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Low Serum Zinc Level May be Related to Higher Doses of EPO in Hemodialysis Patients

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Abstract

Introduction. Anemia is a complication of chronic renal failure observed in patients on hemodialysis (HD) affecting morbidity and mortality of these patients. It is associated with erythropoietin (EPO) deficiency and can be treated by human recombinant erythropoietin (hrEPO). Iron deficiency has been reported as the first line cause for inadequate response to this treatment. Zinc deficiency in hemodialysis (HD) patients was previously reported and the relationship between iron and zinc deficiencies has been known for years. The aim of this study was to find out the relationship between serum zinc level, anemia and rhEPO consuming in HD patients.

Methods. A total of 69 HD patients and 34 healthy individuals were included in the study. Serum zinc levels, clinical, hematologic, biochemical parameters and rhEPO doses were evaluated.

Results. Serum zinc levels were found to be lower in HD patients in comparison to those in the control group (29.92±12.94, 44.82±27.69μg/dL, respectively) ($p<0.001$). There was a positive correlation between serum zinc and hemoglobin (Hb) in the control group ($R^2=0.06$). In HD patients who needed less then 8000U/week rhEPO, Hb levels ($p<0.05$) and serum zinc levels were higher. Serum zinc and Hb levels were found to be higher in patients who were under HD treatment for more than 12 months (10.05±1.06 vs 10.69±1.29; $p=0.022$ and 26.96±13.29 vs 30.88±12.61; $p=0.178$).

Conclusions. HD patients who needed lower dose of EPO had higher serum zinc levels. Although the difference was not statistically significant, these results provide evidence that serum zinc level should be taken into consideration especially in HD patients resistant to EPO therapy.

Key words: serum zinc level, hemodialysis, anemia, erythropoietin

Introduction

The prevalence of anemia in patients with chronic kidney disease is reported to be approximately 50% [1]. In patients on hemodialysis (HD) treatment, a decrease of hemoglobin level by 1g/dl was found to be associated with left ventricular (LV) hypertrophy by 42%, which increases mortality and morbidity [1-3]. The most significant cause of anemia is erythropoietin deficiency due to decreased production of this hormone from kidney tissue [3-5]. The discovery of recombinant human erythropoietin (rhEPO) was a cornerstone in the management of patients with chronic kidney disease [4] and increased their quality of life tremendously. However, this therapy increases the medical expenses whereas some patients do not respond as expected. Iron deficiency, severe secondary hyperparathyroidism, hypo or hyperthyroidism, infectious diseases and some other comorbidities have been implicated as causes of inadequate response to rhEPO [6]. Nevertheless, malnutrition, hypoproteinemia, malabsorption and exposure to large a amount of dialysis solution which does not contain any trace elements might be related to zinc and other trace elements deficiency in HD patients [7]. The relationship between zinc deficiency and hypochrome microcytic anemia is well known since 1960's [8]. In this study, the potential relationship of zinc deficiency to the severity of anemia and to inefficient response to EPO treatment in HD patients has been investigated.

Material and methods

Ninety-six patients with End-Stage Renal Disease (ESRD) (46 males, 50 females, with mean age 56±14 years and HD duration for 44.52±38.00 months) undergoing maintenance HD three times a week, four hours each session, were included in this study. HD treatment was performed using Polysulphone FX60 and FX80 high-flux membranes in Fresenius trademarked 4008 S type machines. The bicarbonate-based dialysate did not contain any zinc supplement. Iron supplementation was performed routinely at

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least once a week to each patient unless a contraindication existed. EPO treatment to each patient was also performed to maintain hemoglobin level between 11-12 g/dL. Samples were drawn before the first HD session of the week. In addition to this group, 34 (14 males, 20 females; mean age 53±10 years) healthy persons were studied as a control group. In addition to demographic, clinical features of these patients and CBC (Combined Blood Count), other hematologic parameters, including serum iron, TIBC (Total Iron Binding Capacity), transferrin saturation, serum B12, ferritin, folate level and zinc levels in both groups were also obtained. Patients with known malignancy, infectious diseases, endocrine abnormalities, gastric problems as well as patients under the age of 18 were excluded from the study.

