Role of Zinc and Copper in Erythropoiesis in Patients on Hemodialysis

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Plasma zinc concentrations are decreased in patients on hemodialysis; zinc supplementation increases hemoglobin levels and reduces erythropoietin-stimulating agent treatments. However, inappropriate zinc supplementation causes copper deficiency. This review discusses the roles of zinc and copper throughout erythropoiesis; it also describes erythropoiesis-stimulating nutritional therapy that avoids copper deficiency, while providing safe zinc supplementation. In early erythropoiesis, erythropoietin regulates erythrocyte precursor proliferation and survival via zinc finger transcription factors. Mature blood cell formation and functional activation are regulated by zinc-mediated hormones, vitamins, and growth peptides. Zinc antagonizes the uptake of divalent cations (e.g., iron and copper) in erythrocyte precursors. Copper is required for iron transfer from cells to blood, ensuring dietary iron absorption and systemic iron distribution. In patients with copper deficiency, copper supplementation is initially performed, followed by zinc supplementation to manage hypozincemia. Serum zinc and copper measurements are needed at 2- to 3-month intervals during zinc supplementation to prevent copper deficiency.

Keywords: copper; erythropoiesis; erythrocyte; ferroportin; GATA-1; hemodialysis; zinc

Introduction and Purpose

THE NUMBER OF patients requiring hemodialysis treatment because of end-stage renal disease is increasing,[1] and plasma zinc concentrations are decreased in patients on hemodialysis.[2] Experimental studies have shown that zinc supplementation stimulates erythropoiesis (i.e., red blood cell [RBC] formation) in rats.[3] Consistent with this finding, zinc supplementation during hemodialysis has been shown to increase hemoglobin levels in patients with end-stage renal disease[4,5]; zinc supplementation reportedly reduces the amount of erythropoietin-stimulating agents used in such patients.[5] The mechanism underlying the association between zinc and hemoglobin production involves the incorporation of zinc into GATA-1 (a zinc finger protein transcription factor that regulates various genes involved in RBC synthesis[6]) (Fig. 1A). Therefore, when zinc is deficient, the GATA-1 transcription factor activity in hematopoiesis decreases, leading to reduced hemoglobin production.[7,8] Furthermore, zinc interacts with signaling cascades such as the growth hormone (GH) to insulin-like growth factor-1 (IGF-1) pathway to influence RBC synthesis[9] (Fig. 1B). Accordingly, zinc is regarded as an important erythroid differentiation factor. However, the precise roles of zinc and copper (a closely related trace mineral) throughout erythropoiesis, as well as the prevention of copper deficiency during zinc supplementation for hypozincemia, have not yet been fully investigated.

Although a considerable portion of the literature regarding erythropoiesis has focused on iron and erythropoietin, this review article discusses the roles of zinc, copper, and other nutrients (e.g., vitamins, folic acid, and selenium) during erythropoiesis (Fig. 1C). This article also describes safe zinc supplementation that does not cause copper deficiency in patients on hemodialysis.

Zinc and Copper Concentrations in Patients on Hemodialysis

Meta-analysis of trace elements in whole blood, serum, or plasma[10] has shown that zinc levels in patients on chronic maintenance hemodialysis appear to be lower than those in the general population, while copper levels tend to be higher (see Table 1 for detailed comparison of healthy, hemodialysis, and end-stage renal disease populations). However, there are some limitations of using whole blood, serum, or plasma to assess overall zinc status; for example, serum zinc levels exhibit circadian dynamics[19] and strong associations with serum protein levels.[20] Furthermore, zinc levels are related to inflammatory status.[21] The types of zinc deficiency in patients on hemodialysis include insufficient intake, impaired absorption, and excess loss. The causes of insufficient zinc intake are related to aging, polypharmacy, and inadequate dietary protein and energy

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Financial Disclosure: The authors declare that they have no relevant financial interests.

