



Circulatory Imbalance of Essential and Toxic Trace Elements in Pre-dialysis and Hemodialysis Patients

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Abstract

The status of essential and toxic trace elements in patients with different stages of chronic kidney disease (CKD) is still unclear and not well characterized. The present study examined the circulatory levels of a wide panel of trace elements (Al, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Pb, and U) in hemodialysis patients (HD group) and pre-dialysis patients with stage 3 CKD (PD group). Comparisons were made between groups of patients and healthy individuals from the control group (CG). The levels of Al, Mn, Co, Ni, Cu, As, Se, Sr, and Pb were higher, while the levels of Cr, Zn, Rb, Cd, and U were lower in HD patients than in our CG. Higher levels of Al and Se, as well as lower levels of As, Sr, Zn, Rb, and U were significant and distinguished HD from PD. Among other analyzed elements, Co, Se, and U are the only trace elements that did not distinguish PD from CG at a statistically significant level. The HD group had lower serum U levels than the PD group, and this could be a result of hemodialysis. This study also revealed that the Cu/Zn ratio could be used as a marker for early and late detection of renal failure. Marked changes of essential and toxic trace element levels in sera indicate additional pathophysiological events in CKD, which could additionally contribute to the preexisting increased morbidity of HD patients. Measurement of trace elements in HD patients should be performed routinely.

Keywords Pre-dialysis patients · Hemodialysis patients · Essential trace elements · Toxic trace elements

Introduction

The homeostasis of trace elements depends on both biliary excretion and optimal renal excretory function [1]. When normal excretory ability becomes inadequate as a result of chronic kidney disease (CKD), hemodialysis (HD) is one of the interventional treatment methods enabling the preservation of vital functions [2]. HD removes uremic toxins based on the concentration gradient between blood plasma and dialysate across a semi-permeable membrane. Depletion of essential substances can occur if the level of the substance in the dialysate is lower than in the blood. The same effect can occur if they are not included in the dialysate. Conversely, if the substance is present in the dialysate but not in the blood, then clinically significant toxicity can occur as a result of substance transfer across the membrane into the circulatory system [3]. Furthermore, the lack of renal clearance in HD patients can lead to an overload of toxic trace elements that originate via ingestion and/or inhalation, even when not present in the dialysate [4]. Therefore, HD patients are at risk for both deficiency and metal intoxication

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effects, depending on residual renal function, dietary intake, and quality of the HD procedure [5, 6]. According to the glomerular filtration rate (GFR) values, five stages of CKD are defined. Patients with end-stage renal disease (ESRD), which is denoted as stage 5 CKD, are particularly susceptible to these effects, since the GFR is lower than 15 mL/min/1.73 m². Stage 3 CKD is characterized by GFR values in the interval 30–59 mL/min/1.73 m²; at this stage, HD is initially considered as a therapeutic option [7].

The amounts of biologically important trace elements (such as Mn, Cu, Zn, and Se) in a patient's body can be abnormal as a result of poor dietary intake (anorexia nervosa, bulimia, and dietary restrictions) or due to the HD procedure itself [7]. Different trace elements have diverse binding affinities for plasma proteins, indicating their unequal loss during HD. For example, Sr and Mn were found to have 77% and 71% binding rates, respectively, while Se and Zn had 25 and 15% binding rates. Therefore, these two latter trace elements are more susceptible to losses during HD compared to Sr and Mn. In contrast, xenobiotics such as toxic trace elements (As, Cd, Pb, Sr, U, Rb, and others) tend to accumulate in the body as a result of excretory mechanism losses or due to contamination with large amounts of dialysis fluid (> 120 L/week) [6]. Thus, environmental factors, exposure, and dialysis water quality should be monitored.

Changes in the levels of essential and toxic trace elements in serum samples of CKD patients are an important issue that remains to be resolved. Additionally, the loss of trace element homeostasis in ESRD patients significantly contributes to their increased morbidity and mortality. In this regard, this study aimed to examine the levels of a wide panel of trace elements (Al, Cr, Mn, Co, Cu, Zn, As, Se, Rb, Sr, Cd, Pb, and U) in the serum samples of hemodialysis patients with ESRD (HD group) and in pre-dialysis patients (PD group). Comparisons were made between groups of patients as well as with healthy people with normal kidney function (control group — CG). This design of experimental groups enables the evaluation of gradation of trace element homeostasis starting from healthy individuals, followed by an intermediate stage of CKD and the terminal ESRD cases.