Appropriate methodology was used for the hematologic and biochemical parameters. The serum zinc level was determined quantitatively by using Inductive Comparable Plasma Optic Emission Spectrophotometric (ICP-AES) colorimetric method and on 206.200 nM wavelength [9].

Kocaeli University Ethicsl Committee approved that this study was in accordance with the ethical standards of the Committee on human experimentation with the Declaration of Helsinki and its revisions. Oral inform consents were obtained from patients and control subjects.

Statistical analysis

For the statistical analysis SPSS 15.0 program (SPSS Inc., Chicago Ill., USA) was used. All data are displayed as mean ± standard deviation. Student's t-test was applied to compare means of continuous data and data with a normal distribution, otherwise Mann-Whitney U test was used. Variables were compared using the Chi-square test, and Pearson's regression test was used to examine correlation between zinc concentration and hematologic data.

Results

Age, body mass index and gender were compared between HD patients and healthy subjects (Table 1). The mean zinc level was found to be lower in patients undergoing HD in comparison to that observed in the control group (29.92±12.94/ 44.82±27.69; p<0.001) (Table 1). In HD patients no statistically significant correlation was observed between serum zinc level and hemoglobin (Hb) (p>0.05) or hematocrit (Hct) levels (p>0.05). However, serum zinc and Hb levels were positively correlated in the control group (R^2=0.06) (Figure 1). When the patients were evaluated in terms of rhEPO doses, in the group who needed less than 8000IU rhEPO, mean Hb level was significantly higher and serum zinc level was also higher (Table 2), but this finding was not statistically significant. However, a positive but week correlation between Hb and serum zinc levels (R=0,003) was observed in this group (Figure 1). Patients undergoing HD treatment for more than 12 months had higher Hb, Hct and serum zinc levels than patients whose dialysis duration was less than 12 months (30.88±12.61 and 26.96±13.29, respectively) (Table 3).

Table 1. Demographic, clinical and biochemical features of patients and control subjects

	Patients (n=96)	Controls (n=34)	p
Age	55.98±13.95	53.15±10.56	0.290
Gender (F/M)	50/46	20/14	0.687
BMI(kg/m²)	26.88±5.22	28.78±5.07	0.320
WBC(X10³/uL)	6.69±2.47	7.184±1.87	0.297
Hb (g/dL)	10.51±1.25	13.92±1.64	<0.001
Hct (%)	33.49±3.77	41.50±4.60	<0.001
MCV (fL)	92.34±5.23	88.12±5.67	<0.001
PLT ($X10^3$/mL)	200.27±72.68	287.94±67.92	<0.001
Ferrum (mcg/dL)	46.64±17.61	78.50±34.98	0.006
Ferritin (ng/mL)	719.09±92.66	351.73±87.11	<0.001
TIBC (mcg/dL)	178.90±41.06	336.78±58.52	<0.001
Transferin saturation (%)	26.71±9.18	23.88±10.50	0.43
Vitamin B12 (pg/mL)	1497.81±2415.19	322.94±134.59	0.007
Folic acid (ng/mL)	48.14±79.75	14.84±35.69	0.066
ESR (mm/h)	34.70±13.91	16.64±12.54	<0.001
CRP (mg/dL)	1.67±1.46	1.22±2.44	0.565
Glucose (mg/dL)	136.99±87.47	103.64±68.62	0.051
Urea (mg/dL)	141.22±36.28	30.31±8.67	<0.001
Creatinin (mg/dL)	7.60±2.52	0.74±0.13	<0.001
Total protein (g/dL)	6.61±0.60	7.394 ± 0.50	<0.001
Calcium (mg/dL)	8.50±0.79	9.61 ± 0.56	<0.001
Phosphate (mg/dL)	5.27±1.41	3.38 ± 0.57	<0.001
Magnesium (mg/dL)	2.37±0.34	2.18± 0.17	0.021
Zinc (µg/dL)	29.92±12.94	44.82±27.69	<0.001

