Serum Zinc and Malondialdehyde Concentrations and Their Relation to Total Antioxidant Capacity in Protein Energy Malnutrition

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Summary The aim of present study was to assess the association between serum zinc and oxidant/antioxidant status in children with protein energy malnutrition. Serum zinc, total antioxidant capacity and malondialdehyde were measured spectrophotometrically in 100 children (6 mo to 5 y); out of these, 50 children were malnourished and 50 children served as controls. Serum zinc levels were found to be significantly low in the malnourished ($p < 0.001$). Serum zinc levels in Grade I and Grade II malnourished were 82.7 and 67.7 $\mu\text{g/dL}$ respectively and in Grade III and IV combined was 53.2 $\mu\text{g/dL}$ as compared to 109.5 $\mu\text{g/dL}$ in the control group. These levels were significantly lower in children who had skin lesions than in those without such lesions ($p < 0.001$). Total antioxidant capacity was found to be significantly lowered in malnourished children (Grade I = 1.3 mmol/L, Grade II = 1.1 mmol/L, Grade III and IV = 0.5 mmol/L) as compared to 2.0 mmol/L in the control group ($p < 0.001$). The malondialdehyde concentration in malnourished children was significantly higher ($p < 0.001$) (Grade I = 1.6 nmol/mL, Grade II = 1.9 nmol/mL, Grade III and IV = 2.9 nmol/mL) as compared to 1.3 nmol/mL in controls. Total antioxidant capacity and hypoalbuminaemia were also correlated positively with low serum zinc level. Serum trace element deficiency leading to depleted antioxidant protection may be a contributing factor to the pathophysiology of protein energy malnutrition and replacement of these elements in the management of this condition might be important.

Key Words zinc, total antioxidant capacity, malondialdehyde, protein energy malnutrition

Protein energy malnutrition (PEM) is one of the most common health problems among children of developing countries, including India. Growth retardation due to this condition occurs in children of post weaning age, which may be a result of dietary deficiency of specific nutrients.

Among them, zinc (Zn) deficiency may play an important role, as it is critical for the functioning of metalloenzymes including Zn-superoxide dismutase (SOD), which form an integral part of the antioxidant defense system (1).

The clinical features of Zn deficiency like poor appetite, growth failure, skin lesions, diarrhea, poor wound healing and impaired immune response (2) are also observed in children with severe PEM. Inadequate Zn intake may limit the growth of these children during recovery from malnutrition (3). Nutritional deficiency in infants and children may occur as a result of inadequate intake, impaired absorption, hyper excretion or the occurrence of diseases that affect the metabolism of the nutrient, increased losses due to diarrhea, along

with a lack of breast feeding (4). Although clinical features of PEM are well defined, its pathophysiology is still poorly understood. Recently, free radicals have been implicated in the pathophysiology of PEM (5).

Oxidants or free radicals are atoms or molecules capable of independent existence that contain one or more unpaired electrons, making these species highly reactive. A delicate balance exists between the formation of the reactive oxygen species (ROS) and the endogenous protective mechanisms, such as antioxidants.

Antioxidants transform free radicals into less reactive species, thereby limiting their toxic effects. There are several endogenous anti-oxidant systems to deal with the production of ROS. These systems can be divided into enzymatic and non-enzymatic groups. The enzymatic subgroups include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). These enzymes also require trace metal cofactors for maximal efficiency including selenium for GPX; copper, zinc, or manganese for SOD; and iron for CAT. The non-enzymatic group includes a variety of biologic molecules, such as vitamin E, A, C, FAD (vitamin B₂) and glutathione.

Table 1. Serum Zn, albumin, TAC and MDA of different groups of malnourished children. Results are expressed as mean $\pm$ SD.

	Control (n=50)	Grade I (n=20)	Grade II (n=15)	Grade III and IV (n=15)	F*	p value
Serum Zn (μ g/dL)	109.5 $\pm$ 17.3	82.7 $\pm$ 3.6	67.7 $\pm$ 1.6	53.2 $\pm$ 7.8	95.7	$\leq$ 0.001
Albumin (g/dL)	3.9 $\pm$ 0.3	3.2 $\pm$ 0.3	2.9 $\pm$ 0.3	2.5 $\pm$ 0.2	75.0	$\leq$ 0.001
TAC (mmol/L)	2.0 $\pm$ 0.5	1.3 $\pm$ 0.2	1.1 $\pm$ 0.1	0.5 $\pm$ 7.2	56.9	$\leq$ 0.001
MDA (nmol/mL)	1.3 $\pm$ 0.1	1.6 $\pm$ 0.4	1.9 $\pm$ 0.5	2.9 $\pm$ 3.4	101.8	$\leq$ 0.001

Number in parentheses shows number of samples analyzed.

