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Research Article

Is the Iron Treatment Important for Lowering Erythrocyte Zinc in Non-Dialyzed Patients with Chronic Kidney Disease?

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Abstract

Introduction: Zinc (Zn) protoporphyrin (ZPP) presents in red blood cells (RBC) in small amounts, but increases in iron deficiency or lead poisoning. Formation of heme - a component of hemoglobin - finished with insertion of an iron into the molecule of protoporphyrin. In iron deficiency, or inhibition of iron insertion (lead poisoning), zinc replaces iron to form ZPP, which cannot bind to oxygen. Plasma zinc is lower in patients with chronic kidney diseases (CKD), but it's not yet clear whether it's a real deficit or is due to redistribution.

Aim: The aim of this study was to evaluate the effects of iron supplementation on zinc redistribution in the body tissues of non-dialyzed chronic kidney disease (CKD) patients.

Material and Methods: Zn and Fe status of 38 non-dialyzed patients with iron-deficit anemia (17 males; 21 females);mean GFR - 43.3 mL/min(32.5 to 57.3 mL/min), was evaluated before and after 5 i.v. injections of 100 mg iron each. The following parameters were tested: RBC and plasma Zn, ZPP, ferritin (F), transferrin saturation (TFS), and total iron (Fe).

Results: F and TFS increased from 86.5+/-61.2 ng/mL to 132.4+/-64.3 ng/mL and from 21.5+/-9.4% to 26.8+/-8.3% (P<0.05), respectively, after iron supplementation. Absolute iron deficiency (ferritin <100 g/L and TFS < 20%) was present in 44% of the patients and decreased to 25% after iron treatment. After supplementation, patients with low plasma zinc decreased from 49.5% to 30.8% (p N.S.). At start of the study 29 of the patients had high erythrocyte zinc. After iron treatment, erythrocyte zinc decreased significantly in 24 patients (p< 0.001), and only a weak lowering was observed in ZPP (p N.S.).

Conclusions: The study suggests that iron deficiency may participate in the inadequate distribution of zinc in patients with CKD and iron supplementation may decrease the abnormal elevated erythrocyte zinc levels.

Keywords: Chronic kidney disease (CKD); Chronic renal failure (CRF); Zinc (Zn); Zn protoporphyrin (ZPP); Iron (Fe); Ferritin (F) Transferin saturation (TFS)

1. Introduction

The morbidity and mortality risks of long-term CKD can be attributed to many factors including wasting, inflammation, oxidative stress, and impaired immune responsiveness [1, 2]. Patients in the early stages of CKD have some alterations essential trace minerals, predisposing them to such complications [2-4]. In particular, some of these patients have decreased serum zinc (Zn) concentrations [5, 6]. Zn has antioxidant and anti-inflammatory properties and regulates T- and B-lymphocyte function, making it vital for the maintenance of normal immune function and resistance to infection [7, 8]. HD patients have elevated levels of plasma copper (Cu) and a markedly increased Cu/Zn ratios [9, 10]. Cu also acts as an antioxidant and anti-inflammatory agent and is required for immune function [11]; accordingly, increased plasma levels of Cu and elevated Cu/Zn ratios are associated with nutritional abnormalities, oxidative stress, inflammation, and immune dysfunction [9, 10, 12]. The disruption of Zn and Cu levels has been suggested as a cause of clinical deterioration and adverse outcomes in HD patients. However, the mechanisms that mediate the homeostasis of Zn and Cu remain unknown. The most common symptoms of zinc deficiency are anorexia, growth retardation, hypogonadism, skin lesions, impaired taste, and decreased cell-mediated immunity [1, 2]. Plasma zinc is lower in patients with chronic kidney diseases (CKD), but it's not yet clear whether it's a real deficit or it's due to a redistribution in the cells, because erythrocyte zinc concentration in these patients is found frequently elevated [2-4].

