EABR
Experimental and Applied Biomedical Research
ISSN 2956-0454
eISSN 2956-2090
VOL 25 No 3
September, 2024.

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)

Address:

Experimental and Applied Biomedical Research, Faculty of Medical Sciences,
University of Kragujevac 69 Svetozara Markovica Street, 34000 Kragujevac, PO Box 124, Serbia

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

EABR is published four times annually

Experimental and Applied Biomedical Research is categorized as a scientific journal of M51 category by the Ministry of Education, Science and Technological Development of the Republic of Serbia

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

61

EABR : Experimental and Applied Biomedical Research / editor in chief Vladimir Zivkovic. - Vol. 25, no. 3 (sep. 2024)- . - Kragujevac : Faculty of Medical Sciences, University of Kragujevac, 2023- (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

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OXIDATIVE STRESS IN HEMODIALYSIS PATIENTS: PATHOPHYSIOLOGICAL MECHANISMS, CLINICAL CONSEQUENCES AND BASIC PRINCIPLES OF TREATMENT

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Received: 27.01.2019.
Accepted: 15.02.2019.

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ABSTRACT

Microinflammation is a non-traditional risk factor for the development of cardiovascular diseases in patients on hemodialysis. It occurs in 30-50% of these patients, and its main causes are: uremic toxins, oxidative stress, metabolic acidosis, vitamin D deficiency, overhydration, altered intestinal microbiome, impaired intestinal epithelial barrier integrity, increased translocation of endotoxin from the intestinal lumen into the systemic circulation, occult infection of the vascular approach for hemodialysis, periodontal disease, bioincompatibility of the hemodialysis membrane and the presence of endotoxin in the hemodialysis solution. The main clinical consequences of microinflammation are: accelerated atherosclerosis, malnutrition, anemia, resistance to the action of erythropoietin, hemoglobin variability and dialysis-related amyloidosis. Postdilution online hemodiafiltration, extended and adsorptive hemodialysis prevent the development of microinflammation. Optimal control of microinflammation prevents the development of cardiovascular diseases, improves the quality of life and the outcome of patients who are treated with regular hemodialysis.

Keywords: *Microinflammation, postdilution online hemodiafiltration, extended hemodialysis, adsorptive hemodialysis.*

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UDK: 616.61-008.6-78-06
UDK: 616.12:616.61-78-056.24
Eabr 2024; 25(3):199-205
DOI: 10.2478/sjecr-2019-0008

INTRODUCTION

Cardiovascular diseases are the leading cause of death in patients on regular hemodialysis treatment (1). They are the leading cause of death of patients treated with kidney replacement methods. In this patient population, the prevalence of traditional and non-traditional risk factors for the development of cardiovascular diseases is high. Non-traditional risk factors include: anemia, inflammation, oxidative stress, hyperhomocysteinemia, hypervolemia, metabolism of calcium and phosphate, and lack of vitamin D (1, 2).

Oxidative stress and pathophysiological mechanisms of its generation

Oxidative stress is defined as organ damage caused by the balance disturbance between the formation of free radicals and the function of antioxidant systems (3, 4). A free radical is any atom or molecule with one or more unpaired electrons. The processes of protein, carbohydrate, lipid and nucleic acid oxidation cause damage to the structure and function of organ tissue cells (3, 4). Free oxygen radicals are produced in polymorphonuclear leukocytes under the action of NADPH (nicotinamide-adenindinukleotide-phosphate oxidase), which transforms the molecular oxygen into the superoxide anion (3, 4). The superoxide anion is transformed into hydrogen peroxide (H_2O_2) under the action of superoxide dizmutase (SOD). The superoxide anion and hydrogen peroxide are precursors in the formation of stronger oxidants. The superoxide anion radical ($O_2^{\cdot-}$) reacts with nitric oxide (NO) and, in that case, toxic nitrogen products, such as peroxynitrite (ONOO-) (nitrosative stress), are formed (3). Hydrogen peroxide (H_2O_2) reacts with intracellular iron (Fe^{2+}) forming the hydroxyl radical (OH^-), and that reaction is known as the Fenton reaction (classical oxidative stress) (3, 4). Within the Haber-Weiss reaction, the hydroxyl radical (OH^-) is also generated by the interaction between the superoxide anion and hydrogen peroxide. Under the action of myeloperoxidase polymorphonuclear leukocytes (MPO), hydrogen peroxide is converted into hypochloric acid ($HOCl^-$) in the presence of chlorine anions (Cl^-). Hypochloric acid can react with endogenous amines ($R\text{-}NH_2$) causing the production of chloramines (RNH-Cl) (chlorinated stress) (3, 4). The natural antioxidant system consists of an enzyme and a non-enzymatic component. Superoxide dismutase (SOD) is a representative of the first line of the antioxidant system**.** It accelerates the degree of superoxide anhydration in hydrogen peroxide. Catalase (CAT) converts hydrogen peroxide into water, and this also works with glutathione peroxidase (GSH-Px), but in the presence of glutathione, a hydrogen source (3). The non-enzymatic components of defense include: vitamin C, vitamin E, N-acetylcysteine, coenzyme Q10 (3, 4).

Oxidative stress induced by dialysis

Patients in the final stage of chronic kidney failure treated with hemodialysis exhibit increased free oxygen radicals levels due to prooxidative factors (age, diabetes mellitus, chronic inflammatory status, uraemia, bioincompatible dialysis membrane, presence of endotoxins in the hemodialysis solution) and the reduced activity of antioxidant mechanisms (lack of vitamin C and selenium, lack of vitamin E, reduced glutathione system activity) (4, 5).

Hemodialysis is itself a trigger for the increased formation of free oxygen radicals. The two major pathophysiological mechanisms for the increased formation of free oxygen radicals during the hemodialysis session are: bionicompatibility of the dialysis membrane and the presence of endotoxin in a hemodialysis solution (4, 5). Dialysis membranes play a central role in the hemodialysis and hemodynamic therapy process. They can be natural and artificial (synthetic). Natural membranes are cellulose derivatives, "low-flux", have low clearance of medium molecular weight uremic toxins and a lower degree of biocompatibility compared to synthetic membranes. Synthetic membranes (polysulphon, polyamide, polyacrylonitrile) are highly permeable ("high-flux"), biocompatible, have good clearance of uremic toxins of medium molecular weight and are highly water-permeable (high coefficient for ultrafiltration - Kuf) (6, 7). The parameter for the evaluation of the efficiency of the dialysis membrane is the coefficient of mass transfer - KoA. It represents the product of the coefficient of transmission (Ko) and the surface of the membrane (A). Depending on KoA dialysers can be: low-efficient dialysers KoA <300, moderately effective dialysers - KoA = 300-600 and high-efficiency dialysers - KoA > 600-700 (6, 7). The ultrafiltration capacity of the dialyser (provides clearance of uremic toxins of medium and high molecular weight) is quantified based on the ultrafiltration coefficient - Kuf. Depending on the ultrafiltration coefficient, the dialysers can be: "low-flux" (Kuf < 10 ml/h x mmHg) and "high-flux" (Kuf > 20 ml/h x mmHg) (6, 7). High-flux semipermeable dialysis membranes are used for on-line hemodialysis, with an ultrafiltration coefficient greater than 20 ml/h x mmHg (≥ 50 ml/h x mmHg, high water permeability and water-soluble secondary molecular weight substances) (6, 7). When the patient's blood is touched by the hemodialysis system, the complement and blood coagulation systems, platelets, mononuclear and polymorphonuclear cells of the immune system are activated, and can also signal hypersensitivity reactions (6, 7). During the hemodialysis session, due to direct contact of the blood and the surface of the membrane for hemodialysis, there is a direct activation of the polymorphonuclear leukocytes, which, due to activated myeloperoxidase (MPO), increase the free acidic radicals' levels [8]. The measurement of myeloperoxidase concentration released from the serum neutrophils during the hemodialysis is an indicator of the severity of oxidative stress induced by the use of membranes for hemodialysis of a different degree of bionicompatibility (8). Liquid that enters the dialyser is a combination of dialysis water and electrolyte solution and is called a dialysis solution (dialysate, dialysis fluid), and the fluid coming out of the dialyser is the combination of dialysis fluid and toxic molecules removed from the patient's blood (9, 10). Water mixed with electrolytic solution, with prior treatment in the water treatment system, is called dialysis water. During standard hemodialysis (3x

weekly for 4h), the patient's organism is exposed to approximately 360 liters of dialysis solution. Therefore, high microbiological quality of the dialysis solution (ultra-pure dialysis solution) is required, and clinical trials show its beneficial effect on the outcome of the treatment of patients (9, 10). According to the European Best Practice Guidelines/European Renal Best Practice, ANSI/AAMI RD52 (American National Standards Institute/Association for the Advancement of Medical Instrumentation RD 52) and ANSI/AAMI/ISO 11663 for the Advancement of Medical Instrumentation ISO 11663), the ultra-pure dialysis solution is defined as a solution in which the number of colonies of bacteria is < 0.1 CFU/mL and the endotoxin concentration is E < 0.03 EU/mL. The ultrafine solution is used for high-flux hemodialysis (HFHD) and hemodynamic filtration (HDF) (9, 10). For hemodialysis with the low-flux membrane (LFHD), according to current recommendations, the concentration of endotoxin should be ≤ 0.50 EU/mL (≤ 0.25 EU/mL) and the number of colonies ≤ 100 CFU/mL (≤ 50 CFU/mL) (9, 10). Endotoxin and other bacterial products, backdiffusion/backfiltration processes, pass from a dialysis solution, through a dialysis membrane (pore size on the dialysis membrane, the ability of the membrane to adsorb endotoxins, the thickness of the membrane) into the patient's blood and activate the mononuclears and polimorfonuclears to produce free oxygen radicals and proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor - TNFα), all of which results in the development of oxidative stress, microinflammatory and accelerated atherosclerosis (9, 10). To detect bacterial products in a hemodialysis solution (dialysis), a biological assay of peripheral blood mononuclear cell induction is used to produce cytokines - PBMC (cytokine induction in peripheral blood mononuclear cells), and for the detection of lipopolysaccharide (LPS) and endotoxin, LAL (Limulus-amebocyte-lysate test) test (9, 10). Ultrapure dialysis solution prevents the development of oxidative stress, microinflamation, slows down the decrease in residual renal function of the kidney, improves the nutritional status of patients, increases the sensitivity of the red blood cell line to the effect of erythropoietin, reduces the cardiovascular morbidity and mortality of patients treated with regular dialysis (9, 10).

In patients treated with regular hemodialysis, the activity of enzymatic and non-enzymatic antioxidative systems is reduced. The decreased activity of antioxidant enzymes (superoxide dizmutase, glutathione peroxidase) is due to reduced concentration of trace elements, such as selenium, copper and zinc. Concentration of trace elements is reduced due to insufficient input, but also increased loss during hemodialysis session (11). Because of the lack of vitamin C and vitamin E, the capacity of non-enzymatic antioxidative protection systems is reduced (11, 12).

Clinical consequences of oxidative stress

The main clinical consequences of oxidative stress include the development and acceleration of the atherosclerosis process, the development of anemia and the resistance to erythropoietin activity, malnutrition and amyloidosis associated with hemodialysis (13). The superoxide anion oxidizes tetrahydrobiopterin (an endogenous cofactor necessary for the activity of nitric oxide (NO) synthetase enzyme) and in this way reduces the production of NO. Nitric oxide is continuously produced in endothelial cells by the action of NO synthetase on L-arginine. It has a protective effect on the cardiovascular system (blocking the proliferation of vascular smooth muscle cells, platelet aggregability and adhesion of monocytes on endothelium). The activity of NO synthetase can be blocked by endogenous methylarginins. Asymmetric dimethylarginine is the most important endogenous blocking agent of the NO synthesis. It is mostly excreted through the kidneys, and partly under the influence of dimethyl-diaminohydrolase (DDAH), it is degraded to citrulline. Oxidative stress blocks the activity of DDAH, which reduces the degradation of asymmetric dimethylarginine, and its accumulation in endothelial cells blocks the nitric oxide synthase, which begins the process of atherosclerosis (13). Increased serum homocysteine concentration is another significant blocker of the activity of dimethyl-diamino-hydrolysis enzyme (DDAH) in endothelial cells of arterial blood vessels. Increased serum homocysteine concentrations are present in 80% of patients treated with regular hemodialysis. It is defined as the concentration of homocysteine in the serum higher than 15 μmol/L and is the result of a reduced activity of the enzymes crucial in the metabolism of homocysteine, such as the 5-methyltetrahydrofolate reductase, methionine synthase, and beta-synthesis of cystation. The decreased activity of these enzymes is due to the decreased concentration of vitamins B6, B12 and folic acid (cofacture of the enzymes mentioned) (14, 15). The lack of vitamin B6 occurs when the concentration of vitamin B6 in the serum is < 20 nmol/L, the lack of vitamin B12 when the concentration of vitamin B12 in the serum is < 200 pg/mL, and the lack of folic acid as the serum folate concentration is < 2.2 mg/mL (14, 15). In healthy population, the normal concentration of ADMA in plasma is 1.0 μmol/L, in hemodialysis patients 2.2 μmol/L, and at the concentrations of 3-15 μmol/L, ADMA blocks the formation of NO in the endothelial cells of the blood vessels and begins the atherosclerosis process (16). In addition to oxidative stress and hyperchomocysteinemia, a significant role in the development of atherosclerosis in patients treated with regular hemodialysis is the role of microinflammation. It is present in 30-50% of these patients and is defined as the concentration of C-reactive protein in the serum ≥ 10 mg/L. A significant role in causing and maintaining chronic low-level microinflammation in this population of patients belongs to bio-compatibility of dialysis membrane, water quality for hemodialysis and vascular approach for hemodialysis (17-19). Microinflammation causes the accumulation of neutrophils and monocytes in the atherosclerotic plaque, and the release of free radicals of oxygen (oxidative stress), cytokines and metalloproteinases can lead to rupture of the atherosclerotic plaque cap and the development of acute coronary events (19, 20).

In 10-30% of patients treated with regular hemodialysis, there is a resistance to erythropoietin activity. According to the European recommendations, the resistance to

erythropoietin activity is defined as the inability to achieve the target hemoglobin concentration in the blood (Hb = 110-120 g/L) using erythropoietin at a dose of ≥ 300 IU/kg/week (≥ 20.000 IU/week) or darbepoetin-a at a dose of ≥ 1.5 μg/kg/week (≥ 100 μg/week) or as a constant need for high doses of erythropoietin in order to maintain the target hemoglobin concentration (21). For the measurement of the severity of resistance to erythropoietin, the erythropoietin resistance index - ERI (Erythropoietin Resistance Index) is used (21). It represents the ratio of weekly dose of erythropoietin depending on body weight and blood hemoglobin (EPO/kg/weekly/Hb). Erythropoietin resistance index ≥ 0.02 μg/kg/week/g of Hb indicates the presence of erythropoietin resistance (21). The main risk factors for the development of resistance to erythropoietin activity are iron deficiency, inflammation, oxidative stress, lack of vitamin D and secondary hyperparathyroidism, lack of vitamin C, vitamin B12, folic acid and L-carnitine, anti-EPO antibodies (21). Iron deficiency, oxidative stress, microinflammation and lack of vitamin C block the proliferation and differentiation of red cell precursor cells, reduce the synthesis of endogenous erythropoietin, stimulate the secretion of hepcidin and the development of a functional iron deficiency (22, 23).

Parameters of oxidative stress in patients with hemodialysis

Free oxygen radicals have a very short half-life (one second), so the clinical evaluation of oxidative stress is measured by measuring stable oxidation products. Oxidative stress parameters include lipid peroxidation products (such as: acrolein, malonyldialdehyde, 4-hydroxynonenal, TBARS, F2-isoprosthenes), lipid oxidation products (oxLDL, anti-oxLDL antibodies), oxidatively altered proteins (final product of protein oxidation - AOPP), final protein glycation products (AGE), evaluation of the activity of antioxidant enzymes (SOD and glutathione peroxidase in erythrocytes), evaluation of non-enzymatic anti-oxidants (plasma vitamin C, glutathione and vitamin E content in erythrocytes) and inflammatory proteins CRP, albumin (23). 8-hydroxy-2'-deoxiguanosine (8-OHdG) is used as the parameter of nucleic acids oxidation, and its concentration in the serum and leukocytes is increased in patients treated with regular hemodialysis (23, 24).

Treatment of oxidative stress in hemodialysis patients

The antioxidant treatment strategy consists of the supplementation with vitamin C, vitamin E (α-tocopherol), selenium, N-acetylcysteine, and coenzyme Q10. Patients treated with regular hemodialysis have a deficiency of vitamin C due to reduced dietary intake (fresh fruits and vegetables in addition to vitamin C also contain significant amounts of potassium) and its elimination during the hemodialysis session (low molecular weight - MW = 176.1 Da, in a small percentage it is bound for plasma proteins - PB = 25%, hydrosoluble vitamin) (24, 25). During the hemodialysis session, 100-300 mg of vitamin C is removed (vitamin C concentration after hemodialysis is reduced by 30-50%) (24, 25). Normal serum vitamin C concentration is 30-60 μmol/L, and patients treated with regular hemodialysis often have a severe lack of vitamin C (vitamin C concentration in the serum < 10 μmol/L) and require the substitution of this vitamin (24, 25). In patients treated with regular hemodialysis, vitamin C is administered per os at a dose of 100-200 mg/day, and can also be applied i.v. at a dose of 300-500 mg after each hemodialysis session over a period of 8-12 weeks, with a defective monitoring for early detection of systemic oxalosis (measurement of serum oxalate concentration required) (24, 25). Intravenous use of vitamin C reduces the concentration of ferritin and proinflammatory mediators in the serum, reduces oxidative stress and resistance to the effect of erythropoietin in patients on regular hemodialysis treatment (24, 25).

Vitamin E (α-tocopherol) has a very strong antioxidant effect. It is administered per os, and the dose of vitamin E can be expressed in international units or milligrams: 100 IU = 67 mg of natural vitamin E (26). When applied in a dose of 400-800 mg/day over a period of 8-12 weeks it significantly reduces the concentration of malodialdehyde, oxLDL and TBARS in the plasma of patients treated with regular hemodialysis. The use of vitamin E reduces oxidative stress, prevents the development and acceleration of atherosclerosis and reduces the corpulence of intima-media of carotid arteries in the population of patients treated with regular hemodialysis (26).

N-acetylcysteine (NAC) increases the production of glutathione, which plays an important role in the function of antioxidant enzymes, such as glutathione peroxidase (GSH-Px). Applied at a dose of 600-1200 mg/day for 3-6 months it significantly reduces the concentration of malondialdehyde (MDA) and asymmetric dimethylarginine (ADMA) in the plasma, reduces the resistance index to the effect of erythropoietin and compensates for the treatment of anemia in patients treated with regular hemodialysis (23, 26).

Coenzyme Q provides homeostasis of mitochondria and reduces oxidative stress (prevents oxidation of lipids, proteins, and nucleic acids). Applied in a dose of 1200-1800 mg daily for 4-6 months, it significantly reduces the concentration of final protein oxidation products (AOPP) and malondialdehyde (MDA) in the plasma of patients treated with regular hemodialysis (26, 27).

The choice of the modality of hemodialysis, the type of dialysis membrane and the type of solution for hemodialysis can significantly reduce oxidative stress, prevent the development of accelerated atherosclerosis, and correct the treatment of anemia in patients treated with hemodialysis (28-41).

On-line hemodiafiltration reduces the resistance to erythropoietin activity. The reduction of resistance to erythropoietin activity results from an increased removal of hepcidin, inflammatory mediators, and lipid, protein and nucleic acid oxidation products during a hemodynamic filtration session. Treatment *on-line* by hemodiafiltration over a period of three to six months significantly reduces inflammation, oxidative stress, serum hepcidin concentration, which increases the

availability of iron for erythropoiesis and reduces the resistance to erythropoietin activity (28, 29).

Vitamin E coated hemodialysis membranes reduce serum lipid peroxidation parameters such as malondialdehyde (MDA), thiobarbutyric acid reactive compounds (TBARS) and oxidized LDL cholesterol (oxLDL). Studies have shown that these membranes also reduce the concentration of oxidative nucleic acid parameters, such as 8-OHdG, as well as the concentration of microinflammatory parameters (CRP, interleukin-6) (30-37). Vitamin E coated dialysis membranes reduce the content of 8-OHdG in leukocytes in patients treated with regular hemodialysis (reduces DNA leukocyte oxidation). These membranes provide good control of the function of leukocytes, exhibit an antioxidant and antiinflammatory effect (30-37). The treatment of high-flux hemodialysis with polysulphonic membrane-bound vitamin E over a period of six months significantly reduces oxidative stress, microinflammation, an erythropoietin resistance index and corrects the treatment of anemia in patients treated with regular hemodialysis (30-37).

The treatment of anemia in patients on hemodialysis involves the use of erythropoiesis (ESA) stimulating agents and intravenous iron (iron sucrose). After the administration of intravenous iron at a dose of 100 mg (within 15-30 minutes of infusion), the concentration of free oxygen radicals in these patients significantly increased (38-41). In order to prevent the development of oxidative stress after i.v., Ferrous Pyrophosphate Citrate (FPC) for adult patients treated with hemodialysis, administered through a solution for hemodialysis, was approved by the US Food and Drug Administration in 2015 (38-41). One 5 ml FPC (TrifericTM) ampoule is added to every 2.5 gallons of bicarbonate concentrate so that the final FPC concentration in the hemodialysis solution is 110 μg/L (2.0 μmol/L). FPC is used in every hemodialysis treatment, and the serum ferritin concentration and iron transfer (TSAT) saturation should be measured every three months. In patients with a serum ferritin concentration greater than 1000 ng/mL, and TSAT greater than 50% should be discontinued, FCD should be used, standard bicarbonate solution for hemodialysis should be used. In patients whose serum ferritin concentration is less than 200 ng/mL, i.v. iron 400-500 mg, during the next 4-5 hemodialysis treatments (100 mg/HD), and the FPC should be continuously applied. When the target serum ferritin concentration and saturated transfer of iron transfer is achieved, FPC should be applied continuously because 5-7 mg of iron (maintenance of target values of ferritin and TSAT) is lost during each hemodialysis session (38-41). Iron application through hemodialysis solution reduces oxidative stress and reduces the resistance to erythropoietin (dose of erythropoietin decreases by 35%) (38-41).

CONCLUSION

Cardiovascular diseases are the leading cause of patients with chronic kidney disease treated with kidney replacement methods. In this patient population, there is a high prevalence of traditional but also new non-traditional risk factors for the development of cardiovascular diseases. Oxidative stress is a significant non-traditional risk factor for the progression of chronic kidney disease and the development of cardiovascular diseases in the population of patients on regular hemodialysis treatment. The main clinical effects of oxidative stress are: atherosclerosis, amyloidosis associated with hemodialysis, resistance to erythropoietin activity and malnutrition. The antioxidant treatment strategy consists of replenishing vitamin C, vitamin E, selenium, N-acetylcysteine and coenzyme Q10. *On-line* hemodialysis, a biocompatible vitamin E-coated dialysis membrane and an ultra-pure solution for hemodialysis prevent oxidative stress. Early detection of oxidative stress and timely application of appropriate antioxidant therapy can prevent the development of cardiovascular diseases, reduce the rate of cardiovascular morbidity and mortality, and improve the life quality of patients treated with renal replacement methods.

ACKNOWLEDGMENTS

Authors would like to express their deepest gratitude to the Ministry of Education, Science and Technological Development of the Republic of Serbia for the Grant N0175014 and also to the Faculty of Medical Sciences University of Kragujevac for their Junior Grant N002/19 from which the funds were used as one of the sources to financially support this paper.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

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ONLINE TEACHING AND LEARNING IN BIOMEDICAL SCIENCES: STUDENTS' AND STAFF' ATTITUDES AND EXPERIENCES ON THE NEW EDUCATIONAL ENVIRONMENT IMPOSED BY THE COVID 19 OUTBREAK

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Received: 05.10.2020.
Accepted: 06.03.2021.

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ABSTRACT

The aim of this pilot study is a preliminary evaluation of previous models / modalities of online teaching at the Faculty of Medical Sciences in Serbia and to examine the attitudes of students and academic staff about education during the COVID-19 pandemic, as well as their previous experiences. The research was designed as an observational qualitative epidemiological study which was conducted on a population of students and academics staff at the Faculty of biomedical sciences, University of Kragujevac during the pandemic of SARS-CoV-2 infection in Serbia. The first phase is a pilot study which included 332 participants performed between December 2020 and January 2021. The pilot study questionnaire is formed for the purposes of the research and consists of 17 closed-ended questions with graduated answers. Students and academic staff completed the questionnaire through an online learning platform in all environments and from all electronic devices. The importance of this study is reflected in the fact that it provides detailed and valid data that can serve the purpose of improving the efficiency of online teaching at the faculties of medical sciences in Serbia In general, the results of our study indicate that in addition to great inexperience, both students and academic staff cope well during online education and the changed environment and learning conditions despite all the difficulties.

Keywords: *Online education, medical education, COVID-19, attitudes, experiences.*

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UDK: 37.018.43:077]:61-057.875
UDK: 616.98:578.834
Eabr 2024; 25(3):207-217 DOI:
10.2478/sjecr-2021-0041

INTRODUCTION

The outbreak of the SARS-CoV-2 virus pandemic declared by the World Health Organization in early 2020 affected the health system, the economy, education, and other aspects of life (1). The first case of SARS-CoV-2 infection was registered in the Republic Serbia on March 6, 2020, and after only 9 days, due to the increase in the number of infected, the Government of the Republic of Serbia declared a state of emergency. The declaration of a state of emergency has led to a number of restrictive measures, including the closure of state borders, restrictions on the movement of citizens and the suspension of all educational institutions indefinitely (2).

Due to the introduction of such measures, the teaching process at all educational levels has reoriented from the classical model of teaching to different types of online teaching. Accordingly, the Faculty of Medical Sciences of the University of Kragujevac has adapted the teaching process to the current epidemiological situation and an online platform has been formed. The platform for online teaching (studije.fmn.rs) was created with the aim of providing quality support to students but also to teachers and associates during teaching activities with the possibility of checking students' knowledge and testing them at a distance. Students of all study programs of the Faculty of Medical Sciences, as well as teachers and associates had the opportunity to access the teaching content on the platform by creating a user account. Other medical faculties in Serbia have also, in various technical and organizational formats, introduced online teaching during the epidemic.

Due to the transition from the classic to the online way of student education and teacher work, the quality and efficiency of this form of teaching have become the subject of research. An additional challenge is the specificity of the teaching process at the faculties of health care, as well as insufficient previous experience of both students and teachers. Currently, there are only a few publications that assess the impact of the pandemic and its consequences on the educational process in the field of medical sciences, especially in terms of the attitudes and experiences of students and teachers. Previous problems that students have encountered in relation to teaching online methods described in the literature are difficult communication and inability to discuss, inadequate assessment of student knowledge, difficult use of technological tools necessary for this type of education, time management and anxiety and stress due to quarantine and isolation (3, 4). As the most significant shortcoming of this model of education, students state the impossibility of replacing it with classical education, which enables direct contact of the student with the patient. Also, students' attitude is that they cannot learn practical skills through online education (5). The advantages that students notice are reduced costs during their studies and time savings. Also, as positive aspects, they mention the availability and quick access to learning materials, safety and reduced risk of infection. Previous research has shown that students have a positive attitude towards online teaching and believe that it should be in addition to the classical form of education (4). The literature states that more attention should be paid to practical and individual learning needs of students during online education in medical sciences. and clinical practice (6).

By analyzing the advantages and disadvantages of the online learning model that students and teachers of the faculties of medical sciences encounter, this type of teaching can be improved. It is necessary to consider and further adapt this form of education to the needs of all participants in the teaching process due to the tendency to repeat pandemics over time and the inability to predict their scale. Also, in the long run, a mix model of on-site online teaching (hybrid learning) seems to be suitable for teaching in medical and health sciences (7, 8).

The aim of this pilot study is a preliminary evaluation of previous models / modalities of online teaching at the Faculty of Medical Sciences in Serbia and to examine the attitudes of students and academic staff about education during the COVID-19 pandemic, as well as their previous experiences.

PARTICIPANTS AND METHODS

Ethical concerns

Participation in the survey was voluntary, and completely anonymous, identification data of the technical approach of survey participants are available only to in accordance with the standard operating procedure of the Faculty of Medical Sciences, University of Kragujevac, Serbia. The conduct of the study is in line with the principles of the Declaration of Helsinki (revision 2013) and the protocol of the study was approved by institutional ethics committee Faculty of Medical Sciences (Number 01-3491).

Participants and protocol of study

The research was designed as an observational qualitative epidemiological study which was conducted on a population of students and academics staff at the Faculty of biomedical sciences, University of Kragujevac during the pandemic of SARS-CoV-2 infection in Serbia. The first phase is a pilot study which included 332 participants performed between December 2020 and January 2021. The second phase at the national level is in progress. The main including criteria for participants were the following: undergraduate or vocational or postgraduate student or teacher/associate of all faculties of medical sciences in Serbia, the existence of the participant's user account on the appropriate online platform or appropriate online approach and voluntary participation. No special exclusion criteria were defined.

Based on the results of the analysis in the first phase, the redesign / improvement of the survey questionnaire will be approached in order to improve its validity and feasibility. Thus, the new format of the survey questionnaire-assessment

instrument will be submitted again to the Ethics Committee for consideration, and before the implementation of the second phase, in the form of an Amendment to the Protocol. The second phase of testing will be conducted according to the same principles as the pilot study, and upon approval of the protocol amendments. It is planned that in the second phase, the participants will be students, teachers and associates of medical faculties in Kragujevac, Belgrade, Novi Sad, Nis and Pristina-Kosovska Mitrovica, as well as the Faculty of Pharmacy and the Faculty of Dentistry of the University of Belgrade. The implementation of the study in this phase will be coordinated by the Association of Medical Faculties of Serbia, through coordinators appointed by each faculty that accepts to participate in the study, with the main coordinator-manager being a representative of the Faculty of Medical Sciences, University of Kragujevac.

Instrument

The pilot study questionnaire is formed for the purposes of the research and consists of 17 closed-ended questions with graduated answers. Students and academic staff completed the questionnaire through an online learning platform in all environments and from all electronic devices.

The first three questions relate to the socio-demographic characteristics of the participants such as gender, age and academic status. Other questions were formed based on the existing published studies with the same or similar topic. The answers to each of the 14 questions were ranked on a five-point Likert scale (9), where respondents answered by choosing one of the 5 answers offered (I very much support /I support; I do not support; dispute I do not support / I do not support very much; or There are very large ones; There are big ones; There are moderates; There are small ones or They do not exist at all). The questionnaire was modified according to similar research methods (3, 4). In the second phase of the study, a redesigned / improved questionnaire will be used, according to the results of this pilot study.

Before completing the survey, the participants will be explained the purpose of the research and given brief instructions in text form. The technical format and content of the informed consent of the respondents with full information was adjusted to the online environment, in accordance with the relevant recommendations (7).

Statistical analysis

The data were analyzed using IBM SPSS version 22.0. All categorical variables were expressed in frequencies (N) and percentages (%). The $\chi 2$ goodness of fit test was used to determine the differences in the distribution of the participant responses. Differences between the groups were analyzed with the $\chi 2$ test of independence or by Fisher's exact test if assumptions for the $\chi 2$ test were not met. The data were presented in tabular and graphical form. A p-value less than 0.05 was considered to be a measure of statistical significance for all statistical tests used.

The study sample was calculated according to the assumption of a margin error of 5% and confidence interval of 95%. The total estimated available sample of participants at the Faculty of Medical Sciences, University of Kragujevac was 2700 and the response rate was estimated at 30%. Using the online Raosoft sample size calculator (10), a sample of 289 respondents was calculated, which was rounded up to at least 300 participants.

RESULTS

Basic characteristic of study population

This pilot study included 332 participants from Faculty of Medical Sciences, University of Kragujevac in Serbia who filled study online questionnaire between December 2020 and January 2021. Study population consisted of total 332 participants, of which 302 were students (91.96%) and 30 academic staff (9.04%). In relation to gender, of the total number of participants 76.8 % were female and 23.2 % male (χ^2=95.434; df=1; p=0.000). All participant were divided into three categories according to years old: below 19 years old (46 (13.9%)), 20-29 (260 (78.3)) and above 30 years old (26 (7.8%)).

