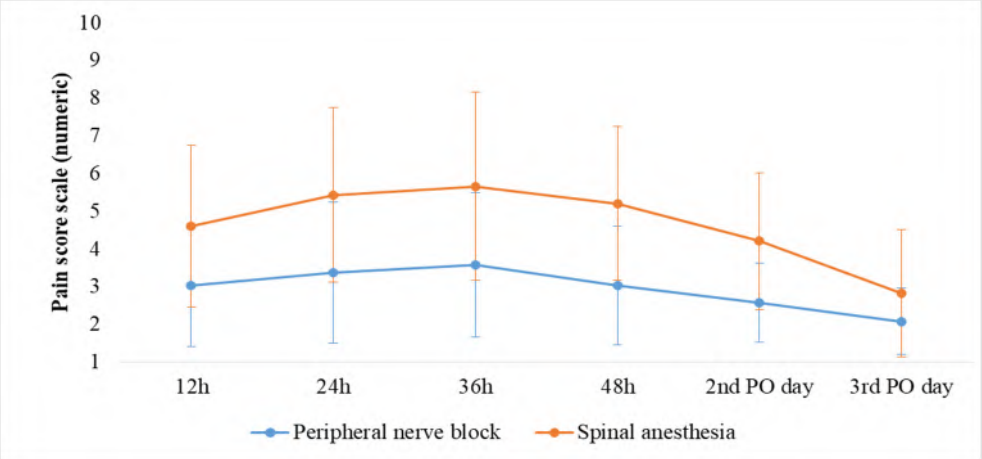


Figure 5. Pain scale for SA group and PNB group at six distinct points: 12h, 24h, 36h, 48h, the second and third postoperative day. Data are presented as the mean ± standard deviation (SD).



*PO - postoperative

DISCUSSION

In this study, we found that the patients that were given PNB had longer analgesia time. Pain scores were lower during the first 48h after the operation, but after that time, this difference was not significant. The patients that received SA had significant and prolonged hypotension more often that was treated with fluids and vasopressors as necessary in comparison to those receiving PNB.

It has been reported that most of the analgesic benefit of PNB seems to occur during the early postoperative period after TKA, with very little effect extending beyond 48 hours (6,7). Good postoperative pain control has been reported to accelerate rehabilitation (6). Our data were in accordance to these findings. We have also found a significant difference between the patients in the treatment groups with regard to the time of the first request for opioids. The patients receiving PNB consistently reported lower pain scores, faster discharge from hospital, and lower opioid consumption (8-10).

Peripheral block anesthesia for knees has traditionally involved the use of a femoral nerve block (FNB), either in isolation or in conjunction with a sciatic nerve block (SNB). This combined femoral and sciatic block has been shown to provide superior pain relief over an isolated FNB alone as SNB provides posterior analgesia that FNB cannot provide (11). While perhaps not beneficial in isolation, they may aid to improve pain control as part of the so-called three-in-one nerve block (12). Nonetheless, SA is associated with more complete, denser surgical block and thus less chance of a patchy block resulting in pain during surgery, compared to other forms of regional anesthesia.

The use of regional anesthesia is common in our institution. In our hands, the technique was effective for providing surgical anesthesia. Athough considered quite safe, all used techniques should be repeatedly evaluated for any associated risks. There is always a debate about the risk/benefit ratio of using the regional anesthesia. When considering the risk profiles associated with each of the two methods used here, there are some recognized differences. As previously mentioned, SA offers rapid onset analgesia with minimal systemic toxicity and prolonged duration in the postoperative period (13). However, SA intraoperatively provides more cardiovascular instability and sudden cardiac death may happen, especially among elderly and diabetic patients with autonomic neuropathy, due to impaired compensation to the vasodilatory effects of anesthesia (14). Even if these complications are considered to be rare, severe ones such as headache, cauda equina syndrome and risk of infection have also been reported for spinal anesthesia (15). On the other side, PNBs are not without disadvantages. These include motor inhibition and the potential risk of falls as a result of quadriceps weakness (16,17). Furthermore, there is a small but significant risk of neurologic and vascular compromise as a result of the procedure (18). While neurologic symptoms are usually limited to nerve dysesthesias that are resolved within weeks or months, more significant nerve injuries are possible (17). Also, a negative effect such as an uncomfortable position of the patient in both PNBA and SA should be acknowledged when compared to general anesthesia.

We observed a significant drop in blood pressure in 15.38% of patients in SA and 3.84% in PNB group and on the other side, the use of vasopressores was also more common in the same groups (9.61% vs 0%). Hypotension can result in cardiac ischaemia, cerebral hypoperfusion, acute tubular necrosis and renal injury. A study by Monk et al. showed that intraoperative hypotension is associated with an increased 30-day operative mortality (19). Therefore, intraoperative hypotension is undesirable. PNBs have been shown to result in more stable hemodynamics as compared to general anesthesia (20) and neuraxial block (21).

Therefore, significant hemodynamic changes would lead in some high risk patients, such as dose in ASA score 3 to exarcebation of their cardiovascular status and potentially fatal outcomes. With these results, our study showed that PNB resulted in stable BP and HR, and would, therefore, be a good choice of anesthesia for patients undergoing TKR.

It is also interesting to see a combination and concentration of different local anesthetics used in a different center for both PNB and SA. In comparison to other studies, (22-24)we used maximally 45 ml 0.25% bupivacaine and 35ml 1.3% lidocaine for lumbar plexus, sciatic and femoral block altogether. For SA, we used a combination of 2 ml 2% lidocaine and 3 ml 0.5% bupivacaine. Although the volume of local anesthetics used in our center is higher, the concentration is significantly lower, which reduces the possibility of systemic adverse events. These authors found similarly that PNB provided more stabile hemodynamic effect during the surgery, longer sensory blockade and lower postoperative pain scores (22-24).

While both periarticular injections and PNBs provide superior pain relief when compared to narcotic use alone, they appear to provide similar pain relief postoperatively. Recent meta-analysis of 10 randomized, controlled trials that included the total number of 744 total knee arthroplasties comparing PNBs to periarticular injections found similar results in regard to the pain control and postoperative function (25). Hannon et al. in their recent survey about current analgesia and anesthesia practices in USA containing 28 questions have found that there was no consensus regarding the optimal multimodal anesthetic and analgesic regimen for TKR (26). In our research, all patients received intravenous infusion of Paracetamol 12 hours after the surgery and 100 mg of Trodon, if the average numeric pain score was six or higher.

Perineural continuous infusion catheters have been both used in knee arthroplasty. The results from 2 recent meta-analyses suggest that continuous intra-articular infusion catheters may improve the short-term pain control following total knee arthroplasty, but given the heterogeneity of studies, both studies were unable to offer any firm conclusions (27,28). Prolonged perineural continuous infusion catheters (the so-called continuous blocks) may offer extended pain relief and more rapid progression of function following surgery when compared to placebo (29) and shorter-term infusion catheters (30,31). However, when compared to a single-injection neural blockade or even periarticular injection alone, several randomized, controlled trials have demonstrated no benefit in using the infusion catheter (32-34).

The discussion is ongoing concerning the possible benefits of ultrasound-guided peripheral nerve blocks compared to blocks performed with the use of a nerve stimulator, particularly with respect to the analgesic quality. However, although some studies have shown a trend toward a possibly better outcome when blocks are performed under the ultrasound guidance, no study has shown yet a significant benefit when compared to the nerve stimulator-guided techniques (35). In our study, all blocks were performed by a highly experienced anesthesiologist.

Limitations and strengths of the study

The study has several limitations. First, it was a single centre study. Second, although spinal anesthesia resulted in hypotension, it is not known if this led to a higher rate of cardiac adverse events after the hospital discharge. Only the increased length of hospital stay was noted among these patients. The main strength of this study is a large number of patients with a detailed clinical information, which provides statistical power to make comparisons among groups and valid conclusions.

CONCLUSION

In conclusion, both anesthesia methods demonstrated sufficient analgesic efficacy in TKR, although there was less pain severity and longer analgesic effect of PNB in patients who were 60 years old or older. SA showed a significantly higher degree of hypotension than in those receiving PNB. PNB provided a hemodynamic stability and therefore should be considered, whenever possible, as an option of anesthesia for TKR. Continued research is warranted to determine the most optimal anesthesia in terms of analgesia and hemodynamics following TKR.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Ethics Committee of the institution and was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all patients

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

FUNDING

None.

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STERILITY TESTING OF PLATELETS CONCENTRATE WITHIN QUALITY CONTROL: EXPERIENCES AND OPPORTUNITIES TO EXTEND THE APPLICATION

Dragana Gojkov[1], Nemanja Borovcanin[1] and Dusan Vucetic[1,2]

[1] *Institute for Transfusiology and Hemobiology, Military Medical Academy, Belgrade, Serbia*
[2] *Faculty of Medicine, Military Medical Academy, University of Defence, Belgrade, Serbia*

Received: 10.02.2020.
Accepted: 22.02.2020.

Corresponding author:

Dragana Gojkov, MD

Institute for transfusiology and hemobiology
Military Medical Academy, Crnotravska 17,
Belgrade, Serbia

Phone: +381 11 360 9177

E-mail: jovidrag@gmail.com

sciendo

UDK: 616.94-074
Eabr 2023; 24(1):27-32
DOI:10.2478/sjecr-2020-0014

ABSTRACT

Despite numerous measures, bacterial sepsis associated with the transfusion remains a major threat. The incidence of septic events induced by platelets transfusion is approximately 10 times higher than with transfused red blood cells due to their storage temperature. This caused new Standard that implements the methods for the detection and reduction of bacteria in the platelet concentrates (PC). The aim is to consider the possibility of wider application of this tests in order to extend the shelf-life of PC. Sterility testing of PC is done once or twice per month using BacT/Alert BPA and BacT/Alert BPN bottles. If positive, all products from the initial unit were tested to confirm or deny the status. During six years period, 67236 PC units were made and 872 of them were tested. Only two were found initially positive. After testing the other products from the same initial unit, results were negative so, final results proclaimed false positive. Pretransfusion bacterial detection is an important potential method for reducing the risk of bacteriemia and transfusion-associated septic reactions. In addition to routine measures, Mirasol PRT pathogen inactivation system, could be included. This allows certain amount of PC to be inactivated during the first 32 hours. Untreated PC units would be stored in standard conditions and for given time (three days) potentially present bacteria would reach a detectable level. This way the quantity of samples for sterility testing could be reduced, taking only 2 ml of each of four units of PC. Samples would be planted at the same vial-aerobic bottle, which would also, double the capacity in BacT/Alert 3D automated system.

Keywords: *Platelets, bacterial sepsis, sterility control, shelf life.*

INTRODUCTION

Introduction of the routine use of a closed system of plastic bags for blood collection, processing under sterile conditions that such system allows and adequate storage, enables significantly enhanced sterility of blood components. Despite these measures, bacterial sepsis associated with the transfusion remains a major threat and is a topic constantly repeated in the literature over the past 40 years. The incidence of septic events induced by platelets transfusion is approximately 10 times higher than with transfused red blood cells (1). Platelet concentrates (PC) are the most common cause of bacterial infections. A retrospective study conducted at the National Blood Service, North London Centre, found that the rate of bacterial contamination is 0.7% and 0.4 % in pooled and apheresis platelets respectively (2).

Possible strategies in order to reduce bacterial contamination and transmission include detailed screening of donors, improvement of disinfection of the venipuncture site, removing the initial blood into sampling pouch (the first 10-20 ml of blood) and sterility controlling in automated systems (1, 2).

Given the frequency of bacterial contamination, on March 2004 the American Association of Blood Banking (AABB) announces a new Standard that implements the methods for the detection and reduction of bacteria in the PC (3, 4). At the Institute for Transfusiology and Hemobiology of Military Medical Academy (MMA) sterility control of PC was first performed in 1997. Routine sterility testing as the part of the quality control of PC started in 2008. Implementation of screening of PC on bacterial contamination, according to the global data (5-9), successfully identified the contaminated products and reduced transfusion of such products to the patients.

The aim of this study was to evaluate the results of routine PC sterility testing as part of the quality control. Also, according to these results and data from the literature, regarding the frequency of bacterial transmission by PC transfusion, consider the possibility of wider application of this test in order to extend the shelf-life of PC from five to seven days.

MATERIALS AND METHODS

Whole blood (450 ± 45 ml) was collected from random healthy donors, age 18-65 years, after adequate disinfection of venipuncture site and removing the initial blood into sampling pouch (the first 10-20 ml of blood). We used quadruple bags Macopharma (Macopharma, France) and Terumo (Terumo, Japan) with citrate-phosphate-dextrose (CPD) as anticoagulant-conserving solution and SAGM (sodium chloride-adenine-glucose-mannitol) as additive solution for red blood cells (RBC). Primary separation (separation of RBC, fresh frozen plasma - FFP and buffy coat - BC) of whole blood units was performed after 2-8 hours of collection by centrifugation in a centrifuge JOUAN (Jouan, France). Parameter settings were: speed 3603 rpm (3890 g) for 10 minutes at 22 ± 2 °C (the radius - R = 268 mm, acceleration - A = 6, brake - B = 4). Separation was performed on the automatic separator T-ACE II (Terumo, Japan). Secondary separation was performed after BC resting overnight at ambient temperature. Parameter settings: speed 1170 rpm (377 g) at 22 ± 2 °C for 5 minutes (R = 245 mm, A = 2, B = 0). The resulting platelet concentrate from the buffy coat (PC-BC) were kept in a shaker for platelets at a temperature of 22 ± 2° C. Donors were non-reactive for hepatitis B and C virus (HBV and HCV), human immunodeficiency virus (HIV) and lues markers - investigated by chemiluminescent immunoassay (Architect, Abbott, USA), as well as by NAT testing (Cobas AmpliPrep and Amplicor Analyzer, Roche, Germany)

Sterility testing of PC-BC was done once or twice per month to ten samples minimum. Samples were collected in BacT/Alert BPA and BacT/Alert BPN bottles for liquid culture of aerobic and anaerobic bacteria (BioMerieux, Durham, NC, USA). After proper marking, plastic caps were removed from bottles and rubber top disinfected with 70% ethyl alcohol and left to dry for 30 seconds. Hose of PC-BC bag whose sterility is examined, is cleaned with 70% ethyl alcohol and allowed to dry. On the clip corresponding to the diameter of the bottle is placed a sterile disposable needle and when the hose is dry, needle entered disinfected part. In the clip is placed BacT/Alert bottle in which is withdrawn (due to negative pressure) 4-8 ml of the bag contents. After that, the bottle is removed from the clip. In the same way is taken the content into the other bottle and before the needle is pulled out, the hose is sealed between the needle and the bag. The bottles were placed in the BacT/Alert 3D automated system for liquid cultures for the incubation period of five days. Capacity of device is 60 bottles at the time. Samples were taken from the PC-BC unit fifth or sixth day of their storage, provided that the blood collecting day counts as day zero.

Really positive PC-BC unit is considered if the same microorganism, detected in the PC-BC sample, is isolated from all available products derived from the same whole blood. A false positive PC-BC unit is in the case other products obtained from the initial whole blood unit remain sterile, ie. microorganism was not isolated. In this case probable reason for sample PC-BC positivity is inadequate disinfection and manipulation during sampling.

RESULTS

Routine sterility testing of PC in our Institute began in 2008 year. Until July 2010, except for sampling, the whole process took place at the Institute of Microbiology, and after that, when the Institute for Transfusion and Hemobiology got its own BacT/Alert 3D automated system for liquid bacterial culture, the process is handled entirely by the Department of blood products with quality control.

During the 2008 109 samples were tested on sterility, 2009 only 35 (due to the small capacity of the incubator and major requirements for blood culture testing from other clinics), 2010 172 sample, 2011 209 samples, 2012 159 samples and 2013 188 (Table 1). Among these 872 samples, positivity was detected only twice in aerobic microflora detection bottles (ones 2008 and 2011 second time). In both cases, Coagulasa positive Staphylococcus was isolated, but positivity was false, as other products from the same initial blood unit remained sterile after five days of incubation.

In the same period (since 2008 till 2013) 67236 units of PC-BC were made. Not one really positive sample was detected. The percentage of false positives was 0.2%.

Table 1. Number of PC-BC tested during five years

YEAR PC-BC	2008.	2009.	2010.	2011.	2012.	2013.
Total PC-BC number	10015	10324	10767	10816	12264	13050
Nº PC-BC tested	109	35	172	209	159	188
Nº PC-BC tested pos.	0	0	0	0	0	0
Nº falsely positive (%)	1 (0.92)	0	0	1 (0.48)	0	0

DISCUSSION

In the United States during the period from 1986 to 1991 years, bacterial contamination was the cause of 15.9% of all fatalities regarding transfusion. In France, between 1994 and 1998 there were 18 deaths as a result of bacterial contamination of blood components. In the UK, from 1995 to 2000, there were 26 transfusion transmitted infections, in 15 cases the cause was bacterial infection (2). In the most of these cases, bacterias are detected in units of PC (1 in 2,000 to 1 in 1,000) (10, 11). From the data on the incidence of bacterial contamination of blood products, the governments of many countries, have given the recommendation to test for bacterial contamination within the "Quality Control". This is a good step to improve the safety of blood products, but the clinical significance (benefit for a medical institution) is still limited. If we assume that the degree of platelet contamination is 0.2% (3), then in our case, when tested 1.3% of all PC units (872 out of 67236), the chance to detect a contaminated product is 1.3% × 0.2% = 0.0026%. This means that the possibility of contaminated product to be given to the patient is still very high, although the role of quality control of the process is undeniable, since, in the case of continued failure in the production process as a possible source of contamination, the center would be alerted that something was wrong with the process.

However, the real answer to potential bacterial contamination of PC and transfusion of such products to patient is screening. Screening means testing 100% of the PC units on bacterial contamination (12). Four elements are necessary for transmission of infectious agents by blood transfusion:

1. Asymptomatic phase while the microorganism (MO) is present in the blood,
2. MO viability must be preserved during storage,
3. There must be a population of seronegative recipients,
4. MO must be able to cause disease.

A unique aspect of most bacterial species compared with viruses is their ability to easily proliferate in, for them, very nourishing blood products during storage. This is especially true for PC units that are stored at ambient temperature. It has been calculated that the level of contamination at the time of collection is generally low, probably only 1-10 colony forming units (CFU)/ml or less. However, when the product is contaminated, the bacteria can multiply so quickly that in a few hours reaches the level of 10^6/ml or more. If product with such amount of bacteria was transfused, in a short time could cause bacteremia, which can progress to sepsis and death. The outcome of the contaminated blood transfusion depends of the amount of bacteria, types of bacteria and its pathogenicity for man, transfusion rate and clinical status of the patient. Immunosuppressed patients and elderly people with poor nutritional status are the most vulnerable populations, although fatal deaths in healthy people are not rare, especially in case of transfusion of large amount of Gram-negative bacteria that produce endotoxin.

Possible sources of bacteria in blood components may be: donor's bacteremia, contamination during collection and processing of blood, as well as the contamination of blood collection sets.

Blood donors with asymptomatic bacteremia or in recovery from a bacterial infection, can regardless of transient bacterial episode be eligible for donation, of which there are numerous data in the literature (sepsis caused by Yersinia enterocolitica) (13, 14). Short-term bacteremia may occur after certain dental manipulations, especially the extraction of teeth, and there were cases of contamination of PC with Staphylococcus aureus as a result of this situation (12).

However, the greatest risk of bacterial contamination is during blood collection, so, the most of the microorganisms isolated from contaminated PC is actually normal microflora of the skin, as it is virtually impossible to completely

decontaminate the venipuncture site. It is also thought that a small amount of skin can enter the needle during venipuncture and allow the entry of viable bacteria from the deeper layers of the skin despite adequate disinfection of surfaces (15).

Improperly sealed or damaged hoses, micro damages of collection bags, were associated with episodes of bacterial sepsis as well (16).

Therefore, measures for the prevention of bacterial contamination of blood products would be:

A. Reducing the risk of contamination during blood collection: an improved screening of blood donors; enhanced disinfection of venipuncture site; removing the initial blood into sampling pouch,
B. Optimization of processing and storage of blood components: optimization of storage temperature; limited storage time; universal leukoreduction,
C. Reducing the exposure of the patient to blood donors,
D. Pretransfusion bacterial detection: visual inspection of components prior to issuance; direct staining of bacteria; bacterial ribosomal essay; assay for bacterial endotoxin; nucleic acid testing (NAT) for bacterial DNA; measurement of CO_2 production or O_2 consumption by bacteria; direct bacterial cultures,
E. Introduction of pathogen reduction / inactivation.

Pretransfusion bacterial detection is an important potential method for reducing the risk of bacteremia and transfusion-associated septic reactions, but none of currently available laboratory techniques is ideal. Automatic systems for liquid cultures are the most sensitive method used in transfusion centers and are currently widely used (17). Two of these systems are granted in the United States:

1. BacT/Alert (BacT/Alert; BioMerieux, Durham, NC) uses the bottles with the substrate and colorimetric sensor that changes color as a result of increased production of CO_2 generated during cell growth. Two bottles (aerobic and anaerobic) are used; in each is inoculated 4-8 ml of PC. Since the needle is used for inoculation, the system is not completely closed. The system is extensively validated with a wide range of potentially contaminating MO. The method reliably detects PC contamination when 10 CFU/ml, in many cases, even 5 CFU/ml of bacteria is inoculated (eg, B.cereus, S.marcescens, C. perfringens, S.epidermidis, S.pyogenes, E.coli, K.pneumoniae, S.aureus and S.viridans) for 12 to 26 hours, while for the detection of Propionibacterium acnes (whose clinical significance is debatable), require longer incubation time (1, 2, 10, 18-21). In most works this system is used as a reference (22).
2. Another system for bacterial culture is Pall Bacterial Detection System (BDS). Sampling is done so that the system remains effectively closed: about 5-6 ml of leukoreduced platelet rich plasma is taken from the bag and passed through a filter (to remove residual leukocytes, platelets, and about 50% of bacteria) in the incubation bag. Incubation bags containing sodium polianetol sulfonate that enhances the growth of gram-negative organisms. Incubation at 35°C is for at least 24 hours before the measurement of oxygen concentration in the upper part of the bag, which decrease to 19.5% or lower indicates bacterial growth. This means that the system does not detect the obligatory anaerobes. In trials with 10 well-known platelet contaminants at a dose of 100 to 500 CFU/ml, 96.5% were detected in the first 24 hours, and 100% for 30h. However, some external validation study showed a lower than expected sensitivity of *S.epidermidis* and *S.agalactiae* (3).
3. BACTEC FX System is a recently introduced automated system for haemoculture, used in Korea; detects growth of MO with fluorescent sensors for CO_2.

