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Influence of secondary hyperparathyroidism in management of anemia in patients on regular hemodialysis

Uticaj sekundarnog hiperparatireodizma na lečenje anemije kod bolesnika na hroničnom programu hemodijalize

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Abstract

Background/Aim. Anemia is a common complication in hemodialysis patients. Treatment of anemia is affected by iron deficiency, insufficient dose of erythropoietin, microinflammation, vitamin D deficiency, increased intact parathyroid hormone concentration and inadequate hemodialysis. The aim of the study was to determine the prevalence of vitamin D deficiency and its impact on hemoglobin con-centration, iron status, microinflammation, malnutrition, dialysis adequacy and erythropoietin dose in patients on regular hemodialysis. **Methods.** The study involved 120 patients divided into three groups: severely deficient of vitamin D: 25-hydroxyvitamin D [25(OH)D] < 10 ng/mL; deficient – 25(OH)D within range of 10–20 ng/mL, and insufficient – 25(OH)D > 20 ng/mL. For statistical analysis Kolmogorov-Smirnov test, the single-factor parametric analysis of variance – ANOVA and Kruskal-Wallis test were used. **Results.** The prevalence of vitamin D deficiency in patients on regular hemodialysis was 75.83%, while the prevalence of severe vitamin D deficiency was 24.7%. Patients with severe vitamin D deficiency had lower blood concentration of hemoglobin, hematocrit, serum concentration of total proteins and albumin, and dialysis indices were also lower compared to the other two groups of patients. The level of C-reactive protein was significantly higher in the group of patients with severe vitamin D deficiency than in the two rest groups. **Conclusion.** Hemodialysis patients with severe vitamin D deficiency have lower hemoglobin, lower dialysis adequacy, significant microinflammation, malnutrition, bone meta-bolism disorders and need higher dose of erythropoietin than patients whose vitamin D was higher than 10 ng/mL. Vitamin D is important risk factor for development of anemia in hemodialysis patients and important factor that can affect treatment of anemia in these patients.

Key words:
anemia; erythropoietin; renal dialysis; risk factors; vitamin d.

Apstrakt

Uvod/Cilj. Anemija je česta komplikacija kod bolesnika na hemodijalizi. Na lečenje anemije utiču: nedostatak gvožđa, nedovoljna doza eritropoetina, mikroinflamacija, nedostatak vitamina D, povećana koncentracija intaktnog paratireoidnog hormona i neadekvatna hemodijaliza. Cilj rada bio je da se utvrdi prevalenca nedostatka vitamina D, kao i njegov uticaj na koncentraciju hemoglobina u krvi, status gvožđa, mikroinflamaciju, malnutriciju, adekvatnost hemodijalize i dozu eritropoetina kod bolesnika na redovnoj hemodijalizi. **Metoda**. Ispitivanjem je bilo obuhvaćeno 120 bolesnika podeljenih u tri grupe: grupa sa teškim deficitom vitamina D – koncentracija 25-hidroksi vitmina D [25(OH)D] < 10 ng/mL; grupa sa deficitom vitmina D – koncentracija 25(OH)D 10–20 ng/mL; grupa sa nedovoljnim nivoom vitamina D – koncentracija 25(OH)D > 20 ng/mL. Za statističku analizu korišćeni su: Kolmogorov Smirnov test, jednofaktorska parametarska analiza varijanse-ANOVA i Kruskal-Wallis-ov test. **Rezultati**. Prevalenca snižene koncentracije 25(OH)D u serumu bolesnika koji su se lečili redovnom hemodijalizom iznosila je 75,83%, a prevalenca teškog nedostatka vitamina D 24,17%. Bolesnici sa teškim deficitom vitamina D u serumu imali su statistički značajno nižu koncentraciju hemoglobina u krvi, hematokrita, koncentraciju ukupnih proteina i albumina u serumu i vrednosti

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parametara adekvatnosti hemodijalize. Koncentracija C-reaktivnog proteina u serumu bila je statistički značajno viša kod bolesnika sa teškim deficitom vitamina D u serumu, u odnosu na bolesnike sa koncentracijom 25(OH)D u serumu ≥ 10 ng/mL. **Zaključak**. Bolesnici sa teškim deficitom vitamina D u serumu imaju manju koncentraciju hemoglobina u krvi, manje adekvatnu hemodijalizu, značajnu mikroinflamaciju, malnutriciju, poremećaj metabolizma koštanog tkiva i zahtevaju veću dozu eritropoetina u odnosu na bolesnike sa koncentracijom 25(OH)D ≥ 10 ng/mL. Vitamin D je značajan faktor rizika od razvoja i lečenja anemije kod bolesnika na redovnom programu hemodijalize.