Table 1 shows the distribution of study participants in relation to gender, but also gender representation in general in the student and teaching population of the Faculty of Medical Sciences in January 2021 which represent a similar gender-distribution in study population to the official student-academic staff population at the Faculty of Medical Sciences in Kragujevac.

Table 1. Distribution of male and female participants in study population and in total student and academic population at the Faculty of Medical Sciences.

	Total number	Female (%)	Male (%)
Study population	332	255 (76.8)	77 (23.2)
Students at the Faculty of Medical Sciences	1572	1181 (75.1)	391 (24.9)
Academic staff at the Faculty of Medical Sciences	293	165 (56.3)	128 (43.7)

Results are presented as frequency in percent from total number of selected participants.

Previous experiences of the study population with online learning

In terms of previous experience with online education in study population, the answers were expected. Figures 1-3 show distribution of response in study population. On question (Q4): have you had previous experiences with online education? Most of participants responded that they had a little or no experience. Female participants answered that they had

no experience or very little in as many as 71.8%, while male respondents gave the same answer in 67.5% (Figure 1). On the other hand, students denied the presence of previous experience in 72.2% and academic staff in 56.7% (Figure 2). In general, the majority of the study population had no experience or had very little (70.8%) and only a small number of participants had extensive previous experience in viewing online education (11.7%) (Figure 3). Using Chi squared test, we not confirmed statistically significant differences between distribution of response in relation to gender (χ^2=0.513; p=0.774) nether in relation to academic status (χ^2=3.990; p=0.125).

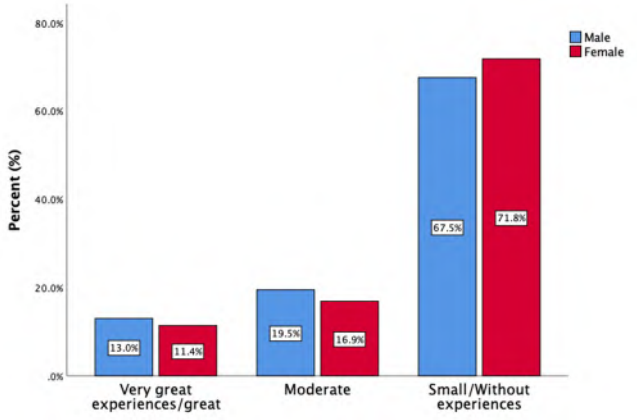

Figure 1. Distribution of responses on Question (Q4): Have you had previous experiences with online education? in relation to gender in study population. The answers are categorized into the following categories: Very great experiences/great, Moderate experiences and Small/without experiences. Results are presented as frequency in percent (%).

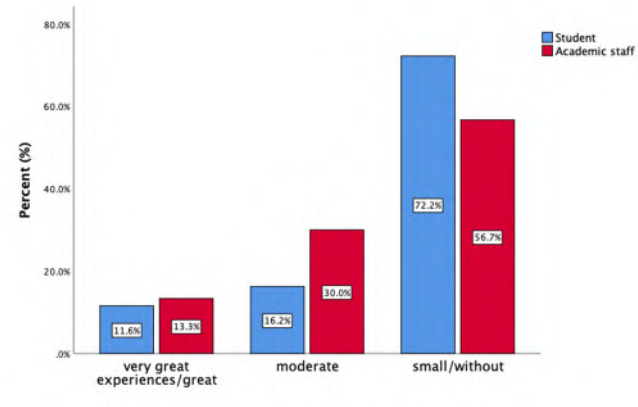

Figure 2. Distribution of responses on Question (Q4): Have you had previous experiences with online education? in relation academic status in study population. The answers are categorized into the following categories: Very great experiences/great, Moderate experiences and Small/without experiences. Results are presented as frequency in percent (%).

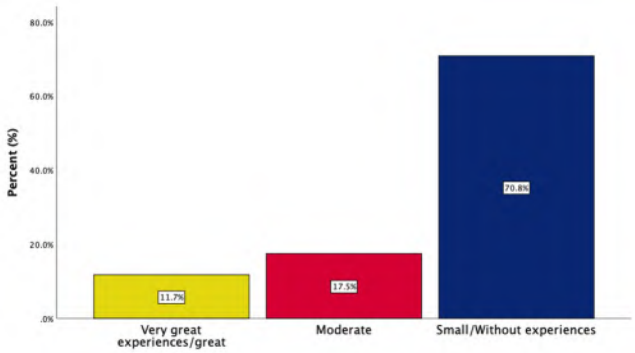

Figure 3. Distribution of responses on Question (Q4): Have you had previous experiences with online education? in study population in general. The answers are categorized into the following categories: Very great experiences/great, Moderate experiences and Small/without experiences. Results are presented as frequency in percent (%).

Attitudes and opinions during online teaching in study population in COVID-19 pandemic

In the second part of study, we evaluated the attitudes on online education with direct, online or combining contact of the male and female students and academic staff. We have founded that almost all responses on Q5, Q6 and Q7 were significantly different in relation to academic status, but only on Q7 in relation to gender of participants (Table 2). Interestingly,

The majority of respondents largely supported the combined way of learning (50% and 86.7%), as well as online learning in student population (56%). On the other hand, the attitude towards learning only through direct contact was divided among students and academic workers with similar distribution of support for each type of education. In relation to gender, in almost all questions there were no statistically significant differences between response and attitudes on online education with direct, online or combining contact of male and female participants. Interestingly, students (56.0%) and female participants (58.0%) are more supportive of online learning than other participants, which is to be expected, given the larger number of female participants in the study population (Table 2).

Table 2. Distribution of male and female participants and in relation to academic status in responses about attitudes on online education. Results are presented as frequency in percent from total number of selected participants.

Variables	**Students (n=302) (%)**	Academic staff **(n=30) (%)**	χ^2/p	**Male participants (n=77) (%)**	Female participants **(n=255) (%)**	χ^2/p
(Q5) **Attitude on combining online teaching with direct contact teaching ***						
I very much support / I support	151 (50.0)	26 (86.7)	**14.814/ 0.001**	42 (54.5)	135 (52.9)	0.071/ 0.965
I do not support or dispute	70 (23.2)	2 (6.7)		16 (20.8)	56 (22.0)	
I do not support / I do not support very much	81 (26.8)	2 (6.7)		19 (24.7)	64 (25.1)	
(Q6) **Attitude about teaching only in direct contact**						
I very much support / I support	105 (34.8)	15 (50.0)	5.971/ 0.055	29 (37.7)	91 (35.7)	4.333/ 0.115
I do not support or dispute	70 (23.2)	9 (30.0)		24 (31.2)	55 (21.6)	
I do not support / I do not support very much	127 (42.1)	6 (20.0)		24 (31.2)	109 (42.7)	
(Q7) **Attitude about teaching only online**						
I very much support / I support	169 (56.0)	10 (33.3)	**7.379/ 0.027**	31 (40.3)	148 (58.0)	**8.316/ 0.016**
I do not support or dispute	49 (16.2)	10 (33.3)		20 (26.0)	39 (15.3)	
I do not support / I do not support very much	84 (27.8)	10 (33.3)		26 (33.8)	68 (26.7)	

Difficulties and problems during online education in study population in COVID-19 pandemic

Furthermore, questions from Q8 to Q16 are relate to difficulties during the duration of online education and concern various aspects. All responses from study population are shown in the form of Table 3. The main differences were observed in testing knowledge, gaining of professional experience, assessing the quality of study during online teaching and other difficulties ($p<0.05$). A large number of students and academic staff reported no or no difficulties at all in testing the knowledge during online education. Then, most of students reported that gaining professional experience during online classes was went with large difficulties but not for academic staff in the same time. Also, most of the student population reported that in assessing the quality of study during online teaching were present some difficulties.

On the other hand, participants reported that difficulties regarding the communication during classes, technical and psychological aspects, organizing time as well as the mastering new health technologies were equally present in student and academic staff or in male and female participants ($p>0.05$) (Table 3).

In relation to gender, all responses on Q8-Q16 are similarly distributed in male and female participants ($p>0.05$) (Table 3).

Table 3. Distribution of male and female participants and in relation to academic status in responses about difficulties during online education.

Variables	**Students (n=302) (%)**	Academic staff **(n=30) (%)**	χ^2/p	**Male partic-ipants (n=77) (%)**	Female partici-pants **(n=255) (%)**	χ^2/p
(Q8) **Difficulties in communication during online classes ***						
There are very large ones	16 (5.3)	0 (0.0)	5.178/ 0.242	40 (5.2)	12 (4.7)	6.371/ 0.173
There are big ones	29 (9.6)	1 (3.3)		10 (13.0)	20 (7.8)	
There are moderates	96 (31.8)	15 (50.0)		30 (39.0)	81 (31.8)	
There are small ones	84 (27.8)	9 (30.0)		21 (27.3)	72 (28.2)	
They do not exist at all	77 (25.5)	5 (16.7)		12 (15.6)	70 (27.5)	
(Q9) **Difficulties in testing knowledge during online classes ***						
There are very large ones	30 (9.9)	2 (6.7)	**10.352/ 0.027**	10 (13.0)	22 (8.6)	6.371/ 0.173
There are big ones	32 (10.6)	5 (16.7)		8 (10.4)	29 (11.4)	
There are moderates	63 (20.9)	10 (33.3)		18 (23.4)	55 (21.6)	
There are small ones	72 (23.8)	10 (33.3)		24 (31.2)	58 (22.7)	
They do not exist at all	105 (34.8)	3 (10.0)		17 (22.1)	91 (35.7)	
(Q10) **Difficulties in technical aspects during online teaching ***						
There are very large ones	15 (5.0)	0 (0.0)	2.912/ 0.556	4 (5.2)	11 (4.3)	1.120/ 0.905
There are big ones	16 (5.3)	0 (0.0)		2 (2.6)	14 (5.5)	
There are moderates	84 (27.8)	8 (26.7)		22 (28.6)	70 (27.5)	
There are small ones	114 (37.7)	12 (40.0)		30 (39.0)	96 (37.6)	
They do not exist at all	73 (24.2)	10 (33.3)		19 (24.7)	64 (25.1)	
(Q11) **Difficulties in gaining professional experience during online classes ***						
There are very large ones	94 (31.1)	4 (13.3)	**11.809/ 0.015**	24 (31.2)	74 (29.0)	7.405/ 0.116
There are big ones	63 (20.9)	10 (33.3)		24 (31.2)	49 (19.2)	
There are moderates	50 (16.6)	11 (36.7)		12 (15.6)	49 (19.2)	
There are small ones	50 (16.6)	3 (10.0)		11 (14.3)	42 (16.5)	
They do not exist at all	45 (14.9)	2 (6.7)		6 (7.8)	41 (16.1)	

Variables	**Students (n=302) (%)**	Academic staff **(n=30) (%)**	χ^2/p	**Male partic-ipants (n=77) (%)**	Female partici-pants **(n=255) (%)**	χ^2/p
(Q12) **Difficulties in psychological aspects during online teaching ***						
There are very large ones	32 (10.6)	0 (0.0)	7.348/ 0.096	5 (6.5)	27 (10.6)	2.374/ 0.676
There are big ones	20 (6.6)	0 (0.0)		4 (5.2)	16 (6.3)	
There are moderates	48 (15.9)	6 (20.0)		10 (13.0)	44 (17.3)	
There are small ones	70 (23.2)	11 (36.7)		21 (27.3)	60 (23.5)	
They do not exist at all	132 (43.7)	13 (43.3)		37 (48.1)	108 (42.4)	
(Q13) **Difficulties in mastering new health technologies during online teaching ***						
There are very large ones	47 (15.6)	2 (6.7)	5.000/ 0.275	12 (15.6)	37 (14.5)	4.648/ 0.325
There are big ones	40 (13.2)	8 (26.7)		10 (13.0)	38 (14.9)	
There are moderates	67 (22.2)	7 (23.3)		23 (29.9)	51 (20.0)	
There are small ones	66 (21.9)	7 (23.3)		17 (22.1)	56 (22.0)	
They do not exist at all	82 (27.2)	6 (20.)		15 (19.5)	73 (28.6)	
(Q14) **Difficulties in organizing time during online classes ***						
There are very large ones	17 (5.6)	0 (0.0)	5.139/ 0.230	4 (5.2)	13 (5.1)	5.448/ 0.239
There are big ones	12 (4.0)	2 (6.7)		6 (7.8)	8 (3.1)	
There are moderates	35 (11.6)	5 (16.7)		8 (10.4)	32 (12.5)	
There are small ones	67 (22.2)	10 (33.3)		22 (28.6)	55 (21.6)	
They do not exist at all	171 (56.6)	13 (43.3)		37 (48.1)	147 (57.6)	
(Q15) **Difficulties in assessing the quality of study during online teaching ***						
There are very large ones	45 (14.9)	1 (3.3)	**11.139/ 0.020**	12 (15.6)	34 (13.3)	7.948/ 0.093
There are big ones	47 (15.6)	7 (23.3)		12 (15.6)	42 (16.5)	
There are moderates	68 (22.5)	13 (43.3)		27 (35.1)	54 (21.2)	
There are small ones	63 (20.9)	6 (20.0)		12 (15.6)	57 (22.4)	
They do not exist at all	79 (26.2)	3 (10.0)		14 (18.2)	68 (26.7)	

Variables	**Students (n=302) (%)**	Academic staff **(n=30) (%)**	χ^2/p	**Male partic-ipants (n=77) (%)**	Female partici-pants **(n=255) (%)**	χ^2/p
(Q16) **Other difficulties during online classes ***						
There are very large ones	20 (6.6)	0 (0.0)		8 (10.4)	12 (4.7)	
There are big ones	16 (5.3)	2 (6.7)		3 (3.9)	15 (5.9)	
There are moderates	67 (22.2)	16 (53.3)	**13.409/ 0.006**	21 (27.3)	62 (24.3)	9.438/ 0.047
There are small ones	101 (33.4)	7 (23.33)		30 (39.0)	78 (30.6)	
They do not exist at all	98 (32.5)	5 (16.7)		15 (19.5)	88 (34.5)	

Results are presented as frequency in percent from total number of selected participants.

Future intention of study population regarding the online education

By filling in the questionnaire according to the principle of self-evaluation of the participants, we followed the intentions of students and academic staff to implement the acquired knowledge, skills, and attitudes into professional practice (Q17) (Figs. 4-6). A large number of students (8.630; p=0.055) and female participants (8.707; p=0.069) reported that there was a very large or large intention for the future intentions but statistically insignificant. In general, more than half of all participants said yes to future intentions (Figure 6).

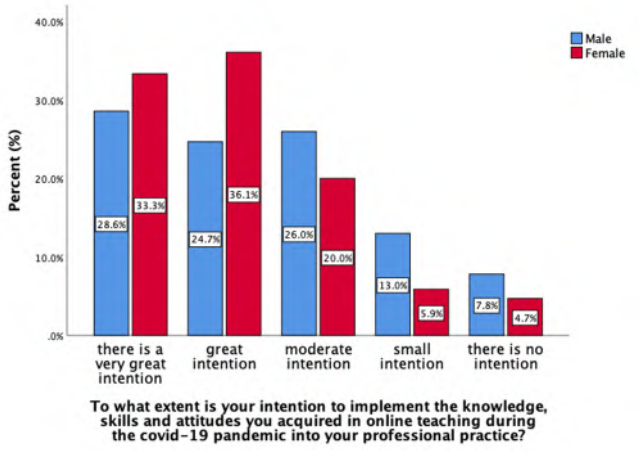

Figure 4. Distribution of responses on Question (Q17): Intention to implement the acquired knowledge, skills, and attitudes into professional practice, in relation to gender in study population. The answers are categorized into the following categories: Very great intention, Great intention, Moderate intention, Small intention and without intention. Results are presented as frequency in percent (%).

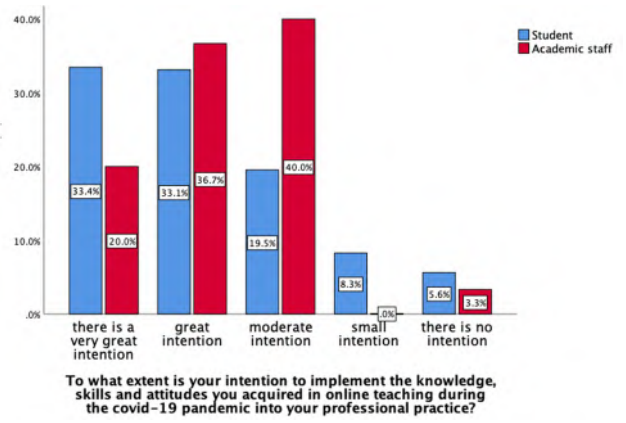

Figure 5. Distribution of responses on Question (Q17): Intention to implement the acquired knowledge, skills, and attitudes into professional practice, in relation to academic status in study population. The answers are categorized into the following categories: Very great intention, Great intention, Moderate intention, Small intention and without intention. Results are presented as frequency in percent (%).

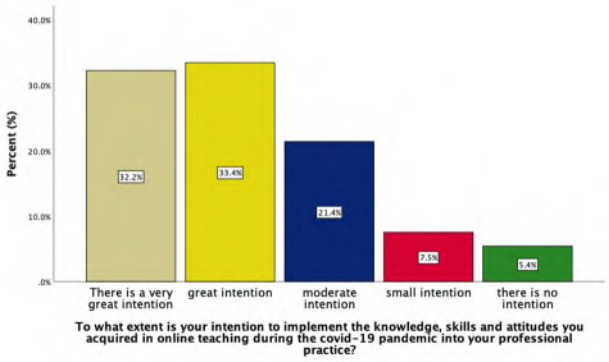

Figure 6. Distribution of responses on Question (Q17): Intention to implement the acquired knowledge, skills, and attitudes into professional practice, in study population at all. The answers are categorized into the following categories: Very great intention, Great intention, Moderate intention, Small intention and without intention. Results are presented as frequency in percent (%).

DISCUSSION

The importance of this study is reflected in the fact that it provides detailed and valid data that can serve the purpose of improving the efficiency of online teaching at the faculties of medical sciences in Serbia. In this way, you will gain insight into previous experiences in educating students during the COVID-19 pandemic. Also, their attitudes and perceived difficulties can serve as a guide for improving the quality of online education in a specific field such as medical sciences (11, 12).

This unique study indicated the attitudes and experiences of students and teachers about the current model of education at the faculties of medical sciences during the COVID-19 pandemic. Also, provided the analysis of the obtained research results which shows what difficulties students and teachers encounter during the teaching process in order to take them into account when improving the existing model. The results will be used as a basis for recommendations for improving teaching as well as teaching competencies in the field of medical and health sciences (13-17). Beside that, using the Likert scale as a technique for the measurement of attitudes we are sure that obtained results are good instrument (9).

Our study collected online responses from a large number of participants students and academic staff and provided interesting results. The majority of respondents largely supported the combined way of learning (50% and 86.7%), as well as online learning in student population (56%). On the other hand, the attitude towards learning only through direct contact was divided among students and academic workers with similar distribution of support for each type of education. That means that academic staff is very despite the sudden changes in the way of education, it can be said that the academic staff was well prepared and therefore had an attitude that supports all ways of education. On the other hand, the student population, as the most promising part of society, mostly supported modern technologies and thus online learning methods (Table 2).

Furthermore, a large number of students and academic staff reported no or no difficulties at all in testing the knowledge during online education. Then, most of students reported that gaining professional experience during online classes was went with large difficulties but not for academic staff in the same time. Also, most of the student population reported that in assessing the quality of study during online teaching were present some difficulties.

On the other hand, participants reported that difficulties regarding the communication during classes, technical and psychological aspects, organizing time as well as the mastering new health technologies were equally present in student and academic staff or in male and female participants ($p>0.05$) (Table 3).

This study highlighted that faculty members, especially students, were generally unsure if they are in favor of online education (18). Ambivalence during the change process is an expected response during the transition. According to Kurt Lewin's 3 Stage Change Model, transition during change is typically accompanied by feelings of hesitation and confusion (18, 19). The ambivalent attitude of faculty may possibly be due to the fact that while faculty seem to have concerns about online teaching and learning to include but not limited to depersonalize instruction and proliferation of academic dishonesty, faculty are left with less options as they are required to adopt the new normal of education (19).

Currently, there is a little literature data about the attitudes and experiences of students and academic staff in the field of biomedical sciences on the online education during the COVID-19 pandemic.

One of the previous studies was conducted in the North Macedonia. A significant number of teachers in North Macedonia disagreed with changing the traditional teaching method with e-learning (20).

Another study also included institutional, interpersonal, training and technology, and cost/benefit analysis barriers to online education (21). The finding of this research suggests that problems and challenges associated with online education must be addressed, and online courses must be carefully planned and regulated (21, 22).

Unger Sema and coworkers examined responses of the undergraduate student attitudes towards rapidly shifting to an entirely online learning environment were assessed due to COVID-19 in Pakistan. Also, they have founded that many students (75.6%) responded they held some level of anxiety towards rapidly shifting to finishing a semester online, with 84.2% having discussed disease transmission actively and yet only 64.6% felt well prepared for emergency situations (23).

Also, in Cyprus, *Umut Akcil et al* tested by establishing a relationship between digital citizenship and e-learning. The

study was conducted among higher education students. It has been observed that there is a positive relationship between digital citizenship behaviors and e-learning attitudes. In addition, it has been observed that the negative anxiety of students due to the pandemic is reflected in their e-learning processes. However, overall results show that digital citizenship behavior digital learning process could be a positive response to COVID-19 closure period (24).

In Poland, Michal Baczek et al examined the students' perception of online learning during the COVID-19 pandemic. Eight hundred four students answered the questionnaire. According to respondents' answers, the main advantages of online learning were the ability to stay at home (69%), continuous access to online materials (69%), learning at your own pace (64%), and comfortable surroundings (54%). The majority of respondents chose lack of interactions with patients (70%) and technical problems with IT equipment (54%) as the main disadvantages (25).

In Indonesia was evaluated the teachers' attitude towards online learning during Covid-19 pandemic. There were 71 teachers with different demographic background from different state and private education levels in several parts of Indonesia voluntarily participating the self-administered survey using online google form. The results showed that 64.8% of the institution the teachers worked provided online facilities, but 73.2% of the respondent still used their own addition media. 52.1% of the respondent did not feel have problem with online learning, but 23.9% were happy teaching in online mode. Moreover, 22.5% respondent agreed and 16.9% strongly agreed that online teaching must be implemented in addition to conventional mode after COVID-19 pandemic is over (26).

All these studies, as well as our study, confirmed that online education is a powerful tool for teaching medical students and useful tool education for academic staff. However, successful implementation of online learning into the curriculum requires a well thought-out strategy and a more active approach (27).

In general, the results of our study also indicate that in addition to great inexperience, both students and academic staff cope well during online education and the changed environment and learning conditions despite all the difficulties.

Our study has a little limitations and further huge researches are necessary to confirm these results. First, results were obtained by online survey, and second, response rate could be limited by this way of research. Although the scope of the questionnaire is limited to fourteen questions, and the number of the respondents represents a small group appropriate for a pilot study, the analysis of the answers the respondents wrote yields some consistent patterns which are repetitive throughout the entire corpus, highlighting some important aspects related to the students' attitudes towards the nature of online education. Anyway, the outcome of this study can be used as an input in the future educational and research plans of the faculties of biomedical sciences.

CONCLUSION

In summary, the lack of past experience on using online tools and education, were identified by students as the main barrier to online educations but ability to adapt to modern technologies as a characteristic of students as a vital part of society were identified as main advantage of online teaching model in biomedical sciences.

Although the pandemic of COVID-19 appeared as uncommon catalyst for promoting online education, further research is needed to assess whether learners are ready and willing to make greater use of online education to obtain high quality teaching and learning opportunities, which could totally change educators' and students' attitudes and impression, and subsequently the general themes of online education.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTERESTS

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

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THERAPEUTIC OPTIONS AND PROGNOSTIC FACTORS IN TREATMENT OF ANAPLASTIC GLIOMAS

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Received: 07.06.2021.
Accepted: 10.09.2021.

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ABSTRACT

Anaplastic gliomas compromise about 5.9% of primary CNS tumors. The main goal of the operation is the maximum removal of the tumor, reduction of the tumor mass and reduction of the increased intracranial pressure. Different pathohistological subtypes of anaplastic gliomas show significantly different prognosis depending on the applied oncological therapeutic protocol as well as the modality of the applied radiotherapy. The study was designed as a retrospective, clinical observational study. The study included 34 participants who were diagnosed with anaplastic glioma in the followed time period. Survival rates were calculated based on the localization, modality of therapy and complications. We concluded that 20,4% of anaplastic gliomas were formed by transformation from previously operated lower grade gliomas. The initial sign of the disease is the appearance of epileptic seizures. Anaplastic gliomas most oftenly occur in the frontal region, with a frequency of 47%. The incidence of anaplastic gliomas in the temporal lobe is 23,5%. The length of survival is in relation to the localization of tumor expansion($p<0.05$). The overall survival in the group of anaplastic gliomas operated on in the Department of Neurooncology KCS in the follow-up period of five years is 52.9%. The application of different chemotherapy modalities is not significant predictor in the length of survival. The radical nature of the operation has significance in the length of patient survival, which confirms the conclusions of most of the conducted studies cited in oncology textbooks.

Keywords: *Anaplastic glioma, surgical treatment, survival rate, neurooncology.*

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UDK: 616.831-006
Eabr 2024; 25(3):219-224
DOI: 10.2478/sjecr-2021-0043

INTRODUCTION

Classification and treatment of anaplastic gliomas (WHO gr III) is the subject of numerous debates and discrepancies in the field of neurooncology. [1] The incidence and prevalence of brain tumors has been inaccurately documented for a long period of time, no benign tumors or metastatic diseases were reported before the beginning of 2003. A database from the U.S. Central Brain Tumor Registry from 2006 to 2010 indicates that the incidence of brain tumors in children aged 0 to 19 was 5.26 per 100,000, and 27.38 per 100,000 for adults. [2] Secondary brain tumors are, depending on the literature data, three to ten times more common than primary tumors. The classification of brain tumors lists about one hundred subtypes of CNS malignancies. As testing for genetic disorders is routinely used in diagnostic procedures for gliomas, clinicians and pathologists also encounter certain practical problems, such as conflicting outcomes due to different interpretations of genetic alterations, this segment may be a somewhat variable part of diagnostic data. Within the classification in which "genotype dominates over phenotype", the accuracy of molecular tests is crucial, which requires adequate knowledge of the pathologist.[3] Gliomas account for 80% of primary malignant brain tumors [4] The group of anaplastic gliomas includes astrocytomas, oligodendrogliomas, oligoastrocytomas, pleomorphic xanthoastrocytomas. Anaplastic gliomas compromise about 5.9% of primary CNS tumors. [5]

Therapy involves multimodal therapy that includes maximal surgical reduction, followed by chemotherapy and radiotherapy. The main goal of the operation is the maximum removal of the tumor, reduction of the tumor mass and reduction of the increased intracranial pressure, as well as providing the tissue for pathological analysis in a way that minimizes the risk of neurological deficit. Anaplastic gliomas are infiltrative tumors, which makes complete surgical resection virtually impossible. Nevertheless, the operation achieves the effect of reducing the mass effect, edema and the potential for development of hydrocephalus. Retrospective evidence from the literature indicates that better treatment outcomes are associated with radical surgery, because cells that are out of the cell cycle and thus resistant to chemotherapy and radiotherapy are removed. Cytoreduction initiates the proliferation of G0 tumor cells into a more vulnerable phase of the cell cycle. Technological advances in surgical approaches, techniques, and instrumentation have made most tumors available for resection. [2] Different pathohistological subtypes of anaplastic gliomas show significantly different prognosis depending on the applied oncological therapeutic protocol as well as the modality of the applied radiotherapy. The median survival after surgery for anaplastic astrocytoma is about 19 months Survival estimates for these tumors vary widely and seem to be associated with age,[6,7] Karnofsky Performance Score (KPS) status,[8,9] extent of surgical resection,[10] use of adjuvant radiotherapy,[11] ki-67 immunohistochemical markers,[12-14] and sensitivity to chemotherapy as determined by genetic mutations such as IDH1(Isocitrate dehydrogenase 1) [15], PTEN, EGFR amplification,[16] and 1p19q codeletion.[17].

MATERIALS AND METHODS

Study design

The study was designed as a retrospective, clinical observational study. The study used clinical and diagnostic data from the patients that were treated surgically in the Clinic for neurosurgery of Clinical center of Serbia diagnosed with brain tumors that after PH verification confirmed to be anaplastic gliomas. Clinical data were collected retrospectively, by reviewing medical histories and conciliatory decisions recorded in an electronic database, and then analyzed prospectively. All data were obtained from period of 2011 to 2014.

Examined variables:

The study included data on demographic and socio-economic parameters, personal and family history, localization, pathohistological subtype of tumor and applied oncological protocol.

Pathohistological analysis was performed at the Clinic for Neurosurgery KCS, using standard hematoxylin-eosin staining, as well as immunohistological staining.

Research instruments:

- Preoperative CT and MR of the endocranium
- Postoperative CT of the endocranium used to determine the degree of tumor resection.
- Periodic control MRI of the endocranium used to determine the success of applied chemotherapy and radiotherapy.
- ECOG PS(Eastern Cooperative Oncology Group Performance Score) and Karnofsky performance score

Statistical data processing

Data processing was performed in SPSS Windows 20.0. All data are presented and analyzed by adequate mathematical and statistical methods appropriate to the type and type of data, the results will be presented in tables and graphs. For normal variables, mean and standard deviations were calculated. P-values less than 0.05 were considered statistically significant.

RESULTS

The study included 34 patients diagnosed with anaplastic gliomas that were treated in at the Department of Neurooncology. In the group of patients with anaplastic gliomas there were 15(44%) patients with anaplastic astrocytoma, 9(27%) patients with anaplastic oligodendroglioma and 10(29%) patients with anaplastic oligoastrocytoma. Patients whose pathohistological diagnosis would confirm the finding of

anaplastic pleomorphic xanthoastrocytoma were not operated on in the monitored period.

The age median was 43 years. The youngest patient who underwent surgery was 23 years old, while the oldest patient was 63 years old. Of the total number of operated patients, 26(76.5%) of patients were female, 8(23.5%) were male.

By analyzing the data on previous hospitalizations and previously applied surgical treatment, as well as insight into pathohistological findings obtained in previous operations we concluded that 20,4% of anaplastic gliomas were formed by transformation from previously operated lower grade gliomas.

The initial, dominant sign of the disease is the appearance of epileptic seizures, followed by signs of elevated ICP(intracranial pressure), hemiparesis, psychoorganic syndrome, diplopia and other symptoms. The basic sociodemographic and clinical data can be seen in **figure 1**.

Table 1. Basic sociodemographic and clinical data of the patients.

Gender	Male 8(23.5%)
	Female 26(76.5%)
Age	Minimum 23
	maximum 63
	median 43
Anaplastic glioma subtype	astrocytoma-15 (44%)
	oligodendroglioma-9(27%)
	oligoastrocyoma-10(29%)
	xanthoastrocytoma-0(0%)
Classification based on previous existence of gliomas	de novo tumor 79.4%
	previously operated lower grade glioma-20.6%
Dominant symptoms	epileptic seisure 44.1%
	Hemiparesis 11.8%
	signs of elevated ICP 20.6%
	psychoorganic syndrome 8.8%
	diplopia 2.9%
	other symptoms 11.8%

According to the localization of anaplastic gliomas, they most often occurred in the frontal region, with a frequency of 47%. When examining on which side tumors appeared more often, we concluded that more often tumors appear the right side, then on the left side and then on both sides of the frontal region. percentual representation of most common tumor localizations in this study can be seen in **figure 1**.

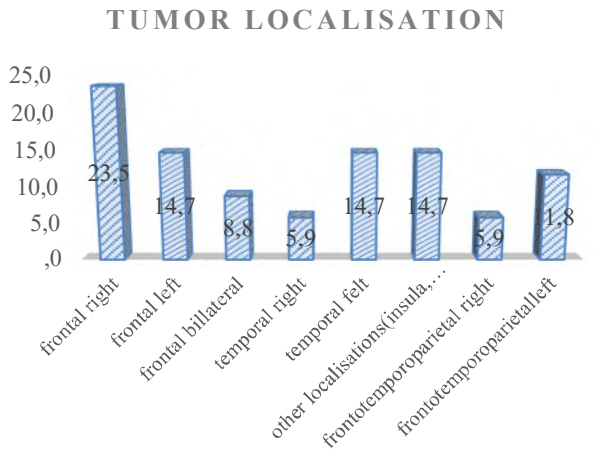

Figure 1: percentual representation of most common tumor localizations in this study.