Material and Methods

Sample Collection

The study included 37 HD patients (female/male = 10/27; mean age = 56.7 ± 13.1 years), 34 patients with stage 3 CKD (female/male = 8/26; mean age = 57.1 ± 9.93 years), and 50 healthy blood donors (female/male = 14/36; mean age = 55.2 ± 11.2 years) in the CG. The definitive diagnosis of CKD was confirmed by fulfilling clinical, radiological, and/or histopathological criteria for diagnosis, according

to the Clinical Practice Guidelines for Chronic Kidney Disease of National Kidney Foundation (2012 KDIGO clinical practice guideline for the evaluation and management of CKD) [7]. The average time patients had been on HD was 6 ± 4 years. Primary causes of CKD in PD patients are hypertension (60%), diabetic nephropathy (23.3%), glomerulonephritis (10%), and polycystic kidney disease (6.67%). Diagnoses that lead to end-stage CKD and hemodialysis procedures were hypertension (52.38%), diabetic nephropathy (23.81%), glomerulonephritis (14.29%), and polycystic kidney disease (9.52%). Patients were dialyzed three times per week for 4 h using a polysulfone dialysis membrane. The water used for HD was prepared according to international standard ISO 13959: 2014. In order to analyze the parameters, blood samples were taken before the second weekly dialyses. The CG consisted of untreated healthy volunteers recruited for routine general examination without renal failure or any other medical disorder that could significantly affect the homeostasis of trace elements. These individuals were matched by age with patients from both the HD and PD groups.

Blood samples from HD patients were taken in the middle of the week, after 12–14 h of overnight fasting and immediately prior to dialysis. Blood samples from patients with stage 3 CKD and healthy individuals were collected in the morning after overnight fasting. All blood samples were collected into trace element-free evacuated tubes (BD Vacutainer). At the time of blood sampling, 10% of patients were receiving magnesium supplements, without taking any other minerals. The collected whole peripheral blood (5 mL) from each subject was left for approximately 30 min to coagulate, and the obtained serum was separated after centrifugation (3000 × *g*) into decontaminated, trace element free, Eppendorf tubes. All serum samples were frozen at – 80 °C until analysis.

In order to avoid confounding factors that could influence the distribution of trace elements, patients who had other medical conditions, such as gastrointestinal, inflammatory, hepatic, respiratory, malignant, and autoimmune diseases, who excessively used alcohol or who had a history of substance abuse were excluded from this study. The patients were clinically stable, with adequate nutrition status, and with no recorded cardiovascular events. None of the patients was receiving corticosteroid, anti-inflammatory, or cytotoxic drugs. Moreover, patients who were employed in any chemical industry or in similar places that might interfere with the ingestion/inhalation of trace metals were also excluded. All study participants signed written informed consent to participate in this study, which was approved by the Ethical Review Board of the Military Medical Academy, Belgrade, Serbia, in accordance with the principles of the Declaration of Helsinki.

Laboratory Procedures

Serum samples were prepared according to Stojšavljević et al. [8]. Briefly, serum samples were diluted 10 times with an aqueous solution containing 0.05% nitric acid, 0.1% Triton X-100, and 3% n-butanol. All elements were quantified by inductively coupled plasma mass spectrometry (ICP-MS) (iCAP Qc, Thermo Scientific, UK) in standard mode and by adding an internal standard solution, containing ^{45}Sc at a concentration of 50 $\mu\text{g/L}$ and ^{71}Ga , ^{89}Y , ^{115}In , ^{159}Tb , and ^{208}Bi at concentrations of 10 $\mu\text{g/L}$, in equal amounts to each test solution. Linearity of the calibration curve above 0.99 was obtained for each element in the range from 1 to 50 $\mu\text{g/L}$ (Al, Cr, Mn, Co, Ni, As, Sr, Cd, Pb, and U) or from 1 to 250 $\mu\text{g/L}$ (Cu, Zn, Se, and Rb). Accuracy was assessed by analysis of certified reference material (CRM) Seronorm™ Trace Elements Serum L-1. According to the results obtained with CRM, the following isotopes were selected: ^{27}Al , ^{53}Cr , ^{55}Mn , ^{59}Co , ^{60}Ni , ^{65}Cu , ^{67}Zn , ^{75}As , $^{78/82}\text{Se}$, ^{85}Rb , ^{88}Sr , ^{111}Cd , ^{208}Pb , and ^{238}U (obtained recovery values for selected isotopes ranged from 92.4 to 119.2%).

Data Analysis

Statistical analysis was conducted using SPSS statistical software (IBM Statistics v.20). Data were summarized as the mean and standard deviation (*SD*), median and range (min–max), as well as geometric mean (*GM*) and percentiles (*Ps*) from the 5th to the 95th. The normality of data was tested using the Shapiro–Wilk test. Comparisons between groups were performed using the Kruskal–Wallis one-way analysis of variance and Mann–Whitney *U* test. *P* value below 0.05 was considered statistically significant. Principal component analysis (*PCA*) was performed in the software package PLS ToolBox, v. 6.2.1, Matlab 7.12.0 (R2011a); all data were auto-scaled before the multivariate analysis.

