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Research Article

EFFECT OF ZINC STATUS ON ALKALINE PHOSPHATASE ACTIVITY IN CHILDREN WITH PROTEIN ENERGY MALNUTRITION

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ABSTRACT
Kwashiorkor and marasmus are the two types of under nutrition, most commonly seen in children, especially of rural origin. As per the World Health Organisation (WHO), currently protein energy malnutrition (PEM) affects about half the young children in South Asia, which is 6.5 times higher than that of western hemisphere. Children with protein energy malnutrition in addition are affected by micronutrient deficiencies, which also have detrimental effect on growth and development. Protein-energy malnutrition cases and age & gender matched normal healthy controls were identified in Anganwadi centres based on Indian Association of Pediatrics (IAP) guidelines. 34 children with protein energy malnutrition constituted the cases group while 30 healthy children served as control group. Serum was analysed for total protein, albumin, aspartate transaminase, alanine transaminase, alkaline phosphatase and zinc. Statistical analysis has been done on the basis of mean values, standard deviation and t-test. Partial correlation co-efficient was compared with a constant. It was observed that total protein and albumin levels were significantly lower in cases of protein energy malnutrition as compared to healthy controls ($p < 0.01$). Similarly, there was a decreased alkaline phosphatase activity in cases of PEM as compared to controls ($p < 0.05$). Low serum zinc levels was seen in cases of malnourished children as compared to control group ($p < 0.001$), showing high significance. Zinc levels showed positive correlation with alkaline phosphatase levels among controls ($r = 0.438$). Zinc deficiency in malnutrition affects alkaline phosphatase activity, thereby the growth and development of these children.

Key Words: *Alkaline Phosphatase, Malnutrition, Zinc*

INTRODUCTION
Kwashiorkor and marasmus are the two types of protein energy malnutrition, the distinction being made by the presence or absence of oedema. Kwashiorkor is primarily attributed to ‘protein’ deficiency in the diet and marasmus is as a result of ‘energy’ deficiency. Although significant clinical differences between marasmus and kwashiorkor exist, it is now thought that marasmus represents an adaptation to starvation and kwashiorkor represents a dysadaptation to starvation Beers and Berkow (1999).
Prevalence of malnutrition is spread worldwide. As per the WHO, currently PEM affects about half the young children in South Asia, which is 6.5 times higher than that of western hemisphere. Malnutrition is directly responsible for 3,00,000 deaths per year in children younger than 5 years in developing countries Onis M de (1993).
Malnutrition is known to affect virtually every organ system in the body. Impaired physical growth, cognitive function, immune functions, brain development have been shown to be associated with malnutrition. Other pathologic changes observed in malnutrition include fatty degeneration of liver and heart, skeletal changes, small bowel atrophy and decrease in intravascular volume Etukudo *et al.*, (1999).
Children with protein energy malnutrition in addition are affected by micronutrient deficiencies, which also have detrimental effect on growth and development. Micronutrient deficiencies commonly encountered with PEM are iron, zinc and vitamin A Ghai OP *et al.*, (2006). Hence this study was undertaken to know the zinc status of children with PEM and its effect on enzyme alkaline phosphatase activity in them.

MATERIALS AND METHODS

Twelve Anganwadi centres which are under Kuppam Mandal, Chittoor District, Andhra Pradesh State, India were visited. A preliminary survey of nutritional status was done of all children between 3 years & 5 years attending respective Anganwadi centres by measuring their height & weight. Protein-energy malnutrition cases and were identified in each of these centres based on IAP guidelines. 34 children with protein energy malnutrition constituted the case group while 30 healthy children served as control group. Informed consent for the study was taken from the parents/guardians of these children before the collection of sample. Children with recent history of fever, jaundice, liver disorders, cardiac abnormalities, bleeding diathesis or any such illness known to affect protein, enzymes of liver origin or zinc levels in serum were excluded from the study.

Under aseptic precautions, 4 ml venous blood was collected from children from the median cubital vein. The sample was transferred to a test tube & the blood was allowed to clot. The sample was then centrifuged at 3000 rpm for 10 minutes to separate the serum. Analysis of serum was carried out in batches, with inclusion of Bio-Rad internal quality control materials for each batch. Serum was analysed for total protein, albumin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase and zinc concentrations using 'Chemwell' fully automated analyser at Clinical Biochemistry Laboratory, P.E.S. Institute of Medical Sciences & Research, Kuppam. Total protein in serum was determined by biuret method Gornall AG *et al.*, (1949) and serum albumin by BCG-dye binding method Doumas BT *et al.*, (1971). Enzymes in serum were estimated by the standard IFCC methods Expert panel of the IFCC on enzymes (1976), McComb RB and Bowers GN (1972). Serum zinc was estimated colorimetrically by the reaction with Nitro-PAPS Akita A and Yiamashita S (1989), Makino T (1991).

