ORIGINAL SCIENTIFIC ARTICLE ORGINALNI NAUČNI RAD ORIGINAL SCIENTIFIC ARTICLE

INFLUENCE OF DIALYSIS MODALITY ON THE TREATMENT OF ANEMIA IN PATIENTS WITH END-STAGE KIDNEY DISEASE

Nedim Hamzagic[1], Marija Andjelkovic[2,3], Marijana Stanojevic Pirkovic[2,3], Petar Canovic[3], Milan Zaric[3] and Dejan Petrovic[3,4]
[1]Center of Hemodialysis, Medical Center Tutin, Tutin, Serbia
[2]Center of Laboratory Diagnostics, Clinical Center Kragujevac, Kragujevac, Serbia
[3]University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia
[4]Clinic of Urology, Nephrology and Dialysis, Clinical Center Kragujevac, Kragujevac, Serbia

UTICAJ MODALITETA DIJALIZE NA LEČENJE ANEMIJE KOD BOLESNIKA SA ZAVRŠNIM STADIJUMOM BOLESTI BUBREGA

Nedim Hamzagić[1], Marija Anđelković[2,3], Marijana Stanojević Pirković[2,3], Petar Čanović[3], Milan Zarić[3] i Dejan Petrović[3,4]
[1]Centar za hemodijalizu, Zdravstveni centar Tutin, Tutin
[2]Služba za laboratorijsku dijagnostiku, KC Kragujevac, Kragujevac
[3]Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Kragujevac
[4]Klinika za urologiju, nefrologiju i dijalizu, KC Kragujevac, Kragujevac, Srbija

Received / Primljen: 15. 10. 2018. Accepted / Prihvaćen: 13. 11. 2018.

ABSTRACT

Anemia is a common complication among the patients with end-stage kidney disease. Management of anemia is influenced by several factors: iron deficiency, subtherapeutic dosage of erythropoietin, microinflammation, vitamin D deficiency, increased iPTH levels and inadequate hemodialysis.

The aim of the study was to examine impact of dialysis modality on blood hemoglobin level as well as status of iron, status of vitamin D, hemodialysis adequacy and erythropoietin dose.

The study included 120 patients which were divided into two groups: the group of patients treated with hemodiafiltration and the group of patients treated with standard hemodialysis. For statistical analysis Kolmogorov-Smirnov test, Student's t-test and Mann-Whitney U-test were used.

Blood hemoglobin level and parameters of hemodialysis adequacy (Kt/V index, spKt/V index, URR index), hematocrit ad protein catabolic rate (nPCR) were statisticaly significant lower in patients treated with regular hemodialysis compared to patients treated with regular hemodiafiltration. Serum ferritin level, C-reactive protein level and average monthly dose of intravenous iron were higher in the patients treated with regular hemodialysis compared to patients treated with hemodiafiltration.

Patients treated with hemodiafiltration have lower grade of microinflammation, better iron status and better control of anemia compared to the patients treated with regular hemodialysis. Dialysis modality is an important factor that influences management of anemia in the patients with end-stage kidney disease.

Keywords: *hemodialysis, hemodiafiltration, erythropoietin, anemia.*

SAŽETAK

Anemija je česta komplikacija kod bolesnika sa završnim stadijumom bolesti bubrega. Na lečenje anemije utiču: nedostatak gvožđa, nedovoljna doza eritropoetina, mikroinflamacija, nedostatak vitamina D, povećana koncentracija iPTH, neadekvatna hemodijaliza.

Rad je imao za cilj da ispita uticaj modaliteta dijalize na koncentraciju hemoglobina u krvi, status gvožđa, vitamin D, adekvatnost hemodijalize i dozu eritropoetina.

Ispitivanje je uključilo 120 bolesnika. Bolesnici su podeljeni u dve grupe: lečeni hemodijafiltracijom i lečeni standardnom hemodijalizom. Za statističku analizu korišćeni su: Kolmogorov Smirnov test, Student-ov T test, Mann-Whitney U test.

Bolesnici koji se leče redovnom hemodijalizom imaju visoko statistički značajno (p < 0.01) manju: koncentraciju hemoglobina u krvi, vrednost parametara adekvatnosti hemodijalize (Kt/V indeks spKt/V indeks, URR indeks), statistički značajno (p < 0.05) manju vrednost hematokrita i brzinu razgradnje proteina (nPCR), kao i statistički značajno (p < 0.05) veću: koncentraciju feritina u serumu, C-reaktivnog proteina i prosečnu mesečnu dozu i.v. gvožđa, u odnosu na bolesnike koji se leče redovnom hemodijafiltracijom.

Bolesnici koji se leče redovnom hemodijafiltracijom imaju manji stepen mikroinflamacije, bolji status gvožđa u organizmu i optimalnu kontrolu anemije, u odnosu na bolesnike koji se leče standardnom hemodijalizom. Modalitet dijalize je značajan faktor za lečenje anemije kod bolesnika sa završnim stadijumom bolesti bubrega.