Ključne reči:
anemija; eritropoetin; hemodijaliza; faktori rizika; vitamin d.

Introduction

Chronic kidney disease (CKD) is a progressive disease and loss of renal function is followed by various complications of which anemia, secondary hyperparathyroidism (SHPTH) and cardiovascular diseases are the most important [1, 2]. Ninety percent of patients starting with hemodialysis are diagnosed with anemia caused by lack of endogen erythropoietin (EPO) which stimulates erythropoiesis in the bone marrow [3]. Among other causes of anemia the most important is hemorrhage [3]. Screening for anemia should be performed when gromelular filtration rate (GFR) is less than 60 mL/min/1.73 m^2. Measured parameters include hemoglobin concentration (Hb), hematocrit (Hct), red blood cell indices, serum iron (Fe^{2+}) and ferritin concentration (FER), transferrin saturation (TSAT) and serum concentration of C-reactive protein (CRP) [1–3].

Anemia is independent risk factor for CKD progression and cardiovascular complications [4–10]. In hemodialysis patients treatment of anemia with EPO should be set when Hb concentration is less than 100 g/L while target Hb concentration should be within range 100–120 g/L [11]. Prior to treatment with EPO optimum iron status in hemodialysis patients should be ensured (TSAT = 20–40% and FER = 100–500 ng/mL) [12–15]. After applying EPO the target Hb concentration in the blood is not achieved in 10–20% of patients. The risk factors that influence the treatment of anemia in hemodialysis patients include: iron deficiency, insufficient dose of EPO, microinflammation, malnutrition, the lack of vitamin D, SHPTH, inadequate hemodialysis, and the existence of antibodies on EPO [16–21].

SHPTH is a common and significant complication in hemodialysis patients. The lack of vitamin D, the reduced production of the active metabolite of vitamin D [1,25(OH)D], hypocalcaemia and hyperphosphatemia are the main causes of the development of SHPTH in these patients [22].The risk factors that reduce the production of vitamin D in hemodialysis patients include: reduced vitamin D synthesis in the skin, reduced filtration and reabsorption of vitamin D in epithelial cells of proximal renal tubules, reduced intake of vitamin D-rich foods, reduced absorption of vitamin D from the gastrointestinal tract [22]. The main clinical consequences of vitamin D deficiency are: development of SHPTH, reduced bone density and increased risk of fractures, reduced iron availability for Hb synthesis in erythrocytes ("functional" iron deficiency), reduced response to EPO, atherosclerosis, hypertrophy of the left heart ventricle, vascular calcification, cognitive impairment, progressive loss of residual renal function and increased mortality rate [22]. The potential mechanisms of the impact of vitamin D deficiency on the development of anemia include increased production of proinflammatory mediators in the cells of the immune system: interleukin (IL)-1, IL-6, interferon-γ, tumor necrosis factor alpha (TNFα). Proinflammatory mediators block proliferation and differentiation of erythrocyte precursor cells in the bone marrow, and IL-6 stimulates formation of hepcidin in liver, which has been proved to cause a "functional" iron deficiency [22, 23]. SHPTH causes anemia through both direct and indirect ways. The direct effects of intact parathyroid hormone (iPTH) include: blocking the formation of endogenous EPO, blocking the proliferation and differentiation of erythroid progenitors (EP) and the shortened erythrocyte life span. Indirect effects of PTH have been mainly based on the inducing revival of the bone marrow (the loss of erythrocyte precursor cells) [22, 23].

The aim of this study was to determine the prevalence of anemia, disorders of the metabolism of minerals, vitamin D and PTH, as well as to examine the effect of vitamin D deficiency and enhanced secretion of iPTH on the blood concentration of Hb, the status of iron, microinflammation, malnutrition, the adequacy of hemodialysis and EPO dose in patients on regular hemodialysis.