Anemia in this population is predominantly due to decreased erythropoietin production and Fe deficit. In the non-dialytic stage of CKD, Fe stores are low because of a combination of anorexic reduction of Fe intake, low protein diet, lowered gastrointestinal absorption of Fe, use of phosphate binders, increased bleeding accidents, proteinuria etc. [5, 6]. The treatment of anemia includes Fe supplementation and erythropoietin stimulation, if anemia is more severe. Intake of i.v. Fe probably replaces Zn with Fe in ZPP, decreasing RBC content of Zn and removing Zn to the plasma [7, 8]. Our study was constructed to prove this hypothesis, and to evaluate the needs of Zn supplementation in CKD.

2. Material and Methods

Hematocrit, hemoglobin, ferritin, and TFS are currently used to assess Fe deficiency. One more parameter, proposed for valid marker is Zn protoporphyrin (ZPP), as the concentration of ZPP is proved inversely proportional to that of plasma Fe. ZPP increases in Fe deficiency, because zinc is incorporated into the protoporphyrin IX ring of

heme instead of iron, increasing ZPP, respectively Zn in the red blood cells [9, 10].

Fasting blood was collected in plastic Zn-free tubes with an anticoagulant in 38 non-dialyzed patients with iron-deficit anemia (17 males; 21 females); mean GFR - 43.3 mL/min (32.5 to 57.3 mL/min). Samples were centrifuged and kept frozen at -20°C until Zn level determination by AA Spectrophotometry (Hitachi Instruments Inc, San Jose, CA). Blood samples for hematological, and biochemical parameters were obtained in the same day by standard techniques. ZPP measurement was performed by an haematofluorometer; ferritin was measured by a chemiluminescence immunoassay method [9], TFS - calculated as: plasma Fe: total iron binding capacity (TIBC) × 100. Iron deficiency was considered absolute when plasma ferritin concentration was lower than 100 mcg/L and transferrin saturation was lower than 20% [10]. An i.v. injection of 100 mg iron (Iron(III)-Hydroxide Sucrose Complex 100 mg/5 ml) was given every 7 days, each patient was given 5 injections without any side effects. The hematological and biochemical parameters were obtained before the treatment and two weeks after the last injection. Statistical analysis were performed using SPSS, Windows (8.0, 1998, SPSSInc, Chicago, IL).

3. Results

The demographic, clinical and biochemical date of the patients are shown in Table 1. The effect of Fe supplementation on iron and Zn parameters are shown in Table 2.

Parameters	**Values**
Numbers	38
Gender (m/f)	17/21
Mean Age	54 (18 – 78)
GFR	43.3 (38.5 – 57.3) mL/min

Table 1: Demographic, clinical and biochemical parameters of the patients.

Parameter	**Before therapy**	**After therapy**	**p value**
Hb (g/dL)	11.3+/-1.2	11.6+/-1.3	N.S
sFe (ng/L)	9.9+/- 2.1	10.3+/-3.2	N.S
Ferritin (ng/mL)	86.5+/- 61.2	132.4+/-64.3	0.05
TFS (%)	21.5+/-9.4	26.8+/-8.3	0.05
Zn in RBC (mcg/gHb)	52.3+/-5.5	47.2+/-4.8	0.001
ZPP (mcmol/mol heme)	63.7+/-27.4	61.01+/-25.2	N.S

Table 2: Iron and Zinc status of the pts. Before and after Fe supplementation.

There was slight improvement in hemoglobin and plasma iron concentrations after iron supplementation. However, transferrin saturation and plasma ferritin increased significantly, associated with a decrease in total iron binding capacity. Before supplementation, transferrin saturation was below 20% in 44% of the patients, and this frequency decreased to 25% of the patients after the iron

supplementation ($p<0.01$). Plasma ferritin concentration increased in 73% of the patients after iron supplementation.

