

Effect of Recommended Dietary Intake versus Higher Doses of Supplemental Zinc on Iron and Copper Deficiency Anemia Among Patients with Chronic Kidney Diseases, A Double-Blinded, Randomized Clinical Trial

Zahra Nazari-Taloki¹, Ebrahim Salehifar², Atieh Makhloogh³, Simin Dashti-Khavidaki^{1*}

¹Department of Clinical Pharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

²Department of Clinical Pharmacy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

³Department of Nephrology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

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Abstract

Background: Complex interplays happen in absorption and function of iron, zinc and copper. Both zinc deficiency and excess may lead to anemia. In Iran, commonly available supplements for chronic kidney disease (CKD) patients contain 25 mg-zinc (Zn). This study compared 25 mg versus 7.5 mg dose of zinc in anemia of CKD patients, the latter dose approximates to recommended dietary intake (RDI) of zinc.

Methods: In this double-blinded clinical trial, 51 non-dialysis CKD patients were randomized to continue previous formulation (25 mg-Zn group) or change to a new preparation (7.5 mg-Zn group) for three months. Blood counts and serum iron, zinc and copper status were compared between and within the groups.

Results: At the end of the study, serum copper and ceruloplasmin concentrations were significantly higher in 7.5 mg-Zn group compared with those in 25 mg-Zn arm (115.04± 23.05 vs. 102.48±14.98 µg/dL; P= 0.02 and 29.97±7.94 vs. 25.42±4.23 mg/dL; P= 0.01, respectively). Serum zinc levels did not differ between two groups (76.73±15.35 vs. 77.68±18.07 µg/dL for 7.5 mg-Zn and 25 mg-Zn groups, respectively; P= 0.84). After three months, patients in 7.5 mg-Zn group experienced increase in their Hb (11.11±1.17 vs. 10.72±1.03 g/dL; P= 0.04), HCT (35.28± 4.01 vs. 33.96± 3.74%; P= 0.03), MCV (86.30 (81.40-90.82) vs. 86.00 (80.35-88.77) fL; P= 0.01) and ferritin (202.60 (79.29-298.97) vs. 129.07 (42.25-225.87) ng/mL; P<0.001) compared to their baseline values.

Conclusion: Reducing zinc content to its RDI value in supplement for CKD patients led to increased serum copper and ceruloplasmin concentrations. Moreover, patients who switched to RDI zinc-containing formula experienced a significant rise in blood hemoglobin, hematocrit, mean corpuscular volume (MCV), and ferritin concentration.

Introduction

Anemia is common among patients suffering from chronic kidney diseases (CKD) with an estimated incidence of about 60% among non-dialysis CKD patients.¹ Anemia of CKD patients is multifactorial, among them abnormal iron (Fe) homeostasis, erythropoietin deficiency, and reduced red blood cell (RBC) life span are well-known causes.²

In addition to iron, zinc and copper are two other cations that play role in hematopoiesis. Zinc deficiency is also prevalent among CKD patients.³ There are complex interplays between iron, zinc, and copper in their absorption and function⁴ that are briefly presented below and in Figure 1.

Dietary iron may be in heme or non-heme form. Plant-

source, non-heme iron is usually in ferric form (trivalent Fe) that is reduced to ferrous form (divalent Fe) by duodenal cytochrome B (DCYTB) and picked up in the apical membrane of intestinal epithelial cells by divalent metal transporter 1 (DMT1). Ferrous iron is transferred to ferritin by poly C binding protein 2 (PCBP2) where it is oxidized to ferric iron. PCBP2 is also bound to DMTI and ferroportin (FPN), the latter exports ferrous iron from the basolateral membrane of the intestinal epithelial cells to the portal bloodstream. This exported divalent Fe is oxidized to trivalent Fe by hephaestin (a multicopper ferroxidase) and binds to transferrin and is released into various peripheral tissues (Figure 1). In addition to iron absorption, iron availability from its body stores is regulated by hepcidin.