In clinical microbiology laboratories a five-day incubation protocol is generally used (17). As the level of bacteria that is present at the time of blood or PC donation is low, detection is limited by several factors, including the initial bacterial titer, size and time of sample collection for testing and the kinetics of growth of some bacteria. Low levels of bacteria at the time of donation, especially those slow growing, is a challenge for most methods of cultivation and requires preincubational period of at least 24 hours before sampling and subsequent 24 hours for incubation. However, several studies have shown that the percentage of undetected bacterial contamination, when the initially negative units sowed the fifth or seventh day of storage, range from 13% to 43%. As of slow growing bacteria in question was mainly Propionibacterium acnes who is considered to be clinically insignificant, the rate is reduced to 6% (2, 18, 23-25).

Another important factor of sampling is the sample size. Unlike apheresis units that contain 200-250 ml of products, PC units extracted from random donors' blood units, have volume of 45-65 ml. Therefore, the amount of sample needed to inoculate the aerobic and anaerobic bottle (total of 8-16 ml), would significantly reduce the amount of products for transfusion (2, 18, 24). On the other hand, McDonald and colleagues showed in their study that the inoculation of 2 ml of PC-BC in Pedi-BacT (pediatric, aerobic) Bact/Alert bottle, was sufficient to detect any bacteria in the reporting period (other than P. Acnes), present in quantity of 10-100 CFU/ml (18). Also, of the most commonly isolated bacterial pathogens that are related with transfusion induced bacteremia and sepsis: Gram-positive *Staphylococcus epidermidis, S. Aureus, Streptococcus agalactiae, B. Cereus, E. faecalis, S. Pneumoniae, and Gram-negative E. coli, Enterobacter aerogenes, E. cloacae, P. rettgeri* and *Y. enterocolitice*, only *Cl.perfrigens* is anaerobic MO. Fortunately, these bacteria grow in aerobic and anaerobic bottles (2, 11, 18, 23, 24, 26) and Mastronardis study (20) showed a lower sensitivity of the anaerobic bottle.

The frequency of clinically diagnosed septic episodes as a result of transfusion of contaminated products is significantly reduced with respect to the existing data on bacterial

contamination of blood products, particularly platelets (3). The probable reason is that serious, even fatal sepsis cases go unrecognized as a result of transfusion, which means that the actual prevalence of septic transfusion reactions is significantly higher than reported. An aggravating circumstance is that the septic reactions due to transfusion of contaminated products can easily be unrecognized as such because of the high incidence of febrile nonhaemolytic transfusion reactions (FNHTR), especially after platelet transfusion. Incidence rate of FNHTR after platelet transfusion may be up to 15%. Since the symptoms of septic transfusion reactions are similar to those of FNHTR, many mild septic reactions may remain unrecognized as such. In addition, most MO isolated from contaminated unit PC are normal skin flora (eg, Gram-positive cocci) and is often considered a consequence of contamination during the sampling process, ie. false positive (3, 14, 23).

CONCLUSION

Since there is a great need for platelets in our hospital, we think that common practice of PC sterility control could be used to extend the shelf-life of platelets. Specifically, in addition to routine measures, such as detailed donors' screening, good disinfection of venipuncture site and removing the initial blood into sampling pouch, Mirasol PRT pathogen inactivation system, used in our facility, could be introduced. Certain amount of PC, could be inactivated during first 32 hours after separation, as recommended by the manufacturer, and be ready for clinical use. Unused PC units would be stored in standard conditions and for given time (three days) potentially present bacteria have time to reach a detectable level. In this way we could reduce the quantity of samples for sterility testing (and thus provide an adequate amount of product for transfusion), taking only 2 ml of each of four units of PC, which would be inoculated at the same vial. Based on data from previous studies, only one, aerobic bottle, would be sufficient, which would also, double the capacity in BacT/Alert 3D automated system. Since all PC units are treated/tested, shelf-life could be extended from five to seven days.

ACKNOWLEDGMENT

This work was supported by the Faculty of Medicine, Military Medical Academy, University of Defence, Belgrade (Project MFMMA9/17-9).

ETHICS APPROVAL

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

FUNDING

None.

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THE OCCURRENCE OF LOCAL RECIDIVE IN PATIENTS WITH PLANOCELLULAR CARCINOMA OF THE LARYNX

Dejan Jovanovic[1], Biserka Vukomanovic[2], Vanja Jovanovic[3] and Nemanja Rancic[4]

[1]*Military Medical Academy, Institute of Radiology, Belgrade, Serbia*
[2]*Military Medical Academy, Institute for Pathology, Belgrade, Serbia*
[3]*Military Medical Academy, Institute of Occupational Medicine, Belgrade, Serbia*
[4]*Military Medical Academy, Centre for Clinical Pharmacology, Belgrade, Serbia*

Received: 24.02.2020.
Accepted: 07.04.2020.

Corresponding author:

Dejan Jovanovic, MD

Military Medical Academy, Institute of Radiology, Belgrade, Serbia

E-mail: jovanovicdrdejan@gmail.com

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UDK: 616.22-006.6-074
Eabr 2023; 24(1):33-40
DOI:10.2478/sjecr-2020-0037

ABSTRACT

Early diagnosis and adequate surgical treatment are the key factors for the course of the laryngeal cancer of the head and neck and the outcome of the surgical treatment in these patients. The aim of the paper is to compare the characteristics of the surgically treated patients with the larynx carcinoma who developed recidivism to those who did not. The study was done as a retrospective observational case-control study on a total of 90 patients with the laryngeal cancer who had been successfully operated on and whose cases had been followed over a period of at least five years after the surgery. After that, they were shorted into two groups based on the development of recidivism, and clinical characteristics of the patients with and without recidive disease were compared. 19 out of 90 patients developed recidivism. Maximum diameter of the tumour was significantly larger in patients without local recidivism. In patients without local recidivism, most common clinical stages were 3 and 4 (32.4%, 40.8%, respectively), whereas in patients with local recidivism stages 1 and 2 were the most common (47.7%, 26.3%, respectively). The average time to disease recurrence in the 19 patients who developed local recidive disease was 648.10 (384.67-911.54) days. Pathohistological analysis showed that samller the size of the tumour and lower T grade at the moment of surgery are precautionary factors for further monitoring of patients with laryngeal cancer.

Keywords: *Larynx, planocellular carcinoma, survival, recidivism.*

INTRODUCTION

Head and neck tumours are a heterogenous group of malignant diseases, with different clinical courses and prognoses, and their pathogenesis includes a lot of etiological factors. Planocellular carcinomas, tumours which form from the cells of covering, stratified squamous epithelium of the mucosa of the upper aerodigestive tract, account for 90% of these diseases (1). The digree of their cellular and tissual differentiation varies from well differentiated to poorly differentiated and anaplastic forms. Their biological behavior depends on the cytological and histological characteristic of the tumorous tissue. When it ocmes to localization, these tumorous include oral cavity carcinomas, all three sections of the pharynx (oropharynx, nasopharynx, hypopharynx), larynx, nasal cavity and sinuses (1-4).

Laryngeal cancer is the most common head and neck tumour (33.9%) (1). Epidemiological studies show that middle-aged and elderly men are predominantly affected. Various data point at the fact that health education, i.e. campains against smoking and alcoholism, lowers the number of addicts, and simultaneously the number of people suffering from laryngeal cancer, which underlines its importance in the prevention of the disease. Humanpapilloma virus infection (HPV) is another risk factor, which can lead to the development of carcinoma in younger population (2, 3). The mot common histological type of carcinoma (in 95%) is the planocellular carcinoma (4).

Early diagnosis and adequate surgical treatment are the key factors for the course of illness in patients with planocellular carcinoma of the head and neck (5). There is ample evidence that there is a direct relationship between the presence of malignant cells in surgical margins and a higher risk for the development of recidivism (5).

The diagnosis of malignant tumour of the larynx is multidisciplinary, it lies in anamnesis, clinical otorhinolaringological examination, endoscopic examination, radiographic and pathohistological findings. Endoscopic examinations include a flexible nasopharingolaryngoscopy, endovideostroboscopy, laryngomicroscopy and aesophagoscopy. Laryngomicroscopy (LMS) is the examination of the larynx under the surgical microscope. It is done in general endotracheal anesthesia. Laryngomicroscopy is the most reliable method for the determination of the local tumour spread. In case of infiltrative tumours, there is edema, which makes tumour boundaries less clear, so they are not always easy to determin. LMS is the optimal method for obtaining tumour specimen for histological examination.

Histopathological diagnostics is the golden standard for making the diagnosis of a planocellular carcinoma. Tumours are classified based on the degree of histological and nuclear differentiation, with or without a degree of extracellular keratinization, from well to poorly differentiated degree. Today, immunohistochemical methods are very useful additional methods in pathohistological diagnostics. P16 as a surogat marker for human papillomavirus infection is of great importance for the diagnosis (6).

It is necessary that the diagnostics in case of patients with laryngeal tumour be completed with computerized tomography (CT), although the examination with nuclear magnetic resonance (NMR) is more and more common. CT does not provide sufficient data regarding very small lesions, nor regarding the spread of big tumorous lesions, but it is very useful in determining the spread of tumours to subglottis and cricoarytenoid region, to lymph nodes of the neck, and it provides insight into the surrounding bone structure. NMR can point to the early spread to cartilage and anterior commissure, as well as to submucous and transglottic spread of the tumour. NMR has the advantage over CT when it comes to determining the stage of the primary tumour, while a positive aspect of CT scan is that it lasts shorter. Meta analysis which included 63 studies, 24 retrospectives and 39 prospective cases and which was conducted in 2015, compared CT and NMR in diagnosing verification of the metastases of the neck, in case of head and neck region carcinoma, for the evaluation of the lymph node of the neck. It showed that CT has higher sensitivity, whereas NMR has higher specificity. NMR is superior when it comes to diagnosing pathological, metastatic lymph nodes of the neck, especially for confirming the diagnosis, while CT has better efficacy to exclude the metastatic lymph node (7).

The choice of the method for treatment depends on the degree of the histological diagnosis with the parameters of the spread of the illness in local structures, the invasion through blood and lymph vessels and nerves, i.e. locoregional, distant spread of the tumour, the patient's general medical condition, social conditions, and the motivation of the patient to accept the recommended way of treatment. The decision about the treatment is made for every patient individually by the Oncology consilium for malignant tumours of the head and neck region. The Oncology consilium consists of an otorhinolaryngologist, a radiation oncologist, a medical oncologist and a pathologist.

Determining the stage of the planocellular carcinoma of the head and neck influences greatly the right choice of treatment. After the processing and pathohistological verification of the planocellular carcinoma, but before the beginning of the treatment, the illness needs to be classified according to the TNM classification (*TNM staging system 8-edition, 2016*) by The Union for International Cancer Control (*franc. Union Internationale Contre le Cancer, UICC*) (8). According to this classification, small primary tumours of the head and neck, up to 2 cm in size (T1) and 2 cm to 4 cm (T2), are treated either surgically or with radiotherapy. The main advantage of radiotherapy is the fact that the function of the organ remains intact. In case of an advanced illness, when the tumour is more than 4cm in size or there is an infiltration to the surrounding tissues (T3 and T4 or N+, spread to the lymph nodes), a combined approach is necessary, i.e. surgical

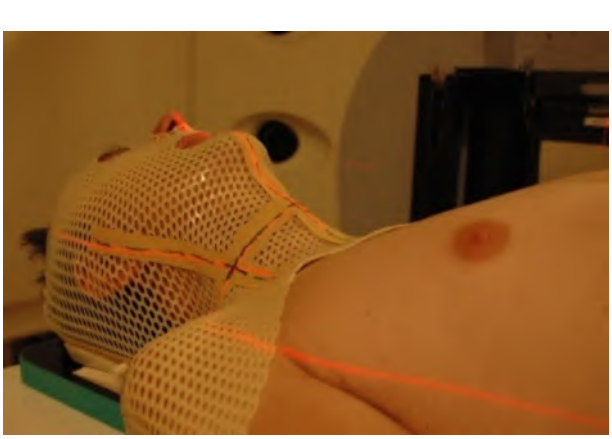
procedure, chemotherapy and radiotherapy. In case there are no clinical signs that there is spread to the lymph nodes of the neck (N0), it is nonetheless necessary to treat the neck with radiotherapy if the risk of occult metastases is greater than 20%. In that case, along with the radiation of the primary tumour, elective radiation of the neck needs to be done as well (9).

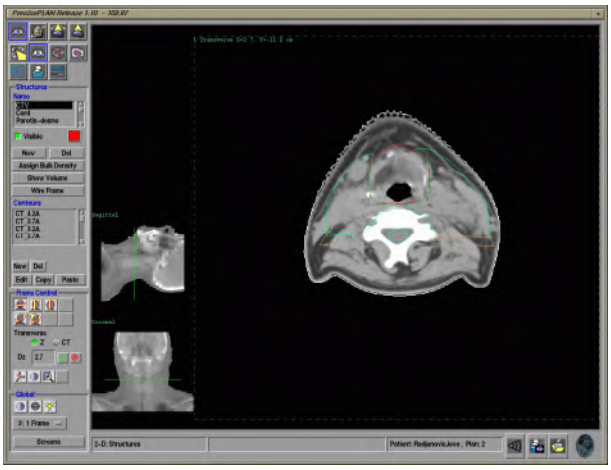
In case of laryngeal cancer, radiotherapy can be used as a primary adjuvant or neoadjuvant method of treatment, most often postoperavively. Indications for postoperative radiation are: the surgery was not radical, assumption that the surgery caused the spread of the process, due to the localization of the infiltrative process, the presence of blood and lymph vessels and nerve endings, or the presence of occult regional metastases or when, upon the dissection of the neck, a lymph node whose capsule was pierced by the tumorous process was found.

The main point of postoperative radiation is the sterilization of the operating filed to eliminate possible lingering occult malignant cells, which reduces local recidivism or the possibility of the appearance of close or distant metastases. We can conclude that radiotherapy is performed postoperatively on the primary tumour location, i.e. the neck, in patients with a high risk of locoregional recidive, and this way locoregional control of the illness is improved and survival is more imminent. Postoperative radiotherapy is recommended for any T (1-4) carcinoma with positive N (nodal) status (the illness is present in lymph nodes) or if surgical margines are positive for tumorous tissue. Also, extracapsular extension of the disease (extracapsular spread of the tumour to a metastatic lymph node) is an indication for postoperative chemoradiotherapy, as well as other unfavourable risk factors such as perineural ivasion and vascular invasion, which are relative indications for postoperative radiotherapy. The addition of cisplatin during chemoradiotherapy shows a better control of the illness than radiotherapy alone in high-risk patients and in case of locally advanced illness (10). Postoperative radiation is done about six weeks after the surgery. The opinion is unanimous that during the locoregional radiation therapy treatment, the dose should be from 45Gy to 50 Gy, then, targeted RT to the affected LN (extracapsular extension of the illness) from 56Gy to 60Gy, as well as additional radiation of the tumour bed of up to 65Gy, or 70Gy (11, 12).

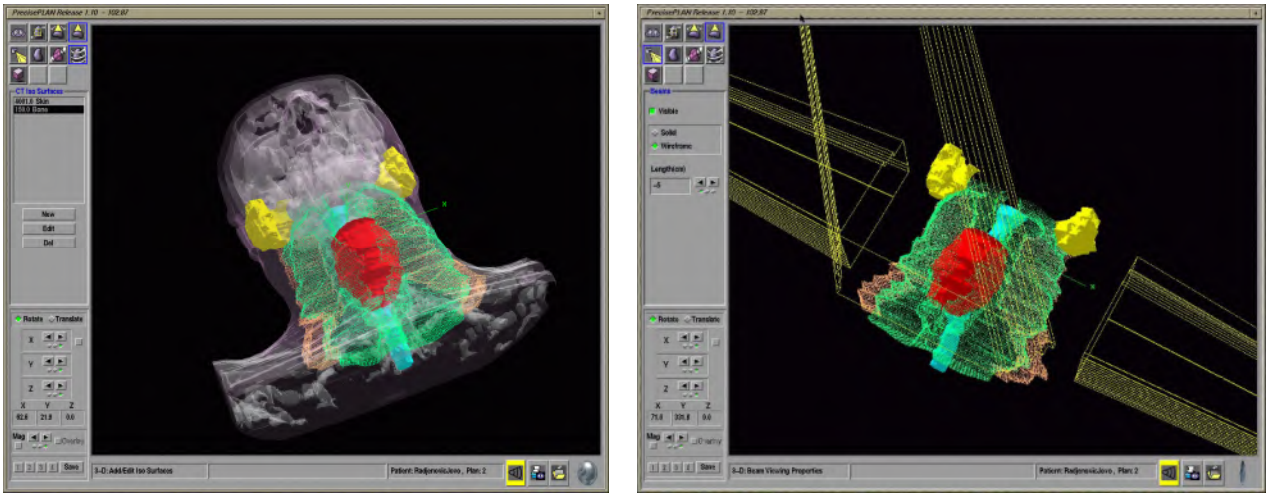
Radiotherapy can be radical and palliative. Paliative radiotherapy is performed on patients who are not able to undergo other methods of specific oncological therapy and it is temporary help for the patient. In case of palliative radiation, doses are lower, 2/3 of the radical dose which is delivered in hypofractionated mode. Radical radiotherapy in the head and neck region is the greatest challenge when applying conformal radiotherapy, because of a complex geometry of anatomical structures and potential complications caused by radiation. Often, the distance between the gross tumour volume (GTV) and the clinical target volume (CTV) and the organ at risk (OAT), such as the optical nerv, spinal cord or salivary gland, is not more than a few millimeters (13). Because of the potential escalation of the tumorous dose and a better locoregional control, conformal radiotherapy technique for the treatment of head and neck tumours is introduced, and its planning is based on computerized tomography - CT. At the moment, CT-NMR imaging is used in order to provide the radiation onclolgist with more precise directions for delineation, i.e. for outlining the target volumes (14). Optimal delineation of the primary tumour and involved lymphonoduses is the prerequisite for the curative radiotherapy of the planocellular carcinoma of the head and neck. When planning radiation therapy, it is important to localize the majority of peripherally embedded tumorous cells, with the aim to perform radiation of tumorous cells as precisely as possible, while sparing organs at risk. Three-dimensional conformal radiotherapy (3D RT) is a radiation technique with which the radiation dose, with its shape adjusted to the shape of the target volume, is distributed. This should enable more precise application of the therapy dose to the tumour, with minimal harm to the healthy tissue.

Figure 1. Preparatory and therapy procedures 3D CRT (conformal radiotherapy)





Immobilisation - making the mask and contouring volumes of interest



Virtual patient and beam combination

METHODS

At the Radiology Institute of Military Medical Academy, in cooperation with Otorhynolaryngology Clinic and Pathology Institute, a retrospective observational case-control study of surgically treated patients over a period of time from 2008 to 2016 and those treated with radiotherapy from 2011 to 2016 was conducted. All the patients with laryngeal cacer who received radiotherapy after the surgery were the subject of analysis. All the patiens were surgiclly treated and have postoperative radiation terapy, according to indications which it were listed in introduction. Postoperative radiation is done about six weeks after the surger in to the dose from 65 Gy to 70 Gy. They were divided into two groups: the group which developed recidive after the treatment and the group without recidive.

Sociodemographic characteristics (gender and age) and pathohistological characteristics of the tumour after surgical resection (Maximal tumours diameter, Ceratoticum, Perineural invasion, Perivascular invasion, Perilymphatic invasion, Histologic grade, Nuclear grade, T grade, N grade, Clinical TNM stage) were analysed. MSCT was also used to measure tumour diameter. Average time to recidive disease was then calculated, as well as the percentage of patients who developed recidive after one, three, five and ten years.

Complete statistical analysis was done with the statistical software package, PASW Statistics 18. Atribute variables were presented as frequency of certain categories, while statistical significance of differences was tested with the Chi-square test. Numerical variables were presented as mean with standard deviation or median with interquartile range (25-75 percentile), while statistical significance of differences was tested with the Mann-Whitney test or Independent samples t test (normal or not normal distribution). All the analyses were estimated at $p<0.05$ level of statistical significance. Unadjusted time to recurrence disease or disease-free survival was calculated using Kaplan-Meier plots and p-values derived from the univariate Log-rank test.

RESULTS

The study encompassed the total of 90 patients with laryngeal cancer who were operated on and who received radiotherapy over a period of time from 2008 to 2016 at the aforementioned clinics and institutes of the Military Medical Academy. Local recidive disease appeared in 19 patients, i.e. 21.11%. Statistically significat difference regarding gender and age of the patients who developed recidive and those who did not was not found (Table 1).

Table 1. Sociodemographic characteristics of the treated patients with laryngeal cancer

	Patients without recidive (n=71)	Patients with recidive (n=19)	p value
Gender: male/ female	58 (81.7%) / 13 (18.3%)	17 (89.5%) / 2 (10.5%)	0.644[1]
Age (years); M ± SD	65.45 ± 9.60	62.16 ± 12.06	0.212[2]

M ± SD- mean and standard deviation;
[1]- Chi-square test; [2]- Independent Samples t test

Table 2 shows pathohistological characteristics of the tumours after the surgical resection. There is a great difference with regart to the maximal tumour diameter, which was

significantly bigger in patients without local recidive, compared to the patients with local recidive disease (median; 25 vs. 15 mm). There is also a significant difference with regard to T stage of the disease, where the patients without local recidive were usually stage 2, 3 and 4 (25.4%, 36.6%, 25.4%, respectively), while the ones with local recidive disease were usually stage T1 (47.7%).

