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Serum Zinc and Copper Levels in Malnourished Pre-School Age Children in Jos, North Central Nigeria

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Abstract: Serum zinc and copper were determined in thirty (30) malnourished pre-school-age children (age, 0-60 months) and thirty (30) age-and sex-matched apparently healthy well nourished controls to evaluate the effect of protein-energy malnutrition on serum zinc and copper. Mean serum zinc and copper were significantly reduced ($p<0.05$) in malnourished than in well-nourished children. While serum total protein was significantly lower ($p<0.05$) in malnourished than the controls, and comparable ($p>0.05$) among kwashiorkor and marasmus, serum albumin was significantly lower ($p<0.05$) in kwashiorkor than in marasmus. Mean haemoglobin concentration was significantly ($p<0.05$) lower in malnourished than in the controls while total white blood cell count (TWBC) did not differ significantly ($P>0.05$). This study shows that malnourished children have deficient serum zinc and copper with anaemia and leucopenia. For effective management of protein-energy malnutrition, zinc and copper supplementation should be part of treatment regimen, however, in order to prevent zinc and copper deficiency and its health implications in pre-school age children, food fortification should be promoted.

Key words: Zinc, copper, deficiency, malnutrition, pre-school age

Introduction

Reports on global nutrition showed that the scope of malnutrition is still unacceptably high and efforts at improving it in most region of the world has been a challenge for decades (ACC/SCN, 2000; Kwena *et al*., 2003; De-Onis *et al*., 2004). Defined as the cellular imbalance between supply of nutrients and energy and the body's demand for them to ensure growth, maintenance and specific functions (WHO, 2000), malnutrition is recognized globally as the most important risk factor for illness and death, contributing to more than half of deaths in children worldwide (Grigsby and Shashidhar, 2006). About 300,000 deaths occur per year as a result of direct consequence of malnutrition and about half of all deaths in young children has indirectly been attributed to malnutrition (Nemer *et al*., 2001; Müller *et al*., 2003; Black *et al*., 2003).

In 2000, an estimated 182 million of pre-school children or a third of children less than five years old in developing countries were stunted, reflecting long-term cumulative inadequacies of health and nutrition (WHO, 1995; De-Onis, 2000). In children PEM is defined by measurements that fall below 2 standard deviations under normal weight-for-age (underweight), height-for-age (stunting) and weight-for-height (wasting). It has been shown that of all children under the age of five years in developing countries, about 31% are underweight, 38% have stunted growth and 9% show wasting (Brabin and Coulter, 2003). In Nigeria the prevalence of stunting ranges from 34.9% in the north (Zoakah *et al*., 2000) to about 60.8% in the south (Adelekan *et al*., 1997).

In addition to deficiencies of energy and proteins, children with protein-energy malnutrition (PEM) have been found to be deficient in micronutrients (Bryan *et al*., 2004; Malouf and Grimley, 2003; Muller and Krawinkel, 2005; Singla *et al*., 1996; Thakur *et al*., 2004). Malnutrition, particularly that related to micronutrients (vitamins, trace minerals, essential amino acids, polyunsaturated fatty acids), is certainly one of the most easily preventable causes of death and disability (Pelletier *et al*., 1995). Causes of malnutrition include, inadequate intake as result of insufficient or inappropriate supply of food, early ceassation of breast-feeding, cultural and religious believes, poor sanitation, increased armed conflicts and chronic diseases (Brabin and Coulter, 2003; FAO., 2004; De Waal and Whiteside, 2003; Salama *et al*., 2004; Young *et al*., 2004). Adverse effects of malnutrition include physical and developmental manifestations such as poor weight gain (Doherty *et al*., 1998; Ninh *et al*., 1996), impaired immunologic factors (Ambrus and Ambrus, 2004), irritability, apathy, delayed to permanent cognitive deficit (Grantham-Mc-Gregor and Ani, 1999; Malouf and Grimley, 2003) depending on the severity and duration of nutritional compromise and the age at which malnutrition occur. The physiological and biochemical functions of some micronutrients have been described

in health and diseases (Baylin *et al.*, 2005; Villamor *et al.*, 2005; Jaimton *et al.*, 2003).

Zinc, iron and vitamin A play critical role in immune competence (Beard, 2001; Shankar and Prasad, 1998) and linear growth (Bahl *et al.*, 1997; Brown *et al.*, 1998). Copper is known to be involved in many metalloproteins and cuproenzymes for example superoxide desmutase (SOD), an important antioxidant enzyme used in combating the effects of reactive oxygen species. Few studies on serum zinc and copper in malnourished children were encountered (Singla *et al.*, 1996; Thakur *et al.*, 2004) both were done in Asian countries and showed a decrease in mean serum zinc and copper. Although micronutrients deficiencies are common in developing countries (Muller *et al.*, 2001), the levels of these micronutrients are rarely determined in malnourished children. The aim of this study is to determine the serum levels of zinc and copper in malnourished children of pre-school age in Jos, the Plateau state capital, North central Nigeria.

