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The influence of vitamin E-coated dialysis membrane on oxidative stress during a single session of on-line hemodiafiltration

Uticaj dijalizne membrane obložene vitaminom E na oksidacioni stres u toku pojedinačne seanse *on-line* hemodijafiltracije

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Abstract

Background/Aim. Oxidative stress is an important risk factor for the development of cardiovascular atherosclerotic diseases in the population of patients treated with regular hemodialysis. Bioincompatibility of the dialysis membrane and increased concentration of endotoxin in the hemodialysis solution are two main factors that can trigger oxidative stress. This paper was intended to examine the effect of a vitamin E-coated membrane on oxidative stress during a single session of on-line hemodiafiltration. **Methods.** Twenty-four patients undergoing hemodiafiltration with vitamin E-coated polysulfone dialysis membrane (Leoceed 21H) were examined, followed by a polysulfone dialysis membrane treatment without vitamin E (FX800). The following parameters of oxidative stress were measured: superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), thiobarbutyric acid reactive substances (TBARS), nitric oxide (NO_2^-), catalase (CAT), superoxide dizmutase (SOD), and reduced glutathione (GSH) activity. Statistical analysis included the Kolmogorov-Smirnov test, Student-*t* test and Wilcoxon test. **Results.** On-line hemodiafiltration using a high-flux polysulfone vitamin E-coated membrane led to significant reduction of TBARS concentration and SOD activity, while the on-line hemodiafiltration session using a high-flux polysulfone membrane that is not vitamin E-coated induced a significant increase in H_2O_2 concentration in the serum and a decrease in SOD activity. There was no statistical significance among the other parameters of oxidative stress. **Conclusion**. A single session of on-line hemodiafiltration using a vitamin E-coated polysulfone membrane significantly affects oxidative stress. After a single session of on-line hemodiafiltration using a vitamin E-coated membrane, the concentration of TBARS has significantly decreased. The decreased activity of superoxide dismutase could be a consequence of an increased loss of microelements during an on-line hemodiafiltration session using a high-flux polysulfone membrane. Patient selection, continuous on-line hemodiafiltration using a vitamin E-coated polysulfone membrane over a 3–6 month period and increased antioxidant protection capacity could possibly reduce the risk of cardiovascular morbidity and mortality in patients treated by hemodialysis.

Key words:
hemodiafiltration; membranes, artificial; oxidative stress; vitamin e.

Apstrakt

Uvod/Cilj. Oksidacioni stres je značajan faktor rizika od razvoja kardiovaskularnih aterosklerotskih bolesti u populaciji bolesnika koji se leče redovnom hemodijalizom. Bioinkompatibilnost dijalizne membrane i povećana koncentracija endotoksina u rastvoru za hemodijalizu su dva glavna faktora koja mogu da podstaknu oksidacioni stres. Rad je imao za cilj da ispita uticaj membrane obložene vitaminom E na oksidacioni stres u toku pojedinačne seanse *on-line* hemodijafiltracije. **Metode.** Ispitana su 24 bolesnika koja su lečena *on-line* hemodijafiltracijom sa polisulfonskom dijaliznom membranom obloženom vitaminom E (Leoceed 21H), a zatim i polisulfonskom dijaliznom membranom neobloženom vitaminom E (FX800). Glavni parametri oksidacionog stresa, koji su praćeni, bili su: superoksidni anjon (O_2^-), vodonik peroksid (H_2O_2), reaktivne supstancije vezane za tiobarbituričnu kiselinu