The incidence of anaplastic gliomas in the temporal lobe is 23.5%. Statistical analysis revealed a statistically significant difference ($p < 0.05$) in the length of survival in relation to the localization of tumor expansion.

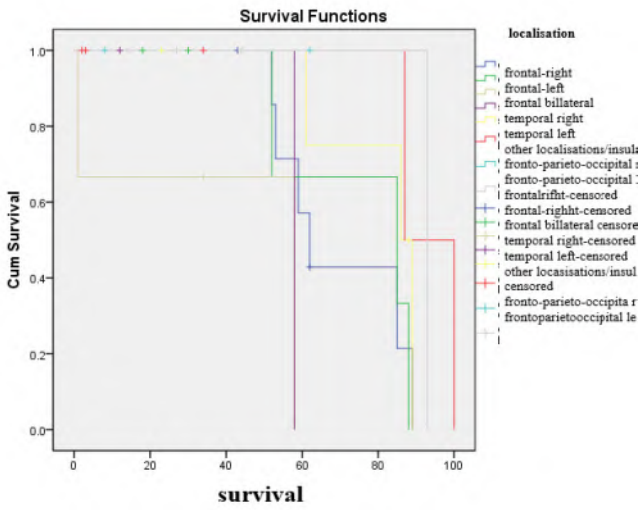

Figure 2. Survival functions in patients with anaplastic gliomas depending of localization.

In over 50% of cases, tumor extirpation was achieved intraoperatively, based on data from operative lists and discharge letters, as well as postoperative computed tomography, in the early postoperative period. Intraoperative subtotal reduction was achieved in 32% of cases. In the group of operated patients with anaplastic gliomas, early postoperative complications were verified in 14 patients. In 5 patients, bleeding in the operative lodge was observed in the early postoperative course, which was the reason for reoperation. In 3 patients, internal hydrocephalus developed, due to which the cerebrospinal fluid drainage system was placed, in 3 patients, postoperative cerebrospinal fluid was observed at the site of the operative wound, which was resolved by repeated

lumbar punctures. Statistical analysis showed that there was no significant difference in the length of survival in relation to the occurrence of postoperative complications (p> 0.05).

Postoperative chemotherapy and radiation therapy were not administered to five patients, due to the Karnofsky score below 60 and the inclusion of symptomatic therapy. In 67.6% of cases, chemotherapy was used as BCNU monotherapy. The combined PCV protocol was applied in 14.7% of cases (Figure 3). Statistical analysis did not determine the significance in the length of survival, in relation to the applied chemotherapy modality. 3D conformal radiotherapy was applied in 85% of cases.

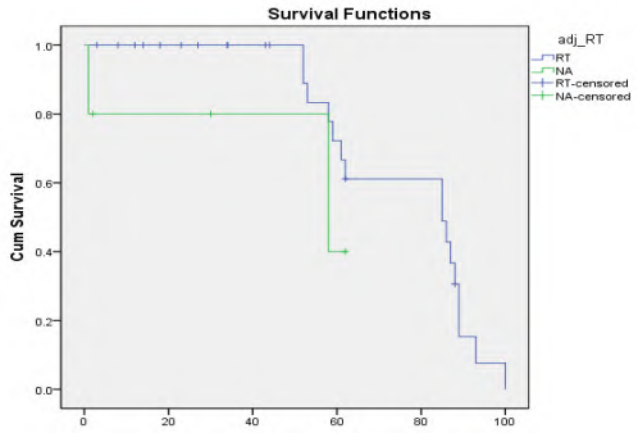

Figure 3. Overall survival in the group of anaplastic gliomas in patients with adjuvant radiotherapy.

The overall survival in the group of anaplastic gliomas operated on in the Department of Neurooncology KCS in the follow-up period of five years is 52.9%.In the group of astrocytomas 80% of patients survive for one year, while the three-year survival is 46.67%. A five-year follow-up resulted in a survival result of 40%.

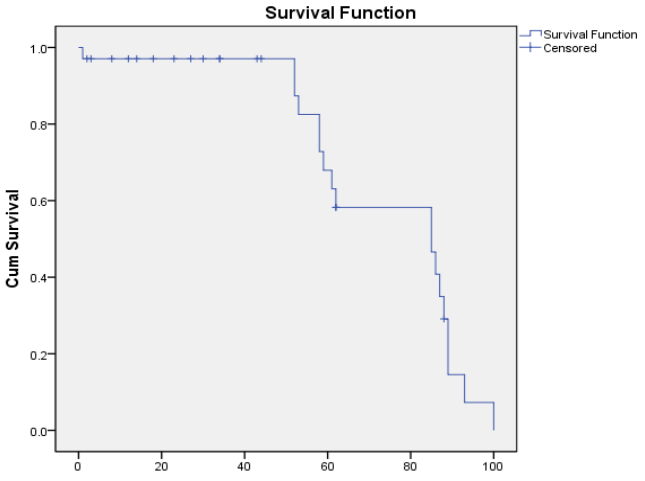

Figure 4. Overall survival in patients with anaplastic gliomas.

In the group of oligodendrogliomas, 88.89% survived the first year after surgery, while the three-year survival in our series is equal to the five-year survival and amounts to 55.56%.

DISCUSION

Our paper studied the data on patients treated for anaplastic gliomas in the period from October 2011 to December 31, 2014, who were treated at the Clinic for Neurosurgery, Department of Neurooncology. All patients were operated by the same team of neurosurgeons, which contributes to the uniformity of the approach. Decisions on the modality of treatment were made by the Council for CNS Tumors, which consists of a multidisciplinary team of neurosurgeons, medical oncologists, radiation oncologists and pathologists.

According to the literature, the frequency of gliomas in the group of primary brain tumors is 26.4% of all tumors, while the so-called high-grade tumors (grade III and grade IV) make up 19.9% of primary brain tumors. Glioblastoma, according to the US Central Registry, occur with 15.6%, while the incidence of anaplastic gliomas is 4.3%. From this we conclude that about 21.6% of high-grade gliomas are anaplastic gliomas.[2]

According to our results, in a three-year period, in the Department of Neurooncology of the Clinic for Neurosurgery KCS, in the group of operated patients there were 22.7% of patients with anaplastic gliomas, in the group of patients with high - grade gliomas, grades 3 and 4.

In our study, the group of anaplastic astrocytoma makes up 10% of high-grade tumors, while anaplastic oligodendroglioma make up to 6% of high-grade gliomas. According to some published studies, astrocytomas occur with an incidence of 1.7% of primary brain tumors while oligodendrogliomas occur with an incidence of 0.5% of all primary brain tumors. The ratio of astrocytomas and anaplastic oligodendrogliomas in our study is 1.6:1. oligoastrocytoma, NOS (Not Otherwise Specified), is a rare tumor because most high-grade gliomas with mixed or ambiguous histology can be classified as IDH mutated anaplastic astrocyte or IDH mutated anaplastic oligodendroglioma with 1p / 19q correlation [2]. Bearing in mind that in the analyzed period we did not perform, systematically, cytogenetic molecular analyzes, in our work the incidence of anaplastic oligoastrocytomas is identical to the incidence of astrocytomas. histological material is classified in the group of oligoastrocytomas, which is why their incidence in our work is statistically significantly higher than the data from the literature. The results obtained in this study indicate that 76.5% of patients are female, in contrast to studies cited in the literature where 55% of patients with high grade gliomas are male. Until the discovery of the IDH mutation as a molecular marker, the diagnosis of anaplastic glioma was based only on histological evidence.[18] In this era, the peak age was considered to be 45

years of age. In our work, the median occurrence of anaplastic gliomas is 43 years.

The initial symptomatology in terms of the occurrence of EPI seizures is a better prognostic factor, compared to the appearance of other symptoms and signs of the disease. The localization of the tumor in the frontal region has a favorable prognostic significance, which can be associated with the possibility of more radical surgical treatment, as well as more frequent localization of oligodendroglioma in this region. Our study also confirms a statistically significantly worse prognosis in astrocytomas compared to other histological forms of anaplastic gliomas. Proliferative activity, determined with Ki-67 index, is not prognostically significant for astrocytomas. are not connected with the outcome of astrocytoma. Genetic changes of IDH 1/2 mutations are associated with a better outcome, while IDH wild-type anaplastic astrocytoma has an outcome similar to that of IDH wild-type glioblastoma. (19) EGFR amplification as well as genotype with 7q amplification and 10q deletion were associated with a poorer outcome. Proliferative activity, determined with Ki-67 index, is not prognostically significant for astrocytomas. are not associated with outcome in astrocytoma. Genetic changes of IDH 1/2 mutations are associated with better outcome, whereas IDH wild-type anaplastic astrocytoma has an outcome similar to that of IDH wild-type glioblastoma. EGFR amplification as well as genotype with 7q amplification and 10q deletion are associated with a poorer outcome. By monitoring the impact of surgical complications in the early postoperative course, we do not obtain their statistical significance as a prognostic factor. We seem to explain the seemingly illogical result by the need for continuous monitoring of patients and timely resolution of complications.

Due to the low performance score in 5 patients, no adjuvant radio or chemotherapy was applied. Having that in mind in our study there is no comparation between survival rates in these patients and patients with high performance status and had adjuvant radio and chemotherapy. Other studies showed that patients with Karnofsky Performance Score (KPS) of ≥70 have a significantly better 5-year OS(Overall Survival) as compared to those with KPS <70 (33%) . The use of adjuvant temozolomide (TMZ) shows longer 5-year OS compared to 36% in patients who did not receive adjuvant chemotherapy (20)

Unfortunately, we cannot get a general conclusion about the epidemiological characteristics of anaplastic gliomas on the entire territory of the Republic of Serbia, due to the lack of precise unified data. Radical surgery, which is not an end in itself, is one of the basic prognostic factors. In addition to age, general condition, IDH mutational status, and the extent of surgical resection affect the outcome of treatment, which is confirmed by statistical processing of the results obtained in our work. The use of a new generation operating microscope, cavintron ultrasound aspirator, intraoperative navigation and neuromonitoring contribute to the radicality of the operative approach. In that sense, uniform use of the same in all neurosurgical centers in Serbia is necessary. Genetic changes of the IDH 1/2 mutation are associated with a better outcome, whereas the IDH wild-type anaplastic astrocytoma has an outcome similar to that of the IDH wild-type glioblastoma. [21] In the period we analyzed, and unfortunately not even today, genetic mutations are not systematically examined in all patients operated on for anaplastic gliomas in our country, which is why we cannot compare our results with data from the literature. What is necessary to introduce into routine use in our country is to increase the number of cytogenetic laboratories, bearing in mind the results that indicate that molecular and genetic analysis directly determine the prognosis in the treated patient, but also oncological therapy of anaplastic gliomas directly depends on its cytogenetic characteristics. which would exclude a general approach to the treatment of anaplastic gliomas, but we would apply different therapeutic modalities depending on the characteristics of the tumor, i.e., an individual approach to each patient.

CONCLUSION

The incidence of anaplastic gliomas operated on in the Department of Neurooncology of the Clinic for Neurosurgery of the Clinical Center of Serbia corresponds to their frequency, which is published in the available literature.

During the follow-up period, a significantly higher incidence of oligoastrocytomas is observed, which is explained by the impossibility of determining the isocitrate dehydrogenase mutation and 1p / 19q codeletion, which could be used to classify most high-grade gliomas with mixed or ambiguous histology as anaplastic IDH mutant oligodendroglioma with 1p / 19q codeletion.

The application of different chemotherapy modalities does not show statistical significance in the length of survival, in the follow-up period.

Bearing in mind that RT was applied in all patients in whom the Karnofsky score was over 70, we were not able to estimate the prognostic value of RT application.

The radical nature of the operation has statistical significance in the length of patient survival, which confirms the conclusions of most of the conducted studies cited in oncology textbooks.

ACKNOWLEDGEMENTS

None

CONFLICT OF INTERESTS

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

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THE PREVALENCE OF HELICOBACTER PYLORI INFECTION IN PATIENTS WITH CHRONIC KIDNEY DISEASE UNDERGOING HEMODIALYSIS

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Received: 02.08.2021.
Accepted: 28.09.2021.

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ABSTRACT

The aim of this study was to analyze the prevalence of Helicobacter pylori infection and gastroduodenal lesions in Montenegrin patients with chronic kidney disease on hemodialysis. The study included 55 hemodialysis patients with dyspeptic symptoms and 50 control subjects with normal kidney function who had also dyspepsia. After dyspepsia assessment by an interview, all subjects underwent gastroduodenoscopy and histopathological analysis of biopsy specimens, taken from the corpus and antrum of the stomach. Helicobacter pylori was confirmed in 50.9% of corpus mucosa i.e. 60.6% of antrum mucosa of maintenance hemodialysis patients. There were no significantly differences in endoscopic findings of the stomach and bacterial presence between the two study groups of participants ($p = 0.451$), while duodenal lesions were prevalent in control subjects ($p < 0.001$). The atrophy of corpus mucosa was more common in hemodialysis patients ($p = 0.007$), especially in those who have been on hemodialysis for a longer time ($p < 0.001$) and had lower pH ($p = 0.011$). The prevalence of Helicobacter pylori infection shown an inverse relationship with dialysis duration. Contrary, a positive relationship between Helicobacter pylori and the concentration of bicarbonate was demonstrated ($p = 0.031$). The prevalence of Helicobacter pylori and atrophic mucosal changes in Montenegrin hemodialysis patients depends on dialysis duration and acid-base balance.

Keywords: *Chronic kidney disease, dialysis, Helicobacter pylori.*

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UDK: 616.33/.34-022.7:579.84
UDK: 616.61-06
Eabr 2024; 25(3):225-231
DOI: 10.2478/sjecr-2021-0044

INTRODUCTION

Chronic kidney disease (CKD) is a progressive loss of kidney function that is presented by glomerular filtration rate below 60 ml/min/1.73m^2 for a period longer than 3 months (1). Because of growing prevalence, it has been recognised as one of the main death causes among the chronic non-communicable diseases (2). Data from 2017 testify the age-standardised morbidity rate for Montenegrin population was 13.1% (3).

Progressive CKD has been associated with a number of serious complications including gastrointestinal disturbances (4, 5). Most often it is about dyspeptic symptoms like occasional or constant epigastric pain, early satiety, bloating, nausea, excessive belching and postprandial fullness (6, 7). The pathogenetic aspects of dyspepsia have been sufficiently unexplored. Many factors are thought to be involved in pathogenesis of this condition like uremic toxins (8), digestive hypomotility (9), amyloid protein deposition (10), sensory disturbances (11), and *Helicobacter pylori* (HP) infection (12).

The prevalence of HP infection and its relationship to gastroduodenal lesions in hemodialysis patients have been extensively studied (13). Nevertheless, the available literature data on the HP presence in hemodialysis patients are quite unequal with a wide range of variation (20-80%) (14, 15). There are also different opinions on the frequency of gastroduodenal lesions in hemodialysis CKD patients compared to non-CKD subjects (16). Some authors reported that hemodialysis patients suffered more often from gastroduodenal disorders while the others not found the differences between hemodialysis and the general population (13, 17, 18).

Thus, the aim of our study was to determine the prevalence of HP infection and gastroduodenal lesions as well as their connections with certain biochemical findings and hemodialysis quality in Montenegrin CKD patients with dyspeptic symptoms.

PATIENTS AND METHODS

Study population

The investigation has been conducted as a single center, cross-sectional study with one-time point evaluation during 2019 and early 2020s in Clinical Center of Montenegro, Podgorica. The study initially enrolled 70 patients with diagnosis of CKD undergoing hemodialysis, but further research was continued with 55 patients in whom dyspeptic symptoms have been identified for at least 3 months. The control group consisted of 50 consecutive, age and gender matched, subjects with preserved kidney function referred for endoscopic examination due to dyspepsia. The exclusion criteria for participation in the study were: (1) recent active infections, (2) chronic inflammatory or autoimmune diseases; (3) previously diagnosed gastroduodenal structural changes like inflammation or ulcer; (4) contraindications for performing upper gastrointestinal endoscopy, (5) therapy with H_2 inhibitors, proton pump antagonists and/or nonsteroidal anti-inflammatory drugs; (6) *under 18 years of age* and (7) pregnancy.

The study was planned according to the Declaration of Helsinki. All patients and control subjects gave informed consent for the participation. The investigation has been approved by the institutional Ethical Committee according to local research regulations.

Measurements

We performed an interview about dyspeptic symptoms in all study subjects as well as physical and biochemical examination. Gastroduodenoscopy with pathohistological analysis of biopsy specimens was applied only in the cases of confirmed dyspepsia. The study also concerned to dialysis parameters such as the duration of hemodialysis (in months) and its efficiency.

Dyspepsia assessment

Dyspepsia was defined based on Roma III criteria (19): the presence of certain symptoms, which exist for the last 3 months onset and continue at least 6 months prior to diagnosis (excluding structural gastroduodenal disease). A questionnaire included the following dyspeptic symptoms: constant and occasional epigastric pain, bloating, early satiety, nausea, vomiting and heartburn.

Biochemical parameters

Biochemical parameters (hemoglobin, urea, creatinine) were investigated by standard laboratory methods, while the acid-base balance was assessed by pH and bicarbonate (HCO_3^-) concentration.

Chemiluminescent immunoassay and *Architect Intact PTH* reagent by Abbott was used for determination of parathyroid hormone (PTH) level (range 1.6-7.2 pg/ml).

The efficiency of hemodialysis was assessed by calculation of Kt/V with dialysis machine software. Kt/V presents dialyzer clearance of urea (expressed in ml/min) multiplied by the dialysis time (expressed in minutes) and divided by volume of distribution of urea.

Gastroduodenoscopy

Gastroduodenoscopy with Olympus XQ 40 endoscope was performed in all study participants in whom the presence of dyspeptic symptoms was previously determined by the questionnaire. During the procedure, the biopsy specimens were taken from the corpus and antrum of the stomach, and stored in 4% formalin to further processing. The final diagnosis was made by endoscopic and histopathological findings of mucosal lesions regarding the degree of inflammation and its activity, the degree of atrophy, intestinal metaplasia and *Helicobacter pylori* presence.

Statistical analysis

The commercial software SPSS version 22.0 for Windows was used for the statistical analysis. Data are presented as mean ± standard deviation (SD). Differences in the parameters between the groups of patients were estimated by the Independent samples T-test or Mann-Whitney U-test (depending on the distribution). Chi-square (χ^2) test served to compare the frequency of categorical variables. For all tests described above, *p* values less than 0.05 were considered to be statistically significant.

RESULTS

The results of our study are presented in Tables 1-3 and Figures 1-3.

The study included 55 maintenance hemodialysis patients with dyspepsia and 50 control subjects, who underwent endoscopic examination because of dyspeptic symptoms. There were 29 (52.8%) males and 26 (47.2%) females in the group of hemodialysis patients, respectively 27 males (54%) and 23 females (46%) in the control group. The average age of hemodialysis patients was 56.48 ± 13.66ys, while in the control group it was 54.45 ± 11.38ys (Independent samples T test, p = 0.311).

Analysis of dyspeptic symptoms showed that occasional epigastric pain, bloating and nausea were the dominant symptoms in the group of hemodialysis patients, while the control group mostly showed early satiety, bloating and nausea (Figure 1). It estimated the significant difference in the frequency of constant epigastric pain (χ^2 = 4.335, p=0.037) and vomiting (χ^2 = 10.772, p=0.005) between the groups.

Data obtained by gastroduodenal examination did not confirm a statistically significant difference in endoscopic findings of the stomach between hemodialysis patients and control subjects (χ^2 = 5.758, p=0.451).

The most common finding in both study groups was gastritis, confirmed in 27 (49.1%) hemodialysis patients and 28 (56%) control subjects. When it comes to duodenal lesions, majority of hemodialysis patients had a normal endoscopic finding (67.9%), while in control participants duodenitis was the most frequent (42%) (p < 0.001) (Figures 2-3).

The results of pathohistological examination of biopsy samples taken from the corpus and antrum of the stomach are shown in Tables 1-2.

We found that atrophy of corpus mucosa was significantly more prevalent in hemodialysis patients (p = 0.007) compared to control subjects, who mainly showed the signs of inflammatory activity in the corpus. There was no statistically significant difference in the presence of HP infection neither in the samples of a corpus or an antrum between two study groups. HP was confirmed in 50.9% of corpus mucosa i.e. 60.6% of antrum mucosa of hemodialysis maintain patients.

In further research, we analyzed the influence of certain biochemical parameters and dialysis treatment on the occurrence of gastric atrophy and HP positivity. It has been estimated that gastric atrophy was more common to CKD patients that underwent hemodialysis for a longer time (p < 0.001). No influence of Kt/V (p = 0.385), concentration of hemoglobin (p = 0.338), creatinine (p = 0.100) and PTH (p = 0.201) on atrophic changes was observed. The disturbance of acid-base balance (lower pH) had a positive effect on the occurrence of gastric atrophy (p = 0.011).

When it comes to relationships of HP presence and the above-mentioned parameters, the data are shown in Table 3.

HP presence was higher in both corpus and antrum samples of CKD patients that underwent hemodialysis for a shorter period. Also, a positive relationship between bicarbonate concentration and HP infection was demonstrated (p = 0.031).

Table 1. Frequency of pathohistological categories of corpus mucosa in two study groups

Category		Hemodialysis patients n = 55	Control patients n = 50	χ^2	significance
Inflammation activity	Yes	28 (50.9%)	31 (62.5%)	1.084	p = 0.298
	No	27 (49.1%)	19 (37.5%)		
Atrophy	Yes	23 (41.9%)	8 (15%)	7.274	**p = 0.007**
	No	32 (58.1%)	42 (85%)		
Intestinal metaplasia	Yes	0	0	–	–
	No	55 (100%)	50 (100%)		
Helicobacter pylori	Yes	28 (50.9%)	22 (44%)	0.315	p = 0.574
	No	27 (49.1%)	28 (56%)		

Table 2. Frequency of pathohistological categories of antrum mucosa in two study groups

Category		Hemodialysis patients n = 55	Control patients n = 50	χ^2	significance
Inflammation activity	Yes	39 (72.1%)	21 (42.5%)	0.485	p = 0.486
	No	16 (27.9%)	29 (57.5%)		
Atrophy	Yes	24 (44.2%)	21 (42.5%)	0.024	p = 0.877
	No	31 (55.8%)	29 (57.5%)		
Intestinal metaplasia	Yes	2 (4.7%)	0	1.906	p = 0.167
	No	53 (95.3%)	50 (100%)		
Helicobacter pylori	Yes	33 (60.6%)	26 (52.5%)	0.890	p = 0.355
	No	22 (39.4%)	24 (47.5%)		

Table 3. The relationships of HP presence and laboratory parameters

Parameter		Corpus mucosa		Antrum mucosa	
		Mean value	p	Mean value	p
Hemoglobin (g/dl)	HP-	10.47	p = 0.269	10.52	p = 0.508
	HP+	10.89		10.78	
Creatinine (μmol/l)	HP-	1017.0	p = 0.394	1024.3	p = 0.394
	HP+	963.64		969.18	
PTH (pg/ml)	HP-	473.73	p = 0.537	480.59	p = 0.568
	HP+	396.87		407.01	
pH	HP-	7.310	p = 0.133	7.309	p = 0.169
	HP+	7.323		7.322	
HCO_3^- (mmol/l)	HP-	13.70	**p = 0.031**	13.78	p = 0.156
	HP+	14.70		14.47	
Dialysis duration (months)	HP-	91.10	**p < 0.001**	106.38	**p < 0.001**
	HP+	31.68		33.63	
Kt/V	HP-	1.226	p = 0.447	1.199	p = 0.765
	HP+	1.190		1.213	

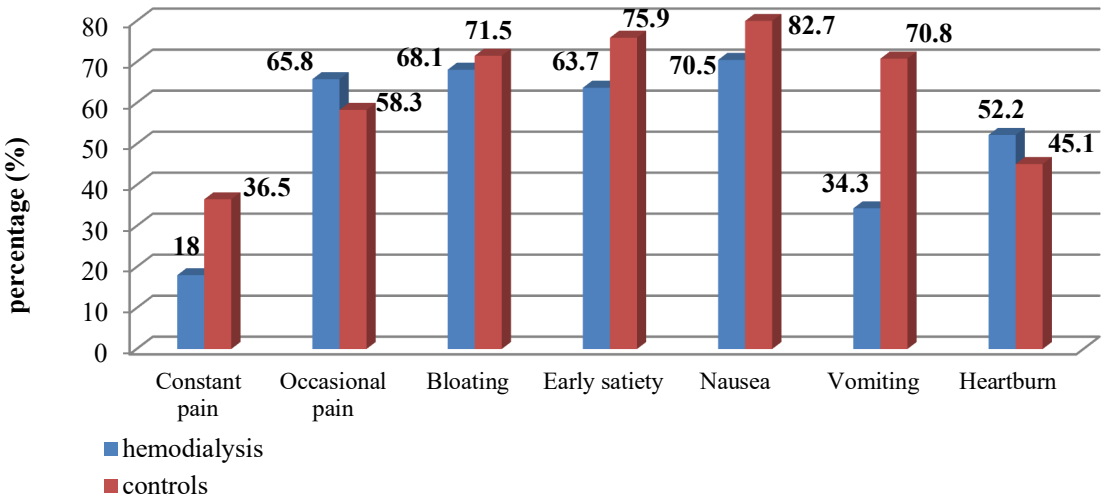

Figure 1. The prevalence of dyspeptic symptoms in hemodialysis patients and control subjects

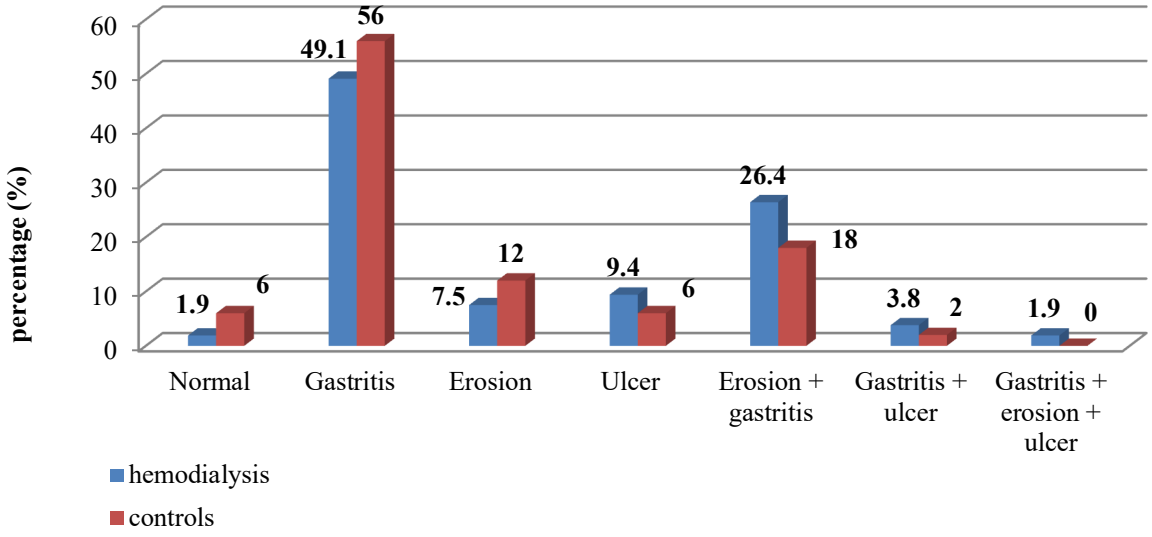

Figure 2. Endoscopic findings of the stomach in hemodialysis patients and control subjects

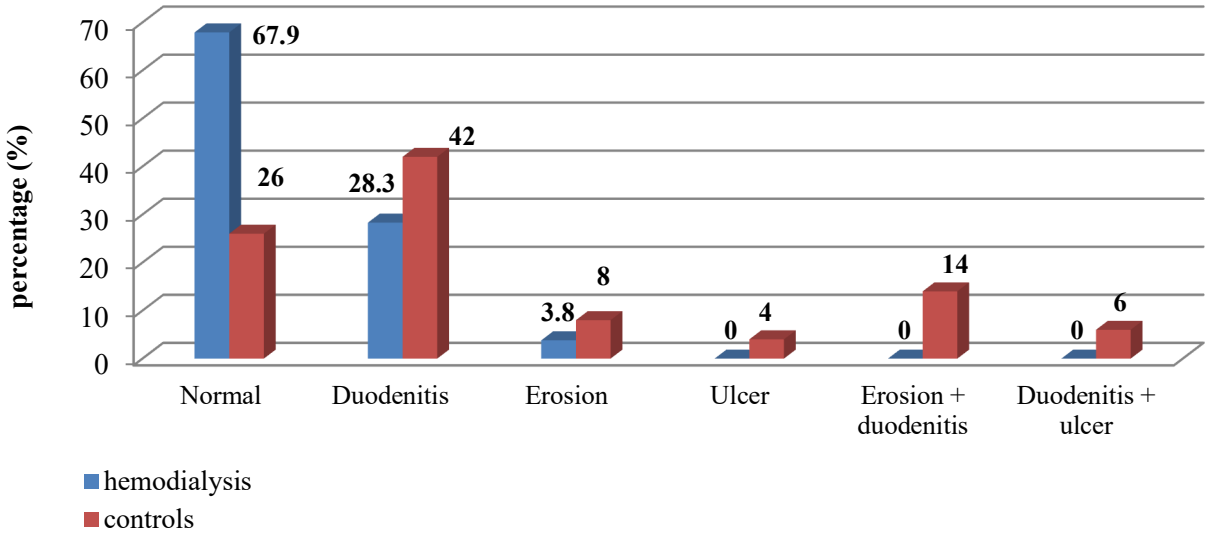

Figure 3. Endoscopic findings of the duodenum in hemodialysis patients and control subjects

DISCUSSION

The current study analyzed the presence of gastroduodenal lesions and Helicobacter pylori infection in Montenegrin hemodialysis patients with dyspeptic symptoms.

We estimated that CKD patients undergoing hemodialysis commonly presented as dyspepsia as occasional epigastric pain, bloating and nausea. However, dyspeptic symptoms were more pronounced in control subjects. There were no significantly differences in endoscopic findings between the two study groups of participants, although control subjects had more often the lesions of the duodenum. Pathohistological analysis demonstrated that atrophy of corpus mucosa was more prevalent in hemodialysis patients and it positively correlated with dialysis duration. Lower pH also stimulated atrophic changes. The prevalence of HP was over 50 percent so that its presence was higher in the patients that underwent hemodialysis for a shorter time.

HP is a gram-negative bacillus that inhabits the gastric mucosa and it seems to have an important role in the pathogenesis of gastroduodenal disorders in hemodialysis patients (20, 21). Namely, HP modulates gastric secretion by

increasing serum gastrin concentration and decreasing the level of somatostatin in the gastric mucosa (22). On the other hand, the inflammatory response to HP can induce the dysfunction of gastric smooth muscles and lower sensation for gastric distension (5, 23).

The results of previous studies on gastroduodenal lesions and HP infection in hemodialysis patients are quite inconsistent (13, 17). There is no single view on the frequency of endoscopic findings in CKD patients that underwent hemodialysis (existence of an equal or higher rate of lesions compared to the general population). Our investigation showed that duodenal lesions were more common in control subjects, who mainly had a histopathological finding of inflammation and a pronounced clinical presentation of dyspepsia. Contrary, atrophic mucosal changes and HP presence was higher in hemodialysis patients. These results indicate the potential effect of HP on the occurrence of mucosal atrophy. Namely, ammonia, a product of urea *hydrolysis,* formed by the action of bacterial urease, has a pro-atrophic effect on the cells of gastric mucosa (24). Additionally, it has been shown that HP could induce the apoptosis of gastric cells by increasing the expression of pro-apoptotic Bax protein (25).

Our results are in agreement with the findings of Khedmat and coworkers (13), who also reported higher prevalence of HP infection in CKD patients. Here, we must emphasize that, although higher in hemodialysis patients, the prevalence of HP did not differ significantly between two groups enrolled in the study. A meta-analysis conducted by Wijarnpreecha (26) estimated HP infection prevalence of 44% in end-stage CKB patients, which is similar to our findings in the stomach (HP confirmed in just over 50% of cases). However, current literature data indicates a wide range of variation in HP positivity in hemodialysis patients (14, 27, 28), which could be a consequence of the different design and methodology of the conducted studies.