*Corresponding Author: Simin Dashti-Khavidaki, E-mail: dashtis@sina.tums.ac.ir

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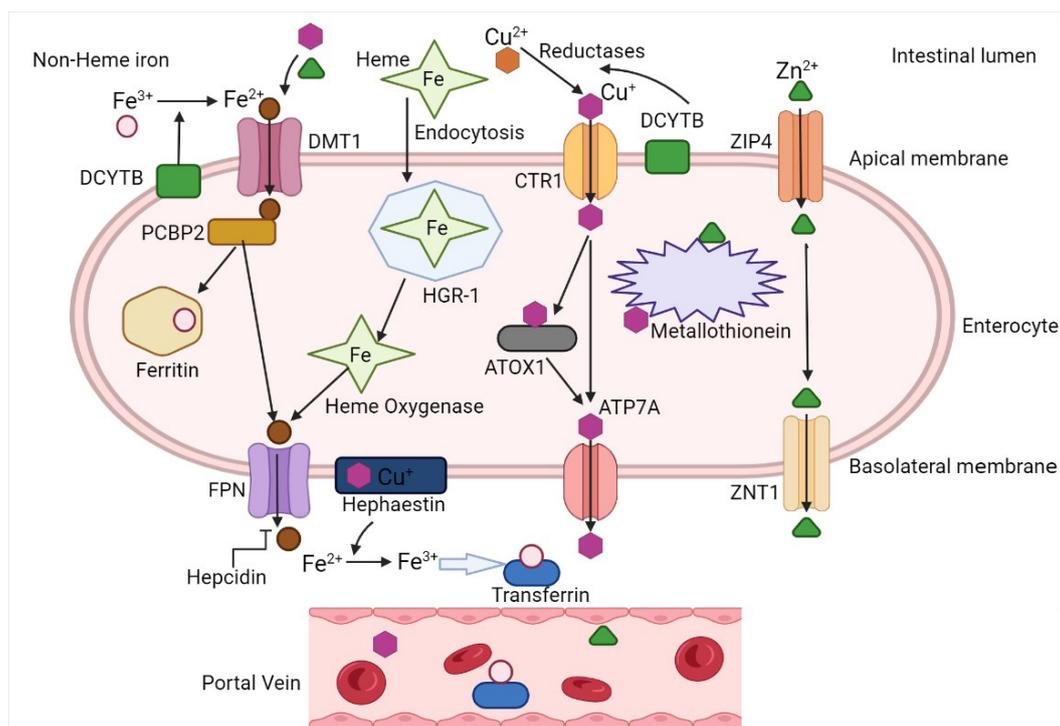


Figure 1. Absorption of iron, zinc, and copper. At apical membrane, ferric iron is reduced to ferrous by DCYTB and picked up by DMT1. Ferrous is transferred to ferritin by PCBP2 where it is oxidized to ferric. FPN exports ferrous from basolateral membrane to the portal bloodstream. Exported divalent Fe is oxidized to ferric by hephaestin, binds to transferrin and is released into various peripheral tissues. Hepcidin binds to ferroportin in enterocytes and causes ferroportin internalization and degradation followed by decreased iron export from enterocytes to the blood circulation. Heme iron is mainly harvested by HRG-1 into the cytosol via endocytosis and degraded by heme oxygenase to produce ferrous iron. The iron released from heme is transported to the portal vein in the same way as non-heme iron. Dietary or supplemental zinc is picked up by ZIP4 and transported to the basolateral membrane or bound with metallothionein. Zinc is excreted into the portal vein by ZNT1. Bivalent copper is reduced by several reductases and picked up by CTR1, transferred by ATOX1 and exited by ATP7A to the portal vein, or is bound to cytosolic metallothionein. Fe: iron; Zn: zinc; Cu: copper; DCYTB: duodenal cytochrome B; DMT1: divalent metal transporter 1; PCBP2: poly C binding protein 2; FPN: ferroportin; HRG-1: heme responsive gene 1; ZIP4: Zrt-, Irt-related protein 4; ZNT1: zinc transporter 1; CTR1: copper transporter 1; ATOX1: copper chaperone antioxidant 1; ATP7A: copper-transporting P-type ATPase.

Hepcidin binds to ferroportin in enterocytes, macrophages and hepatocytes and causes ferroportin internalization and degradation followed by decreased iron export from enterocytes and macrophages to blood circulation.^{4,5}

Dietary heme iron from animal protein sources is mainly harvested by heme responsive gene 1 (HRG-1) into the cytosol via the endocytosis pathway. After that, heme is degraded by heme oxygenase to produce ferrous iron. The iron released from heme is transported to the portal vein in the same way as non-heme iron (Figure 1).⁴ Apart from dietary sources, iron is also recycled from phagocytized and degraded erythrocytes by macrophages. FPN is highly expressed in macrophages and hepatocytes and plays crucial role in the storage of recycled iron as ferritin in the liver.⁴

Dietary or supplemental zinc is picked up by Zrt-, Irt-related protein 4 (ZIP4) and transported to the basolateral membrane or bound with metallothionein. Zinc is probably excreted into the portal vein by Zn transporter 1 (ZNT1) and released into peripheral tissues (Figure 1).⁴