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1051-2276

https://doi.org/10.1053/j.jrn.2022.02.007

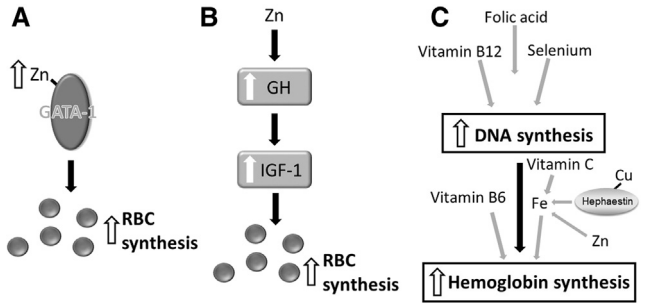

Figure 1. Schema summarizing the effects of minerals on (A) GATA-1 signaling and RBC synthesis, (B) GH and IGF-1 during RBC synthesis, and (C) DNA synthesis and hemoglobin synthesis (in combination with vitamins). Cu, copper; DNA, deoxyribonucleic acid; Fe, iron; GH, growth hormone; IGF-1, insulin-like growth factor-1; RBC, red blood cell; Zn, zinc.

(undernutrition).[22-24] The causes of impaired absorption include ingestion of absorption inhibitors (e.g., oxalic acid, phytic acid, polyphosphoric acid, dietary fiber, calcium, magnesium, and iron) and combined use of zinc chelating agents.[25]

A cause of excess loss of zinc is removal by hemodialysis. A total of 80% of zinc in the blood is found in RBCs, most of which is carbonic anhydrase (zinc-requiring enzyme).[26] The remaining zinc (approximately 20%) is present in serum, 60%-80% of which is bound to albumin and is not removed by hemodialysis.[27] Other zinc in serum is bound to alpha-2 macroglobulin,[28] but a small amount of this is bound to amino acids, such as cysteine, which are removed by hemodialysis.

Zinc levels are positively correlated with levels of albumin, hematocrit, and prealbumin.[29] Zinc levels are thought to be an indicator of nutritional status because zinc levels are high when people are well nourished.[30] There are varying reports of copper concentrations in patients on hemodialysis, such as moderate copper deficiency, high copper levels, or copper toxicity.[31] Cases of copper deficiency caused by zinc supplementation for hypozincemia have been reported.[32,33] Therefore, the balance between zinc and copper is clinically important during zinc supplementation.

Roles of Zinc, Copper, and Other Factors in the Process of Red Blood Cell Hematopoiesis

Iron and erythropoietin are widely regarded as important components in hematopoiesis. To more fully understand the need for careful management of zinc and copper levels in patients on hemodialysis, it is important to consider the roles of zinc, copper, and other factors in hematopoiesis.

Erythropoiesis

In the early stage of erythropoiesis, erythropoietin and IGF-1 act together to initiate hematopoiesis[34] (Fig. 2, top right). IGF-1 is a hormone that is mainly produced in the liver by the action of GH. GH and IGF-1 are also related to zinc (Fig. 2, top right)[9]; the liver stores a small amount of systemic zinc,[35] along with considerable amounts of copper and iron.[36,37] Zinc supplementation for pregnant women with anemia results in an increase in IGF-1 levels,[38] which are correlated with an increase in hemoglobin levels

Table 1. Serum Concentrations of Copper and Zinc in Healthy Controls, Chronic Kidney Disease Patients, and Hemodialysis Patients

		Copper (μg/dL)	Zinc (μg/dL)	References
Healthy	Free serum Cu	10-15		Brewer 2012[11]
	Ceruloplasmin	18-35 (mg/dL)		
	Ceruloplasmin Cu*	54-105		
	Total serum Cu†	64-120		
		105.26 ± 19.63	79.44 ± 10.28	Attar 2019[12]
		102 ± 5	92 ± 3	Zima et al. 1998[13]
		78.5 ± 13.0		Toro-Román et al. 2021[14]
		108.0 ± 8.5	93.3 ± 12.1	Batista et al. 2006[16]
	Male		84.9 ± 0.8	Hennigar et al. 2018[15]
	Female		80.6 ± 0.6	
CKD	DM	130.02 ± 36.7	84.1 ± 17.7	Batista et al. 2006[16]
	NDM	109.7 ± 26.2	81.2 ± 19.8	
	eGFR 30-59	109 ± 20	76 ± 16	Shih et al. 2012[17]
	eGFR 15-29	124 ± 17	69 ± 13	
Hemodialysis		102 ± 6	69 ± 3	Zima et al. 1998[13]
			57.4 ± 2.4	Rashidi et al. 2009[18]

CKD, chronic kidney disease; Cu, copper; DM, patients with type 2 diabetes; eGFR, estimated glomerular filtration rate (mL/min/1.73 m^2); NDM, nondiabetic patients.