Ključne reči: *hemodijaliza, hemodijafiltracija, eritropoetin, anemija.*

sciendo
UDK: 616.155.194:616.61-78-056.24
Ser J Exp Clin Res 2020; 21 (3): 231-238
DOI: 10.2478/sjecr-2018-0050

Corresponding author:
Prof. dr Dejan Petrović
Faculty of Medical Sciences, University of Kragujevac, Kragujevac,
Clinic of Urology, Nephrology and Dialysis,
Clinical Center Kragujevac, Kragujevac
E-mail: dejanpetrovic68@sbb.rs;
Tel.: 064-3741-694

INTRODUCTION

Anemia is present in about ninety percent of patients starting treatment with regular hemodialysis. The main factor that causes anemia among these patients is the lack of endogene erythropoietin which stimulates proliferation and differentiation of erythroid precursors in the bone marrow [1, 2]. Another common cause is a blood loss due to occult gastrointestinal hemorrhage related to uremic gastritis, extracorporeal thrombosis, frequent blood sampling [1, 2]. In order to diagnose anemia on time in hemodialysis patients it is necessary to check hemoglobin concentration, hematocrit, red blood cell indices (MCV, MCH, MCHC), serum iron (Fe^{2+}) and ferritin (FER) concentration, transferrin saturation (TSAT) and serum concentration of C-reactive protein (CRP) [1, 2].

Anemia is independent risk factor for cardiovascular diseases in hemodialysis patients. When hemoglobin concentration is lower than 100 g/L hemodynamic mechanisms of adaptation are activated. Left ventricle is overloaded with volume which leads to development of excentric left ventricle hypertrophy and ischemic heart disease [3-7]. Other clinical consequences of anemia are: deterioration of renal residual function, cognitive disorders, reduced working ability as well as reduced quality of life of hemodialysis patients [8, 9].

Treatment of anemia with erythropoietin is recommended when hemoglobin level is lower than 100 g/L, while target Hb concentration is within range 100-120 g/L [10]. Optimal iron status (TSAT = 20-40%, FER = 100-500 ng/mL) should be achieved prior to treatment with erythropoietin [11-14]. Target Hb concentration is not achieved among about 10-20% of patients [15, 16]. Risk factors that affect treatment of anemia in hemodialysis patients are: iron deficiency, insufficient dose of EPO, microinflammation, malnutrition, vitamin D deficiency, secondary hyperparathyroidism, inadequate hemodialysis, and the existence of antibodies on EPO [15-18].

Hemodiafiltration is dialysis modality that combines diffusion and convection that provide better clearance of uremic toxins of small and medium molecular weight compared to standard "low-flux" hemodialysis [19-23]. Online hemodiafiltration requires ultrapure dialysis solution and polysulphonate "high-flux" membranes with Kuf > 50 ml/h x mmHg [19-23]. This dialysis modality provides hemodynamic stability of patients, delays reduction of residual renal function, improves nutritional and cognitive status, while reduces: microinflammation, resistence on erythropoietin, amount of erythropoietin needed for achievement of target hemoglobin level and cardiovascular morbidity and mortality rate [19-23]. During hemodiafiltration course levels of serum proinflamatore mediators as well as level of hepcidine are reduced. Decreased hepcidine level promotes iron releasing from its depot, increases its availability for erythropoiesis in bone marrow and decreases resistance on erythropoietin treatment [19-23]. Individualization and optimization of dialysis treatment, online hemofiltration, polysulphonate "high-flux" membrane with Kuf > 50 ml/h x mmHg and ultrapure dialysis solution altogether can decrease proinflamatore mediators levels (interleukin-1, interleukin-6, tumor necrosis factor-α), CRP and hepcidine in serum, provide optimal control of anemia and survival of patients on regular hemodialysis [19-23].

AIM OF THE STUDY

The aim of the study was to determine influence of dialysis modality on the treatment anemia in patients with end-stage kidney disease.

PATIENTS AND METHODS

One hundred and twenty patients of Center for nephrology and dialysis, Clinic for Urology, Nephrology and Dialysis of Clinical Center Kragujevac, Kragujevac, Serbia participated in the study. The study protocol was in accordance with the principles of the Declaration of Helsinki and was approved by The Ethics Comitee of Clinical Center Kragujevac. All patients signed informed consent prior to enrollment. All participants were treated with regular bicarbonate hemodialysis, 12 hours per week for period longer than three months on hemodialysis maschines type Fresenius 4008S, 5008S and type Gambro AKA200US and Gambro Artis. Ultrapure dialysis fluid and "high-flux" as well as low-flux polysulfone dialysis membrane were used while for hemodiafiltration "high-flux" polysulphone dialysators with Kuf > 50 ml/h x mmHg were used. Exclusion criteria were active bleeding and active infection.

In order to evaluate impact of dialysis modalityon management of anemia in hemodialysis patients the following parameters were measured: hemoglobin (Hb), hematocrit (Hct), FER, TIBC, unsaturated iron binding capacity (UIBC), transferrin saturation (TSAT), serum calcium (Ca^{2+}), inorganic phosphate (PO_4^{3-}), alkaline phosphatase (ALP), vitamin D and intact parathyroid hormone (iPTH). Parameters of hemodialysis adequacy were also considered.

Blood sampling for laboratory examination was performed prior to starting with hemodialysis and hemodiafiltration and prior to heparin administration. Every laboratory parameter was assigned with the value that was the average of two measuring in two succesive months.