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(TBARS), azotni oksid (NO_2^-), katalaza (CAT), superoksid dizmutaza (SOD) i aktivnost redukovanog glutationa (GSH). Za statističku analizu korišćeni su: Kolmogorov-Smirnov test, Student-ov *t*-test za vezane uzorke i Wilcoxon-ov test. **Rezultati.** *On-line* hemodijafiltracija sa *high-flux* polisulfonskom membranom obloženom vitaminom E statistički je značajno smanjivala koncentraciju TBARS i aktivnost SOD u serumu. Nakon seanse *on-line* hemodijafiltracije sa *high-flux* polisulfonskom membranom koja nije bila oboložena vitaminom E statistički se značajno povećala koncentracija H_2O_2 u serumu, dok se aktivnost SOD značajno smanjila. Kod ostalih ispitivanih parametara oksidacionog stresa nije utvrđena statistički značajna razlika između korišćenih membrane. **Zaključak.** Pojedinačna seansa *on-line* hemodijafiltracije sa polisulfonskom membranom obloženom vitaminom E statistički značajno utiče na oksidacioni stres. Koncentracija TBARS u serumu je statistički značajno niž posle pojedinačne seanse *on-line* hemodijafiltracije sa membranom obloženom vitaminom E. Smanjena aktivnost SOD mogla bi da bude posledica pojačanog gubitka mikroelemanata u toku seanase *on-line* hemodijafiltracije sa *high-flux* polisulfonskom membranom. Izbor bolesnika, *on-line* hemodijafiltracija sa polisulfonskom membranom obloženom vitaminom E kontinuirano u vremenskom periodu od 3–6 meseci i povećanje kapaciteta antioksidacione zaštite mogli bi da smanje rizik od kardiovaskularnog morbiditeta i mortaliteta kod bolesnika na hemodijalizi.

Ključne reči:
hemodijafiltracija; membrane, veštačke; stres, oksidativni; vitamin e.

Introduction

Oxidative stress is a significant risk factor for the development of cardiovascular diseases in the population of patients treated with regular hemodialysis [1]. In these patients, oxidative stress happens as a consequence of increased effect of prooxidative factors and reduced activity of antioxidant protection systems (non-enzyme and enzyme systems). The prooxidation factors include: age, diabetes mellitus, uremic background, chronic inflammatory status, bioincompatible dialysis membrane and presence of endotoxins in the hemodialysis solution. On the other hand, decreased activity of antioxidant non-enzyme mechanisms happens due to a lack of vitamin C and vitamin E, while decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx), is due to a lack of cofactors [increased selenium (Se) and zinc (Zn) loss through the dialysis membrane during hemodialysis] and reduced activity of glutathione (GSH) system [2, 3].

Hemodialysis is itself a trigger for the occurence of oxidative stress. The main pathophysiological mechanisms of the increased formation of reactive oxygen species (ROS) during the hemodialysis session are the bioincompatibility of the dialysis membrane, presence of endotoxins in the hemodialysis solution and increased loss of cofactor oligoelements that are necessary for the activity of antioxidant enzymes [2–6].

Dialysis membranes play a central role in the hemodialysis process (hemodialysis). They can be natural and synthetic. Natural membranes include cellulose derivatives with water permeability coefficient – Kuf < 10 mL/h × mmHg ("low-flux" membrane), low clearance of moderate molecular weight uremic toxins and a lower biocompatibility degree compared to synthetic membranes. Synthetic membranes are biocompatible membranes that have a high water permeability coefficient – Kuf > 20 mL/h × mmHg ("high-flux" membranes) and high clearance of medium molecular weight uremic toxins [3–6]. The evaluation of the dialysis membrane efficacy is based on the coefficient of mass transfer (KoA), which can be calculated by multiplying the coefficient of transmission (Ko) and the surface of the membrane (A). Highly effective dialysis membranes are those that have KoA > 600–700 [3–6]. Highly effective (KoA > 600–700) and highly permeable water membranes with ultrafiltration coefficient of Kuf ≥ 50 mL/h × mmHg are used for on-line hemodiafiltration [3–6].

During a hemodialysis session, there is a direct activation of the polymorphonuclear leukocytes due to direct contact of the blood and the surface of the dialysis membrane, which is the result of myeloperoxidase activity which increases the formation of free oxygen radicals. Measurement of serum myeloperoxidase concentrations released from polymorphonuclears during a hemodialysis session indicates the severity of oxidative stress induced by different bioincompatibility degrees of dialysis membranes [7].