Methods

This study included patients of the Center for Nephrology and Dialysis, Clinic for Urology, Nephrology and Dialysis, Clinical Center Kragujevac, Kragujevac, Serbia. The study was in compliance with the principles of the Declaration of Helsinki and was approved by The Ethics Comitee of the Clinical Center Kragujevac. All patients involved in the study signed informed consent prior to enrollment. All examined patients were treated using bicarbonate hemodialysis 12 h per week for period longer than three months on hemodialysis machines, the type Fresenius and the type Gambro. Ultrapure dialysis fluid and high-flux as well as low-flux polysulfone dialysis membranes were used. Patients with active proved infections were not included in the study.

In order to evaluate impact of SHPTH in management of anemia in hemodialysis patients the following parameters were measured: Hb, Hct, FER, total iron binding capacity (TIBC), unsaturate iron binding capacity (UIBC), TSAT, calcium (Ca^{2+}), inorganic phosphate (PO_4^{3-}), alkaline phosphatase (ALP), vitamin D and iPTH. Parameters of hemodialysis adequacy were also considered.

Serum samples from patients were collected prior to hemodialysis and prior to heparin administration. Every laboratory parameter was assigned with the value that was the mean of two measuring in two succesive months.

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

Nutritional status of the patients was assessed by measuring total protein (TP) and albumin (Alb) concentrations in the serum, as well as by calculating the body mass index (BMI) and normalized protein catabolic rate (nPCR).

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

Serum concentration of iron, FER, TIBC, Ca^{2+}, PO_4^{3-} 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. TSAT was calculated using formula TSAT = Fe/TIBC × 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. A method for FER was turbidimetric one. FER reference range in the patients underwent regular hemodialysis is 100–500 pg/mL.

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

Ca^{2+} concentration in the serum was determined by a photometric test. Normal Ca^{2+} level in the serum is 2.20–2.65 mmol/L. PO_4^{3-} in the serum was determined by a photometric test. The normal PO_4^{3-} in the serum is 0.80–1.60 mmol/L.

Level of vitamin D in the serum was determined by a method of electrochemiluminescence, on the Cobase 411 analyser. Normal level of vitamin D in the 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. The level of iPTH in serum was determined by an immunoradiometric method (IRMA), on the gamma counter WALLAC WIZARD 1470. Normal level of iPTH in the serum is 11.8–64.5 pg/mL. In patients with hemodialysis the upper normal limit is 500 pg/mL.

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

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

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 – BW after hemodialysis (kg). According to K/DOQI guidelines, hemodialysis is adequate if single-pool adequacy of hemodialysis index (spKt/V) ≥ 1.2. The degree of reducing urea (URR) index was calculated using following formula: URR = (1-R) × 100%, where: R is the ratio of urea concentration in the serum after and before the hemodialysis treatment. Hemodialysis is adequate if the URR index = 65%–70%.

Depending on the level of vitamin D [25(OH)D] in the serum, the patients were divided into three groups. The first group involved the patients with the level of 25(OH)D lower than 10 ng/mL, the second group constituted patients with 25(OH)D levels within range of 10–20ng/mL, while the third group constituted of the patients with 25(OH)D levels higher than 20 ng/mL. Depending on the serum level of iPTH, the patients were divided into three groups. The first group consisted of the patients who had the serum level of iPTH lower than 500 pg/mL, the second group constituted patients with iPTH levels within range 150–500 pg/mL, while the third group constituted of the patients with the iPTH levels higher than 500 pg/mL.

The statistical analysis was performed using the Kolmogorov-Smirnov test, the single-factor parametric analysis of variance (ANOVA) and Kruskal-Wallis test. The threshold of significance was the probability of 0.05 and 0.01.

Results

The cross-sectional study was conducted at the the Clinic for Urology, Nephrology and Dialysis, Clinical Center Kragujevac, including the patients treated with regular hemodialysis in a period longer than three months. We examined 120 patients (75 men and 45 women), average age being 63.15 ± 10.39 years, the average length of treatment with hemodialysis 6.18 ± 5.95 years, and the average spKt/V 1.01 ± 12.27. General patient data are shown in Table 1. Patients were treated with short-acting and long-acting EPO with a parenteral iron composition, and their average monthly doses are shown in Table 2.

The average values of parameters of the standard laboratory tests are shown in Table 3.

The prevalence of anemia (Hb < 100 g/L) in the examined patients was 44.17% (53 patients). The average blood concentration of Hb, and average monthly single dose of EPO with short and long effects are shown in Table 2.