Data are expressed as mean ± standard deviation.

*Each 1 mg/dL of ceruloplasmin contributes 3 μg/dL of serum Cu.

†Total serum Cu = Free serum Cu + ceruloplasmin Cu.

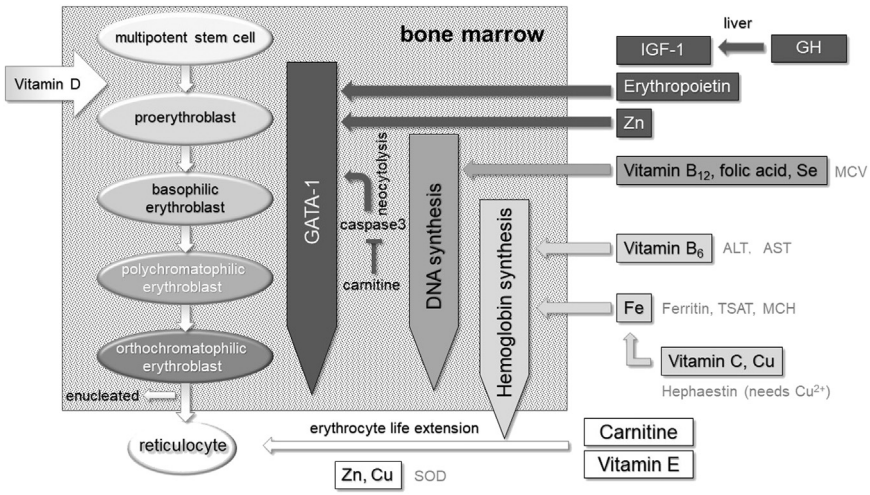

Figure 2. Schema showing zinc, copper, and other factors involved in erythropoiesis.[28] In the early stage of erythropoiesis (top right, dark gray boxes/arrows), Epo and IGF-1 act together to initiate hematopoiesis. IGF-1 is produced by the action of GH, but GH and IGF-1 are also related to zinc. When multipotent stem cells change to proerythroblasts (top left), Epo binds to the Epo receptor, and GATA-1, which is an erythroid differentiation factor, is released (middle, dark gray boxes/arrows). Proerythroblasts change to basophilic erythroblasts, polychromatophilic erythroblasts, and orthochromatophilic erythroblasts (left). There is a zinc finger in the structure of GATA-1. When Epo is stopped or the dose is reduced, caspase 3 is released and it mediates degradation of GATA-1 to cause neocytolysis, but carnitine inhibits caspase 3 and prevents apoptosis (middle, dark gray boxes/arrows). Vitamin D acts as an adjuvant in the early stages of erythroid differentiation (top left). Vitamin D receptor also has a zinc finger as a structural factor. When GATA-1 is released and erythroid differentiation begins, DNA synthesis begins, which requires vitamin B_{12} and folic acid (middle right, lighter gray arrows/boxes). Hemoglobin synthesis also begins; vitamin B_6 is required at the beginning of this synthesis (bottom right, lightest gray arrows/boxes). Zinc antagonizes divalent cations, such as iron and copper, during the absorption process at DMT1. Copper is required for ferroportin. After reticulocytes are formed, copper/zinc superoxide dismutase acts as a scavenger, and carnitine and vitamin E prolong the lifespan of erythrocytes (bottom, white boxes/arrows). ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cu, copper; DNA, deoxyribonucleic acid; Epo, erythropoietin; Fe, iron; GH, growth hormone; IGF-1, insulin-like growth factor-1; MCV, mean corpuscular volume; SOD, superoxide dismutase; Zn, zinc.

and the RBC count. This suggests that IGF-1 plays a role in increasing RBCs.