During a single 4-hour session of hemodialysis, the patient's organism is exposed to approximately 120 liters of dialysis solution. Therefore, a high microbiological quality of the dialysis solution (ultra-pure dialysis solution) is required [8, 9]. 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 bacterial colonies is < 0.1 (colony-forming units – CFU)/mL and the endotoxin concentration is E < 0.03 (endotoxin units – EU/mL). This solution is used for high-flux hemodialysis (HFHD) and hemodiafiltration (HDF), while a solution with endotoxin concentration ≤ 0.50 EU/mL (≤ 0.25 EU/mL) and the number of colonies ≤ 100 CFU/ml (≤ 50 CFU/mL) is used for low-flux hemodialysis (LFHD), according to current recommendations [8, 9]. Endotoxin and other bacterial products can pass from the dialysis solution through the dialysis membrane to the patient's

blood via backdiffusion/backfiltration processes and activate the mononuclear and polymorphonuclear cells to boost the formation and release of free oxygen and pro-cationic cytokine radicals interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), all of this resulting in the development of oxidative stress, microinflammatory and accelerated atherosclerosis [8, 9].

In patients treated with regular hemodialysis, the activity of enzymatic and non-enzymatic antioxidant systems is reduced. Reduced concentration of trace elements, such as Se, Cu and Zn decreases the activity of antioxidant enzymes (SOD, Gpx). This is probably a result of insufficient intake, but also increased loss during HFHD/HDF [10]. Also, because of a lack of vitamins C and E, the capacity of non-enzymatic antioxidative protection systems is reduced [10].

The present investigation aimed to examine the effect of a vitamin E-coated membrane on oxidative stress during a single session of on-line HDF.

Methods

The study included 24 patients treated with on-line HDF at the Center for Nephrology and Dialysis of the Clinical Center Kragujevac. The research was conducted in accordance with the Helsinki Declaration on Medical Research, the approval from the Ethics Committee of the Clinical Center Kragujevac and the consent of the patients.

The patients examined in this study were regularly treated with on-line HDF, three times a week for 4 hours (12 h per week), over a period of more than three months with a convective volume of 17 liters per session, on machines with controlled ultrafiltration type Fresenius 5008S, Gambro AKA200US and Gambro Artis, with the blood flow strength - Qb = 250 mL/min and the dialysis flow strength-Qd = 500 mL/min were examined. A standard ultra-pure solution for on-line hemodiafiltration (endotoxin concentration – E < 0.03 EU/mL) was used. For on-line HDF, the high-flux dialysis membrane was coated with vitamin E-Leoceed 21H (polysulfonic membrane with the effective area of 2.1 m^2, KoA = 1351 mL/min, high-flux - Kuf = 88 mL/h × mmHg, sterilized by gamma rays, Asachi Kasei Medical Europe, Germany) and a high-flux dialysis membrane that is not coated with vitamin E – FX800 (polysulphonic membrane with the effective area of 2.0 m^2, KoA = 1365 mL/min, high-flux - Kuf = 62 mL/h × mmHg, sterilized by steam, Fresenius Medical Care, Germany). Exclusion criteria were as follows: patients with proven active bleeding, active systemic inflammation or infection, with uncontrolled malignancies, as well as patients treated with immunosuppressive and antioxidant drugs.

In order to evaluate the influence of the dialysis membrane coated with vitamin E on oxidative stress during an individual session of on-line HDF, several parameteres were followed: anemia (hemoglobin, hematocrit, erythrocyte indexes), iron status in the patient's organism (iron and ferritin serum concentration, iron saturation of transferin), microinflammation (C-reactive protein), nutritional status (prealbumin, transferin), secondary hyperparathyroidism (iPTH, vitamin D), oxidative stress: (superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), thiorbutyric acid reactive substances (TBARS), nitric oxide in the form of nitrites (NO_2^-), superoxide dizmutase (SOD), catalase (CAT), reduced glutathione activity (GSH)), carotid artery intima media thickness, blood flow through the vascular hemodialysis approach (Qavf) and the dialysis adequacy parameter (spKt/V).

Blood samples for laboratory analyses were taken prior to initiation and after completion of a single on-line HDF, before subcutaneous administration of heparin and before intravenous administration of iron and vitamin B complex at the end of the on-line HDF session. Blood samples were taken from the same group of patients before and at the end of an individual HDF session with a "high-flux" polysulfone membrane of type Leoceed 21H, as well as before and after type FX800 treatment. Routine laboratory analyses were performed using 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 C-reactive protein (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. The 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. The 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.