The prevalence of absolute iron deficiency in the examined patients was 4.17% (5 patients), and the prevalence of functional iron deficiency was also 4.17% (5 patients). One hundred and ten patients (91.66%) had the normal status of iron in the body. Twenty patients with normal level of iron and normal TSAT, had level of FER higher than 1,000 μg/L. The examined patients were given parenteral iron. The average monthly single dose of parenteral iron was 155.83 ± 180.76 mg.

Table 1
The characteristics of patients on regular hemodialysis (n = 120)

Characteristics	Values
Gender (m/f), n (%)	75/45 (62.5/37.5)
Age (years), mean ± SD	63.15 ± 10.43
Duration of treatment with hemodialysis (years), mean ± SD	6.18 ± 5.98
Body mass index (kg/m^2), mean ± SD	24.68 ± 4.59
Lean body mass (kg), mean ± SD	71.46 ± 15.55
Ultrafiltration (L), mean ± SD	2475.00 ± 992.30
Residual diuresis (mL/24 h), mean ± SD	594.17 ± 710.08
Index of adequacy of hemodialysis, mean ± SD	1.01 ± 0.27
Single-pool adequacy of hemodialysis index, mean ± SD	1.01 ± 0.25
Urea reducing ratio (%), mean ± SD	61.91 ± 8.80
Primary kidney disease, n (%)	
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, n (%)	
hypertension	69 (57.50)
hypotension	3 (2.50)
other cardiovascular diseases	30 (25.00)
diabetes mellitus	18 (15.00)

m – male; f – female; SD – standard deviation.

Table 2
Doses of erythropoietin and intravenous iron: average monthly dose and ESA/Hb index

Characteristics of treatment of anemia	Mean ± SD
Average monthly dose of erythropoietin	18,517.24 ± 9,442.79
short-term (IU)	
long-term (μg)	121.07 ± 76.90
ESA/Hb index	
short-term erythropoietin (IU/g)	191.39 ± 110.43
long-term erythropoietin (μg/g)	1.24 ± 0.83
Average monthly dose of intravenous iron saccharose (mg)	155.83 ± 181.52

ESA – erythropoiesis-stimulating agents; Hb – hemoglobin; SD – standard deviation.

Average BMI of examined patients was 24.68 ± 4.57 kg/m^2, and nPCR was 1.69 ± 0.62 g/kg/day (Table 3).

The prevalence of vitamin D deficiency [25(OH)D ≤ 20 ng/mL] on examined patients was 75.83% (91 patients), and the prevalence of severe vitamin D deficiency [25(OH)D < 10 ng/mL] was 24.17% (29 patients). The level of 25(OH)D in the serum of 10–20 ng/mL was present in 62 (51.67%) of patients, 20–30 ng/mL in 19 (15.83%) of patients, and normal level of 25(OH)D (30–80 ng/mL) in 10 (8.33%) of the examined patients. The prevalence of SHPTH (iPTH ≥ 500 pg/mL) in the examined patients was 14.17% (17 patients). The biggest number of the patients (58 or 48.33%) had iPTH ≤ 150 pg/mL, and 45 (37.50%) patients had the serum iPTH level within range of 150–500 pg/mL. Almost all the patients – 110 (91.60%) were treated with the same Ca^{2+}-containing PO_4^{3-} binding agent, 54 (45%) of patients were treated with active vitamin D metabolites and 1 (0.83%) patient was treated with vitamin D. Parenteral form of paricalcitol was given to 14 (11.67%) of patients, and average monthly dose was 30.00 ± 15.20 μg.

The patients with 25(OH)D level in the serum of less than 10 ng/mL had a highly statistically significant ($p < 0.01$) lower concentration of Hb TP, Alb and TSAT and statistically significantly lower Hct and CRP ($p < 0.05$) compared to the patients with 25(OH)D level in the serum of 10–20 ng/mL and higher than 20 ng/mL (Table 4). There was no statistically significant difference between the second and third group of patients in concentrations of Hb, Hct and CRP ($p > 0.05$) (Table 4). There was no statistically significant difference in average monthly dose of short-acting and long-acting EPO among the examined groups of patients (Table 5).

There was no statistically significant difference between the second and third group of patients in the level of TP and Alb and TIBC (Table 4).