If we take a look at the clinical TNM stage, we can also see that stages 3 and 4 are the most common in patients without local recidive disease (32.4%, 40.8%, respectively), while in the patients with local recidive stages 1 and 2 are the most common (47.7%, 26.3%, respectively). In terms of other characteristics, no significant difference was discovered.

Table 2. Initially patohistological characteristics of the tumour in the treated patients with laryngeal cancer

	Patients without recidive (n=71)	Patients with recidive (n=19)	p value
Maximal tumors diameter (according to MSCT); median with IQR- mm	25.00 (17.00-35.00)	10.00 (0.00-25.50)	**0.002**1
Maximal tumors diameter; median with IQR- mm	25.00 (20.00-35.00)	15.00 (10.00-20.00)	**<0.001**1
Ceratoticum: yes/no	44 (62.0%) / 27 (38.0%)	12 (63.2%) / 7 (36.8%)	1.0002
Perineural invasion: yes/no	14 (19.7%) / 57 (80.3%)	2 (10.5%) / 17 (89.5%)	0.5532
Perivascular invasion: yes/no	25 (35.2%) / 46 (64.8%)	4 (21.1%) / 15 (78.9%)	0.3702
Perilymphatic invasion: yes/no	34 (47.9%) / 37 (52.1%)	4 (21.1%) / 15 (78.9%)	**0.065**2
Histologic grade: 1 / 2 / 3	42 (59.2%) / 28 (39.4%) / 1 (1.4%)	13 (68.4%) / 6 (31.6%) / 0 (0.0%)	0.6972
Nuclear grade: 1 / 2 / 3	31 (43.7%) / 38 (53.5%) / 2 (2.8%)	13 (68.4%) / 6 (31.6%) / 0 (0.0%)	0.1432
T grade: 1 / 2 / 3 / 4	9 (12.7%) / 18 (25.4%) / 26 (36.6%) / 18 (25.4%)	9 (47.7%) / 5 (26.3%) / 2 (10.5%) / 3 (15.8%)	**0.005**2
N grade: 0 / 1 / 2	43 (60.6%) / 12 (16.9%) / 16 (22.5%)	15 (79.0%) / 2 (10.5%) / 2 (10.5%)	0.3232
Clinical TNM stage: 1 / 2 / 3 / 4	7 (9.9%) / 12 (16.9%) / 23 (32.4%) / 29 (40.8%)	9 (47.7%) / 5 (26.3%) / 1 (5.3%) / 4 (21.1%)	**<0.001**2

IQR- interquartile range (25-75 percentile);
[1]- Mann-Whitney test; [2]- Chi-square test; MSCT- multislice computed tomography

In the 19 patients who developed recidive disease, the diameter was about 35mm (median) (table 3). Extracellular keratinisation and lymphatic invasion were present in a high percentage of cases. Their clinical stadium was usually TNM 4.

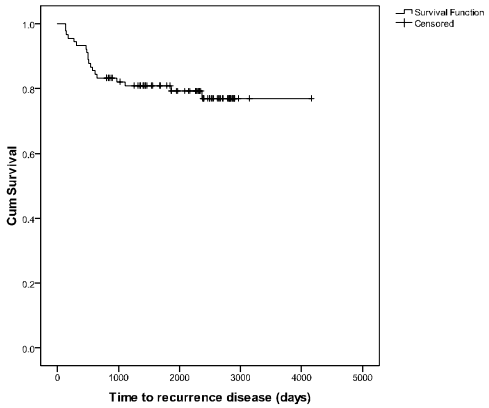
Table 3. Patohistological characteristics of recidivism in the 19 treated patients with laryngeal cancer

Maximal tumors diameter (according to MSCT); median with IQR- mm	35.00 (22.75-45.25)
Maximal tumors diameter; median with IQR- mm	30.00 (20.00-40.00)
Ceratoticum: yes/no	12 (63.2%) / 7 (36.8%)
Perineural invasion: yes/no	7 (36.8%) / 12 (63.2%)
Perivascular invasion: yes/no	7 (36.8%) / 12 (63.2%)
Perilymphatic invasion: yes/no	11 (57.9%) / 8 (42.1%)
Histologic grade: 1 / 2 / 3	1 (5.3%) / 15 (78.9%) / 3 (15.8%)
Nuclear grade: 0 / 1 / 2	1 (5.3%) / 12 (63.2%) / 6 (31.6%)
T grade: 1 / 2 / 3 / 4	2 (10.5%) / 4 (21.1%) / 5 (26.3%) / 8 (42.1%)
N grade: 0 / 1 / 2 / 3	10 (52.6%) / 2 (10.5%) / 5 (26.4%) / 2 (10.5%)
M grade: 0 / 1	18 (94.7%) / 1 (5.3%)
Clinical TNM stage: 1 / 2 / 3 / 4	2 (10.5%) / 1 (5.3%) / 5 (26.3%) / 11 (57.9%)

IQR- interquartile range (25-75 percentile);
MSCT- multislice computed tomography

Time to recurrence disease was 3381.56 (3069.24-3693.87) days on average in all the patients (Figure 3). Distrubution of the patients without recidive after one-year, 3-year, 5-year and 10-year was 93.33%, 82.22%, 81.11% and 78.89%, respectively.

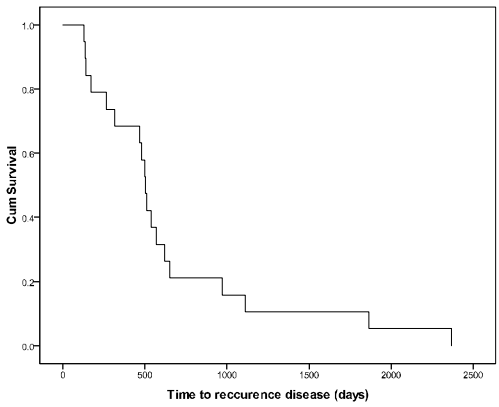
Figure 3. Time to recurrence of the disease in the treated patients with laryngeal cancer; means with 95% confidence interval: 3381.56 (3069.24-3693.87) days



One-year, 3-year, 5-year and 10-year overall time without recidivism were 93.33, 82.22, 81.11 and 78.89%, respectively

On the other hand, time to recurrence of the disease in patients with local recidive was on average 648.10 (384.67-911.54) days (Figure 4). The rate of the appearance of recidive disease after one-year, 3-year, 5-year and 7-year period was 31.58%, 84.21%, 89.47% and 100%, respectively.

Figure 4. Time to recurrence of the disease in the 19 treated patients with laryngeal cancer with recidive disease; means with 95% confidence interval: 648.10 (384.67-911.54) days



Rates of recidive in the one-year, 3-year, 5-year and 7-year period were 31.58, 84.21, 89.47 and 100%, respectively

Based on logistic regression, the initial size of the tumour (maximal diameter) stands out as the only significant predictor of the appearance of the local recidive disease. In case of smaller tumours, there was 1.25 higher probability for the development of local recidive disease (Odss ratio 1.25 (1.04-1.51); p=0.02).

DISCUSSION

Survival of the patients who were operated on and the absence of recidive disease are the criteria for the successful treatment of the laryngeal cancer. Any approach to treating laryngeal carcinoma leads to the development of local or locoregional recidive to a certain point. Regardless of the primary approach, regular control examinations are necessary in all stages of the disease, because recurrence of the

locoregional disease depends chiefly on the stage of the disease (15).

In our study, which analysed a total of 90 patients with laryngeal cancer who had the surgery and recived radiotherapy and who were treated in the aforementioned clinics, local recidive disease developed in 19 patients, i.e. 21.11%. The longest period of time to the development of the local recidive after the primary surgical tretament was nine years. With regard to gender distribution and age, no statistically significant difference was found between the patients who developed recidive and those who did not. Time to the development of local recidive in these 19 patients with local recidive disease was 648.10 days on average, that is about 1.77 years. One study showed that 95% of recidives appear withing the first 3.6 years (16).

Previous results shown that in 22.6% of cases out of the total number of patients, the average time within which the development of recidive was recorded was 12.6 months (17). Recidive is significantly more common in male subjects (23.87%) than in female subjects (12.22%). Time to recurrence of the disease is 36 months. Other authors (16) did not find the difference in the incidence of recidive between the subjects of different gender. Evaluation of the patients in various stages of the disease is what makes our studies different. Namely, their study was limited only to stage IV of the carcinoma in the glottis region, while in our study, patients with laryngeal cancer in all locations and in different stages of the disease were included and analysed, and we also did not find an important connection between gender and the development of recidive.

The initial size of the tumour (maximal diameter) was the only significant predictor of recidive that stood out in our observed group. In case of smaller tumours, probability for the development of recidive was 1.25 higher (p=0.02). This can be explained by the fact that in case of small tumours operated with transoral technique, specific postoperative oncological therapy is not administered. In case of locoregional spread of the tumour, chemoradiotherapy is administered postoperatively, so in this way better locoregional control over the disease is achieved, i.e. there is a smaller number of local recidive diseases. A group of authors (18) analysed 438 patients with planocellular carcinoma of the larynx, who most commonly underwent hemilaryngectomy in T2 stage, and in 76 patients (17.35%) local recidive developed mostly during the first year so these patients received radiation therapy after the surgical treatment of the recidive disease. Another group of authors (19), in their study of 439 primarily surgically treated patients with supraglottic laryngeal carcinoma, over a period of 22 years, showed that local recidives developed in 22 patients (5%). In case of two patients, recidive developed on the base of the tongue, in six patients on the ventricular fold and in five patients on the glottis. Total laryngectomy was performed on these patients followed by postoperative radiotherapy. Five-year survival, with no signs of illness, is 76%.

A group of authors (20) describes local recidive in 12%, and regional recidive in 9% of treated patients. Similarly to the previous one, another group of authors (21) examined 416 patients in stages T1 and T2, and local recidive developed in 7.2% of cases (23) The lower recidive percentage which these authors stated compared to our results can be explained by the fact that our patients with recidive disease were most often stage T1.

In an earlier study (18), it was shown, with regular controls after the priod of five years, that 346 (79.0%) patients were without any sighns of illness and were in good general health at the moment of the examination.

A group of authors (20) stated that five-year survival of their patients after hemilaryngectomy without recidive disease was 81%. Another group of authors (22) stated that in their longitudinal study of 150 patients who underwent hemilaryngectomy, two-year survival was 83%. The results of the stated authors are in accordance with our findings, the distribution of patients without recidive after one-year, 3-year, 5-year and 10-year was 93.33%, 82.22%, 81.11% and 78.89%, respectively.

CONCLUSION

Our study showed that the development of recidive in patients is most commonly diagnosed in stage 4 TNM. Also, in those patients, extracellular keratinisation, over 95%, was present pathohistologically, as well as perilymph invasion within recidive tumour.

Moreover, the initial size of the tumour was the only significant recidive predictor which stood out in our observed group. In case of smaller tumours, probalility for recidive disease was higher. This can be explained by the fact that small tumours transorally operated do not receive specific postoperative oncological therapy. Therefore, initially diagnosed and operated small laryngeal tumours require more regular local status controls. That is to say that in case of patients who were operated using endoscopic surgical technique, regular control examinations by means of indirect laryngoscopy, with which early local recidive can be diagnosed, should be planned.

Our research points at the fact that locoregional control of the disease is very important for a successful treatment of the advanced laryngeal cancer. Only multidisciplinary approach to diagnostics and treatment of laryngeal cancer makes the survival of the patients without local recidive disease within five years from the previous treatment in over 82% of cases possible.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary written and

informed consent was obtained from the patient prior to enrollment in the study

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

FUNDING

None.

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THE RELIABILITY OF VENOUS SINUS STENOSIS USING NON CONTRAST 3D MRV IN PREDICTION OF THE DIAGNOSIS OF IDIOPATHIC INTRACRANIAL HYPERTENSION, A CASE CONTROL STUDY

Noor Abbas Hummadi Fayadh , Noor Kathem Nee'ma Al-Waely and Ammar Mosa Al-Mosawe

Department of Surgery -Diagnostic Radiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Received: 18.12.2021.
Accepted: 18.04.2022.

Corresponding author:

Dr. Noor Abbas Hummadi Fayadh
Lecturer at the College of Medicine/Al-Nahrain University

Department of Surgery-Diagnostic Radiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Phone: +964 780 289 6685

E-mail: noorabbashummadi@nahrainuniv.edu.iq

UDK: 616.831-008.6-073
Eabr 2023; 24(1):41-47
DOI:10.2478/sjecr-2022-0038

ABSTRACT

Idiopathic intracranial hypertension, or shorty IIH, refers to a clinical syndrome in which there is elevated CSF pressure and papilledema in the lack of any focal neurological signs. It is largely a diagnosis of exclusion, but imaging workup is undertaken routinely in order to rule out other pathologies. In this study we tried to evaluate the prevalence of venous sinus abnormalities using the simpler and less invasive non contrast enhanced 3D TOF MRV in group of patients with IIH, using the combined stenosis score, and compare this score with a group of controls, to assess the accuracy of MRV alone as an individual test in predicting the diagnosis of IIH. The study sample included 44 patients and 25 control subjects, following MRV analysis it was found that a combined severity score of cut of value of 4.5 for the combined conduit score (CCS) was associated with 79% sensitivity and 88.6 specificity for the diagnosis of IIH, and a cut off value of 5.5 for the adjusted score method gives a 83% sensitivity and 88.6 % specificity for the diagnosis. MRV is a valuable in the diagnosis of IIH; non contrast 3D MRV is an efficient test and gives valuable diagnostic information.

Keywords: *3D MRV, idiopathic intracranial hypertension.*

INTRODUCTION

Idiopathic intracranial hypertension, or shorty IIH, also termed primary pseudotumor cerebri, refers to a clinical syndrome in which there is elevated CSF pressure and papilledema in the lack of any focal neurological signs. It is largely a diagnosis of exclusion, but imaging workup is undertaken routinely in order to rule out other pathologies that my lead to intracranial hypertension, such as obstructive hydrocephalus, masses, meningitis, vascular malformations and fistulae, or dural venous sinus (1–6).

The importance of this condition is that if left untreated, visual deterioration and even blindness may ensues (1,3).

With routine use of MRI and MRV to exclude other causes of raised intracranial pressure, a significant proportion of patients were noted to have non-thrombotic venous sinus abnormalities (1,7–9).

The most frequent MRV finding is bilateral lateral sinus stenosis, with some working groups showing that nearly all patients showing venous sinus flow abnormalities that are non-thrombotic in nature, namely, bilateral significant focal venous sinus stenosis (6,8,10).

However, venous sinus abnormalities like narrowing and hypoplasia are not infrequent findings noted routinely in MRI imaging including studies on healthy subjects (9); older research have questioned the value of MRV for diagnosis of IIH stating that venous sinus abnormalities may are prevalent in controls, making the diagnostic contribution of MRV limited in cases of IIH, suggesting that MRI and MRV makes no important contribution in confirming or refuting a diagnosis of IIH (11,12).

Moreover, most of the radiological workup of IIH patients with MRI used contrast enhanced MRV, stating higher sensitivity and accuracy in evaluation of sinovenous stenosis , and for this purpose some researchers used a quantitative scoring system that combine the findings from both lateral sinuses to assess the combined stenosis score (8,9,13).

THE AIM OF THE PAPER

In this study, we tried to evaluate the prevalence of venous sinus abnormalities using the simpler and less invasive non contrast enhanced 3D TOF MRV in group of patients with IIH, using the combined stenosis score, and compare this score with a group of controls, to assess the accuracy of MRV in predicting the diagnosis of IIH.

PATIENTS AND METHODS

Patients

In the time from March 2019 to December 2020, cases clinically suspected to have IIH by the hospital's neuromedicnie specialists, were referred to the MRI unit at our hospital, examination by MRI and MRV was done, the MR study was followed by CSF pressure assessment by LP done 24-72 hours by the clinical team and only cases proved to have raised ICP were selected for the patient group; therefore, the inclusion criteria included cases clinically suspected as IIH, with neuroimaging showing no alternate pathology , and confirmed by CSF manometry done in the neuromedicine ward.

The local ethics committee approved the study.

Written informed consent was obtained from all the patients prior to inclusion.

Exclusion criteria for cases were when the MRI showed abnormalities such as mass, infection, intracranial hypotension, patients with previous surgical interventions, cases with venous sinus thrombosis, hydrocephalus, or any abnormality that would label the patient a diagnosis other than Idiopathic intracranial hypertension .

Controls

All MRV studies performed from March 2019 to December 2020 at the MRI unit at our hospital were reviewed and a control group of subjects was identified based on the availability of a brain MRI reported as normal by a single neuroradiologist and with no feature of venous sinus thrombosis on MRV. Patients were not excluded from the control based on MRV abnormalities other than thrombosis.

Unfortunately, no clinical data for the presenting symptoms of the control group was available.

Image acquisition

MR imaging was performed with a Philips healthcare Achieva 3T TX 32CH.

Standard neurovascular coil (8-channel). Routine coronal, axial, and sagittal T2-weighted images (TR=3440–4100 ms; TE=84–88 ms; field of view 23 cm; slice thickness 5mm; interslice gap1mm; echo train length, 16; matrix, 352×256; number of average=2) and 3DPCA MRV (TR=15ms, TE=35 ms; field of view 22cm; slice thickness 1.4 mm; number of average 1; echo train length 1; matrix 256*256; flip angle10°) source, and maximum intensity projection (MIP) images were evaluated. Two radiologists with 10 years of experience in neuroimaging evaluated the radiological images.

Image analysis

For assessment of the venous diameter for both patients and control, we used the combined conduit score method, CCS, which is the method originally adopted by Frab et al

(13), were assessment of the lumen of each transverse sinus was measured and compared to the diameter of the of the distal superior sagittal sinus , regardless of the length of the segment , then graded as: 0 for lack of flow or total aplasia, 1 for severe >75% narrowing; 2 for moderate 50-75% narrowing; 3 for narrowing less than 25-50%; and 4 for normal or very mild less than 25% stenosis (13).

Interpretation of the findings was done by two radiologists with more than 7 years' experience in neuroimaging, and consensus result was recorded, the radiologists were not blinded to the diagnosis of patients and controls.

Based on our belief that venous sinus hypoplasia is a common benign finding that is seen frequently in asymptomatic subjects, and in order not to overcall stenosis, we proposed an additional adjusted score method, in this method , whenever venous sinus hypoplasia was encountered, we used the grade for the better opposite side, and multiplied it by two to calculate the combined score, if the opposite side is worse, we would keep the hypoplastic side score as it is, we tried to examine whether this method would give more accuracy in diagnosis of IIH.

Statistical analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences, version 23.0 (SPSS, Chicago, Illinois).

ROC curves were used to assess the sensitivity, specificity, and cut of values.

RESULTS

Our study sample included a total of 44 patients, 38 of whom are females (86.4%), the control group included 24 subjects, 15 of which (62%) are female (table 1).

Table 1. Demographic data of both groups.

Parameter		Patients	Control	P value
		N=44	N=24	
Age (yr)		29.61±1.13	32.25±3.7	**0.502**
Gender	Female	38 (86.4)	15 (62.5)	**0.033**
	Male	6 (13.6)	9 (37.5)	

Age presented by mean±; standard error of mean, gender presented by frequency and percentage, p value for age by unpaired t-test, p value for gender by Fisher exact test.

For the MRV results, using unpaired t-test, both of the combined score and the adjusted score showed statistically significant difference between patients and controls, with P value less than 0.0001 for both, table (2).

Table 2. Comparison of scores between patients and control by unpaired t-test.

Parameter	Patients	Control	P value
	N=44	N=24	
Combined score	2.09±0.31	6.0±0.34	**<0.001**
Adjusted score for hypoplasia	2.23±0.35	7.08±0.32	**<0.001**

Data presented by mean± standard error of mean

For the Combined score method, ROC curve analysis showed that a cut of value of 4.5 was associated with 79.2% sensitivity and 88.6% specificity between IIH cases and controls (figure 1), table (3).

Figure 1. ROC curve for combined score between patients and controls

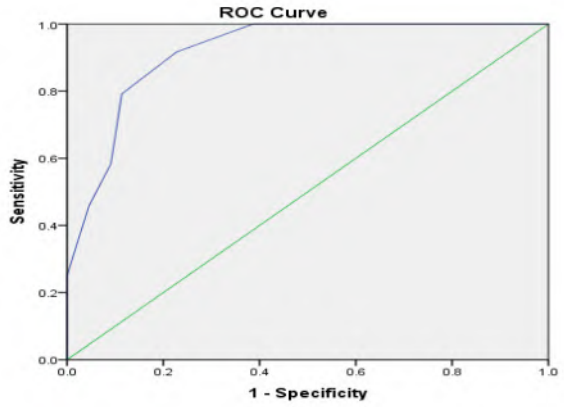


Table 3. Area under curve, sensitivity, specificity and cutoff value for combined score between patients and controls.

Parameter	Area under curve	Sensitivity	Specificity	Cutoff value
Combined score	0.919	79.2%	88.6%	**4.5**

Using CCS, five of our control showed values less than the cutoff, and 5 of the patient showed values higher than the cutoff.

Adopting the adjusted score, a cut of value of 5 was associated with improved sensitivity of 83.3% , without compromising specificity (88.6% as well); (figure 2, table 4).

Figure 2. ROC curve for adjusted score for hypoplasia between patients and controls

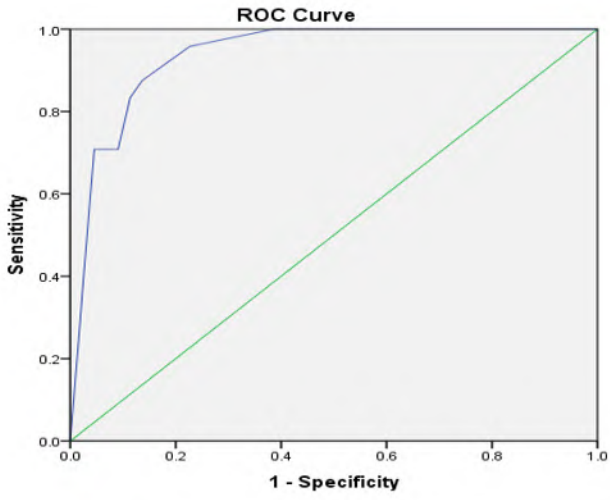


Table 4. Area under curve, sensitivity, specificity and cutoff value for adjusted score between patients and controls.