Interestingly, we have shown the reduction of HP positivity in the patients that underwent hemodialysis for a longer time. In other words, the duration of dialysis was significantly longer in HP negative patients and *vice versa*. The association between HP infection and dialysis duration is a subject of scientific discussion (29). Thus, Nakajima et al. (30) estimated a negative correlation between the duration of dialysis and HP infection, unlike Rasmi (31) who found positive effect of hemodialysis length on HP prevalence. Undoubtedly, the duration of dialysis is an important factor affecting the HP infection. Growing evidence suggests HP eradication in CKD patients over time due to high serum concentration of urea (32), decreased gastric acid secretion and higher pH value (33), an adequate therapeutic approach (34), as well as the secretion of proinflammatory cytokines (14). It is assumed that mentioned factors induce the atrophic changes of gastric mucosa and prevent the survival of HP in altered conditions.

However, further investigations are needed to determine the association between the duration of dialysis and HP prevalence by continuous monitoring of CKD patients at multiple time points. Previous research, including ours, have focused on HP infection in dialysis patients by single sampling and measurement of appropriate variables.

CONCLUSION

The estimated prevalence of Helicobacter pylori in Montenegrin hemodialysis patients was over 50%, with a somewhat greater presence in the antral mucosa. There were no significant differences in endoscopic findings between CKD patients undergoing hemodialysis and non-CKD patients with dyspepsia. Atrophic mucosal changes were more common in hemodialysis patients and they positively correlated with dialysis duration. Helicobacter pylori presence was reduced in the patients that underwent hemodialysis for a longer time.

ACKNOWLEDGMENTS

Authors would like to express their deepest gratitude to the Faculty of Medical Sciences, University of Kragujevac, for their Junior Project grant N0 02/13, which was used as a source to financially support the study.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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THE IMPACT OF NANO-CRYSTAL HYDROXYAPATITES ON THE REGENERATION OF BONE DEFECTS

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Received: 06.05.2021.
Accepted: 19.07.2021.

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UDK: 616.7-089.843
615.46
Eabr 2024; 25(3):233-242
DOI: 10.2478/sjecr-2021-0053

ABSTRACT

Calcium hydroxyapatite is a widely used material for replacing bone defects. However, the effectiveness of nano-crystalline calcium hydroxyapatite produced from eggshells in the replacement of bone defects has not been investigated yet. The study aimed to evaluate the effectiveness of using nano-crystalline calcium hydroxyapatite made from eggshell for the healing of bone defect of the femur in rats. Forty-eight (n=48) rats underwent a surgical procedure to simulate femoral defect. The animals were sub-divided into 4 groups (each with n=12) depending on the methods of bone defect replacement: I control group (CG) (without bone defect replacement); II intervention group (the bone defect was replaced by PRP (PRP); III intervention group (the bone defect was replaced by nano-crystalline hydroxyapatite obtained from eggshell) (HA) and IV interventional group (the bone defect was replaced by a combination of hydroxyapatite and PRP) (HA+PRP). The degree of effectiveness of studied methods was assessed using radiological (on the 14th day), histological (on the 61st day), and biomechanical analysis (on the 61st day). According to radiographic data, the CG group had the lowest level of bone regeneration after 14 days (4.2 ±1.7%). In the HA + PRP group, the level of bone regeneration was 22.1±7.1 %, which was higher in comparison with the rates of consolidation of bone defects in the HA group (20.7± 9.3) (p = 0.023). According to the histo-morphometry data, the rates of bone tissue regeneration in the PRP group (19.8 ±4.2%) were higher in comparison with the CG group (12.7 ± 7.3%), (p>0.05). In the HA+PRP group, bone regeneration rates (48.9±9.4 %) were significantly higher (p=0.001) than in the HA group (35.1±9.8%). According to the results of biomechanical assessment under the maximum stress (121.0722), the maximum bending deformation of the contralateral bone without defect was 0.028746, which was higher than the indicators of the HA+PRP group, where at the maximum stress (90.67979) the bending deformation was 0.024953 (p>0.05). Compared to CG, PRP, and HA, biomechanical bone strength was significantly higher in the HA + PRP group (p≤0.01). At the maximum stress (51.81391), the maximum bending strain in the CG group was 0.03869, which was lower than in the PRP group, where the maximum stress and bending strain were 59.45824 and 0.055171, respectively (p>0.05). However, the bone strength of the HA group was statistically significantly higher compared to the CG and PRP groups (p<0.01). The results demonstrated the effectiveness of the use of nano-crystalline calcium hydroxyapatite obtained from eggshell in the healing of a bone defect. The best results were observed in the group of the combined use of nano-crystalline calcium hydroxyapatite and PRP.

Keywords: *Bone defect, bone fracture, hydroxyapatite, PRP, experimental study.*

INTRODUCTION

Autologous replacement of bone defects is the gold standard of the treatment of bone defects in orthopaedics (1). However, difficulties associated with obtaining donor materials hamper a wide implication of such methods in the clinics (2).

Up to date, different types of bone substitute materials have been proposed (with properties close to autologous materials) (3). It encompasses organic and synthetic compounds such as type I collagen, calcium phosphate, calcium hydroxyapatite and others (4). Materials for bone replacement should demonstrate excellent conductive and biological properties. In addition, the concentration of bone minerals has to be as close as possible to the physiological composition of bone tissue (5). For example, calcium hydroxyapatite, consisting of a complex of phosphorus and calcium, is a key material of skeletal tissue, and for this reason, many types of bone grafts contain it as a basic component (6).

One of the sources of calcium hydroxyapatite is eggshell. The eggshell is the end-product of the food industry, and it has been considered waste, which contributes to pollution. The use of the eggshell as a material for a bone graft is economically beneficial and environmentally friendly (7). It should be noted, that the size and biological properties of the resulting particles of the calcium-phosphate complex (from calcium hydroxyapatite) are very convenient for bio-engineering applications (8). In this study, we utilized nano-crystalline calcium hydroxyapatite obtained from eggshell, which is a biologically soluble film based on nano-sized polymer fibres (9).

The calcium hydroxyapatite obtained from eggshells possesses the ability to degrade rapidly, which leads to the increase in the local concentration of calcium at the site of a bone defect (10). Moreover, it has been shown that it can facilitate sufficient osseo-integration during bone regeneration (11) . However, the level of degradability of synthetic calcium hydroxyapatite is low compared to calcium hydroxyapatite obtained from the eggshell (11). Apart from that, no preclinical study has been carried out to evaluate the effectiveness of a biologically soluble film obtained from eggshells based on nano-sized polymer fibers and calcium hydroxyapatite.

The aim of this study was to assess the effectiveness of the use of nano-crystalline calcium hydroxyapatite made from eggshell for the healing of a critical bone defect in vivo.

MATERIAL AND METHODS

Ethical issues

The study was carried out in the Laboratory of Experimental Medicine of NJSC " S.D. Asfendiyarov KazNMU", Almaty, Kazakhstan. The experimental study protocol was approved by the Local Ethics Committee of S. D. Asfendiyarov Kazakh National Medical University (Approval No. 871), dated January 29, 2020.

The animals were kept in accordance with the international rules "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2011), as well as with the ethical principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 2006).

An experimental study was carried out on outbred rats (n = 48) weighing 175 ± 42 grams. Animals before and after the operation were kept in the conditions of the Vivarium of the Atchabarov Research Institute of Fundamental and Applied Medicine (Republic of Kazakhstan, Almaty) with a standard diet and care.

In order to minimize the number of animals used in the experiment, the required number of samples was adjusted and optimized up to n = 48 outbred rats.

Study design

Laboratory animals (n = 48) were divided by random randomization into 4 groups:

1) group I - Control group (CG), formation of a bone defect, without additional interventions (n = 12);
2) group II - Interventional group (PRP-G): bone defect formation + PRP (n = 12);
3) group III - Interventional group (CH-G): bone defect formation + nanocrystalline calcium hydroxyapatite (n = 12);
4) group IV - Interventional group (PRP + CH): nanocrystalline calcium hydroxyapatite + PRP (n = 12).

PRP preparation

Venous blood obtained from the tail vein in a volume of 1 ml (vacutainer with EDTA, China). Then it was centrifuged for 5 minutes at a force of 2000 xg 1200 rpm (12). After centrifugation, plasma was aspirated from the top of the tube. Quality control of platelet-rich plasma was carried out using a Sysmex XE-2100 hematology analyzer (Sysmex, Kobe, Japan).

Synthesis of nano-crystalline calcium hydroxyapatite

Nano-crystalline calcium hydroxyapatite was obtained from eggshell, which was annealed for 2 - 3 hours at a temperature of 900 ° - 1000 ° C. After annealing, the powder was loaded into an enamel reactor, and then, a 6-9% solution of ortho-phosphoric acid was introduced with constant stirring and heating up to 60 - 80 ° C to accelerate the chemical reaction. The resulting solution was exposed to ultrasound for 1–2 h at a frequency of 32–36 kHz to ensure the same size of calcium hydroxyapatite nanocrystals. The resulting micron-dispersed powder was a material with dimensions equal to 1-2 microns. The Ca / P ratio in the synthesized nano-

crystalline calcium hydroxyapatite was 1.67, which corresponds to the stoichiometric ratio of calcium hydroxyapatite in the human bone structure. The synthesized nano-crystalline calcium hydroxyapatite was used to obtain films based on nano-sized polymer (9).

The modelling of a bone defect

The bone defect was simulated under sterile conditions with a pre-shaved surgical field, under general anaesthesia (ketamine 70 mg / kg + xylazine 6 mg / kg) (13) the dose and time of administration of the substances were recorded. During the procedure, the rats were fixed on the operating table in a lateral position. All animals underwent the formation of a bone defect in the middle third of the right femur using a Strong 204-102L dental drill with a 2 mm drill (Figure 1). The defect was formed by drilling the cortical layer and forming an oval-shaped defect with a length of 3 mm (3, 13) while maintaining the integrity of the bone marrow and medullary vessels (14).

In the group I (CG), a bone defect was formed without additional interventions. In the group II (PRP), PRP was used to replace the formed bone defect. Plasma was injected into the bone defect with an excess of a syringe with a short needle (18G1½) (14).

In group III (CH), a bone defect was formed using nano-crystalline calcium hydroxyapatite in the form of a paste (9).

In group IV (PRP + CH), after the formation of a bone defect, nano-crystalline calcium hydroxyapatite was used in the form of a paste in combination with PRP.

In animals of all studied groups, after manipulations on the bone tissue, the surgical wound was closed with intermittent sutures. After this, the animals were returned to their cages, with no restrictions in activity.

Laboratory animals were withdrawn from the experiment on days 21 and 61 after the formation of a bone defect by the method of cervical dislocation (15).

Animals monitoring

The animals were weighed from the first day (before the simulation of the bone defect) to 14 days every day, the behaviour and physical activity of the rats were monitored. The wound was examined for oedema, hyperaemia, swelling and pain on palpation, according to the described method (16). Pain on palpation of the injured area and with full extension of the injured limb was assessed based on the reaction of the animal according to the method of Meimandi-Parizi et al. (17).

X-Ray procedure

On the 14th day after the surgical procedure, the animals were examined using an X-ray method to assess the process of bone consolidation at the site of the defect (18). From each group, n = 3 rats were randomly selected. The procedure was performed using a PROX-S device, DigiMed (South Korea). For the analysis, a scanner was used with the parameters of the generating tube current value of 2 mA, the exposure range (0.01 s - 1.6 s). For scanning, the animals were preliminarily anesthetized with xylazine hydrochloride at a dose of 0.10 ml / 100 g of body weight in the supine position (19). Bone tissue consolidation was determined by the absence of a bone gap or the presence of a pontine callus on the cortical layer (20).

Morphological evaluation

On days 21 and 61 after removing the animals from the experiment, the femurs were extracted. The bone defects were evaluated depending on the type and density of tissue replaced in the area of the defect. From each experimental group, n = 3 rats were randomly selected.

Histological studies

Prepared slides with histological sections 4 μm thick were stained with hematoxylin-eosin. Microscopic analysis of bone tissue sections of animals from different experimental groups was performed using a Leica DM 2000 binocular light microscope (Leica Microsystems, Wetzlar, Germany) and digital software (Image-Pro plus 6.0; Media Cybernetics, USA).

A histo-morphometric evaluation was conducted by two independent histologists and performed on compiled images showing the entire cavity at x100 magnification (21). The formation of new bone and the line between the old and new bone were investigated. Bone regeneration was measured and averaged over multiple sites. Bone regeneration was expressed as a percentage of the height of the main bone at the periphery of the lesions (22).

Biomechanical analysis

On day 61 after euthanasia, n = 3 rats were randomly selected from each group, the femur was dissected under sterile conditions, and the muscles and surrounding defect site were removed for biomechanical evaluation. After processing, the bones were placed into a PBC solution for further testing for mechanical strength. The contralateral femur without defect was used as a control. A standardized three-point bend test was performed using an LR5K Plus electromechanical universal testing machine. Each bone sample was placed horizontally on two support rods at a distance of 26 mm, and the third rod was lowered into the middle of the defect (23, 24). The applied loads and deformation of the sample were recorded continuously throughout the experiment. The limits of the permissible relative error of the force meter were equal to 0.5 %, the discreteness of the digital reading device was 0.005 % of the rated load of the force meter. The procedures were conducted at the room temperature (20°C). All tests

were performed on the same day to minimize variations due to temperature or biomechanical setting.

The results were evaluated using the NEXYGEN Plus software. The bending rate was 1 mm / min, then the maximum bending stress (σ, MPa) and the maximum bending deformation (relative unit) were determined based on the results of mechanical tests. The maximum bending load leading to rupture was considered as a fracture force.

Statistical analysis

To calculate the sample size of animals, we used the G * Power v. 3.1.9.4. programme (Germany). Statistical analysis was performed using SPSS v 25.0 for Windows. Arithmetic mean value (M), a standard deviation (SD) were derived from the quantitative indicators. Qualitative characteristics were described in absolute (n) and relative values (%). Differences between the considered parameters were considered statistically significant at $p < 0.05$. One-dimensional analysis of variance ANOVA was chosen for the statistical test. This test assumes that the samples from the groups are independent, and the F-distribution was used to test the hypothesis in the case of analysis of variance. Mann-Whitney U-test (Wilcoxon test) was performed to assess deformity.

RESULTS

All animals were clinically stable after wound simulation. In the postoperative period, the rats quickly recovered, returning to routine activities (regardless of the method of bone defect replacement). None of the five groups showed either macroscopic or microscopic signs of cellular inflammation or rejection of bone graft material.

There were n = 48 animals in total, no one died, and no bone fractures were found. Laboratory animals were withdrawn from the experiment only within the established timeframe according to the study protocol, no serious complications or diseases were observed during the study. The monitoring of indicators of the local inflammatory response, such as oedema, redness, pain during palpation, pain during flexing, and limb activity, showed that all groups had identical results (no significant differences were found in these indicators). Although there were minor changes in the severity of symptoms in the control group, they were not statistically significant.

In blood samples taken from laboratory animals, the average haematocrit level was 38 ± 5.4%, the number of leukocytes was 7.9 ± 4.1 · 10^3 / μl, and the average number of platelets was 580 ± 190 10^3 / μl. 0.7-0.8 ml of PRP was obtained, with an average platelet concentration of 1302 ± 480 10^3 / μl. Thus, we managed to achieve a threefold increase in the concentration of platelets in comparison with the initial count, the number of leukocytes in the PRP samples was - 1.1 ± 0.6 · 10^3 / μL.

X-Ray evaluation

Representative radiographic findings from the study showed (Figure 2) that after 14 days, the CG group had the lowest bone regeneration rate of 4.2 ± 1.7 %. In the PRP group in comparison with the CG group, the rates of bone defect healing were almost twice as high, which amounted to 8.4 ± 3.3 %, but there was no statistically significant difference ($p > 0.05$). In the HA + PRP group, the level of bone regeneration was 22.1 ± 7.1 %, which was higher compared to the rates of consolidation of bone defects in the HA group (20.7 ± 9.3 %), which was regarded as a statistically significant difference ($p = 0.023$).

Histological analysis

According to the results of the histo-morphometric analysis carried out on day 61, it was revealed (Table 1) that the percentage of bone tissue regeneration varied from 12.7 to 48.9 % in all groups. In the CG control group, bone regeneration rates of 12.7 ± 7.3 % were lower than the rate of bone healing in the PRP group (19.8 ± 4.2 %). However, there was no statistically significant difference in the regeneration of bone defects in the CG and PRP groups ($p = 0.214$ and $p = 0.095$, respectively). In the HA + PRP group, the regeneration rates (48.9 ± 9.4 %) were significantly higher than in the HA group (35.1 ± 9.8 %) ($p = 0.001$).

In control group I, CG, histological examination (Figure 3, a) showed that scattered fragments of bone trabeculae with periosteocytic voids were located among the connective tissue. In places, the space between the trabeculae was filled out with bone marrow content. Osteoblasts were arranged in chains along the bone beams and vessels. Bone formation was incomplete: a scarce newly formed osteoid without mineralization prevailed.

In the group II, PRP in the area of the wound cut (Figure 3, b), neo-angiogenesis was poorly presented (just a few single vessels). The site of injury was filled with fibro-reticular tissue with scant lymphoid infiltration. Bony trabeculae were scattered, with foci of periosteocytic voids. The space between them was filled out with bone marrow. Osteoid deposits are scarce, with no signs of mineralization. In areas remote from the injury, bone tissue was detected.

In the group III HA, on day 61 of the experiment, the morphological pattern of the fracture zone (Figure 3, c) showed a variegated picture. The bulk of callus consisted of fibro-reticular tissue with foci of incomplete osteogenesis. The osteoid, located among the fibres of the connective tissue, had blurred outlines, and it was surrounded by groups of active osteoblasts. Areas represented exclusively by cartilaginous tissue were recorded. Sites of mineralization were located haphazardly, mainly in distal bone fragments. The lesion was filled out with lamellar spongy bone with small foci of imperfect osteogenesis.

The bone tissue of laboratory animals of group IV HA + PRP on the 61st day of the experiment was normally

structured. The area of the defect was represented by a lamellar bone with a system of osteons and Haversian canals (Figure 3d). The cancellous bone was transformed into a compact one, and the thickness of the cortical layer increased. There were single osteoclasts forming lacunae of resorption in the projection of osteogenesis, as well as in the distal regions.

Biomechanical analysis

On day 61, according to the study protocol, the bones were placed in an LR5K Plus electromechanical universal testing machine (Figure 4).

The results of the biomechanical assessment indicated (Figure 5) that the highest strength of the femur was recorded in the contralateral bone without defect ($p \leq 0.01$), which was selected as a control. It was revealed that at the highest load the maximum bending deformation was 0.028746 (at maximum bending stress equal to 121.0722 MPa).

Apart from that, the bone tissues of the animals of the HA + PRP group also showed a high strength of the regenerated bone in comparison with the rest of the experimental groups ($p \leq 0.05$). In the HA + PRP group, the maximum bending stress of the tested bone was 90.67979, and the maximum bending strain was 0.024953. There was no statistically significant difference in bone strength between the contralateral bone without a defect and the HA + PRP group ($p > 0.05$). However, compared with CG, PRP, and HA, the biomechanical parameters of bone strength were significantly high in the HA + PRP group, which was regarded as a statistically significant difference ($p \leq 0.01$).

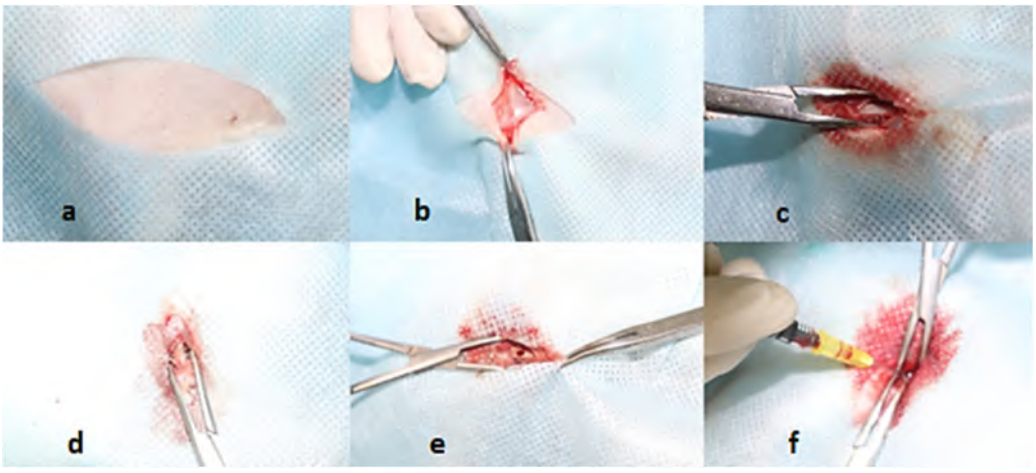
In third place in terms of bone strength, samples of femurs were identified, whose defects were replaced by nanocrystalline calcium hydroxyapatite (group HA) with the maximum bending stress and maximum bending deformation equal to 58.32674 and 0.032192, respectively. In addition, the bone strength of the HA group compared to the CG and PRP groups was statistically significantly higher ($p < 0.05$).

In the CG group with an unsubstituted bone defect in comparison with the PRP group, the resistance and mechanical strength of the bone tissue was slightly lower, where the maximum bending deformation was determined equal to 0.03869, with a maximum bending stress of 51.81391. In the PRP group, when exposed to the maximum load at a maximum bending stress of 59.45824, the highest bending deformation was recorded with an index of 0.055171. However, there was no statistically significant difference in the strength of the regenerated bone between these CG and PRP groups ($p > 0.05$).

Table 1. Histomorphometric analyzes of the level of regeneration of the rat femoral defect.

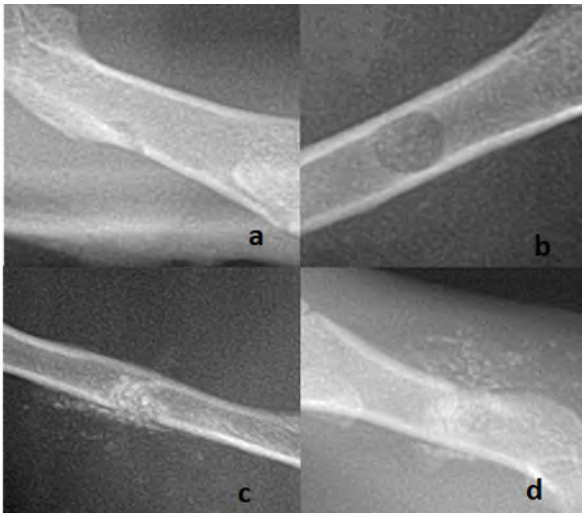
Group number	Bone regeneration (%)	p
Group 1 CG	12,7 ± 7,3	0,214
Group 2 PRP	19.8 ± 4,2	0,095
Group 3 HA	35,1 ± 9,8	**0,028***
Group 4 HA+PRP	48,9 ± 9,4	**0,001***

Measurement of bone formation after 61 days of regeneration



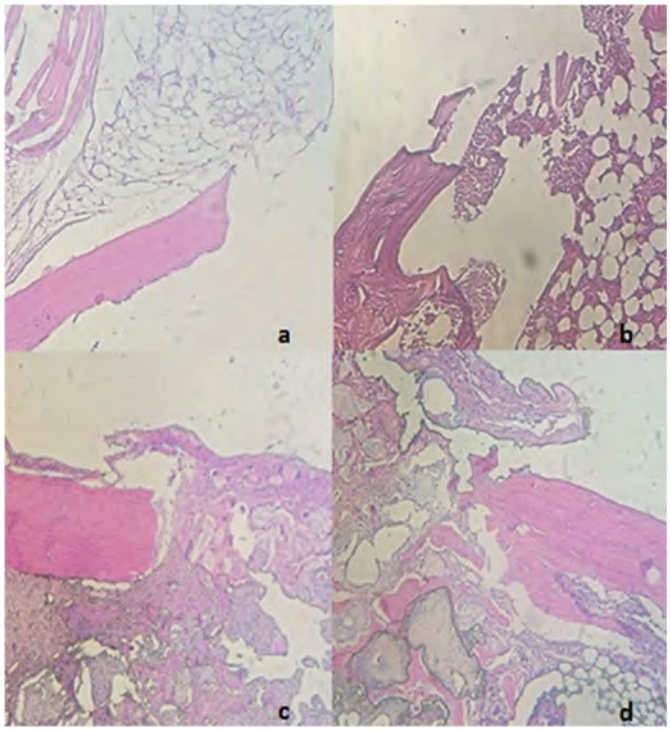
a) Processing of the operating field of the femoral region;
b) Performing a skin incision;
c) Gaining access to the femur;
d) Periosteum removal procedure;
e) Formation of a defect of the rat femur with a diameter of 2 mm;
f) Replacement of the femur defect with the studied components

Figure 1. Stages of surgical formation of bone defects in an experiment on rats.



a) group CG;
b) PRP group;
c) HA group;
d) group HA + PRP

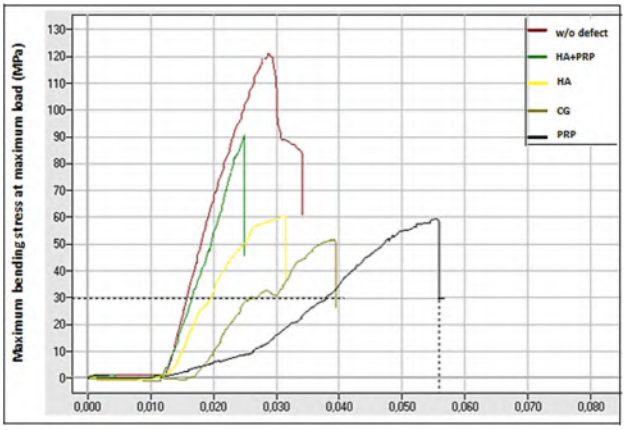
Figure 2. Radiological images of the regeneration of bone defects of the femur in each group on day 14.



a) group CG; b) PRP group; c) HA group; d) HA + PRP group

Figure 3. Histological picture of the femoral bone defect in the study groups on the 14th day.

Figure 4. The process of preparing bone tissue for biomechanical strength testing.



red - contralateral bone without defect; green - group HA + PRP;
yellow - group HA; brown - CG group with bone defect;
black - PRP group

Figure 5. Indicators of biomechanical assessment of bone tissue in the study groups.

DISCUSSION

Normal healthy bone has the ability to self-regenerate during remodelling process or after minor trauma. However, if the site of the defect exceeds the critical size, the bone will not be able to spontaneously heal during life. In this case, bone replacement is required to regenerate new tissue (25).

The use of an animal model to reproduce a prototype of bone defects or other bone pathology is an effective method for comparing the approaches of bone tissue restoration (26). In our study, we investigated the comparative characteristics of the replacement of bone defects with nano-crystalline hydroxyapatite calcium obtained from eggshell, PRP, and their combination.

The results showed the advantage of the application of biological hydroxyapatite obtained from eggshell for tissue regeneration and replacement. The effectiveness of the use of hydroxyapatite from eggshells was described in previous reports, not only in terms of biological properties for replacing a bone defect, but also in connection with economic expediency (27, 28). Biological apatite has been known to be nanostructured material with unique chemical, physical, and electrical properties (29) . Calcium hydroxyapatite, resulting

from the direct action of a living organism, so-called "biogenic" hydroxyapatites, provides a possibility to overcome the limitations of synthetic apatites, including poor adhesion and low mechanical strength. In fact, calcium hydroxyapatite possesses very useful properties such as reduced solubility and convenient particle size (30).

It must be also noted that calcium hydroxyapatite does not contain osteogenic cells and signal substances that are essential for the correct regeneration process of bone tissue (31). In this regard, it should be emphasized that PRP contains a number of different growth factors, such as platelet growth factors (PDGF), vascular endothelial growth factor (VEGF), insulin growth factor (IGF) and transforming growth factor (TGF) (5, 32). These growth factors (in platelets) play an important role in bone regeneration through cascade reactions of angiogenesis and bone repair (33).

The results of the histo-morphometric analysis indicated that the use of nano-crystalline calcium hydroxyapatite on the 61st day after the formation of the defect led to the increase in bone regeneration (equal to 35.1 ± 9.8 %), which was higher than in the CG and PRP groups ($p = 0.028$). However, the percentage of regeneration of bone defects with the combined use of nano-crystalline calcium hydroxyapatite and PRP (48.9 ± 9.4 %) demonstrated a high efficiency in comparison with the data of the HA group ($p = 0.001$).

In addition, our results indicated that the individual use of hydroxyapatite in comparison with the CG and PRP groups provided a comparatively better biomechanical strength of the regenerated bone tissue ($p < 0.05$). The high efficiency of the HA + PRP complex for mechanical stability of the regenerated bone did not statistically significantly different from the high biomechanical properties of the contralateral bone without a defect ($p > 0.05$). In addition, the radiographic picture of the healing of the bone defect of the femur of rats on day 14 in the HA group was almost 2.5 times better than in the PRP group and almost 5 times higher than in the CG group.

The results of previous studies showed that PRP is highly effective in replacing a bone defect only when used together with a bone graft (34, 35). There is also evidence of the effectiveness of the combined use of PRP and synthetic hydroxyapatite, in contrast to the separate use in a rabbit bone defect model (36). Nevertheless, there are some contradictory data on histological and biomechanical bone repair after PRP application without bone grafts (37).

Our results showed their consistency, since the bone defect must certainly be filled with a material acting as a bone framework, for example, calcium hydroxyapatite (38). PRP, due to its known properties, can act as only an auxiliary material that potentiates the process of restoring the strength of bone tissue, but not paramount (39). It can be explained by the fact that hydroxyapatite has the ability to activate platelets, due to its bio-degradability and bio-absorbability, which allows calcium ions to be released (40).

The nano-crystalline calcium hydroxyapatite used in our study had a calcium / phosphorus ratio of 1.67, which is identical to the level of calcium hydroxyapatite in the human bone structure. In fact, the eggshell contained 94 % calcium bicarbonate (9), which indicates its applicability and availability for the effective replacement of bone defects. It has been shown that the use of nano-structured hydroxyapatite in bone tissue replacement increases its bioactivity in comparison with the application of large hydroxyapatite particles (3). The abovementioned properties of nano-crystalline calcium hydroxyapatite might contribute to the effectiveness in the replacement of induced defect of the rat femur observed in our study.

The results of this preclinical study indicate that nano-crystalline calcium hydroxyapatite obtained from eggshells is effective in the regeneration of bone defects. The advantages of the use of natural calcium hydroxyapatite are associated with its good bioavailability, safety and cost-effectiveness. The application of hydroxyapatite in combination with PRP demonstrated the high efficacy in high consolidation of the bone defect, marinating the mechanical strength and improved resistance of the regenerated bone tissue. Our findings indicate the need in further intensive research regarding clinical relevance, safety and effectiveness.

ACKNOWLEDGEMENT

The authors are thankful to the S.D. Asfendiyarov Kazakh National Medical University for the administrative and technical support.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

FUNDING

This research received no external funding.

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THE EFFECTS OF ANTIDEPRESSANT THERAPY ON HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH A CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND DEPRESSIVE SYMPTOMS

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Received: 18.11.2021.
Accepted: 19.12.2021.

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ABSTRACT

Symptoms of depression are often present in patients with chronic obstructive pulmonary disease (COPD) and treatment of depression may substantially improve the quality of life of such patients. The aim of our study was to investigate factors that influence the efficacy of antidepressant therapy in terms of the quality of life in patients with COPD and a depressive disorder. The study was designed as a prospective cross-sectional study and conducted between October 2016 and December 2019 in the Primary Health Center, Kragujevac, Serbia. The study sample included 87 patients. Associations between putative risk factors and change in the quality-of-life score were tested by a multivariate linear regression model and interpreted by the regression coefficients. Our study showed a clear positive effect of therapy with SSRIs on the severity of depression symptoms and the quality of life of patients with co-occurrence of COPD and depression. However, multiple linear regression shows that the effect of SSRIs was more prominent in patients with a higher degree of COPD severity since patients with lower FEV1 values had a more extensive increase in the Q-LES-Q-SF score (B=-0,034; p=0,020). Treatment of depression that accompanies COPD is an important segment of managing such patients, which significantly improves HRQoL. Patients with more severe COPD would especially benefit from such treatment since their response to SSRIs is more pronounced.