Table 3

Laboratory parameters results

Parameters	Mean ± SD
Hb (g/L)	101.79 ± 10.94
Hct (%)	30.71 ± 3.21
TP (g/L)	61.47 ± 4.95
Alb (g/L)	36.45 ± 3.51
nPCR (g/kg/dan)	1.69 ± 0.62
Fe^{2+} (μmol/L)	10.25 ± 3.38
TIBC (μmol/L)	34.23 ± 6.36
UIBC (μmol/L)	23.95 ± 6.56
TSAT (%)	30.78 ± 10.98
F (μg/L)	790.79 ± 354.58
CRP (mg/L)	11.02 ± 19.63
Ca^{2+} (mmol/L)	2.24 ± 0.18
PO_4^{3-} (mmol/L)	1.49 ± 0.37
$Ca^{2+} \times PO_4^{3-}$ ($mmol^2/L^2$)	3.34 ± 0.87
ALP (mg/L)	106.51 ± 141.39
25(OH)D (ng/mL)	15.91 ± 9.68
iPTH (pg/mL)	278.70 ± 381.03

Hb – hemoglobin; Hct – hematocrit; TP – total protein; Alb – albumin; nPCR – normalized protein catabolic rate; TIBC – total iron binding capacity; UIBC – unsaturated iron binding capacity; TSAT – transferrin saturation [TSAT = (Fe^{2+}/TIBC) × 100 (%)]; F – ferritin; CRP – C-reactive protein; $Ca^{2+} \times PO_4^{3-}$ – solubility product; ALP – alkaline phosphatase; 25(OH)D – vitamin D; iPTH – parathyroid hormone.

Patients with severe vitamin D deficiency had statisticaly significant ($p < 0.005$) lower Kt/V as well as URR compared to other groups of patient (Table 5). The two rest groups had no statistically significant difference in the parameters of hemodialysis adequacy (Table 5).

Patients with levels of iPTH > 500 pg/mL had statistically significantly higher level of ALP, a higher PO_4^{3-} level, and a solubility product compared to patients with the serum levels of iPTH lower than 150 pg/mL (Tables 6 and 7).

Discussion

Among the patients with the end-stage kidney disease who begun regular hemodialysis, 90% suffer from anemia. Main clinical consequences of CKD are: progressive loss of residual renal function, cardiovascular complications, cognitive impairment and reduced quality of life of hemodialysis patients [24].

Regardless of appropriate treatment of anemia, which includes parenteral administration of iron and EPO, anemia is still a common complication in the population of patients treated with regular hemodialysis. Anemia, defined as blood Hb concentration lower than 100 g/L, had high prevalence (44.17%) in the examined patients with CKD. The most important risk factor that affect the treatment of anemia in patients on dialysis include: iron deficiency, insufficient dose of EPO, inflammation, infection, SHPTH, increased serum iPTH levels, lack of vitamin D in the serum, malnutrition and inadequate hemodialysis [24].

Table 4

The influence of vitamin D deficiency on the concentration of hemoglobin (Hb), C-reactive protein (CRP), parameters of nutritive status and metabolism of minerals and bone tissue in hemodialysis patients

Parameters	Patients' groups according to 25(OH)D level (ng/mL)			Significance	
	< 10	10–20	> 20	F	*p*
Hb (g/L)[a], mean ± SD	95.31 ± 9.42	103.77 ± 11.08	104.02 ± 9.75	7.431	0.001
Hct (%)[b], mean ± SD	29.16 ± 2.88	31.20 ± 3.28	31.19 ± 2.94	4.728	0.011
CRP (mg/L)[c], mean ± SD	18.42 ± 32.22	8.24 ± 9.96	9.55 ± 17.53		0.026
TP (g/L)[d], mean ± SD	58.76 ± 6.18	62.28 ± 4.18	62.45 ± 4.18	6.273	0.003
Alb (g/L)[e], mean ± SD	33.47 ± 4.18	37.16 ± 2.41	37.91 ± 3.11	18.493	0.0001
nPCR (g/kg/), day	1.52 ± 0.51	1.70 ± 0.64	1.85 ± 0.66	2.112	0.126
Fe^{2+} (μmol/L), mean ± SD	9.00 ± 2.72	10.64 ± 3.44	10.68 ± 3.66	2.686	0.072
TIBC (μmol/L)[f], mean ± SD	30.98 ± 7.40	35.12 ± 5.96	35.55 ± 5.02	5.389	0.006
UIBC (μmol/L), mean ± SD	22.09 ± 7.55	24.44 ± 6.29	24.78 ± 5.82	1.584	0.209
TSAT (%), mean ± SD	30.64 ± 11.41	30.83 ± 10.55	30.84 ± 11.82	0.003	0.940
F (ng/mL), mean ± SD	775.40 ± 405.24	789.82 ± 380.04	808.26 ± 234.34	0.062	0.940
Ca^{2+} (mmol/L), mean ± SD	2.18 ± 0.21	2.27 ± 0.15	2.24 ± 0.21	2.107	0.126
PO_4^{3-} (mmol/L)	1.43 ± 0.40	1.50 ± 0.37	1.53 ± 0.35	0.501	0.607
$Ca^{2+} \times PO_4^{3-}$ ($mmol^2/L^2$)	3.11 ± 0.89	3.40 ± 0.84	3.44 ± 0.89	1.353	0.263
ALP (IU/L)	133.53 ± 251.15	98.42 ± 82.32	96.79 ± 78.28		0.961
iPTH (pg/mL)	278.10 ± 466.71	290.51 ± 362.75	254.06 ± 334.04		0.501

