Estimation of serum zinc and alkaline phosphatase in malnourished children

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Abstract

The serum Zinc, Alkaline phosphatase activity and Albumin levels of 50 protein energy malnourished children (6 months – 5 years) were compared with those of 50 normal children. The levels of zinc was found to be significantly low in malnourished groups and tended to vary with the degree of malnutrition as compared to the control group (p<0.001). These levels were lower in children who had skin lesions than in those without such lesions (p<0.001). The alkaline phosphatase activity was also lower in the malnourished children as compared to control group (p<0.001). There was a positive correlation between the alkaline phosphatase activity and the zinc concentration. The low serum zinc levels were also correlated positively with hypolbuninaemia.

In summary, malnourished children are often in negative zinc balance and may develop systemic zinc deficiency. Replacement with supplemental zinc may be needed to maintain or replete zinc stores.

Introduction

Protein energy malnutrition (PEM) has been known to be a major health and nutrition problem in India, with a high incidence in the pre-school children. According to the recent National family health survey (NFHS-3), 2005-06 the prevalence of PEM in Madhya Pradesh (M.P.) has been increased (60%) as compared to NFHS-2, 1998-99 (54%).

Trace element deficiencies are common in children with PEM [1-5], and, as a result, they may suffer from various nutrient-specific deficiency disorders. Zinc (Zn) has established its importance as an essential micro-nutrient in human health and nutrition, as it is required for the functional activity of several enzyme systems including Alkaline phosphatase (ALP). The clinical features of Zn deficiency like poor appetite, growth failure, skin lesions, diarrhea, poor wound healing and impaired immune responses are also observed in children with severe PEM. Inadequate Zn intake may limit the growth of these children during recovery from malnutrition [6].

The present study was carried out to characterize possible Zn deficiency in children with different forms of malnutrition and to find out its association with serum ALP activity. As assessment of total body Zn status is difficult since measurements of serum, red cell, hair and urinary Zn concentrations are cumbersome and are not always reliable [7], hence the serum ALP may serve as a useful index of Zn status.

Materials and Methods
Blood samples for the study were collected from 100 children aged between six months and five years, 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 Indian Academy of Pediatrics [8] in 4 grades (Grade I, II, III and IV), with various percentage of expected body weight for the age. The cases of grade III and IV (Severe PEM) were combined and 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. 50 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 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 the agreement with the Helsinki Declaration.

Venous blood was drawn, the serum carefully separated and transferred to microtubes and stored at -200C until analysis. Assay of serum Zn concentrations was performed by Double beam spectrophotometer (systronics) using commercial kit (Randox Laboratories, U.K.). Serum ALP and Albumin were also estimated by kit method (Merck Ltd.).

Statistical analysis was performed with the SPSS-8 soft-ware. Blood parameters were analyzed by analysis of variance (ANOVA) followed by a Student-Neumen-Keuls multiple range test. P value ≤0.05 was used as a threshold of significance. Correlation coefficients were calculated by Pearson’s correlation analysis (two-tailed).

Results

The serum Zn levels were found to be significantly low in malnourished groups and tended to vary with the degree of malnutrition (p<0.001). Mean serum Zn levels in grade I and grade II PEM were 82.74 μg/dL and 67.76 μg/dL respectively and in grade III and IV combined was 53.28 μg/dL as compared to 109.52 μg/dL in the control group. The ALP activity was also lower in malnourished children (Grade I = 223.72 U/L, Grade II = 213.62 U/L, Grade III and IV= 284.44 U/L) as compared to control group (513.23 U/L) and the difference was significant (p<0.001). Analysis of serum for albumin levels shows significantly low levels in malnourished (Grade I = 3.28 gm%, Grade II = 2.98 gm%, Grade III and IV = 2.59 g%) as compared to 3.95 gm% in control. (Table 1).

Table 1: Serum Zn, ALP and Albumin levels in control and malnourished children. (mean±S.D)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 50)</th>
<th>Grade I (n = 20)</th>
<th>Grade II (n = 15)</th>
<th>Grade III and IV (n = 15)</th>
<th>F*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Zn (μg/dL)</td>
<td>109.52±17.37</td>
<td>82.74±3.64</td>
<td>67.76±1.69</td>
<td>53.28±7.84</td>
<td>95.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>513.23±18.65</td>
<td>223.72±11.45</td>
<td>213.62±10.11</td>
<td>284.44±103.98</td>
<td>357.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (gm %)</td>
<td>3.95±0.39</td>
<td>3.28±0.30</td>
<td>2.98±0.31</td>
<td>2.59±0.27</td>
<td>75.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Number in parenthesis shows number of samples analyzed.
F* = One way analysis of variance (ANOVA)
P≤0.05 (Student-Neumen-Keuls multiple range test)