Several minerals, namely magnesium, selenium, and zinc, are important determinants of IGF-1 bioactivity.[39] Plasma GH levels are increased during intravenous zinc administration, and IGF-1 and IGF-binding protein 3 levels are increased after oral zinc supplementation.[40] Therefore, GH and IGF-1 levels decrease when zinc is deficient and increase with zinc supplementation (Fig. 1B). Zinc supplementation in older men significantly increases hematocrit levels, RBCs, and testosterone levels.[41] Therefore, an increase in RBCs because of zinc supplementation may occur via androgen metabolism. Erythropoietin then regulates erythrocyte production by delaying deoxyribonucleic acid (DNA) degradation and preventing apoptosis (programmed cell death)[42] of erythroid progenitor cells.[43]

Erythropoietin and Release of GATA-1

When multipotent stem cells change to proerythroblasts (Fig. 2, top left), erythropoietin binds to the erythropoietin receptor, leading to the release of GATA-1 (Fig. 2, middle; Fig. 3A). GATA-1 and friend of GATA-1 (FOG-1; a GATA-1 cofactor) form a complex and interact to regulate transcriptional activity for erythroid differentiation.[44] Proerythroblasts then change to basophilic erythroblasts, polychromatophilic erythroblasts, and orthochromatophilic erythroblasts (Fig. 2, left).[45] GATA-1 and FOG-1 require zinc for bridging by the zinc finger protein in the structure[46,47] (Fig. 3B). Therefore, in the event of zinc deficiency, GATA-1 cannot be produced. Additionally, when erythropoietin stimulation is interrupted or the erythropoietin dose is reduced, caspase 3 (a key component of apoptosis[48]) is released, disrupting GATA-1 and causing neocytolysis (selective destruction of erythrocytes formed during hypoxia to increase the oxygen transport capacity of blood[49]) (Fig. 2, middle). However, the synergistic actions of zinc and carnitine (a branched nonessential amino acid with diverse functions[50]) suppress the cleavage of caspase 3 (Fig. 2, middle)[51]; this suppression prevents apoptosis in RBCs,[52] thus inhibiting neocytolysis.[53] In contrast, combined depletion of zinc and copper promotes apoptosis.[54]

Vitamin D Acts as an Adjuvant

Vitamin D acts as an adjuvant in the early stages of erythroid cell differentiation (Fig. 2, top left).[55] Zinc is also involved in this interaction because the vitamin D receptor contains the zinc finger, which requires zinc as a

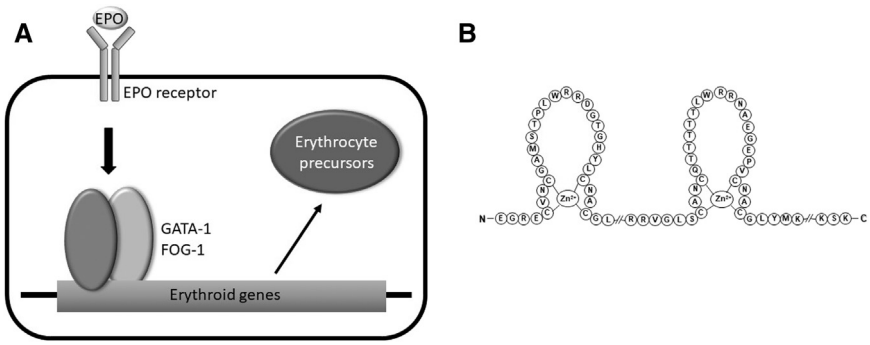

Figure 3. GATA-1: signaling and structure. (A) Schema showing the relationship between erythropoietin and GATA-1 during early erythropoiesis. (B) Zinc finger bridging in the GATA-1 structure.[36]

structural factor.[56] Vitamin D receptor expression is reduced with zinc deficiency.[57] Zinc supplementation increases vitamin D receptor levels and increases the effect of vitamin D, resulting in a significant decrease in parathyroid hormone.[58]

Initiation of DNA Synthesis

When erythroid differentiation is begun by GATA-1, DNA synthesis begins, which requires folic acid (as a source of tetrahydrofolate, a cofactor for nucleic acid synthesis[59]) and vitamin B_{12} (to ensure sufficient levels of active tetrahydrofolate[59]), as well as selenium (to regulate expression levels of DNA repair enzymes[60]) (Fig. 1C; Fig. 2, middle right).[61] If vitamin B_{12} and folic acid are insufficient, mean corpuscular volume (i.e., mean RBC volume) becomes high because cells are unable to divide effectively.[62] Selenium deficiency also induces macrocytosis (i.e., greatly increased RBC volume, indicative of various systemic illnesses).[63] When supplementing vitamin B_{12}, it is poorly absorbed by oral intake.[64] In patients on hemodialysis, administering vitamin B_{12} by intravenous injection from the hemodialysis circuit is possible. Although B vitamins are classified as water-soluble, vitamin B_{12} accumulates in the liver after intravenous injection.[65] Therefore, the maximum effect of vitamin B_{12} supplementation in patients with end-stage renal disease is achieved by injection rather than oral intake.[66] Folic acid supplementation is effective by starting with 5 mg/day and gradually reducing it to 15 mg/week or less.[67]