The concentration of intact parathormone (PTH) in the serum was determined by the immunodiathymetric method (IRMA), on the gamma counter WALLAC WIZARD 1470. The normal concentration of intact PTH in the serum is 11.8–64.5 pg/mL. In patients with hemodialysis the upper normal limit is 300 pg/mL.

The principle of determining the concentration 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. Measurement takes place at a wavelength λ = 550 nm.

The 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.

The determination of the lipid peroxidation index was carried out indirectly through products of the lipid peroxidation reaction with 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). Measurement takes place at a wavelength λ= 530 nm.

Determination of the concentration of nitrogen monoxide (NO_2^-) was carried out on the basis of the amount of released nitrites. 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 takes place at a wavelength λ= 550nm.

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 self-oxidation rate of adrenaline in the alkaline environment, which is dependent on $O_2^{-\cdot}$. Given that $O_2^{-\cdot}$ Is removed by the present SOD, the adrenaline authoxidation 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.

The Beutler method was used to determine the CAT activity. The principle is the spectrophotometric monitoring of the rate of decomposition of H_2O_2 in the presence of CAT at a wavelength of 230 nm, in which H_2O_2 absorbs light.

For the determination of reduced GSH activity, the Beutler spectrophotometric method was used. The principle of the method is based on the oxidation of GSH by 5,5-dithio-bis-6,2-nitrobenzoic acid (DTNB).

The hemodialysis adequacy was assessed on the basis of the single-pool Kt/Vsp index calculated according to Daugirdas 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 - interdialysis yield (L), W - body weight after the hemodialysis (kg). According to K/DOQI guidelines, hemodialysis is adequate if Kt/Vsp ≥ 1.2.

Urea reduction rate (URR) index was calculated using the following formula: URR = (1-R) × 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 = 65–70%.

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 = $r^2\pi/4$ × Vmean × 60 (mL/min), where: r – radius of vascular access, and Vmean – mean blood flow velocity through vascular approach. The blood flow is calculated as the mean of three measurements, 2–4 cm on the vein vascular approach, proximal to the anastomosis site. Blood flow through a vascular approach that provides adequate hemodialysis is 500–1000 mL/min.

The thickness of carotid arterial intimal media (IMT) was determined by the Color Doppler ultrasonic examination, on the Logic P5 apparatus, using a 7.5 MHz 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 ultrasonograph apparatus. The normal thickness of the intima media is defined as a value of less than 0.9 mm.

The tests used for the statistical analyses of the obtained data were following: the Kolmogorov-Smirnov test, Student *t*-test and Wilcoxon test. Significance threshold was a probability of 0.05 and 0.01.

Results

In the Center for Nephrology and Dialysis of the Clinical Centre Kragujevac, a cross-sectional study was conducted, which included patients who have been treated with regular on-line HDF over a period of more than three months. Twenty four patients (19 men, 5 women) were examined (mean age: 60.92 ± 8.20 years, average dialysis length: 9.53 ± 5.45 years, and average spKtV dialysis index: 1.20 ± 0.18). General data on patients are shown in Table 1.

Anemia treatment that was used included short-acting and long-acting erythropoietin, i.v. administration of iron, i.v. preparation of vitamin B and folic acid perorally. The average monthly dose for short-acting erythropoietin was 18,000.00 ± 12,055.43 IU, and for long-acting erythropoietin it was 140.00 ± 36.33 μg. The average monthly intravenous iron dose was 256.25 ± 131.50 mg, the average monthly dose of vitamin B_{12} was 2,916.67 ± 1592.56 μg, and the average monthly folic acid dose was 181.25 ± 62.23 mg. Secondary hyperparathyroidism in the examined patients was treated with calcium-containing phosphate linkers, active metabolites of vitamin D and paricalcitol. The average monthly dose of rocaltrol was 3.08 ± 5.10 μg, and the average monthly dose of i.v. paracalcitol was 2.50 ± 12.25 μg.

The average values of anemia parameters, iron status, microinflammation, nutritional status, secondary hyperparathyroidism and ultrasound examination of carotid arteries are shown in Table 2.

In order to evaluate the influence of the dialysis membrane type on oxidative stress during a single session of on-line hemodiafiltration, the following parameters were examined: $sO_2^{-\cdot}$, H_2O_2, TBARS, NO_2^-, SOD, CAT, and reduced activity GSH. They are shown in Table 3.