Keywords: *Chronic obstructive pulmonary disease, depression, antidepres-sants, quality of life.*

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UDK: 616.24-036:616.89.454-085
615.214.32
Eabr 2024; 25(3):243-247
DOI: 10.2478/sjecr-2021-0068

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease and is the third leading cause of death worldwide (1). COPD adversely affects the quality of life of patients, decreasing the number of Disability Adjusted Life Years (DALY) one can get during lifetime (2). A number of countries organized national registries of COPD patients, showing a relatively high prevalence of this disease, which ranges between 4 and 5% (3-5). Numerous factors contribute to the development of COPD. Long-term tobacco smoking associated with outdoor, occupational and indoor air pollution and genetic predisposition is one of the main factors contributing to the development of obstructive pulmonary disease (1,2,6). The severity of COPD also depends on the age and sex of the patient, frequency of exacerbations, clinical picture, and results of diagnostic procedures (2,7).

There are many other serious diseases and chronic medical conditions that may co-occur in individuals with COPD. Symptoms of depression are often present in COPD patients, with prevalence of 15,2% to 35,7% (8). However, it is difficult to distinguish between true depression and the depressive reaction of COPD patients, because COPD itself affects some basic life functions and roles. A lot of mental and physical symptoms are related to both disorders: increased fatigue, sleep and appetite disorders, reduced physical activity, difficulty with concentration (9,10). People with depression also smoke more often and more intensely, further aggravating COPD. Depression in patients with COPD further reduces their working ability as well as the quality of life (11). Several studies showed that some respiratory and physical symptoms were directly related to depression symptoms in patients with COPD (12,13).

The aim of our study was to investigate the factors that influence the efficacy of antidepressant therapy in terms of the quality of life in patients with COPD and a depressive disorder.

MATERIAL AND METODS

The study was set up as a prospective cross-sectional study and conducted between October 2016 and December 2019 in the Primary Health Center, Kragujevac, Serbia. The inclusion criteria were adulthood, diagnosis of COPD established by a specialist of pulmonology and diagnosis of any type of depression by a specialist of psychiatry. The exclusion criteria were pregnancy and co-morbid malignant diseases. A diagnosis of COPD was established on medical history, current symptoms, suggestive findings from physical examination, and available pulmonary function tests, as per the definitions provided by GOLD criteria. The estimate of COPD severity was based on the post-bronchodilator forced expiratory volume in the 1st second (FEV_1). The study sample included 87 patients. The study was approved by the Institutional Ethical Review Board and Ethics Committee of the Primary Health Center, Kragujevac, Serbia (No: 01-542/2, 2016).

The severity of depression in the study patients was evaluated by the Hamilton Scale (HAM-D), and health-related quality of life was assessed by short form of the Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF) (14-16). The evaluations were made before and after 8 weeks of treatment with selective serotonin reuptake inhibitors (SSRIs) administered according to the preference of psychiatrists who treated the patients. The data about demography, habits, concomitant diseases and concomitant therapy were extracted from the patient files.

The data were described by descriptive statistics, using measures of central tendency (mean or median), variability (standard deviation from the mean) and relative numbers. The differences in the values of continuous variables in the same patients at the beginning and the end of the study were tested by Wilcoxon signed rank test, or by Friedman's test, where applicable.

The differences were considered significant if the probability of the null hypothesis was ≤ 0.05. Associations between putative risk factors and change in the quality of life score were tested by a multivariate linear regression model, and interpreted by the regression coefficients. Previously it was tested whether the data met assumptions for linear regression (linear relationship, homoscedasticity, no-multicollinearity and normal distribution of residuals). All calculations were performed by the SPSS (Statistical Package for Social Science for Windows) software, version 18.

RESULTS

Characteristics of the study group are shown in Table 1, as well as changes in HAM-D scale score and Q-LES-Q-SF score from basal to values after 8 weeks of therapy. Both scores showed statistically significant improvement after 8-week therapy with SSRIs. Patients' overall satisfaction with the treatment and life, in general, were rated with questions 15 and 16 of the Q-LES-Q-SF scale. Friedman's test showed that ratings of both questions significantly improved after treatment with the SSRIs: ($Fr=15.00$, $df=1$, $p<0.001$) and ($Fr=78.00$, $df=1$, $p<0.001$), respectively.

Table 1. Characteristics of the study (n=87) group, and main outcomes.

Variable		Values	
Age (years)		48.84±7.43	
Gender (male)		30 (34.5%)	
FEV_1 (%)		74.79±15.07	
mMRC	Stage 1	34 (39.1%)	
	Stage 2	43 (49.4%)	
	Stage 3	10 (11.5%)	
HAM-D scale score before SSRIs therapy		15.79±4.43	Related samples Wilcoxon signed rank test p<0.001
HAM-D scale score after 8 weeks of SSRIs therapy		10.02±3.97	
Q-LES-Q-SF score before AD		32.43±5.51	Related samples Wilcoxon signed rank test p<0.001
Q-LES-Q-SF score after AD		46.65±4.79	
Difference in Q-LES-Q-SF score (after – before)		14.20 ± 2.02	

A multiple linear regression model was built including the following predictors of difference between values of the Q-LES-Q-SF score after and before the treatment with SSRIs: gender, age, mMRC, FEV_1, HAM-D scale scores before and after 8 weeks of SSRI therapy. The only significant predictor turned out to be the FEV_1 (p=0,020) (Table 2).

Table 2. Significant predictor of difference between values of the Q-LES-Q-SF score after and before the treatment with SSRIs.

Predictor	Adjusted R^2	F	B	t	p	95,0% Confidence Interval for B	
						Lower Bound	Upper Bound
FEV_1	0.051	5.625 (p=0.020)	-0.034	-2.372	0.020	-0.062	-0.005

DISCUSSION

Our study showed a clear positive effect of therapy with SSRIs on both severity of depression symptoms and the quality of life of patients with co-occurrence of COPD and depression. However, the effect of SSRIs is more prominent in patients with a higher degree of COPD severity, since patients with lower FEV_1 values had a more extensive increase in the Q-LES-Q-SF score.

Inverse correlation between the severity of COPD and health-related quality of life (HRQoL) was shown in many studies: dyspnea, nocturnal symptoms, reduced physical activity, and frequent exacerbations affect adversely physical, psychological and social aspects of a patient's life (17). More severe symptoms result in lower quality of life, and vice versa. On the other hand, it is well known that patients with worse depression have more visible improvement after a period of treatment with antidepressant medication, because their baseline was lower in the beginning and maximal therapeutic response cannot be pushed further (18). This effect was first proven for tricyclic antidepressants, and then shown also for SSRIs. It seems that the same holds true for other types of antidepressants, including noradrenaline reuptake inhibitors (NARIs) like reboxetine (19). Our results speak in favor of the fact that improvement in depressive symptoms is more pronounced in patients with more severe COPD; this underlines the necessity to recognize early symptoms of depression in patients with COPD and implement treatment with SSRIs or other antidepressants.

Adherence to prescribed medication is crucial for the beneficial outcome of treatment in COPD patients (20). Since the adherence is improved if the patients are less depressive and anxious (21), if depressive symptoms are recognized early in a patient with COPD, and treatment with SSRIs started without delay, the patients will be more adherent to prescribed bronchodilators and other COPD medication, with resultant improved outcome. Improved COPD would then additionally help with the decrease of symptoms of depression, and a beneficial circle will be established, resulting in the end with minimized COPD symptoms and maximized quality of life.

There are certain limitations of our study. In the first place, our study was based on a non-randomized sample, which may introduce a certain degree of selection bias. Second, the size of the sample was relatively small, so only the factor with really strong statistical influence could have been delimited as significant (severity of COPD), while a number of also important factors were subject to type two statistical error.

In conclusion, our study showed that treatment of depression that accompanies COPD is an important segment of managing such patients, which significantly improves HRQoL. The patients with more severe COPD would especially benefit from such treatment since their response to SSRIs is more pronounced.

ACKNOWLEDGEMENT

None.

FUNDING

None.

CONFLICT OF INTEREST

None of the authors has any conflict of interest regarding the content of this manuscript.

AUTHOR CONTRIBUTIONS

I.J., F.M. and G.M. contributed to the concept and design of the study and collected data. M.F. and S.M. participated in the study design, analyzed and interpreted data and wrote the manuscript.

Each author listed on the manuscript has seen and approved the submission of this version of the manuscript.

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IMPACT OF THE SARS-COV-2 OUTBREAK ON THE EPIDEMIOLOGY AND TREATMENT OUTCOMES OF FRACTURES OF THE PROXIMAL FEMUR IN KAZAKHSTAN

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Received: 24.09.2021.
Accepted: 28.11.2021.

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UDK:616.718.4-001.5(574)"2010/2020
616.98:578.834]:615.863(574)
Eabr 2024; 25(3):249-258
DOI: 10.2478/sjecr-2022-0026

ABSTRACT

The study aimed to assess the impact of isolation and quarantine on the frequency of registration and the treatment of fractures of the proximal femur in Kazakhstan in the context of the COVID-19 pandemic in 2020 (compared to the pre-pandemic period).
This retrospective observational comparative study included all primary patients with injuries (the code S72) in the period 2019-2020 according to the national register.
In 2020, the number of S72 fractures was 6.6 % higher compared to 2019. In comparison with 2019, in 2020 the number of bed-days of patients was reduced to 7.1±3.8 days ($p \leq 0.001$). Both in 2019 and in 2020, the number of women predominated among all patients ($p \leq 0.05$). The frequency of conservative treatment in 2020 compared to 2019 was increased from 26.6% to 35.6%, while the surgical procedure for internal fixation was reduced to 34.2% in 2020. In 2020, the highest number of cases among women with S72 fractures cases were recorded in the age groups 60-74 years and 75-90 years. In 2019 in female patients (42%) with S72 cases were registered in the age group 75-90 years.
The incidence of fractures of the proximal femur did not change significantly in 2020 compared to 2019. However, the number of conservative treatment methods has increased along with the decrease in the frequency of surgical interventions. We observed the growth of the frequency of non-surgical treatment methods in 2020 that might impose the possible risks of mortality of these patients in the long term after conservative treatment.

Keywords: *Fracture, femur hip, epidemiology; COVID-19 pandemic, Kazakhstan.*

INTRODUCTION

In December 2019, an outbreak of a new coronavirus infection (COVID-19) caused by the SARS-CoV-2 virus was reported in the Chinese province of Wuhan (1). The first case outside China was reported on January 13, 2020, in Thailand (2). The outbreak quickly spread from China to many countries around the world, and extremely concerned about the alarming spread of the infection and the severity of the consequences, the World Health Organization (WHO) declared the COVID-19 outbreak as a pandemic on March 11, 2020 (3). In Kazakhstan, since the registration of the first cases of COVID-19 infection on March 13, 2020 (4), despite the anti-epidemiological measures taken, there was a fairly high incidence amongst various age groups of the population (5). Given the severity of the epidemiological situation, health care providers have never faced such a dramatic epidemic wave before. Moreover, most health systems around the world were insufficiently equipped to deal with this serious disease (6).

In order to provide adequate medical care to combat the epidemic and growing need for drugs and intensive care units, almost all departments of hospitals in most countries of the world, including the departments of orthopedics and traumatology, were forced to re-profile into infectious diseases departments (7, 8).

The COVID-19 pandemic forced orthopaedic surgeons to consider alternative treatments for many aspects of orthopaedic emergencies and injuries, by modifying standard management plans to minimize patient exposure and overall impact on resources (9). In many countries, this also led to a deliberate reduction in planned operations, which led to an increase in morbidity rates (10).

The reduction in the number of planned hospitalizations has also led to the freeing of hospital beds and equipment (including ventilators) for the care of patients with COVID-19 (11). Although, due to the severe restrictions on movement associated with the lockdown, there was a significant reduction in the number of some injuries, such as sports, road transport, industrial ones (12). According to some reports, COVID-19 led to a slight decrease in the number of patients with fractures around the world, although according to some reports, it was noted that the frequency of brittle fractures remained unchanged (12). A brittle fracture of the proximal femur, otherwise called a “hip fracture,” is of particular importance in the management of trauma in elderly patients (13). Fractures of the proximal femur are cause of a high mortality, and they require the provision of surgical intervention within 48 hours after injury (12). However, in some cases conservative treatment methods are also applicable (14).

In general, the COVID-19 pandemic has made a negative impact on the healthcare system of all countries, including the Republic of Kazakhstan (15). However, due to an unstable epidemiological situation caused by COVID-19, its impact on the prevalence rate is still unclear. It also encompasses the problems of treatment choice (operative or conservative) for fractures of the proximal femur.

This study was aimed at assessing the impact of isolation and quarantine on the frequency of registration and the nature of the treatment of fractures of the proximal femur in the Republic of Kazakhstan in the context of the COVID-19 pandemic in 2020 (compared to the pre-pandemic period).

MATERIALS AND METHODS

Ethical Issue

To form the study cohort, we used patient data of registered cases of fractures of the proximal femur in the 2019-2020. For this study, anonymous information from the national registry of inpatients was utilized. The study was conducted in accordance with the high ethical standards of the state and national research committee. Due to the retrospective nature of the epidemiological study and the use of anonymous patient data, there was no need to obtain informed consent.

Data collection

Data for this multicenter, observational, retrospective, descriptive study were obtained from the electronic patient registry of trauma hospitals from all 17 regions of the Republic of Kazakhstan. The electronic register is a common base for collecting and storing medical data from all public trauma hospitals. Materials from the electronic register for the period from 01.01.2019 to 31.12.2020 were provided and approved by the "Republican Center for Electronic Health" of the Ministry of Health of the Republic of Kazakhstan.

For the study, the medical information of patients with fractures of the proximal femur under the code S72 was used in accordance with the 10th revision of the International Classification of Diseases (ICD-10). According to the ICD-10 classifier, the following pathologies from the S72 pathology groups were analyzed: S72.0 Fracture of the femoral neck; S72.1 Transtrochanteric fracture; S72.2 Subtrochanteric fracture; S72.3 Fracture of the body of the femur; S72.4 Fracture of the lower end of the femur; S72.7 Multiple fractures of the femur; S72.8 Fractures of other parts of the femur; S72.9 Fracture of the part of the femur, unspecified.

Demographic data such as age and gender of patients were analyzed as well. By age, the patients were divided into 6 age groups: 0-17; 18-44; 45-59; 60-74; 75-90; and 90 years of age or older.

In addition, the following indicators were determined as well: the place of residence (city or village), the capacity of the hospital for hospitalized patients depending on the number of beds, and the month of admission of patients (less than 300; from 300 to 500; more than 500 beds).

By the type of hospitalization: patients with planned or emergency hospitalization were involved in the study.

The patients were divided into 7 groups based on the type of referral of patients to the hospital: referred by ambulance, KDP, another hospital, self-referral, primary care, maternity hospital and others.

According to the outcome of inpatient treatment, the following categories were identified: improvement, recovery, no change, death and deterioration.

The patients received the following assistance in regard to the type of treatment performed: conservative treatment (non-surgical), internal fixation, total arthroplasty, hemiarthroplasty, and more.

Besides, we also studied the gender and age characteristics of patients and the territorial feature of cases of fracture of the proximal femur, depending on the period of hospitalization.

Statistical analysis

Statistical analysis was conducted using SPSS software (version 25.0, IBM SPSS Inc., Chicago, Illinois, USA). A general descriptive analysis was carried out in general and by period, including indicators of the central trend for quantitative variables. Continuous variables were presented as mean and standard deviation, and categorical variables were presented as number and percentage. We utilized demographic information obtained from the Committee on Statistics of the Ministry of National Economy of the Republic of Kazakhstan on the total population of the Republic of Kazakhstan.

RESULTS

20351 patients with fractures of the proximal femur were registered for 2019-2020 in Kazakhstan, of which n = 9514 (46.7 %) in 2019 and n = 10837 (53.3 %) in 2020. Clinical and demographic characteristics of hospitalized patients are presented in Table 1.

The average age of patients in 2019 was 58.8 ± 24.92 years, and in 2020, 57.32 ± 25.2 years. By sex, both in 2019 and in 2020, women prevailed among patients, the number of whom was 56 % (n = 5329) and 55.7 % (n = 6041), respectively.

By place of residence in 2019 and in 2020, urban residents also prevailed, with indicators equal to 59.7 % (n = 5682) and 64.1% (n = 6944), respectively ($p \leq 0.05$).

Depending on the hospital capacity in 2019, 58.5 % (n = 5565) of patients were hospitalized in hospitals with less than 300 beds, and 7.4 % (n = 701) of patients were hospitalized in inpatients with more than 500 beds ($p \leq 0.001$). In 2020, 65.4 % (n = 7087) were hospitalized in hospitals with less than 300 beds, and 3.4 % (n = 371) patients were hospitalized in hospitals with more than 500 beds (n = 371) ($p \leq 0.001$).

According to the admission period, the highest frequency of registration of fractures of the proximal femur was observed in the autumn-winter period. In 2020, the highest patient registration rate was registered in December 10.9 % (n = 1185) compared to December 2019 6.8 % (n = 674) ($p \leq 0.001$). Apart from that, in April, July and October 2020, cases of fractures of the proximal femur were recorded more compared to identical months in 2019, which was regarded as a statistically significant difference ($p \leq 0.05$).

By the type of hospitalization, in 2020 the number of patients 21.6 % (n = 2344), hospitalized in a planned manner, in contrast to 2019, was 4.3 % (n = 408) ($p \leq 0.001$). The number of patients hospitalized for emergency indications in 2019, 95.7 % (n = 9106), was 78.4 % (n = 8493) ($p \leq 0.05$) compared to 2020.

By type of referral to inpatient treatment, the largest number of admissions was for emergency ambulance, which amounted to 47.7 % (n = 4538) in 2019 and 38.3 % (n = 4142) in 2020 ($p \leq 0.05$).

According to the outcome of hospital treatment in 2019, patients were discharged with an improvement of 90.7 % (n = 8632) and 91.4 % (n = 9903).

By type of treatment, in 2020 n = 3848 (35.6 %) patients were treated without surgery, and in 2019 the number of patients treated with a conservative method was less (26.6 %, n = 2530). Internal fixing operations were carried out more in 2019 in 54.7 % (n = 5202) cases, compared to 2020, where this indicator was 34.2 % (n = 3706). Total arthroplasty in 2019 and 2020 was performed in 11.5 % (n = 1091) and 13.6 % (n = 1474) cases, respectively. Statistically significant differences were found for hemiarthroplasty, since in 2020 compared to 2019, the number of this surgical procedure increased from n = 418 to n = 819 cases ($p \leq 0.05$). In addition, the number of other medical measures carried out in 2019 was 2.9 % (n = 273), which was lower than the indicators of this procedure in 2020 equal to 9.1 % (n = 990), with a statistically significant difference ($p \leq 0.05$).

The frequency of registration of various forms of S72 according to ICD-10, depending on the age category of patients in the context of 2019-2020 in the territory of the Republic of Kazakhstan, is presented in Table 2. In the age category 0-17 years in 2019, the number of fractures in general was n = 1150, and in 2020, the number of cases of S72 children was increased to n = 1397, with no statistically significant difference. In pediatric patients in 2020, n = 5 (0.4 %) cases of fracture of the unspecified part of the femur (S72.9) were recorded. In 2019, in children aged 0-17 years, this diagnosis was not made at all, which was considered statistically significant difference ($p \leq 0.001$). In the age group 60-74 years compared to 2019 (n = 2849), the total number of S72 in 2020 was higher, amounting to n = 3398, but no statistically significant difference was found. And in the group of patients 75-90 years old for all groups of nosology S72, both in 2019 and in 2020, there were almost identical rates of registration of these fractures with an insignificant difference, except for the diagnosis S72.9,

which in 2020 was defined as n = 7 (0.2 %) cases in comparison with 2019 without a single registration S72.9 ($p \leq 0.001$).

It should be noted that in the oldest age group of patients (90 years and older) diagnosed with S72.0 (hip fracture) in comparison with 2019. In 2020, there was a decrease in the number of cases of registration of this type of fracture (n = 75 vs n = 58), with a statistically significant difference ($p = 0.041$). However, according to the diagnosis S72.1 (pertrochanteric fracture) in patients aged 90 years and older compared to 2019, in 2020 there was a statistically significant increase in the detection of these injuries from n = 95 (45.0 %) to n = 112 (59.3 %) cases, respectively ($p = 0.012$).

The gender and age characteristics of patients with fractures of the proximal femur are presented in figure 1. In 2019, among all hospitalized female patients, 42 % (n = 2239) of cases of fractures of the proximal femur were recorded in the age group 75-90 years, and among men in a given year, 24 % (n = 1006) of patients were 60-74 years old. In 2020, the highest number of cases of fractures of the proximal femur among women in 37.1 % (n = 2239) and 37.5 % (n = 2265) cases were recorded in the age groups 60-74 and 75-90 years, respectively. And among men in 2020, the highest number of cases of fractures of the proximal femur was determined in the age group 60-74 years with an indicator equal to 24.2 % (n = 1159) cases.

The territorial characteristics of cases of registration of fractures of the proximal femur, in the context of 2019 and 2020 are presented in figure 2. In 2019, in the territory of the Republic of Kazakhstan, the largest number of cases of fractures of the proximal femur was registered in the city of Almaty (n = 1419). However, in Almaty city (2020), there was an increase in the number of these fractures (n = 1545), which was regarded as a statistically significant difference ($p \leq 0.05$). Apart from that, high registrations of fractures of the proximal femur were identified in 2019 (n = 1545) and 2020 (n = 1545), in the Karaganda region, but without a statistically significant difference. A statistically significant difference was determined for cases of femoral fractures in the Almaty region, where in 2020 (n = 1324), this pathology was recorded more in comparison with 2019 (n = 1116), $p \leq 0.05$. In addition, in the Zhambyl region, in comparison with 2030 (n = 651) in 2019 (n = 478), fewer cases of femoral fractures were recorded ($p \leq 0.05$). In the Turkestan region, a high level of registration of this pathology was also determined in 2020 (n = 1011), in contrast to 2019 (n = 847), $p \leq 0.05$.

DISCUSSION

To the best of our knowledge, this is the first analysis of trauma care and treatment outcomes for patients with proximal femur fractures in Kazakhstan in the context of the COVID-19 pandemic in 2020. It should be taken into account that, according to statistical sources, more than 130 nationalities live on the territory of Kazakhstan (14), among ethnic groups Central Asians make up a large share (Kazakhs, Uzbeks, Tatars, Kyrgyz, Uighurs, Tajiks, Turkmen, etc.) (14). This fact provides an opportunity to extrapolate the results obtained from this study for the entire Central Asian region. The statistics available for review shows that in the orthopedic area the largest number of operations is associated with fractures of the proximal femur, which are the most common cause of disability in patients over 65 years of age (16).

According to the results obtained in Kazakhstan in 2020 (regardless of the fact that since March 2020 there was a strict lockdown in the country), the number of cases of fracture of the proximal femur did not decrease in comparison with 2019. Moreover, we observed a slight increase of 6.6 % ($p \leq 0.05$).

Relative growth in S72 cases in 2020 compared to 2019 was observed in both men and women. It should also be noted that, as in 2019 and in 2020, the S72 fracture was more typical for women aged 75-90 years in comparison with men ($p \leq 0.05$). However, the results of our study do not find agreement with a study conducted in Italy, where, thanks to restrictive measures for the free movement of people during quarantine, the number of S72 fractures, like other fractures, was reduced (16). In addition, our results do not conform with the data of another study conducted in Turkey, where the number of fractures in 2020 due to strict restrictive quarantine and due to a fairly high proportion of postponement of planned operations during the quarantine period was reduced by 1/3 of cases compared to COVID period (17).

There is some evidence that there is a link between falling and aging, as the frequency of falls increases significantly with age (18). The increase in the number of femoral fractures among the elderly population revealed in 2020 could be associated with the mechanism of femoral fractures. It is known that such injuries are often the result of low-energy trauma (falling from a standing height and falling from a low height) that usually occur at home, which emphasizes the importance of primary prevention measures (prevention at home). This finding finds an agreement with the results of a retrospective multicenter cohort study conducted in the UK, where the number of femoral fractures in 2020 was almost identical to the rates of this type of fracture in 2019 (19). This reflected the fact that for this category of patients, falling at home is the most common cause for a femur fracture. Therefore, social distancing and isolation during the COVID-19 pandemic could not affect the frequency of its registration (20).

The same features were revealed in Spain, where the absolute volume of hospitalizations with osteoporotic hip fractures remained stable during the state of emergency in Spain (21). In another study conducted in Turkey, an increase in the number of cases of fractures of the proximal femur was noted, which could be explained by a decrease in mobility of people during a pandemic, and it was also published that the etiology of the development of this type of hip fracture of osteoroporotic genesis was associated in 31.2 % as a result of

slipping during ablution (a Muslim ceremony before prayer, which consists of washing the face, neck, hands and feet) (22). Given the fact that a high proportion of residents of Kazakhstan are Muslims (23), there is an assumption that this reason could also affect the increase in the frequency of falls for this reason, which could lead to the development of a fracture of the femur.

During the worst phase of the pandemic in China, according to an earlier study, 87 % of fractures were from standing falls in people with an average age of 76; and 72.7 % of patients suffered their fractures at home (24). Of the 453 fractures analyzed by these authors, 264 (53.8 %) were hip fractures (24).

It should be noted that according to the results of this study, osteoporosis in women and in older men could potentiate the risks of fractures due to low-energy trauma. In fact, the risk of developing any osteoporotic fracture at the age of 50 ranges from 40 to 50 % in women and 13-22 % in men (25). In addition, in patients with osteoporosis after a hip fracture, the risk of developing another hip fracture doubles in women and triplets in men (26). The risk increases to 9% in the first year and up to 20 % in 5 years (27). These findings indicate the necessity of proper prevention and treatment of osteoporosis at all stages of medical care (28). The high rate of osteoporotic fractures increases the risks of non-earmarked expenses from the state budget. Instead of directing funds to prevent fractures, the budget will be spent on the treatment and social benefits for disability (29).

Apart from that, the results of our study demonstrate that the frequency of S72 fractures increased in winter periods (2019-2020). This finding agrees with the results of previous studies (30, 31). It indicates that an activity of elderly in cold period plays an important role in the development of this type of fracture (31).

There was an increase in the rate of hospitalization of patients with S72 fractures in hospitals with fewer than 300 beds (65.4%) in 2020 compared with hospitals with more than 500 beds ($p \leq 0.001$). Such a finding could be explained by the fact that large multidisciplinary hospitals (more than 500 beds) were converted into infectious diseases hospitals in 2020 due to pandemics. In fact, the same temporary reorganization aimed at providing care to patients with COVID-19 (32) was carried in all countries around the world.

The number of conservative treatments of fractures of the proximal femur increased in 2020, with a decrease in the number of internal fixation operations. The rise of the frequency of conservative therapy in the treatment of fractures in 2020 could be associated not only with direct indications, but could also be linked with the epidemiological situation with COVID-19. First of all, it can be caused by lowering of the incidence of invasive surgical procedures. In an earlier study in a rural hospital in the UK, it was also noted that in the COVID period, the frequency of conservative treatment of femoral fractures increased 3 times compared with the pre-COVID period, and the length of stay of these patients in the hospital decreased ($p \leq 0.001$) (33). This finding can be explained by the exacerbation of chronic concomitant diseases, including in a number of patients with unstable cardiac pathology, which required intensive medical intervention (33). Despite the increase in the use of conservative methods in the treatment of S72 fractures in 2020, no statistically significant differences were found in 2019-2020 in treatment outcomes (mortality, etc.) at the time of discharge from the hospital.

The data obtained in our study find some agreement with the results of another study, where, hospital mortality was the same between patients treated promptly (14.9 %) and conservatively (18.1 %) (14). In the same study, when assessing one-year mortality rates, mortality was significantly higher in the group of patients treated conservatively (67.0 %) in comparison with patients who underwent surgery (48.2 %) ($p = 0.005$) (14). However, there is a range of studies where no significant differences were found in one-year mortality after conservative or surgical treatment of fractures of the proximal femur (34). Given that the frequency of conservative treatment was increased in 2020, it should be noted that this circumstance may have some undesirable effect on treatment outcomes in the long-term perspective. In our study, among all S72 pathologies, femoral neck fracture (S72.0) and pertrochanteric femoral fracture (S72.1) were the most frequently reported types of femoral fracture. The results obtained show that in the context of 2019-2020, the age category of people from 60 to 90 years old is a risk group for the development of S72.

The results of study conducted by Lv et al. indicate that the most common mechanism of injury during a pandemic was low energy trauma (i.e. sliding, stumbling, or falling at home), in 79.1 % of cases compared with 34.4 % of low energy fractures in the control group (2019) (35). All these facts demonstrate the need for appropriate preventive measures to prevent osteoporosis (36) and reduce the incidence of low-energy household injuries among the elderly population.

In 2020, compared with the pre-COVID period (2019), the incidence of fractures of the proximal femur did not change significantly. However, the number of conservative treatment methods was increased with a corresponding decrease in the frequency of surgical interventions. Given the increase in the frequency of use of non-surgical methods of treatment in 2020, and the potential risks of mortality in patients in the long term after this type of treatment, the results of this study can be useful for the health care system. It includes the optimization of rehabilitation of patients who underwent conservative treatment at the PHC level. In addition, the findings of this study can be used for forecasting the possible shortage of hospital beds and management of patients with priority diseases in the case of high hospital occupancy caused by an epidemiological emergency.

Table 1. Clinical and demographic characteristics of hospitalized patients with S72.

Characteristics	2019	2020	p
Age	58.8±24.92	57.32±25.2	
Number of bed days	10.8±4.7	7.1±3.8	**0.001***
Sex			
male	4185(44.0)	4796(44.3)	
female	5329(56.0)	6041(55.7)	
Residence			
city	5682(59.7)	6944(64.1)	**0.05**
village	3832(40.3)	3893(35.9)	**0.05**
Number of beds			
less 300	5565(58.5)	7087(65.4)	**0.001**
300 to 500	3248(34.1)	3379(31.2)	
more 500	701(7.4)	371(3.4)	**0.001**
Time of admittance			
January	1004(10.6)	978(9.0)	
February	818(8.6)	1046(9.7)	
March	799(8.4)	935(8.6)	
April	783(8.2)	648(6.0)	**0.05**
May	825(8.7)	891(8.2)	
June	767(8.1)	841(7.8)	
July	754(7.9)	688(6.3)	**0.05**
August	783(8.2)	857(7.9)	
September	802(8.4)	920(8.5)	
October	735(7.7)	942(8.7)	**0.05**
November	770(8.1)	906(8.4)	
December	674(6.8)	1185(10.9)	**0.001**
Type of hospitalization			
Planned	408(4.3)	2344(21.6)	**0.001**
Emergency	9106(95.7)	8493(78.4)	**0.05**
Who is directed to hospitalization			
Emergency team	4538(47.7)	4142(38.3)	**0.05**
Out-patient clinic	600(6.3)	751(6.9)	
Other hospital	801(8.4)	937(8.6)	
Self-admittance	1800(18.9)	2540 (23.5)	
PCP	1453(15.3)	2466(22.7)	
pMaternity Unit	322(3.4)	1(0.01)	**0.001**
Outcome			
improvement	8632(90.7)	9903(91.4)	
convalescence	359(3.8)	319(2.9)	

Characteristics		2019	2020	p
	no changes	395(4.2)	485(4.5)	
	death	124(1.3)	119(1.1)	
	deterioration	4(0.04)	11(0.1)	
Treatment types				
	No operation (standard)	2530(26.6)	3848(35.6)	
	Innner fixation	5202(54.7)	3706(34.2)	
	Total arthroplasty	1091(11.5)	1474(13.6)	
	hemiarthroplasty	418(4.4)	819(7.5)	**0.05**
	Others	273(2.9)	990(9.1)	**0.05**
Total		9514(46.7)	10837(53.3)	

Data presented as a frequency (%);

Table 2. The frequency of registration of various forms of S72 according to ICD-10 in patients standardized by age in the context of 2019-2020 in the territory of the Republic of Kazakhstan.