Results

The parameters of descriptive statistics, together with the *GMs* and selected *Ps* for elements in the serum samples of CG, PD, and HD, are given in Tables 1, 2, and 3, respectively. Copper (*Cu*) was the most abundant element in all three groups, followed by zinc (*Zn*), rubidium (*Rb*),

Table 1 Parameters of descriptive statistics, together with geometric mean (*GM*) and selected percentiles (*Ps*) in serum samples for trace elements ($\mu\text{g/L}$) in the control group (CG)

	Al	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Cd	Pb	U
Mean	5.87	0.54	2.34	0.76	2.59	855	607	0.54	61.8	160	24.6	0.05	0.31	0.021
<i>SD</i>	3.83	0.47	1.95	0.24	1.00	219	125	0.32	14.4	31.5	8.33	0.03	0.24	0.025
Min	1.90	0.15	0.61	0.40	1.26	500	410	0.09	33.8	65.1	10.3	0.01	0.05	0.002
Max	19.4	1.83	7.94	1.59	5.44	1486	940	1.44	99.8	226	48.7	0.19	1.47	0.121
Median	4.56	0.32	1.62	0.69	2.31	826	583	0.46	63.0	157	22.7	0.04	0.23	0.009
<i>GM</i>	5.00	0.41	1.80	0.73	2.43	828	594	0.45	60.1	157	23.3	0.04	0.25	0.012
P5	2.33	0.19	0.66	0.49	1.46	561	436	0.15	40.1	118	14.3	0.02	0.09	0.004
P25	3.35	0.25	1.07	0.64	1.87	677	510	0.35	48.3	137	18.7	0.03	0.17	0.006
P50	4.56	0.32	1.62	0.69	2.31	826	583	0.46	63.0	157	22.7	0.04	0.23	0.009
P75	7.07	0.60	2.64	0.84	3.04	1015	687	0.70	70.9	178	27.6	0.06	0.38	0.023
P95	12.9	1.67	7.33	1.22	4.89	1228	816	1.31	83.7	215	39.4	0.10	0.65	0.073

Table 2 Parameters of descriptive statistics, together with geometric mean (*GM*) and selected percentiles (*Ps*) in serum samples for trace elements ($\mu\text{g/L}$) in the pre-dialysis patients (PD group) with 3rd stage chronic kidney disease

	Al	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Cd	Pb	U
Mean	11.2	0.15	4.06	0.96	8.86	1148	445	0.97	68.7	190	51.8	0.014	3.04	0.013
<i>SD</i>	2.33	0.12	1.02	0.47	1.30	186	82.2	0.15	24.6	39.8	14.4	0.003	1.36	0.009
Min	5.28	0.02	2.55	0.60	5.52	882	249	0.51	44.0	133	31.3	0.006	0.99	0.001
Max	15.0	0.51	7.04	2.44	11.3	1499	635	1.20	157	280	90.6	0.019	7.20	0.039
Median	11.6	0.13	4.01	0.76	9.04	1146	443	1.01	62.0	184	47.0	0.014	2.94	0.014
<i>GM</i>	11.0	0.12	3.95	0.88	8.76	1134	437	0.95	65.6	187	50.0	0.014	2.78	0.009
P5	7.17	0.03	2.67	0.62	6.71	906	318	0.64	45.2	141	35.0	0.009	1.31	0.001
P25	9.93	0.08	3.39	0.71	8.08	984	393	0.98	55.1	160	42.8	0.013	2.13	0.008
P50	11.6	0.13	4.01	0.76	9.04	1146	443	1.01	62.0	184	47.0	0.014	2.94	0.014
P75	12.6	0.18	4.39	0.90	9.70	1277	494	1.04	71.3	210	56.2	0.015	3.71	0.017
P95	14.4	0.39	6.37	2.00	10.5	1469	579	1.08	117	266	81.0	0.018	5.29	0.028

Table 3 Parameters of descriptive statistics, together with geometric mean (GM) and selected percentiles (Ps) in serum samples for trace elements ($\mu\text{g/L}$) in the hemodialysis patients (HD group)