The patients treated with HDF were shown a significantly ($p < 0.05$) lower concentration of TBARS and significantly ($p < 0.01$) lower SOD activity after the on-line HDF session with a vitamin E-coated membrane (Leoceed 21H), as shown in Table 3. When we treated the

same patients in a single on-line HDF session using a membrane without vitamin E-coating (FX800), t H_2O_2 values were significantly higher, while the SOD activity was significantly lower after the treatment ($p < 0.01$) than before the onset of HDF (Table 3). There was no significant difference in values of the rest of oxidative stress parameters: CAT, GSH, O_2^-, NO_2^- ($p > 0.05$), before and after the session of on-line HDF using a vitamin E-coated membrane (Leoceed 21H), as well as a a membrane without vitamin E-coating (FX800) (Table 3).

Table 1

General data on patients

Data	Values
Number of patients	24
Gender (M/F), n (%)	19/5 (79.17/20.83)
Age (years), mean ± SD	60.92 ± 8.20
Length of hemodyalisis treatment (years), mean ± SD	9.53 ± 5.45
Body mass index (kg/m^2), mean ± SD	25.63 ± 3.53
Systolic arterial blood pressure (mmHg), mean ± SD	131.67 ± 13.73
Diastolic arterial blood pressure (mmHg), mean ± SD	77.50 ± 6.76
Mean arterial blood pressure (mmHg), mean ± SD	95.56 ± 8.49
Body weight (kg), mean ± SD	74.21 ± 12.69
Interdialytic weight gain (kg), mean ± SD	2.33 ± 0.92
Interdialytic weight gain (%),mean ± SD	3.22 ± 1.32
Ultrafiltration rate (mL/kg/h), mean ± SD	8.04 ± 3.30
Ultrafiltration (mL/h), mean ± SD	583.33 ± 229.21
Residual diuresis (mL/24 h), mean ± SD	425.00 ± 591.98
Arteriovenous fistula flow (mL/min), mean ± SD	967.08 ± 415.62
Index of hemodyalisis adequacy (Kt/Vsp), mean ± SD	1.20 ± 0.18
Primary kidney disease, n (%)	
glomerulonephritis chronica	3 (12.5)
nephropathia hypertensive	10 (41.66)
nephropathia diabetica	1 (4.16)
nephropathia obstructiva	1 (4.16)
nephropathia chronica	4 (16.67)
renes polycystici	5 (20.83)
Comorbidity, n (%)	
hypertension	22 (91.66)
hypotension	1 (4.17)
diabetes mellitus	1 (4.17)

M – male; F – female; SD – standard deviation.

Table 2

Basic investigation parameters

Parameters	Values (mean ± SD)
Hemoglobine (g/L)	105.88 ± 14.56
Hematocrit (%)	32.09 ± 4.47
Mean corpuscular volume (fL)	93.74 ± 4.86
Mean corpuscular hemoglobin concentration (g/L)	329.50 ± 6.53
Vitamine B_{12} serum concentration (pg/mL)	962.33 ± 503.24
Folic acid serum concentration (ng/mL)	20.42 ± 13.16
Iron serum concentration (μmol/L)	9.62 ± 4.04
Transferrin saturation (%)	24.46 ± 9.77
Feritine serum concentration (ng/mL)	591.96 ± 318.31
C-reactive proteine serum concentration (mg/L)	6.08 ± 6.74
Albumine serum concentration (g/L)	37.96 ± 3.24
Prealbumine serum concentration (g/L)	0.29 ± 0.08
Transferine serum concentration (g/L)	1.57 ± 0.28
Vitamin D serum concentration (ng/mL)	20.16 ± 9.69
Intact parathormon serum concentration (pg/mL)	228.92 ± 287.42
Average right carotid artery intima-media thickness (mm)	1.21 ± 0.24
Average left carotid artery intima-media thickness (mm)	1.19 ± 0.26
Average intima-media thickness of both carotid arteries (mm)	1.20 ± 0.23

SD – standard deviation.