Hct – hematocrit; TP – total proteins; Alb – albumin; nPCR – protein catabolism rate; TIBC – total iron binding capacity; UIBC – unsaturated iron binding capacity; TSAT – transferrin saturation; F – ferritin; $Ca^{2+} \times PO_4^{3-}$ – solubility product; ALP – alkaline phosphatase; iPTH – intact parathiroid hormone; SD – standard deviation.

Statistical analysis: [a] – $p_{I,II} = 0.001$, $p_{I,III} = 0.001$, $p_{II,III} = 1.000$; [b] – $p_{I,II} = 0.013$, $p_{I,III} = 0.043$, $p_{II,III} = 1.000$; [c] – $p_{I,II} = 0.026$, $p_{I,III} > 0.05$, $p_{II,III} > 0.05$; [d] – $p_{I,II} = 0.004$, $p_{I,III} = 0.011$, $p_{II,III} = 1.000$; [e] – $p_{I,II} = 0.0001$, $p_{I,III} = 0.0001$, $p_{II,III} = 1.000$; [f] – $p_{I,II} = 0.010$, $p_{I,III} = 0.016$, $p_{II,III} = 1.000$.

Note: H for CRP, ALP and iPTH are 7.266, 0.080 and 1.382, respectively.

Table 5
The influence of [25(OH)D] deficiency on parameters of hemodialysis adequacy, dose of erythropoietin and iron

Test parameters	Patients' groups according to 25(OH)D (ng/mL)			Significance	
	< 10	10–20	> 20	F	*p*
Kt/V[a], mean ± SD	0.91 ± 0.21	1.08 ± 0.31	0.96 ± 0.21	4.848	0.009
spKt/V, mean ± SD	0.96 ± 0.26	1.04 ± 0.26	0.99 ± 0.22	1.173	0.313
URR (%)[b], mean ± SD	58.37 ± 8.30	64.09 ± 8.94	60.78 ± 7.83	4.770	0.010
ESA/Hb index – KDE, mean ± SD	215.56 ± 131.27	202.00 ± 113.29	178.07 ± 65.28	0.538	0.587
ESA/Hb index – DDE, mean ± SD	1.34 ± 0.54	1.31 ± 1.04	1.13 ± 0.72	0.224	0.801
Monthly dose – KDE, mean ± SD	19631.58 ± 10462.69	18333.33 ± 9619.69	17636.36 ± 6622.28	0.181	0.835
Monthly dose – DDE, mean ± SD	129.44 ± 52.35	125.24 ± 92.09	115.77 ± 65.66	0.095	0.910
PMDG (mg), mean ± SD	184.48 ± 223.25	129.84 ± 142.99	182.76 ± 206.27		0.496

kt/V – index of adequacy of hemodialysis; spkt/V – single pool adequacy of hemodialysis index; URR – urea reducting ratio; ESA – erythropoiesis-stimulating agents; Hb –hemoglobin; SD – standard deviation; KDE – average monthly dose of short-acting erythropoietin; DDE – average monthly dose of long-term erythropoietin; PMDG – average monthly dose of intravenous iron.
Statistical analysis: [a] – $p_{I,II}$ = 0.014, $p_{I,III}$ = 1.000, $p_{II,III}$ = 0.134; [b] – $p_{I,II}$ = 0.011, $p_{I,III}$ = 0.852, $p_{II,III}$ = 0.263;
Note: H for PMDG is 1.402.