Parameter	Area under curve	Sensitivity	Specificity	Cutoff value
Adjusted score for hypoplasia	0.938	83.3%	88.6%	**5.5**

Using adjusted scores, four of controls showed values less than the cutoff, and five patients showed values more than the cutoff.

Therefore, in the majority of the cases, MRV did show venous sinus abnormalities in comparison to controls, most bilateral stenosis, as shown in figures 3 and 4.

Figure 3. MIP coronal (A) and axial (B) images MRV showing bilateral occlusions of the lateral segment of both transverse sinuses in patient with pseudotumor cerebri.

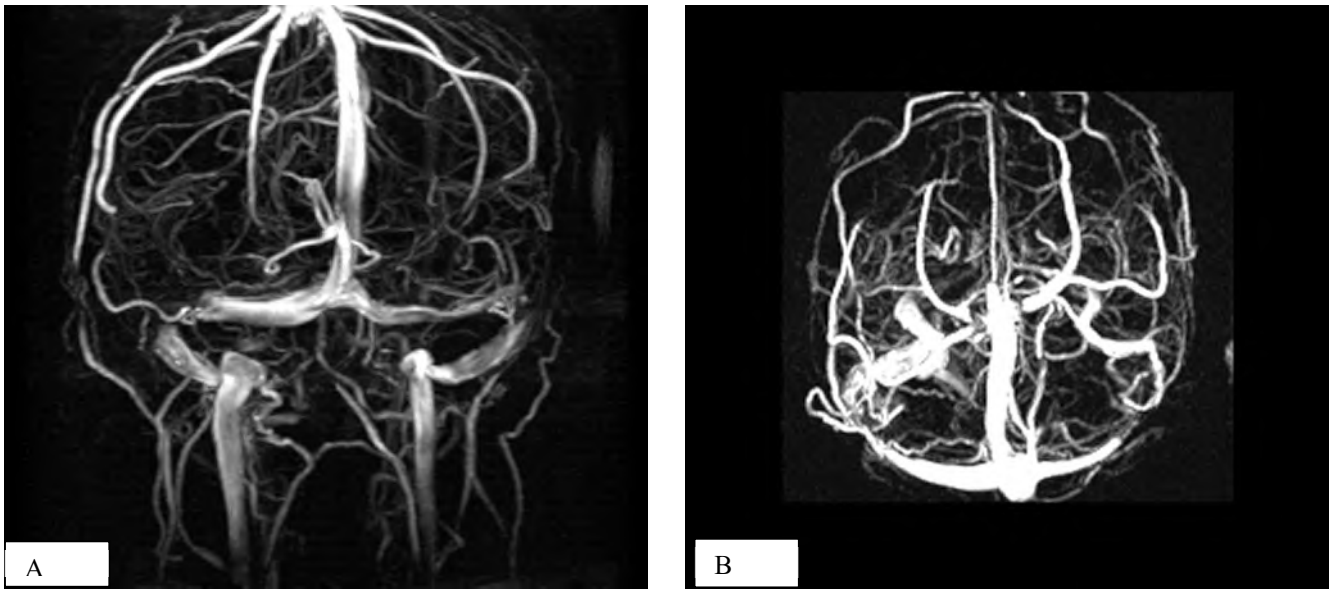
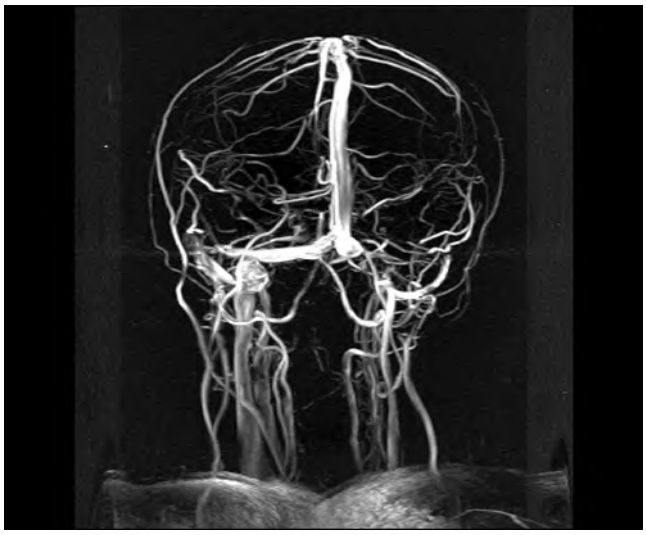


Figure 4. Coronal MIP MRV image shows bilateral transverse sinus occlusions; the patient was diagnosed with IIH clinically.



DISCUSSION

The diagnosis of IIH is supported by multiple radiological findings, and while most of them are variable and may be observed in healthy controls, with no single MRI feature being consistently reported in patients with IIH, but the addition of MRV is frequently reported to raise diagnostic accuracy of MRI (6–8,12,14–17).

We did not attempt to assess the conventional MRI signs for predicting their accuracy alone or in combination with MRV, as this study was mainly targeted at the MRV value in IIH.

Most of the studies used combination of conventional MRI signs such as empty sella turcica and perioptic nerve sheath fullness etc. with MRV, and most of the available studies on the subject used contrast enhanced venography, and concluded that MRV show bilateral venous sinus stenosis in very high percentage of patient with IIH (13,15–17).

In our study, we used our routine non contrast 3 D TOF MRV which is readily available noninvasive technique, and we observed that venous sinus stenosis was far more frequent in patients with idiopathic intracranial hypertension than our control group, and that a combined severity score of cut of value of 4.5 for the CCS was associated with 79% sensitivity and 88.6 specificity, figure 1 table 3, and 5.5 for the adjusted score method gives a 83% sensitivity and 88.6 % specificity figure 2 table 4.

Our use of adjusted scores for hypoplasia has resulted in mild improvement in sensitivity (from 79% to 83%) after adjusting the cutoff point from 4.5 to 5, this method, would prevent overestimation of the score in cases of simple hypoplasia, and while not affecting the specificity, allowed for better sensitivity because of the higher cutoff value of 5.

This result is consistent with the study done by Maralani et at (10) (8), they adopted contrast enhanced MRV, and found that CCS of less than 4 is very specific , nearly 100% but with sensitivity of 62% , and they concluded that finding CCS less than 4, with or without other MRI conventional signs for IIH on MRI, would greatly increase the diagnostic certainty of the test, from 18-46 folds as given in the results (8), however, their results showed sensitivity of 62%, so they concluded also that absence of MRV findings should not dismiss the diagnosis of IIH.

Our data showed overall improved sensitivity in comparison to Maralani et al, this could have been resulted from the higher cut off values for our results, however, the specificity is significantly better in their study , i.e. 100% , our figure of 88.6 % may also have resulted from difference in the calculated cut off, but may also be the result of the technique itself with higher proportion of controls showing venous narrowing that may be artefactual in nature related to slower flow, reflecting the inherently less accurate nature of the non-contrast MRV as compared to contrast studies, but this should not, nonetheless, abandon the value of unenhanced MRV with good sensitivity and specificity, with the advantage of ease of use and lack of contrast medium administration , with its well-known limitations and clinical implications.

Morris et al (16) also found that transverse sinus stenosis was identified on MRV in 94% of patients with idiopathic intracranial hypertension and in 3% of controls and they concluded that MRV is the most sensitive test for IIH (16), these studies were also conducted using contrast MRV. Their data show higher sensitivity than ours, but this may have been largely related to the image analysis they adopted, they regarded unilateral venous sinus stenosis of less than 50% as a positive result, not calculating scores as in our study, which would result in higher positive results, therefore, higher sensitivity.

The variability of MRV findings in normal subjects is well known, and the fact that venous sinus hypoplasia or narrowing may be seen in normal subjects may cast doubt on the significance of MRV findings when IIH is suspected, and earlier research on the topic has even refuted a significant association between IIH and venous sinus abnormalities, stating that they are no more common than in control subjects (12), however, and similar to our results, a study by Kelly et al (9) investigated the prevalence of venous sinus abnormalities in a retrospective study excluding with IIH , their study, with imaging protocol using contrast MRV, indicated that while bilateral significant venous sinus narrowing may exist in subjects without IIH, but this observation is not common, and therefore, finding bilateral lateral sinus narrowing should prompt a thorough search for papilledema (9).

Higgins et al. (18) and similar to our experiment, relied on non-contrast 3D and 2D MRV, and their work on 20 patients with IIH and 40 control subjects, their control group was a group of healthy adult subjects, rather than studies reported as normal, their patients were asymptomatic for headache or any other neurologic complaint, they found that MRV showed significant value in diagnosis of IIH, with 13 out of 20 patients showing bilateral venous sinus stenosis, Higgins et al found that none out of 40 healthy controls were showing significant bilateral transverse sinus narrowing, but 13 of them did show unilateral sinus narrowing, but the study did not attempt to quantitatively assess the degree of stenosis, to enable comparison between patients and controls, or to enable comparison with our work, however, they are obviously in disagreement with the studies refuting the value of non-contrast MRV in the diagnosis of IIH. Higgins et al suggested that image artefacts and poor choice of control may contribute to failure to recognize typical IIH pattern in MRV by the other workers.

The most significant limitation was the nature of the control group, being recruited retrospectively from the radiology department archive using cases with consensus reporting as normal, and not from a group of healthy volunteers.

Also the radiologist was not blinded to the diagnosis, but adapting a score system rather than visual interpretation would have eliminated the potential reading bias.

CONCLUSION

MRV is a valuable in the diagnosis of IIH. Non contrast 3D MRV is an efficient test and gives valuable diagnostic information.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary written and informed consent was obtained from each participant prior to enrollment in the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None.

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THE EFFECT OF TIZANIDINE ON POSTOPERATIVE PAIN MANAGEMENT AFTER LUMBAR FUSION SURGERY

Hossein Meskar[1], Goli Aezzi[1] , Aliyeh Zamani Kiyasari[1], Misagh Shafizad[2], Fatemeh Jalalian[3] and Mohammadali Alderraji[4]

[1]*Mazandaran University of Medical Science, Department of Anesthesiology, Sari, Mazandaran, Iran*
[2]*Assistant professor of Neurosurgery, Orthopedic Research Center, Mazandaran University of Medical Science, Sari, Mazandaran, Iran*
[3]*Mazandaran University of Medical Science, Department of Radiology, Sari, Mazandaran, Iran*
[4]*Shahed University of Medical Science, Tehran, Iran*

Received: 26.12.2019.
Accepted: 26.01.2020.

Corresponding author:

Dr Hossein Meskar

Mazandaran University of Medical Science, Department of Anesthesiology, Sari, Mazandaran, Iran

Phone: +98 9111 556 785

E-mail: sirhossein@gmail.com

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UDK: 616.711-009.7-085.216.5
616.711-001-089.168
Eabr 2023; 24(1):49-56
DOI:10.2478/sjecr-2020-0008

ABSTRACT

Lumbar fusion is one of the most commonly used surgical procedures to improve the pain and instability of the spinal vertebrae. After surgery, patients experience varying degrees of pain. The aim of this study was to determine the effect of tizanidine for the management of postoperative pain after lumbar spinal fusion surgery. This randomized clinical trial study was performed on 50 patients who were selected for spinal fusion surgery. Patients were randomly divided into two groups of 25 patients. Patients in the tizanidine and placebo groups received 4 mg oral tizanidine and placebo one hour before surgery and 24 hours after that. Severity of pain, presence of nausea, vomiting, pruritus, headache, vertigo, xerostomia, somnolence, bradycardia and excess opiate in the two groups were evaluated and recorded prior to exit from recovery and 2, 4, 6, 12, 24 and 48 hours after surgery. Data were analyzed by SPSS software version 24. There was a significant difference in pain score in tizanidine group at 24 and 48 hours after surgery compared with placebo (P <0.005). In the control group, the use of morphine sulfate was more than the tizanidine group. There was no significant difference in the incidence of side effects in the two groups (P> 0.05). Low dose tizanidine in postoperative pain management is effective without significant side effects. Due to its simpler administration, it is recommended to use for postoperative pain control after lumbar fusion surgery.

Keywords: *Tizanidine, lumbar fusion surgery, postoperative pain.*

INTRODUCTION

Lumbar fusion surgery is one of the most common selective neurosurgery procedures to relieve pain, instability or other symptoms by removing movement between the vertebrae (1-3). After all surgeries, patients inevitably feel pain to varying degrees. Despite increasing attention to the pain assessment program and the development of new standard methods of pain management, many patients still endure severe post-operative pain (4, 5). Patients undergoing spine surgery also experience moderate to severe postoperative pain (6, 7).

Postoperative pain can cause undesirable physiological effects such as inadequate breathing, failure to discharge respiratory secretions, atelectasis and other pulmonary complications, increased heart rate, hypertension, ileus and prolonged bed rest. This can eventually increase the incidence of deep vein thrombosis (8, 9). In addition, in patients undergoing vertebral surgery, postoperative pain can delay the onset of walking and physiotherapy, which may increase the length of hospital stay and ultimately the cost of surgery and changes the patient's sense of recovery and surgical outcome (6, 10).

Increasing evidence indicates that postoperative pain is not adequately treated (11). It is essential to treat pain with minimal side effects to increase rehabilitation and decrease postoperative morbidity (12). Side effects, such as prolonged hospital stays, delayed functional improvement, higher readmission rates, and increased health care resources are associated with inadequate pain management (13, 14). In addition, there is evidence that acute postoperative pain reduction may reduce the spread of chronic pain (15, 16).

Finding a way to get the most out of pain reduction and patient relaxation with minimal complications is one of the most important issues after surgery. Opioids, especially their injectable form, have been widely used as the first line of pain relief to relieve acute pain after surgery, including lumbar spine surgery (9, 17, 18). On the other hand, pain is a multifactorial phenomenon that is not completely controlled by monotherapy with opioids. In addition, Opioids use is associated with dose-related complications such as respiratory depression, nausea, vomiting, urinary retention, pruritus, drowsiness or postoperative ileus (19).

Tizanidine is a centrally-acting alpha-2 agonist with muscle relaxant, anxiolytic and sedative properties which has been widely used in the treatment of muscle spasms and more recently in the treatment of musculoskeletal pain (20). The use of tizanidine in anesthesia has been studied in several studies. According to these studies, oral tizanidine reduces the effect of hypertensive response during laryngoscopy and reduces the dose of midazolam induction. It also reduces the maintenance dose of propofol and MAC[1] of sevoflurane and can prolong spinal anesthesia (21-24). The role of postoperative analgesia of tizanidine on animal models (25) as well as in few human studies has been investigated (26).

The effects of tizanidine on pain management after lumbar disc and lumbar fusion surgery have not been studied. Only there is few clinical trials in databases that have been conducted on the effect of tizanidine in pain management in other surgeries (26-28). On the other hand, a significant portion of postoperative pain is due to surgical position as well as muscle spasms, which has received less attention than the pain of incisions and operation.

Therefore, in view of the above, and the importance of pain relief in patients after surgery, including spinal surgery, as well as the few studies to determine the analgesic effects of tizanidine and its potential positive effects on pain control, this study was designed to determine the effect of preoperative oral tizanidine on the severity of pain after lumbar fusion surgery.

METHOD AND MATERIALS

Ethical considerations

Permission was obtained from the research department of the faculty to initiate the project. The information from this research and the responses of the individuals were collected in a confidential and anonymous manner. Subjects participated in the study with informed consent.

Design of study

This study is a double blind randomized controlled clinical trial.

Protocol of study

The study population consisted of patients undergoing elective lumbar fusion surgery referred to Imam Khomeini medical center in Sari. The sample comparison method was used to determine sample size. In addition, based on the study by Ahiskalioglu et al. (26) and according to the sample size calculations, taking into account the 80% power, the 95% confidence level was equal to 20 persons, which was determined to be 25 in each group, given the possible loss of participants. To conduct the study, 50 patients aged between 35-60 years with Class 1 and 2 Anesthesia (ASA) candidates for laminectomy with selective lumbar fusion surgery with 1. Discopathy of more than two lumbar spaces 2. Lumbar spondylolisthesis 3. Multiple lumbar canal stenosis, were selected as available. Initial selection was based on the condition of the study units. Inclusion criteria included: 1- Confirmation of diagnosis by physical examination, CT scan and MRI 2- Patient's willingness to participate in the study and obtaining informed consent 3- Candidate for non-emergency lumbar fusion surgery 4- age between 35 to 70 years old and 5- No

[1] Monitored Anesthesia Care

history of tizanidine hypersensitivity 6- lack of bradycardia (HR <60) 7- No CNS disease 8- No previous lumbar surgery. Exclusion criteria were as follow: 1- Patient's unwillingness at any time to continue the study 2- Opioids use 24 hours prior to intervention 3- Alcohol or drug abuse 4- Incidence of any uncommon side effects during surgery. Selected patients according to the inclusion criteria then were divided into groups A and B using permuted block randomization. Before surgery, patients were provided with detailed instructions on how to determine the severity of pain, nausea, vomiting, and postoperative pruritus using the visual analog scale (VAS) and how to use a PCA pump. Patients in group A received a single oral 4 mg tizanidine tablet (manufactured by Jalinous pharmaceutical company) and patients in group B received an oral placebo one hour before surgery and 24 hours after that. Anesthesia protocol was performed including premedication; midazolam at dose of 0.01 mg/kg and fentanyl at dose of 2 mc/kg; Then induction with sodium thiopental at dose of 4-6 mg/kg/iv and atracurium at dose of 0.5 mg/kg/iv and intubation with armored tube No. 7.5-8 was performed. During surgery, morphine sulfate at dose of 0.1 mg/kg and atracurium at dose of 0.01 mg/kg iv were administered to patients and 50% nitrous oxide gas with oxygen and isoflurane inhalation anesthetic 0.5-1.5 MAC was used as a maintenance anesthetic.

After surgery and in recovery room, an PCA pump containing the same medicine composition as 30 mg morphine sulfate, 3 gram of acetaminophen and the remainder up to 80 cc (total pump volume) of normal saline were applied for all patients. The pump setting specification was as follows: bolus rate of 0.5 cc and 15 min of Lock interval and 2 cc background infusion per hour.

The severity of pain, nausea and vomiting, pruritus and opioids use of patients and other possible side effects including xerostomia, bradycardia, headache, dizziness and drowsiness in the two study groups after consciousness in recovery and then in neurosurgery ward at time 2, 4, 6, 12, 24 and 48 hours after surgery were evaluated and recorded. Morphine would administer intravenously at a dose of 3 mg if the patient has a pain score more than 5 (according to the VAS criteria for pain control). In case of nausea with the score more than 3 or vomiting, ondansetron 4 mg was given up to three doses in 24 hours. In addition, the amount of fentanyl consumed during anesthesia was also assessed and recorded. The primary outcome is the study of patients' pain intensity and opioids use and the secondary outcome is the presence of nausea and vomiting, pruritus, xerostomia, bradycardia, headache, dizziness and drowsiness. The evaluation was done by a project nurse who was unaware of the study groups and had received adequate training in this area. Patients' body mass index (BMI) was calculated and was recorded in the relevant form alongside with other information including age, education, duration of surgery, duration of anesthesia, the number of intervertebral space and number of vertebrae involved in fusion surgery and morphine intake at 48 hours and ondansetron administration at 48 hours (if VAS> 4) After surgery. It is noteworthy that the placebo required in the study was obtained from Sari School of Pharmacy. In addition, all surgeries were performed by a surgeon.

Data collection

Data was collected by field method using information questionnaire.

Statistical analysis

Data analysis was performed using SPSS version 24. The variables were described with percentage, mean, standard deviation, minimum and maximum. Repeated measure analysis of variance, independent and dependent t-tests were used to compare mean pain intensity between groups. Chi-square test was used to compare the frequency of nausea, vomiting, pruritus, xerostomia, bradycardia, headache, dizziness and drowsiness. The significance level was less than 0.05 available to university officials completely and unchanged.

RESULTS

In the present study, 50 patients were enrolled which divided into two groups of 25, one group receiving Tizanidine and the other placebo. In total of two groups were 30 (60%) female and 20 (40%) male. In intervention group 14 (56%) were female and 11 (44%) were male and in placebo group 16 (64%) were female and 9 (36%) were male. There was no significant difference between the two groups in terms of gender ($p = 0.821$).

Age distribution analysis showed that in the tizanidine group 36% were under 45 years old and 64% were over 45. In the placebo group, 44% were under 45 and 56% were over 45 years old. The results of chi-square test showed that the two groups were similar in age distribution, so that the mean age of the tizanidine group was 49.19 ± 9.80 years and the mean age of placebo group was 47.13 ± 11.31 ($p = 0.65$).

The results showed that there was no significant difference between the two groups in terms of other demographic data ($p> 0.05$) (Table 1).

The mean VAS severity measured at the time of recovery (zero hour) was 3.92 ± 1. 1.87 in the intervention group and 3.52 ± 1.78 in the placebo group but the difference between the two groups was not significant ($p= 0.442$). The mean VAS severity measured at 2 hours after surgery was 4.6 ± 1 in the intervention group and 4.52 ± 1 in the placebo group, with no significant difference between the two groups ($p= 0.802$). The mean VAS severity measured at 4 hours after surgery in the intervention group was 4.2 ± 0.91 and in the placebo group was 4.32 ± 0.9 which was not significant ($p= 0.620$). The mean VAS severity measured at 6 hours after surgery was 3.96 ± 1.27 in the intervention group and 3.96 ± 1.2 in the placebo group, which was not significant ($p= 0.992$). The mean VAS severity measured at 12 hours after surgery was 1.58 ± 3.8 in the intervention group and 1.55 ± 3.8 in the placebo group, but the difference between the two groups was not significant ($p = 0.897$). The mean VAS

severity measured in the 24 hours after surgery was 1.88 ± 1.39 in the intervention group and 2.84 ± 1.21 in the placebo group. The difference between the two groups was significant (p= 0.013). The mean VAS severity measured 48 hours after surgery was 1.28 ± 1.13 in the intervention group and 2.12 ± 1.39 in the placebo group. The difference between the two groups was significant (p= 0.024). (Figure 1 and 2).