Total hemoglobin was measured using colorimetric method. The target hemoglobin level in patients on dialysis was 100-120 g/L.

The normalized protein catabolic rate (nPCR) was calculated using formula of National Cooperative Dialysis Study: nPCR = (PCR x 0.58)/Vd. Formula for calculating PCR is PCR = 9.35G + 0.29Vd, where G - urea production rate, Vd - volume of body fluid (Vd = 0.58 x BW). Urea production rate was calculated by formula G = [(C1-C2)/Id] x Vd, where C1 is serum urea concentration prior to dialysis (mmol/L), C2 - serum urea concentration after dialysis (mmol/L), Id - time (hours) between two successive dialysis. Normal range for nPCR is 1.1 ± 0.3 g/kg/day.

Serum concentration of iron, ferritin, total iron binding capacity, calcium, inorganic phosphorus and CRP were measured using Beckman Coulter AU680 analyzer. Serum iron was determined by photometric method using TPTZ [2,4,6-Tri-(2-pyridyl)-5-triazine] as the chromogen. Serum iron reference range is 6.6 - 26.0 μmol/L. TIBC was done indirectly by the Unsaturated Iron Binding Capacity (UIBC) method. TIBC reference range is 48 - 56 μmol/L. Transferrin saturation - TSAT was calculated using formula TSAT = (Fe/TIBC) x 100%. Reference range for TSAT in hemodialysis patients is 20-40%. UIBC was measured using spectrophotometric method. Reference range for UIBC is 28 -54 μmol/L. Method for ferritin was turbidimetric. Ferritin reference range in the patients underwent regular hemodialysis is 100 - 500 pg/mL.

CRP level in the serum was determined by turbidimetric method. Normal CRP level in the serum is ≤ 5 mg/L. Microinflammation is defined as level of CRP in serum higher than 5 mg/L.

Calcium concentration in serum was determined by a photometric test. Normal calcium level in serum is 2.20 - 2.65 mmol/L. Phosphate level in serum was determined by a photometric test. The normal phosphate level in the serum is 0.80 - 1.60 mmol/L.

Level of vitamin D in the serum was determined by the method of electrochemiluminiscence using Cobas e 411 analyser. Normal level of vitamin D in serum is 20 - 40 ng/mL. In hemodialysis patients, normal vitamin D level is ≥ 30 ng/mL (30 - 80 ng/mL). A severe deficit is defined as the level of vitamin D < 10 ng/mL, vitamin D deficiency exists if level is 10 - 20 ng/mL, and the insufficiency is defined as the level of vitamin D in the serum of 20-30 ng/mL.

Level of intact parathormone in serum was determined by immunoradiometric method (IRMA) using gamma counter WALLAC WIZARD 1470. Normal concentration of intact parathormone in serum is 11.8-64.5 pg/mL. Patients on regular hemodialysis had iPTH up to 300 pg/mL.

The adequacy of hemodialysis was assessed on the basis of the single-pool Kt/Vsp index calculated according to the Daugridas second-generation formula:

$$Kt/Vsp = -\ln(C_2/C_1 - 0.008 \times T) + (4 - 3.5 \times C_2/C_1) \times UF/W,$$

with: C1 - the value of urea before dialysis, C2 - the value of urea after dialysis (mmol/L), T - duration of hemodialysis (h), UF - interdialysis yield (L), W - body weight after hemodialysis (kg). According to K/DOQI guidelines, hemodialysis is adequate if Kt/Vsp ≥ 1.2.

The degree of reducing urea - URR index is calculated using following formula: URR = (1-R) × 100%, where: R is the ratio of the urea concentration in serum after and before the treatment with hemodialysis. Hemodialysis is adequate if the URR index = 65-70%

Vascular access blood flow - Qavf was determined by Color-Doppler ultrasound by Logic P5 machine 7.5 MHz, where blood flow were estimated by equation: $Qavf = r^2\pi/4 \times Vmean \times 60$ (mL/min), r - radis of vascular access, Vmean - mean flow rate through vascular access. Blood flow is estimated as average value of three measurements, 2-4 cm on vain that serves as vascular access, proximally of the anasthomosis site. Blood flow rate that provides adequacy of hemodialysis is 500-1000 mL/min.

Depending on dialysis modality patients were divided into two groups. The first group included patients treated with standard hemodialysis, and the second group included patients treated with hemodiafiltration.

The statistical analysis was performed using the Kolmogorov-Smirnov test, Student's t-test and Mann-Whitney U test. The threshold of significance was the probability of 0.05 and 0.01.

RESULTS

At the Clinic for Urology, Nephrology and Dialysis of the KC Kragujevac a cross section study was conducted including patients who were treated with regular hemodialysis and hemodiafiltration over a period of more than three months. 120 patients (75 men, 45 women) were examined, mean age 63.15 ± 10.39 years, average duration of dialysis treatment 6.18 ± 5.95 years and average index of hemodialysis adequacy Kt/Vsp 1.01 ± 0.27. General patient data are shown in Table 1.