Diagnosis according to ICD-10	**Age according to the WHO classification**								
	0-17			18-44			45-59		
	2019	2020	p	2019	2020	p	2019	2020	p
72.0 Fracture of the femoral neck	68 (5.9)	69 (4.9)	0.245	190(18.2)	221(16.9)	0.475	479(34.4)	632(38.4)	0.548
72.1 Transtrochanteric fracture	8 (0.7)	18 (1.3)	0.452	129(12.4)	189(14.4)	0.221	422(30.3)	404(24.6)	0.079
72.2 Subtrochanteric fracture	61(5.3)	105 (7.5)	0.324	104(10.0)	131(10.0)	0.854	106(7.6)	130(7.9)	0.354
72.3 Fracture of the body of the femur	816 (71.0)	973 (69.6)	0.108	384(36.8)	495(37.8)	0.763	160(11.5)	200(12.2)	0.124
72.4 Fracture of the lower end of the femur	153 (13.3)	182 (13.0)	0.789	148(14.2)	168(12.8)	0.367	176(12.7)	214(13.0)	0.842
72.7 Multiple fractures of the femur	13 (1.1)	11 (0.8)	0.452	53(5.1)	51(3.9)	0.471	19(1.4)	20(1.2)	0.541
72.8 Fractures of other parts of the femur	31 (2.7)	34 (2.4)	0.951	33(3.2)	53(4.0)	0.168	27(1.9)	39(2.4)	0.681
72.9 Fracture of part of femur, unspecified	0 (0)	5 (0.4)	**0.001***	2(0.2)	2(0.2)	0.978	2(0.1)	5(0.3)	0.251
Total	1150	1397	0.547	1043	1310		1391	1644	

Diagnosis	**Age according to the WHO classification**		
	60-74	75-90	90 and more

according to ICD-10	2019	2020	p	2019	2020	p	2019	2020	p
72.0 Fracture of the femoral neck	1141(40.0)	1403(41.3)	0.121	1145(39.9)	1193(41.2)	0.321	75(35.5)	58(30.7)	**0.041***
72.1 Transtrochanteric fracture	988(34.7)	1231(36.2)	0.781	1343(46.8)	1289(44.5)	0.135	95(45.0)	112(59.3)	**0.012***
72.2 Subtrochanteric fracture	185(6.5)	194(5.7)	0.982	133(4.6)	138(4.8)	0.148	13(6.2)	5(2.6)	0.2978
72.3 Fracture of the body of the femur	221(7.8)	229(6.7)	0.457	123(4.3)	110(3.8)	0.625	13(6.2)	6(3.2)	0.752
72.4 Fracture of the lower end of the femur	269(9.4)	282(8.3)	0.247	106(3.7)	126(4.3)	0.425	11(5.2)	8(4.2)	0.097
72.7 Multiple fractures of the femur	18(0.6)	16(0.5)	0.657	9(0.3)	11(0.4)	0.177	0(0)	0(0)	--
72.8 Fractures of other parts of the femur	25(0.9)	40(1.2)	0.197	11(0.4)	25(0.9)	0.684	4(1.9)	0(0)	-
72.9 Fracture of part of femur, unspecified	2(0.1)	3(0.1)	0.541	0(0)	7(0.2)	**0.001***	0(0)	0(0)	-
Total	2849	3398		2870	2899		211	189	

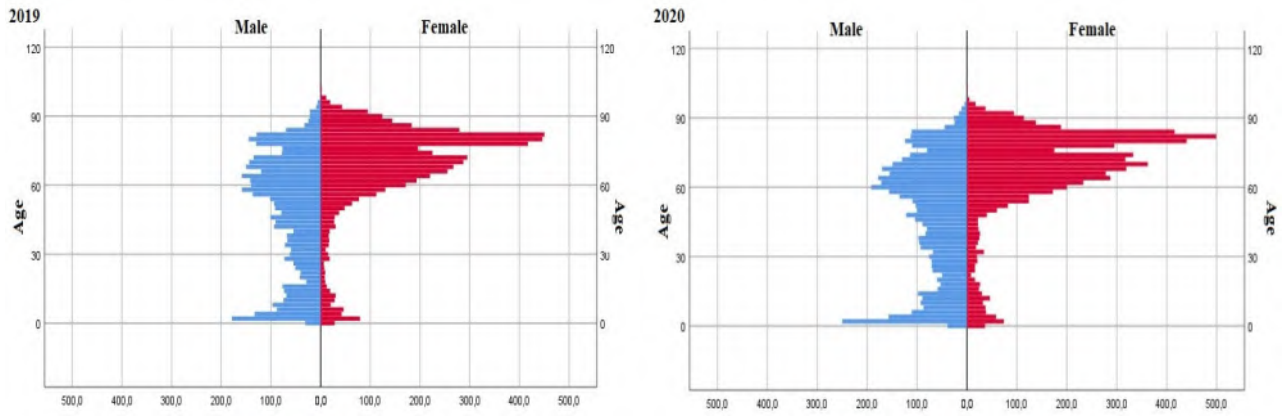

Figure 1. Age and sex characteristics of hospitalized patients with S72 for 2019, 2020.

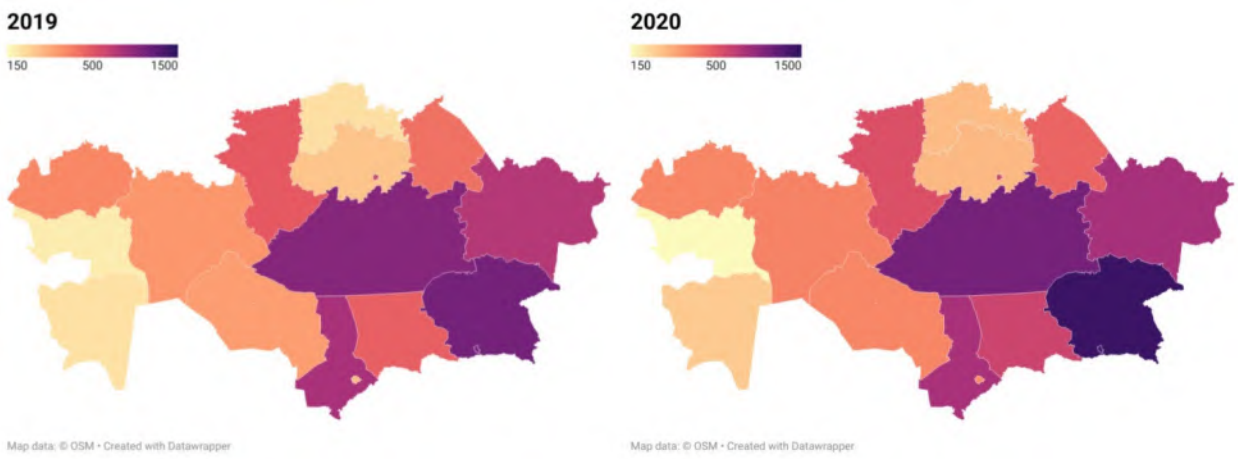

Figure 2. Territorial characteristics of cases of registration of S72 in the Republic of Kazakhstan for 2019-2020.

STRENGTHS AND LIMITATIONS OF STUDY

The retrospective observational design can be considered a limitation of the current study. In addition, the lack of data on long-term outcomes for S72 fractures cannot fully reflect an objective assessment of the impact of the COVID-19 pandemic on the quality of trauma care for this cohort of patients. So that it requires further research in this direction. On the other hand, the study strong sides, including a multicenter approach, a fairly large sample and the fact that this study was conducted for the first time on the territory of Kazakhstan during the pandemics.

ACKNOWLEDGMENTS

The authors are thankful to the S.D. Asfendiyarov Kazakh National Medical University for the administrative and technical support.

FUNDING

The study received no external funding.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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INFLUENCE OF ATRIAL FIBRILLATION ON TWO-YEAR SURVIVAL OF PATIENTS WITH IMPLANTED CARDIOVERTER DEFIBRILLATOR

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Received: 05.02.2022.
Accepted: 19.02.2022.

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UDK: 616.12-085.817"2014/2018"
Eabr 2024; 25(3):259-265
DOI: 10.2478/sjecr-2022-0035

ABSTRACT

Implantable cardioverter defibrillators (ICD) effectively reduce risk of sudden cardiac death in both primary and secondary prevention, but only a small proportion of patients included in the clinical trials had atrial fibrillation. It is still unclear whether patients with atrial fibrillation have the same benefit from ICD implantation as patients in sinus rhythm. This is a clinical, prospective study which included 210 patients in the period 2014-2018. ICD was implanted in the Clinical Center Kragujevac and a two-year follow-up was performed. Patients were divided into 2 groups: a group in sinus rhythm and a group of patients with atrial fibrillation (paroxysmal, persistent and permanent). At the end of the two-year follow-up, there was no difference in survival between the compared groups. The total number of cardioverter defibrillator activations did not differ between the groups, but a significantly higher number of inappropriate cardioverter defibrillator activations was registered in the group with atrial fibrillation. In most patients who had inappropriate defibrillator activation during the two-year follow-up, appropriate defibrillator activation was also registered.

Keywords: *Atrial fibrillation, implantable cardioverter defibrillator, ICD, heart failure, sudden cardiac death.*

INTRODUCTION

Implantable Cardiverter Defibrillator (ICD) is device that is implanted in the patient's body, capable of performing cardioversion, defibrillation and electrostimulation (pacing) of the heart muscle. Today, the use of ICD is the most important primary and secondary prevention of sudden cardiac death (SCD). Primary prevention refers to patients who did not have life-threatening arrhythmia, but based on clinical characteristics, predominantly reduced left ventricular ejection fraction (less than 35%) have an increased risk of life-threatening arrhythmias and SCD. Secondary prevention refers to patients who have previously been successfully resuscitated after cardiac arrest (most commonly caused by ventricular fibrillation) or after life-threatening ventricular arrhythmias, when there is a risk of cardiac arrest recurrence. Large clinical studies have shown superiority of ICD in reducing the incidence of SCD over drug therapy in both primary and secondary prevention (1-5). Atrial fibrillation as the most common arrhythmia is a common comorbidity in patients with reduced left ventricular EF who underwent ICD implantation. Epidemiological studies have shown that atrial fibrillation is associated with increased overall mortality (6), but it is still unclear to what extent it affects mortality in patients with implanted ICD. As one of the potential causes, researchers state a higher frequency of heart failure in the population of patients with atrial fibrillation (7, 8). Heart failure and left ventricular dysfunction affect the development of atrial fibrillation, and atrial fibrillation itself leads to electrical and structural remodeling of the atrial and ventricular myocardium, thus closing the vicious circle. Accelerated heart rate during an atrial arrhythmia, especially in combination with interventricular conduction disorders, may lead to inappropriate activation of the ICD. Inaproppriate ICD activation is device activation and delivery of therapy when there is no life-threatening heart rhythm disorder (e.g. supraventricular arrhythmia or atrial fibrillation) in contrast to appropriate ICD activation. In the MADIT II study 13% of patients with ICD had inappropriate defibrillation shock, where in 44% of cases atrial fibrillation was identified as the main cause (5). Inaproppriate defibrillation shocks as well as appropriate defibrillation shocks may be associated with increased mortality in patients with implanted ICD (9, 10) indicating the need for programming, treatment of underlying disease and heart rhythm disorders to minimize the number of shocks delivered.

Despite the fact that the effective use of ICD devices has been studied in a large number of clinical studies, only a small proportion of patients included in the trials had atrial fibrillation, so it is still unclear whether patients with atrial fibrillation benefit equally from ICD implantation as the patient in synus rhythm.

THE AIM OF THE RESEARCH

To examine whether the presence of atrial fibrillation in patients with implanted cardioverter defibrillators has led to increased mortality and frequency of ICD activation in comparison to patients in sinus rhythm.

PATIENTS AND METHODS

Clinical, prospective study which included 210 patients in the period 2014-2018. who underwent ICD implantation in Clinical Center Kragujevac and had completed two-year follow-up after the implantation. Patients were divided into 2 groups depending on the registered presence of atrial fibrillation. The group of patients with atrial fibrillation consisted of patients who were diagnosed atrial fibrillation (ECG recording of atrial fibrillation lasting minimal 30 seconds), whether paroxysmal, persistent or permanent at any time during follow-up. Group of patients in sinus rhythm consisted of patients without recordings of atrial fibrillalion or other forms of atrial arrhythmias (atrial flutter/atrial tachycardia). All the patients have to meet criteria for ICD implantation (primary or secondary SCD profilaxis). For each ICD implantation, the patient was hospitalized in the Kragujevac Clinical Center, where hospitalization could be elective or urgent. During hospitalization, the following data were collected for each patient: demographic data, risk factors, echocardiographic findings, indications for the implantation of ICD, previous arrhythmic events, associated cardiovascular diseases, lab analysis (blood count, cardiac enzymes, NT-proBNP, CRP, BUN, electrolytes).

ICD device implantation was performed in local anesthesia. Venous approaches were used for placement of electrode catheters: ligation and incision of the cephalic vein or via the subclavian or axillary vein by the puncture method. The positioning of the electrode catheter in the right ventricle was performed under radioscopic control, usually in the interventricular septum or the apex of the right ventricle. The pulse generator is placed subcutaneously prepectorally. Patients who underwent an ICD device implantation came for regular, quarterly check-ups during the next period of 2 years. Exept regular controls urgent or symptom driven check-ups of the petents were also recorded. The following data were collected each time the patient arrived: ICD device function parameters, recorded arrhythmic events, data on the patient's subjective sympthoms, findings of subsequent diagnostic and therapeutic procedures. Controls of ICD devices are performed with the help of appropriate programmers of device manufacturers. All data obtained during the control examination were recorded in the patient's card and in electronic form. In case of absence of follow-up examinations patient data were obtained by telephone or by insight in the hospital's electronic information system.

In the statistical analysis we used descriptive methods and methods of analytical statistics.To compare the categorical variables we used Chi square test. For the analysis of numerical variables T test or Mann-Whitney test were used. In order to assess the survival between different groups of patients, we used Kaplan-Maier curve and Log-rank test.

RESULTS

The study included 210 patients (47,1% in sinus rhythm and 52.9% in the atrial fibrillation). Demographic characteristics of the groups are shown in Table 1.

Table 1. Demographic characteristics of patient groups

Group		Sinus rhythm	Atrial fibrillation	p
Number(%)		99 (47,1%)	111 (52,9%)	
Age (years)		67,7 ± 11,3	67,7 ± 9,6	p=0,517[a]
Gender	Man	68 (68,7%)	78(70,3%)	p=0.062[b]
	Woman	31 (31,3%)	33 (29,7%)	
Hypertension		77,8%	88,3%	p=0.064[b]
Diabetes mellitus		41,4%	28,8%	p=0.056[b]
Hyperlipidemia		65,6%	60,0%	p=0.399[b]
Smoking		27,3%	21,6%	p=0.340[b]
Heredity		27,2%	21,6%	p=0.340[b]
[a] T test, [b] Xi square test, * significance level 0.05				

The groups did not differ statistically significant according to the age of the patients, gender, nor the presence of risk factors. There is trend of higher prevalence of diabetes mellitus in the sinus rhythm group, but statistical significance has not been achieved.

The values of laboratory parameters monitored in patients are shown in Table 2.

Table 2. Value of laboratory parameters between compared groups

Group		Sinus rhythm	Atrial fibrillation	p
NT-proBNP (pg/mL)	Mean ± SD	4037.5 ± 5474.5	4852.3 ± 6645.1	p=0.177[c]
	Median	1519.5	2036	
Troponin (pg/mL)	Mean ± SD	0.065 ± 0.138	0.065±0.20	p=0.978[c]
	Median	0.019	0.019	
Hemoglobin (g/L)	Mean ± SD	125.1 ± 18.5	129.4 ± 22.9	p=0.113[c]
	Median	129	132	
CRP (mg/L)	Mean ± SD	8.39 ± 12.28	8.33 ± 10.8	p=0.815[c]
	Median	4.95	4.20	
Urea (mmol/L)	Mean ± SD	9.00 ± 4.01	8.38 ± 4.25	p=0.362[c]
	Median	8.15	7.6	
Creatinine (µmol/L)	Mean ± SD	125.9 ± 157.5	114.1 ± 52.0	p=0.104[c]
	Median	96	104	
Na^+ (mmol/L)	Mean ± SD	139.6 ± 3.8	135.42 ±14.25	p=0.794[c]
	Median	140	140	
[c] Mann-Whitney test * significance level 0.05				

The values of laboratory parameters: NT-proBNP, troponin, hemoglobin concentrations, CRP, urea, creatinine and serum sodium ion concentrations did not differ significantly between the compared groups. The prevalence of cardiovascular drug therapy is shown in Table 3.

Table 3. Prevalence of cardiovascular drug groups in patient groups.

Group	Sinus rhythm	Atrial fibrillation	p
Amiodarone	**49.5%**	**68.5%**	**p=0.005[b*]**
Beta blocker	84.5%	91.0%	p=0.154[b]
ACE inhibitor	74.7%	71.2%	p=0.481[b]
Anticoagulant therapy	**61.0%**	**95.5%**	**p=0.000[b*]**
ASA	**56.1%**	**25.2%**	**p=0.000[b*]**
Thienopyridine	**19.1%**	**9.0%**	**p=0.028[b*]**
Statin	67.7%	58.6%	p=0.143[b]
[b] Xi square test, * significance level 0.05			

A statistically significantly higher prevalence of amiodarone was observed in atrial fibrillation group in comparison to sinus rhythm group (p = 0.005). Patients with atrial fibrillation had a statistically significantly higher prevalence of oral anticoagulant drugs in therapy, while patients with sinus rhythm had a statistically significantly higher prevalence of antiplatelet drugs (ASA and thienopyridines) (p=0.000).

Out of the total number of patients, 46.5% (99 patients) had myocardial disease of ischemic etiology, while 53.5% of patients (111 patients) had non-ischemic etiology of heart disease. The prevalence of associated cardiovascular diseases between the compared groups is shown in Table 4.

Table 4. Prevalence of associated cardiovascular diseases.

Group	Sinus rhythm	Atrial fibrillation	p
Ischemic heart disease	**60.6%**	**35.1%**	
Non-ischemic heart disease	**39.4%**	**64.9%**	**p=0.000[b*]**
Peripheral artery disease	**21.2%**	**9.9%**	**p=0.023[b*]**
Cerebrovascular event	**5.0%**	**14.4%**	**p=0.024[b*]**
Chronic renal failure	7.1%	10.8%	p=0.346[b]
[b] Xi square test, * significance level 0.05			

Myocardial disease of ischemic etiology and peripheral artery disease were significantly more prevalent in the group of patients in sinus rhythm compared to atrial fibrillation group. The group of patients with atrial fibrillation had a significantly higher number of patients who had cerebrovascular event, compared to sinus rhythm group, as well as a significantly higher number of patients with non-ischemic etiology of heart muscle disease.

At the end of the two-year follow-up 89.9% of patients in the sinus rhythm group and 91.0% of patients with atrial fibrillation were alive. There was no statistically significant difference in patient survival between the compared groups during the two-year follow-up of patients (Log rank, p = 0.785). Patient survival curves are shown in Figure 1.

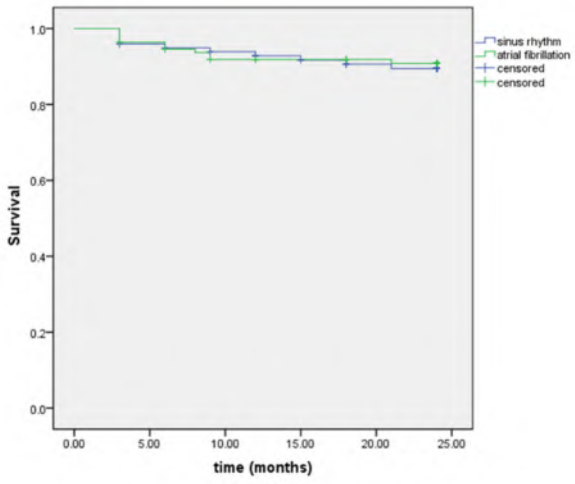

Figure 1. Patient survival curves.

During a two-year follow-up of a total of 210 patients enrolled in the study, 39 patients (18.3%) had activation od ICD in the form of antitachycardia pacing and/or defibrillation shock. Table 5. shows the distribution of patients with ICD activation between the groups.

Table 5. Distribution of patients with ICD activation between the compared groups.

Group	Sinus rhythm	Atrial fibrillation	p
Number of patients with ICD activation	16 (16.2%)	23 (20.7%)	P=0.396[b]
[b] Xi square test, * significance level 0.05			

There was no difference in the number of ICD activation between the compared groups at the end of the two-year follow-up.

Out of total of 39 patients in whom ICD activation occurred during the two-year follow-up, in 25 patients (64.1%) the appropriate ICD activation was registered. In 14 patients (35.9%), inappropriate activation of the ICD was registered. Appropriate and inappropriate defibrillation shock may be reported in same patients during follow-up. Figure 2 shows the types of activation of the ICD during the two-year follow-up.

Most patients with ICD activation had appropriate ICD therapy. In patients who had inappropriate therapy in most cases during two-year follow-up in addition to inappropriate an appropriate ICD therapy was registered. Distribution of ICD activation types between the groups was shown in Table 6.

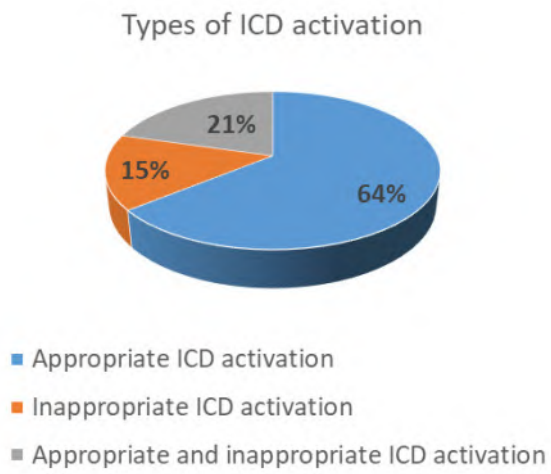

Figure 2. Types of activation of the implanted cardioverter defibrillator.

Table 6. Distribution of types ICD activation between the groups.

Group	Sinus rhythm	Atrial fibrillation	P
Incidence of appropriate ICD activation	16 (48.5%)	17 (51,5%)	p=0.863[b]
Incidence of inappropriate ICD activation	**3 (21,4%)**	**11 (78,6%)**	**P=0.046[b*]**

There was no difference between compared groups in the number of patients with appropriate activation of ICD. The group of patients with atrial fibrillation had significantly more patients with inappropriate ICD activation compared to the sinus rhythm group (p=0.046).

DISCUSSION

Out of the total number of patients included in the investigation, 52,9% had atrial fibrillation (paroxysmal and permanent), while 47,1% of the patients were in sinus rhythm. The prevalence of atrial fibrillation in our investigation can be compared with study of authors from Leiden (11) which included 913 patients (27% with atrial fibrillation: paroxysmal, persistent, permanent) followed for an average of three years after ICD implantation. This study revealed higher mortality of patients with permanent atrial fibrillation compared to patients in sinus rhythm (35% vs. 12%), while patients with paroxysmal and persistent atrial fibrillation had similar mortality compared to patients in sinus rhythm. Patients with permanent atrial fibrillation in this study had significantly higher NYHA class of heart failure, lower creatinine clearance, wider QRS complexes, more frequent use of antiarrhythmics and diuretics than sinus rhythm group, which points association between patients risk profile and survival rather than the impact of atrial fibrillation itself. The presence of ischemic heart disease was more common in patients with sinus rhythm, paroxysmal and persistent atrial fibrillation, while the presence of non-ischemic heart disease was more common in the permanent atrial fibrillation group, similar to our research. In our study, there was no difference in mortality between the groups, but there was no difference in the risk profile that existed in the Leiden study. Investigators from Leiden have included patients in the study since 1996, while our study included patients who had an ICD implanted since 2014. so it should be taken into consideration that significant progress has been made in the treatment of heart

failure in recent years which could improve survival of the patients.

In the MADIT II study (12) patients with atrial fibrillation accounted for only 8% of patients (102 patients) and had significantly higher risk profile than sinus rhythm patients: elderly patients, higher incidence of chronic renal failure, wider QRS complex, higher NYHA class of heart failure. After twenty months follow-up patients with atrial fibrillation had higher cumulative frequency of hospitalizations and mortality compared with patients in sinus rhythm, noting that the presence of atrial fibrillation was not in itself an independent predictor of increased mortality, but affected more frequent hospitalizations. Sub-analysis of the MADIT II study (13) identified risk factors which reduced the benefit of ICD in reducing SCD such as: advanced age (over 70 years of age), severe heart failure, presence of chronic renal failure, presence of atrial fibrillation and wider QRS complexes. In our trial risk profile did not differ significantly between patient groups (exept higher serum concentration of NT-proBNP in patients with permanent atrial fibrillation), which may explain the equally two-year survival in all patient groups.

Sub-analysis of another important study which examined the optimal programming of ICD algorithms (PREPARE study), assesed the impact of atrial atrial fibrillation/tachycardia on mortality and ICD function (14). After one year follow-up 8% patients experienced ICD shock with higher number of ICD shocks in patients with atriall fibrillation and inappropriate shocks accounting for the majority of the difference (6.9% vs 2.6%, P = 0.02), but the mortality was similar in patients with and without atrial fibrillation/tachycardia.

In accordance with the current recommendations for the treatment of heart failure, most patients in our study were treated with beta-blockers, statins and ACE inhibitors. There was no statistically significant difference in the prevalence of these drugs between the compared groups of patients. Prevalence of amiodarone was significantly higher in the group of patients with paroxysmal and permanent atrial fibrillation compared to patients in sinus rhythm, which can be explained by strategy of rate and rhythm control, beside its use to suppress ventricular arrhythmias in both groups. According to the recommendations for the treatment of atrial fibrillation, use of anticoagulant therapy in order to reduce ischemic thromboembolic events is the basis of treatment. As the patients with atrial fibrillation included in our investigation were already with significant structural myocardial disease, even without the presence of other risk factors they had elevated CHA2DS-VA2SC score which justifies anticoagulant therapy.

As the presence of atrial arrhythmias may be one of the reasons for inappropriate activation of ICD devices, in our study we examined the causes that led to device activation and delivery of therapy, based on which we classified the delivered antitachycardia therapy as appropriate and inappropriate. During the two-year follow-up of patients with ICD in our study, out of the total number of patients who had ICD activation and delivery of antitachycardia therapy, 36% of patients had inappropriate device activation. There was no statistically significant difference in the number of patients with appropriate ICD activation between the groups, but in the group of patients with atrial fibrillation in our study we observed significantly higher number of patients with inappropriate ICD activation and defibrillation shock delivery (11 patients versus 3 patients). In the already mentioned Leiden group study (15), during three-years follow-up there was singifficantly higher number of patients with inappropriate ICD activations in permanent atrial fibrillation group (32% of patients) and paroxysmal atrial fibrillation group (28% of patients) in comparisson sinus rhythm group (13% of the patients). This trial has also reported higher inicedence of appropriate ICD activations in the permanent atrial fibrillation group in comparison to other forms of atrial fibrillation and to sinus rhythm group, which could be attributed to higher risk profile of patients and more severe form of heart failure in this group.

Higher rate of inappropriate defibrillator activations in patients with atrial fibrillation in our investigation could be explained by the effort to keep patients with paroxysmal atrial fibrillation in sinus rhythm for as long as possible, but also to feel good in daily activities, which affects the choice and dose of antiarrhythmic drugs. In patients with atrial fibrillation, attacks of paroxysmal atrial fibrillation, even in the short term, could lead to an acceleration of the heart rate, entry into the ICD therapeutic zone and delivery of antitachycardia therapy. In our study, we found that the majority of patients (57%) who had inappropriate ICD activation and defibrillation shock delivery had also appropriate defibrillator activation during the two-year follow-up, which may explain the fact that there was no difference in morality between the compared patient groups. Patients in whom inappropriate activation of ICD devices occurred in most cases benefited from implantation because they also had appropriate defibrillator activations which interrupted life-threatening heart rhythm disorders.

CONCLUSION

1. In patients with ICD, there was no statistically significant difference in mortality after two years follow-up between patients with atrial fibrillation and patients in sinus rhythm.

2. In patients with ICD, there was no statistically significant difference in the number of patients with activation of ICD after two years follow-up between patients with atrial fibrillation and patients in sinus rhythm.

3. During the two-year follow-up of patients with ICD, inappropriate activation of the ICD was registered in 36% of patients who had activation of ICD. There was significantly higher number of patients with inappropriate ICD activation in patients who had atrial fibrillation compared to patients in sinus rhythm.

4. The majority of patients (57%) who had inappropriate activation of the ICD and delivery of defibrillation shock, also had appropriate ICD activation during the follow-up.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

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QUALITY OF LIFE IN PATIENTS AFTER STROKE

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Received: 18.08.2021.
Accepted: 20.01.2022.

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UDK: 613-056.24:616.127-005.8
Eabr 2024; 25(3):267-273
DOI: 10.2478/sjecr-2022-0002

ABSTRACT

The goal of all medical treatments is a better quality of life for patients. Post-stroke rehabilitation is a long process with uncertain result. The aim of this study was to explore the factors which affect the quality of life of patients recovering from a cerebrovascular disease. This is a prospective study evaluating the quality of life of one hundred patients one month and six months after a stroke, and patients also answered questions retrospectively, of how they felt before the stroke. As assessment tools we used a questionnaire on general and clinical data and Medical Outcomes Study Short Form (SF-36) questionnaire. Physical functioning and Physical role domains of SF-36 show significant differences in both measured periods ($p<0.001$). Emotional role, Social functioning, Mental health, Vitality and General health domains show a statistically significant change during first six months, while Bodily pain domain did not change ($p>0.05$). Physical summary score has changed significantly during 6 months ($p <0.001$). Mental summary score showed no significant difference in both periods ($p <0.687$; $p <0.958$). The brain localization is important factor ($p<0.0002$). Gender, age, education, employment status and previous strokes did not have a statistically significant influence ($p> 0.05$). Post-stroke physical impairment is not always accompanied by emotional impairment. Emotional functioning impairments generally return to the premorbid level during the period of six months, while physical impairments continue to occur. Further research is needed for better understanding of these relationships.

Keywords: *Quality of life, stroke, SF-36.*

INTRODUCTION

Medical science is in a period of transition from biomedical to socio-economic phase. In this context, the main objective of medicine is not merely prolongation of human life, but also the achievement of a higher quality of life (1, 2).

Quality of life as a technical term has been present in the literature for more than three decades. Quality of life can also be understood as an individual perception of one's own position in life in the context of cultural and the system of social values in which the individual lives and it is always closely related to goals, expectations, standards and interests of the person (3).

All questionnaires for assessing the quality of life include a large number of questions from various fields of human life and they assess its functioning in terms of physical, mental and social recovery, especially in terms of personal perception of patient's health (4).

Medical Outcomes Study questionnaires (Short Form SF-8, SF-12, SF-20, SF-36) are most commonly used among the general health questionnaires (5, 6). The major disadvantage of general health questionnaires is the fact that they do not have the appropriate sensitivity for specific diseases (7).

Cerebrovascular diseases, which are the subject of this study, represent more or less a heterogeneous group of diseases associated with various forms of brain circulation disorders.
The aim of this study was to explore the most important factors which affect the quality of life by analyzing physical and mental state, as well as the social capability of patients recovering from a cerebrovascular disease.

MATERIAL AND METHODS

Research methodology

Examinees completed questionnaires one month and six months after their cerebral stroke. Questionnaires were completed under the code, in accordance with the ethical principles of scientific research. Participation in the study was voluntary after signing the voluntary consent. The research was carried out after the approval of the Ethics Committee of the Clinical Center of Vojvodina.

Instruments

Two questionnaires were used: a questionnaire on general and clinical data and a questionnaire about the overall quality of life (Short Form SF-36).

The general information questionnaire was designed for this research and included socio-demographic data (gender, age, education and employment status), the data about previous strokes or transient ischemic attacks (TIA), as well as data about the type and localization of a stroke. We used the SF-36 questionnaire which assesses eight domains of the quality of life within the last year through 36 items.

Assessments were performed on two occasions: before the incident, one month and six months after the cerebral stroke. Patients also answered questions retrospectively, of how they felt before the incident. (8, 9).

Sample

The sample consisted of 100 patients diagnosed with cerebral stroke who were treated at the Clinic of Neurology, Clinical Center of Vojvodina from 1st January 2019 to 1st June 2019. The inclusion criteria implied that the patients suffered from a cerebral stroke, belonged to both genders, were older than 30, lived on the territory of Novi Sad, were cooperative (regularly attended control check-ups and signed the Informed consent form). Before all the study procedures we evaluated examinee's cognitive state using Mini-Mental State Examination (MMSE) and excluded patients whose scores were less than 19 because those patients were unable to meet the study demands. The exclusion criteria were uncooperativeness due to severe motor disorders and other medical states which could affect the quality of life of subjects.