Morphine sulphate intake during the 48 hours was 27.5 mg in the placebo group and 22 mg in tizanidine group, which was statistically significant ($p < 0.05$).

5 patients in the tizanidine group and 4 in the placebo group had nausea during the first 6 hours after surgery. No cases were reported at 12, 24 and 48 hours. There was no statistical difference between the two groups in terms of incidence and severity of nausea during different hours of operation ($p > 0.05$).

One person in both groups had vomiting, which was not statistically different between the groups. These two were given extra ondansetron. Considering the presence of one case of vomiting in both groups and the use of ondansetron, the mean of administered ondansetron was compared in both groups. Statistical calculations showed no significant difference between the two groups in terms of ondansetron intake.

2 patients in the placebo group and 1 in the tizanidine group had pruritus, which was not statistically significant. It also showed that there was no pruritus during the 4 hours after the operation.

According to Table 2, drowsiness in the tizanidine group was seen in 6 patients (24%) and in the placebo group was seen in 7 patients (28%), which was not statistically significant. Headache rate in tizanidine group was 4% and in placebo group 2 was 8% which the difference was not statistically significant. Also, dizziness was 12% in the tizanidine group and 4% in the placebo group, which was not statistically significant. Xerostomia in both groups was seen in 7 patients (28%), which was not statistically significant. Bradycardia hasn't occurred in none of the patients in both groups.

In overall, the results showed that the pain intensity was lower in the tizanidine group at 24 and 48 hours after surgery compared to the other group. There was no significant difference between the two groups in terms of possible complications such as nausea, vomiting, pruritus, headache, dizziness, xerostomia, bradycardia and drowsiness. Morphine sulfate intake was lower in the tizanidine group during 48 hours than in the placebo group and this difference was significant. However, the two groups did not differ in the amount of ondansetron intake.

Table 1. Demographic characteristics and clinical information of patients

Variable	Groups		P value
	Tizanidine (n= 25)	Control (n= 25)	
age	49.19 ± 9.8	47.11 ± 13.31	0.65
BMI (kg/m^2)	29.35 ± 3.98	30.88 ± 2.56	0.36
Operation time (minute)	232.46 ± 65.22	201.12 ± 78.71	0.74
Duration of anesthesia (minutes)	291.25 ± 56.41	261.32 ± 82.76	0.17
Number of fused vertebrae	3.20 ± 0.96	3.31 ± 1.17	0.96

Figure 1. The process of pain score changes within 48 hours after receiving the medications

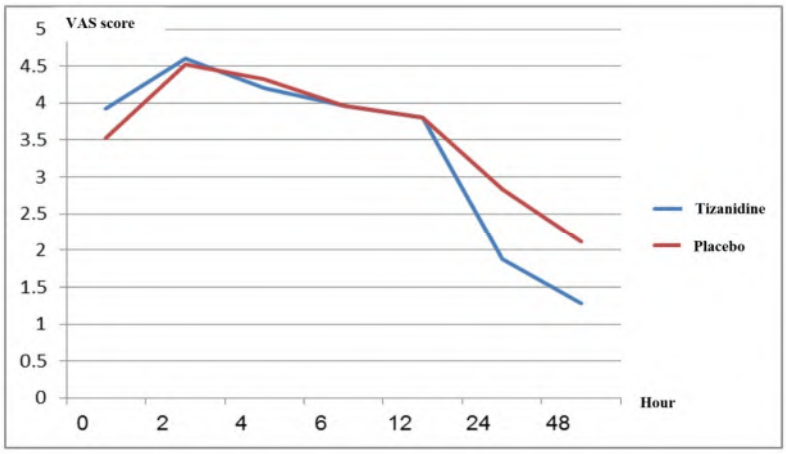

Figure 2. Comparison of mean pain score within 48 hours after medications administration

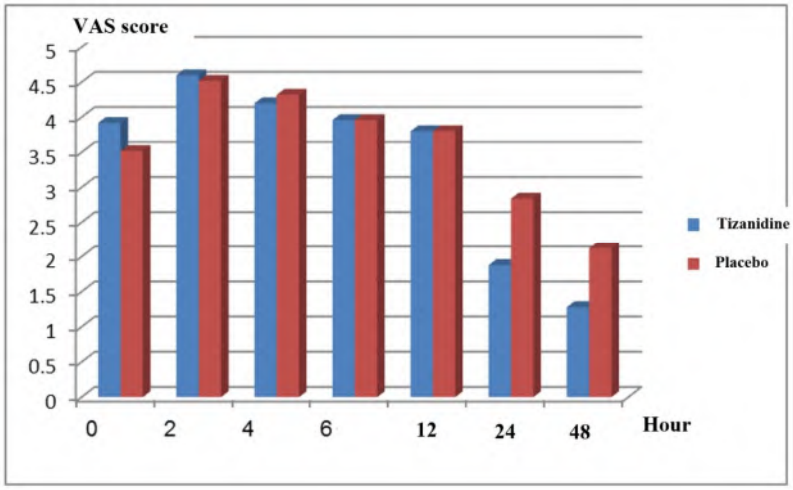

Table 2. Other qualitative variables and their comparison between intervention and placebo groups

Variables	Tizanidine group N (%)	Placebo group N (%)	Total N (%)	p value
Drowsiness	6 (24 %)	7 (28 %)	13 (26 %)	0.327
Headache	1 (4 %)	2 (8 %)	3 (6 %)	0.327
dizziness	3 (12 %)	1 (4 %)	4 (8 %)	0.35
Bradycardia	0	0	0	1.00
Xerostomia	7 (28 %)	7 (28 %)	14 (28 %)	1.00

* The date is expressed as frequency (percent)

DISCUSSION

Our study results showed that the VAS score was significantly lower in patients treated with low-dose tizanidine at 24 and 48 hours postoperatively than those receiving placebo. Postoperative morphine sulfate intake was significantly lower in the tizanidine group than in the placebo group. Earlier use of tizanidine or higher doses can lead to better and faster efficacy. Also, given prone position of patient in lumbar fusion surgery and long period of time which patient stays in this position, there may be muscle spasms consequently which tizanidine intake as prophylactic or therapeutic can improve the pain, due to its antispasmodic properties.

Clonidine is a prototype of a2-agonist. The role of clonidine in postoperative anesthesia is quite clear (29). Clonidine, when administered injectable, relieves post-operative pain and acts as a topical anesthetic. The pain then subsides and reduces the need for opioids during anesthesia. (7, 27) Clonidine and tizanidine were not previously compared in the terms of postoperative anesthesia, but the antinociceptive properties of tizanidine are weaker than clonidine and side effects of tizanidine are well tolerated (19, 28)

The sedative and hemodynamic effects of tizanidine and clonidine have been studied in healthy volunteers. Both medications have sedative and hypotensive effects and reduce salivary secretion, however; tizanidine effect is short-term. 4mg tizanidine decreased the SBP (systolic blood pressure) by 9% (30). Overall, adverse events were low with our pain management strategy; severity was mild and no treatment was required. Changes in heart rate and mean arterial pressure were within the prescribed range of operation and there was no required treatment and intervention in both groups.

In a study by Talakob *et al* on the effect of tizanidine on postoperative pain in patients undergoing laparoscopic cholecystectomy, 4mg oral tizanidine preoperatively reduced postoperative pain score during recovery hours 1, 2, 3, 6, 12 and 24, as well as opioids use duration of recovery in patients. While the reduction of pain score in our study was at 24 and 48 hours postoperatively and there was no significant difference between the study groups before that. In this study, 4 patients in the tizanidine group experienced drowsiness, which was statistically significant and other complications such as bradycardia, nausea and vomiting were not significant. However, in our study the incidence of complications was not significantly different between the two groups (27).

In the study of Rupani *et al*, the effects of oral Tizanidine on pain after anorectal surgery under spinal anesthesia were investigated. 60 minutes before admission to the operating room, placebo was given to control group and study group received 4 mg oral tizanidine. Pain onset and meperidine intake 24 hours after surgery and side effects were recorded. The mean duration of analgesia was 332.5 and 144 minutes, in the tizanidine and placebo groups, respectively, which was significantly longer in the tizanidine group than in the control group. Also, the mean dose of meperidine in the 24 hours after spinal anesthesia in the control group was 42.17 mg and 26.67 mg in the tizanidine group which was statistically significant. Same as our study, morphine sulfate intake levels were significantly lower in the tizanidine group at 48 hours postoperatively (28).

In a study by Ahiskalioglu *et al*, the single-dose effect of 6mg Tizanidine preoperatively and bilateral cervical superficial block (BSCPB) comparing with the effect of BSCPB alone and placebo on postoperative total thyroidectomy pain was evaluated. In the tizanidine+BSCPB group, there was a significant difference in postoperative pain and opioid use compared to the placebo group. Fentanyl consumption was lower in the group 0-4 and 4-8 hours postoperatively than in the placebo group. Fentanyl consumption was also lower in the tizanidine+BSCPB group than in the BSCPB group alone during the first 4 hours postoperatively. Apart from the subgroup receiving tizanidine, occipital headaches and back pain were less common (28). In his study, a single 6mg dose of tizanidine significantly reduced pain in the PACU at 1, 2, 4, 8, 12, and 24 hours. However, in our study, there were significant differences in pain reduction at 24 and 48 hours. In their study, one person had xerostomia and one had drowsiness in the tizanidine group (n = 20) (26).

A study by Yazicioglu et al investigated the effect of tizanidine on acute pain after inguinal hernia surgery. The tizanidine group received 4 mg of tizanidine 1 hour before surgery and continued the treatment twice daily for one week, with the other group receiving placebo instead of tizanidine. The tizanidine group had lower pain scores at 6, 12, and 24 hours and 2, 3, and 4 days postoperatively, both at rest and during exercise. Excess analgesic consumption was also lower in the tizanidine group than in the control group. The return to normal activity and quality of life in the tizanidine group was also better than the control group (30).

Imanaga et al showed that 3 mg oral tizanidine as premedication can reduce the pain of infiltration and anesthetic diffusion due to epidural catheterization (31). In this study, the analgesic effects of tizanidine during epidural infiltration anesthesia was evaluated, but we studied its effect on postoperative pain in patients who underwent anesthesia.

Norouzi et al showed that premedication with 4 mg of tizanidine prior to lower extremity plaque removal surgery caused less pain during surgery and less opioid use after surgery, which in our study also reduced opioid use.

Side effects of tizanidine are dose dependent. Xerostomia and drowsiness are among the most common (26). In our study, there was no significant difference between the tizanidine group and placebo group in terms of side effects.

Several features of the study protocol require discussion. This was the first study to investigate the role of tizanidine in postoperative pain management of lumbar fusion surgery.

The effective dose and timing of tizanidine are not known in the field, so only a small dose of this medication intentionally was used. In addition to the low side effects of tizanidine, the low dose used in our studies and the timing of the first dose of tizanidine may be the cause of reduction in side effects incidence that we have encountered. One goal of using tizanidine is to avoid postoperative analgesic side effects by reducing their dose. In addition to impairing the patient's functioning, these side effects can interfere with the side effects of tizanidine. We believe that the low-dose side effects of tizanidine were similar in the two groups and were not significant.

The limitations of the study are as follows. Initially, two doses of 4 mg tizanidine were used in this study. More and more doses can have better analgesic effects, but may have more side effects. Second, the study protocol prevented the participation of patients who were prone to develop various side effects associated with pain management. Third, there were no fixed nursing staff at the time of hospitalization and there may be potential interference with information. In addition, patients were not assessed after discharge in terms of fatigue or sleep schedule, and it is unclear whether their pain score varied during the week following surgery.

CONCLUSION

Tizanidine has been shown to reduce postoperative pain in 24 and 48 hours after surgery and reduce analgesic administration without any significant side effects. Postoperative pain relief results in a faster return of patients to their normal daily activities. Obviously, the quality of life has also improved in terms of pain relief.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary written and informed consent was obtained from the patient prior to enrollment in the study.

CONFLICT OF INTEREST

The authors declare that there is no potential conflict of interest regarding this manuscript.

FUNDING

None.

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PERIODONTAL DISEASE: CORRELATION WITH HISTOLOGICAL AND IMMUNOLOGICAL PARAMETERS

Bojan Kujundzic[1], Zlatibor Andjelkovic[2], Radmila Maric[3], Ruzica Lukic[4], Veljko Maric[3] , Helena Maric[3], Miroslav Obrenovic[3] and Sinisa Kojic[3,5]

[1]*University of East Sarajevo, Faculty of Medicine Foca, Department of Dentistry, Bosnia and Herzegovina*
[2]*University of Pristina, Faculty of Medicine, Department of Histology and Embryology, Serbia*
[3]*University of East Sarajevo, Faculty of Medicine Foca, Department of Surgery, Bosnia and Herzegovina*
University of East Sarajevo, Faculty of Medicine Foca, Department of Microbiology, Bosnia and Herzegovina
[5]*Special clinic for plastic, reconstructive and aesthetic surgery Varis, Belgrade, Serbia*

Received: 02.06.2020.
Accepted: 21.06.2020.

Corresponding author:

Veljko Maric, MD, PhD,
ANURS correspondent member

University of East Sarajevo, Faculty of Medicine Foca, Department of Surgery, Bosnia and Herzegovina, Studentska 5, 73300 Foca, Republika Srpska, BiH

Phone: +387 58 210 420

E-mail: veljko_maric@yahoo.com

sciendo

UDK: 616.314.17-002
Eabr 2023; 24(1):57-62
DOI:10.2478/sjecr-2020-0024

ABSTRACT

Periodontal disease is inflammatory pathological conditions in the gingiva and dental support structures that usually results in extracellular matrix and connective tissue destruction. During periodontitis, inflammatory cells facilitate collagen and connective tissue loss, affects the number and activity of fibroblasts and its production of local collagen networks. Aim of this study was to evaluate collagen density and accumulation of collagen producing fibroblast and macrophages in affected tissue of periodontal disease. Histological and immunohistochemical analyzes were performed on paraffin embedded tissue sections of gingival biopsies, obtained from 30 patients with diagnosis of periodontal disease and 10 healthy donors. Tissue sections of gingival of patients with periodontal disease had significantly decreased collagen volume density and visible fragmentation and lysis of the collagen fibers, decreased number of fibroblasts, accompanied with increased accumulation of macrophages. Presented data implicate that macrophages accumulation may be the cause of enzyme mediated collagen destruction

Keywords: *Periodontal disease, collagen, fibroblasts, macrophages.*

INTRODUCTION

Periodontal disease presents group of pathological conditions in the gums and dental support structures, produced mainly by infective agents (1). Approximately 15%–35% of the adult population has periodontal disease (2-3). One of the hallmarks of periodontal disease is inflammation in periodontium (1). During inflammation, immune cells accumulate in gingival tissue in order to control invading micro-organisms. Periodontitis usually results in the destruction of the teeth supporting structures such as alveolar bone and connective tissue attachment to teeth (4).

Extracellular matrix and connective tissue destruction in gingival are sequel of lytic enzymes activity. These enzymes are partly produced by bacteria, but, in general, main source are host cells. During periodontitis, inflammatory cells secrete a variety of substances, such as enzymes. Enzymes responsible for periodontal destruction are matrix metalloproteinases (MMPs) (5). Matrix metalloproteinases play important functions within the setting of inflammation and tissue injury and remodeling (6). The main source of this enzymes are connective tissue cells and inflammatory cells (6,7). Activated inflammatory cells, that infiltrate gingival tissue produce large amounts of metalloproteinases and facilitate collagen and connective tissue destruction (6). Macrophages represent common immune cells that accumulate into affected gingival tissue (8). Bacterial LPS activates macrophages through Toll-like receptors (9). Once activated, macrophages may produce matrix metalloproteinases that contributes to local extracellular matrix destruction (8). Main source of collagen in gingival connective tissue are fibroblast (10-12). Local inflammation affects the number and activity of fibroblasts and its production of local collagen networks.

Main goal of this study was to evaluate collagen density and accumulation of collagen producing fibroblast and macrophages in affected tissue of periodontal disease. Obtained results revealed loss of collagen fibers accompanied with decreased number of fibroblast and increment in macrophages infiltration in affected gingival tissue.

MATERIAL AND METHODS

Human gingival tissues

Each diagnose was estimate according to periodontal disease classification (13-5) and confirmed by a pathologist, on hematoxylin and eosin stained slides, using standard histopathologic criteria. Control group consist of tissue specimens taken from patients who had an indication for dental extraction due to orthodontic reasons. These sections had no visible inflammation and tissue injury observed.

Hystological and immunohistochemical analyzes

Van Gieson staining was performed for collagen fibers detection (16), while immunohistochemical staining was performed for macrophages detection. Immunohistochemistry was performed on a single representative block from each case, as previously described (17). Primary polyclonal antibodies were directed against CD68 (Sigma-Aldrich, USA). The slides were examined and analyzed at x100, x200 and x400 magnification, using conventional light microscopy (Olympus, Japan). Only brightness and contrast were adjusted. Collagen volume density and number of fibroblast and macrophages in tissue sections was estimated using the M42 test system calibrated for proper magnification.

Statistical analysis

The data were analyzed using commercially available software (SPSS version 23.0). All results were analyzed using the Student's t test or Mann-Whitney U test, where appropriate. All reported P values were 2-sided and $p<0.05$ was considered statistically significant and highly significantly different when $p<0.01$. Data are presented as mean ± SEM.

RESULTS

Lower collagen volume density in periodontal lesions

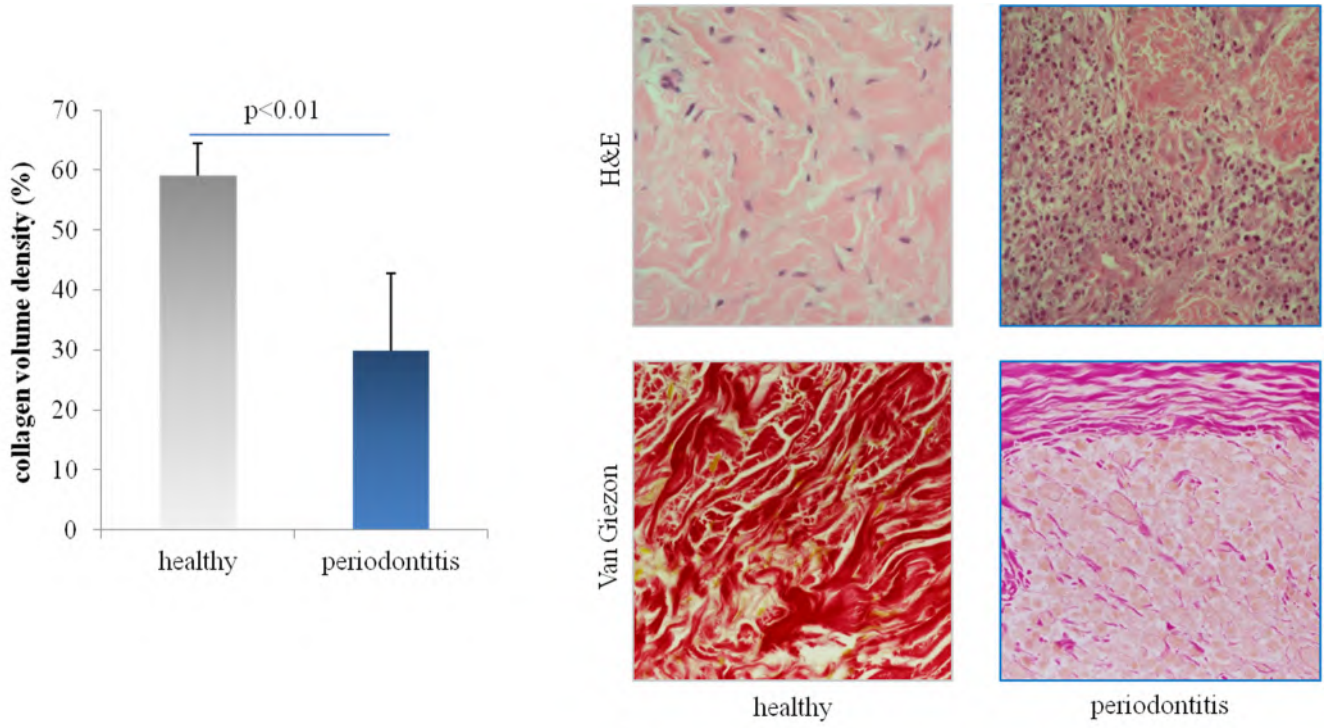
All of recruited patients in experimental group met the criteria for periodontitis, according to periodontal disease classification, while control subjects had no visible inflammation and local tissue injury. There was no significant difference in gender distribution between groups. Patients with diagnosed periodontal disease were significantly elder ($p<0.05$) in comparison to healthy patients (data not shown).

Tissue samples of gingiva derived from patients with periodontal disease had evident destruction of basal lamina with intense accumulation of inflammatory cells (Figure 1, right panel). In the same samples we found significantly decreased collagen volume density ($p<0.01$) and visible fragmentation and lysis of the collagen fibers. This finding is confirmed with pathohistological characteristics shown in figure 1.

