ORIGINAL SCIENTIFIC ARTICLE

RELATION BETWEEN OXIDATIVE STRESS AND CAROTID ARTERY ATHEROSCLEROSIS IN HEMODYALISIS PATIENTS

Svetlana Antic[1], Nevena Draginic[2], Milena Jovanovic[3], Tomislav Nikolic[3,4], Nevena Jeremic[2,5] Vladimir Živkovic[5], Ivan Srejovic[5], Dejan Petrovic[4,6] and Vladimir Jakovljevic[5,7]

[1]*Clinic for Nephrology, Military Medical Academy Belgrade, Belgrade, Serbia*
[2], *University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Kragujevac, Serbia*
[3] *University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia*
[4]*Center for Nephrology and Dialysis, Clinic for Urology and Nephrology, Clinical Center Kragujevac, Kragujevac, Serbia*
[5]*University of Kragujevac, Faculty of Medical Sciences, Department of Physiology, Kragujevac, Serbia*
[6]*University of Kragujevac, Faculty of Medical Sciences, Department of Internal Medicine, Kragujevac, Serbia*
[7]*1st Moscow State Medical University IM Sechenov, Department of Human Pathology, Moscow, Russia*

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Corresponding author:

Dejan Petrovic

University of Kragujevac, Faculty of Medical Sciences, Department of Internal Medicine, Center for Nephrology and Dialysis, Clinic for Urology and Nephrology, Clinical Center Kragujevac, Kragujevac, Serbia

Phone: +381 64 3741 694

E-mail: dejanpetrovic68@sbb.rs

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ABSTRACT

Oxidative stress represents a significant risk factor for the accelerated development of atherosclerosis in a population of patients on regular hemodialysis. Oxidative stress induced by hemodialysis can be triggered with both the bioincompatibility of dialysis membrane and increased endotoxin concentration in a hemodialysis solution. The aim of this study was to investigate the correlation between the parameters of oxidative stress, microinflammation, nutrition, secondary hyperparathyroidism and carotid artery intima-media thickness in patients on regular hemodialysis.One hundred and twenty five patients treated with standard hemodialysis and on-line hemodiafiltration with "high-flux" polysulfone dialysis membrane were examined. The following parameters of oxidative stress were measured : index of lipid peroxidation - measured as TBARS, nitric oxide in the form of nitrite - NO_2-, super oxide anion radical - O_2- and hydrogen peroxide - H_2O_2, catalase, superoxide dismutase (SOD) and reduced glutathione activity. For statistical analysis of results, the following tests were used: the Kolmogorov-Smirnov test, the Spirman test and the Pearson correlation test. Oxidative stress affects atherosclerosis of the carotid arteries in patients treated with regular hemodialysis and online hemodiafiltration. There is a statistically significant positive correlation between H_2O_2 concentration and the thickness of the carotid arteries` intima-media. High statistically significant positive correlation was found between TBARS concentration and carotid arteries intima-media thickness, while a high statistically significant negative correlation was found between SOD activity and a carotid artery intima-media thickness. There is a statistically significant negative correlation between the serum albumin and prealbumin concentration and a carotid artery intima-media thickness. Oxidative stress may be a significant risk factor for the carotid artery atherosclerosis development in patients treated with regular hemodialysis.

Keywords: *Oxidative stress, atherosclerosis, hemodialysis.*

INTRODUCTION

Cardiovascular disease remained the leading cause of death in patients treated with regular hemodialysis. Atherosclerotic heart disease and congestive heart failure are two clinical conditions that are the cause of death in more than 50% of these patients. There are traditional and non-traditional risk factors for the development of cardiovascular disease in this patient population. Non-traditional risk factors are associated with impaired renal function and hemodialysis procedures and they include the oxidative stress, microinflammation, malnutrition, endothelial dysfunction, uremic toxins, hyperhomocysteinemia, anemia, hypervolemia, vitamin D deficiency and secondary hyperparathyroidism. Oxidative stress is one of the most significant non-traditional risk factors for the development of accelerated atherosclerosis in a population of patients treated with regular hemodialysis (1).

In these patients, the oxidative stress occurs due to the bioincompatibility of the hemodialysis membrane, the increased concentration of endotoxins in the hemodialysis solution, the intravenous application of iron solutions and reduced activity of enzymatic and non-enzymatic antioxidant protective mechanisms (2-4). During the hemodialysis session, activated cells of the innate immune system in peripheral blood (neutrophils, monocytes) generate reactive oxygen species (ROS) intensely and the capacity of endogenous antioxidant protective mechanisms is reduced due to loss of water-soluble antioxidants (vitamin C) and oligoelements (antioxidant enzyme cofactors). Malnutrition also contributes to reduction of antioxidant protective mechanisms activity in the population of patients undergoing hemodialysis. Main clinical consequences of oxidative stress are the development of atherosclerosis, resistance to erythropoetin and the development of cardiovascular disease in patients treated with regular hemodialysis (4-7).