*F: One way analysis of variance (ANOVA).

p $\leq$ 0.05 (Student-Neumen-Keuls multiple range test).

Zn=zinc; TAC, total antioxidant capacity; MDA, malondialdehyde.

Table 2. Comparative data of serum Zn in severe PEM with and without skin lesions. Results are expressed as mean $\pm$ SD.¹

	Marasmus (n=5)	Marasmic kwashiorkor with skin lesions (n=4)	Marasmic kwashi- iorkor without skin lesions (n=6)	F*	p value
Serum Zn (μ g/dL)	61.3 $\pm$ 1.3 ^a	43.3 $\pm$ 3.6 ^b	53.1 $\pm$ 4.2 ^c	31.5	$\leq$ 0.001

Number in parentheses shows number of samples analyzed.

¹ Means within the row with different superscript letters are significantly different (^{ab} is $\leq$ 0.001, ^{ac} and ^{bc} are $\leq$ 0.05).

*F: one way analysis of variance (ANOVA).

p $\leq$ 0.05 (Student-Neumen-Keuls multiple range test).

Zn, zinc; PEM, protein energy malnutrition.

When free radicals are above certain levels, the oxidant/anti-oxidant balance deteriorates and free radicals attack lipids, carbohydrates and enzymes and cause harmful effects, like peroxidation of lipids resulting in formation of various products including malondialdehyde (MDA). Oxidants and anti-oxidants have well defined functions and reside in specific cellular compartments. It has been shown that some alteration in the oxidant and anti-oxidant balance may cause several pathologic conditions in diabetes mellitus, infectious diseases and cancer (6).

Since free radicals play an important role in immunological responses (7), correlations between trace element deficiency and antioxidant defense would lead to new insight into the management of PEM.

MATERIALS AND METHODS

Blood samples for the study were collected from 100 children aged between 6 mo and 5 y, out of which 50 children were presenting for the first time with various forms of PEM in the Pediatrics Department of our hospital. These children were examined for clinical signs of malnutrition, diagnosed and classified according to the Nutrition Subcommittee of the Indian Academy of Pediatrics (8) in 4 grades (Grade I, II, III and IV), with various percentages of expected body weight for the age. The children were classified using the standard value (100%) as 50th percentile of the Harvard growth standard (Normal=more than 80% of standard weight for age; Grade I=71–80%; Grade II=61–70%; Grade III=51–60% and Grade IV=50% or less of standard weight for

age). The cases of Grade III and IV (severe PEM) were further classified into marasmus, kwashiorkor and marasmic kwashiorkor and latter again with and without skin lesions. Anthropometric measurements of mid-arm circumference, head circumference, chest circumference, weight and height were taken. Fifty children with an apparently normal and healthy physique and presenting with no clinical or anthropometric signs or symptoms suggestive of any form of malnutrition were used as the control group. A questionnaire (responded to by the mothers of the children) was used for each of the children to obtain as much information as possible about their feeding habits, their qualitative food intake, and about the educational and socio-economic background of the parents. Informed consent was obtained from the parents of subjects and the research protocol was approved by the Institutional Ethics Committee and was in agreement with the Helsinki Declaration.

Venous blood was drawn, and the serum was carefully separated and transferred to micro tubes and stored at -20°C until analysis.

Assay of serum Zn concentration was performed by a double beam spectrophotometer (Systronics), using a commercial kit (Randox Laboratories, UK). Total antioxidant capacity (TAC) was estimated by the method described by Koracevic et al. (9). MDA estimation was done in serum by the spectrophotometric method of Hunter et al. (10). Statistical analysis was performed with the SPSS-8 software (SPSS Inc.). Blood parameters were analyzed by analysis of variance (ANOVA) followed by a Student-Neumen-Keuls multiple range test.

Table 3. Correlation analysis of serum Zn concentration with albumin level and TAC.

	Control (n=50)	Grade I (n=20)	Grade II (n=15)	Grade III and IV (n=15)
Albumin level	0.2	0.5 ^b	0.4	0.8 ^a
TAC	0.6 ^a	0.6 ^a	0.7 ^a	0.8 ^a

Number in parentheses shows number of samples analyzed.

^a Correlation is significant at the 0.001 level.

^b Correlation is significant at the 0.05 level.