Bivalent copper in the food or supplements is probably reduced by several reductases and picked up by the copper transporter 1 (CTR1) in the plasma membrane, transferred by copper chaperone antioxidant 1 (ATOX1) and exited

by a copper-transporting P-type ATPase (ATP7A) to the portal vein (Figure 1). In the liver, the copper is loaded to ceruloplasmin. This loading is mediated by another copper transporting ATPase (ATP7B). Excess cytosolic copper is bound to metallothionein to reduce copper toxicity.⁴

A review on interplays between iron, zinc and copper is important to understand the results of their interactions on anemia treatment or development. Data on the interaction between iron and zinc are contradictory. Zinc is transported from apical membrane of enterocytes by DMT1 that transports iron as well; however, the affinity of DMT1 to iron is much greater than to zinc (Figure 1). Therefore, zinc and iron competition for DMT1 at absorption phase may not be significant. Moreover, zinc regulates DMT1 and ferroportin expressions and functions and by this mechanism improves iron absorption.⁵ However, some authors reported that zinc in high doses reduces iron absorption.⁶ Experimental and human studies revealed that zinc deficiency can induce iron deficit by blocking the intestinal absorption of iron via reducing the expression of DMT1 and ferroportin and also inhibiting the transportation of iron from its storage tissues to the blood.⁷ Zinc also downregulates hepcidin production and by this mechanism regulates iron absorption and availability from

its storages. Matrix metalloproteinases 2, as a regulator of hepcidin function, is a zinc-dependent endopeptidase.^{5,8-9} Zinc deficiency can cause systemic inflammation; the latter is a main stimulator of hepcidin production in the liver and as a result suppresses iron absorption by enterocytes and iron availability from body stores.⁹ Moreover, zinc acts as a catalyst for enzymes such as alpha-aminolevulinic acid dehydratase that is necessary for heme production and in the structure of growth factor protein that regulates erythroid cell growth.¹⁰ Regarding the impact of iron on zinc homeostasis, iron may interfere with zinc absorption by competitive binding to the DMT1 protein or by reducing the expression of this protein which also involves in the absorption of zinc to some extent (Figure 1).¹¹ Studies have shown that iron supplementation with a dose of more than 25 mg can impair zinc absorption.¹²

Regarding the interplays between copper and iron, these two cations interfere with each other in gastrointestinal absorption, storage and utilization in the body. Both synergistic and antagonistic interactions may happen between copper and iron in the intestinal tract. Both metals are reduced by DCTYB before intestinal absorption that may be a site of interaction between them (Figure 1). ATP7A under-expression and subsequent disrupted intracellular copper homeostasis may reduce DCTYB expression.¹³ DMT1, an important iron transporter in the apical membrane of enterocytes, is also a copper transporter in some situations such as iron deficiency, suggesting that these two metals may compete for absorption into enterocytes. High iron intake may block copper transport via DMT1 and/or CTR1 leading to copper deficiency (Figure 1).^{4,13} On the other hand, copper promotes iron absorption from the enterocytes.¹³ Copper is a cofactor for many redox enzymes including those converts ferrous to ferric iron that is necessary for iron absorption, release, and hematopoiesis. Ferroportin action is also influenced by copper. Hephaestin that is required for dietary iron absorption and transferrin loading is also a multi-copper ferrioxidase (Figure 1).⁴ In addition, the expression of ATP7A that is necessary for copper export to portal vein is markedly upregulated by iron deficiency. After that, increased copper in erythrocytes and liver stabilizes hypoxia inducible factor activity leading to increased whole body and intestinal iron metabolism.¹³ Iron-copper interaction is not limited to the intestinal tract. Interplays between these two cations may also happen in the liver. Hypocupremia-induced reduction in ceruloplasmin biosynthesis leads to impaired hepatic iron release and causes hepatic iron accumulation. Copper is also needed for hemoglobin synthesis possibly by its role for iron importing into and utilization within mitochondria.¹³

Copper and zinc also interact with each other in different ways. Oral zinc can cause hypocupremia by competitive reduction of gastrointestinal absorption of copper. Oral zinc induces metallothionein in intestinal mucosal cells. Binding of copper and zinc to this protein inhibits their intestinal absorption (Figure 1). This protein tends to bind

to copper more than zinc, so high administration of zinc inhibits copper absorption. Although controversial, some investigators showed that people taking 50 mg zinc per day for several weeks to months experience copper deficiency.¹³

Taken together and based on the interplays among these three elements, it is thought that zinc can affect anemia in different ways. Zinc deficiency by decreasing iron availability and erythroid growth factor and zinc excess via interference with gastrointestinal absorption of iron (either directly or by reducing serum copper levels), and inducing erythropoietin-resistance can influence anemia.