In addition to oxidative stress, a significant role in the development of a carotid artery atherosclerosis in patients on regular hemodialysis has the microinflammation, hyperhomocysteinemia, and malnutrition (4-8). Main factors for the development of microinflammation in these patients are: bioincompatibility of the extracorporeal circulation, the presence of endotoxin in the hemodialysis solution (reverse diffusion), asymptomatic infection of the arteriovenous fistula for hemodialysis, the periodontal disease and dislocation of bacteria and endotoxins from intestines to circulation, as a result of disruption of the gut microbiome (7, 8). Blood contact with the synthetic material of the extracorporeal circulation during the hemodialysis session causes constant leukocyte and the complement system activation, release of elastase, myeloperoxidase, pro-inflammatory mediators and ROS (8). Increased serum neutrophil elastase concentrations have been associated with microinflammation, shortened erythrocyte lifetime (erythrocyte membrane disorder), resistance to erythropoietin and adverse outcomes in patients on regular hemodialysis (8). Oxidative stress and microinflammation are key factors for the development and progression of atherosclerotic cardiovascular disease in patients undergoing regular hemodialysis. Optimal control of the oxidative stress and microinflammation is a key step in reducing cardiovascular morbidity and mortality in this patients' population (8).

In patients who are on the regular hemodialysis program, the hyperhomocysteinemia is defined as a plasma homocysteine concentration of > 15 μmol/L, resulting from the decreased activity of enzymes crucial in homocysteine metabolism, such as 5-methyl-tetrahydrofolate reductase, methionine synthase, and γ- cistation synthase. The reduced activity of these enzymes occurs due to a deficiency of vitamins B6, B12 and folic acid, which serve as cofactors of enzymes mentioned (insufficient intake, increased loss during the hemodialysis session) (9). Increased homocysteine concentration, oxidative stress (superoxide anion radical) and microinflammation block the activity of the dimethyl diamino-hydrolase enzyme (DDHA) in endothelial cells, which degrades asymmetric dimethylarginine - ADMA (dimethylarginine) to L-citrulline and methionine. Asymmetric dimethylarginine is the most significant endogenous nitric oxide synthase blocker while the reduced nitric oxide formation in endothelial cells plays a key role in initiating the process of atherosclerosis. In addition, the superoxide anion oxidizes tetrahydrobiopterin (an endogenous NO synthase cofactor) and thus further reduces NO synthase activity and contributes to accelerating the process of atherosclerosis. Endothelial dysfunction plays a key role in the pathogenesis of atherosclerotic cardiovascular disease in a population of patients undergoing regular hemodialysis. Clinical trial results indicate a statistically significant positive association between the asymmetric dimethylarginine concentration and carotid artery intima-media thickness (10).

Malnutrition as a result of protein deficiency - PEW (Protein-Energy Wasting) is present among 30-60% of patients undergoing regular hemodialysis. According to the International Society of Renal Nutrition and Metabolism (ISRNM) guidelines, PEW exists if: the serum albumin concentration is<0.38 g/L, the serum prealbumin concentration<0.30 g/L, the total cholesterol concentration<100 mg/dL, the body mass index less than 23 kg/m2, the unintentional weight loss ≥ 5% over three months and the protein intake less than 0.8 g/kg/day. PEW results from reduced intake and increased protein catabolism. Risk factors that promote protein catabolism in patients undergoing regular hemodialysis include: the metabolic acidosis, microinflammation, and oxidative stress (11).

Carotid artery atherosclerosis is defined as an intima-media thickness (IMT) of > 0.90 mm, as measured by the ultrasound examination of carotid arteries. Based on the color Doppler ultrasonography, the atherosclerotic carotid artery disease is classified into four stages: stage I: IMT<0.9 mm, grade II: IMT > 0.9 mm, stage III: the presence of atherosclerotic plaques with a stenosis ≤ 50% and stage IV: the presence of atherosclerotic plaques with stenosis > 50%. The presence of plaque is defined as a structure that protrudes into the carotid artery lumen at least 0.5 mm (5).

Hemodialysis membranes play a key role in preventing the development of oxidative stress, microinflammation, and malnutrition in patients treated with regular hemodialysis. High-flux hemodialysis membranes have a greater number of advantages over low-flux membranes: they are more biocompatible, better remove small and medium molecular weight uremic toxins, have a less degree of neutrophil activation and complement systems (less potential to produce proinflammatory cytokines and acute phase proteins inflammation), provide less resistance to erythropoietin and better preserve residual renal function (8). High-flux membranes improve quality of life and provide a better long-term outcome for patients treated with regular hemodialysis (8). Vitamin E-coated membranes for the hemodialysis reduce microinflammation (reduce serum interleukin 6 concentration), prevent lipid peroxidation (decrease serum TBARS concentration), increase iron availability for erythrocytopoesis (reduce hepcidin concentration in erythropoietic) and reduce resistance to erythropoetin.