Decreased number of fibroblasts is accompanied with increased accumulation of macrophages in periodontal lesions

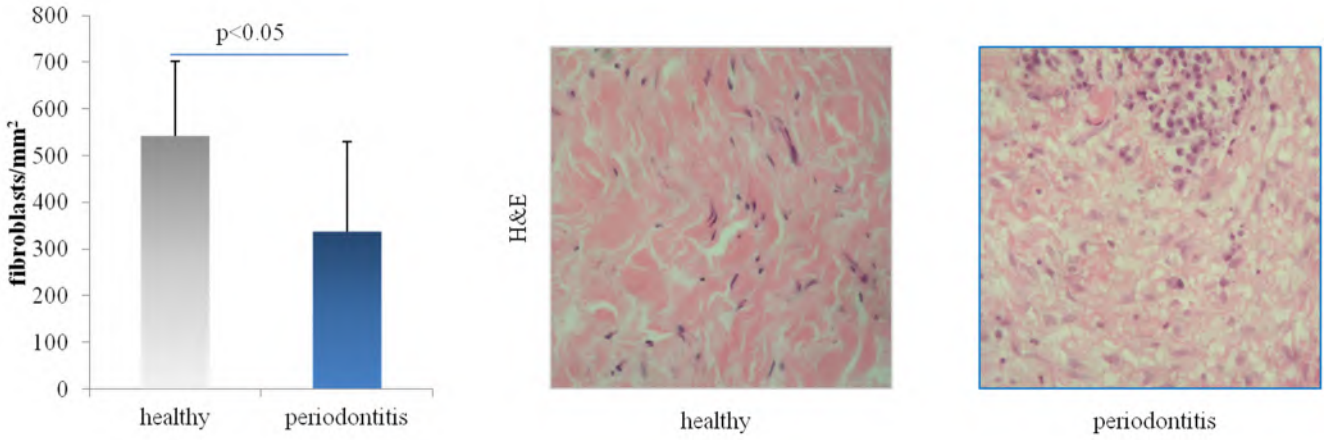
Further histological analyzes of gingiva were focused on fibroblasts. Number of fibroblasts was significantly lower in affected gingival tissue of patients with periodontal disease in comparison to control ($p<0.05$). In addition, in healthy gingiva fibroblasts were present predominantly in papillary layer, while in diseased tissue there was lesser number of fibroblasts, as presented in figure 2, right panel. Immunohistochemical analyzes showed increment of number of $CD68^+$ cells in periodontal lesions, compared to healthy control ($p<0.05$; Figure 3). $CD68^+$ macrophages are rare and evenly distributed throughout the healthy tissue. In affected gingival tissue $CD68^+$ macrophages are located predominantly into inflammatory infiltrates, as shown on figure 3, right panel.

Figure 1. Collagen volume density. Collagen fibers were detected by Van Gieson staining.



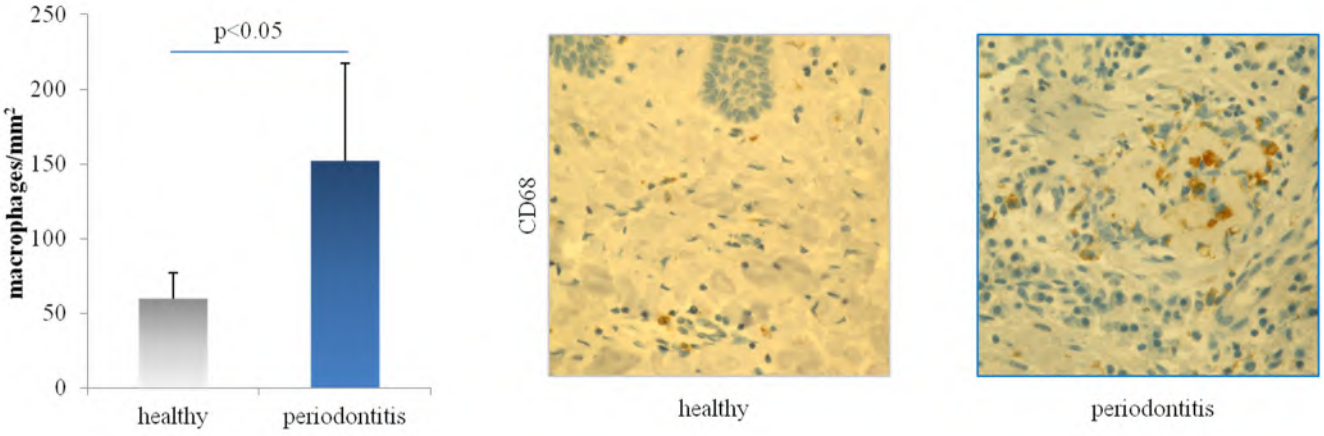
Representative images of human gingival tissue, healthy and periodontitis: upper right panel (H&E, original magnification: x400), lower right panel (Van-Gieson, original magnification: x400).

Figure 2. Number of fibroblasts. Fibroblast were detected by H&E staining.



Number was estimated by using the M42 test system. Representative images of human gingival tissue, healthy and periodontitis (H&E, original magnification: x400).

Figure 3. Number of macrophages.



Macrophages were evaluated by immunohistochemistry for each patient. Number was estimated by using the M42 test system. Representative images of human gingival tissue, healthy and periodontitis (Anti-CD68, original magnification: x400).

DISCUSSION

The aim of this study was to evaluate collagen density and accumulation of collagen producing fibroblast and macrophages in affected tissue of periodontal disease. We found significant decrement in collagen volume density, lower number of fibroblast and higher number of local infiltrating macrophages in affected gingival tissue, compared to healthy gingiva.

Firstly, we investigate extracellular matrix composition and find significantly decreased collagen volume density (Figure 1). In line with this finding, recent studies also revealed loss of the extracellular matrix during periodontitis (18,19). Significant decrement in collagen volume density implicate it`s destruction in affected tissue. Possible explanation for this phenomenon is inflammation as a cause of collagen loss, as recruited leukocytes represent a significant source of MMPs, enzymes that present major factors for collagen lysis (19-21). During inflammation, inflammatory cells accumulate in gingival tissue in order to control invading micro-organisms. Among inflammatory cells, macrophages present important players in establishing inflammation and tissue remodeling (22). Namely, they produce various pro-inflammatory mediators as well as MMPs. They are the main source of several types of MMPs (23). Increment of number of macrophages detected in affected gingival tissue (Figure 3) revealed that enhanced accumulation of macrophages and subsequent secretion of MMPs may be the cause of collagen volume density decrement. This finding can also explain visible fragmentation and lysis of the collagen fibers in same group of tissue samples (Figure 1). Degradation of collagen is probably due to gingival infiltration of inflammatory cell populations, such as macrophages.

Factors that limit collagen repair, such as decrement in number of fibroblasts may be as important as those that initiate its loss. Fibroblasts are known for their role in synthesizing and re-modelling the ECM in tissues (22). It is well established that lysis of collagen fibers is accompanied with the loss of fibroblast (24). Namely, number of fibroblasts was significantly lower in affected gingival tissue (Figure 2). Possible mechanism that underlies this finding is the stimulation of apoptosis in fibroblasts (25). Previous studies revealed that bacteria induce apoptosis of fibroblast *in vitro* (26-29), but inflammation and immune cells have a more prominent role in apoptosis of fibroblasts (30). Study by Moodley Y et al. demonstrated that apoptotic fibroblasts release a potent chemoattractant for macrophages that subsequently phagocytose fibroblast (31). Increment in number of macrophages (Figure 3) accompanied with decrement in number of fibroblasts (Figure 3) supports previous study and implicate that loss of fibroblast is also probably due to gingival infiltration of inflammatory cell populations, such as macrophages.

CONCLUSION

Taken all together, presented data reveal that periodontal disease results in the destruction of the collagen. Collagen loss is accompanied with intense macrophage infiltration, followed by decrement of number of fibroblasts in affected gingival tissue. Possible explanation of this finding is that macrophages accumulation may be the cause of enzyme mediated collagen destruction as well as removal of apoptotic fibroblast. Clarifying of suggested interplay requires further studies.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary written and informed consent was obtained from each participant prior to enrollment in the study. The protocol of the study was approved by the local ethics committees.

DECLARATION OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

This work was supported by grant from the Faculty of Medical Sciences Kragujevac (JP11/18), Serbia.

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WORK-RELATED MUSCULOSKELETAL DISORDERS OF TEACHING STAFF IN HIGHER EDUCATION

Elvis Mahmutovic , Emir Bisevac and Zana Dolicanin

State University of Novi Pazar, Department of Biomedical Sciences, Novi Pazar, Serbia

Received: 21.04.2020.
Accepted: 14.06.2020.

Corresponding author:

Mahmutovic Elvis

State University of Novi Pazar, Department of Biomedical Sciences, Novi Pazar, Serbia

E-mail: ehmahmutovic@gmail.com

UDK: 616.7-057:378
613.62:378-051
Eabr 2023; 24(1):63-68
DOI:10.2478/sjecr-2020-0022

ABSTRACT

Work-related musculoskeletal disorders are among the most common disorders of the musculoskeletal system. The aim of this paper is to determine the prevalence of the musculoskeletal disorders (MSD) for the teachers in higher education. Examinees and methods: 100 teachers (66% men and 34% women with average age of 38.8 ± 13.1) were included in the prospective study. The information about the state of the teachers' musculoskeletal system was gathered using a questionnaire for the MSD analysis. Potential risk factors for MSD were analyzed. MSD was observed in 74 (74%) teachers and associates (68.9% male and 31.1% female, $p<0.05$). 32% Teachers had pain within the first five years of teaching work, while the rest of MSD occurred after that period. 73% of teachers were diagnosed with rigor in the lower back, 62% were diagnosed with neck pain, 45.9% were diagnosed with upper back pain, while 27% had pain in their ankles/feet. Lower pain prevalence was noticed in wrist, hands, hips and elbows. The prevalence of the musculoskeletal disorders for teachers is 74% and it is higher for men than for women. MSD, whose samples are multifactorial, are the most common in the lower back, neck, upper back and shoulders. Ergonomics and ergonomic education are the primary factors in the prevalence and treatment of MSD.

Keywords: *Musculoskeletal disorders, work-related diseases, back pain, neck pain, ergonomic measures.*

INTRODUCTION

The word "ergonomics" comes from the Greek word ergo, which means "to work", and nomos, which encompasses natural laws or systems (1). Hence, the ergonomics is defined as a science which studies people in relation to their work environment, i.e. the adaptation of the devices and general conditions which should suit an individual in order to be fully efficient (2).

The positions in which teachers and administrative workers find themselves while doing their everyday activities are often forced positions with sympathogenic potential, which is why they are highly susceptible to musculoskeletal disorders. These disorders affect muscles, ankles, ligaments and nerves located between feet and neck. The symptoms vary from feeling uncomfortable and having weak or strong pain, to more serious health conditions which can cause social and economic consequences. They encompass lowered quality of work services, frequent absence from work or even retirement (3). The pain and physical disability brought about by MSD affects social functioning and mental health, further diminishing the patient's quality of life (4). The most common symptoms are back pain, affecting primarily the lumbar and cervical part of the spine, followed by shoulders, knees and feet. Factors contributing to the back and neck pain are: staying in the same position for a long time (sitting, standing), sudden body movements, obesity, spinal stud shape, aging, weak muscles, lack of exercising, the nature of the movements, techniques of lifting objects, mechanical load, stress, etc. (5).

The goal of this paper is to confirm the prevalence of the musculoskeletal disorders for teachers and associates with different years of service at the State University of Novi Pazar.

METHODS

One hundred teachers and associates of the State University of Novi Pazar took part in the analysis. "The standardized Nordic questionnaire for the analysis of the musculoskeletal symptoms" was used to gather the information about the prevalence of the musculoskeletal symptoms for the teachers (5, 6). A couple more questions were used as an addition to the questionnaire (2, 5, 6). They referred to the: gender of the examinees, age and years of service, working conditions (duration and frequency of the work-related activities), working hours, body position while working, taking breaks, existence of the musculoskeletal rigor and history of the rigor. The questions were formulated and asked so as to question the working conditions.

Possible risk factors for MSD (gender, age, years of service, number of working hours, posture - body position while working, body height) were also analyzed. There were 66% men and 34% women with the average age of 38,8±13,1, with 9,9±11,6 years of service in total. The data about the state of the musculoskeletal system of teachers and associates were gathered using the questionnaire, and they were input in a special database. All the gathered data was processed using SPSS, version 14.0 (SPSS Inc., Chicago, II, USA). The results are displayed in frequencies and percentages (5, 6).

RESULTS

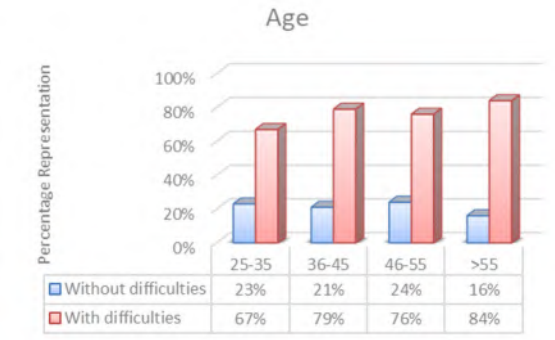
Musculoskeletal rigor was found in 74 teachers and associates (74%) out of 100 examinees; 51 were male (68.9%) and 23 (31.1%) female. For male teachers, the highest percentage of the rigor was discovered in the lower back (78,4%), followed by the neck rigor (65%) and upper back rigor (53%), and the lowest percentage was in the hips (21,5%) and elbows rigor (7,9%). For female teachers, the highest rigor percentage was also in the lower back (69.8%), followed by the neck rigor (61.2%) and upper back rigor (48%), while they felt the least amount of rigor in the hips (17.4%) and elbows (8,7%) (Table 1).

Table 1. The frequency of pain in different parts of the body relative to the gender of the teachers

Body parts	Men		Women	
	Without pain	With pain	Without pain	With pain
Neck	18 (35%)	33 (65%)	9 (38,8%)	14 (61,2%)
Shoulders	45 (89,3%)	6 (11,7%)	16 (69,5%)	7 (30,5%)
Elbows	47 (92,1%)	4 (7,9%)	21 (92,3%)	2 (8,7%)
Wrist joints/hands	46 (90,2%)	5 (9,8%)	18 (78,3%)	5 (21,7%)
Hips	40 (78,5%)	11 (21,5%)	19 (82,6%)	4 (17,4%)
Upper back	24 (47%)	27 (53%)	12 (52%)	11 (48%)
Lower back	11 (21,6%)	40 (78,4%)	7 (30,2%)	16 (69,8%)
Knees	43 (84,3%)	8 (15,7%)	15 (65,2%)	8 (34,8%)
Ankles/Feet	42 (82,4%)	9 (17,6%)	14 (60,9%)	9 (39,1%)

Among younger teachers (25-35 years old) rigor was discovered in 67% of the cases, with values which were similar to the age groups 36-45, i.e. 46-55. For teachers older than 56, rigor was discovered in more than 80% of cases (Graph 1). There were no statistically significant differences in

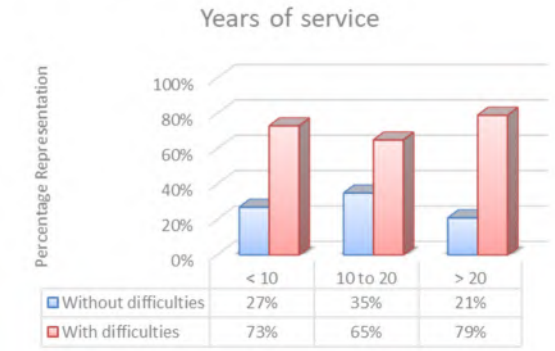
musculoskeletal pain occurrence for the examinees of different ages.



Graph. 1. The frequency of pain in subjects of different ages

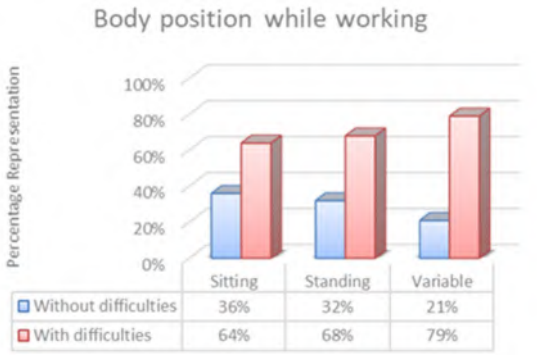
Half of the examinees felt pain in the first three years of employment. For the teachers with up to 10 years of service, for those between 10 and 20 years of service, as well as for those with more than 20 years of service, rigor was discovered in more than 60% of cases. There was no statistically significant difference in musculoskeletal pain occurrence for the examinees with different years of service (Graph 2).

Most of the teachers (77.5%) worked 5 days a week. Working hours varied from 1 to 12. 28.1% worked 8 hours a day, and 20.2% worked 6 hours a day. As much as 95% of the teachers constituted in the group who worked more than 10 hours a day.



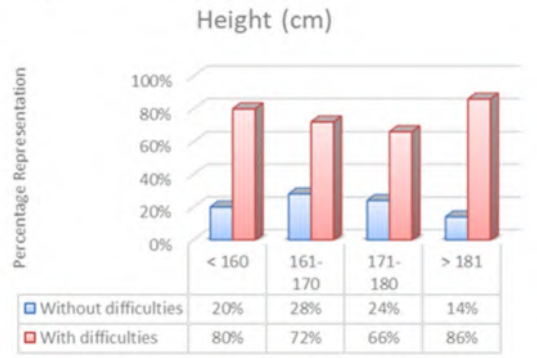
Graph. 2. The frequency of pain in subjects depending on length of service

29,2% of them sat while working and had rigor in almost 70% of the cases, while 30,3% of them stood, but still had the same percentage of the registered rigor (70%). Still, the highest number of the teaching personnel (40,4%) changed their positions and had the highest rigor percentage (around 80%) (Graph 3). 95,9% of the teachers took breaks.



Graph. 3. The frequency of pain in subjects depending on body position

The average height of the examinees was 173.87 cm, with a standard deviation of 10.26 cm. The median was 172 cm. The percentage of the teaching personnel with painful rigor was almost the same (75%) for the different height examinees. Students' t-test did not show any statistically significant difference for the musculoskeletal pain occurrence in the different height examinees (Graph 4).



Graph 4. The frequency of pain in subjects depending on height

According to the standardized Nordic questionnaire, the highest number of the teaching personnel felt the lower back pain (75,7%), followed by the neck pain (63,5%); 51,3% had upper back pain; 24,3% had ankle/foot rigor; 21,6% had knee rigor; 20,3% had hip rigor and 17,6% had shoulder rigor. The lowest pain prevalence was discovered in the elbows area (8,1%) (Table 1).

DISCUSSION

The standardized Nordic questionnaire for the analysis of the musculoskeletal symptoms is internationally recognized questionnaire for evaluating the degree of the musculoskeletal rigor (5, 7) and it was used in our examination. Musculoskeletal pain prevalence among the examined teaching personnel was 74%.

The study showed that all the examinees took positions which were not optimal while working and it significantly contributed to the MSD occurrence, as Kaljic (1) showed in

his paper. The theory that workers (teachers) are the most common patients due to the more frequent mechanic change on their spine is supported by the study (1) done in a policlinic for physical therapy and rehabilitation Stari Grad in the period from April 1st 2004 to December 31st 2009, where they treated 913 patients with pain in the lumbar part of their spine. They came to a conclusion that clerks were the most common (466 or 51% in total). Out of 913 patients in the same study, the highest number of the treated patients were between 45 and 54 years old (283 or 30.99%). The age structure of the examinees shows the frequency of the painful lumbar syndrome for the ages between 25 and 65, which represents work-related active group of examinees.

Some authors showed that the pain occurrence is influenced by the years of service, teachers' age, and the working hours. The pain prevalence can also be influenced by the body height of the teaching personnel (3, 5). Our examination showed that working hours are an important risk factor for pain occurrence.

Some examinations show (8-11) that most of the teaching personnel who take the sitting position have kyphotic posture. Ergonomically looking, the teaching personnel should take positions which preserve the physiological curves of the spine. Such positions, besides being considered neutral, diminish the tension of the soft tissue structures, stabilizers and spine and thus, prevent their elongation and micro-traumas, which can lead to consequential degenerative changes at the spinal unit level due to their repetitive nature, and can later spread to the entire spine segment. Ergonomic chairs which follow the physiological spinal curves are the most suitable and they diminish the need for taking the discogens positions while working (12).

The common teacher's posture while working is protraction, head and shoulders flexion, with shoulders bent forward. That can cause shortening of m. sternocleidomastoideus, m. scalenus, m. serratus anterior and m. pectoralis minor; while the middle and lower bundles m. trapezius can be elongated due to the adaptation to that posture. Such muscle imbalance can play a role in developing not localized chronic pain (13, 14). Muscle imbalance between abs and lower back muscles, which is very common for the teaching personnel, can cause additional rigor. Repetitive bending forward, while bending our backs, can cause exhaustion in the top extensors of the lower back, while deep abs (m. tranversus abdominis and m. obliquus abdominis) tend to get weaker (15).

McKenzie's institute for mechanic diagnosis and therapy in New Zealand recommends taking neutral posture while doing everyday activities, and doing exercises in the direction of the primary movements (direction opposite to the one usually done while working) in order to prevent the musculoskeletal rigor. Head should be in a neutral position, with the chin parallel to the ground. Neck should be in the flexion from 0 to 10 degrees, without rotation and bending to the side, or in protraction. Shoulders and the pelvis should be horizontal, and we should put a stand under one of our feet, so that the pressure can be transmitted equally and unload bone joints of the lower limbs (2, 16).

The main torso and shoulder muscles are supposed to offer a firm and secure support to our arms. Strengthening these muscles is the basis of Pilates. Pilates is an exercising program that can contribute to musculoskeletal health of the teaching personnel, and it requires using light weights and a high number of repetitions. While doing these exercises we should pay attention to strengthening the deep torso muscles using isometric weight volume. Muscle strengthening exercises should be done three times a week, taking a one-day break after exercising. We should start with a small number of trainings and repetitions and increase them over time (16).

Ergonomically speaking, chairs are very important in classroom (15). Chairs should support the teachers' bodies in neutral positions. Backrest convexity should keep the natural lumbar position while sitting, so that's why it's called lumbar support. This lumbar support should be approximately 20 cm high, not thicker than 3-5 mm, convex bottom to the top, so it can imitate the natural back posture. If it was thicker, it could create additional pressure to the lumbar vertebra. When it comes to the high backrests, the top part should not create pressure on the pectoral part of our back and thus push it forward. All the backrests that surpass the lower border of the blade bone can lower the usage of the lumbar support by transferring the pressure to the blade bone, so they should be about 6 cm below the bottom border of the blade bone (17). Chairs without backrests are also considered to be ergonomic, since while sitting in such chairs our pelvis is in almost neutral position, like in a saddle or while standing. Such pelvis position helps balance the spine while performing various movements, but such chair design could still create pressure on the peritoneal region (15).