A clinical study on healthy, adult Chilean females exhibited that daily supplementation of 20 mg zinc between meals for two months exert no impact on their iron absorption or iron status.¹⁴ While studies in children revealed that chronic supplementation by physiologic or higher doses of zinc decrease serum iron levels without significant changes in blood hemoglobin (Hb) concentration, mean corpuscular volume (MCV), ferritin or transferrin saturation that means no profound anemia following zinc supplementation and decline in serum iron concentration.^{15,16} KDOQI clinical practice guideline suggests receiving recommended dietary intake (RDI) of zinc in CKD patients and does not suggest routine zinc supplementation in these patients.¹⁷ RDI of zinc is 8 to 11 mg.¹⁸ In Iran, commonly available supplement formulations for CKD patients contain water soluble vitamins, vitamin E and usually 25 mg of elemental zinc in each tablet. To assess the impact of this high daily supplemental dose of zinc on anemia of CKD patients and the need for formulation revision by pharmaceutical companies this study compared 25 mg versus RDI dose of zinc in supplement formulations for CKD patients.

Methods

Patients and setting

This single-center, double-blinded, randomized clinical trial was conducted on non-dialysis CKD patients who were regularly visited at a nephrology clinic affiliated to Mazandaran University of Medical Sciences, Mazandaran, Iran, from September 2021 to the end of August 2022.

Adult, non-dialysis CKD stage 3–5 patients with anemia (*i.e.*, serum hemoglobin (Hb) concentrations of less than 13.0 and 12.0 g/dL in males and females, respectively) were eligible to be included in this research. As the use of CKD patients-specific supplement (Nephrotonic®, Zahravi Pharmaceutical Company, Tabriz, Iran) is prevalent among Iranian CKD patients and almost all visited patients were using this supplement at the time of screening and due to the limited number of supplement-naïve patients, those who were taking this supplement for at least 3 months were included in the study. Patients were requested not to make significant changes in their diet during the study period.

Patients with a history of hospitalization, infection, or blood product transfusion within last month, those taking anemia-inducing drugs (*e.g.*, linezolid, isoniazid, antiretroviral agents, non-steroidal anti-inflammatory

drugs, chemotherapeutic agents, hydroxyurea, azathioprine, sulfasalazine, trimethoprim-sulfamethoxazole or methotrexate), those taking corticosteroids or other immunosuppressive medications and patients with cancer and alcohol use were not included.

Patients who experienced acute infection, inflammatory diseases, surgery, or moved to dialysis treatment during the study period, and participants who did not wish to continue the study were excluded from the study.

Intervention

Patients were randomly assigned to receive either currently available CKD supplement formulation containing 25 mg elemental zinc (25 mg-Zn group) or the newly developed formulation containing 7.5 mg elemental zinc (7.5 mg-Zn group) using the block randomization at a proportion of 1:1. They took one supplement tablet daily for 3 months. Both supplement formulations were prepared by Zahravi Pharmaceutical Company, Tabriz, Iran and were identical in shape, color, odor, and packaging. In addition to zinc, both formulations contained 1.5 mg vitamin B1, 1.7 mg vitamin B2, 20 mg niacin amide, 10 mg vitamin B5, 10 mg vitamin B6, 6 mcg vitamin B12, 300 mcg biotin, 1 mg folic acid, 50 mg vitamin E, and 60 mg vitamin C. Above mentioned amounts of vitamins and 25 mg zinc in each tablet is a commonly available product in Iranian pharmacies and in real work clinical practice is administered to all Iranian CKD patients.

Data collection

All demographic and medical characteristics of the patients including gender, age, weight, height, medical and drug history at baseline and at the end of 3-month study were obtained by a questionnaire.

Venous blood samples were collected from all patients after overnight fasting at baseline and at the end of 3 months of supplementation. Two tubes were gathered from each participant, one for the instant measurement of hematological markers using an automated analyzer. The other one was centrifuged and the collected serum was stored at -40°C for measurement of other biomarkers. Complete blood counts (CBC) were measured with an automatic counter (Sysmex XN-2000 Hematology Analyzer, SYSMEX Corporation, Kobe). Serum concentrations of iron, ferritin, total iron binding capacity, zinc, copper and creatinine were quantified with an automatic counter (Mindray BS-800 Chemistry Analyzer, Mindray Corporation, UK). Serum ceruloplasmin level was measured with Roche Cobas Integra 400 plus Chemistry Analyzer.