Table 6
The influence of intact parathyroid hormone (iPTH) on the concentration of hemoglobin (Hb), C-reactive protein (CRP), parameters of nutritional status, metabolism of minerals and bone tissue, hemodialysis adequacy, erythropoietin and iron dosage

Parameters	Patients' groups according to iPTH (ng/mL) level			Significance	
	150	150–500	> 500	F	*p*
Hb (g/L)[a], mean ± SD	101.85 ± 10.09	101.80 ± 13.03	101.53 ± 7.07	0.006	0.994
Hct (%)[b], mean ± SD	30.66 ± 2.95	30.70 ± 3.83	30.87 ± 2.03	0.028	0.972
CRP (mg/L)[c], mean ± SD	11.61 ± 24.50	8.29 ± 8.50	16.23 ± 21.77	1.061	0.349
TP (g/L)[d], mean ± SD	62.24 ± 5.12	60.33 ± 5.03	61.85 ± 3.35	1.977	0.143
Alb (g/L)[e], mean ± SD	36.51 ± 3.65	36.42 ± 3.67	36.32 ± 2.46	0.052	0.950
nPCR (g/kg/day)	1.74 ± 0.54	1.61 ± 0.73	1.76 ± 0.53	0.652	0.523
Fe^{2+} (μmol/L), mean ± SD	9.91 ± 2.59	11.10 ± 4.09	9.17 ± 3.25	2.657	0.074
TIBC (μmol/L)[f], mean ± SD	34.30 ± 7.47	34.47 ± 4.93	33.32 ± 5.68	0.205	0.815
UIBC (μmol/L), mean ± SD	24.46 ± 6.78	23.28 ± 6.25	24.00 ± 6.66	0.406	0.668
TSAT (%), mean ± SD	29.73 ± 8.23	33.03 ± 13.13	28.41 ± 12.10	1.625	0.201
F (ng/mL), mean ± SD	768.40 ± 339.46	806.22 ± 398.23	826.35 ± 273.33	0.241	0.786
Ca^{2+} (mmol/L), mean ± SD	2.27 ± 0.17	2.18 ± 0.17	2.26 ± 0.21	3.621	0.030
PO_4^{3-} (mmol/L), mean ± SD	1.37 ± 0.34	1.56 ± 0.38	1.72 ± 0.30	7.649	0.001
$Ca^{2+} \times PO_4^{3-}$ ($mmol^2/L^2$), mean ± SD	3.12 ± 0.84	3.39 ± 0.82	3.91 ± 0.80	6.014	0.003
ALP (IU/L), mean ± SD	69.34 ± 26.64	93.46 ± 39.86	267.91 ± 329.43	16.795	0.0001
25(OH)D (pg/mL), mean ± SD	15.09 ± 8.24	17.26 ± 11.26	15.12 ± 9.46	0.696	0.500

Hct – hematocrit; TP – total proteins; Alb – albumin; nPCR – protein catabolism rate; TIBC – total iron binding capacity; UIBC – unsaturated iron binding capacity; TSAT – transferrin saturation; F – ferritin; $Ca^{2+} \times PO_4^{3-}$ – solubility product; ALP – alkaline phosphatase; 25(OH)D – 25-hidroxy vitamin D; SD – standard deviation.

Statistical analysis: [a] – $p_{I,II}$ = 0.028, $p_{I,III}$ = 1.000, $p_{II,III}$ = 0.393; [b] – $p_{I,II}$ = 0.027, $p_{I,III}$ = 0.002, $p_{II,III}$ = 0.337; [c] – $p_{I,II}$ = 0.324, $p_{I,III}$ = 0.003, $p_{II,III}$ = 0.093; [d] – $p_{I,II}$ = 1.000, $p_{I,III}$ = 0.0001, $p_{II,III}$ = 0.0001; [e] – $p_{I,II}$ = 0.0001, $p_{I,III}$ = 0.0001, $p_{II,III}$ = 1.000; [f] – $p_{I,II}$ = 0.010, $p_{I,III}$ = 0.016, $p_{II,III}$ = 1.000.