The treatment of anemia of examined patients included short-acting and long-acting erythropoietins, iron (i.v.), folic acid (p.o.), vitamin B complex (i.v.). The average monthly dose of short-acting erythropoietin was 18517.24 ± 9361.04 IU, long-acting erythropoietin 121.07 ± 75.98 mg, intravenous iron 155.83 ± 180.76 mg, folic acid 153.75 ± 23.52 mg, and the average monthly number of Beviplex ampules was 11.37 ± 1.47 (vitamin B12 45.48 ± 5.88 g).

In order to evaluate the influence of dialysis modality on the treatment of anemia the following parameters were examined: the concentration of hemoglobin in blood (Hb), hematocrit (Hct), the concentration of iron in the serum (Fe^{2+}), total iron binding capacity (TIBC), free iron binding capacity (UIBC), saturation of transferin with iron (TSAT), the concentration of ferritin in the serum (FER), the concentration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) in the serum, the concentration of vitamin D, intact parathormone (iPTH), nutritive status parameters, the concentration of total proteins (TP) and albumin (Alb) in the serum, the concentration of uric acid in the serum (UA), the rate of decomposition of proteins (nPCR), the parametres of hemodialysis adequacy (Kt/V index, spKt/V index, URR index), as well as the average monthly dose of short-acting (KDE-M) and long-acting erythropoietin (DDE-M), the index of resistance of short-acting (KDE/Hb) and long-acting erythropoietin (DDE/Hb), and the average monthly dose of i.v. iron (PMDG). Depending on the type of dialysis, the patients were divided into two groups. The first group consisted of patients treated with regular hemodialysis (HD),

Table 1. General patient data

GENERAL DATA Xsr ± SD		Statistical parameters
Number (N)		120
Pol (m/f, %)		70/45 (62.5/37.5%)
Age (yr.)		63.15 ± 10.43
Length of Treatment with Hemodialysis (yr.)		6.18 ± 5.98
Body Mass Index - BMI (kg/m^2)		24.68 ± 4.59
Systolic Arterial Blood Pressure - STA (mmHg)		121.83 ± 14.61
Diastolic Arterial Blood Pressure - DTA (mmHg)		72.82 ± 10.33
Medium Arterial Blood Pressure - SAP (mmHg)		89.16 ± 10.80
Dry Body Mass of the Patient - W (kg)		71.46 ± 15.55
Ultrafiltration - UF (L)		2475.00 ± 992.30
Residual Diuresis - RD (mL/24h)		594.17 ± 710.08
Blood Flow through a Vascular Approach - Qavf (mL/min)		841.33 ± 433.48
Hemodialysis Adequacy Index - Kt/V		1.01 ± 0.27
Single-pool Index of Hemodialysis Adequacy - spKt/V		0.01 ± 0.25
Urea Reduction Ratio - URR (%)		61.91 ± 8.80
Primary Kidney Disease	Glomerulonephritis Chronica	12 (10.00%)
	Nephropathia Hypertensiva	39 (32.50%)
	Nephropathia Diabetica	16 (13.33%)
	Nephropathia Obstructiva	8 (6.67%)
	Nephropathia Endemica	1 (0.83%)
	Nephropathia Chronica	18 (15.00%)
	Pyelonephritis Chronica	3 (2.50%)
	Renes Polycystici	21 (17.50%)
	Nephritis Tubulointerstitialis	2 (1.67%)
Comorbidities		
Hypertension		69 (57.50%)
Hypotension		3 (2.50%)
Diabetes Mellitus		18 (15.00%)
Cardiovascular Disease		30 (25.00%)

whereas the second group consisted of patients treated with regular hemodiafiltration (HDF). The average values of examined parameters are shown in Table 2.

Based on the Kolmogorov-Smirnov test, the Student's T test for two independent samples was used to examine the significance of the difference between the examined groups (hemodialysis:hemodiafiltration) for the following parameters: hemoglobin (Hb), hematocrit (Hct), mean erythrocyte volume (MCV), mean hemoglobin concentration in erythrocyte (MCHC), the number of leukocyte (Le), the concentration of iron in the serum (Fe^{2+}), total iron binding capacity (TIBC), free iron binding capacity (UIBC), the saturation of iron with transferin (TSAT) the concentration of uric acid (UA), total serum protein (TP) and serum albumin (Alb), the rate of decomposition of proteins (nPCR), the concentration of aspartate aminotransferase in serum (AST), the concentration of calcium (Ca^{2+}), phosphate (PO_4^{3-}) and vitamin D in serum, the parametres of hemodialysis adequacy (Kt/V index, spKt/V index, URR index), the average monthly dose of long-acting erythropoietin (DDE-M), the index of resistance of short-acting erythropoietin (KDE/Hb), the index of resistance of long-acting erythropoietin (DDE/Hb), Table 2. To determine the statistical significance of the difference between the examined groups for the average amount of hemoglobin in the erythrocyte (MCH), ferritin (FER), C-reactive protein (CRP), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), intact parathormone (iPTH) and the average monthly dose of i.v. iron (PMDG) Mann-Withney-U-test was used, Table 3.