Materials and Methods

Subjects and controls: Altogether sixty, (60) children aged 0-60 months were involved in the study. They include thirty, (30) with protein-energy malnutrition (based on clinical findings and anthropometric parameters) who were admitted into the paediatric ward of Jos University Teaching Hospital (JUTH). The children were classified into kwashiorkor (n=15), marasmic (n=9) and marasmic kwashiorkor (n=6) in accordance with Wellcome classification (Welcome, 1970).

Thirty (30) age-and sex-matched well nourished and apparently healthy children attending Paediatric out-patient Department (CHOPD) of JUTH for medical examination served as the controls. Subjects were selected with the help of a consultant paediatrician. The purpose of the study was explained to mothers of the children after which their consents were obtained for enrolment of their children. Socio-demographic data such as age, sex, and parents' level of education were obtained via oral interview. The ethical committee of JUTH approved the study protocol for this study. The approval was on the agreement that patient anonymity must be maintained, good laboratory practice/quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only in compliance with the Helsinki declaration.

Sample collection and analyses: Five millilitres of blood was collected via the antecubital fossa by venepuncture or via femoral vein for babies less than one year old. Two (2ml) millilitres of the blood was dispensed into EDTA bottles for haematological assays while the remainder was emptied into clean dry glass test tubes for clotting and retraction. Serum was isolated by centrifugation at 200Xg for five minutes and the samples frozen prior to analyses.

Haemoglobin concentrations and white blood cell counts were determined as in a standard haematological textbook (Dacie and Lewis, 1995). Nutritional status of the subjects was assessed by measuring total serum protein using colorimetric Biuret method (Reinhold, 1953) and albumin levels by the bromocresol green method (Hill, 1985). Serum zinc and copper concentrations were determined by flame atomic absorption spectrophotometer (Cornelis *et al.*, 1996). Each parameter was done in duplicate and the mean determined.

Statistical analysis: Data were analyzed for mean and standard deviation. Differences in parameters were tested for statistical significance at $p<0.05$ using student's t-test.

Results

Table 1 show the age and sex distribution of the test and control subjects. Majority of the subjects were in the age-group 0-24 months although the tests and controls were matched for both age and sex. Malnourished children and their healthy control counterparts show significant differences in anthropometrics (Table 2). Significantly higher proportions of our subjects were from parents with lower economic status based on parents' level of education (data not shown). Table 3 show the mean serum concentrations of copper and zinc in malnourished children compared with healthy controls. Mean serum copper concentration in marasmus, kwashiorkor and marasmic-kwashiokor were significantly lower than in the controls (marasmus, $p<0.05$; kwashiorkor, $p<0.05$; marasmic-kwashiokor, $p<0.05$). Mean serum zinc concentration were also significantly lower in malnourished children than in healthy controls (marasmus $p<0.05$; kwashiorkor, $p<0.05$; marasmic-kwashiokor, $p<0.05$).

Table 4 show that the mean haemoglobin concentrations were significantly lower in malnourished children than in healthy controls ($p<0.05$). However, the mean total white blood cell count (TWBC) did not differ significantly between the malnourished and the healthy controls, although there were decreases in count in marasmus and kwashiorkor with an increase of about 10% above that of the healthy controls in marasmic-kwashiokor. Mean total serum protein and albumin concentrations were significantly lower in malnourished children than in healthy controls (albumin, $p<0.05$; total protein, $p<0.05$). However, while total protein in marasmus. and kwashiorkor were comparable ($p>0.05$), albumin was significantly lower in kwashiorkor than in marasmus (Table 5).

Table 1: Age and sex distribution of test and control subjects

Age	Test			Controls		
(Months)	Males	Females	Total	Males	Females	Total
0-24	10	12	22	9	10	19
25-60	2	6	8	7	4	11
Total	12	18	30	16	14	30

Table 2: Anthropometrics of test and control subjects (Mean±S.D)

	Tests	Controls	t-values
Weight (Kg)	6.03±1.06	9.97±2.21	8.5652*
MUAC (cm)	9.86±1.42	12.46±1.77	6.1905*
OFC (cm)	29.45±2.02	33.00±3.05	5.2206*
Height (cm)	70.37±4.00	73.40±2.84	3.3297*

Legend
MUAC: Mid upper arm circumference
OFC: Occipito-frontal circumference
*Difference statistically significant (p-values less than 0.05).