BMI=body mass index; WBC= white blood cell count; Hb= hemoglobin; Hct= hematocrit; MCV=mean corpuscular volume; PLT= platelet; TIBC= total iron binding capacity; ESR= erythrocyte sedimentation rate; CRP=C-reactive protein

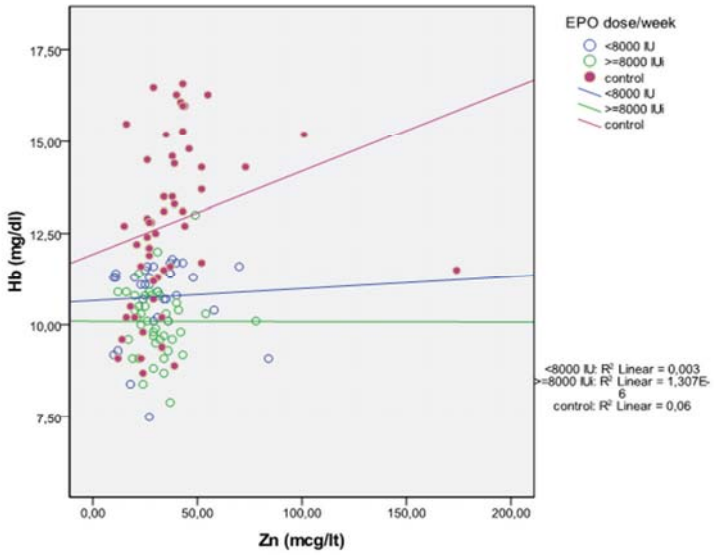

Fig. 1. Relationship between EPO dose, Hb and serum zinc level in control subjects and HD patients

Table 2. Differences between patients who needed higher and lower EPO dose

n	≥8000IU/week 42	<8000IU/week 33	p
Age	58,30±13,12	56,44±17,07	0,595
Dialysis duration (month)	46,69±29,96	41,69±33,64	0,409
Hb (g/dL)	10,09±0,94	10,77±1,03	<0,001
Hct (%)	32,64±2,80	33,93±3,41	0,078
MCV (fL)	91,16±5,63	94,08±4,80	0,020
Hb≤10g/dL	42/19(45%)	33/5(15%)	<0,001
Zn (µg/dL)	30,86±11,60	31,91±16,23	0,852
Ferrum (mcg/dL)	34.25±11.58	56.50±16.78	0.033
TIBC (mcg/dL)	151.00±40.14	195.33±37.74	0.201
Trasferrin saturation (%)	23.92±9.35	29.81±9.34	0.201
Ferritin (ng/mL)	831.95±512.02	639.07±268.49	0.522

Table 3. Hemodialysis duration shorter and longer than 12 months

	HD duration ≤12months	HD duration >12months	p
Zinc (µg/dL)	26.96±13.29	30.88±12.61	0.178
Hb (g/dL)	10.05±1.06	10.69±1.29	0.022
Hct (%)	32.25±3.51	34.01±3.81	0.038
rhEPO dose (IU/week)	7726±3240	8316±4668	0.533
T.protein (mg/dL)	6.69±0.63	6.56±0.59	0.358
Albumin (mg/dL)	3.68±0.57	3.78±0.46	0.422
Phosphate (mg/dL)	5.03±1.33	5.44±1.36	0.158

Discussion

Treating anemia in chronic kidney disease (CKD) is very important since cardiovascular mortality, morbidity and quality of life of these patients are very much related to better management of anemia. Human rhEPO has been used successfully for years in order to treat anemia in patients with CKD [10]. However, in some cases rhEPO failed to correct anemia for several reasons. The most important cause of inefficient response to rhEPO treatment is iron deficiency. The relationship between iron deficiency anemia and zinc deficiency was pointed out in 1961 by Prasad, *et al.* [11] in a child characterized by geophagia, iron deficiency anemia, hepatosplenomegaly, hypogonadism and dwarfism [12]. They claimed that consumption of a large amount of phythate rich grain could inhibit the absorption of both iron and zinc leading to iron deficiency anemia that does not respond to iron therapy unless if this is combined with zinc supplementation [12,13].