The aim of this study was to investigate the correlation between parameters of oxidative stress, microinflammation, malnutrition, secondary hyperparathyroidism, and carotid artery intima-media thickness in regular hemodialysis patients.

PATIENTS AND METHODS

The study included 125 patients treated with regular hemodialysis and on-line hemodiafiltration at the Center for Nephrology and Dialysis of the Clinical Center Kragujevac. The study was conducted in accordance with the Declaration of Helsinki for Medical Research (revised in 2013.), the consent was obtained from patients and the Ethics Committee of the Clinical Center Kragujevac. Patients treated with regular hemodialysis and on-line hemodiafiltration were examined 2-3 times a week for 4 hours (8-12h per week), for a period longer than three months, with "high-flux" membranes (for regular hemodialysis: polysulfone high-flow membrane with surface area 1.4-1.7 m^2, for hemodiafiltration: polysulfone "high-flux" membrane with surface area 2.0-2.4 m^2). Dialysis machines used in study were with controlled ultrafiltration type Fresenius 5008S, Gambro Artis and BBraun, with average blood flow rate - Qb=222.80 ± 25.89 mL/min and average dialysate flow rate - Qd=500 mL/min. A standard ultrapure hemodialysis solution (endotoxin concentration - E<0.03 EU/ml) was used, with a calcium concentration of 1.75 mmol/L (PGS21), 1.50 mmol/L (PGS25) and 1.25 mmol/L (PGS27) while convective volume in patients treated with on-line hemodiafiltration was Vconv=17 liters per session. Unfractionated heparin was used for anticoagulation of the extracorporeal circulation. The average monthly dose of unfractionated heparin for single hemodialysis was 4418.00 ± 525.34 IU. The study did not include patients with the manifested active bleeding, active systemic inflammation or infection (average leukocyte count was 7.06 ± 1.56 x 10^9/l), with uncontrolled malignancies, or patients treated with immunosuppressive and antioxidant medications.

In order to evaluate the effect of oxidative stress, microinflammation, nutrition and secondary hyperparathyroidism on the intima-media thickness of the carotid artery the following parameters were investigated: index of lipid peroxidation - measured as TBARS (thiobarbituric acid reactive substances), nitric oxide in the form of nitrite - $NO2^-$, super oxide anion radical - O_2^- and hydrogen peroxide - H_2O_2., catalase (CAT), superoxide dismutase (SOD) and reduced glutathione activity (GSH), hemoglobin, hematocrit, iron, ferritin, transferrin saturation with iron, C-reactive protein, albumin, prealbumin, transferrin, intact parathormone (iPTH) and vitamin D. Hemodialysis adequacy was evaluated based on the spKt/V urea index.

Determination of serum laboratory analyzes

Blood samples were taken before and after each hemodialysis session, before administration of heparin. Routine laboratory analyzes were determined by standard laboratory tests and calculated as an average value of three measurements over three consecutive months.

The serum ferritin concentration was determined by the turbidimetric method, on the Beckman Coulter AU680 apparatus. In patients treated with regular hemodialysis, the normal serum ferritin concentration is 100-500 ng/mL. The serum CRP concentration was determined by the turbidimetric method on the Olympus AU680 and calculated as the average value of two measurements over two consecutive months. Normal serum CRP concentration is ≤ 5 mg/L. Microinflammation is defined as the concentration of CRP in the serum of > 5 mg/L. The concentration of vitamin D in the serum was determined by electrochemiluminescence, on the Cobas e 411. Normal vitamin D concentration in the serum is 20-40 ng/mL. In patients treated with regular hemodialysis, the normal vitamin D concentration is ≥ 30 ng/mL (30-80 ng/mL). A severe deficit is defined as the concentration of vitamin D<10 ng/mL, vitamin D deficiency exists if the concentration is 10-20 ng/mL, and the insufficiency is defined as the concentration of vitamin D in the serum of 20-30 ng/mL. Concentration of an intact parathormone in the serum was determined by the immunodiathymetric method (IRMA), on the gamma counter WALLAC WIZARD 1470. Normal concentration of the intact parathormone in the serum is 11.8-64.5 pg/mL. In patients with hemodialysis the upper normal limit is 300 pg/mL.