Many researches have confirmed that armrests help prevent the neck, shoulder and lower back pain, since they lower muscle activity, especially for the trapezius of the dominant hand. Armrests should be highly adjustable and should offer support in the neutral position. Well-adjusted armrests will prevent the development of the neck and shoulders pain. Elbow-rests lower the activity of the m. rhomboideus, as well as that of peritoneal and cervical part of the m. errector spinae (15, 18).

Except for the position, another factor is the working hours. It has been proven that even a low level of pressure, which lasts for a while, can cause muscle tiredness and chronic pain (6). Hence, a number of studies have shown promising results for the provision of work breaks of varying durations. However, studies that would aim to identify the optimal duration of work breaks by comparing different break durations are still lacking (7).

Several short breaks while working (no longer than 5 seconds) are more practical and offer complete recovery of the tired muscles. During these micro-breaks, the tense muscles

get more blood and time to recover (13). Bearing in mind that we should avoid static positions, it would be good to adjust the backrests and chairs every now and then, so that the pressure would be transferred from one tissue to another and thus the micro-trauma would be minimized (16).

MSD are a major medical, social and economic problem because of their high prevalence and the ever-increasing number of patients. The results of many studies showed that MSD do not only affect an individual, but also their families, workplace and the health care system (19-21).

Another problem is the lack of effective prevention which would reduce the incidence of MSD. Additionally, there are social and economic consequences of MSD as many people with musculoskeletal problems leave the labor market, either temporarily or permanently, usually with adverse effects on their family life and socio-economic status (22).

The significance of our study is that reflected prevention of MSD related to work is necessary in terms of providing ergonomically designed working environment, taking correct body posture during work, regular pauses and posture changes during work, doing physical activities (exercises) as well as health promotion which would contribute to preventing absence from work due to MSD, increase labor productivity, but most importantly, it would improve the life quality of the teaching staff.

Many studies indicate the importance of ergonomic measures in the prevention of work-related MSDs (23-25).

CONCLUSION

Our study showed that the prevalence of the musculoskeletal rigor for the teaching staff of the State University of Novi Pazar was 74%, and it was higher for men than for women. The highest number of the examinees had musculoskeletal rigor in the lower back, neck, upper back and shoulders. To prevent the work-related musculoskeletal diseases (MSD), it is necessary to do the appropriate MSD risk evaluation at the work place, which will be used to give ergonomic solutions along with the evaluation of the given suggestions.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary written and informed consent was obtained from each participant prior to enrollment in the study. The protocol of the study was approved by the local ethics committees of State University of Novi Pazar, Novi Pazar, Serbia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None.

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RELATIONSHIP BETWEEN MALIGNANT BRAIN TUMORS AND VALUES OF HOMOCYSTEINE, FOLIC ACID AND VITAMIN B12

Zivanka Djurovic[1], Slavica Mutavdzin[2], Milos Drobnjakovic[3], Marko Djurovic[4], Irena Cvrkota[4] and Vladimir Jovanovic[4,5]

[1]*Clinic for Orthopaedic Surgery and Traumatology, Clinical Center Serbia, Belgrade, Serbia*
[2]*University of Belgrade, Institute of Physiology, Medical Faculty, Serbia*
[3]*Faculty of Chemistry, University of Belgrade, Serbia*
[4]*Clinic of Neurosurgery, Clinical Center of Serbia, Belgrade, Serbia*
[5]*Medical Faculty, University of Belgrade, Serbia*

Received: 20.07.2018.
Accepted: 25.07.2018.

Corresponding author:

Zivanka Djurovic

Clinic for Orthopaedic Surgery and Traumatology, Clinical Center Serbia, Belgrade, Serbia

E-mail: zdjurovic63@gmail.com

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UDK: 616-006.6-074:577.1
Eabr 2023; 24(1):69-74
DOI:10.2478-sjecr-2018-0045

ABSTRACT

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults. Homocysteine (Hcy) has a detrimental influence on human neurons, considering that human GBM cells undergo cell death already at D,L-Hcy concentrations in culture medium of 50 μM. This data demonstrate that Hcy is a potent gliotoxic agent capable of inducing the death of human glial cells already at concentrations reached in brain during hyperhomocysteinemia. The one retrospective study found that the serum vitamin B12 level can be used to predict survival time in metastatic cancer patients including neurological cancer. Cancer risk increases with elevated vitamin B12 level, mostly within the first year of the follow-up period, suggesting that vitamin B12 level could be used as a cancer diagnostic marker. In addition, the relationship between elevated vitamin B12 level and poor cancer survival time has been reported. Previous investigation suggests that the folate supplementation could be used as an adjuvant in antiglioma therapy to limit the low DNA methylation level because this confers a poor prognosis in glioblastoma multiforme patients. Taking into account all presented data, it can be concluded that effect of homocystein, folic acid and vitamin B12 on formation, development and outcome of treatment in patients with carcinoma is very intriguing question, whose response requires additional both experimental and clinical research. There lack of data in the literature on the incidence of elevated levels of Hcy in the blood, as well as the disorders of folic acid and vitamin B12, at malignant tumors of the brain.

Keywords: *Malignant tumors of the brain, homocysteine, folic acid, vitamin B12.*

RELATIONSHIP BETWEEN CARCINOGENESIS AND VALUES OF HOMOCYSTEINE, FOLIC ACID AND VITAMIN B12

Homocysteine (Hcy) is an amino acid containing sulfur and participates in methionine metabolism. It is an essential amino acid that is metabolized in one of two ways: by remethilation and transsulfuration. Abnormalities of these pathways lead to hyperhomocysteinaemia. If the accumulated Hcy does not convert immediately into methionine and cysteine, it is excreted, with strictly regulated cellular mechanisms. The excess Hcy is removed from the blood by the liver and kidneys. The importance of high blood Hcy in clinical practice is still the subject of discussion. Hyperhomocysteinemia is present in about 5% of the general population and is associated with an increased risk of developing many diseases such as vascular and neurodegenerative disorders, autoimmune diseases, congenital anomalies, diabetes, kidney disease, osteoporosis, neuropsychiatric disorders and malignancies. The central question is whether it is clinically justifiable to measure and treat elevated Hcy levels (1, 2). While some authors consider that Hcy is merely a marker of certain diseases, but not a causative agent, or ignores Hcy by attributing to it a role of metabolite appearing with other risk factors, most scientific evidence points to the contrary.

Carcinogenesis is a long-term process of gradual accumulation of genetic abnormalities; cloned cell populations are experiencing progressive genetic alterations that lead to malignancy and selective progression in growth. It is known that carcinogenesis is associated with metabolic alterations that accelerate or develop from neoplastic progression (3). Metabolic reactions involving folic acid are called one-carbon metabolic reactions (4). Changes in single-carbon metabolism reflect on DNA synthesis, repair, and methylation, which can lead to carcinogenesis (5). Folic acid is involved in the synthesis of serine and purine and pyrimidine bases and it is a donor of the methyl group in the methionine synthesis cycle. Hcy is an intermediate metabolite in the methionine metabolism and is metabolized to methionine and cysteine. During these metabolic cycles, S-adenosyl methionine (SAM), the main donor of the methyl group in the human organism, is formed. The concentration of Hcy in the serum is a sensitive indicator of folate status of the organism; folate deficiency is often associated with hyperhomocysteinemia, and the folate input can lower the level of Hcy. Hcy has been shown to inhibit methyltransferases, to suppress DNA repair and to facilitate apoptosis when accumulated inside the cells. Autooxidation of Hcy metabolites results in H_2O_2 accumulation and challenging neurons to Hcy metabolites for longer period leads to necrotic cell death (6). Folate deficiency leads to a reduction in incorporation of uracil into DNA, which leads to DNA instability that may lead to carcinogenesis. It was found that elevated values of Hcy in adults were associated with colorectal cancer (7) and cervical cancer of the uterus (8). Alterations in the methionine cycle are also present in breast (9), pancreatic (10), and larynx (11) cancer. A moderate deficit of folate does not have to be mutagenic *in vivo*. However, it was observed that its interaction with other risk factors, such as genetical environmental, led to tumor progression (12). Increased values of Hcy are associated with chromosome damage, even in the absence of folic acid and vitamin B12 deficiency, and this mechanism can not be explained by the metabolic disorder of uracil methylation in thymine. It has been proven that Hcy can directly damage the DNA in the presence of numerous reactive oxidants, and there is also the opinion that the metal-dependent DNA damage through H_2O_2 is the mechanism how elevated level of Hcy in plasma lead to carcinogenesis (13).

During the folate remedylation cycle, catalyzed by methionine synthase (MS), Hcy receives a methyl group of 5-methyltetrahydrofolate (5-methylTHF). In this reaction, the cofactor is vitamin B12 (cobalamin). 5-methylTHF was previously formed from 5,10-methyltetrahydrofolate by the action of methylenetetrahydrofolate reductase (MTHFR) (14). Folate is the major source of methyl groups. They originate from ingested foods and have the significance of biological methylation processes (methylation of DNA, RNA, proteins). Folic acid depots are constantly renewed and found in the liver, kidneys and intestinal mucosa. Within the folate cycle, MTHFR participates in the formation of 5-methylTHF, which is the predominant circulating form of folate. It is the major donor of the methyl group in the process of removing Hcy into methionine. In this way MTHFR is active at the most important point of the folate cycle, essential for the restoration of the folate level to the account of DNA and RNA biosynthesis. It is crucial in the distribution of folate between the two main pathways: biological methylation and nucleotide synthesis (15).

MECHANISMS OF CARCINOGENESIS INDUCED BY DISORDERS OF HOMOCYSTEINE METABOLISM

There are two mechanisms where folate deficiency can increase the risk of malignancy:

1. Causing DNA hypermethylation and thereby activating proto-oncogene;
2. Incorrectly induced uracil incorporation during DNA synthesis, which further leads to discontinuation of DNA strands and major chromosomal damage (16).

DNA hypermethylation was associated with tumor formation and was the focus of numerous studies in the field of oncogenetics (17). Global hypomethylation and gene-specific demethylation can lead to aberrant expression and activation of the genes, which can lead to tumor formation (18). The significance of MTHFR is confirmed by the fact that in a number of adults with hyperchomocystinemia there is polymorphism within folate-binding groups (such as C → T substitution on nucleotide 677). These changes lead to the formation of dysfunctional thermolabile MTHFR. This leads to a pathological accumulation of Hcy, which leaves the cell and passes into the circulation. Variant 677 C → T is associated with high basal Hcy levels in the serum. Lack of vitamin

B12, insufficient folate availability and C677T polymorphism of MTHFR lead to reduced disintegration of Hcy, its accumulation, and consequently increased circulatory hypersensitivity. Many tissues have an alternative pathway of Hcy removing, while in the central nervous system, Hcy remediation is performed only by MS. The collected Hcy, in addition to its negative effect, also results in reduced availability of methionine the main donor of the metal group for a variety of molecules responsible for the function, development and differentiation of cells (DNA, RNA, phospholipids, catecholamines). Likewise, the reduced cycle of Methilentetrahidrofolate leads to a decrease in the concentration of the active tetrahidrofolate form involved in the synthesis of purine and nucleic acids (19).

Table 1. Summary of some studies testing the effects of homocysteine or vitamines B on glioblastoma

Model	↑ Hcy or ↑B-vitamines	Mechanisms	Study
Glioblastoma T98G	D,L-Hcy 0-5 mM (50 μM)	↑ Cell death	Škovierová H et al. (2015) (34)
Human neuroblastoma	Folate and B12 deficient media	Hypomethylation of nt 451-454 in the promoter region of PS1	Fuso A et al. (2005) (44)
Glioblastoma U373	↑ Folate	Limit the low level of DNA methylation, and promote the MGMT gene methylation.	Hervouet E et al. (2009) (39)
Mice bearing human glial tumor xenograpfts (D-54, SWB77, SWB40,U-87)	↓ Methionine	Dietary MET restriction induced regression of some tumor lines	Kokkinakis DM et al (2002) (37)
Patients with glioma	↑ Hcy	C677T polymorphism of MTHFR lead to ↑ Hcy	Sciacca FL et al. (2004) (38)
Retrospective study (on humans)	↑ Vitamine B12	Prognostic factor for survival time in glioblastoma patients	Oh HK et al. (2018) (40)

Methylthioadenosine phosphorylase (MTAP) is a key enzyme essential for the methionine salvage pathway. MTAP catalyzes the cleavage of 5-methylthioadenosine into adenine and 5-methylthioribose-1-phosphate (MTR-1-P). Adenine is then used to generate AMP, whereas MTR-1-P is converted into methionine. In normal mammalian tissues, MTAP recycles purines and methionine consumed during polyamine synthesis. MTAP deficiency has been reported in both liquid and solid tumors, including gliomas. Previous studies have reported gliomas are frequently associated with abnormalities in chromosome 9 and the MTAP locus is located at 9p21.3. The shortest region of overlap of the deletions maps in the interval between the centromeric end of the interferon gene cluster and MTAP gene. This deletion is associated with high grade and recurrent gliomas suggesting that these alterations could contribute to the progression of glioblastoma (GBM). Further, MTAP resides approximately 100 kb telomeric of p16INK4A. MTAP is usually codeleted with p16 (*cdkN2a/ARF*). Homozygous deletions of human chromosome 9p21 occur frequently in malignant cell lines and are common in primary gliomas. In general, GBMs lack expression of the enzyme MTAP, due to either deletion or methylation of the MTAP promoter (20).

RELATIONSHIP BETWEEN MALIGNANT BRAIN TUMORS AND VALUES OF HCY, FOLIC ACID AND VITAMIN B12

Malignant brain tumors are among the most feared types of cancer, not only for their poor prognosis, but also because of the direct repercussions on quality of life and cognitive function (21). GBM is the most common primary malignant brain tumor in adults. It can

occur anywhere in the central nervous system, but primarily occurs in the white mass of the large brain (22). Histopathological presentation includes nuclear atypia, cell pleomorphism, mitotic activity, vascular thrombosis, microvascular proliferation, and necrosis. The World Health Organization (WHO) classification system groups gliomas into 4 histological grades defined by increasing degrees of undifferentiation, anaplasia, and aggressiveness (23). The majority of these tumors occurs *de novo*, without obvious precursors - the primary GBMs. Secondary GBMs develop slowly from diffuse glioma II or anaplastic glioma III. GBM with its variants is graded as grade IV (24).

Despite the deadliness of this disease, much is still unknown about the metabolic pathology of GBM because tumor heterogeneity and genetic alterations cause an altered metabolism and metabolic profile within the same tumor, making it difficult to correlate metabolic signatures. In recent years, metabolomic-based approaches have been recognized as an emerging tool to discover products of cellular biochemical reactions that fuel cell proliferation in a variety of malignancies (25-27). Furthermore, metabolomic profiling has led to the discovery and identification of numerous key cellular pathways (28, 29).

Applications of metabolomics in clinical oncology have shown strong potential in the early detection, diagnosis, and prognosis of cancer as well as being a predictive/pharmacodynamic biomarker of drug efficacy.

There is almost no worldwide clinical literature on clinical studies that link malignant brain tumors with Hcy, folic acid, and vitamin B12 levels in the blood.

INFLUENCE OF METHIONINE ON DEVELOPMENT OF GLIOBLASTOMA

Methionine is an essential neutral amino acid that can readily cross the blood-brain barrier through neutral amino acid transporters and accumulates in an active tumor. Cellular uptake studies of methionine in GBM, glioma stem cells (GSC), and normal human astrocytes (NHAs) show a significant uptake of methionine by GBMs and GSCs when compared to NHA. The uptake of methionine in a normal brain is relatively low as compared to those with gliomas. Normal cells can survive and proliferate without methionine, while cancer cells would not due to the deregulation of various enzymes in methionine metabolic pathway (20). The methionine dependency and its role in cancer growth control can be achieved using the methionine restriction strategy, particularly in cancers that require methionine for survival and proliferation (30). The deficiency of MTAP has been reported in glioma (31). The aggressiveness of tumors has been linked with MTAP deficiency. Though there are challenges and advantages of targeting tumors which lack MTAP activity, MTAP deficiency in human GBM could be a potential target for tumor-specific chemotherapy.

The metabolic discovery data from study conducted by Palanichamy K and Chakravarti A. shows the expression of MTA in the extracellular compartment of GBM cell lines. MTA is excreted by GBM cell lines, but not by NHA (20). Additionally, MTAP rescue studies in xenografts suggest MTAP is a tumor suppressor (32).

Exploiting this metabolic vulnerability could lead to better therapeutic intervention in cancers while sparing normal cells (20). Collectively, all these studies suggest that methionine dependency can be exploited for therapeutic benefit.

INFLUENCE OF HOMOCYSTEINE ON DEVELOPMENT OF GLIOBLASTOMA

Previous investigations had shown that Hcy has a detrimental influence on human neurons (33). Human GBM cells undergo cell death already at D,L-Hcy concentrations in culture medium of 50 μM. Since only the L enantiomer is biologically active and occurs naturally this corresponds to a concentration of 25 μM of the L enantiomer of Hcy. This data demonstrate that Hcy is a potent gliotoxic agent capable of inducing the death of human glial cells already at concentrations reached in brain during hyperhomocysteinemia. The available data suggest that Hcy, even at moderate levels, can exert its neurotoxic effect by:

1) several mechanisms that trigger neuronal cell death, e.g., increased oxidative stress
2) acting as an agonist for glutamate receptors
3) its capability to elicit a rise in the level of cytosolic Ca^{2+} and
4) inducing DNA damage and changes in energy metabolism associated with reduced availability of ATP (34).

Methionine and Hcy depleted diets prevent metastasis in tumor bearing animals. Methionine depletion induces many modifications in tumor cells, including cell arrest in the S and G2 phases of the cell cycle, apoptosis, decrease of glutathione content and reduced activity of *O*-methylguanine-methyltransferase (35, 36). A G2 cell cycle blockade in tumors and extension of the life span of the animals bearing human tumor xenografts also has been demonstrated. Impressive tumor regressions can be induced by reduction of the plasma methionine level to a steady state of under 5 μM in athymic mice bearing human tumor xenografts (37).

Homocysteine levels correlated with the genotype carriage, being higher in individuals carrying MTHFR C677T genotypes, both in patients and healthy controls. Patients, anyway, had homocysteine values significantly higher than healthy controls; homocysteine levels are strongly influenced by environmental factors, particularly diet and chemotherapy, and thus the higher values observed in patients compared with controls could partly be attributable to the tumor itself but possibly also to the lifestyle and drug treatment of patients with tumor (38).

INFLUENCE OF FOLIC ACID ON DEVELOPMENT OF GLIOBLASTOMA

Results of investigation that conducted Eric et al. suggests that the folate supplementation could be used as an adjuvant in antiglioma therapy to limit the low DNA methylation level because this confers a poor prognosis in glioblastoma multiforme patients. Their work identifies the use of folate as a promising alternative resource to the use of DNA hypomethylating agents as an anticancer treatment including an epigenetic-based adjuvant. Consistently with this results, this opens a new door to a better management of glioblastoma multiforme because the folate-induced methylation seems to be an efficient tool to limit the low level of DNA methylation, a biomarker conferring poor prognosis in glioblastoma multiforme patient and to promote the MGMT gene methylation, a biomarker associates with a benefit from temozolomide treatment (39).

VITAMIN B12 AS PROGNOSTIC AND DIAGNOSTIC MARKER IN MALIGNANT BRAIN TUMORS

The one retrospective study found that the serum vitamin B12 level can be used to predict survival time in metastatic cancer patients including neurological cancer. Cancer risk increases with elevated vitamin B12 level, mostly within the first year of the follow-up period, suggesting that vitamin B12 level could be used as a cancer diagnostic marker. In addition, the relationship between elevated vitamin B12 level

and poor cancer survival time has been reported (40). Serum vitamin B12 levels might increase because of decreased cellular uptake of vitamin B12 from the blood. In this case, excess vitamin B12 in the blood cannot be fully utilized by the cells, which leads to a vitamin B12 deficiency-like state, including problems in methionine synthesis, as indicated by elevated levels of homocysteine, an intermediate in methionine metabolism (41-43).

CONCLUSION

GBM is the most common brain tumor, and accounts for about 12-15% of all malignant intracranial neoplasms and 6075% of all astrocytic tumors. A large number of organizations are engaged in the monitoring of incidence of GBM, based on the use of data from the civil service for the monitoring of malignant diseases or using the health system record.

The question remains whether the elevated values of Hcy are a consequence of the defensive mechanism of organism on tumor cells, since Hcy acts cytotoxic to GBM, or chronic hyperchomocysteinemia leads to brain tumors. There is lack of data in the literature on the incidence of elevated levels of Hcy in the blood, as well as the disorders of folic acid and vitamin B12, with malignant tumors of the brain. Further investigations are necessary to determine of the importance of Hcy, folic acid and vitamin B12 blood levels as predictive factors for the outcome of the malignant brain tumors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None.

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ACUTE HEMORRHAGIC EDEMA OF INFANCY - CASE REPORT

Rasa Medovic[1], Gordana Kostic[1,2], Slavica Markovic[1,2], Marija Medovic[3], Gordana Ristic[3,4], Dragana Savic[1,2], Aleksandra Simovic[1,2], Sanja Knezevic-Rangelov[1,2] and Zoran Igrutinovic[1,2]

[1] *Pediatric Clinic, Clinical Centre Kragujevac, Serbia*
[2] *University of Kragujevac, Faculty of Medical Science, Department of Pediatrics, Serbia*
[3] *Clinic for Dermatovenerology, Clinical Centre Kragujevac, Serbia*
[4] *University of Kragujevac, Faculty of Medical Science, Department of Pediatrics, Serbia*

Received: 08.12.2019.
Accepted: 09.02.2020.