Table 3: Mean serum copper and zinc in healthy and malnourished children (mean±S.D)

	Copper (umol/L)	Zinc(umol/L)
Control (n=30)	3.61±0.10	15.15±0.19
Marasmus (n=9)	2.20±0.11	13.01±0.17
Kwashiokor (n= 15)	2.00±0.12	14 98±0.20
Marsmic-kwashiokor (n= 6)	2.17±0.10	12.66±0.15

Mean serum copper and zinc concentrations were significantly lower in malnourished children than in controls ($p<0.05$).
Note: p-values were calculated by student's t-test.

Table 4: Mean serum haemoglobin and total white blood cell count in healthy and malnourished children (mean±S.D)

	Haemoglo-bin (g/L)	Wbc (X 10^9-cells/L)
Control (n=30)	120.0±8.0	9.43±3.1
Marasmus (n= 9)	93 0±7.0	9.22±3.4
Kwashiokor (n= 15)	87.0±6.0	8.14±3.6
Marasmic-kwashiokor (n- 6)	91.0±4.0	10.41±6.0

The mean haemoglobin concentrations were significantly lower in malnourished children than in the controls ($p<0.05$) while total white blood cell count (TWBC) in the controls and malnourished children were comparable ($p>0.05$).

Table 5: Mean serum total protein and albumin in healthy and malnourished children (Maen±S.D)

	Total protein (g/L)	Albumin (g/L)
Control (n = 30)	69.12±6.1	32 20±6.0
Marasmus (n = 9)	62.50±7.6	27.90±5.0
Kwashiokor (n = 9)	60.04±6.2	21.20±4.0

Mean serum total protein and albumin were lower in malnourished children than in healthy controls ($p<0.05$). Also mean serum albumin was significantly lower in kwashiorkor than in marasmus ($p<0.05$).

Discussion

Studies have associated protein-energy malnutrition with micronutrient deficiencies (Sommer and West, 1996; Kjolhede and Beisel, 1996). In the present study, there were significantly lower mean serum copper and zinc concentrations in malnourished children than the healthy controls. This is in accord with previous studies (Singla *et al.*, 1996; Thakur *et al.*, 2004). We also observed that lowest level of copper was found in children with kwashiorkor in corroboration with the finding of Ambrus and Ambrus (2004). Copper and zinc deficiency like other micronutrient deficiencies has been attributed to increase losses, inadequate intake and/or poor bioavailability (Aggett and Comerford, 1995; Sandstead, 1995). For example, copper deficiency has been found in children with diarrhoea (Ambrus and Ambrus, 2004), in patients receiving long-term parenteral nutrition (Ito *et al.*, 2005), during excess zinc ingestion and in population with high intakes of cereals. Phytate in cereals, which constitute the most important sources of nutrients of children in our environment, may be responsible for copper and zinc deficiency as a result of poor availability (Dijkhuizen *et al.*, 2004). The mechanism by which lower levels of copper and zinc occurred concurrently is not known, since the two elements exhibit antagonistic relationships (Smith *et al.*, 1998). Excess zinc levels induce the synthesis of the intracellular ligand metallothionine (MTO) in enterocytes, which then binds zinc. The excess zinc bound to MTO then is excreted in the faeces through enterocyte shedding. However, copper, with its higher affinity for MTO, displaces zinc and also is excreted, reducing the amount of copper delivered to the enterocyte (Webb and Cain, 1982). On the other hand copper with its higher affinity for metallothionine binds preferentially and therefore sequestered, making it unavailable for absorption (Ajayi, 2005). That the concentrations of the two elements were lower in malnourished children in the present study might be an indication that both were present at lower levels in the diet of our subjects or that both were possibly poorly available. It has been established that copper and zinc contents of diet in a given population depends on the soil contents of these elements (Milne, 1999). Additionally, the degree and distribution of protein-energy malnutrition (PEM) and micronutrient deficiencies in a given population depends on other factors, which include the political and economic situation, the education and sanitation, the season and climatic condition, food production, cultural, and religious food custom, breast-feeding habit, existence and effectiveness of nutrition programmes and the availability and quality of health services (De Waal and Whiteside, 2003; Salama *et al.*, 2004; Young *et al.*, 2004). In the present study, majority of our subjects were within the age 0-24 months, which coincides with the age of weaning. After weaning, children derive nutrition from a monotonous diet, depending heavily on the cereals with little nutritious accompaniment (Cohen and Atieno-Odhiambo, 1989). Also malnutrition and infectious diseases may likely interact during this period, thus resulting in compromised nutritional status (Kwena *et al.*, 2003). Numbers of factors induced by infection are known to further impair nutritional status
(Kinney, 1995; Blackburn, 1997). These include

