Copper and Copper/Zinc Ratio in Cystic Fibrosis Patients

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Cystic fibrosis (CF) patients require a stable and sufficient supply of micronutrients. Since copper is an essential micronutrient for human development, studies are carried out to investigate the serum copper levels, serum copper/zinc (Cu/Zn) ratios, and their relationship with nutritional indicators in a group of CF patients. There was no significant correlation between the serum copper concentrations and respiratory and pancreatic function, respiratory colonization, and the results of the abdominal ultrasound.

Keywords: hypocupremia ; hypercupremia ; inflammatory response ; risk of zinc deficiency

1. Introduction

Cystic fibrosis (CF), also called mucoviscidosis, is recognized as an important genetic disease worldwide [1]. It is an autosomal recessive disorder that commonly affects white people with an annual incidence of approximately 1 in 3500 live births [2]. This multisystem disorder is characterized by genetic mutations in the CF transmembrane conductance regulator (*CFTR*) gene on chromosome 7, which encodes a protein that is essential for the regulation of transmembrane chloride reabsorption [3]. Mutations in *CFTR* result in channelopathy, with impaired sodium and chloride conductance obstructing the mucosa of the exocrine glands [4], affecting a variety of organs, including the lungs, pancreas, intestine, and hepatobiliary tract [5]. More importantly, CF is characterized by a progressive lung infection and exocrine pancreatic dysfunction due to the production of altered sweat and increased mucus production in the lungs and digestive system [6]. Lung disease is the most closely associated cause of morbidity and mortality in these patients [7]. However, pancreatic disease presents the highest penetrance regarding the severity and consequences of CF, as the pancreas is one of the first organs to be affected by this disease [8].

In CF, there is a strong association between nutritional status and lung function, and therefore life expectancy [9]. With increasing longevity, the burden and prevalence of comorbidities increase, which includes CF-related diabetes (CFRD), CF-related liver disease (CFLD), and CF-related kidney and bone disease, along with the increased chance for obesity and overweight, which were all reported in CF patients [5][10][11]. CF is closely related to a poor nutritional status, which is linked directly to factors associated with the genetic mutation underlying this disease [12]. In addition to a decreased nutrient intake, especially during periods of acute illness [13], the risk of nutritional deficiencies in CF patients is likely to be due to several coexisting factors, such as the malabsorption of fat, protein, energy, and micronutrients that are secondary to pancreatic insufficiency, the alteration in bile salts, the increase in energy needs due to the deterioration in lung function, chronic inflammation, and not only microbial colonization but also recurrent lung infection [14]. Although the prevalence of fat-soluble vitamin deficiencies (vitamins A, D, E, and K) and the need for their supplementation in CF is well known [13], other micronutrient deficiencies, such as minerals and trace elements, are not well established, especially during acute exacerbations [15].

Copper is an enigmatic ion that has an important role in biological systems [16]. It is a transition metal that exists in two forms: Cu^{+} is the reduced cuprous form and Cu^{2+} is the oxidized cupric form of copper [17][18]. This important dietary ion fulfills essential structural functions in enzymes [16], such as cytochrome oxidase, superoxide dismutase, monoamine oxidase, and lysyl oxidase [19]. Furthermore, it is considered a critical cofactor for a group of cellular transporters, namely, the cuproenzymes [17]. Organ meats, nuts, seeds, chocolate, and shellfish are rich sources of copper [18]. However, due to the ability of copper to alternate between two oxidation states, and since free copper is toxic, most of the cellular copper is tightly bound [16]. Copper is essential for the proper functioning of the human body, as it mainly intervenes in metabolic processes, such as the synthesis of hemoglobin, the function of neurotransmitters, the oxidation of iron, cellular respiration, amidation of antioxidant peptides, and the formation of pigments and connective tissue [17]. Copper is

necessary for growth, defense mechanisms, bone mineralization, the maturation of red and white blood cells, iron transport, cholesterol metabolism, myocardial contractility, glucose metabolism, and brain development [20].