Characteristics of a group:

The average MMSE score one month after the stroke was 26.8 ± 2.3 (range 21-30) and 28.1 ± 1.2 (range 23-30) six months after the stroke.

There were 46 women and 56 men in the study sample. The average age was 57.7 ± 7.6 years (41 - 77 years). The average age was 58.9 for men and 56.4 for women. 11% of the group finished elementary school, 64% high school and 25% college. There were employed subjects with regular income (37%) and unemployed subjects without regular income (63%). In terms of etiology of the stroke, most of the group had ischemic stroke (93%) and others had hemorrhagic stroke. Supratentorial localization of the stroke was more frequent (67%) than infratentorial (33%). Brain stem lesions were observed in 29% and cerebellar lesions in 3% of the sample. Every fifth (20%) patient had previous insult without permanent neurological deficit and 19% of patients previously experienced transient ischemic attack (TIA).

Collected data was verified by the author and entered in a specially created database. During the database entry additional validation of the data was performed. Statistical analysis included: comparison of distribution of responses in relation to the time point of measurement ($\chi 2$ test), then the calculation of the standard values for domains, subgroup analysis and hypotheses testing concerning the respondents' state during the follow-up (Pearson's t-test, paired t-test, general linear model (GLM) and ANOVA for repeated measures).

RESULTS

Comparison of the average values of Physical health and Role limitation in both time points of the study is statistically significant at p <0.001.

Comparison of the average Role limitations due to emotional problems and Social functioning domain values are significantly different after 1 month and after 6 months, while do not differ in the period before and six months after the stroke.

Comparison of the average values of Bodily pain domain in both time points do not differ.

The average values of the Mental health, Vitality and General health domain show statistically significant differences between the time points 1 month and 6 months after the stroke, while there is no difference between the domains before and 6 months after the stroke.

Analysis of the Physical summary score and Mental summary score

In order to facilitate the interpretation of the results we created a mathematical model that synthesized the values of all eight domains into two summary scores: Physical summary score (PSS) and Mental summary score (MSS).

Physical summary score consists of Physical functioning, Role limitations due to physical health, Bodily pain and General health domains, while Mental summary score consists of Role limitations due to mental health, Social relations, Mental health and Vitality domains (Table 1).

Table 2 shows the average values for Physical summary score presented in relation to the time points of the study. Comparison of the average values of Physical summary score in both points (1 month vs. 6 months after the stroke and before vs. 6 months after the stroke) show a significant difference at p <0.001.

Average values for Mental summary score do not show a significant difference in relation to the time points of the study (Table 2).

With respect to the age, the average value was observed (57,7 years). Values of one standard deviation more or less (from 50 to 60 years) formed the group of the average age, and above and over that younger and older group. Analysis of demographic and neurological parameters in relation to the Physical summary score indicated that there was a statistically significant difference only in relation to the location of the stroke (Table 3).

There is statistically significant difference in the Physical summary score between all three time points of the study (Table 4).

Statistically significant difference is between the time points for Physical summary score in supratentorial left, both sided and brain stem localization of the stroke, while there is no difference in this score in right or cerebellar localization of the stroke (Table 5).

Table 1. Average values of SF-36 scale domains

Domain	Time point	Min	Max	Average	SD	p
PF	before the stroke	40.00	100.00	92.2	13.4	-
	1 month after	0.00	100.00	61.7	30.4	1 m / 6 m **< 0,001**
	6 months after	10.00	100.00	78.5	22.8	6 m / before **< 0,001**
RP	before the stroke	25.00	100.00	87.1	21.7	-
	1 month after	0.00	100.00	46.2	33.0	1 m / 6 m **< 0,001**
	6 months after	0.00	100.00	68.1	27.1	6 m / before **< 0,001**
RE	before the stroke	25.00	100.00	92.2	17.1	-
	1 month after	0.00	100.00	86.1	26.0	1 m / 6 m = 0.037
	6 months after	50.00	100.00	91.9	13.4	6 m / before = 0.149
SF	before the stroke	12.50	100.00	92.5	22.4	-
	1 month after	0.00	100.00	82.1	25.1	1 m / 6 m = 0.034
	6 months after	25.00	100.00	86.7	20.7	6 m / before = 0.170
BP	before the stroke	12.00	100.00	94.4	17.7	-
	1 month after	0.00	100.00	84.4	25.7	1 m / 6 m = 0.062
	6 months after	12.00	100.00	91.6	21.3	6 m / before = 0.126
MH	before the stroke	10.00	100.00	82.5	17.1	-
	1 month after	0.00	100.00	76.7	21.3	1 m / 6 m = 0.021
	6 months after	35.00	100.00	81.2	18.8	6 m / before = 0.227

Domain	Time point	Min	Max	Average	SD	p
VT	before the stroke	18.75	100.00	81.4	18.2	-
	1 month after	0.00	100.00	71.0	21.2	1 m / 6 m = 0.011
	6 months after	25.00	100.00	72.1	21.9	6 m / before = 0.807
GH	before the stroke	35.00	100.00	75.6	19.1	-
	1 month after	0.00	100.00	61.5	20.9	1 m / 6 m **< 0,001**
	6 months after	25.00	100.00	66.0	19.3	6 m / before = 0,214

* PF - physical functioning; RP - role limitations due to physical health;
RE - role limitations due to emotional problems; SF - social functioning; BP – bodily pain;
MH - mental health; VT - vitality, GH - general health.

Table 2. Results of Physical summary score and Mental summary score.

	Data point	Average value	Min	Max	SD	p
PHYSICAL SUMMARY SCORE	Before	53,7	37,5	61,6	5,6	-
	1 month after	41,6	23,4	57,4	9,4	1 m / 6 m **< 0,001**
	6 months after	47,7	32,1	58,0	6,7	6 m / before **< 0,001**
MENTAL SUMMARY SCORE	Before	55,2	29,4	65,8	9,1	-
	1 month after	54,8	17,9	64,7	10,1	1 m /6 m < 0,687
	6 months after	55,1	21,0	65,1	8,6	6 m / before < 0,958

Table 3. Results of general and neurological caracteristics in relation to the Physical summary score results.

Variable	SS	degree	MS	F	P
Gender	2.75	1	2.75	2.017	0.158
Age	0.66	4	0.16	0.470	0.757
Education	1.56	4	1.39	1.993	0.241
Employment	0.98	1	0.76	0.376	0.845
Stroke localization	11.43	8	1.429	4.711	**0.0002**
Previous cerebral incidents	0.74	4	0.187	0.520	0.714

Table 4. Analysis of the Physical summary score in relation to the time point of the study and the location of the stroke.

	Time point	DF	SS	MS	F	P
PHYSICAL SUMMARY SCORE	before	4	6.158	1.540	3.150	**0.017**
	1 month after	4	12.325	3.081	3.048	**0.020**
	6 months after	4	9.563	2.390	6.484	**0.000**

Table 5. Mean value, standard deviation and the significance of differences of the Physical summary score in relation to the location of stroke

Localization of the stroke	Before stroke	SD	After 1 month	SD	After 6 months	SD	P 1m / 6 m	P 6 m / before
Left	4.454	1.175	4.181	1.424	4.818	0.583	**0.000**	**0.000**
Right	5.000	0.000	4.777	0.427	5.000	0.000	0.209	1.000
Cerebellum	5.000	0.000	4.647	0.485	4.764	0.430	0.190	0.945
Both sides	4.750	0.452	4.250	1.356	4.000	1.279	0.505	**0.046**
Brain stem	5.000	0.000	3.000	0.000	4.000	0.000	**0.000**	**0.047**

DISCUSSION

Patients have better results 6 months after than one month after the stroke, but some domain scores do not reach the values prior to the cerebrovascular incident.

Lowest achievements can be observed in the field of Physical role and General health domains, and the best-preserved domain is Emotional role. Results suggest that physical damage is not necessarily accompanied by an emotional damage. Kong and Singaporean group of authors assessed the quality of life in patients after stroke and stated that there was a significant impact of depression on the outcome (10). Our research did not confirm that claim.

Physical functioning and Physical role dimensions showed a significant difference in both time points of the study. There was a significant recovery six months after the stroke, but the quality of life measured by these dimensions was still significantly lower than before the stroke. All other dimensions showed a significant improvement after six months and came to the state similar to the state before the insult. Bodily pain is approximately the same during the whole period, which means that there is no significant difference between the period before and after the stroke in terms of pain. Widar et al. investigated the impact of pain on the quality of life in patients who suffered from a stroke with long-lasting pain (11).

The average value of the Physical summary score one month after the stroke is significantly reduced compared to the value before the stroke and is significantly improved after six months, but still does not reach the premorbid level.

This result is in accordance with scores of individual domains that make the Physical summary score. Identical results were found by Gallien et al, Pickard et al, Carod-Artal, and Xie (12-15).

Comparison of the average values of the Mental summary scores are no statistically significant changes in mental function of patients. This result is probably a consequence of the previously mentioned selection of patients. This is already mentioned shortcoming of the SF-36 questionnaire in terms of the inability to be applied on patients with severe mental deterioration after the stroke, which Hagen and his associates also noted (16). They also noted lower sensitivity of the questionnaire in the period of 3-6 months after the stroke, which was not detected in this study.

Physical summary score is dependant on the location of the cerebral stroke, whereas gender, age, education, employment status and history of previous strokes do not have a significant influence.

There were slightly fewer women (46%) in the sample. According to the data from the world reference cerebral stroke is more common in male, but mortality is higher in female population. Increasing trend of the incidence of stroke has been noted in the female population, and it is approaching male incidence during the last decade. Lai with a group of authors from the Pamela Duncan team examined the differences in the quality of life with respect to gender and reached the conclusion that women recovered more slowly, especially if they had depressive symptoms (17). Gall and associates, as well as Bushnell and Appelros found that women have worse functional outcome and a lower quality of life after the stroke, especially if their occupations involve physical work, but this was not confirmed by our research (18, 19).

Divani and associates studied the effect of subject's age on the outcome and came to the conclusion that the occurrence of stroke in later years have a worse outcome. However, age did not prove to be a significant factor in our study (20).

Hardie gives data on the importance of recurrent strokes for the outcome of patients. Earlier strokes did not have a significant impact on the quality of life in our study, which can be explained by the fact that patients already had a personal experience with stroke and thereby less fear of the unknown (21). Mackenbach et al. found positive correlation between the level of education and outcome after stroke. However, educational level and employment status showed no influence on the quality of life of patients after the stroke in our study (22).

Location of the cerebral stroke has significant impact on the quality of life in all observed time points in our study. Brain stem localization is associated with lower scores and supratentorial right with higher scores of the quality of life questionnaires. Our results indicate that brain stem, supratentorial left and bilateral localizations produce significant changes of the quality of life, while these changes are not so prominent in supratentorial and cerebellar localizations. Hinduja and Eminovic have not found a relation of the outcome and localization of the stroke (23, 24). De Haan found that patient with left-sided lesions have worse quality of life probably because of the speech disabilities, that is confirmed in our study. Infratentorial lesions have a better outcome according to his research (25).

CONCLUSION

This study shows that the stroke leads to the decline in the quality of life of patients as a rule. Physical symptoms, which are dominant, are not always accompanied by the emotional symptoms. Emotional functioning deficits generally withdraw to the premorbid level after 6 months, while physical deficits are retained. Change in the quality of life after stroke is not dependant on gender, education, employment status and previous strokes. However, the localization of the lesion is substantial in terms of the quality of life, and is most affected in patients with bilateral lesions, left-sided lesions and lesions of the brain stem. Further research will lead to better understanding of these relationships with special emphasis on the risk factors that diminish the quality of life.

ACKNOWLEDGMENTS

None.

FUNDING

The study received no external funding.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

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NEW INSIGHTS IN THE PATHOGENESIS OF CISPLATIN-INDUCED NEPHROTOXICITY

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Received: 23.01.2019.
Accepted: 17.02.2019.

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UDK: 615.38.099
UDK: 616.61-091.8
Eabr 2024; 25(3):275-284
DOI: 10.2478/sjecr-2019-0012

ABSTRACT

Cisplatin (cis-diamminedichloroplatinum II) is a widely used chemotherapeutic agent. However, efficacy and clinical utility of this drug is significantly limited by severe side effects such as nephrotoxicity which develops due to renal accumulation and biotransformation in proximal tubular epithelial cells. Cisplatin-induced nephrotoxicity can be manifested as acute kidney injury (AKI), or as different types of tubulopathies, salt wasting, loss of urinary concentrating ability, and magnesium wasting. The attenuation of cisplatin-caused AKI is currently accomplished by hydration, magnesium supplementation or mannitol-induced forced diuresis. However, mannitol treatment causes over-diuresis and consequent dehydration, indicating an urgent need for the clinical use of newly designed, safe and efficacious renoprotective drug, as an additive therapy for high dose cisplatin-treated patients. Accordingly, we emphasized current knowledge regarding molecular mechanisms responsible for cisplatin-caused nephrotoxicity and we described in detail the main clinical manifestations of cisplatin-induced renal dysfunction in order to pave the way for the design of new therapeutic approaches that can minimize detrimental effects of cisplatin in the kidneys. Having in mind that most of cisplatin-induced cytotoxic effects against renal cells are, at the same time, involved in anti-tumor activity of cisplatin, new nephroprotective therapeutic strategies have to prevent renal injury and inflammation without affecting cisplatin-induced toxicity against malignant cells.

Keywords: *Cisplatin, nephrotoxicity, acute kidney injury, apoptosis, inflam-mation.*

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II) is one of the most potent antitumor drugs, which is used to treat various types of malignancies, such as head and neck, gastrointestinal, urogenital, breast and lung cancers (1-13). Nevertheless, efficacy of cisplatin is significantly limited by severe side effects, including nephrotoxicity, ototoxicity and myelosuppression (14,15). Cisplatin-induced nephrotoxicity is a result of renal accumulation and biotransformation of cisplatin and is manifested as acute kidney injury (AKI), or as different types of tubulopathies, salt wasting, loss of urinary concentrating ability, and magnesium wasting (16,17). AKI is dose-dependent complication, noticed in 25-30% of cisplatin-treated patients (18-20). The attenuation of cisplatin-caused renal injury is currently accomplished by hydration, magnesium supplementation or by mannitol-induced forced diuresis (20). However, mannitol treatment may cause over-diuresis and consequent life-treating dehydration (16), indicating an urgent need for the clinical implementation of newly designed, safe and efficacious nephroprotective drug, as an additive therapy for cisplain-treated patients. Until now, amifostine [(ethanethiol, 2-[(3-aminopropyl)amino] dihydrogen phosphate ester)] was most usually used against cisplatin-induced renal injury, but its adverse effects, such as ototoxicity, hypotension, vertigo, hypocalciemia, nausea and vomiting, significantly limited its clinical use (16,17). Accordingly, there still remains an unmet need for the development of new, nephroprotective agent against cisplatin-caused AKI. Having in mind that most of cisplatin-induced cytotoxic effects against renal cells are, at the same time, involved in anti-tumor activity of cisplatin (13), this new, nephroprotective agent will have to prevent AKI without affecting cisplatin-induced toxicity against malignant cells. Design of novel therapeutic strategies against cisplatin-provoked nephrotoxicity requires understanding of molecular mechanisms which are involved in cisplatin-induced renal injury and inflammation and which are responsible for the development of main clinical manifestations and complications of cisplatin treatment. Accordingly, in this review article, we emphasized current knowledge about signaling pathways and cellular mechanisms which are responsible for the development of renal inflammation and activation of detrimental immune response elicited after cisplatin-caused injury of proximal tubular epithelial cells (PTECs). Additionally, herewith we described in detail the main clinical manifestations of cisplatin-induced renal dysfunction in order to pave the way for the design of new therapeutic approaches that can minimize detrimental effects of cisplatin in the kidneys. Our hope is that clinicians and scientists will use information presented herein as a starting point for the design of novel, effective nephroprotective strategies against cisplatin-induced nephrotoxicity.

Molecular mechanisms responsible for the development of cisplatin-induced nephrotoxicity

Cisplatin is mainly excreted by the kidneys, by both glomerular filtration and tubular secretion (21). During renal excretion cisplatin accumulates in the kidneys, and levels of this drug in PTECs are about five times greater than in the blood (18). Accordingly, toxic effects occur primarily in PTECs (21,22). The copper transporter 1 and 2 (Ctr1 and Ctr2), the P-type copper-transporting ATPases (ATP7A and ATP7B), the organic cation transporter 2 (OCT2), and the multidrug extrusion transporter 1 (MATE1) are the most important membrane transporters involved in the cellular uptake of cisplatin (23). Although cisplatin may enter PTEC through passive diffusion, Ctr1 and OCT2-mediated uptake of cisplatin are mainly responsible for the import of cisplatin in PTECs and for high accumulation of cisplatin in the kidneys (21). Accordingly, genetic deletion of Ctr1 significantly reduced cisplatin-induced apoptosis of PTECs (24). Similarly, deficiency of OCT2 notably attenuated toxicity of cisplatin (25), while cimetidine, an OCT2 substrate, reduced cisplatin uptake by PTECs and alleviated nephrotoxicity (26).

Cisplatin-induced toxicity is a consequence of cisplatin conversion into several nephrotoxic molecules within the PTECs. Several studies have shown that glutathione-conjugate of cisplatin in the kidneys is metabolized via gamma glutamyl transpeptidase (GGT) expressed on the surface of PTECs. These conjugates are further degradated into highly reactive thiols by the activity of aminopeptidase N (APN) and cisteine-S-conjugate beta-lyase (CCBL). Since among all tissues, GGT, APN and CCBL have the highest activity in the kidneys, these enzymes were considered as potential targets for the attenuation of cisplatin-induced nephrotoxicity (20,27). Accordingly, several ongoing experimental and clinical trials investigate potential therapeutic effects of GGT, APN and CCBL inhibition as new approach for alleviation of cisplatin-induced AKI.

Once cisplatin enters the PTECs, it forms intrastrand crosslinks among two adjacent guanine residues within DNA affecting replication and transcription which results in the activation of DNA repair mechanisms. Accordingly, an increased activity of nucleotide excision repair (NER) and mismatch repair (MMR) system has been associated with resistance to cisplatin-induced AKI and enhancement of NER and MMR system activity is considered a new approach for prevention of cisplatin-induced AKI (28).

In addition, regulators of cell cycle play an important role in the development of cisplatin-induced AKI and these molecules could also be taken into account as potential targets for prevention of cisplatin-induced renal injury. After cisplatin administration, normally quiescent kidney cells enter the cell cycle and consequently the cell cycle-inhibitory proteins (p21, 14-3-3 and p27), which coordinate cell cycle and play a protective role against toxicity, become induced. Accordingly, deletion of the p21 or 14-3-3 genes resulted in enhanced nephrotoxicity elicited by cisplatin (29-31). Since cisplatin treatment causes an increased activity of Checkpoint kinase 2 (Cdk2), p21-mediated protection from cisplatin-induced injury is relied on inhibition of Cdk2 (32). Accordingly, several lines of evidence suggested the importance of

Cdk2-inhibitory drugs in prevention of cisplatin-induced nephrotoxicity (32).

Oxidative stress is crucially involved in cisplatin-induced AKI. Within the cell, cisplatin is converted into a highly reactive form, which can rapidly react with thiol-containing antioxidant molecules, such as glutathione, methionine and metallothionein (33,34). Consequently, depletion of glutathione and similar antioxidants leads to increased accumulation of endogenous reactive oxygen species (ROS) resulting in induction of oxidative stress within cisplatin-injured PTECs. ROS may target and modify cell components, including lipids, proteins, and DNA, resulting in cellular stress (35). Additionally, cisplatin may promote ROS generation by direct binding to P450 (CYP) system in microsomes (18,36) or may induce mitochondrial dysfunction by distorting respiratory chain (37). In line with these observations, several studies demonstrated that treatment with antioxidants significantly attenuated cisplatin-induced AKI (38-41), indicating crucial role of oxidative stress in the pathogenesis of cisplatin-induced AKI.

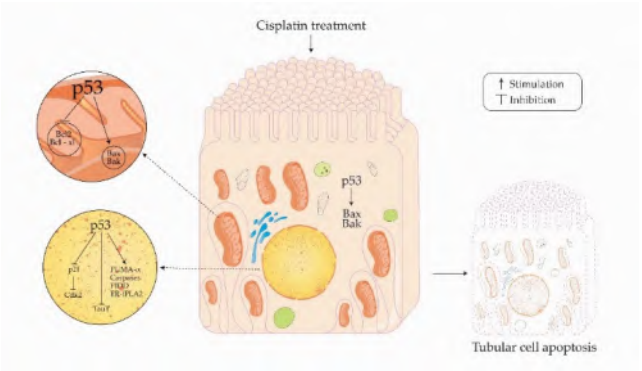
Importantly, cisplatin-induced extensive generation of ROS and free radicals in PTECs accelerate production of advanced glycation end products (AGEs) which further contribute to the progression of renal injury and inflammation (35). Since kidneys have a crucial role in AGEs disposal, cisplatin-induced renal dysfunction increases AGE levels in injured kidneys resulting in the development of glomerulosclerosis, interstitial fibrosis, and tubular atrophy (35).

Treatment with cisplatin *in vivo* causes a great increase in both necrosis and apoptosis of PTECs (42). Extrinsic, intrinsic (mitochondrial) and endoplasmic reticulum (ER)-stress pathway are involved in cisplatin-induced AKI. Cisplatin treatment of PTECs resulted in translocation of Bax to mitochondria and releasing of cytohrome c (43,44), apoptosis-inducing factor (AIF) (45) and endonuclease G (46) from mitochondria, accompanied by activation of caspase-3,-8 and -9 (47) and caspase-12 which has been designated as initiator caspase in ER-stress pathway regulated by the expression of calcium-independent phospholipase A2 (ER-iPLA2) (48-50).

Cisplatin is known to activate all three mitogen-activated protein kinases (MAPKs) in the kidney, including p38, extracellular signal-regulated kinase (ERK), and Jun N-terminal Kinase/Stress-Activated Pathway Kinase JNK/SAPK (51). The ERK was shown to mediate cisplatin-induced nephrotoxicity via phosphorylation of the proapoptotic p66 shc protein (52). Furthermore, inhibition of ERK resulted in attenuated expression and activation of caspase-3, and consequently decreased apoptosis in cisplatin-treated renal cells (51).

P53 protein has an important role in cisplatin-induced AKI (53-55). Cisplatin treatment induces a DNA damage, leading to the activation of molecular sensor for DNA damage- ataxia telangiectasia and Rad3-related protein (ATR), which activates Chk2. Both ATR and Chk2 can phosphorylate p53 for its activation. Also, ROS may promote activation of p53 by inducing DNA damage or through ATR, Chk2, nuclear factor kappa B (NF-kB) or p38 activation (53). Activated p53 increases transcription of pro-apoptotic genes, such as PUMA-α and ER-iPLA2, and down-regulates expression of anti-apoptotic genes (p21 and taurine transporter (TauT)) (53-55). Additionally, p53 promotes apoptosis of PTECs through the interactions with Bcl-XL, Bax, Bak proteins in mitochondria and/or cytosol (53) (Figure 1).

Figure 1. Cisplatin-induced activation of p53 results in apoptosis of tubular cells.



Activated p53 increases transcription of pro-apoptotic genes, such as PUMA-α and ER-iPLA2, and down-regulates expression of anti-apoptotic genes (p21 and taurine transporter (TauT)). Additionally, p53 promotes apoptosis of tubular cells through the interactions with Bcl-XL, Bax, Bak proteins in mitochondria and/or cytosol. **Abbreviations:** Bcl-2: B-cell lymphoma 2; Bcl-xL: B-cell lymphoma-extra large; Bax: Bcl-2-associated X protein; Bak: Bcl-2 homologous antagonist killer; PUMA-α: p53 upregulated modulator of apoptosis; PIDD: p53-induced protein with a death domain; ER-iPLA2: Ca2+-independent phospholipase A2; Cdk2: Cyclin-dependent kinase complex; TauT: taurine transporter.

In addition to the regulation of apoptosis, p53 may contribute to the development of cisplatin-caused nephrotoxicity by modulating autophagy which, as an adaptive mechanism, promotes PTECs survival during AKI (56). Within hours after cisplatin administration, markers of autophagy, such as Beclin 1, LC3, and Atg5 are significantly up-regulated in injured PTECs (56-58). Importantly, DNA damage, activation of p53, and mitochondrial injury are increased in proximal tubules of autophagy-deficient mice, suggesting protective role of autophagy in cisplatin-injured kidneys (57,59-62).

It is important to highlight that, in addition to apoptosis and autophagy, necroptosis was also detected in renal tubules after injection of cisplatin (63,64). Receptor-interacting protein 1 (RIP1) and mixed lineage kinase domain-like protein (MLKL) – deficiency, as well as pharmacological inhibition of necroptosis, significantly reduced cisplatin-induced AKI (63,64).

The important role of inflammation in the development of cisplatin-induced aki

Inflammation plays a key role in the progression of cisplatin-induced AKI (65-72). After cisplatin treatment, several alarmins are produced by injured renal cells such as mesangial cells, glomerular cells, endothelial and renal tubular cells which can initiate enhanced production of inflammatory cytokines (70). Among them, tumor necrosis factor alpha (TNF-α) was significantly elevated in the serum as well as urine of cisplatin-treated animals, indicating important pathophysiological role of TNF-α in cisplatin-induced nephrotoxicity (65,66). The biological activities of TNF-α are mediated by two different receptors, TNFR1 and TNFR2 (71,72). Although TNFR1 was responsible for TNF-α-induced systemic and anti-tumor effects (71), several lines of evidence demonstrated that TNFR2 rather than TNFR1 mediates cytotoxic and inflammatory actions of TNF-α in cisplatin-injured kidneys (72). Accordingly, inhibition of TNFR2 should be further explored in up-coming experimental studies as a potentially new therapeutic approach that could reduce AKI without affecting anti-tumor effects of cisplatin mediated by TNF-α.

Some research demonstrated an important role of TLR-4 for enhanced production of TNF-α in resident and renal-infiltrating immune cells. One of possible endogenous molecules which can bind TLR-4 and initiate an innate immune response after cisplatin-treatment is gp96, which is increased after cisplatin administration (68). Activation of TLR-4 promotes p38 MAPK dependent signaling which induces enhanced secretion of TNF-α in cisplatin-injured kidneys (67,68). In addition to TLR-4 dependent production of TNF-α, cisplatin treatment also activates inflammasome complex in renal infiltrated leukocytes, resulting in enhanced secretion of interleukin (IL)-1β (69).

Elevated levels of TNF-α and IL-1β are isially accompanied with enhanced production of other inflammatory cytokines, particularly IL-18, and IL-6 resulting in the increased recruitment of circulating immune cells into cisplatin-injured kidneys (73-76). Intercellular adhesion molecule-1 (ICAM-1) has been considered a crucially important adhesion molecule for migration of immune cells into cisplatin-injured kidneys since the inhibition of this integrin significantly reduced total number of renal-infiltrated leukocytes (77).

Although expression of IFN-γ, well known inflammatory cytokine, is increased in cisplatin-injured kidneys, neutralization of this cytokine had no impact on renal dysfunction, suggesting the existence of IFN-γ-independent development of cisplatin-induced AKI (78). Recently published studies indicated important pathogenic role of IL-33 in cisplatin-induced AKI. Serum levels of IL-33 were increased in cisplatin-injured animals. Mice with cisplatin-induced AKI injected with fusion protein, which neutralized IL-33, had a significant decrease in creatinine, pathohistological score, and showed reduced apoptosis of cisplatin-induced PTECs, while injection of recombinant IL-33 notably aggravated cisplatin-induced AKI (79).

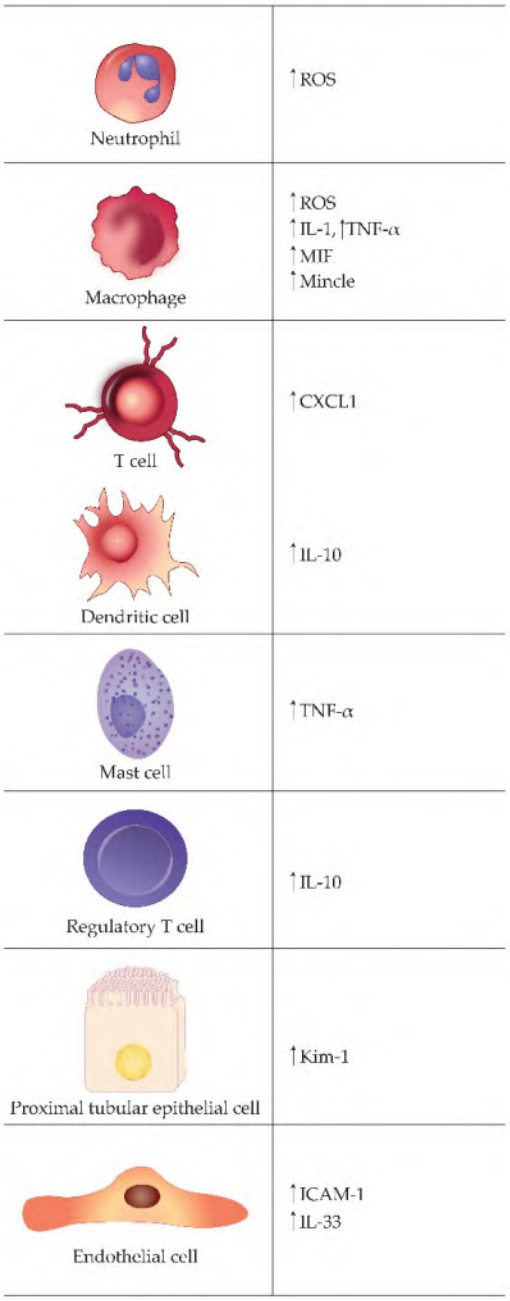
IL-10 is a cytokine with potent anti-inflammatory properties that suppresses the activation of leukocytes and the production of proinflammatory cytokines and chemokines in cisplatin-injured kidneys (80). Several studies demonstrated that IL-10, secreted mainly by regulatory T cells (Tregs), tolerogenic dendritic cells, and alternatively activated macrophages, reduces cisplatin nephrotoxicity, and may act, in part, by inhibiting the maladaptive activation of genes that cause leukocyte activation and adhesion, and induction of iNOS (78,80-82).

Different types of immune cells, including neutrophils, macrophages, mast cells, natural killer (NK) cells, T and B cells produce inflammatory cytokines or anti-inflammatory IL-10 and other immunomodulatory factors which play an important role in the pathogenesis of cisplatin-induced AKI (Figure 2).

Mast cells have important pathogenic role in cisplatin-induced nephrotoxicity. Depletion of mast cells resulted in significantly reduced renal injury in cisplatin-treated mice (82). Deficiency of mast cells was accompanied by lower number of renal-infiltrated leukocytes and notably down-regulated serum levels of TNF-α, suggesting that mast cells mediated cisplatin-induced AKI by promoting recruitment of circulating immune cells in the kidneys in TNF-α-dependent manner (82,83).

Renal-infiltrated neutrophils produce large amounts of ROS, proteases, and inflammatory cytokines, leading to renal epithelial injury (84-86). In contrast, an inhibition of TNF-α or TLR-4 signaling pathways, administration of IL-10 as well as inhibition of ICAM-1 (68,77,80,87) are associated with a decreased number of activated neutrophils in renal parenchyma of cisplatin-treated animals which corresponds to the attenuation of renal injury and inflammation. It has to be noted that neutrophils, which infiltrate cisplatin-injured kidneys, may alter their inflammatory phenotype depending on the cross-talk with immunosuppressive cells (86).

Figure 2. Immunomodulatory molecules which expression is enhanced in renal parenchymal and immune cells upon cisplatin treatment.



Cisplatin treatment induces enhanced expression of reactive oxygen species (ROS), inflammatory cytokines and chemokines as well as integrins in the kidneys enabling crosstalk between cisplatin-injured proximal tubular epithelial cells, endothelial cells and renal-infiltrated innate and adaptive immune cells.
***Abbreviations*:** ROS: Reactive oxygen species; IL: Interleukin; TNF-α: Tumor necrosis factor alpha; MIF: Macrophage migration inhibitory factor; Mincle: Macrophage-inducible C-type lectin; CXCL1: Chemokine (C-X-C motif) ligand 1; Kim-1: Kidney injury molecule-1; ICAM-1: Intercellular adhesion molecule-1.