Transferrin saturation (TSAT) was calculated using $\text{TSAT} = (\text{serum iron} \times 100) / \text{TIBC}$ equation. Erythropoietin resistance index (ERI) was computed as weekly dose of erythropoiesis-stimulating agent (ESA) (IU)/body weight (kg) divided by blood Hb content (g/dL).¹⁹ Absolute zinc deficiency was defined as a serum zinc concentration of less than $60\mu\text{g/dL}$.³

Patients received their supplements from one of the investigators of the study. Their compliance to taking the supplements was assessed by pill count. Patients were considered adherent to their medication if they took at least 80% of their supplement tablets during the study follow-up.

Sample size estimation

For type 1 and 2 errors of 5% and 20%, respectively, a sample size of 30 patients in each group was estimated to detect a 0.4 standard deviation (SD) change in serum Hb concentrations between baseline and endpoint values using following formula:

$$n = [z^2 \times p(1-p)] / \epsilon^2$$

Where z is the z score, ϵ is the margin of error, n is population size, and p is the population proportion (CKD prevalence in Iran that is reported to be 3.7%).²⁰

Ethics

The study was approved by the local ethics committee of Tehran University of Medical Sciences (IR.TUMS.TIPS.REC.1400.048). The trial was registered in the Iranian Registry of Clinical Trials (identification no. IRCT20100111003043N15). All patients signed informed consent form.

Statistical analysis

Data were analyzed using SPSS 26 statistical software. Normal distribution of continuous variables was evaluated by Kolmogorov-Smirnov test. Quantitative variables are presented as means \pm SD or medians (interquartile range (IQR)) according to their distribution. Categorical data are expressed as frequencies and percentages. Univariate end points between and within the two groups were compared using Pearson chi-square test or Fisher exact test for categorical variables and t-test or Mann-Whitney/Wilcoxon signed-rank test for continuous variables. P value of less than 0.05 was considered as statistically significant.

Results

From 853 screened CKD patients of the clinic, 60 patients who met the inclusion criteria were enrolled in the research. Of them, 9 patients were excluded from the trial due to the different causes (Figure 2). Hence, the per-protocol statistical analysis was performed on 25 participants in the 25 mg-Zn arm and 26 patients in the 7.5 mg-Zn group. All patients who were included in data analysis were found to be adherent to taking their supplement.

As shown in Table 1, no statistically significant differences were observed between the two groups in terms of demographic data and baseline clinical characteristics such as duration, severity and cause of CKD, and the time taking routine supplement for CKD patients containing 25 mg zinc before the study enrollment.

As summarized in Table 2, the two groups did not differ regarding baseline CBC, iron status, zinc, copper and