Initiation of Hemoglobin Synthesis

After DNA synthesis begins, hemoglobin synthesis also begins, but vitamin B_6 is required in this early stage (Fig. 2, bottom right). Before hemoglobin is made from globin and heme, iron binds to the center of protoporphyrin, which is the skeleton of heme, and heme is formed. Protoporphyrin is made from succinyl-coenzyme A and glycine in the mitochondria. Vitamin B_6 is required as a coenzyme of aminolevulinic acid synthase during its synthesis.[68] Because water-soluble vitamins are removed by hemodialysis, alanine aminotransferase levels may be low in patients on hemodialysis owing to vitamin B_6 deficiency.[69]

Iron is required in the subsequent hematopoietic stage, but zinc antagonizes divalent cations, such as iron and copper, in the absorption process at divalent metal transporter 1 (DMT1; a transporter of ferrous iron and some divalent metal ions across plasma and endosomal membranes[70]) (Fig. 4, left).[71] The total amount of ionic species affects absorption of zinc, and a total dose of iron exceeding 25 mg may produce a measurable effect on zinc absorption.[72]

Because patients on hemodialysis often take iron-containing phosphorus adsorbents continuously for a long period, regular serum zinc concentration tests may be necessary. Additionally, when iron is pumped from ferroportin (an exporter protein that transfers iron from cells to blood,[73] ensuring iron distribution between tissues and iron absorption from dietary intake), copper is required for hephaestin (a transmembrane copper-dependent ferroxidase that transports iron from intestinal enterocytes into blood,[74] ensuring iron absorption from dietary intake) (Fig. 4, left).[75] Therefore, care must be taken because copper deficiency anemia may occur if excessive zinc supplementation is administered.[76]

Erythrocyte Maturation After Reticulocyte Formation

After reticulocyte formation, copper/zinc superoxide dismutase (an enzymatic antioxidant that neutralizes superoxide[77]) acts as a scavenger, and carnitine and vitamin E prolong the lifespan of erythrocytes (Fig. 2, bottom).[53] Copper/zinc superoxide dismutase reduces oxidative stress caused by iron metabolism,[78] but its effect is thought to be reduced in hypozincemia and copper deficiency. Therefore, zinc and copper play important roles in the hematopoietic process of erythrocytes.

Safe Zinc Supplementation Without Copper Deficiency

With regard to the balance of zinc, copper, and iron, zinc and iron have a competitive antagonistic effect during absorption. Oral iron supplementation inhibits zinc

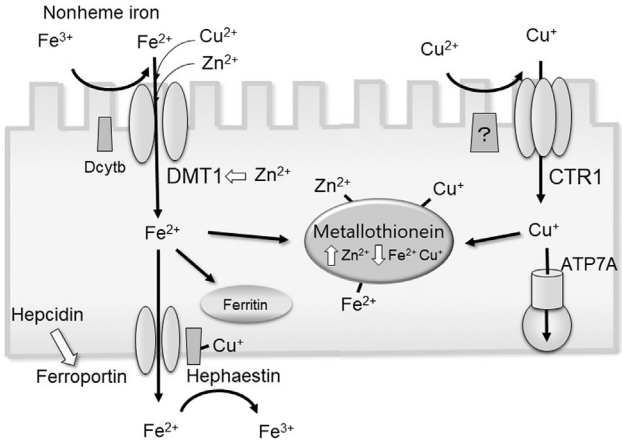

Figure 4. Absorption mechanism of iron, zinc, and copper in the digestive tract.[55,60] In addition to iron (Fe^{2+}), DMT1 transports divalent cations, such as zinc (Zn^{2+}) and copper (Cu^{2+}); it provides a common pathway for gastrointestinal absorption of these metal ions (left). When iron is pumped from ferroportin, copper is required for hephaestin. Metallothionein has the ability to bind to heavy metals via the thiol group of cysteine residues; it typically binds to zinc, other heavy metals can compete for binding with metallothionein. Metallothionein is removed by hemodialysis; as zinc levels increase, iron and copper levels decrease. DMT1, divalent metal transporter 1; Dcytb, duodenal cytochrome B; CTR1, copper transporter 1; ATP7A, ATPase copper transporting alpha.