Folate and vitamine B_{12} were determined on the Access 2 Analyzer by Beckman Coulter using chemilumminescent immunoassay. Prealbumine and albumine were determined on the Abbott an Architect analyzer using prealbumin and transferrin immunoturbidimetric method. Normal concentration of prealbumine in hemodyalisis patients is ≥ 0.3 g/L (≥ 30 mg/dL)

Determination of parameters of oxidative stress in plasma

The principle of the determination of superoxide anion ($O_2^{-\cdot}$) in blood plasma samples uses the O_2 reaction with nitro

tetrazolium blue (Nitro Blue Tetrazolium - NBT) to nitroformase blue. The measurement was performed at a wavelength λ=550 nm (12).

Method for determining the concentration of hydrogen peroxide (H_2O_2) is based on the oxidation of phenol red by the hydrogen peroxide reaction, which catalyses Horse Radish Peroxidase (HRPO). The final result of this reaction is the formation of a compound with a maximum absorption λ max=610 nm (13). Determination of the lipid peroxidation index was carried out indirectly through products of the lipid peroxidation reaction with thiobarbituric acid (Thiobarbituric Acid Reactive Substances- TBARS). The principle of this method is based on the determination of lipid peroxide levels based on the reaction of one of them, malonildialdehyde (MDA) with thiobarbutyric acid (TBA). The measurement was performed at a wavelength λ=530 nm (14). The concentration of nitrogen monoxide (NO) was determined based on the amount nitrites released. The principle of this method involves the use of a Griess reagent, which builds a diazo complex with nitrites, which gives the purple color. Measurement was performed at a wavelength λ=550 nm (15).

Determination of parameters of antioxidant defense system

An adrenaline method was used to determine the activity of SOD. The principle of this method, which normally belongs to the group of the "negative" type is to monitor the reduction in the rate of adrenaline autoxidation in the alkaline environment, which is O_2^- dependent. Considering that O_2^- is removed by the present SOD, the adrenaline autoxidation reaction is inhibited. The system monitors the rate of adrenaline autoxidation change through the change in absorbance at 480 nm, which is inversely proportional to SOD activity (16). The Beutler method was used to determine the catalase activity. The principle is the spectrophotometric monitoring of the rate of the hydrogen peroxide decomposition in the presence of catalase at a wavelength of 230 nm, in which hydrogen peroxide absorbs light. For the determination of reduced glutathione (GSH) activity, the Beutler spectrophotometric method was used. The principle of the method is based on the oxidation of glutathione GSH by 5,5-dithio-bis-6,2-nitrobenzoic acid (DTNB) (17).

Determination of parameters of hemodialysis adequacy

Hemodialysis adequacy was assessed on the basis of the single-pool Kt/Vsp index calculated according to Daugridas second-generation formula: Kt/Vsp=-ln (C2/C1 - 0.008 x T) + (4 - 3.5 x C2/C1) x UF / W (mmol/L), T - hemodialysis duration (h), UF – interdalytic weight gain (L), W - body weight after the hemodialysis (kg). According to KDOQI guidelines, hemodialysis is adequate if Kt/Vsp is ≥ 1.2. Urea reduction rate index - URR index is calculated using the following formula: URR=(1-R) x 100%, where: R represents the ratio of urea concentration in the serum after and before the hemodialysis treatment. Hemodialysis is adequate if the URR index is=65-70%.

Determination of blood flow and thickness of a carotid artery intima-media

The blood flow through the vascular approach - Qavf was determined by the Color Doppler ultrasound scan, on the Logic P5 apparatus, using a 7.5 MHz probe, wherein the blood flow is calculated from the formula: $Qavf=r^2p\pi/4$ x Vmean x 60 (mL/min), r - radius of vascular access, and Vmean - mean blood flow velocity through vascular approach. The blood flow is calculated as the average of three measurements, 2-4 cm on the vein vascular approach, proximal to the anastomosis site. The blood flow through a vascular approach that provides adequate hemodialysis is 500-1000 mL/min. The thickness of a carotid artery intima-media (IMT) was determined by the Color Doppler ultrasonic examination, on the Logic P5 apparatus, using a 7.5MHz probe, as the average value of three individual measurements on the right and left carotid arteries. Measurements were performed 1-2 cm below the bifurcation of carotid arteries by the same ultrasound. The normal thickness of the intima-media is defined as a value of less than 0.9 mm.

Statistical analysis

Tests used for the statistical analyses of the obtained data were as follows: the Kolmogorov-Smirnov test, the Spirman test and the Pearson correlation test. Significance threshold was set on 0.05 and 0.01.

RESULTS

A cross-sectional study was conducted at the Center for Nephrology and Dialysis of the Clinical Center Kragujevac. The study included patients treated with regular hemodialysis and on-line hemodiafiltration for a period longer than three months. Investigated population (n=125) included 78 male and 47 female patients, an average age of 62.83 ± 10.49 years with the mean dialysis treatment length 6.51 ± 6.12 years, the mean nutrition status 25.86 ± 4.60 kg/m2 and with an average dialysis adequacy index spKtV 1.24 ± 0.29. General data on patients are presented in Table 1.