Patients treated with regular hemodialysis have a high statistically significant ($p < 0.01$) lower: concentration of hemoglobin in blood (Hb), values of adequacy parameters of hemodialysis (Kt/V index, spKt/V index, URR index), statistically significant ($p < 0.05$) lower value of hematocrit (Hct) and protein decomposition rate (nPCR), as well as statistically significant ($p < 0.05$) larger: concentration of ferritin in serum (FER), C-reactive protein (CRP), and the average monthly dose of i.v. iron (PMDG) compared to the patients treated with regular hemodiafiltration, Table 2 and Table 3. There is no statistically significant difference ($p > 0.05$) between patients treated with hemodialysis and hemodiafiltration in other parameters of the study.

DISCUSSION

Anemia is present in 90% of patients in the end-stage of chronic kidney disease who begin their treatment with regular hemodialysis. Its main clinical consequences are: progressive decline in residual kidney function, development of cardiovascular complications, cognitive function disorders, and reduced quality of life [24, 25].

Regardless of the appropriate treatment of anemia, which involves parenteral application of iron and erythropoietin, anemia is still a common complication in the population of patients treated with regular hemodialysis. The prevalence of anemia, defined as a hemoglobin concentration in the serum lower than 100 g/L, is high - 50% of examined patients. The most significant risk factors that affect the treatment of anemia in patients with hemodialysis are: iron deficiency, insufficient dose of erythropoietin, inflammation, secondary hyperparathyroidism, lack of vitamin D, increased concentrations of parathormone in the

Table 2. The influence of the type of dialysis modality on the treatment of anemia in patients treated with regular dialysis (Student Test)

Test parameters	Type of dialysis		Significance differences (p)
	Hemodialysis	Hemodiafiltration	
	Xsr ± SD	Xsr ± SD	
Hb (g/L)	100.93 ± 11.06	106.88 ± 7.32	t = -2.667, p = 0.009
Hct (%)	29.46 ± 3.22	32.63 ± 2.93	t = -2.607, p = 0.040
MCV (fL)	94.91 ± 4.23	94.25 ± 4.87	t = 0.696, p = 0.488
MCHC (g/L)	331.78 ± 6.13	331.57 ± 6.11	t = 0.160, p = 0.873
Le (x 10^9/L)	7.09 ± 1.87	6.42 ± 1.96	t = 1.639, p = 0.104
Fe^{2+} (mmol/L)	10.44 ± 3.45	9.64 ± 3.14	t = 1.093, p = 0.277
TIBC (mmol/L)	33.91 ± 6.18	35.25 ± 7.11	t = -0.974, p = 0.277
UIBC (mmol/L)	23.46 ± 6.26	25.55 ± 7.38	t = -1.484, p = 0.140
TSAT (%)	31.58 ± 11.24	29.16 ± 8.62	t = 1.049, p = 0.296
UA (mmol/L)	373.63 ± 73.26	372.00 ± 66.29	t = 0.105, p = 0.916
TP (g/L)	61.47 ± 5.20	61.48 ± 4.09	t = -0.014, p =0.989
Alb (g/L)	36.54 ± 3.76	36.16 ± 2.56	t = 0.479, p = 0.620
nPCR (g/kg/day)	1.61 ± 0.65	1.95 ± 0.46	t = -2.605, p = 0.010
AST (IU/L)	16.45 ± 5.97	16.39 ± 4.29	t = 0.048, p = 0.962
Ca^{2+} (mmol/L)	2.23 ± 0.18	2.28 ± 0.19	t = -1.370, p = 0.173
PO_4^{3-} (mmol/L)	1.49 ± 0.38	1.50 ± 0.35	t = -0.187, p = 0.852
$Ca^{2+}xPO_4^{3-}$ ($mmol^2/L^2$)	3.30 ± 0.86	3.44 ± 0.90	t = -0.723, p = 0.471
Mg^{2+} (mmol/L)	1.17 ± 0.25	1.25 ± 0.24	t = -1.569, p = 0.119
Vitamin D (ng/mL)	16.32 ± 10.47	16.68 ± 4.44	t = -0.174, p = 0.862
Kt/Vindex	0.95 ± 0.24	1.20 ± 0.30	t = -4.381, p = 0.0001
spKt/Vindex	0.97 ± 0.26	1.13 ± 0.19	t = -2.862, p = 0.0050
URR (%)	60.18 ± 8.84	67.58 ± 5.89	t = -4.152, p = 0.0001
KDE-M (IU)	18.883.72±9971.89	18133.33±7614.52	t = 0.265, p = 0.792
DDE-M (mg)	115.44 ± 73.40	149.38 ± 89.38	t = -1.130, p = 0.265
KDE/Hb (IU/g)	201.03 ± 118.94	174.38 ± 74.35	t = 0.812, p = 0.420
DDE/Hb (mg/g)	1.15 ± 0.83	1.28 ± 0.97	t = -0.419, p = 0.678

Abbreviations: Hb - hemoglobin, Hct - hematocrit, MCV - average erythrocyte volume, MCHC - mean hemoglobin concentration in erythrocyte, Le - number of leucocytes, Fe^{2+} - iron, TIBC - total iron binding capacity, UIBC - free iron binding capacity, TSAT - saturation of transferrin with iron, UA - uric acid, TP - total proteins, Alb - albumin, nPCR - rate of protein decomposition, Ca^{2+} - calcium, PO_4^{3-} - phosphate, Ca^{2+} x PO_4^{3-} - product of solubility, Kt/V - index of hemodialysis adequacy, spKt/V - index of hemodialysis adequacy, URR - index of hemodialysis adequacy, KDE-M - average monthly dose of short-acting erythropoietin, DDE-M - average monthly dose of long-acting erythropoietin, KDE/Hb - index of short-acting erythropoietin, DDE/Hb - index of long-acting erythropoietin