increased protein metabolism and negative nitrogen balance, depletion of carbohydrate stores, increased energy consumption, increased gluconeogenesis, relative insulin resistance, altered lipid metabolism and redistribution of minerals between nutrient compartments (including iron, zinc and copper) and these factors further increase the vicious cycle between malnutrition and infections. Infections no matter how mild, have adverse effect on nutritional status and conversely, almost any nutrient deficiency, if sufficiently severe will impair resistance to infection (Scrimshaw and SanGiovanni, 1997). Although describing the cause of malnutrition is beyond the scope of this study, our findings suggest inadequate intake or increased losses of nutrients or both as the possible causes of malnutrition in this environment.

It has been suggested that intestinal malabsorption can result in copper deficiency and should be considered in differential diagnosis of severe anemia and neutropenia (Hayton, 1995).

Zinc and copper are important components of many enzymes and metalloproteins, which are involved, in many metabolic processes in the body (Shankar and Prasad, 1998). Zinc is essential component of enzymes involved in RNA and DNA synthesis. Thus it could be inferred that zinc deficiency is partly contributory to the lower levels of serum total protein and albumin observed in the present study. On the other hand circulating levels of copper and zinc closely correlate with the major carrier proteins, albumin thus the lower concentrations of these elements in the present study may reflect depressed plasma binding (Vulpe and Packman, 1995). However, caution should be exercised in interpreting low serum copper in protein-energy malnutrition, as low circulating concentrations of copper may not necessarily reflect copper deficiency, but rather due to protein deficiency which in turn results in decreased serum copper due to reduced synthesis of caeruloplasmin in the liver (Beshgetoor and Hambidge, 1998).

Although, there was no significant difference in the levels of total protein in the marasmus and kwashiorkor, serum albumin was lower in kwashiorkor in conformity with the findings of (Fongwo *et al.*, 1999). This suggests greater losses and inadequate intakes in kwashiorkor than in marasmus and this may in part explain the presence of oedema in kwashiorkor (Brabin and Coulter, 2003).

Zinc is also known to play central role in the functioning of the cells mediating non-specific immunity, such as neutrophils and natural killer cells and is needed for specific immune processes, such as balancing T-helper cell function (Shankar and Prasad, 1998). Thus reduced serum zinc might partly account for the depression of total white blood cell count in the present study. Although the mechanism for neutropenia in copper deficiency is unknown, neutropenia has been demonstrated experimentally in copper-deficient mice and is associated with arrested maturation (Karimbakas *et al.*, 1998). Also white blood cell (WBC) has been reported to be four-times richer in copper than red blood cells and that TWBC is reduced in copper deficiency (Maurice, 1997).

Reduction in the white blood cell count might affect all the subtypes of the cells especially the neutrophils (Hayton *et al.*, 1995; Olivares and Uauy, 1996; Ito *et al.*, 2005; Harless *et al.*, 2006), which in turn may impair the phagocytic functions of the neutrophils and predispose to infectious pathogens.

Studies have shown that copper is required for infant growth, host defense mechanisms, bone strength, red and white cell maturation (Hirase *et al.*, 1992; Olivares and Uauy, 1996). Copper has an essential role in several enzymatic reactions in RBCs, and copper deficiency interferes with iron transport and utilization and, therefore, with heme synthesis. Specifically, ceruloplasmin (which incorporates copper) is a ferroxidase that converts ferrous (+2) to ferric (+3) iron, allowing it to bind transferrin and be transported. The copper-dependent enzyme cytochrome-c oxidase also is required for the reduction of ferric iron to incorporate it into the heme molecule (Maurice, 1997; Frieden, 1983). In addition to interference with heme synthesis, there is approximately 85% reduction of superoxide dismutase activities in the RBC membrane in copper deficiency, which decreases RBC survival time. On the other hand, copper may be needed for the formation of bone marrow necessary for the formation of red cells.

Hence too low a copper status will result in iron deficient anaemia, which is characterized by a low haemoglobin level (Percival, 1998), as evidenced by the present finding of significant reduction in haemoglobin concentration in malnourished copper deficient children. We conclude that malnourished pre-school age children in our environment are copper and zinc deficient with associated anaemia and leucopenia. Supplemental copper and zinc should be part of nutritional rehabilitation of malnourished children in order to achieve optimal results and avoid clinical complications associated with zinc and copper deficiencies. However, fortification of food with zinc and copper remains the best way to prevent deficiencies in those at risk.

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