Tolerogenic renal DCs and T regulatory cells (Tregs), in juxtracrine and paracrine manner, promote enhanced expression of IL-10 in renal-infiltrated neutrophils resulting in their differentiation into immunosuppressive and anti-inflammatory cells. Accordingly, depletion of neutrophils as well as their adoptive transfer may result in either alleviation or aggravation of cisplatin-induced AKI, depending on the cellular-make up and microenvironment of the cisplatin-injured kidneys (86).

Macrophages play an important inflammatory role in the initial phase of cisplatin-caused AKI. Cisplatin treatment induces activation of inflammasome, p38 MAPK and NF-kB pathways in renal macrophages resulting in enhanced production of superoxide anions, nitric oxide (NO), IL-1 and TNF-α (73,89). In addition, cisplatin-induced activation of TLR-4 induces expression of macrophage-inducible C-type lectin (Mincle) in renal infiltrating macrophages. Mincle promotes generation of inflammatory M1 macrophages which are capable to produce large amounts of inflammatory cytokines. Accordingly, cisplatin-induced activation of Mincle on macrophages results in exacerbation and progression of renal inflammation (90). In line with these findings, suppressed expression of Mincle on renal macrophages completely abrogates their inflammatory phenotype and adoptive transfer of Mincle-silenced macrophages protects against cisplatin-induced nephrotoxicity (90). Accordingly, Mincle is considered a potential molecular target for macrophage dependent attenuation of cisplatin-induced AKI and its therapeutic potential is going to be explored in future experimental studies. In addition to Mincle, macrophage migration inhibitory factor (MIF) plays an important pathogenic role in cisplatin-induced nephrotoxicity. Deletion of MIF suppressed influx of M1 macrophages and reduced concentration of macrophage-derived inflammatory cytokines and chemokines in the kidneys, attenuated recruitment of circulating immune cells in the cisplatin-injured kidneys which resulted in alleviation of AKI (91).

CD4+ and CD8+ T cells are crucially important in orchestrating immune response during cisplatin-induced AKI (66,79,92). Cisplatin-injured renal cells release IL-33 which activates CD4+ T cells and increase production of inflammatory cytokines, such as TNF-α, and chemokine CXCL1 (79). CXCL1 induces enhanced recruitment of neutrophils and may directly induce apoptosis of tubular epithelial cells (66,79). Cisplatin treatment increases expression of Fas receptor on renal tubular cells enabling apoptosis of these cells due to their interaction with FasL expressing renal infiltrating CD8+ T lymphocytes and NK cells (92).

Cisplatin treatment provokes enhanced expression of T cell immunoglobulin mucin 1 (Tim-1) on PTECs which acts as a costimulatory molecule for activation of renal-infiltrated T cells (93). Consequently, use of Tim-1-blocking antibody suppressed activation of renal-infiltrated CD4+ helper T cells and their cross-talk with CD8+ cytotoxic T cells, significantly reduced apoptosis of PTECs and protected against cisplatin-induced AKI (93). Due to its important role in the development and progression of AKI, Tim-1 was designated as kidney injury molecule-1 (Kim-1) and has been considered

as potential molecular target for the attenuation of T cell-driven renal inflammation (93).

Among immunosuppressive cells, Tregs and tolerogenic DCs have the most important role for the attenuation of detrimental inflammatory response in cisplatin-injured kidneys (94-96). Soon after cisplatin injection, forkhead box P3 (FoxP3)-expressing CD4+CD25+ circulating Tregs migrate into the injured kidneys where, in IL-10 dependent manner, suppress activation of M1 macrophages, inflammatory neutrophils, Th1 and Th17 cells (94). Accordingly, adoptive transfer of Tregs significantly attenuated renal dysfunction and mortality of cisplatin-treated T cell–deficient mice (94). Treg-induced alleviation of cisplatin-caused AKI was accompanied by down-regulated serum levels of inflammatory cytokines (TNF-a and IL-1b), reduced number of M1 macrophages and inflammatory IFN-γ and IL-17-producing leukocytes in the injured kidneys, indicating therapeutic potential of Treg-based therapy for attenuation of cisplatin-induced nephrotoxicity.

Tolerogenic DCs represent specific sub-population of resident renal immune cells which have important immunosuppressive and nephroprotective role in cisplatin-induced AKI (95,96). Damage associated molecular patterns (DAMPs) and alarmins, released from cisplatin-injured PTECs, down-regulate expression of costimulatory molecules and enhance expression of anti-inflammatory IL-10 in renal DCs (78,88,95,96). These immunosuppressive DCs, mainly in IL-10 dependent manner, inhibit activation of M1 macrophages, inflammatory neutrophils and Th17 cells in the cisplatin-injured kidneys contributing to the alleviation of inflammation (78,88,95,96). Accordingly, depletion of tolerogenic DCs significantly enhanced inflammatory response in the kidneys and aggravated cisplatin-induced AKI (88,95).

Clinical manifestations of cisplatin-induced renal injury

Acute renal failure, thrombotic microangiopathy, hypomagnesemia, anemia, salt wasting and Fanconi-like syndrome have been the most usually observed clinical manifestations of cisplatin-induced nephrotoxicity (97).

Generally, acute renal failure begins a few days after cisplatin treatment. It is manifested by an increase in the blood urea nitrogen and serum creatinine concentrations and decrease of glomerular filtration rate. Cisplatin-treated patients usually develop non-oliguric AKI, and glucosuria and minimal proteinuria might be observed as a result of PTEC injury (20).

Significant decrease in renal blood flow occurs three hours after cisplatin administration, resulting in deterioration in glomerular filtration rate (GFR). Increased sodium chloride delivery to macula densa and tubulo-glomerular feedback are related to an increased vascular resistance and reduced GFR (98).

The proximal renal tubules are the major site of sodium and water reabsorption (98). Cisplatin treatment causes reduction activity of ATPase, disturbed transport of water and electrolytes, mitochondrial dysfunction and altered cation balance in PTECs, leading to the decreased reabsorption of sodium and water and increased salt and water excretion (98). Accordingly, renal salt wasting syndrome occurs in 10% patients and represents one of the most usually observed complication of cisplatin therapy (99). Renal salt wasting syndrome is associated with intensive polyuria, hyponatremia, hypovolaemia, severe orthostatic hypotension, and prerenal AKI accompanied by dysfunction of the renin-aldosterone system which regulate salt and water wasting in the kidneys (98-101).

Also, cisplatin treatment may decrease the reabsorption of the filtered magnesium which causes refractory hypomagnesemia. This complication occurs in 90% of patients, and depends on the cumulative dose of administered cisplatin (17,99). Having in mind that hypomagnesemia may be observed in some patients 6 years after initial cisplatin treatment, there is a possibility that cisplatin-induced hypomagnesemia has been developed in two phases. The first phase, which is manifested by malabsorption of magnesium, happens due to the cisplatin-caused damage of calcium/magnesium-sensing receptor (99), while the second phase, characterized by patchy necrosis of tubular cells, is manifested by progressive renal injury and extensive magnesium loss (99). It has to be noted that magnesium deficiency may be associated with hypokalemia and hypocalcemia and, accordingly, malignant cardiac arrhythmias and neuromuscular dysfunction may be observed in cisplatin-treated patients (99).

Renal Fanconi syndrome has also been reported in some of cisplatin-treated patients (20). This syndrome is characterized by glycosuria, urinary lack of low molecular weight proteins (β2- microglobulin, retinol- binding protein and α1-macroglobulin), aminoaciduria (loss of amino acids such as alanine, valine, leucine, methionine), proximal tubular acidosis, phosphate and potassium wasting (97,98,102,103). Additionally, long-term exposure to cisplatin may result in the development of tubulointerstitial injury and interstitial fibrosis which may be life-thretening complication of cisplatin (98,104).

CONCLUSIONS

Cisplatin treatment induces apoptosis and oxidative stress in PTECs eliciting strong inflammatory response in the injured kidneys. Having in mind that these cisplatin-induced effects are, at the same time, involved in anti-tumor activity of cisplatin, newly designed renoprotective strategies against cisplatin-caused nephrotoxicity should rely on the identification of the structural and functional differences between cisplatin-injured renal and tumor cells. In that way, the prevention of cisplatin-induced nephrotoxicity will not affect antitumor effects of cisplatin and will significantly improve its clinical utility.

ACKNOWLEDGEMENTS

This study was supported by the Faculty of Medical Sciences University of Kragujevac (MP 01/18) and the Serbian Ministry of Education, Science and Technological Development (ON175069, ON175103).

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

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DEVELOPMENT OF THERAPY APPROACH IN PATIENT WITH CHRONIC GRANULOCITY LEUKEMIA: CASE REPORT

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Received: 17.12.2018.
Accepted: 30.12.2018.

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UDK:616.155.392-085
Eabr 2024; 25(3):285-288
DOI: 10.2478/sjecr-2018-0074

ABSTRACT

Chronic granulocytic leukemia (CGL), Ph + is a chronic myeloproliferative disorder, which, due to its specificity for many decades, has attracted the attention of researchers of numerous specialties. This disease was among the first malignant haemopathies that received their "ID card" since molecular analyzes precisely defined the basic pathological substrate, that is, the origin of the disease. Over the past decades we have witnessed the evolution of the therapeutic approach in the treatment of CGL from oral cystostatic therapy, radiotherapy, through recombinant interferon alpha (IFN-alpha), haematopoeza stem cell transplantation, to the targeted molecular therapy of the tyrosine kinase inhibitor (TKI). In this report we present patient with a diagnosis of CGL, Ph + in the early chronic phase at the age of 13 years based on all relevant analyzes. During the past 26 years, all therapeutic modalities of CGL treatment were applied in patients, according to the time period, concluding with the second generation TKI, resulting in complete remission of diseases with excellent quality of life and identical expectations for the future, as well as in the healthy population.

Keywords: *Chronic granulocytic leukemia, Ph + (CGL), Therapeutic approaches, case report.*

INTRODUCTION

Chronic granulocytic leukemia (CGL) is a highly specific disease that is defined by strict hematologic parameters that include a pathognomonic differential leukocyte count. Usually CGL is accompanied by the presence, in bone marrow cells, of the Ph chromosome, the first chromosomal anomaly to be regularly associated with a human neoplastic disease (1). CGL is predominantly a disease of the productive middle years of life, which maximizes its adverse impact on family life and family economics. The disease is of worldwide distribution and there is a slight male preponderance. The disease is characterized by an initial chronic phase when it behaves as a differentiated neoplasm and responds very well to simple, nonintensive therapy (1-3).

CASE PRESENTATION

We present male patients with whom he was diagnosed with CGL, Ph + in early chronic phase in 1992, when he was 13 years old. Clinical characteristics of the disease at the presentation were signs of anemia syndrome in the presence of organomegaly (hepatosplenomegaly). In haematological parameters, leukocytosis up to 275x109 / L was detected with the presence of all forms of white lines in the peripheral blood smear, with anemia with hemoglobin of 89 g / L and mild thrombocytosis up to 499x109 / L. Biopsy of the bone marrow confirmed the diagnosis of CGL with a chronic phase image, and cytogenetic analysis was confirmed by Ph chromosome. Introductory treatment was carried out with Hydroxiureom in a dose dependent on haematological parameters, which achieved cytoreduction, and in the next five months, the patient received recombinant IFN alpha at a dose of 9 U per week. Clinical and hematologic remission of the disease with the maintenance of the cytogenetic marker was achieved on the applied therapy. Bearing in mind the nature of the underlying disease, allogeneic haemopoietic stem cell transplant (MHH) transplantation from the family matched (in the HLA and ABO system) of the donor, born brother, was done in the future. The conditional regimen was conducted according to the myeloablative protocol in combination with oral formulation of Busulphan-Cyclophosphamide 2 with complete supportive therapy (adequate hydration, urotoprotection, prevention of mucositis and possible forms of infection). The source of the MSH was the bone marrow, with the patient on 27.12.1993 receiving a total of 1.56x108 cells with a nucleus (total nucelar cells -TNC) / kgTM in a suspension of 1170 ml of bone marrow. Prevention of graft versus host disease (GvHD) was carried out with dual immunosuppressive therapy (Cyclosporine A + Methotrexate according to the "short Seatle" protocol). The period of iatrogenic bone marrow aplasia was accompanied by oropharyngeal mucositis of grade 2. The acceptance of the allogeneic coil, measured by the parameters of the blood count (neutrophil and platelet counts), was recorded from +18. days after transplantation. Dana +40. After transplantation complete donor chimerism has been proven. No signs of acute or chronic GvHD are registered. Remission of the disease to the cytogenetic level was maintained until August 1996. During this period, relapses of the disease were confirmed on cytogenetic and then haematological level, 32 months after the primary allogeneic transplantation of the MSH. Treatment of the first relapse begins with recombinant INF alpha at a dose of 9 MU per week for three months. The therapy did not achieve the desired response at the cytogenetic level, so treatment was continued using immunoadaptive therapy, by infusion of lymphocytes taken from the same donor. The patient received three units of donor lymphocytes (DLI) on 10.12. and 30.12.1996. and February 17, 1997, in escalating doses (2.6x107, 4.1x107, 4.1x107 / kg/bw). Clinical monitoring did not record signs of acute GvHD. Hematological monitoring detects a gradual occurrence of pancytopenia, and in April 1997, bone marrow biopsy and myocardial infarction confirmed bone marrow aplasia as a potential adverse effect of DLI. Further treatment was carried out by the secondary allogeneic transplantation of the MSH from the identical family donor. In a conditioned regimen, according to bone marrow aplasia, the patient received only antitomocyte globulin (ATG - Fresenius) at a dose of 5 mg / kg/bw for four days. The MSH source was the peripheral blood of the donor after a five-day preparation of recombinant G-CSF at a dose of 5 mcg /kg/bw. Secondary transplantation was done on May 19, 1997. where the patient received 5.3x108 TNC / kgTM, i.e., 15.5x106 / kg/bw CD34 + cells). Prevention of GvHD was not applied. Acceptance of the allogeneic coil is recorded via the number of neutrophils and platelets of +15. days after transplantation, while cytogenetic and molecular analysis determine the complete remission of the disease with complete donor chiberism. Clinical monitoring did not show signs of acute or chronic GVHD. A complete remission of the disease was maintained until December 2002 (67 months after secondary allogeneic MHH transplantation). During this period, late relapses of the disease were recorded at the cytogenetic level, and the treatment continued with the first generation tyrosine kinase inhibitor (Glivec) at a dose of 400 mg per day. The desired therapeutic response (parsing of the disease at the cytogenetic level) was not achieved, and since October 2004 the preparation of the second generation TKI - Nilotinib was included in the therapy. This form of treatment achieves a complete remission of the disease down to the molecular level that has been maintained over the past 14 years.

DISCUSSION

Chronic granulocytic leukemia is a chronic myeloproliferative disease, a hematopoietic stem cell disease, characterized by a specific Phyladelphia chromosome, or reciprocal translocation between chromosomes 9 and 22 (1-3). Thanks to the significant advances in basic diagnostic procedures, CGL is one of the rare hematological diseases that has its own ID card from clinical symptoms and signs, through physical findings, haematological parameters, peripheral blood smear, bone marrow biopsy, cytogenetics to molecular markers (4). The incidence of CGL is 10-15 people per 100 000 inhabitants per year and occurs most frequently in the age between 60-65 years (5).

The disease passes through three characteristic clinical phases, chronic, phase of acceleration and blast transformation phase, which are clearly defined on the basis of clinical, hematological and cytogenetic-molecular criteria (5, 6). Over the past decades, the therapeutic approach to treating patients with CGL has been reduced and evolved along with a more precise clarification of the pathogenesis of the disease. The end of the 19th century was marked by the use of a arsenic preparation in the treatment of CGL or X radiation of enlarged spleen. The fifties of the 20th century began with the use of chemotherapy, initially Busulphanom, and then Hydroxiuree in patients with CGL. Hydroxiurea has been the most effective cytoreduction agent for this disease for decades, as was the case with our patients at the initial stage. Using various forms of cytoreductive therapy, clinical and hematologic remission of the disease in a negligible percentage of patients could be achieved, while the causative action on the disease itself was absent. In the early 1990s, patients with CGL were treated with recombinant IFN-alpha, a powerful immunomodulatory agent that opened a new era in the control of this malignant disease. Namely, for the first time, one agent acted at the cytogenetic level, bringing in the truth in a small group of patients, and a complete cytogenetic response. The next period was marked by the establishment of new cytogenetic response scales (from minimal to complete), which dictated the therapeutic approach (monotherapy with IFN alpha or combinations with other cytoreductive agents) (7). In the case of our patient, the proposed CGL treatment algorithm was followed in full according to the time period, and immunomodulatory therapy with IFN-alpha was also applied. Transplantation of stem cell hematopoiesis since 1979 (single transplantation) over the eighties and nineties of the 20th century was the standard in the treatment of patients with CGL, and this diagnosis was the most common indication for allogeneic transplantation from a related matched donor.

Based on clinical experience, the European Bone Marrow Transplantation Group(EBMT) has designed an EBMT prognostic score for predicting mortality rates in patients with CGL-treated allogeneic MSH transplantation based on a combination of a number of parameters related to patients and potential donors (7-9). In the case of our patient, since the initial treatment with cytoreductive and immunomodulatory therapy has achieved clinical and hematological control of the disease, without the desired response at the cytogenetic level, an indication is given that the treatment will continue with the allogeneic transplantation of the MSH from the family fully matching donor. In accordance with the current attitudes and the fact that it is a hematopoietic stem cell disease, the preparatory regime was myeloablative, thesource of the stem cells of the bone marrow, and the prevention of GvHD double immunosuppressive therapy (8, 9). In the post-transplant period, a timely engrafment was confirmed in our patient with the achievement of complete donor chimerism, and no clinicalor acute GvHD signs were recorded by clinical monitoring. Opinions regarding the monitoring of patients with CGL in whom allogeneic transplantation of the MSH occurred in the 1990s implied a three-month analysis of the cytogenetic finding, and then over the years and a molecular marker of the disease in order to timely diagnose the possible relapse of the underlying disease. In 1990, for the first time, infusion of donor lymphocytes (DLI) was used in the treatment of relapse of the disease after allogeneic transplantation (7, 10). Clinical observations have unambiguously confirmed that CGL disease is in the first place by degree of sensitivity to applied DLI, that is, that this therapeutic procedure can achieve a significant degree of secondary remission of the disease (11). The most common side effects of DLI include GvHD and bone marrow aplasia (12, 13). In our patient, three years after allogeneic transplantation, a rough relapse of the disease was diagnosed, and treatment was performed with three escalating doses of DLI from the original family donor, according to current recommendations for treatment in these situations. Newborn bone marrow aplasia with potentially fatal infectious complications can be interpreted as an adverse effect of DLIapplication. In this situation, the therapeutic approach for our patients was extremely limited. It is known that secondary allogeneic transplantation of the MSH in the case of leukemia relapse is an extremely complicated procedure, followed by a high degree of post-transplant morbidity and mortality, especially if one of the modalabative preparatory regimes (13, 14) is applied. In the late nineties of the last century, in order to overcome these complications, conditioned regimens of "reduced" intensity were designed primarily for secondary allogeneic transplantation of the MHH or elderly patients with associated illnesses (2, 13-15). In the phase of bone marrow aplasia after DLI our patient was only prepared with ATG, and the source of the MSH from the original family donor was peripheral blood in order to achieve the "graft-versus-leukemia" (GvL) effect needed to control the minimum residual illness. No additional immunosuppressive therapy has been applied to generate GvL effects. This treatment regimen again achieved a multi-year remission condition.

Further development of the therapeutic approach in the treatment of CGL patients afterthe MSH transplantation and the DLI application was based on the understanding of the molecular mechanism of the disease, and implied the use of tyrosine kinase inhibitors (TKI), starting in 1998 (15-17). The application of different generations of TKI led to the revolutionary control of CGL, concluding with the achievement of complete molecular remission of the disease (4, 10, 14). Thanks to the introduction of TKI in CGL therapy and the achievement of molecular disease control, it can rightly be said that patients with CGL have identical life expectations as well as a healthy population. After the onset of the secondrelapse of the disease due to secondary allogeneic transplantation, our patient was initially included in the first, and then due to the incomplete response and the second generation TKI with long-term complete control of the disease and excellent quality of life.

CONCLUSION

Current treatment of CGL over the past 130 years significantly evolved follow-up achievements in medicine and basic biological sciences. Due to a more precise view of the pathogenetic process in the onset and progression of CGL from the chronic phase to the form of acute leukemia, concurrently with molecular mechanisms, targeted drugs have been synthesized that stop the proliferation of malignant clones in this disease (TKI). The displayof our patients fully supports the development of a therapeutic approach in the treatment of CGL, cytoreductive therapy, immunomodulatory agents, MHC transplantation, application of cell therapy such as DLI to first and second generation TKI.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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APPENDICEAL MUCOCELE - A REVIEW OF LITERATURE WITH A CASE REPORT

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Received: 20.04.2021.
Accepted: 20.04.2021.

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ABSTRACT

Appendiceal mucoceles represent neoplastic and non-neoplastic, dilatated, mucus filled appendix vermiformix. Appendectomy is obligatory due to a possible malignancy. It is crucially important to avoid rupturing of the mucocele because it can result in pseudomyxoma peritonei, with high morbidity and mortality. We presented a 52-year-old man with pain and palpable mass in the lower right quadrant of the abdomen. The mucocele was removed without a rupture, and the patient was discharged from the surgical department one day after the surgery without a complication. The resection must be done very carefully, because the rupture of a mucocele can cause pseudomyxoma peritonei, a very dangerous and often lethal condition. Due to the concern of rupture, we performed the classical resection through laparotomy. It is very important, especially for young, inexperienced surgeons to be aware of this rare diagnosis and perform a surgical intervention according to the guidelines of good clinical practice.

Keywords: *Appendiceal Mucocele, Pseudomixoma Peritonei, Case Report.*

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UDK:616.346.2-002-089
Eabr 2024; 25(3):289-292
DOI: 10.2478/sjecr-2021-0027

BACKGROUND

Appendiceal mucocele, first described in 1842 by Rokitansky, is a rare diagnosis, with less than 1% of pathologies related to the appendiceal disease. It represents a distended appendix filled with mucus, not some specific disease. Mucoceles of the appendix are diagnosed as incidentaloma in about 50% of cases. The symptoms can include the abdominal pain or mass, weight loss, nausea, changes in bowel habits, or they can be presented as acute appendicitis (1). Sometimes, appendiceal mucocele can be presented as acute appendicitis or an adnexal mass, mimicking the true nature of a disease (2). US and CT exams are usually sufficient imaging diagnostic tools. Colonoscopic findings may be characteristic with a "volcano sign", presenting the appendiceal orifice elevated and covered by normal mucosa or sometimes with a lipoma-like submucosal tissue (3).

CASE PRESENTATION

We presented an otherwise healthy 52-year-old man with the symptoms of the right-lower quadrant abdominal pain and palpable mass. The abdominal ultrasonography revealed suspicion of appendiceal mucocele, which was confirmed by MSCT of the abdomen (Figure 1). CEA, CA 19-9 and CA 125 were within a normal range.

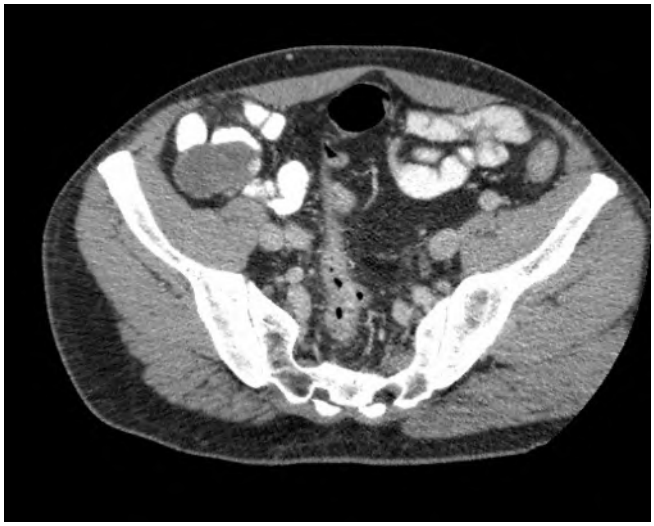

Figure 1. MSCT findings of appendiceal mucocele.

During the open access appendectomy, we clearly identified basis of the appendix, whose diameter was cc 5mm and after careful manipulation, the appendix and mucocele were removed intact, without a rupture of the mucocele wall or mucin leakage (Figure 2). A carefully maintained exploration of the peritoneal cavity excluded any mucin deposits. We performed the "ex tempore"- frozen section biopsy which revealed benign characteristics of the specimen and simple appendectomy was a sufficient treatment. The patient was discharged from the surgery department one day after the surgery without any complaint.

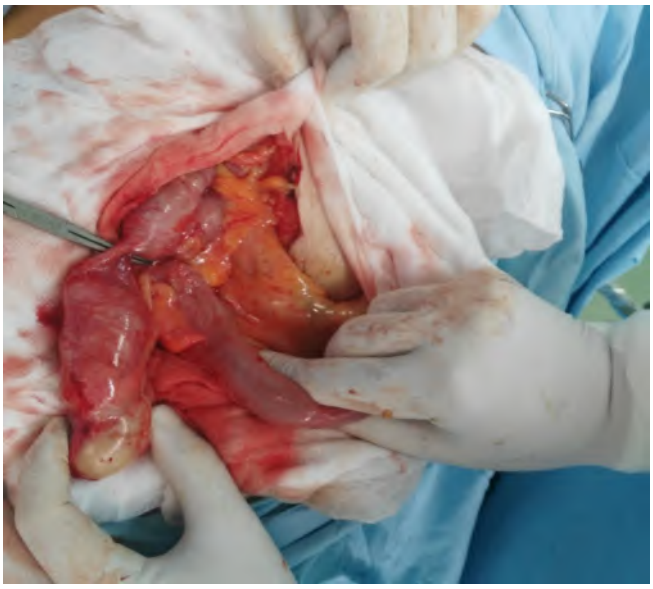

Figure 2. Surgical specimen of AM with an intact wall, basis of the appendix is not affected.

Consecutive pathology demonstrated appendiceal mucocele, with the wall covered by a single-line epithelium of benign histomorphological characteristics, and the lumen filled with a non-structural eosinophilic mucin (Figure 3 and 4).

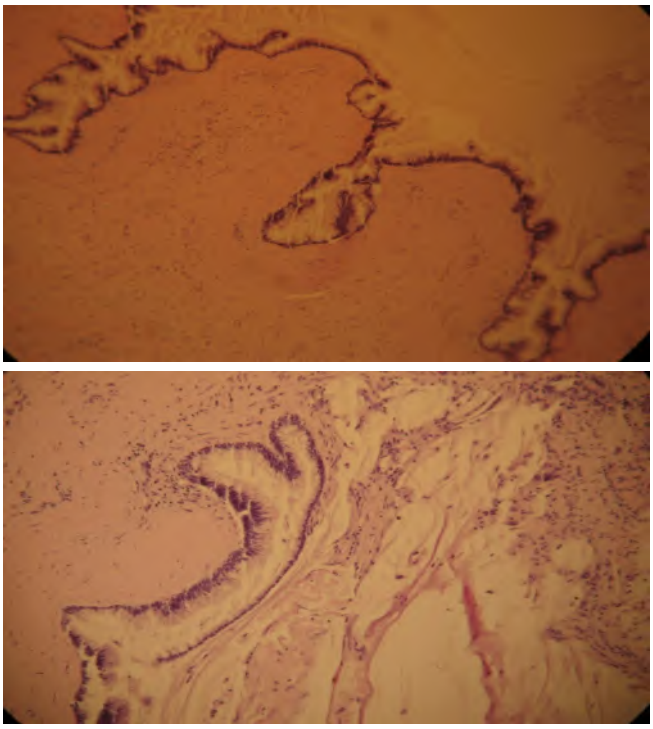

Figure 3 and 4. The wall of the mucocele coated with a single-stranded epithelium of benign histomorphological characteristics and the lumen filled with a non-structural eosinophilic mucin.

DISCUSSION

Historically, appendiceal mucoceles have been classified into four types: A. Non-neoplastic, including (1) a simple mucocele-retention cyst often caused by a fecalith or structure obstructing the appendiceal outflow, (2) mucosal hyperplasia, and B. neoplastic, including (3) a benign mucinous cystadenoma, and (4) a mucinous cystadenocarcinoma. The cystadenomas are typically referred to as low grade appendiceal mucinous neoplasms (LAMNs) (4).

A recent international Delphi consensus has suggested that the term "cystadenoma" should no longer be used for appendiceal tumors. "Mucinous adenocarcinoma" should be used to describe mucinous tumors with infiltrative invasion, "low-grade appendiceal mucinous neoplasm" for describing those with spreading growth but without destruction, "high-grade appendiceal mucinous neoplasm" describes tumors with low-grade architectural features but high-grade cytological features, and term "signet ring carcinoma" for those with over half of the cells with the signet ring morphology. Pseudomyxoma peritonei can be of low-grade, high-grade, and signet ring cell histological type (5).

Mucoceles <2 cm are usually benign, while sizes >6 cm are more often associated with malignancy and a higher rate of perforation (6).

A mucocele is usually diagnosed by the abdominal CT scan. The typical finding of appendiceal mucocele is a low attenuated, well-encapsulated, thin-walled cystic mass in the right lower quadrant of the abdomen (7).

The most feared complication, occurring secondary to a spontaneous or iatrogenic rupture, is pseudomyxoma peritonei (PMP), which represents mucinous deposits within the peritoneal cavity. PMP is poorly understood; however, it is known to develop insidiously as a result of mucin producing neoplastic, epithelial goblet cells forming mucinous implants throughout the abdominopelvic peritoneum and it is associated with significant morbidity and mortality (8).

Both benign and malignant mucoceles can produce mucinous peritoneal deposits known as pseudomixoma peritonei (PMP). A 5-year survival rate in the first case is about 91–100%, while the prognosis for a malignant mucocele is much worse, with only 25% of a 5-year survival rate (9).

With some aggressive regimens, such as HIPEC (heated intraperitoneal chemotherapy) and cytoreductive surgery, 10-year survival can raise close to 50% (10).

For a long time, laparoscopy has been considered as a contraindication for the surgical treatment of appendiceal mucocele. Also, some authorities suggested laparotomy for better exploration of the peritoneal cavity searching for mucin deposits (11).

However, with technical development and general improvement in surgical skills, there is a raising number of recent reports describing safe laparoscopic or robotic assisted removal of appendiceal mucocele (12, 13).

Apart from the dilemma regarding the surgical approach (open vs laparoscopic surgery), there is still a debate concerning the extent of resection, from appendectomy as the only treatment to a right hemicolectomy. To determine indications for the right hemicolectomy, González Moreno and Sugarbaker recommended the use of a sentinel lymph node biopsy, with a frozen section examination. According to them, the indications for the right hemicolectomy are: (1) inability to achieve total removal of the primary tumor or complete cytoreduction by appendectomy, (2) appendiceal or ileocolic lymph nodes involvement, and (3) a non-mucinous neoplasm confirmed by the histopathological examination (14).

A useful practical algorithm, proposed by Filho and collaborators, suggests appendectomy with excision of the mesoappendiceal fat and lymph nodes if appendiceal base is not affected. If the base is involved, typhlectomy or partial right hemicolectomy is indicated. The frozen section is mandatory in both cases, and if a specimen is benign, the right colectomy is not indicated. A malignant specimen indicates an oncologic right hemicolectomy (15).

If a rupture of AM occurs, followed by adenomucinosis, a 5-yearfollow-up period with CT scans and CEA and CA19-9 levels monitored every six months are recommended. In the case of AM perforation with a confirmed diagnosis of mucinous adenocarcinoma, a second-look surgery is recommended, six months after the initial intervention (16).

CONCLUSION

The diagnosis of AM should always be considered in all cases of cystic lesions of the right lower quadrant. The surgical resection of a mucocele is the therapy of choice, open approach or laparoscopy, depending on the surgeon's skills. Benign and especially malignant AMs rupture must be avoided due to PMP and a consequent high mortality rate. The management of appendiceal mucocele is simple, ranging from appendectomy only to the right hemicolectomy followed by HIPEC, depending on the intraoperative and pathological findings. Clear understanding of this rare condition and optimal surgical treatment based on algorithms of good clinical practice are critical.

ACKNOWLEDGMENTS

The authors are grateful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support (Projects No. III41010, III41007). We would like to thank Miss Sanja Dugić for her help with the translation of this manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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INFORMATION FOR AUTHORS

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