	Al	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Cd	Pb	U
Mean	30.4	0.12	4.41	0.95	10.5	1045	399	0.83	81.8	106	45.0	0.015	4.13	0.0014
SD	13.5	0.06	1.97	0.38	3.43	245	92.1	0.26	26.1	41.9	11.3	0.009	2.72	0.0006
Min	12.4	0.01	1.71	0.62	6.10	633	262	0.43	44.7	55.7	26.7	0.006	1.25	0.0001
Max	57.8	0.27	11.1	2.67	21.0	1747	682	1.65	185	272	91.9	0.051	14.1	0.0035
Median	26.6	0.12	4.00	0.86	9.32	1021	384	0.73	80.1	92.6	42.8	0.013	3.17	0.0012
GM	27.6	0.10	4.04	0.90	9.99	1018	390	0.80	78.4	99.6	43.9	0.013	3.51	0.0012
P5	15.4	0.03	2.17	0.66	6.60	702	286	0.48	53.8	67.3	32.8	0.007	1.55	0.0006
P25	19.3	0.07	2.81	0.72	7.78	927	336	0.65	62.9	81.3	39.2	0.009	2.72	0.0011
P50	26.6	0.12	4.00	0.86	9.32	1021	384	0.73	80.1	92.6	42.8	0.013	3.17	0.0012
P75	44.2	0.15	5.28	1.01	13.1	1154	440	1.05	94.2	117	48.2	0.021	4.72	0.0017
P95	53.6	0.23	7.64	1.46	15.4	1484	571	1.17	114	186	62.3	0.028	9.90	0.0021

selenium (Se), strontium (Sr), aluminum (Al), nickel (Ni), manganese (Mn), chromium (Cr), lead (Pb), arsenic (As), cobalt (Co), cadmium (Cd), and uranium (U) (Fig. 1a and b). According to the Kruskal–Wallis test, the HD group differed from CG for each analyzed trace element (Table S1, Suppl. material). The levels of Al, Mn, Co, Ni, Cu, As, Se, Sr, and Pb were higher, while the levels of Cr, Zn, Rb, Cd, and U were lower in HD compared to CG. Higher levels of Al and Se, as well as lower levels of As, Sr, Zn, Rb, and U, were significant to distinguished HD from PD. Among other analyzed trace elements, Co, Se, and U are the only elements that did not distinguish PD from CG ($P > 0.05$) (Tables 1, 2, and 3). We also analyzed the impact of dialysis vintage (below and above 6 years) on the concentration of trace elements and did not find significant differences for all studied trace elements (Table S2, Suppl. material).

PCA was carried out to obtain a more detailed insight into the data structure and to identify influential variables responsible for certain patterns of the grouping of CG, PD, and HD samples. The PCA model obtained had a pattern with four main components that explain 68.43% of the total data variance. This model is graphically presented as mutual projections of factor scores and their loads for the first and third main principal components (PCs) (Fig. 2). The score graph revealed the samples grouped into three distinctive clusters according to the levels of the trace elements (Fig. 2a). The first cluster, consisting of CG samples, was distinguished from the other two groups on the left side of the plot, along the PC1 axis, while the separation of the second and third clusters formed from the PD and HD samples, respectively, was evident along the PC3 axis. The loading plot (Fig. 2b), a plot of the direction vectors that define the model, revealed that Zn, Cr, Cd, Rb, and U had the greatest negative impact on PC1. The high level of these trace elements in the samples of the CG mainly affected their separation from the other groups of samples. The variables that potentially had the greatest influence on the discrimination along PC3 were Se and Al, indicating higher levels of these metals in the

HD patients, while the PD cluster showed higher levels of Sr, As, U, and Rb.