Even though its deficiency is more commonly an acquired condition that is induced by the imbalance between need and dietary copper supply [21], it has been reported in subjects with malabsorption of copper due to malabsorption syndromes, such as celiac disease, tropical and nontropical sprue, CF, and short-bowel syndrome [22]; resulting from intestinal resection (gastric surgery, including gastric bypass or gastrectomy) [23]; the excessive use of copper chelators, antacids, zinc supplement overuse, parenteral overdosing, and denture cream ingestion with zinc; chronic parenteral nutrition without proper copper supplementation and prolonged jejunal enteral feeding; a diet low in copper; other unknown causes [24]. Furthermore, copper deficiency can occur in premature infants who are fed formulas with inadequate copper content, newborns with chronic diarrhea or malnutrition, and patients undergoing prolonged dialysis or who have suffered severe burns [25]. Since copper is involved in many bodily functions, its deficiency can cause a wide range of symptoms [26] that occur in stages of increasing severity (marginal, moderate, and severe clinical deficiencies) [27]. Anemia, neutropenia, and bone abnormalities are the most frequent clinical manifestations of copper deficiency [21].

2. Copper and Copper/Zinc Ratio

Cystic fibrosis is a multisystem disorder involving the pulmonary, gastrointestinal, endocrine, musculoskeletal, and the male genitourinary systems, as well as the sinuses [4]. PI is one of the main factors of CF morbidity. More than 85% of CF patients show evidence of malabsorption from exocrine PI [28], leading to fat malabsorption, predisposing them to a severe deficiency of fat-soluble vitamins (A, D, E, and K) and trace elements, such as calcium, magnesium, iron, copper, and zinc [29]. Although the information on serum copper levels in patients with CF is scanty [4], the mucus (sputum) of patients with CF reveals that there are traces of metals, mostly iron and copper, but also zinc [6], and in separate in vitro studies, these metals have been shown to induce *Pseudomonas aeruginosa* resistance to carbapenem antibiotics [30]. Additionally, deficiencies of copper can result in iron deficiency anemia [31], osteoporosis and joint problems [32], and increased susceptibility to infection that is secondary to poor immune function [33].

Milne et al. reported that serum copper changes according to age and sex [34]. Regarding sex, although females had a higher serum copper (116 µg/dL) than males (109 µg/dL), this difference was not significant. Similarly, other studies showed that women had significantly higher serum copper levels ($p < 0.05$) than men [35]. Although mean serum copper was higher in children (125 µg/dL) than adults (114 µg/dL) and adolescents (96 µg/dL), these differences were not significant. However, it was observed that the serum copper decreased slightly with increasing age in a similar way to those reported by Lin et al. [36]. Moreover, although Best et al. found a moderate copper deficiency in CF patients [37], in this group of patients, there was only one 15-year-old male teenager (6%) with hypocupremia (69 µg/dL). This result contrasts with 44% of CF children with copper deficiency reported by Yadav et al. in twenty-seven north Indian children with CF [38].

As far as nutritional status was concerned, 29% of CF patients displayed malnutrition (four adolescents and one adult), and patients with PI had a higher BMI than patients with PS ($p = 0.020$). Interestingly, mean serum copper was significantly lower in undernourished CF patients (90 µg/dL) than eutrophic patients (122.7 µg/dL, $p = 0.004$). Not one patient had stunted growth, but there were two overweight patients (an 8- and a 13-year-old) and obesity (a 2- and a 25-year-old) according to the waist-to-height index. This fact is interesting because it has been shown in a meta-analysis that a higher level of serum copper could be associated with the risk of obesity in children and adults [39]. Furthermore, in this series, linear regression analysis showed that there was a direct association between serum copper levels and BMI ($R^2 = 0.236$, $p = 0.048$). This result is not surprising because, in a large-scale sample of 2233 15–65-year-old subjects, a strong positive correlation was found between serum copper and BMI ($R = 0.85$, $p < 0.001$) [40].