Table 3. The influence of the type of dialysis on the treatment of anemia in patients on regular dialysis (Mann-Whitney U test): I - hemodialysis, II - hemodiafiltration

Examined parameters	Statistical parameters								Significance-p-value
	Med-I	Med-II	Min-I	Min-II	Max-I	Max-II	IQR-I	IQR-II	
MCH (pg)	31.70	31.15	27.90	27.60	65.10	35.15	2.13	2.56	Z = -0.760 p = 0.447
FER (ng/mL)	836.00	716.50	102.00	19.50	2325.00	1062.00	402.80	310.80	Z = -1.970 p = 0.049
CRP (mg/L)	5.58	4.28	0.30	0.40	171.60	14.10	9.90	6.20	Z = -1.973 p = 0.048
ALT (IU/L)	13.00	13.50	6.00	9.00	34.50	53.00	7.50	8.50	Z = - 0.714 p = 0.475
GGT (IU/L)	18.50	18.00	8.00	10.00	371.50	71.00	15.40	25.30	Z = -0.732 p = 0.464
ALP (IU/L)	73.50	73.50	28.00	34.50	1404.00	630.00	37.60	59.00	Z = - 0.174 p = 0.862
iPTH (pg/mL)	155.00	129.50	7.70	1.00	1866.00	1643.00	221.30	506.00	Z = -0.037 p = 0.970
PMDG (mg)	200.00	100.00	50.00	50.00	800.00	800.00	250.00	100.00	Z = -2.095 p = 0.036

MCH - mean hemoglobin content in red blood cell, FER - serum ferritin concentration, CRP - C-reactive protein, ALT - alanin aminotransferase, GGT - gama glutamil transferase, ALP - alkaline phosphatase, iPTH - intact parathormone, KDE-M - average monthly dose of erythropoietin, PMDG - average monthly dose of i.v. iron, Med - mediana, Min - minimum, Max - maximum, IQR - interquartile range

serum, malnutrition and inadequate hemodialysis (type and dose of dialysis modality) [26-34].

In the last decade, the number of patients treated with hemodiafiltration has increased significantly. The examination included 28 patients treated with regular hemodiafiltration over a period of more than three months (23.33%). Hemodiafiltration is better at removing uremic toxins of a moderate molecular weight, which have been shown to block erythropoiesis in the bone marrow, compared to standard hemodialysis. It also reduces microinflammation and increases the availability of iron for bone marrow erythropoiesis [35-37]. The results of this study have shown that patients undergoing hemodiafiltration have statistically significantly higher concentration of hemoglobin in blood, the value of hematocrit and parameters of hemodialysis adequacy, as well as statistically significantly lower concentration of C-reactive protein and ferritin in the serum, compared to patients treated with standard hemodialysis. These results are in accordance with the previously reported results of studies carried out so far which show that patients who are being treated with hemodiafiltration have a statistically significant higher concentration of hemoglobin in blood and a lower concentration of C-reactive protein and interleukin-6, compared to patients treated with conventional hemodialysis [35-37]. Patients treated with regular hemodialysis with microinflammation require a higher dose of erythropoietin in order to optimally control anemia (achieving and maintaining the target value of hemoglobin of 100-120 g/L) [35-37]. Patients treated with standard hemodialysis have a higher average amount of short-acting erythropoietin and an average higher index of resistance of short-acting erythropoietin compared to patients treated with hemodiafiltration, but this difference is not statistically significant. Considering that patients who are treated with regular hemodialysis have a statistically significantly lower of blood hemoglobin concentration, and that there is no statistically significant difference in the average monthly doses of short-acting and long-acting erythropoietin, it can be concluded that these patients require a higher dose of short-acting erythropoietin to achieve and maintain the target values of hemoglobin (optimal control of anemia). This indicates that hemodiafiltration reduces microinflammation, improves the availability of iron for the synthesis of hemoglobin in erythrocytes and corrects the response to erythropoietin activity. These results are in accordance with the results of other authors who have shown that hemodiafiltration is better at cleansing the blood of patients from uremic toxins of a moderate molecular weight, reduces microinflammation, increases the sensitivity to erythropoietin activity and is better at providing optimal control of anemia, compared to the patients treated with standard hemodialysis [35-37]. The results of the clinical study of REDERT show that online hemodiafiltration statistically significantly reduces inflammation, oxidative stress, concentration of β2-microglobulin in serum and hepcidin in serum, and the resistance to erythropoietin compared to patients treated with “low-flux” bicarbonate hemodialysis, [35-37]. The results of this examination showed that patients treated with hemodiafiltration had a statistically significantly lower concentration of serum ferritin (better iron availability), compared to the patients treated with standard hemodialysis. These results are in accordance with the results of other authors who have shown that there is a statistically significant positive correlation between the concentration of 25-hepcidin and ferritin in serum, and a statistically significant positive correlation was also found between the concentration of 25-hepcidin and the index of resistance to the erythropoietin effect. Reducing the concentration of 25-hepcidin in serum in patients treated with hemodiafiltration results in the resistance to erythropoietin activity [35-37]. The examined patients treated with hemodiafiltration have statistically significantly lower level of C-reactive protein in serum. These results are in accordance with the results of the clinical study CONTRAST (CONvective TRAnsport STudy), which also show that online hemodiafiltration with ultra-pure dialysis solution reduces microinflammation, compared to conventional hemodialysis [35-37]. Patients treated with online hemodiafiltration during the period of 3-6 months have a statistically significantly lower concentration of C-reactive protein and interleukin-6 in the serum, compared to the patients treated with standard bicarbonate "low-flux" hemodialysis [35-37]. The results of clinical studies show that patients who are treated with online hemodiafiltration have a statistically significant lower mortality rate compared to patients treated with standard "low-flux" hemodialysis [38-40].