Discussion

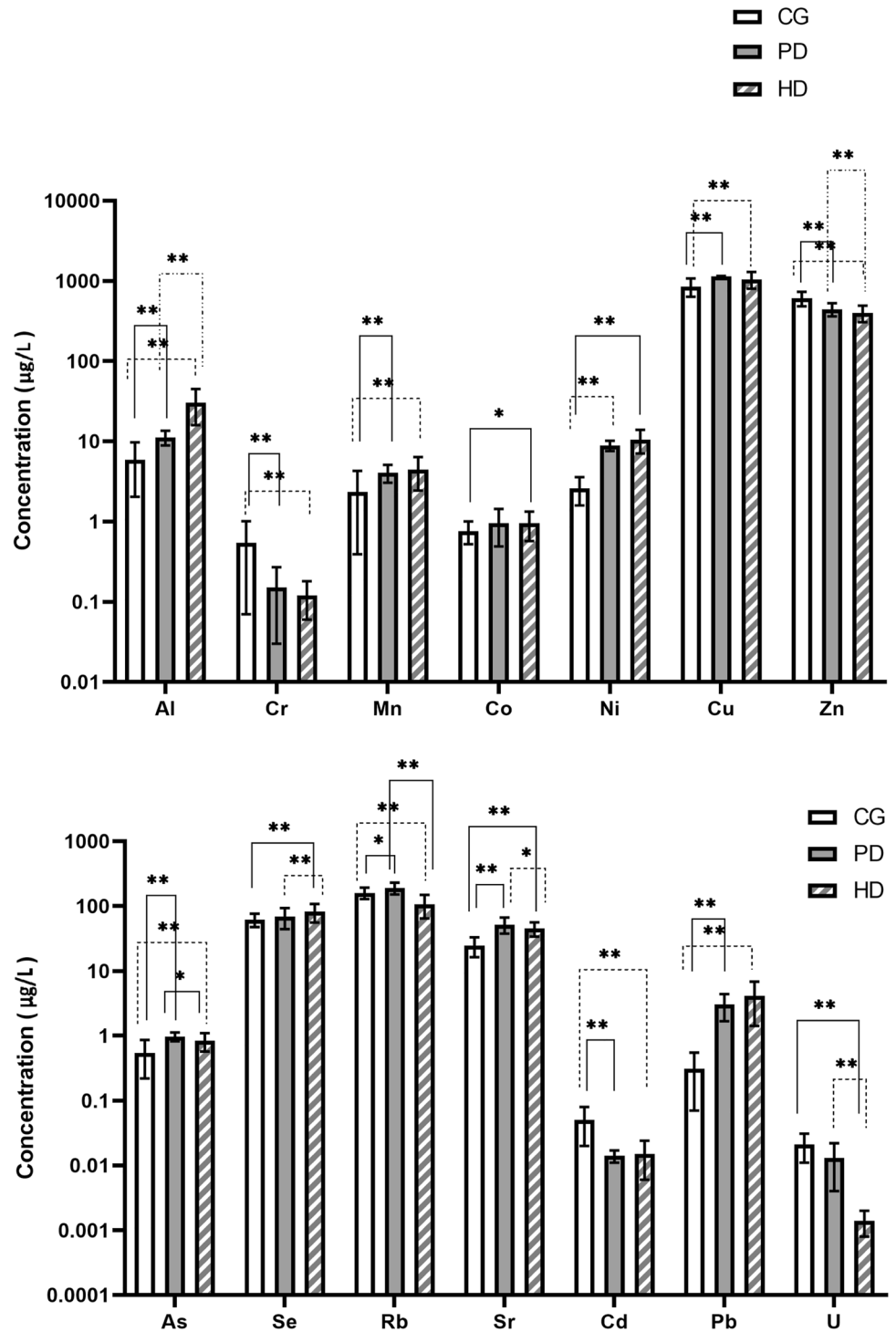
Patients on maintenance HD are predisposed to increased risk of trace element imbalance because they have lost the major route of metal excretion and are in close and prolonged contact with an important source of potential systemic exposure, such as dialysis fluid [5]. However, environmental factors that affect each population should also be considered, due to the well-known differences in trace element levels between countries [9].

This study aimed to investigate a wide panel of trace elements and determine whether there is justification for measuring the concentrations of trace elements in PD and HD patients. Given the growing interest in trace elements in CKD, especially in patients on HD, we aimed to determine the serum levels of essential (Cr, Co, Mn, Cu, Zn, and Se) and toxic trace elements (Al, Ni, As, Rb, Sr, Cd, Pb, and U).

Zinc Levels in Hemodialysis Patients

Zinc is the most analyzed trace element in serum samples of HD patients. Practically, all investigations confirm reduced serum Zn levels in HD patients [3, 10]. Our results agree with the previous studies. Some of them also confirmed that serum Zn levels significantly decreased after HD [2, 10]. Our results reinforce this finding based on the observed lower serum Zn in HD group compared to the PD group. A recent study by Damianaki et al. [11] showed not only lower circulating Zn levels in CKD patients, but also higher levels of Zn in daily urine compared to non-CKD participants. Ching-Tang et al. [12] noted serum Zn levels had a statistically decreasing trend in patients in the advanced stages of CKD from stages 1 to 4. These results also agree with the

Fig. 1 a Graphical view of mean and standard deviation (*SD*) values, together with the differences obtained by the Kruskal–Wallis test for concentrations of Al, Cr, Mn, Co, Ni, Cu, and Zn ($\mu\text{g/L}$) between the control group (CG) ($n=50$), pre-dialysis patients with stage 3 CKD (PD group) ($n=34$), and hemodialysis patients (HD group) ($n=37$). Significance: $*P<0.05$; $**P<0.01$. **b** Graphical view of mean and standard deviation (*SD*) values, together with the differences obtained by the Kruskal–Wallis test for concentrations of As, Se, Rb, Sr, Cd, Pb, and U ($\mu\text{g/L}$) between the control group (CG) ($n=50$), pre-dialysis patients with stage 3 CKD (PD group) ($n=34$), and hemodialysis patients (HD group) ($n=37$). Significance: $*P<0.05$; $**P<0.01$



differences we measured in serum Zn levels in PD and HD groups.