Table 3

The influence of the dialysis membrane type on oxidative stress parameters during a single session of on-line hemodiafiltration (HDF)

Parameters	On-line HDF membrane					
	Leoceed 21H (vitamin E-coated)		*p*	FX800 (without vitamin E-coating)		*p*
	before HDF	after HDF		before HDF	after HDF	
$O_2^{-\cdot}$	3.45 ± 3.51	1.87 ± 2.06	0.078	1.96 ± 1.23	1.83 ± 1.14	0.548
H_2O_2	4.82 ± 1.99	4.74 ± 1.56	0.878	7.14 ± 1.72	7.95 ± 1.54	0.003
TBARS	1.20 ± 0.26	1.07 ± 0.10	0.031	0.88 ± 0.15	0.90 ± 0.24	0.567
NO_2	3.65 ± 1.24	3.34 ± 0.80	0.223	7.79 ± 2.29	8.09 ± 2.00	0.174
SOD	37.99 ± 27.12	19.33 ± 11.46	0.003	27.13 ± 13.72	18.66 ± 11.38	0.010
CAT	2.38 ± 1.51	1.86 ± 1.28	0.107	1.79 ± 1.23	2.15 ± 1.52	0.626
GSH	110,615.52 ± 27,561.68	106,438.65 ± 32,204.63	0.992	90,988.86 ± 14,909.96	92,846.14 ± 12,470.16	0.364

***Note*: Results are given as mean ± standard deviation.**

$O_2^{-\cdot}$ – superoxide anion radical (nmol/mL); H_2O_2 – hydrogen peroxide (nmol/mL); TBARS – thiobarbituric acid reactive substances (μmol/L); NO_2^- nitric oxide in the form of nitrites (nmol/mL); SOD – superoxide dismutase (U/gHb × 10^3); CAT – catalase (U/gHb × 10^3); GSH – reduced glutathione (U/gHb × 10^3).

Discussion

Cardiovascular diseases have remained the leading cause of death in patients with a final stage of chronic kidney disease treated with regular dialysis. Oxidation stress is considered to be a non-traditional risk factor for the development of cardiovascular disease in this population of patients [11, 12]. It occurs as a disbalance between formation of free oxygen radicals and activity of antioxidant protection systems. In the population of patients treated with hemodialysis, four types of oxidative stress can be distinguished: standard oxidation stress, chlorinated stress, nitrosative stress and carbonyl stress. Antioxidant protection against oxidative stress includes enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic systems of protection which include hydrophilic vitamin C and lipophilic vitamin E [11–13]. The main clinical consequences of oxidative stress in patients with a terminal stage of chronic kidney disease treated with regular hemodialysis are: accelerated atherosclerosis, erythropoietin resistance (anemia) and amyloidosis caused by β_2-microglobulin [11–13].

Biocompatibility degree of the dialysis membrane significantly affects oxidative stress in patients with a terminal stage of chronic kidney disease treated with hemodialysis. The results of the conducted studies that compared the influence of two different dialysis membranes on oxidative stress indicate that the hemodialysis session with cuprophane membrane significantly increases the serum MDA concentration relative to the dialysate session with the polysulfone membrane [14]. After a hemodialysis session using a polysulfone and cuprophane membrane, the activity of antioxidant enzymes increases, but this increase is significant only for CAT in patients dialysed using a polysulphonic membranes [14]. However, comparing the effects of the hemodialysis session usingdifferent membranes, hemophane and polysulfone, it has been found that the polysulfone membrane significantly increases the concentration of MDA in the serum and significantly reduces selenium concentration and Gpx activity relative to the hemophane dialysis membrane [15]. The hemodialysis session using the "high-flux" polysulfone membrane significantly lowers the formation of free oxygen radicals during hemodialysis compared to the "low-flux" polysulfone membrane [16]. The hemodialysis session with the "high-flux" polysulfone membrane also provides better control of the neutrophil function compared to the "low-flux" polysulfone membrane [17].