CONCLUSION

Patients treated with regular hemodiafiltration have a lower level of microinflammation, a better status of iron in the body (smaller functional defect) and an optimal control of anemia compared to the patients treated with standard hemodialysis. The modality of dialysis is a significant factor for the treatment of anemia in patients with end-stage kidney disease.

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 N^{0}175014 and also to the Faculty of Medical Sciences University of Kragujevac for their Junior Grant N^{0}11/17 from which the funds were used as one of the sources to financially support this paper.

REFERENCES

1. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease. Kidney Int Suppl 2012; 2(4): 279-335.
2. Rossert JA, Wauters JP. Recommendation for the screening and management of patients with chronic kidney disease. Nephrol Dial Transplant 2002; 17(Suppl 1): 19-28.
3. Levin A. Anaemia and left ventricular hypertrophy in chronic kidney disease populations: A review of the current state ofknowledge. Kidney Int 2002; 61(Suppl 80): 35-8.
4. Stojimirović B, Petrović D, Obrenović R. Hipertrofija leve komore kod bolesnika na hemodijalizi: značaj anemije. Med Pregl 2007; LX (Supl 2): 155-9.
5. Petrović D, Miloradović V, Poskurica M, Stojimirović B. Dijagnostika i lečenje ishemijske bolesti srca kod bolesnika na hemodijalizi. Vojnosanit Pregl 2009; 66(11): 897-903.
6. Petrović D, Miloradović V, Poskurica M, Stojimirović B. Slabost srca bolesnika na hemodijalizi: procena i lečenje. Srp Arh Celok Lek 2011; 139(3-4): 248-55.
7. Petrović D, Trbojević-Stanković J, Stojanović-Marjanović V, Nikolić A, Miloradović V. Iznenadna srčana smrt bolesnika na hemodijalizi: procena rizika i prevencija. Ser J Exp Clin Res 2013; 14(1): 29-32.
8. Murray AM, Knopman DS, Tupper DE, Kane R. Cognitive impairment in hemodialysis patient is common. Nephrology 2006; 67(2): 216-23.
9. Murray AM, Pederson SL, Tupper DE, Hochhalter AK, Miller WA, Li Q, et al. Acute variation in cognitive function in hemodialysis patients: a cohort study with repeated measures. Am J Kidney Dis 2007; 50(2): 270-8.
10. National Kidney Foundation K/DOQI. Clinical Practice Guidelines for Anemia of Chronic Kidney Disease: Update 2000. Am J Kidney Dis 2001; 37(Suppl 1): 182-238.
11. Goodnough LT. The role of iron in erythropoiesis in the absence and presence of erythropoietin therapy. Nephrol Dial Transplant 2002; 17(Suppl 5): 14-18.
12. Cavill I. Iron and erithropoetin in renal disease. Nephrol Dial Transplant 2002; 17(Suppl 5): 19-23.
13. Hörl WH. Clinical Aspect of Iron Use in the Anemia of Kidney Disease. J Am Soc Nephrol 2007; 18(2): 382-93.
14. Wish J. Assessing Iron Status: Beyond Serum Ferritin and Transferrin Saturation. Clin J Am Soc Nephrol 2006; 1(Suppl 1): 4-8.
15. Drüke T. Hyporesponsiveness to recombinant human erythropoietin. Nephrol Dial Transplant 2001; 16 (Suppl 5): 50-5.
16. Good LT, Nemeth E, Ganz T. Detection, evaluation, and management of iron-restricted erythropoiesis. Blood 2010; 116(23): 4754-61.
17. Kiss Z, Ambrus C, Almasi C, Berta K, Deak G, Horonyi P, et al. Serum 25(OH)-cholecalciferol concentration is associated with hemoglobin level and erythropoetin resistance in patients on maintenance hemodialysis. Nephron Clin Pract 2011; 117(4): 373-8.
18. Icardi A, Paoletti E, De Nicola L, Russo R, Cozzolino M. Renal anemia and EPO hyporesponsiveness associated with vitamin D deficiency: the potential role of inflammation. Nephrol Dial Transplant 2013; 28(7): 1672-7.
19. Tattersal JE, Ward RA. Online haemodiafiltration: definition, dose quantification and safety revisited. Nephrol Dial Transplant 2013; 28(3): 542-50.