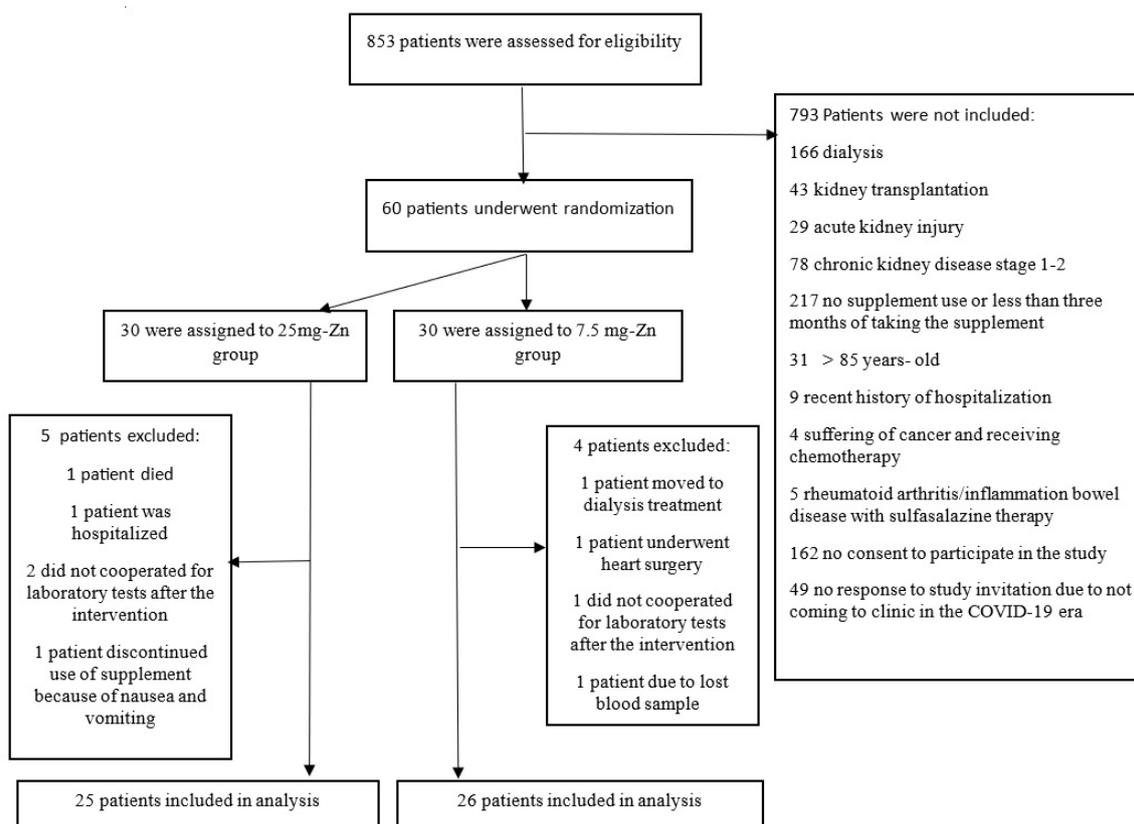


Figure 2. Patients' selection and randomization.

ceruloplasmin concentrations, weekly doses of iron and ESA, ERI and prevalence of zinc deficiency.

After the end of three-month study, mean serum copper and ceruloplasmin concentrations were significantly higher among patients in 7.5 mg-Zn group compared with those of patients in the 25 mg-Zn group. Serum zinc levels did not change significantly between the two groups despite remarkable difference in supplemental zinc doses of 25 mg and 7.5 mg between the two groups (Table 2).

Although blood counts and iron status including serum

iron, TIBC, and ferritin concentrations and TSAT did not significantly differ between the two groups at the end of the study, within group comparisons showed significant increase in blood Hb, HCT, MCV and serum ferritin concentration in patients in 7.5 mg-Zn group ($P=0.04$; $P=0.03$; $P=0.01$; $P<0.001$, respectively), while, no significant within group changes happened among patients in 25 mg-Zn group regarding these parameters from baseline to the end of the study. The weekly dose of elemental iron and ESA remained constant in both groups (Table 2).

Table 1. Baseline demographic and clinical characteristics of the patients in both groups.

Variable	25 mg-Zn group (25)	7.5 mg-Zn group (26)	P
Sex, female/male	18/7 (72/28)	12/14 (46.2/53.8)	0.06
Age (years)	67.16 ± 10.95	64.73 ± 10.15	0.41
Body weight (kg)	70.32 ± 13.75	76.54 ± 10.19	0.07
BMI (kg/m ²)	26.89 ± 4.32	28.69 ± 4.37	0.14
Duration of CKD (years)	6.36 ± 3.73	6.46 ± 4.45	0.90
Duration of taking supplements containing 25mg Zn before enrollment (months)	44.40 ± 24.19	35.58 ± 29.79	0.36
Cause of CKD			
DM	3 (12)	3 (11.5)	0.97
HTN	3 (12)	3 (11.5)	
DM & HTN	13 (52)	15 (57.7)	
Others	6 (24)	5 (19.2)	
SCr (mg/dL)	1.85 (1.70-2.77)	1.91 (1.70-3.21)	0.63
eGFR (mL/min/1.73m ²)	28.75 ± 9.77	29.04 ± 10.05	0.92

Data has been presented as N (%), mean ± SD or median (IQR) which is appropriate.

BMI: body mass index; CKD: chronic kidney disease; DM: diabetes mellitus; eGFR: estimated glomerular filtration rate using MDRD formula; HTN: hypertension; SCr: serum creatinine concentration.

Table 2. Complete blood count; iron, zinc and copper status; weekly ESA and iron doses of patients in both groups at the beginning and at the end of the study.