Zn, zinc; TAC, total antioxidant capacity.

$p \leq 0.05$ was used as the threshold of significance. Pearson's correlation coefficients were calculated and the corresponding p values were provided (two-tailed).

RESULTS

Serum Zn levels were found to be significantly lower in the malnourished ($p < 0.001$). Mean serum Zn levels in Grade I and Grade II PEM were 82.7 and 67.7 $\mu\text{g/dL}$ respectively and in Grade III and IV combined was 53.2 $\mu\text{g/dL}$ as compared to 109.5 $\mu\text{g/dL}$ in the control group. Analysis of serum for albumin levels showed significantly lower levels in the malnourished (Grade I = 3.2 g/dL, Grade II = 2.9 g/dL, Grade III and IV = 2.5 g/dL) as compared to 3.9 g/dL in controls. TAC was found significantly lowered in malnourished children (Grade I = 1.3 mmol/L, Grade II = 1.1 mmol/L, Grade III and IV = 0.5 mmol/L) as compared to 2.0 mmol/L in the control group ($p < 0.001$). The MDA concentration in malnourished children was significantly higher ($p < 0.001$) (Grade I = 1.6 nmol/mL, Grade II = 1.9 nmol/mL, Grade III and IV = 2.9 nmol/mL) as compared to 1.3 nmol/mL in controls (Table 1).

Serum Zn levels were significantly lower in children who had skin lesions than in those without such lesions ($p < 0.001$) (Table 2). Hypoalbuminaemia and TAC were also correlated positively with low serum Zn levels (Table 3).

DISCUSSION

The levels of Zn were found to be significantly low in malnourished groups and tended to vary with the degree of malnutrition ($p < 0.001$) (Table 1). Previous studies (5, 11–13) also found low serum Zn levels in malnourished children. Several factors might have contributed to these low levels. The incidence of measles and diarrhea was a common precipitating factor of malnutrition in most of these children and it is likely that abnormal quantities of Zn might have been lost in their stools, thus leading to non-availability of dietary Zn to the tissues. The feeding practice for the malnourished infants and children may also contribute to the low levels of Zn. Breast milk is a good source of Zn for infants and young children being breastfed, but usually its deficiency occurs when breast milk is either insufficient or

no longer given to the baby. Since Zn is mainly (60–70%) bound to albumin and albumin transfers Zn to various tissues through blood stream, it is likely that the occurrence of hypoalbuminaemia might have contributed to the low levels of Zn.

Also we found significantly lower Zn levels in children who had skin lesions than in those without such lesions ($p < 0.001$) (Table 2). It is well known that Zn helps in the healing process of surgical wounds (14), and therefore, it is quite possible that Zn concentration in tissue in the area around the skin lesions of patients with PEM would be relatively higher. Zn would obviously be derived from the plasma and would lead to further lowering of the plasma Zn level. Increased loss of Zn from the wound area has also been suggested and this further contributes to the lowered levels of plasma Zn.

TAC was found significantly lowered in malnourished children as compared to the control group ($p < 0.001$) (Table 1). Kocaturk et al. (15) and Shaaban et al. (16) found lower erythrocyte SOD activity in malnourished children as compared to control groups. SOD traps the superoxide anion to yield H_2O_2 and acts as the primary quencher of superoxide. The low TAC in malnourished children might be due to low SOD activity. The trace element Zn plays an important role as a nutritional antioxidant cofactor. There was a positive significant correlation between serum Zn levels and TAC in malnourished children (Table 3).

MDA concentration in malnourished children was significantly higher ($p < 0.001$) as compared to the control (Table 1). Tatli et al. (6) also found increased lipid peroxidation in marasmic children. Reduced antioxidant defense status of serum may result in increased peroxidation of membrane lipids and enhanced concentrations of lipid peroxidation. These results suggest increased oxidative damage and lipid peroxidation in children with PEM. Serum trace element deficiency leading to depleted antioxidant protection may be a contributing factor to the pathophysiology of PEM and replacement of these elements in the management of this condition might be important. ROS has been implicated in the pathogenesis of many conditions including PEM-associated edema or anemia (4, 17).

In summary, our study shows that the anti-oxidant defense system is affected in PEM. It provides further evidence that a weakened anti-oxidant defense system and increased lipid peroxidation are important pathophysiologic events occurring in PEM. Disturbances of this system in malnourished children may result from a dietary insufficiency of nutritional antioxidants, proteins and minerals including Zn. Early replacement of antioxidative nutrients, proteins and Zn supplement could be useful in the therapy of this disease.

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