Zn deficiency can have serious health consequences, such as erythropoietin-resistant anemia, increased susceptibility to kidney damage in diabetes mellitus, immunoglobulin A nephropathy, and risk of atherosclerosis (that has a role in CKD progression and complications) [11]. As an antioxidant

and anti-inflammatory mediator, Zn regulates the function of T- and B-lymphocytes, making the body's immune system less susceptible to various infections [13]. Recently, the protective role of Zn in HD patients with COVID-19 has been mentioned [14]. Since it has been noted that Zn deficiency could increase oxidative stress and atherosclerotic complications, particularly in patients in late-stage renal failure, a

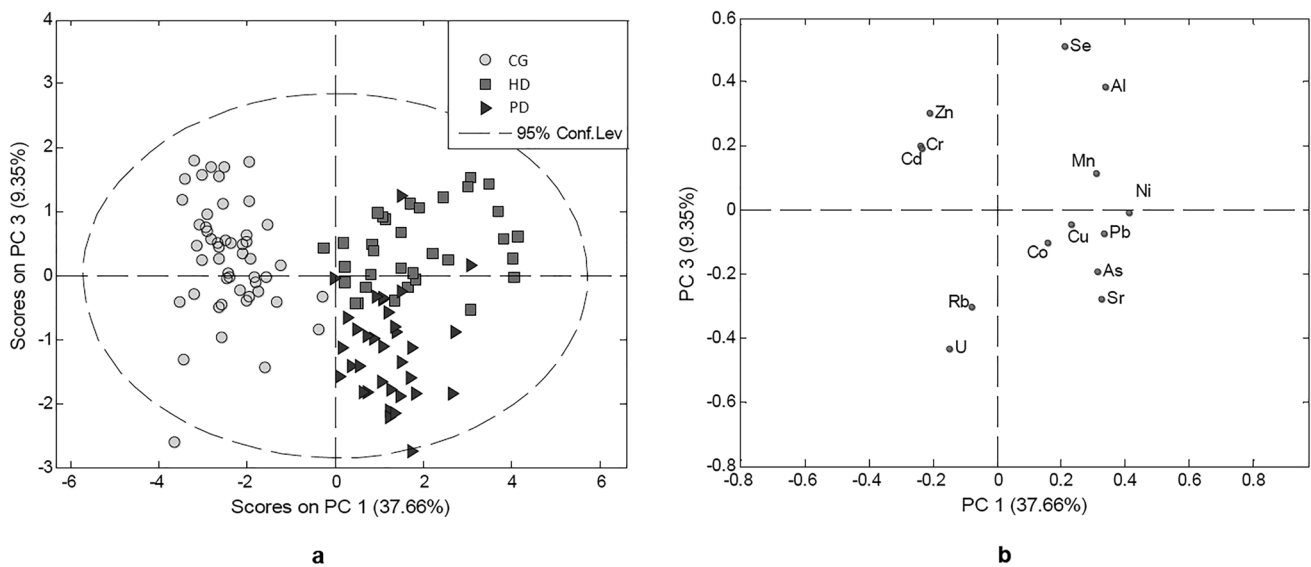


Fig. 2 Classification and differentiation of CD ($n=50$), PD ($n=34$), and HD ($n=37$) according to concentrations of analyzed trace elements by principal component analysis (PCA): **a** score plot; **b** loading plot

practical response would be to start timely Zn supplementation at the moment when patients require initial HD treatment. Dvornik et al. [15] noted that 64% of HD patients with ESRD had low serum Zn levels and that Zn supplementation could only be recommended in patients with a proven deficiency. Fukushima et al. [16] showed that an anti-ulcer agent containing 34 mg Zn could be used for zinc deficiency ($< 800 \mu\text{g/L}$). Guo and Wang [13, 17] found that patients, before receiving Zn supplements, had decreased Zn plasma levels and increased Cu levels. After treatment with 78 mg of zinc gluconate (11 mg of elemental Zn per day) during 2 months, the reverse finding was obtained. According to our Serbian database on food composition, the average dietary intake of Zn was below 8.8 mg/day for 33.3% of patients with HD; the optimal daily requirement for Zn is 15 mg. This finding could also explain hypozincemia in our HD patients.