Variable	At the beginning of the study			At the end of the study		
	25 mg-Zn group (25)	7.5 mg-Zn group (26)	P	25 mg-Zn group (25)	7.5 mg-Zn group (26)	P
RBC ($\times 10^4/\mu\text{L}$)	4.19± 0.75	4.11± 0.61	0.68	4.18± 0.65	4.22 ± 0.69	0.83
Hb (g/dL)	10.78± 1.03	10.72 ± 1.03	0.83	10.99± 0.99	11.11± 1.17	0.68
HCT (%)	34.11± 3.76	33.96± 3.74	0.89	34.72± 2.83	35.28± 4.01	0.57
MCV (fL)	86.00 (81.35-88.00)	86.00 (80.35-88.77)	0.96	86.00 (83.15-88.70)	86.30 (81.40-90.82)	0.77
MCH (pg/cell)	27.20 (25.25-28.45)	27.45 (25.42-29.07)	0.79	27.30 (26.20-28.20)	27.45 (25.55-29.37)	0.61
MCHC (g/dL)	31.71± 1.07	32.19± 1.41	0.17	31.73± 1.02	31.88± 1.40	0.66
Fe ($\mu\text{g/dL}$)	63.96± 30.17	58.31± 24.64	0.47	59.56± 21.40	58.27± 28.30	0.85
TIBC ($\mu\text{g/dL}$)	316.00 (268.50-358.50)	323.00 (308.75-361.00)	0.21	310.00 (284.50-339.50)	302.50 (272.25-351.75)	0.80
TSAT (%)	19.24± 6.24	17.37± 6.92	0.32	19.44± 7.74	19.22± 10.89	0.93
Ferritin (ng/mL)	145.81 (88.42-295.55)	129.07 (42.25-225.87)	0.27	166.50 (83.98-306.16)	202.60 (79.29-298.97)	0.98
Zn ($\mu\text{g/dL}$)	78.84± 16.71	79.35± 15.86	0.91	77.68± 18.07	76.73± 15.35	0.84
Cu ($\mu\text{g/dL}$)	104.08± 16.81	98.00± 18.18	0.22	102.48± 14.98	115.04± 23.05	0.02
Ceruloplasmin (mg/dL)	27.22± 4.71	27.09± 6.40	0.94	25.42± 4.23	29.97± 7.94	0.01
ERI (IU.Kg ⁻¹ /g.dL ⁻¹)	5.19 (2.56-6.00)	4.13 (2.40-6.93)	0.76	5.03 (3.22-6.89)	4.40 (2.49-5.67)	0.36
Zn deficiency n (%)	2 (8)	2 (7.7)	0.97	3 (12)	1 (3.8)	0.29
Weekly dose of elemental iron (mg)	350 (0-385)	200 (0-350)	0.58	350 (0-420)	200 (0-350)	0.13
Weekly dose of ESA (IU)	4000 (2000-4000)	4000 (2000-5000)	0.83	4000 (3000-4000)	4000 (2000-4000)	0.91

Data has been presented as N (%), mean± SD or median (IQR1-IQR3) which is appropriate.

Cu: serum copper level; ERI: erythropoietin resistance index; ESA: erythropoiesis stimulating agent; Fe: serum iron level; HB: hemoglobin; HCT: hematocrit; IU: international unit; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; RBC: red blood cell; TIBC: total iron binding capacity; TSAT: transferrin saturation; Zn: serum zinc level.

Discussion

Iron, zinc and copper are three cations that play role in hematopoiesis and show complex interplays with each other in their absorption and function.⁴ Anemia including iron deficiency type is common among CKD patients.² While KDOQI guideline does not recommend routine zinc supplementation among CKD patients,¹⁷ some studies found that serum zinc concentrations are lower among non-dialysis and dialysis CKD patients compared with control groups, and zinc deficiency (defined as serum zinc level of less than 60 $\mu\text{g/dL}$) is as prevalent as approximately 30% to 50% among these patients.^{3,21-22} Several supplemental dosage forms containing different types and amounts of vitamins and elements are available worldwide for CKD patients. They differ in the presence and amounts of iron, zinc and copper. Commonly-used supplement preparations for CKD patients in Iran contain 25 mg of elemental zinc in each tablet while lack iron and copper. Iron is usually administered separately for these patients, whilst copper supplements are not generally administered for them. The current dietary zinc recommendation is 11 mg for men and 8 mg for women with an upper safety limit of 40 mg total zinc intake per day¹⁸, therefore, this study compared the

most commonly used supplement by Iranian CKD patients with a new product by the same pharmaceutical company. The two formulations were similar in all components except for zinc that was decreased from 25 mg in each tablet in the original form to 7.5 mg in each tablet in the new form. Results of this clinical trial showed that after three months of using these two forms of supplements, serum zinc concentrations and prevalence of zinc deficiency did not differ between the two groups despite significant decrease in daily intake of zinc from 25 to 7.5 mg. Serum copper and ceruloplasmin concentrations were significantly higher among patients who received supplement containing 7.5 mg zinc compared with those who continued supplements consisting 25 mg zinc in each tablet. Regarding serum iron storage and anemia parameters, although between groups analysis showed no statistically significant differences at the end of the 3-month study, within group analyses revealed significant increase in blood Hb content, HCT, MCV and serum ferritin concentration three months after decrease in daily supplemental zinc intake compared with their baseline values among patients in 7.5 mg-Zn group.

Kaneko *et al.*²² reported significant positive correlation between serum zinc and ferritin concentrations among

peritoneal dialysis CKD patients. In contrast, Maruyama *et al.*²³ found no correlation between serum zinc level and iron status including ferritin and TSAT among non-dialysis CKD individuals, while they reported that lower MCV was independently associated with higher serum zinc concentration. In accordance with Maruyama finding²³, in our study mean MCV was lower at baseline compared with the end of 3-month intervention in patients whose daily zinc dose decreased from 25 mg to 7.5 mg.