Analysis has been done by calculating mean values, standard deviation and performing t-test. The values are compared for the corresponding degree of freedom at 5% and 1% levels of significance. Partial correlation co-efficient was compared with a constant. The percentage of significance was obtained on the basis of 'r' values and 'p' values.

RESULTS

In the present study, liver enzymes namely aspartate transaminase, alanine transaminase and alkaline phosphatase activities were analysed along with total proteins, albumin and micronutrient zinc concentrations in serum of malnourished Anganwadi school children and healthy controls.

The age group of the cases and controls groups were between 3 & 5 years. The total number of cases included in the study was 34 with 30 age and gender matched controls. Table 1shows age and gender wise distribution of cases and controls.

Table 1: Age and gender distribution of study population

Trait		3 – 4 Years	% Distribution	4 – 5 Years	% Distribution
Cases	**Males**	5	15	11	32.5
(n = 34)	**Females**	6	18	12	34.5
Controls	**Males**	3	10	15	50
(n = 30)	**Females**	4	13.4	8	26.6

Table 2 shows the nutrition status of the controls based on weight for age. 13.3 % were between 81 – 85%, 30 % were between 86 – 90%, 30% were between 91 – 95%, while 26.7% were > 96%. IAP guideline was followed to grade the nutrition status based on weight for age of malnourished children, which is depicted in Table 3. It reveals that % of cases came under Grade I PEM and of children came under Grade II PEM. None of the children in the study were under Grade III PEM.

Table 2: Nutritional status of children in controls

Trait	81 – 85 %	86 – 90 %	91 – 95 %	> 96%
Controls (n = 30)	4	9	9	8
% Distribution	13.3	30	30	26.7

Table 3: Distribution of cases in different grades of PEM

Trait	Grade I (71 – 80%)	Grade II (61 – 70%)	Grade III (< 60%)
Cases (n = 34)	19	15	00
% Distribution	55.9 %	44.1 %	0 %

Table 4 shows the mean and standard deviation in cases and controls of biochemical parameters namely total protein, albumin, aspartate transaminase, alanine transaminase, alkaline phosphatase and zinc.

Table 4: Mean and standard deviation of biochemical parameters

Trait	Total Protein (g/dl)	Albumin (g/dl)	AST (U/L)	ALT (U/L)	Alkaline Phosphatase (U/L)	Zinc (μg/dl)
Cases n = 34	5.87* ± 1.38	3.53* ± 0.83	39.79 ± 13.02	18.97 ± 6.99	171.18** ± 51.35	72.29# ± 26.14
Controls n = 30	6.897 ± 0.92	4.153 ± 0.45	42.83 ± 9.22	17.57 ± 5.28	230.37 ± 85.50	96.47 ± 23.38
t – Value (Cases Vs Controls)	3.467	3.456	1.148	- 0.966	3.191	4.974

* Significant: $p < 0.01$ ** Significant: $p < 0.05$ # Signicicant: $p < 0.001$

Correlation coefficients were determined by comparison of zinc levels with height and alkaline phosphatase, and height with alkaline phosphatase, which is shown in Table 5.

Table 5: Correlation of zinc with alkaline phosphatase

Parameter	Group	'r' Value
Zinc Vs Alkaline Phosphatase	Cases (n = 43)	0.103
	Controls (n=30)	0.438*

** p < 0.05*

DISCUSSION

In developing countries like India, protein energy malnutrition is a problem of major concern, affecting children in infancy and childhood. Mild degrees of malnutrition usually go unnoticed and over a period of time, it may progress to severe forms resulting in high morbidity and mortality. Alterations in many biochemical parameters contribute to the pathogenesis, leading to various manifestations in malnutrition Müller and Krawinkel (2005).

In the present study, it was observed that total protein and albumin levels showed significant lower levels in cases of protein energy malnutrition as compared to healthy controls ($p < 0.01$). Similarly, there was a decreased alkaline phosphatase activity in cases of PEM as compared to controls ($p < 0.05$). Low serum zinc levels was seen in cases of malnourished children as compared to control group ($p < 0.001$), showing high significance. The enzymes of hepatocellular origin namely aspartate transaminase and alanine transaminase activities noted in cases and controls were similar and did not show any statistical significance. This indicates that the effect on under-nutrition on liver was not profound in these children. It was observed that a positive linear correlation existed between zinc and alkaline phosphatase levels in controls, stating the essentiality of zinc for the enzyme activity.