Several factors can lead to Zn deficiency in HD patients, including reduced food intake, the HD procedure per se, increased expression of some transporters during inflammation, the deficit of Fe (antagonistic effects), impaired gastrointestinal absorption of Zn in uremic patients, a deficit of calcitriol, antagonistic effects of Cu on their common carrier, metallothionein, due to its higher affinity for Cu than for Zn, etc. [4, 7]. Although many studies have reported that serum levels below $800 \mu\text{g/L}$ should be considered deficient, clinical symptoms of deficiency have been observed primarily in individuals with serum Zn levels below $400 \mu\text{g/L}$ [7]. Thus, the serum Zn levels obtained in HD patients in this study ($399 \pm 92.1 \mu\text{g/L}$) could be considered as indicative for Zn supplementation. The type of HD procedure, its frequency, and the presence of other comorbidities should be

evaluated in future studies, which would determine their effects on Zn turnover. Furthermore, the serum Zn in PD ($445 \pm 82 \mu\text{g/L}$) and CG ($607 \pm 125 \mu\text{g/L}$) also deserve attention, because both levels were below the proposed reference range of $800\text{--}1200 \mu\text{g/L}$ [7]. However, this reference range should be taken with some reserve due to the previously mentioned deficit of this element in soil and food in our country [18]. Therefore, the determined reference interval for serum Zn in the adult Serbian population, which is from 400 to $825 \mu\text{g/L}$, strongly implies that our healthy adult populace could be at risk of Zn deficiency [19].

Other Analyzed Trace Elements

Studies for other analyzed trace elements in HD patients are contradictory. Most studies agreed that Cu levels were higher in HD patients than in healthy individuals [20, 21], although there are other studies that showed insignificant [12] or even statistically lower serum Cu in HD patients than in healthy controls [5]. Tonelli et al. [22] reported an independent association between elevated Cu levels and risk of death in HD patients. The significantly high serum Cu levels in patients with HD in this study could be explained by their Zn deficiency, as it is well known that these two trace elements are antagonists. We also found the Cu/Zn ratio could be considered as a marker for HD patients, since a statistical difference was found between the CG ($1.41 \pm 0.88 \mu\text{g/L}$) and both PD (2.66 ± 0.57) and HD (2.67 ± 0.62). Interestingly, the serum Cu/Zn ratio of the PD group did not separate these patients from the HD group, indicating the selectivity of this ratio in regard to disease prognosis.

Tonelli et al. [3] reported that there are associations in blood profiles between HD patients and healthy controls. They found that Cd, Cr, and Pb were higher and that Mn and Se concentrations were lower in patients with HD compared with controls. Agreement with our study was found only for Pb. This discrepancy in the results can be explained by the fact that the study by Tonelli et al. [3] included serum, plasma, and whole blood samples. It is well known that essential trace elements, such as Mn, Se, and Cr, are naturally higher in whole blood than in serum or plasma samples [23]. These facts could also explain the opposite results for Mn, Se, and Cr found by Tonelli et al. [3] compared to our results. Another potential factor that could contribute to these differences is environmental exposure to the mentioned trace elements, which is geographically specific. Thus, the serum Se measured in this study should be carefully interpreted due to the significant lack of this element in the soil and food in our country. The reference interval (P5-P95) for serum Se in the adult Serbian population was 45–98 µg/L [19], which is about half the reference level in other countries [24, 25]. However, our results for HD agree with Gómez de Oña et al. [20]. Greater Se levels in HD than in PD patients suggest the phenomenon of prolonged Se accumulation in their circulatory systems and could be a direct result of their extent of renal failure.

Although the results for Mn are contradictory between published studies, perhaps primarily due to the use of different types of samples, we found agreement with the results of Almeida et al. [5] for this essential trace element. A possible explanation for the elevated Mn in our HD patients could be the inability to effectively remove it during the dialysis water purification process and/or the fact that the main route of elimination of Mn from the body is feces rather than urine [26, 27]. The current study also found that Co levels were higher in HD than in CG. Considering serum Co levels were slightly higher than the accepted reference limit (<0.90 µg/L) reported by Almeida et al. [5], the elevated levels of this element we measured in HD patients (0.95 ± 0.38 µg/L) could be associated with increased gastrointestinal absorption or exposure through dialysis fluid, due to contact with Co-containing metal alloys.