Table 7
The influence of intact parathiroid hormone (iPTH) on parameters of hemodialysis adequacy, erythropoietin and iron dosage

Test parameters	Patients' groups according to iPTH (pg/mL) level			Significance	
	150	150–500	> 500	F	*p*
Kt/V[a], mean ± SD	1.05 ± 0.23	0.95 ± 0.25	1.04 ± 0.42	1.712	0.185
spKt/V, mean ± SD	1.06 ± 0.25	0.97 ± 0.23	0.96 ± 0.29	2.031	0.136
URR (%)[b], mean ± SD	63.82 ± 7.636	59.73 ± 8.90	61.13 ± 10.89	2.909	0.058
ESA/Hb index – KDE, mean ± SD	187.42 ± 113.49	228.38 ± 112.39	145.88 ± 74.75	1.686	0.195
ESA/Hb index – DDE, mean ± SD	1.29 ± 0.87	1.20 ± 0.81	1.36 ± 0.99	0.103	0.903
Monthly dose – KDE, mean ± SD	18281.25 ± 9609.25	21000.00 ± 9708.24	14500.00 ± 6989.79	1.350	0.268
Monthly dose – DDE, mean ± SD	124.71 ± 76.25	118.61 ± 74.85	130.63 ± 88.90	0.070	0.932
PMDG (mg), mean ± SD	137.07 ± 180.29	176.67 ± 196.24	164.71 ± 135.51		0.436

Kt/V– index of adequacy of hemodialysis; spKt/V – single pool adequacy of hemodialysis index; URR – urea reducting ratio; ESA – erythropoiesis-stimulating agents; Hb – hemoglobin; SD – standard deviation.
KDE – average monthly dose of short-term erythropoietin; DDE – average monthly dose of long-term erythropoietin; PMDG – average monthly dose of intravenous iron.
Note: H for PMDG is 1.659.

Results of recent clinical studies show that vitamin D deficiency plays an important role in the development of anemia in patients treated with regular hemodialysis. Vitamin D deficiency is defined as the serum level of 25(OH)D < 20 ng/mL, while severe vitamin D deficiency is defined as the serum level of 25(OH)D < 10 ng/mL [24]. Vitamin D insufficiency is defined as the serum level of 25(OH)D within range of 20–30 ng/mL. Normal serum level of 25(OH)D is ≥ 30 ng/mL. Target level in the serum of 25(OH)D in patients treated with regular hemodialysis is higher than 30ng/mL (30–80 ng/mL), while the level higher than 80 ng/mL can lead to toxic effects [24]. Prevalence of severe vitamin D deficiency in our study group was 24.17%. Vitamin D deficiency was present in 75.83% patients, vitamin D insufficiency in 15.83% (19 patients), while normal serum level of vitamin D had 8.33% patients. These results are similar with those demonstrated by former studies that showed vitamin D deficiency prevalence in hemodialysis patients of about 80% [24].

Our results point to a difference in Hb concentration among groups of patients with different vitamin D levels; patients with 25(OH)D level lower than 10 ng/mL had lower blood Hb concentration than the group with serum 25(OH)D level of 10–20 ng/mL or the group with serum 25(OH)D level higher than 20 ng/mL. These two groups of patients had no difference regarding average dose of short-term and long-term EPO indicating that patients with severe vitamin D deficiency require higher dose of EPO for the treatment of anemia. Other authors have also demonstrated that vitamin D deficient hemodialysis patients have significantly lower blood Hb concentration than patients without vitamin D deficiency and require a significantly higher dose of EPO than patients having target level of 25(OH)D [24–26]. Prevalence of SHPTH in our study was 14.17%. The examined groups of patients did not differ in Hb concentrations as well as in average monthly dose of short-term and long-term EPO. Patients with high levels of serum iPTH had higher level of $PO4^{3-}$ and ALP in the serum, and also higher $Ca^{2+} \times PO_4^{3-}$. Former studies had similar findings – patients with SHPTH had higher $Ca^{2+} \times PO_4^{3-}$ and increased risk of vascular and valvular calcifications [27–30].

Conclusion

Prevalence of vitamin D deficiency in hemodialysis patients was high – 75.83%. Severe vitamin D deficiency was present in 24.17% of patinets, while normal vitamin D level had 8.33% of patients. Patients with vitamin D deficiency level lower than 10 ng/mL had significantly lower Hb concentration and adequacy of hemodialysis indices, as well as microinflamation, malnutrition and bone metabolism disorders present and needed higher dose of EPO compared to the patients with the serum 25(OH)D level higher than 10 ng/mL. Accordingly, vitamin D could be a significant risk factor for development of anemia in hemodialysis patients.

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