Table 2: Comparative data of serum Zn in severe PEM with and without skin lesions. (mean±S.D)
Marasmus (n = 05)

Marasmic kwashiorkor with skin lesions. (n = 04)

Marasmic kwashiorkor Without skin lesions. (n = 06)

Serum Zn (µg/dL)

61.39 ± 1.35  43.35b±3.66  53.14a± 4.24

F*  P value

31.5  ≤0.001

Number in parenthesis shows number of samples analyzed.

*Means within the row with different superscript letters are significantly different.

F٭ = One way analysis of variance (ANOVA)

P≤0.05 (Student-Neumen-Keuls multiple range test)

**Table 3: Correlation analysis between serum Zn concentration and 1. ALP activity 2. Albumin level**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 50)</th>
<th>Grade I (n = 20)</th>
<th>Grade II (n = 15)</th>
<th>Grade III and IV (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ALP activity</td>
<td>0.73a</td>
<td>0.81a</td>
<td>0.80a</td>
<td>0.25</td>
</tr>
<tr>
<td>2. Albumin level</td>
<td>0.21</td>
<td>0.53b</td>
<td>0.46</td>
<td>0.86a</td>
</tr>
</tbody>
</table>

Number in parenthesis shows number of samples analyzed.

aCorrelation is significant at the 0.001 level.
bCorrelation is significant at the 0.05 level.

Serum Zn levels were found to be lower in children who had skin lesions than in those without such lesions (p <0.001) (Table 2). There was a positive correlation between the ALP activities and the Zn concentrations. The lower serum Zn levels were also correlated positively with hypoalbuminaemia (Table 3).

**Discussion**

The results of our study show that malnourished children have a markedly low serum Zn status (Table 1). The low levels are in agreement with those of other studies carried out in Cairo [9], Cape town [10], Hyderabad [11], Nigeria [12], Dar es Salaam [13], Delhi [14], Turkey [15] and Lucknow [16]. 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 practices of the malnourished infants and children may also contribute to the low levels of Zn. Breast milk is good source of Zn for infants and young children being breastfed, but usually PEM occurs when breast milk is either insufficient or no longer given to the baby.

A decrease in Zn status leads to a decrease in the activity of some Zn dependent enzymes such as lactate dehydrogenase, glutamate dehydrogenase, ALP, pyridoxal phosphokinase and thymidine kinase. Thus Zn can also modulate protein, energy and nucleic acid metabolism by affecting enzymes containing or requiring Zn for their activities. Similarly Zn deficiency can restrict cell proliferation. Earlier studies had shown that ALP activity decreases by as much as 48% in rats fed on a Zn deficient diet [17]. In the present study we observed a significant decrease in activity of serum ALP in all the groups of malnourished children as compared to control (Table 1). These low levels of activity are similar to those obtained by investigators in India [18] and other countries [12,19, 20]. It is likely that low intake of Zn coupled with the high incidence of low protein intake are contributory factors for the reported low enzyme activity. Also a significant and positive correlation was obtained between serum Zn and ALP activity (Table 3).
Also we found significantly lower Zn levels in children who had skin lesions than in those without such lesions (Table 2). It is well known that Zn helps in the healing process of surgical wound [21], and therefore, it is quite possible that Zn concentration of tissue in the area around the skin lesions of patients with PEM would be relatively higher. This would obviously be derived from the plasma itself and would lead to further lowering of 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.

A significant and positive correlation was obtained between serum albumin and Zn levels in malnourished children (Table 3). Zn in plasma is distributed between two major fractions-albumin (60-70%) and Zn α-macroglobulin. Results of albumin determination suggest a state of hypo-albuminaemia in all the malnourished children (Table 1). This has been shown to be present in protein deficiency states in children [12]. It is likely that the occurrence of hypo-albuminaemia might have contributed to the low levels of serum Zn.

Conclusion

Our study shows that there is a positive correlation between the ALP activities and the Zn concentrations in the serum in each of the group of PEM. The metalloenzyme ALP is an easily obtainable assay as it may provide an accurate reflection of total body Zn status.

In children with PEM, it would be advantageous to add Zn to food supplements in order to achieve normal levels of serum Zn, promote growth, enhance clinical recovery and restore immuno competence.

References


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