To the best of our knowledge, there are only a few papers on the status of toxic trace elements in HD patients. The most well-known example of intoxication of HD patients is related to Al. Our results confirm previous findings. It should be noted that hemodialysis is not highly effective in eliminating Al due to its ability to bind to transferrin (about 90%) [28]. The findings of our study strongly indicate the justification for considering even stricter quality control of dialysis water, given that the serum Al levels (30.4 ± 13.5 µg/L) were above 20 µg/L, which is characteristic of the symptoms of osteomalacia and/or encephalopathy. Recently, Almeida et al. [5]

also reported reduced Rb and increased Sr levels in HD patients. These results agreed well with ours. Canavese et al. [29] not only reported similar results, but also pointed out that Rb could play a role in neurobehavioral functions, based on the results obtained for the analyzed neural tissues. D'Haese et al. [30] found that HD patients with bone fractures had elevated serum Sr levels compared to other osteodystrophies. Twofold higher serum Sr levels in HD (45 ± 11.3) than in CG (24.6 ± 8.33 µg/L) could be explained by the release of Sr from damaged bones into the vascular system. Gómez de Oña et al. [20] found elevated levels of Ni, As, and Pb in HD patients, which agree with our results. Hypernickemia has been reported in HD patients. Ramprasad et al. [31] also found increased levels of Ni in HD patients. Our results confirm these previous findings, since the serum Ni levels of 8.86 ± 1.30 and 10.5 ± 3.43 µg/L for PD and HD, respectively, were above the toxicologically relevant limit in HD patients, 10 µg/L, as reported by Almeida et al. [5]. Moreover, our results showed that even our CG (2.59 ± 1.00 µg/L) has serum Ni levels above the reference limit (<2 µg/L). Although As levels are increased in HD patients, Zhang et al. [32] reported that foods can affect As levels in HD patients, due to the presence of arsenobetaine (AsB), the most common form of As present in the bloodstream. This indicates that As most likely does not pose a significant danger to HD patients, because AsB is non-toxic. In contrast, the high levels of Pb we measured in the blood of HD patients should be further investigated, as it is well known that Pb is distributed to the kidneys after gastrointestinal absorption. Atieh Makhloogh et al. [33] noted that elevated Pb levels in HD patients could be due to almost complete loss of renal function and difficulty in removing Pb during hemodialysis. The status of serum U in patients on HD is unknown. A positive finding of our study is that U levels were 10 times lower in HD than in PD patients and the CG. This discovery could indicate the importance of HD as a part of therapeutic protocols which are applied if intoxication with this heavy metal occurs.

It would be instructive to quantify the essential and toxic metals in the circulatory system of HD patients, in order to determine these metals' physiological levels and to prevent any toxicity that could occur at higher levels. We stress that HD patients carry a substantial burden of comorbidities and susceptibility to the more severe symptoms caused by trace elements deficiency and/or toxicity, so any measures contributing to damage mitigation would be welcome.

The limitations of this study are primarily related to the insufficient number of subjects to conduct a deeper statistical analysis in relation to sex, age, and other internal/external factors. However, strict selection of patients without the mentioned chronic diseases limited the number of subjects adequately covered by this study.

Conclusion

This study showed significant alterations of circulating trace elements in the bloodstream of HD patients, an important issue that requires attention due to the potential deleterious effects of these metals. The levels of Al, Mn, Co, Ni, Cu, As, Se, Sr, and Pb were higher, while the levels of Cr, Zn, Rb, Cd, and U were lower in HD patients than in our CG. Higher levels of Al and Se, as well as lower levels of As, Sr, Zn, Rb, and U were significant to distinguish HD from PD. Among other analyzed elements, Co, Se, and U are the only trace elements that did not distinguish PD from CG at a statistically significant level. The HD group had lower serum U levels than the PD group, and this could be a result of hemodialysis. This study also revealed the Cu/Zn ratio could be used as a marker for early and late detection of renal failure. Marked changes in serum levels of essential/toxic trace elements indicate additional pathophysiological events in CKD, which could additionally contribute to the preexisting increased morbidity of HD patients. Measurement of trace elements in HD patients should be performed routinely.

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Author Contribution A.S. designed the investigation, conducted the experimental study, analyzed the data, and wrote the first and last version of the paper; S.R. and B.T. collected clinical specimens; D.R.M., S.R., B.R., Đ.K., and D.M. participated in writing through reviewing and editing. All authors contributed critically to the draft and gave final approval for publication. We thank Dr. Marija Takić for her assistance.

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Data Availability Data and material are available from the corresponding author on a reasonable request.

Code Availability Not applicable.

Declarations

Ethics Approval The approval for the study was granted by the Ethical Review Board of the Military Medical Academy, Belgrade, Serbia, in accordance with the principles of the Declaration of Helsinki.

Consent to Participate All patients and healthy blood donors voluntarily participated in this study, and written informed consent from all study participants was obtained according to the ethical standards defined by the Declaration of Helsinki.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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