A review by Takahashi²⁴ stated that although serum zinc concentration is lower among hemodialysis patients and zinc supplementation decreases the need for erythropoiesis stimulating agent, judicious zinc supplementation is necessary in these patients because high zinc doses may induce copper deficiency and induce anemia in other ways.²⁴ A patient with end stage kidney disease taking zinc at a daily dose of 50 mg for several months has been reported that was diagnosed with zinc-induced copper deficiency and erythropoietin-resistant anemia.²⁵ A hemodialysis patient who received high dose of 50 mg zinc three times a day for about six months experienced hypocupremia-induced pancytopenia.²⁶ Another investigation on 65 hemodialysis CKD patients showed that oral taking of 50 mg zinc acetate twice daily could increase the risk of copper deficiency. Those authors proposed the upper limit of 109.7 µg/dL for zinc concentration to prevent copper deficiency.²⁷ A randomized, controlled clinical trial on maintenance hemodialysis Japanese patients which compared zinc supplementation of 34 mg per day versus no supplementation over 1 year found that zinc supplementation resulted in a decrease in serum copper level. Although blood Hb, serum iron and TSAT did not change by zinc supplementation in that study, serum ferritin level decreased significantly. Furthermore, that study reported higher ERI among Zn-supplemented patients.¹⁹ Accordingly, the results of our study showed that reducing zinc supplementation dose to approximately RDI level led to increased serum copper level and increased hemoglobin content and ferritin concentration, while no changes in TSAT was observed. In contrast to Kobayashi *et al.*¹⁹ finding, no decrease in ERI and required doses of iron and ESA were observed in our patients after reduction of zinc supplementation.

A study by Sato *et al.*²⁸ on 21 hemodialysis patients with zinc deficiency who were administered zinc acetate hydrate 50 mg (reduced to 25 mg if needed) for 6 months, found that serum zinc concentration significantly increased but blood Hb did not change significantly. Moreover, they reported that the ESA dose significantly decreased from 0–12,000 IU/week (5630 ± 3351 IU/week) to 0–9000 IU/week (4428 ± 2779 ; $p = 0.04$); and ERI significantly decreased from 0.0–18.2 (8.1 ± 5.1) to 0.0–16.0 (6.3 ± 4.3 ; $p = 0.04$).²⁸ Our results were not compatible with those findings.

Conclusion

This study concluded that changing the components of supplement formulations for CKD patients to contain

lower amount of zinc that is near to its recommended dietary intake resulted in increased serum copper and ceruloplasmin concentrations of the non-dialysis CKD patients. Besides, significant rise in blood hemoglobin content, hematocrit, MCV and serum ferritin level were observed among patients who were switched to this lower zinc-containing product. This study might inspire pharmaceutical companies to avoid high zinc content in supplement preparations for CKD patients who take these products chronically for months to years. In addition to avoiding zinc excess-associated hypocupremia and anemia, this decrease in zinc content also reduces the product cost. To the best of our knowledge, this study is the only available clinical trial comparing RDI versus higher doses of supplemental zinc in anemia of non-dialysis CKD patients that are a growing patient population worldwide and found no need for high doses of zinc in CKD-specific supplement products to maintain normal serum zinc concentrations and even high zinc doses may impair anemia control in these patients. However, this study suffers by small sample size. The coincidence of this study with sixth COVID-19 surge in Iran resulted in poor cooperation of the patients for completing the study. This limitation did not affect significantly on power of the study in detecting differences in copper ($\beta=96\%$) concentrations between the two groups of the study, while resulted in insufficient power of the study for detecting differences in blood hemoglobin concentration that was the primary outcome of the study (calculated power of 40%).

Ethical Issues

The study was approved by the local ethics committee of Tehran University of Medical Sciences (IR.TUMS.TIPS.REC.1400.048). The trial was registered in the Iranian Registry of Clinical Trials (identification no. IRCT20100111003043N15). All patients signed informed consent form.

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Data Availability

Data would be available upon request from corresponding author.

Author Contributions

Zahra Nazari-Taloki: Investigation, Formal Analysis, Writing - Original Draft. Ebrahim Salehifar: Methodology, Formal Analysis, Writing - Review & Editing. Atieh Makhloogh: Resources, Writing - Review & Editing. Simin Dashti-Khavidaki: Conceptualization, Methodology, Resources, Funding Acquisition, Writing - Original Draft.

Conflict of Interest

The authors report no conflicts of interest.

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