CF-related bone disease has increased with life expectancy [5][10][11]. Copper is a micronutrient present in almost every cell in the human body. Approximately 50% of the copper content is stored in bones and muscles (approximately 25% in skeletal muscle), 15% in the skin and bone marrow, 8 to 15% in the liver, and 8% in the brain [41]. In addition, it plays an important role in the synthesis of collagen in the bones and connective tissue [42]. According to Turk, a bone mineral density examination in CF patients should be performed at the age of 8–10 y [5]. Surprisingly, the BCS (0.3 ± 0.9) Z-score was normal and no patient with CF had a low BCS. That is, bone densitometry measured using ultrasound was normal and no patient with CF was at risk of osteoporosis [43]. However, linear regression analysis showed that BCS had a positive correlation with serum copper. In contrast, Chase et al. showed that 44% of children with CF, particularly adolescent girls, have bone demineralization [44]. Among the main risk factors for bone loss in CF are poor nutritional status, vitamin D and K deficiencies, calcium, hypogonadism, glucocorticoid use, physical inactivity, *CFTR* dysfunction,

and exacerbations of lung infections. To a lesser extent, deficiencies of copper, phosphorus, magnesium, zinc, essential fatty acids and proteins, and an excess of vitamin A may have etiological roles [45].

The mean EE was adequate according to WHO's recommendation (p = 0.074). The mean diet was high in protein with adequate carbs, fiber, and EI. The diet was adequate except for the low iodine intake. Serum copper did not correlate with zinc, calcium, magnesium, and iron intakes. Nevertheless, CF patients with RI had more zinc intake (112.5% DRI) than RS (81.5% DRI, p = 0.015). Surprisingly, only vitamin C intake had a negative association with serum copper, and all three CF patients with hypo- and hypercupremia had low vitamin C intake (χ^2 = 0.046). Various dietary factors, such as carbohydrates, iron, zinc, certain amino acids and proteins, molybdenum, and vitamin C, can have adverse effects on the bioavailability of ingested copper [27]. In experimental animals, supplementation with vitamin C can induce a copper deficiency, but it is not clear whether this also occurs in humans [46]. Nevertheless, two studies in healthy men showed that the activity of ceruloplasmin oxidase may be impaired by relatively high doses of supplementary vitamin C [47]. Likewise, vitamin C inhibits copper absorption, binds or chelates copper, and facilitates its removal [48].

The usual pathophysiological features of copper deficiency include anemia, leukopenia, and neutropenia [20]. Copper plays a role in the production of hemoglobin, myelin, melanin, and the normal functioning of the thyroid gland [49]. Furthermore, despite normal serum iron levels, copper deficiency affects the production of hemoglobin because copper is required for the use of iron in bone marrow [50]. In this series, it was found that the 6-year-old girl with RI, PS, and mesenteric adenopathy and was colonized by *Pseudomonas aeruginosa* had hypercupremia, iron deficiency, and slightly high CRP; the 9-year-old girl with RS and PI and was colonized by *Hemophilus influenzae* had hypercupremia; the 15-year-old male with PI and RS and was colonized by *Aspergillus fumigatus* had hypocupremia, prealbumin deficiency, and lymphopenia (1610 cell/mm^3).

Copper may be important for immune system function, where its deficiency is frequently associated with an increased risk of infection and disturbances in copper homeostasis alter immune system function in rodents [25]. Copper may be necessary for the destruction of bacteria by macrophages and copper deficiency can disrupt factors of the cellular and humoral immune system [51]. However, in this series, there were no significant differences in serum copper by bacterial colonization. In contrast, according to Yadav (2014), serum copper was lower (57 µg/dL) in cases with exacerbation of the disease compared to levels in stable cases (p = 0.03) [38]. Likewise, in this series, although 17.6% of the patients were colonized by *Pseudomonas aeruginosa* and *Candida spp.*, and 23.5% by *Staphylococcus aureus*, their lung function was no worse than that of those without such colonization [52]. Songchitsomboon et al. observed a significant increase in serum copper levels in patients with infectious diseases [53]. However, Lee et al. reported that serum copper levels increased significantly several months after recovery from an acute pulmonary exacerbation in CF patients [54].