For anemia treatment short-acting and long-acting erythropoietins, an intravenous iron preparation, vitamin B preparation and folic acid (per os) were used. The average monthly dose of short-acting erythropoietin was 20538.46 ± 10716.42 IU, long-acting erythropoietin 134.89 ± 71.88 μg, the average monthly dose of intravenous iron was 273.91 ± 162.38 mg, the average monthly dose of i.v. of vitamin C was 1420.00 ± 184.04 mg, the average monthly number of ampoules of Beviplex was 11.36 ± 1.47, the average monthly dose of vitamin B12 was 3060 ± 1874.70 μg and the average monthly dose of folic acid was 187.20 ± 65.04 mg. The secondary hyperparathyroidism was treated with calcium-containing phosphate binders, active vitamin D metabolites, and paricalcitol. The average monthly dose of rocaltrol was 2.56 ± 3.86 μg, and the i.v. paricalcitol 1.04 ± 6.70 μg. For the treatment of arterial hypertension, 81 (64.80%) patients used

renin-angiotensin system blockers (mainly angiotensin converting enzyme blockers), 62 (49.60%) beta blockers, 46 (36.80%) loop diuretics and 44 (35.20%) calcium channel blockers.

A hundred and one patients (80.80%) were treated with the standard intermittent high-flux hemodialysis, and 24 (19.20%) patients were treated with the postdilution on-line hemodiafiltration. For the postdilution on-line hemodiafiltration treatment, 24 (19.20%) patients were using dialysers with a high-flux polysulfone membrane of 2.0-24m^2, while other patients (101 patients, 80.80%) were treated with a "high-flux" hemodialysis using a high-flux polysulfone dialyser membrane 1.4-1.8m^2. While 113 patients used hemodialysis solution with a calcium concentration of 1.75 mmol/L (PGS21), 9 patients had Ca^{2+} concentration in the hemodialysis solution was 1.50 mmol/L (PGS25), and only 3 patients used a solution with Ca^{2+} concentration 1.25 mmol/L (PGS27). The Na^+ concentration in the hemodialysis solution was 140 mmol/L and the K+ concentration was 2.0 mmol/L.

The average values of the anemia parameters, iron status, microinflammation, nutritional status, secondary hyperparathyroidism and ultrasound examination of the carotid arteries are shown in Table 2. In order to assess the effect of oxidative stress on atherosclerosis of the carotid arteries, the following parameters were examined: the hydrogene-peroxide (H_2O_2), thiobarbituric acid reactive substances (TBARS), nitrites (NO_2^-), the superoxide dismutase (SOD), catalase (CAT), the reduced glutathione activity (GSH) and the carotid artery intima-media thickness. Average values of the investigated parameters of oxidative stress are shown in Table 2.

There was a statistically significant ($p<0.05$) positive correlation noticed between the serum H_2O_2 concentration and the carotid intima-media thickness. In addition, the high statistically significant ($p<0.01$) positive correlation was found between the serum TBARS concentration and the carotid artery intima-media thickness, while a significant ($p<0.01$) negative association was found between the SOD activity and intima-media thickness of carotid arteries, (Table 3). Significant ($p<0.01$) positive correlation between the prealbumin and serum albumin concentrations was found, while the high statistically significant ($p<0.01$) negative correlation was noticed between the serum albumin and prealbumin concentration and carotid intima-media thickness (Table 4). Among the other parameters tested and carotid artery intima-media thickness, no statistically significant correlation was found (Table 4).

Table 1. Basic characteristics of study population

GENERAL DATA		Statistical parameters
		Xsr ± SD
Number (N)		125
Gender (m/f %)		78/47 (62.40%/37.60%)
Age (years)		62.81 ± 10.44
Hemodialysis treatment length (years)		6.51 ± 6.12
Body mass index - BMI (kg/m^2)		25.86 ± 4.60
Sistolic arterial blood pressure - SBP(mmHg)		127.64 ± 15.77
Diastolic arterial blood pressure - DBP (mmHg)		76.16 ± 7.49
Mean arterial blood pressure- MBP (mmHg)		93.32 ± 9.54
Body weight - W (kg)		72.02 ± 14.70
Interdiaytic weight gain - IDWG (kg)		2.44 ± 1.11
Interdiaytic weight gain - IDWG (%)		3.45 ± 1.58
Ultrafiltration rate - UFR (ml/kg/h)		8.64 ± 3.94
Ultrafiltration rate - UF (mL/h)		610.93 ± 275.26
Residual diuresis - RD (mL/24h)		652.40 ± 683.40
Arteriovenous fistula flow - Qavf (mL/min)		845.60 ± 433.35
Hemodialysis adequacy index - Kt/V		1.10 ± 0.24
Single pool hemodialysis adequacy index - spKt/V		1.24 ± 0.29
Urea reduction ratio - URR (%)		63.87 ± 8.62
Primary kidney disease	Glomerulonephritis chronica	11 (8.80%)
	Nephropathia hypertensiva	40 (32.00%)
	Nephropathia diabetica	19 (15.20%)
	Nephropathia obstructiva	8 (6.40%)