On-line HDF, a biocompatible highly permeable dialysis membrane and an ultrapure solution for hemodialysis could ameliorate oxidative stress and slow the development of atherosclerosis [17, 18]. During a single session of on-line HDF, using membranes that have a large surface and high water permeability (Kuf > 50 mL/h × mmHg), there is an increased loss of trace oligoelements (selenium, zinc, copper), which are significant cofactor of enzymatic antioxidant enzymes (SOD). A significantly lower concentration of oxidative stress parameters is achieved on-line by HFD with a vitamin E-coated membrane in continuity over a 3–6 months period relative to standard hemodialysis using a “low-flux” membrane without vitamin E-coating. The results of clinical trials suggest that treatment with on-line HDF, with “high-flux” membranes over a period of 3–6 months, significantly reduces inflammation, oxidative stress and resistance to erythropoietin activity, compared to hemodialysis with “low-flux” membranes [17, 18]. Hemodialysis membranes (vitamin E-coated hemodialysis) have also shown the reduction of lipid peroxidation parameters values in the serum, such as: MDA, TBARS and oxidized low density lipoprotein (oxLDL) cholesterol [19–24]. Some studies highlight that these membranes also reduce the concentration of oxidative nucleic acid parameters such as 8-hydroxydeoxyguanosine (8-OHdG) and the concentration of microinflammatory parameters (CRP, interleukin-6) [19–24]. Treatment with “high-flux” hemodialysis using polysulphonic membrane-bound vitamin E over a period of three to six months

significantly reduces oxidative stress, microinflammation, and erythropoietin resistance index. It also corrects the treatment of anemia, reduces the amount of erythropoietin and the thickness of intima-media of carotid arteries in a population of patients treated with regular hemodialysis without affecting hemodialysis adequacy parameters (Kt/V index) [19–24]. The results of this study showed that after a single session of on-line HDF using a "high-flux" polysulfone membrane coated with vitamin E (Leocced 21H), the concentration of TBARS and the SOD activity are significantly reduced. This can be explained by the effect of vitamin E, but also by the loss of microelements, the cofactor of SOD during on-line HDF session [25, 26]. The results of the tests carried out so far show that a significant amount of microelements (selenium, zinc, copper) is lost during the on-line HDF and hemodialysis using a "high-flux" polysulfone membrane, which results in a decrease in the activity of antioxidant enzymes, such as SOD and Gpx [27–30]. Normal serum zinc concentration is 70–110 μg/dL, in erythrocytes 40–44 μg/g Hb, and normal activity of SOD in erythrocytes is 1.102–1.601 IU/g Hb. In patients treated with regular dialysis, zinc is administered at a dose of 100 mg/day for 8 weeks. The results of the study show that the zinc applied in this dose significantly increases the activity of SOD in erythrocytes, while the serum MDA concentration significantly decreases [31, 32]. In addition to the oligoelements, during the dialysis session, hydrosoluble vitamins that exhibit an antioxidant effect (vitamin C) are lost. Vitamin C clearance during the hemodialysis session is 30–50%, and during "high-flux" hemodialysis and hemodynamic filtration over 50%. Due to reduced antioxidant capacity and reduced GSH concentration during HDF using "high-flux" polysulphonic vitamin E-coated membranes, oligoelements, vitamin C and vitamin E should be used to prevent these events. Vitamin C should be administered at a dose of 300 mg i.v. after each individual hemodialysis session for 8–12 weeks (with monitoring of serum oxalate concentration), while vitamin E is administered perorally at a dose of 400–800 IU/day for 12–16 weeks for secondary prevention of cardiovascular events; a strong antioxidant effect is achieved at a dose of 1000 mg/day perorally for 8 weeks (alpha tocopherol: 1.0 mg = 1.5 IU) [28–30].

Conclusion

A single session of on-line HDF using a "high-flux" large-surface polysulfone vitamine E-coated membrane ($A \geq 2.0$ m^2) significantly affected parameters of oxidative stress relative to the individual session of the on-line HDF using the "high-flux" polysulfone membrane with surface ≥ 2.0 m^2, which is not coated with vitamin E. After a single session of on-line HDF using the "high-flux" polysulfone vitamine E-coated membrane with surface ≥ 2.0 m^2, the concentration of TBARS was significantly decreased, while the activity of SOD was decreased with both dialysis membranes, probably as a consequence of the increased loss of trace elements, which are the cofactors of enzymatic antioxidative system components (SOD). On-line HFD using "high-flux" vitamine E-coated polysulfone membrane should be applied in a 3–6 months period, with an appropriate assessment of the antioxidant protection capacity during treatment with this dialysis modality. In order to achieve the optimal efficiency of the treatment, individualization of HDF prescription is needed.

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