Copper/Zinc Ratio

Copper and zinc deficiencies are common and underdiagnosed health risks [55]. Both micronutrients are required for cellular metabolism and antioxidant defense systems [56]. Acute infections alter metabolism, while deficiencies increase the risks of infection. While acute infections cause an increase in serum copper in the context of an acute phase response [57], they cause a decrease in serum zinc due to its redistribution in the liver and other tissues [55]. Physiological conditions, such as age and sex, as well as malabsorption, inflammatory condition, and genetics, significantly influence the concentrations of both trace elements [58]. In this series, the median serum zinc (86 µg/dL) was in the normal range of 70 to 120 µg/dL [52]. Although 23% of patients had inadequate zinc intake and 17% serum zinc deficiency, none of the patients with deficient intake had hypozincemia. This situation of deficient zinc intake without hypozincemia alerts to a state of a marginal deficiency of around 41%. In addition, serum zinc was associated with BMI and W/H Z-score and zinc intake was associated with EI and weight-for-age Z-score [52]. Nevertheless, no patient with abnormal serum copper had hypozincemia. Only the teenager with hypocupremia had high calcium and iron intake but low magnesium and zinc intake. Although the serum copper and zinc levels did not correlate with each other and there was no association of their serum levels with the age of the patients, linear regression analysis showed that serum copper had a significant association with serum zinc when adjusted according to age.

One of the most common trace metal imbalances is elevated copper and depressed zinc [58][59]. The optimal plasma or serum ratio between these two elements is 0.70–1.00 [60], and the normal Cu/Zn ratio in children and adults is close to 1:1 [58][59]. In this series, the mean Cu/Zn ratio was high (1.32) with a range from 0.73 to 2.00, and 94% of CF patients had a Cu/Zn ratio > 1.00. The highest Cu/Zn ratio of 2.00 was in the 6-year-old girl with hypercupremia (158 µg/dL) and normal serum zinc (80 µg/dL). A pattern of high copper and low zinc is characteristic of an inflammatory condition [61], and a Cu/Zn ratio greater than 2 means there is a severe bacterial infection [62]. This situation might indicate that an inflammatory status was prevalent in most CF patients in this series. Conversely, the mean Zn/Cu was 0.75 ± 1.9 and ranged from 0.5 to 1.38. A tissue Zn/Cu ratio < 4 is often associated with increased susceptibility to bacterial and viral

infections [48]. In addition, the Cu/Zn ratio had a direct and significant correlation with protein, monosaturated lipids, and niacin intake, as well as triglycerides and gamma-glutamyl transpeptidase, and a negative association with polyunsaturated lipids intake and monocytes. In contrast, the Zn/Cu ratio presented almost the same associations but in an inverse relationship as the Cu/Zn ratio.

According to Osredkar et al., the Cu/Zn ratio is clinically more important than the concentration of either trace metal [49]. It has been reported that the Cu/Zn ratio is a good indicator of various diseases [63] and was proved to be a better predictor of disease severity and/or mortality than copper levels [64]. When high levels of copper and low levels of zinc coexist, they can contribute to diseases such as schizophrenia, hypertension, autism, fatigue, muscle and joint pain, headaches, infantile hyperactivity, depression, insomnia, senility, and premenstrual syndrome [58]. The Cu/Zn ratio has also been related to childhood neurological disorders [59] and assaultive individuals [65]. Additionally, the Cu/Zn ratio is an indicator of the nutritional status of zinc in patients [62]. Zinc deficiency should be highly suspected in individuals with high serum Cu/Zn ratios. Previous studies revealed the validity of the Cu/Zn ratio for the severity of nutritional status, inflammation, oxidative stress, immune dysfunction, and infection associated with zinc deficiency [66]. This fact agrees with the results published previously, where 41% of the cases would have an elevated risk of zinc deficiency [52].

There are four highlights. First, the median serum copper was normal (113 µg/dL) and the prevalence of abnormal serum copper levels was low (6% of CF patients had hypocupremia and 12% had hypercupremia). Second, this demonstrated that the serum copper level had a significant association with several of the nutritional parameters studied (body mass index and bone conduction speed, vitamin C intake, serum zinc, complements C3 and C4, and lymphocytes NK CD16+56). Third, the mean Cu/Zn ratio was high (1.32) and 94% of CF patients in this series had a high Cu/Zn ratio > 1.00, and only one patient had a high Cu/Zn ratio of 2. These correspond with a high inflammatory response and severe bacterial infection, respectively. Finally, there was a high risk of marginal zinc deficiency (41%), and although no patient with abnormal serum copper had hypozincemia, serum copper significantly correlated with serum zinc.

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