	Nephropathia chronica	27 (21.60%)
	Renes polycystici	20 (16.00%)
Comorbidity		
Hypertensio arterialis		75 (60.00%)
Cor hypertensivum compensatum		20 (16.00%)
Cardiomyopathia dilatativa		5 (4.00%)
Hypotensio arterialis		5 (4.00%)
Diabetes mellitus complicatus		20 (16.00%)

Table 2. General investigated final parameters of oxidative stress

INVESTIGATED PARAMETERS	Statistical parameters
	Xsr ± SD
Hemoglobin - Hb (g/L)	104.05 ± 12.28
Hematocrit - Hct (%)	31.52 ± 3.83
Mean corpuscular volume - MCV (fL)	93.89 ± 4.51
Mean corpuscular hemoglobin - MCH (pg)	30.98 ± 1.62
Mean corpuscular hemoglobin concentration - MCHC (g/L)	329.96 ± 5.09
Vitamine B12 serum concentration - VitB12 (pg/mL)	999.78 ± 516.38
Folic acid serum concentration - FOL (ng/mL)	22.48 ± 11.49
Iron serum concentration - Fe^{2+} (μmol/L)	10.05 ± 4.16
Transferrin saturation - TSAT (%)	28.30 ± 11.48
Feritin serum concentration - F (ng/mL)	745.50 ± 344.60
C-reactive protein - CRP (mg/L)	10.57 ± 12.23
Serum protein concentration - P (g/L)	64.36 ± 4.66
Albumine serum concentration - Alb (g/L)	38.10 ± 3.03
Prealbumina serum concentration - Palb (g/L)	0.28 ± 0.09
Transferin serum concentration - Trsf (g/L)	1.56 ± 0.34
Uric acid serum concentration - UA (μmol/L)	366.92 ± 59.06
Normalised protein catabolic rate - nPCR (g/kg/dan)	1.76 ± 0.66
Vitamine D serum concentration - VitD (ng/mL)	17.88 ± 9.63
Intact parathormon serum concentration - iPTH (pg/mL)	175.13 ± 199.85
Superoxide anion radical - O_2^- (nmol/mL)	3.58 ± 4.90
Hydrogene peroxide - H_2O_2 (nmol/mL)	4.65 ± 1.62
Thiobarbituric acid - TBARS (μmol/mL)	1.14 ± 0.23
Nitrites - NO_2^- (nmol/mL)	3.81 ± 1.33
Reduced glutathione - GSH (nmol/mL)	119500.29 ± 17525.20
Catalase - CAT (U/gHb x 10^4)	2.22 ± 1.88
Superoxide dismutase SOD (U/gHb x 10^4)	32.82 ± 20.67
Average intima-media thickness DKA - IMT (mm)	1.24 ± 0.29
Average intima-media thickness LKA - IMT (mm)	1.27 ± 0.31
Average intima-media thickness KA - IMT (mm)	1.25 ± 0.28

Table 3. Correlation between carotid artery intima-media thickness and oxidative stress parameters

Measured parameters	Basic statistical parameters		Significance (p-value)
	Xsr ± SD	N	
Superoxide anion radical - O_2^- (nmol/mL)	3.58 ± 4.90	125	r_{emp}=0.002 p=0.984
Intima-media thickness- IMT (mm)	1.25 ± 0.28		
Hydrogene peroxide - H_2O_2 (nmol/mL)	4.65 ± 1.62	125	r_{emp}=0.190 p=0.034
Intima-media thickness- IMT (mm)	1.25 ± 0.28		

Measured parameters	Basic statistical parameters		Significance (p-value)
	Xsr ± SD	N	
Thiobarbituric acid - TBARS reactive substances (μmol/mL)	1.14 ± 0.23	125	r_{emp}=0.550 p=0.0001
Intima-media thickness- IMT (mm)	1.25 ± 0.28		
Nitrites - NO_2^- (nmol/mL)	3.81 ± 1.33	125	r_{emp}=-0.131 p =0.144
Intima-media thickness- IMT (mm)	1.25 ± 0.28		
Reduced glutathione - GSH (nmol/mL)	119500.29 ± 17525.20	125	r_{emp}=0.112 p =0.214
Intima-media thickness- IMT (mm)	1.25 ± 0.28		
Catalase - CAT (U/gHb x 10^4)	2.22 ± 1.88	125	r_{emp}=0.04 p =0.963
Intima-media thickness- IMT (mm)	1.25 ± 0.28		
Superoxide dismutase - SOD (U/gHb x 10^4)	32.82 ± 20.67	125	r_{emp}=-0.310 p =0.0001
Intima-media thickness- IMT (mm)	1.25 ± 0.28		