absorption, but not copper.[79] Notably, zinc antagonizes divalent cations, such as iron and copper, in the process of absorption by DMT1 (Fig. 4, left). In addition to iron, DMT1 in the gastrointestinal mucosa transports divalent cations, such as zinc and copper, and is a common pathway for absorption in the gastrointestinal tract.[71] When iron is absorbed and ferritin increases, hepcidin binds to ferroportin, and ferroportin is internalized and degraded, leading to decreased export of cellular iron.[80]

When a patient on hemodialysis is unable to maintain a target hemoglobin value (hemoglobin level of 100-120 g/L), despite erythropoiesis-stimulating agent treatment, if the patient has a serum ferritin level of <100 ng/mL (<224.7 pmol/L) and a transferrin saturation of <20%, iron supplement therapy is recommended.[81] Mean corpuscular hemoglobin is controlled to 30-35 pg, and if it is <30 pg, iron is replenished. The RBC count is controlled in the range of 300-350 (10^{12}/L), and if it is <300 (10^{12}/L), erythropoietin is considered to be insufficient and use of an erythropoiesis-stimulating agent is increased.[82]

After iron has been pumped from ferroportin, hephaestin requires copper (Fig. 4, left). Iron is converted from the divalent to trivalent form by hephaestin and is carried by transferrin. Copper is a divalent copper ion, which has a pathway that competes with iron and zinc (mentioned above); it also has a pathway that involves reduction from the divalent to monovalent form, followed by absorption in the duodenum.[83]

In the relationship between zinc and copper, absorption of copper is inhibited by induced metallothionein (a metal-binding protein in the Golgi apparatus[84]).[85] Metallothionein has the ability to bind to heavy metals via the thiol group of cysteine residues, which make up almost 30% of its constituents. Metallothionein plays a protective role against metal toxicity and oxidative stress, and is involved in the regulation of zinc and copper.[86] Metallothionein is capable of binding 7 divalent (Zn^{2+}) and up to 12 monovalent (Cu^{+}) metal ions in vivo.[87] Metallothionein normally binds to zinc, but cadmium, mercury, and copper replace zinc because they have stronger binding strength. The molecular weight of metallothionein is approximately 6,500 Da and it is removed by hemodialysis.[88] Therefore, as zinc levels increase, iron and copper levels decrease (Fig. 4, middle). When replenishing zinc, careful monitoring is needed regarding zinc concentrations, as well as iron and copper concentrations.

The most common clinical symptoms associated with copper deficiency include anemia, leukopenia, and bone lesions (scorbutic-like bone changes and occipital horn). When copper deficiency is suspected, the serum ceruloplasmin (Cp) level and the serum copper level should be measured. Except in newborns and small infants, serum Cp levels may be interpreted as follows: 10-20 mg/dL, mild decrease; 5-10 mg/dL, moderate decrease; and 5 mg/dL or less, marked decrease. And serum copper levels may be interpreted as follows: 60-80 μg/dL, mild decrease; 40-60 μg/dL, moderate decrease; 40 μg/dL or less, marked decrease.[89] Copper deficiency may occur when serum zinc concentrations exceed 250 μg/dL (38.23 μmol/L) after long-term administration of zinc.[90] However, in patients on hemodialysis, copper deficiency occurs when serum zinc concentrations exceed 120 μg/dL (18.35 μmol/L). Therefore, reducing or discontinuing zinc supplementation is desirable if serum zinc concentrations exceed 120 μg/dL (18.35 μmol/L).[91] Furthermore, in cases where serum zinc levels are ≥100 μg/dL (15.29 μmol/L) after zinc supplementation, most patients have copper deficiency after 3 months when zinc supplementation is continued (Fig. 5). Nishime reports a stricter recommendation that in the hemodialysis group, the upper limit of zinc to avoid copper deficiency is 109.7 μg/dL and the upper limit of safety is 78.3 μg/dL. He also recommends checking both zinc and copper values monthly after prescribing zinc acetate.[92]

In the case of copper deficiency, recovery of serum copper concentrations is observed only by withdrawal of zinc acetate hydrate tablets. Therefore, when zinc supplementation is performed, serum concentrations of both zinc and copper should be measured at least every 3 months.