Although there is a variation in zinc levels depending on the geographic origin of patients, it has been reported that zinc level in the Turkish population is generally low. For example, zinc levels have been found to be 91.34 µg/dl in the south region of Turkey and 64.22 µg/dl in the east region of the country [14,15].

In this study, the mean serum zinc levels were 44.82± 27.69 µg/dl in the control group. The control subjects were from the northwest part of the country, which is well developped socio-economically. Thus, low zinc levels were not expected in this group of subjects. This result was considered to be related to their nutrition and/or the method used in this study for determination of serum zinc level.

It has been reported that trace elements, especially serum zinc levels were found to be lower in HD patients [15-17]. A marked decrease in serum zinc levels has been reported in CKD patients despite normal zinc levels in many tissues, which means that it may be due to re-distribution rather than total body deficiency [18]. Zinc deficiency in patients with CKD may be related to protein res-

tricted diet, malnutrition, hypoproteinemia, proteinuria, failure of tubular reabsorption, impairment in the formation of 1.25-dihydroxycholechalsiferol which plays a role in the intestinal zinc absorption. Furthermore, it has been discussed that HD patients are exposed to large amounts of highly purified dialysis solutions which do not contain zinc or other essential trace elements (manganese, copper, selenium). The removal of these elements with HD may lead to clinically relevant deficiency [19]. As a controversy to this theory, serum zinc level was lower in patients who were under HD treatment for less than 12 months and increased over time which was interpreted as zinc removal from these patients through the dialysis solution that is not significant in long term. Also, an efficient dialysis and rhEPO therapy corrects patient's nutritional status and mending zinc deficiency (Table 3).

Furthermore, it has been documented that red blood cell (RBC) survival is markedly reduced in patients with chronic renal failure [20,21]. There is extensive experimental evidence suggesting that uremia affects the mechanical properties of RBCs, such as deformability and fragility [20]. Zinc is an essential trace element, a structural and functional constituent of several enzymes which have important roles in the metabolism of nucleic acids, proteins and carbohydrates. It has been suggested that zinc also plays a significant role in the structure and function of biological membranes [21]. Therefore, it can be postulated that zinc deficiency in HD patients might contribute to some degree to the development of anemia in these patients.

In our study group, the serum zinc level of HD patients was 29.92±12.94 μg/dl, which was significantly lower than in the control group (Table 1, $p<0.05$). This finding is very similar to that reported in literature. It has been previously reported that there is a significant positive correlation between anemia parameters and serum zinc levels [22,23].

In this study HD patients were treated with a mean dose of rhEPO of 8077±33.73 U /week. We have observed that patients who used less than 8000 U/week dose of EPO had statistically significant higher Hb and serum zinc levels (Table 2). This finding was interpreted that the patient with a high serum zinc level would also have higher Hb levels. Thus, patients with higher serum zinc level would require less rhEPO doses to maintain the desired Hb level. Even though every patient received iron supplementation, those who needed EPO doses more than 8000U/week had a lower serum iron level as well as a lower serum zinc level. Consequently, serum zinc and iron levels were related to each other, and if there was a zinc deficiency, it would be difficult to correct iron parameters and to receive better results to EPO treatment [11-13].

There was a significant positive correlation between Hb and serum zinc level in the control group. In contrast, no correlation was detected among HD patients, which might be due to increasing Hb levels by using more rhEPO. Further studies should be planned to investigate the consequences of trace element deficiencies in HD patients and their effect on rhEPO and other treatments. Based on these observations, zinc deficiency may occur in HD patients more often due to their protein restricted nutrition and malnutrition because of uremic environment. When zinc deficiency develops, treatment of anemia becomes more difficult; patients need more amount of EPO to maintain the desired Hb level. In addition, treating the anemia complications increases the cost. When treating anemia in HD patients, it is important to take into consideration malnutrition and deficiency of trace elements, especially zinc along with many other factors.

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