Table 4. Correlation between parameters of microinflammation, nutrition and secondary hyperparathyreoidism and carotid artery intima-media thickness

Measured parameters	Basic statistical parameters		Significance (p- value)
	Xsr ± SD	N	
C-reactive protein - CRP (mg/L)	10.57 ± 12.23	125	r_{emp}=0.038 p=0.673
Intima-media thickness - IMT (mm)	1.25 ± 0.28		
Albumine -ALB (g/L)	38.00 ± 3.02	125	r_{emp}=-0.245 p=0.006
Intima-media thickness- IMT (mm)	1.25 ± 0.28		
Prealbumine - PALB (g/L)	0.28 ± 0.09	125	r_{emp}=-0.243 p=0.009
intima-media thickness -IMT (mm)	1.25 ± 0.28		
Transferine - TRSF (g/L)	1.50 ± 0.34	125	r_{emp}=-0.139 p =0.123
Intima-media thickness- IMT (mm)	1.25 ± 0.28		
Vitamin D - VitD (ng/mL)	17.88 ± 9.63	125	r_{emp}=-0.148 p =0.099
Intima-media thickness - IMT (mm)	1.25 ± 0.28		
Intact parathormon - iPTH (pg/mL)	175.13 ± 199.85	125	r_{emp}=0.051 p =0.574
Intima-media thickness - IMT (mm)	1.25 ± 0.28		
Albumine - ALB (g/L)	38.00 ± 3.02	125	r_{emp}=0.479 p=0.0001
Prealbumine - PALB (g/L)	0.28 ± 0.09		

DISCUSSION

Cardiovascular diseases are the most common complications and a leading cause of death in patients with the end-stage chronic kidney disease treated with regular hemodialysis. Cardiovascular mortality in these patients is up to 20 times higher than in the general population. The first step in the prevention and treatment of these patients is good understanding of non-traditional risk factors for the development of the cardiovascular disease (18). Uremic toxins, oxidative stress, microinflammation, malnutrition and endothelial dysfunction are the most significant non-traditional risk factors for the development of atherosclerotic cardiovascular disease in patients undergoing regular hemodialysis. Uremic toxins cause enhanced leukocyte activation (increased leukocyte oxidation and proinflammatory activity), the upregulation in

leukocyte-endothelial interactions and mononuclear cell infiltration into atherosclerotic vascular lesions. Asymmetric dimethylarginine (ADMA), indoxyl sulfate (IP) and p-cresyl sulfate (pCS) are uremic toxins that exert a prooxidative and proinflammatory effect through inhibition of nitric oxide production in endothelial cells and have high atherogenic potential (via accelerating the progression of endothelial dysfunction) (18, 19).

The degree of biocompatibility of the dialysis membrane significantly impacts oxidative stress and microinflammation in patients treated with regular hemodialysis. Results of the studies that have compared the effect of two different dialysis membranes on oxidative stress have shown that the hemodialysis session with a high-flux polysulfone membrane significantly decreases the formation of oxygen free radicals during hemodialysis compared to a low-flux polysulfone membrane (21). The hemodialysis session with a high-flux polysulfone membrane provides better control of the neutrophil function than a low-flux polysulfone membrane (22). Besides the biocompatibility of the dialysis membrane and the presence of endotoxin in the hemodialysis solution, the development of uncontrolled and permanent microinflammation in the population of patients treated with regular hemodialysis is also affected by: uremic toxins, asymptomatic infection of the arteriovenous fistula for hemodialysis, periodontal disease and dislocation of bacteria and endotoxins from intestines to circulation as a result of disruption of the gut microbiome (23).