Absolute iron deficiency (transferrin saturation 20% and ferritin 100 ng/mL) was present in 45% of the patients and decreased to 24% of them after iron supplementation ($p<0.05$). After supplementation, patients with low plasma zinc decreased from 49.5% to 30.8% (p N.S.) At start of the study 29 of patients had high erythrocyte zinc. After iron treatment, erythrocyte zinc decreased significantly in 24 patients ($P<0.001$), but only a weak lowering was observed in ZPP (p N.S). Considering the data before iron supplementation, positive correlations between plasma zinc levels and transferrin saturation ($r=0.42$, $p<0.01$) and plasma zinc and plasma iron levels ($r=0.43$, $p<0.005$) were observed. A weak but significant inverse correlation was found between plasma iron and erythrocyte zinc levels ($r=0.26$, $p<0.05$) and between ferritin and ZPP ($r=0.28$, $p<0.01$). After Fe supplementation an inverse correlation was found between Zn in plasma and in RBC ($r=0.36$, $p<0.01$).

4. Discussion

Absolute Fe deficiency occurs in non-dialyzed patients, when chronic renal failure advances, and maybe Zn is transferred from plasma to RBC in these patients. Moreover, increased expression of intracellular metallothioneins following oxidative stress or up-regulation of Zn-importing proteins by pro-inflammatory cytokines in CKD can reduce the plasma Zn levels. Inflammatory process also increases urinary albumin excretion, and induces oxidation of albumin and hemoglobin [5, 6]. Improvements in nutritional status can attenuate inflammation and oxidative stress, which correlate with the decline of renal function [15]. In our study we tested if the Fe supplementation and the correction of Fe deficiency could decrease the elevated Zn in RBC, often found in CKD patients [2-4]. In Fe deficiency status, Zn instead of Fe is incorporated into the heme, leading to an increase in the concentration of ZPP in RBC. A number of studies have proved an inverse association between parameters of Fe status and ZPP [11-13]. The increase in ZPP in RBC in Fe-deficiency status could explain at least one of the reasons for the elevated Zn level in erythrocytes in CKD patients [9, 11].

In the present study, we found a high prevalence of Fe deficiency considering either TFS or ferritin concentration. Moreover, absolute iron deficiency was present in 44% of the patients. This condition was also observed by other investigators in non-dialyzed CKD patients [5, 6]. Principally the reasons of iron deficiency in this population are multiple, but low iron intake plays an important role [5, 14, 15]. The reduced iron intake may be a consequence of both low food intake because of anorexia and the low-protein diet, a phenomenon that is found also in other pathological statuses [16, 17]. After 5 injections of i.v. iron, an improvement in the majority of the iron status parameters such as ferritin and transferrin saturation was observed, but the correction of iron deficiency was not complete - iron parameters remained out of normal ranges in a significant number of the patients. Possibly a longer treatment period, higher dosages would have been necessary to completely correct iron deficiency. Before iron supplementation, the zinc status of our patients was very similar to what has been found by our group and other investigators [1, 4, 5, 8]. That means a normal to low concentration of zinc in plasma but high in the erythrocytes. The causes for this abnormal distribution are poorly understood. This condition makes the assessment of zinc status of these patients quite difficult because it is not possible to be sure whether this represents a true zinc deficiency. Aside from the expected changes in the iron parameters, the use of 300 mg i.v. iron also caused a

significant decrease in the erythrocyte zinc level, and the plasma zinc level was correlated inversely with the erythrocyte zinc level, in the population as a whole, and in a more relevant manner in those patients with absolute iron deficiency. This result suggests a possible shift between plasma and erythrocyte zinc levels. Our results have shown a significant effect of iron treatment on lowering Zn in RBC, but slight effectiveness on the decreasing ZPP concentration. Possibly, the incomplete correction of iron deficiency because of the short period of treatment could explain this result.

Conflicts of Interest

The authors declare that there is no conflict of interest, regarding the publication of this paper.

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