Zinc is a constituent of more than 100 metalloenzymes and is essential for growth, protein synthesis, epithelial repair and synthesis of DNA & RNA Sanstead (1994). Zinc plays an important role in growth and development, immune response, neurological functions and reproduction. Important zinc containing enzymes includes – Superoxide, Carbonic anhydrase, Leucine aminopeptidase, Carboxypeptidase, Alcohol dehydrogenase, Retinine reductase, Alkaline phosphatase, Glutamate dehydrogenase, δ - ALA dehydratases and Lactate dehydrogenase Chatterjea and Rana Shinde (2005). Zinc plays an important role in the structure of proteins and cell membranes. A finger like structure known as 'Zinc-finger' motif stabilises the structure of a number of proteins. Zinc finger proteins have been found to regulate gene expression by acting as transcription factors, involved in cell signalling influencing hormone release & nerve impulse transmission, and apoptosis o' Dell BL (2000) Truong-Tran AQ *et al.*, (2000).

Alkaline phosphatase (EC 3.1.3.1) is the enzyme catalysing the alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates. Alkaline phosphatase activity is present in most organs of the body and is especially associated with the membranes and cell surfaces located in the mucosa of the small intestine and proximal convoluted tubules of the kidney, in bone (osteoblasts), liver and placenta. The skeletal, or bone-specific, isoform of alkaline phosphatase is a tetrameric glycoprotein found on the surface of osteoblast cells. Alkaline phosphatase activity is thought to be associated with lipid transport in the intestine and calcification process in bone.

Alkaline phosphatase was the first zinc enzyme to be discovered in which three closely spaced metal ions are present at the active center. Two Zn ions and one Mg ion at all three sites produce a maximally active enzyme. Divalent ions such as Mg^{2+}, Co^{2+} and Mn^{2+} are the activators of the enzyme. Zn^{2+} is a constituent metal ion and correct ratio of Mg^{2+}/Zn^{2+} is necessary for the optimal activity of the enzyme. Physiological bone growth increases alkaline phosphatase levels in serum from 1.5 to 7 times than that in healthy adult serum, the maximum being earlier in girls than in boys. Since the enzyme is zinc dependent, decreased zinc levels in serum contributes to decreased activity of enzyme, thus leading to poor osteoblastic activity and decrease in skeletal growth and development of an individual Burtis CA and Ashwood ER (1999). Several studies have indicated a decrease in zinc levels in serum in malnourished individuals. In a study done on 30 malnourished pre-school children in Jos, Ugwuja and his associates observed that the serum zinc and copper levels were significantly less than in well-nourished children Ugwuja EI *et al.*, (2007). Singla PN and his co-workers also observed significantly low levels of zinc and copper in children with severe malnutrition Singla PN *et al.*, (1996). Trace element deficiency and its contribution to pathophysiology in malnutrition was shown in a study on 80 malnourished children by Savitri Thakur and her colleagues Thakur S *et al.*, (2004). Anuradha Jain and her associates in their study on malnourished children have implicated that negative zinc balance may develop systemic zinc deficiency and alter alkaline phosphatase activity in these children Jain A *et al.*, (2008). Few other studies have demonstrated the increase in alkaline phosphatase activity following zinc replacement therapy Kasarskis EJ and Schuna A (1980), Coleman JE (1998). Hence zinc supplementation Shingwekar AG *et al.*, (1979), Shrivastava SP *et al.*, (1993) in the malnourished children would contribute to attain a proper growth and development of children who are under-nourished.

Conclusion

Protein energy malnutrition is a multi-dimensional disorder affecting many systems. It results not only in decreased levels of protein and energy in the body, but also several micronutrients, the prime one among them is zinc. Zinc plays a very important role in several body functions and zinc deficiency has demonstrated several manifestations amongst which growth retardation being a prominent one. Since zinc is a constituent of the enzyme alkaline phosphatase enzyme, which is involved in osteoblastic activity in bones, its optimum concentration contributes a lot towards achievement of optimum growth and development of an individual. Zinc supplementation needs a strong consideration along with protein and energy rich foods, which helps in the achievement of proper growth and development process as well as healthy outcome of under-nourished children.

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