20. Canaud B, Barbieri C, Marcelli D, Bellocchio F, Bowry S, Mari F, et al. Optimal convection volume for improving patient outcomes in an international incident dialysis cohort treated with online hemodiafiltration. Kidney Int 2015; 88(5): 1108-16.
21. Marcelli D, Scholz C, Ponce P, Sousa T, Kopperschmidt P, Grassmann A, et al. High-Volume Postdilution Hemodiafiltration Is a Feasible Option in Rutine Clinical Practice. Artif Organs 2015; 39(2): 142-9.
22. De Roij van Zuijdewijn CLM, Chapdelaine I, Nube MJ, Blankestijn PJ, Bots ML, Konings CJAM, et al. Achieving high concentration volumes in postdilution online hemodiafiltration: a prospective multicenter study. Clin Kidney J 2017; 10(6): 804-12.
23. Rosati A, Ravaglia F, Panichi V. Improving Erythropoiesis Stimulating Agent Hyporesponsivenessin Hemodialysis Patients: The Role of Hepcidin and Hemodiafiltration Online. Blood Purif 2018; 45(1-3): 139-46.
24. Rossert JA, McClellan WM, Roger SD, et al. Contribution of anaemia to progression of renal disease : a debate. Nephrol Dial Transplant 2002; 17(Suppl 1): 60-6.
25. Tamura MK, Vittinghoff E, Yang J, Go AS, Seliger SL, Kusek JW, et al. Anemia and risk for cognitive decline in chronic kidney disease. BMC Nephrol 2016; 17(1): 13. doi:10.1186/s12882-016-0226-6.
26. Roger SD. Practical considerations for iron therapy in the management of anemia in patients with chronic kidney disease. Clin Kidney J 2017; 10(Suppl 1): 9-15.
27. Drüeke TB, Eckardt KU. Role of secondary hyperparatireoidism in erythropoetin resistance of chronic renal failure patients. Nephrol Dial Transplant 2002; 17(Suppl 5): 28-31.
28. Brancaccio D, Cozzolino M, Gallieni M. Hyperparathyroidism and Anemia in Uremic Subjects: A Combined Therapeutic Approach. J Am Soc Nephrol 2004; 15(Suppl 1): 21-4.
29. Jean G, Souberbielle JC, Chazot C. Vitamin D in Chronic Kidney Disease and Dialysis Patients. Nutrients 2017; 9(4): 328.
30. Stenvinkel P. The role of inflammation in the anaemia of end-stage renal disease. Nephrol Dial Transplant 2001; 16(Suppl 7): 36-40.
31. Akchurin OM, Kaskel F. Update on Inflammation in Chronic Kidney Disease. Blood Purif 2015; 39(1): 84-92.
32. Nassar GM, Fishbane S, Ayus JC. Occult infection of old non functioning arteriovenous grafts: A novel cause of erythropoietin resistance and chronic inflammation in hemodialysis patients. Kidney Int 2002; 61(Suppl 80): 49-54.
33. Chawla LS, Krishnan M. Causes and consequences of inflammation on anemia management in hemodialysis patients. Hemodialysis Int 2009; 13(2): 222-34.
34. De Oliveira Junior WV, de Paula Sabino, Figueiredo RC, Rios DRA. Inflammation and poor response to treatment with erythropoietin in chronic kidney disease. J Bras Nefrol 2015; 37(2): 255-63.
35. Panichi V, Rocchetti MT, Scatena A, Rosati A, Migliori M, Pizarelli F, et al. Long term variation of serum levels of uremic toxins in patient treated by post-dilution high volumen on-line hemodiafiltration in comparison to standard low-flux bicarbonate dialysis: results from the REDERET study. J Nephrol 2017; 30(4): 583-91.
36. Den Hoedt CH, Bots ML, Grooteman MPC, Der Weerd NC, Mazairac AHA, Penne EL, et al. Online hemodiafiltration reduces systemic inflammation compared to low-flux hemodialysis. Kidney Int 2014; 86(2): 423-32.
37. Panichi V, Scatena A, Rosati A, Giusti R, Ferro G, Malagnino E, et al. High-volume online hemodiafiltration improves erythropoiesis-stimulating agents (ESA) resistance in comparison with low-flux bicarbonate dialysis: results of the REDERT study. Nephrol Dial Transplant 2015; 30(4): 682-9.
38. Maduell F, Moreso F, Pons M, Ramos R, Mora-Macia J, Foraster A, et al. Desing and patient characteristics of ESHOL study, a Catalonian prospective randomized study. J Nephrol 2011; 24(2): 196-202.
39. Maduell F, Moreso F, Pons M, Ramos R, Mora-Macia J, Carreras J, et al. High-Efficiency Postdilution Online Hemodiafiltration Reduces All-Cause Mortality in Hemodialysis Patients. J Am Soc Nephrol 2013; 24(3): 487-97.
40. Peters SA, Bots ML, Canaud B, Davenport A, Grooteman MP, Locatelli F, et al. Haemodiafiltration and mortality in end-stage kidney disease patients: a pooled individual participant data analysis from four randomized controlled trials. Nephrol Dial Transplant 2016; 31(6): 978-84.