B-mode ultrasonography of the carotid arteries has been used as a non-invasive diagnostic procedure for the evaluation of atherosclerosis. Patients treated with regular hemodialysis have a significantly higher thickness of the carotid artery intima-media than the general population (absence of kidney disease) (24). Hypoalbuminemia is a good predictor of cardiovascular mortality in patients treated with the regular hemodialysis. In patients with hypoalbuminemia, microinflammatory and oxidative stress, parameters were significantly increased. Results of this study showed that there was a highly statistically significant ($p<0.01$) positive association between the serum albumin and prealbumin concentrations. A high statistically significant ($p<0.01$) negative correlation was found between the serum albumin and prealbumin and intima-media thickness, which is consistent with the results of studies to date, which also confirmed a statistically significant negative correlation with nutritional parameters in patients undergoing the regular hemodialysis (24, 25). In the population of patients undergoing the regular hemodialysis, the thickness of the carotid artery intima-media has a statistically significant positive correlation with the concentration of C-reactive protein and proinflammatory cytokines in serum (24). Results of this study showed no significant positive correlation with the carotid artery intima-media thickness, indicating the need to measure more sensitive microinflammatory parameters in a population of patients undergoing the regular hemodialysis, such as pro-inflammatory cytokines (IL-6, TNF-α). Lipid peroxidation plays a significant role in the process of atherosclerosis. Results of this study are in line with the results of other studies done so far, which also showed a statistically significant positive relationship between the concentration of thiobarbituric acid reactive substances (TBARS) and the carotid intima-media thickness in patients treated with the regular hemodialysis (26, 27). A highly statistically significant ($p<0.01$) negative association was demonstrated between the superoxide dismutase activity in erythrocytes and the carotid artery intima-media thickness, which is in accordance with results of the other authors (25). Between the parameter of oxidative DNA damage (Deoxyribonucleic Acid), 8-OhdG/dG (8-hydroxy-2-deoxyguanosine/deoxyguanosine) ratio, serum malondialdehyde concentration, and carotid artery intima-media thickness in hemodialysis patients a statistically significant positive association was found (28). Carotid artery ultrasound, measurement of cITM (Carotid Intima-Media Thickness) and detection of atherosclerotic plaques are important for assessing the health of the entire arterial vasculature in patients undergoing the regular hemodialysis (29). According to the recommendations of the EAEMP (European Agency for the Evaluation of Medicinal Products), cITM is accepted as a surrogate marker of atherosclerosis, while according to the recommendations of the National Kidney Foundation Kidney Disease Quality Initiative (NKF-DOQI), the carotid artery ultrasound is used for the accurate assessment of cardiovascular artery status in patients treated with the regular hemodialysis, and it can also be used to evaluate vascular calcifications (28, 29). Several investigations have suggested and proved that the dialysis modality and the type of hemodialysis membranes have effects on oxidative stress and microinflammation. For example, the post-dilution on-line hemodiafiltration reduces microinflammation, increases the availability of iron for erythropoesis, reduces erythropoetin resistance, improves nutritional status, the quality of life and the patient outcome compared to the conventional hemodialysis (30). Hemodialysis with a vitamin E-coated membrane over a six-month period significantly reduces the concentration of serum C-reactive protein, interleukin-6, Soluble Intercellular Adhesion Molecule-1 (slCAM1), TBARS and the oxidized LDL cholesterol - ox-LDL. During follow-up, no significant effect on the endothelial cell apoptosis was achieved (31). During the high-flux hemodialysis session and post-dilution on-line hemodiafiltration, a significant amount of vitamin C and oligoelements, which are cofactors of antioxidant enzymes are lost (32). Results of the studies so far have shown that zinc supplementation at a dose of 100 mg/day for eight weeks significantly improves the superoxide dismutase (SOD) activity and significantly reduces the serum malodialdehyde (MDA) concentration. Additionally, selenium supplementation at a dose of 200 μg/day for 12 weeks significantly enhances the glutathione peroxidase (Gpx) activity in erythrocytes (32).

CONCLUSION

Oxidative stress and malnutrition play a significant role in the development of a carotid artery atherosclerosis in patients undergoing the regular hemodialysis. Statistically significant positive correlation was found between the thickness of carotid arteries intima-media and TBARS plasma concentration, while a high statistically significant negative correlation was found between the SOD activity in erythrocytes and the thickness of carotid arteries intima-media. This indicates the importance of lipid peroxidation in the development of atherosclerosis and atherosclerotic cardiovascular disease in a population of patients treated with the regular hemodialysis. A high statistically significant positive correlation between the serum prealbumin and albumin concentration and the carotid artery intima-media thickness highlights the importance of malnutrition in development. Thus, oxidative stress and malnutrition, both individually and together, significantly contribute to the development and acceleration of atherosclerosis and increase the risk of cardiovascular morbidity and mortality in patients undergoing the regular hemodialysis. A high-flux hemodialysis and on-line post-dilution hemodiafiltration could ensure an optimal control of oxidative stress and malnutrition. The control of oxidative stress might be better when using membranes for hemodialysis coated with antioxidants such as vitamin E, along with the substitution of vitamin C and oligoelements, zinc and selenium.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki for Medical Research (revised in 2013.). The consent was obtained from patients and the Ethics Committee of the Clinical Center Kragujevac.

CONFLICT OF INTEREST

There are no conflicts of interest.

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