If copper deficiency is observed, it should be treated with oral or intravenous copper replacement in the form of

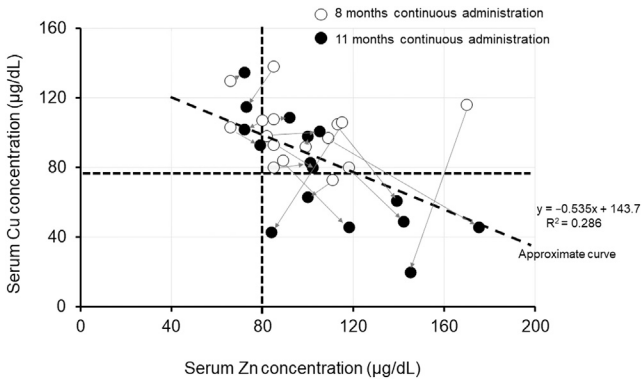

Figure 5. Scatter plot of serum zinc and copper concentrations after continuous administration of zinc acetate hydrate 50 mg/day.[67] Results of a retrospective study on serum zinc and copper concentrations in 36 outpatients on hemodialysis in the morning. The patients had serum zinc concentrations of <80 μg/dL (12.23 μmol/L) and were supplemented with zinc acetate 50 mg/day (17 men, 19 women, mean age: 69.6 ± 9.6 years). The approximate curve of the entire scatter plot intersects the lower limit of the normal serum copper concentration of 78 μg/dL (12.27 μmol/L) and serum zinc concentration of approximately 120 μg/dL (18.35 μmol/L).

copper gluconate, copper sulfate, or copper chloride.[93] A total of 1.5-3 mg/day of copper is usually orally administered as copper sulfate. Because patients on hemodialysis with copper deficiency are often malnourished,[94] intradialytic parenteral nutrition may be utilized to provide additional protein and energy.[95] However, simultaneous administration of a trace element preparation is necessary to prevent copper deficiency.[96] The use of copper-containing dietary supplements is also an option. Foods high in copper levels include liver (12,400 μg per 3-ounce serving of beef liver), cocoa (938 μg per 2-ounce serving of unsweetened baking chocolate), potatoes (675 μg per medium potato), mushrooms (650 μg per half-cup serving of shiitake mushrooms), and nuts (629 μg per 1-ounce serving)[94,97]; because these foods are also sources of phosphorus and potassium,[98,99] their consumption should be carefully considered to ensure safety in patients on hemodialysis. Many types of seafood (e.g., squid, octopus, shrimp, crab, and shellfish) are also rich in copper, since they use hemocyanin[100] (a copper-containing respiratory pigment in mollusks and arthropods[101]) to carry oxygen instead of hemoglobin. When oral zinc supplementation is used, dietary guidance should focus on copper rather than zinc.

Conclusions

In patients on hemodialysis, serum zinc levels are often low, and proper zinc supplementation increases hemoglobin levels, leading to a reduction in erythropoiesis-stimulating agent prescriptions. It is important to understandhow zinc and copper act in the process of RBC hematopoiesis; it is also important to supplement zinc and copper when necessary. Because copper deficiency occurs when zinc is inappropriately supplemented, serum levels of both zinc and copper should be measured every few months. Before administering zinc, it is important to ensure that no copper deficiency is present. In patients with copper deficiency, copper supplementation should initially be performed, followed by treatment for zinc deficiency.

Practical Application

In patients on hemodialysis, serum levels of zinc and copper should be measured at intervals of ≤3 months. Careful monitoring is needed to prevent copper deficiency in patients receiving oral zinc supplementation. The use of intravenous zinc preparations can help to avoid zinc-induced copper deficiency.

Author contributions

A.T. was involved in conceptualization, design, original draft preparation, review, and editing of the manuscript. A.T. takes responsibility that this review has been reported honestly, accurately, and transparently, and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Acknowledgments

The author thanks Ellen Knapp, PhD, and Ryan Chastain-Gross, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

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