Corresponding author:

Medovic Rasa

Pediatric Clinic, Clinical Centre Kragujevac, Serbia
Zmaj Jovina 30, 34000 Kragujevac

Phone: +381 69 2772927

E-mail: rasamedovic@gmail.com

sciendo

UDK: 616-005.1-053.2
Eabr 2023; 24(1):75-78
DOI:10.2478/sjecr-2020-0005

ABSTRACT

Acute hemorrhagic edema of infancy (AHEI) is a rare vasculitis of small dermal vessels with characteristic presentation in infants aged up to 24 months. It manifests as a sudden occurrence of palpable purpuric skin lesions, swellings in hands, feet, face and auricles, and mild fever. The children affected with AHEI are almost always in good general health and with normal laboratory parameters. Approximately 400 cases have been described in the literature so far. However, the etiology is still unknown. Most evidence suggests infections or vaccination as the principal triggers. Extra cutaneous manifestations are recorded in only about 10% of AHEI patients. The majority of the affected children undergoes recovery spontaneously and without any complications within 1-3 weeks, with or without any treatment. AHEI is usually diagnosed on the clinical grounds only and the diagnostic procedure rarely requires a skin biopsy. The current literature indicates the use of corticosteroids and/or antihistamines as a therapy, but there is still a lot of controversy about these therapeutic measures. This paper presents the case of AHEI with its typical clinical manifestations that resolved in a rapid spontaneous recovery without the use of any treatment within a week. AHEI is a rare syndrome that pediatricians should be well familiar with in order to differentiate it from other potentially severe diseases that have similar cutaneous manifestations, but also to avoid unnecessary investigations and therapy.

Keywords: *Acute hemorrhagic edema, infancy.*

INTRODUCTION

Acute hemorrhagic edema of infancy (AHEI) manifests as a sudden onset of painless, non-itching, localized, palpable, purpuric oval skin lesions on extremities, face and auricles, limb and facial swelling and mild fever. It affects children aged up to 24 months who are otherwise found to be in good general condition [1, 2]. AHEI is a rare form of leukocytoclastic vasculitis of small dermal vessels that involves inflammation and endothelium fibrinoid necrosis, and consequently also the presence of perivascular infiltrates and erythrocytes extravasation [3, 4]. The etiology of the syndrome is still unknown even though the certain causal correlations with recent bacterial or viral infections, immunization or drug intake have been reported [5, 6]. In the majority of the patients, most of the laboratory analysis have been recorded as normal [7-9].

The disease has a benign, self-limiting course and usually resolves without any further complications [1, 2, 5-13]. The presence of extra cutaneous manifestations, such as glomerulonephritis, abdominal pain with gastrointestinal bleeding, arthralgia, testicular torsion, intussusception, scarring and hyperpigmentation has been recorded in less than 10% of the patients [12-15]. The current literature indicates the use of corticosteroids and/or antihistamines as a therapy, but there is still a lot of controversy about these therapeutic measures [10, 11, 16, 17]. Most authors find it unnecessary because patients generally recover spontaneously within 1-3 weeks [5-9, 11-14].

This paper will describe one case of AHEI that manifested with its typical clinical presentation and that resolved in spontaneous full recovery without any treatment in a week. Since the syndrome has dramatic manifestations, it is a great source of panic, anxiety and concern to parents. Thus, it is extremely important to recognize it promptly and point out its benign nature [7-9, 11].

CASE REPORT

Patients consent

From parents of patient was obtained written and informed consent for taking data and publishing scientific article. All procedure were described and manuscript was done in according to the *CaRe* guidelines.

Case presentation

A seven-month-old male infant, with no prior health issues reported, was referred to a hematology department on the first day of illness onset. The child had been born after the second full-term pregnancy with normal vaginal delivery. His psychomotor development was evaluated as adequate for his age. The child had had a mild fever (37.8°C) during the previous day and the bruises had appeared on the various body parts. The baby was active, playful, and with stable vitals. However, numerous small hematomas (up to 2 cm in diameter) were present on the lower legs and forearms, as well as on the dorsum of his hands and feet. The mild swelling was detected on the right feet (Fig. 1). There were also several small hematomas on the knees, elbows and cheeks that soon formed larger surfaces whose diameter ranged from 5 to 8cm (Fig. 1-3). All the lesions were purple, irregular, circular, skin-leveled and had well-defined peripheral edges. The child also had an associated mild nasal congestion. Except for the symptoms described above, the complete systematic examination showed no abnormalities.

The parents reported that the baby had been upset on the day of the admission and that they had administered Paracetamol. The child was being breastfed. Reportedly, he had had an infection of the middle ear a month before treated with Amoxicillin ®. The baby had been vaccinated regularly with the last vaccination against Diphtheria, Tetanus and Pertussis, Hemophilus influenza type B and Polio taking place the previous month. Negative family history of bleeding disorders was reported. The possibility of child abuse was eliminated.

All the laboratory tests were in the normal ranges - complete blood count (CBC) and hemostasis examinations, including a test for congenital and acquired thrombophilia, fibrinogen, C-reactive protein, erythrocytes sedimentation rate, liver and kidney function tests, electrolytes, serum proteins and albumins, immunoglobulins, complements, hormones and antibodies of the thyroid gland, antinuclear antibodies, anti-streptolysin O, cellular and perinuclear anti-neutrophilic cytoplasmic antibodies and rheumatoid factor, and urine analysis and culture. The ultrasound of the abdomen and brain appeared normal for the given age. All the available virusology analyses were negative. The only laboratory parameter that was beyond the proscribed level was D-dimer that equaled 6.30 ng/mL and that normalized as the illness regressed.

At first, we suspected the *Henoch-Schönlein* purpura. After consulting the relevant literature, we concluded that we are dealing with AHEI. Skin biopsy and immunofluorescence study were not performed because the child was soon afebrile and the changes on the skin started spontaneously to regress. No therapy was administered; the patient only underwent the elimination diet for vasculitis. He did not have any systemic complications and the skin lesions completely healed in five days. No sequelae were detected or reported during the follow-up visits that took place in the year to follow.

DISCUSSION

AHEI (also known as Finkelstein-Seidlmayer disease, rosette form purpura, medallion-like purpura or infantile postinfectious iris-like purpura) was initially considered to be a variant of Henoch-Schönlein purpura [18]. Later on, it became recognized as a separate clinical entity [3, 4, 19]. There are no exact data on its incidence. According to the systematic review of the recorded AHEI cases conducted by Fiore et al. [13] in 2008, approximately 300 cases of AHEI have been reported, with male patients being predominant (2:1

ratio). The sex of our patient follows this trend. The authors also claim that the number of the described cases has increased to approximately 400 in the following years, probably due to better knowledge on the issue.

All the consulted authors recognized AHEI as a condition which manifests through the specific triad of symptoms. A low-grade fever occurs in the prodrome and is followed in the next few hours by the onset of red macules or urticarial lesions and then by symmetrically arranged large palpable ecchymotic lesions on the skin. Finally, asymmetrical swelling appears on auricles, face and extremities (most frequently dorsum of hands and feet). In all the cases described in the literature, the general condition of the patients was evaluated as good [1-11]. The onset and the further course of the disease in our patient completely corresponded to the typical clinical form described in the literature. Fortunately, except for the mild edema on the right foot, no edema was found elsewhere. The skin lesions did not progress and necrosis or bullas were absent in our case although they reportedly occur in AHEI [12].

The literature source we have consulted have argued that AHEI can be associated with viral infections (Coxsackie, Cytomegalovirus, Rota virus, Hepatitis A), bacterial infections (Escherichia coli, Campylobacter, Streptococcal and Staphylococcal), vaccination (MMR, DTP) or drug intake (antibiotics, non-steroidal anti-inflammatory drug, cough syrup) [3-5, 17, 20]. In 10% of the described cases, it could be linked to immunization [7, 19]. According to Chesser et al., in 75% of the patients infectious agents could be seen as triggering factors, which is further supported by the fact that AHEI occurs more frequently during the winter months [20]. Our patient had had a history of middle ear inflammation for two months and the last vaccination had taken place a month before the onset of the disease. Consequently, we cannot make confident claims about causal factors and what triggered the immune process.

The AHEI diagnosis is initially made on the clinical basis without the necessity for a skin biopsy [3, 4, 12-14]. However, there are a lot of diseases with similar dermatological manifestations, but potentially more severe clinical presentations, which must be promptly differentiated and eliminated. The following diagnoses that should be also considered: meningococcemia, Kawasaki disease, purpura fulminans and skin lesions in septicemia, drug eruptions, Henoch-Schönlein purpura, rheumatoid purpura, Sweet syndrome, erythema multiforme, angioedema, child abuse, idiopathic thrombocytopenia, nephrotic syndrome and acute hemorrhagic urticaria [7, 19]. When skin biopsy is performed, it shows a leukocytoclastic vasculitis of small dermal blood vessels with perivascular infiltrates composed of neutrophils, and occasionally eosinophils [3, 4]. The direct immunofluorescence testing shows vascular deposits of C1q, C3, fibrinogen and immunoglobulins. IgM deposits are the most common of all immunoglobulins while IgA, the most common finding in Henoch-Schönlein purpura, is present in approximately one-third of the patients [3, 4, 18].

According to the majority of the consulted authors, skin biopsy should be performed only if the disease keeps progressing, the further complications start occurring or the diagnosis appears unclear [3, 4, 12-15]. The similar stand has been taken by numerous authors when it comes to not introducing any therapy [8-11, 16]. However, the rapid progression of the syndrome could be an indication for the introduction of the systemic steroid therapy and/or antihistamines [16]. In cases of extensive bullous lesions, secondary infections of ruptured bullae or similar, patients should be treated with local and systemic antibiotics, and also monitored for any signs of infant dehydration [15]. The opinions about putting patients on the elimination diet are also contradictory and thus controversial. Despite all the disagreements, we introduced the elimination diet for vasculitis [21] and intravenous hydration, but decided not to administer any therapy and not to perform skin biopsy given the good clinical course of the disease in our patient.

CONCLUSION

Although AHEI is a rare syndrome, pediatricians should be well informed about its presentation and proper management. The early diagnosis of AHEI is important in order to avoid unnecessary and/or invasive medical investigations and therapy. Due to the dramatic clinical image of AHEI, parents may seek medical help in emergency departments where the condition should be also promptly recognized and adequately treated.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

From parents of patient was obtained written and informed consent for taking data and publishing scientific article. All procedure were described and manuscript was done in according to the CaRe guidelines.

CONFLICT OF INTEREST

The authors declare that there is no potential conflict of interest regarding this manuscript.

FUNDING

None.

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HYPERBARIC OXYGENATION IN TREATMENT OF FEMORAL PSEUDOARTHROSIS CAUSED BY OSTEOMYELITIS

Gordan Stojanovic

University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia

Received: 04.11.2019.
Accepted: 10.02.2020.

Corresponding author:

Gordan Stojanovic

University of Kragujevac, Faculty of medical sciences, Svetozara Markovic 69, Kragujevac, Serbia

E-mail: stgordan@gmail.com

UDK: 616.718.4-002:615.835
616.718.4-001.5-06
Eabr 2023; 24(1):79-83
DOI:10.2478/sjecr-2020-0009

ABSTRACT

Post-traumatic osteomyelitis is a common complication of open fractures. Most infections can be resolved by aggressive wound debridement, antibiotics, and normal wound drainage. However, the eradication of infection can be challenging in patients with chronic infection. The pseudarthrosis caused in this way is maintained and difficult to treat even when the infection is cured. Persistent infection may sometimes require suppressive therapy or even limb amputation to control the disease. Improvements in medical treatment have led to increased survivorship and reduced disability in these patients, posing new challenges in their post-traumatic care. This case report describes the use of HBO therapy as an adjunct treatment in a compromised host with recurrent post-traumatic osteomyelitis and refractory pseudarthrosis despite aggressive wound debridement, removal and replacement of the affected bone by autologous bone graft, and several courses of intravenous and oral antibiotic therapy. Hyperbaric oxygen therapy may be considered as an adjunct to standard treatment protocols for refractory osteomyelitis in compromised hosts.

Keywords: *Post-traumatic osteomyelitis, Pseudarthrosis, Hyperbaric oxygen therapy.*

INTRODUCTION

Unless there are other pathological conditions, femoral fractures are caused by a strong force and are an important factor of morbidity and mortality in mechanical damage to the locomotor apparatus, representing 15% of all fractures (1). The femoral fracture may be classified as open and closed. Open fractures are classified into types I, II, IIIA, IIIB and IIIC, according to Gustilo open fracture classification system, with higher morbidity, where type III implies massive soft tissue damage, compromised vascularization, extensive wound contamination, and fracture instability even after healing. Also, open fractures of type III are divided by the severity of the prognosis into: type IIIA, preserved coverage of the fractured bone, despite profuse laceration of the surrounding soft tissue, type IIIB where there is loss of periosteum with loss soft tissue most commonly associated with massive contamination, and type IIIC - open fracture associated with arterial injury and requiring surgical reconstruction. Bone infection in these three subtypes is as follows: type IIIA 4%, type IIIB 52%, type IIIC 42% (2). Internal fixation used to treat open fractures is sometimes complicated by infection - osteomyelitis and later by pseudarthrosis (3). One of the major complications of open femur fracture is acute post-traumatic osteomyelitis as the first clinical manifestation of acute inflammation and infection of the traumatized femoral region together with increased leukocyte count, increased erythrocyte sedimentation and elevated C-reactive protein levels. These clinical signs abate upon successful treatment, whereas in chronic post-traumatic osteomyelitis the indicators decrease but the bacterium persists (4). The duration of the infectious process adversely affects the prognosis and development of further complications, resulting in pseudarthrosis. Persistent infection causes progression of the sclerosis together with the formation of scar tissue between the two ends of the fractured bone, making the infection more resistant to therapeutic procedures (5). The infection also directly impairs fracture healing process in such a way that bacteria easily colonize the surfaces of osteosynthesis agents and use the compromised immune system response of an already traumatized patient, thus forming an impenetrable glycocalyx film. This makes bacteria resistant to both the acquired immune response of the host as well as antibiotics (6).

Hyperbaric oxygen (HBO) therapy was first used in the treatment of chronic refractory osteomyelitis in 1965 and its effects have been demonstrated in several in vitro and in vivo studies (7). Hyperbaric oxygen is a treatment modality that enhances blood perfusion and improves innate immunity at the site of injury. It encourages osteogenesis, neovascularization as well as collagen production (8 - 11). The purpose of this case study is to suggest that a patient with compromised local immunity, poorly fit for surgery, with refractory osteomyelitis may benefit from early HBO as an adjunct to standard treatment.

CASE REPORT

The patient is a man, aged 73, with acquired and refractory pseudarthrosis, as a possible consequence of chronic osteomyelitis, admitted for treatment in a hyperbaric chamber. Namely, the patient had a severe car accident over 40 years ago, causing polytrauma, and one of the injuries was an open fracture localized in the middle of the left femur. The case presented has Gustilo open fracture classification of IIIB subtype. After several surgical procedures (undertaken immediately after the fracture) and rehabilitation treatments (underwent over the course of two years), the fracture was repaired, while function and full reliance on the injured leg were restored. Thirty-one years after completion of treatment, a new fracture occurred at the same site, without the effect of strong mechanical force on the femur. That was apparently a pathological fracture since the analysis of the wound swab (taken intraoperatively during the surgery undertaken immediately after the fracture) confirmed the presence of Staphylococcus aureus- the most common cause of osteomyelitis. Osteosynthesis of the femur was undertaken and antibiotic therapy was implemented. Over the last two years, osteosynthesis material broke on three occasions while an intramedullary rod, inserted during the last surgery, endured over the next four years. There was no sign of complete fusion of the bone ends visible on radiographs through all this time. Osteosynthesis was occurring at the extremities of the femoral bone ends, resulting in a pseudarthrosis as a diagnosis (Figure 1).

Figure 1. Femur radiograph with visible pseudoarthrosis after implantation of osteosynthetic material

The swab taken during the last surgery was sterile and the wound was always dry, with no signs of inflammation and healed per primam.

Hyperbaric oxygen treatment was provided three and a half years after the fifth surgery. A single-person hyperbaric chamber was used, with an oxygen pressure of 1.8 ATA, one

hour per day for ten days. Due to the possibility of the continued existence of Staphylococcus aureus, antibiotic-clindamycin 600mg was administered in two daily doses for one week prior to HBO therapy. The x-ray was taken six months after the HBO therapy and showed healing zones without engaging the entire pseudarthrosis zone.

A year and a half after the HBO therapy, a new breakage of the osteosynthesis material and intramedullary rod occurred. After the surgical procedure, the healing zone was also impaired. This was the sixth surgery undertaken one year ago after which the wound swab remained sterile and showed no signs of local or bone infection. Finally, a year and a half after this surgery, a new breakage of the intramedullary rod occurred, followed by the seventh surgery. At that time, early HBO therapy was performed, exactly one month after the surgery, in the same way as the previous one. The results of the control are expected in 5 months.

DISCUSSION

This case reports the effects of the HBO therapy as an adjuvant treatment for two specific disorders manifested on the femur: possible chronic osteomyelitis and confirmed acquired pseudarthrosis.

Chen et al. pointed out that in addition to adequate surgical debridement and appropriate antibiotic selection, HBO was effective for chronic osteomyelitis of the femur. Their case series consisted of 13 patients who underwent a mean 4.6 surgeries, were mean 40 years of age (range: 21-61), and had Cierni-Mader classifications of III-IVA or B. The presented case suggests that a patient with significant medical comorbidities (Cierni-Madar classification, IIC) (14), and thus a poor surgical candidate may benefit from early initiation of HBO therapy. This case utilized earlier HBO treatment as a means of delaying surgery, and corollary likely contributed to the resolution of the disease in the compromised host (12). The plausibility and low number of side effects from HBO therapy make it acceptable as an adjunctive treatment for osteomyelitis (13).

Needless to say, what must precede the implementation of HBO therapy is an adequate surgical treatment, both of osteomyelitis and pseudarthrosis zones, as described above. This primarily refers to the removal of necrotic and infected parts of the bone, where intraoperative treatment of surgical margins is crucial, ie. borders of zones in which bone is alive or dead (15). In addition to this treatment, parenteral antibiotic therapy is required initially, followed by per os antibiotic therapy six weeks later (preferably according to antibiogram results). It has been suggested, however, that even with modern chemotherapy regimens, the degree of resection is still crucial (16). Since bacteria easily colonize the surfaces of osteosynthesis agents in an infected fracture, the fixator itself can cause the onset of infection while stabilizing the fracture. Therefore, treatment also requires removal of the fixator at the right time, as premature removal could affect bone stability too (27).

The duration of the infectious process also affects the prognosis. Its longer duration increases the growth of the scar tissue and progression of sclerosis which contribute to further development of pseudarthrosis. They proved to be aggravating factors for the HBO therapeutic approach (16).

As for the approach to the treatment of pseudarthrosis, the current gold standard is the surgical application of autologous bone grafts together with the fixation of separate parts of the bone. In the past decade, there have been several descriptive approaches designed to induce bone tissue formation/regeneration including extracorporeal shock wave therapy (ESWT), ultrasound, electromagnetic, bone morphogenic proteins (BMPs) and platelet-rich-plasma (PRP) (17).

BMPs are parts of the transforming growth factor-beta (TGF-b) superfamily with high osteoinductive potential. They induce a sequential cascade of events for chondro-osteogenesis during bone formation and bone healing processes, including chemotaxis (18), proliferation of mesenchyme and osteoprogenitor cells as well as their differentiation into chondrogenic or osteogenic lineages (18 - 20). Nowadays, several BMP homogeneous human molecules have been used in clinical trials and are commercially available (21,22). The point of using HBO for the treatment of pseudarthrosis would be to induce the above-mentioned cascade for chondro-osteogenesis and develop these factors by revascularization together with the effects of hyperoxia on the affected tissue.

Accordingly, neither optimal surgical results nor proper administration of antibiotic therapy are sufficient to prevent recurrent infection and achieve complete bone strength.

The normal oxygen pressure in healthy bone is about 45 mmHg under normal atmospheric pressure (23). Infected bone and necrotic tissue produce lower oxygen pressure region (24). The oxygen pressure level in chronic osteomyelitis is 23 mmHg or less (23). The causes of low oxygen pressure in chronic osteomyelitis are initial trauma, compromised vascularization, dense fibrous scars and insufficient debridement of infected bone. Oxygen pressure of 30-40-mm Hg is continuously required for ischemia-induced neovascularization (25). Enhanced blood perfusion to the area also leads to a higher concentration of administered antibiotic (28). Furthermore, the access of the aminoglycoside to the bacterial cell wall is oxygen dependent and inhibited in a hypoxic environment. Thus, HBO therapy can enhance transport and increase antibiotic efficacy (29). Similarly, increasing the oxygen pressure above 40 mmHg further improves the ability of leukocytes to kill microorganisms, and most clearly, it eliminates the anaerobic environment responsible for the development of anaerobic microorganisms (26) which cause osteomyelitis.

CONCLUSION

Chronic osteomyelitis, especially together with acquired pseudarthrosis, is a very serious complication of bone fracture, resistant to most therapeutic procedures.

In this case report it is evident that HBO, like all other treatments, did not completely repair pseudarthrosis in order to obtain a complete firmness of the femur that would provide complete support to the diseased leg. However, signs of osteomyelitis were no longer present, and the swab remained sterile, so this can be attributed, among other things, to the beneficial effects of HBO therapy. The incomplete effect of HBO therapy can be explained by its late administration (in comparison with those proposed in previous research and clinical work), as well as the poor condition, ie the great exhaustion of the entire segment of the injured/diseased knee. The patient should be always subjected to diagnostic methods of determining the presence of senile osteoporosis, vitamin D deficiency, and secondary parathyreoidism, and that would require another type of therapy. In a future therapeutic approach, the combination of HBO and antibiotics should be administered as early as possible postoperatively, which has been mentioned several times in the discussion. Future research should also focus on the effect of HBO therapy on bone morphogenic proteins (BMPs) in relation to the time of fracture healing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary written and informed consent was obtained from the patient prior to enrollment in the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None.

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