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## Plasma zinc status of protein energy malnourished children in Nigeria

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### Summary

The plasma zinc status, albumin level and alkaline phosphatase activity of 44 protein energy malnourished Nigerian children were compared with those of 10 normal children. Results were classified according to the type and degree of malnutrition. Plasma zinc levels were low in the malnourished groups and tended to vary with the degree of malnutrition as indicated by the level of growth retardation. Levels in the 'normal' group of children suggest a possible presence of marginal zinc nutrition among Nigerian children.

**Key words:** plasma zinc; protein energy malnutrition; growth retardation.

### Introduction

The dietary essential nature of zinc for experimental as well as farm animals has been recognised (Underwood, 1971). Its importance as an essential trace metal nutrient in man is being increasingly investigated and it is already evident that zinc is important in human health (Sandstead et al., 1976). Reports of several experiments carried out in animals or in humans show the importance of zinc as a component of several metalloenzymes and as an essential nutrient for DNA and RNA synthesis and protein metabolism (Williams and Chester, 1970; Terhune and Sandstead, 1972; Tao and Hurley, 1971).

Human zinc deficiency may result in growth retardation in infants and young children (Sandstead et al., 1967). Deficiency states in infants and children may occur as a result of inadequate intake, impaired absorption, hyperexcretion or the occurrence of diseases that affect the metabolism of the nutrient.

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Many infants and children, particularly in developing countries, suffer from severe retardation of growth between the ages of six months and two years. In general, growth retardation occurs among children of postweaning age and this may be as a result of dietary deficiency of specific nutrients. Protein energy malnutrition (PEM) is one of the most common health problems among children of developing countries including Nigeria (Collie and Janez, 1968; Morley, 1968; Gurney and Omololu, 1971; FSAN, 1976) and growth retardation is one of its main consequences. Zinc deficiency occurs in PEM and persists even after clinical cure in some cases (Sandstead et al., 1965) and may contribute to the growth retardation of PEM.

This study was carried out to characterize possible zinc deficiency as it occurs in Nigerian children with different forms of malnutrition.

### **Patients, Materials and Methods**

Blood samples for the study were collected from children aged between six months and three years presenting for the first time with various forms of protein energy malnutrition (PEM) in the Children's Out-patient Clinic of the University College Hospital, Ibadan. Such children were examined for clinical signs of malnutrition, diagnosed and grouped as suffering from kwashiorkor, marasmus, marasmic kwashiorkor and undernutrition using the Wellcome criteria for classification (Classification of Infantile Malnutrition, 1970). Anthropometric measurements of mid-arm circumference (MAC), weight, height, and tricep skinfold thickness (TSFT) were made according to methods described in the literature (Gurney, 1969; Gurney et al., 1972). 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 at the clinic) 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.

Venous blood drawn from the femoral vein using disposable zinc-free syringes and needles was collected in zinc-free heparinised tubes and centrifuged at 3000 rpm for 20 min to ensure a good separation of plasma from the blood cells. Plasma was carefully transferred into well cleaned polythene vials and stored at  $-20^{\circ}\text{C}$  until required for analysis.

Analysis of plasma for zinc concentrations was by atomic absorption spectrophotometer (AAS). Frozen samples were allowed to thaw at room temperature and specific volumes of these were diluted with glass distilled and deionised water in the ratio of 1:3. The Perkin Elmer atomic spectrophotometer model 403 was used in the reading of the zinc levels. Working zinc standards solution obtained from BDH Chemicals Limited, Poole, England (Product No. 14150) were diluted to specific concentration with 5% glycerol and used in standardising the instrument. All glasswares and polyethylene containers used had been thoroughly cleaned by soaking them in acid overnight and washed with detergent and rinsed in deionised water before leaving in EDTA for four hours followed by further rinsing thrice with deionised water. Containers so treated were found to be free of any detectable zinc contaminants. The Biuret method of estimating plasma proteins (Wolfson et al., 1948) as modified by ICNND (1963) was used for albumin determination. In the modified technic, only 0.2 ml of plasma is used. STAT-Pack TM reagents (obtained from Calbiochem Co., La Jolla, California) consisting of P-nitrophenyl phosphate substrate and 2 amino-2 methyl-1-propanol buffer were used for the assay. Substrate was reconstituted in distilled water to a concentration of  $8.3 \times 10.3$  moles/litre while the buffer was similarly diluted to a concentration of 0.75 moles/litre and pH 10.3.  $100\ \mu\text{l}$  of plasma was added to a cuvet containing 3.0 ml of substrate and buffer mixed in the ratio of 1:2 and incubated for 30 min at a constant temperature of  $30^{\circ}\text{C}$  in a water bath. After

an additional incubation period of 2 min the cuvet was transferred to a temperature controlled spectrophotometer (PYE UNICAM SP 500) and the absorbance read at 420 nm. A second absorbance reading was taken after 3 min and the difference between the two absorbances was used for calculating the activity of the enzyme. Muscle area (MA) and fat area (FA) estimations were derived by calculations using anthropometric measurements of arm circumference and tricep skinfold thickness according to the method of Gurney (1969) and Gurney et al. (1972). One way analysis of variance as well as correlation analysis were carried out.

## Results

### *Anthropometry*

Results of anthropometric measurements are as shown in Table 1. Means and standard deviation of weight, height, MAC and TSFT measurements and calculated estimates of MA and FA of the children in each group are shown in Table 1. Weight, MAC, TSFT, MA and FA were significantly different ( $P < 0.05$ ) from those of the control group. A positive correlation was obtained between weight and the MAC and between weight and the MA. When malnourished children were regrouped according to the Gomez system of PEM classification (Gomez et al., 1955), 75% of the children were found to be in the second and third degree of malnutrition, i.e. with less than 75% weight for age.

### *Plasma zinc concentration*

Results of plasma zinc concentrations, albumin levels, and plasma alkaline phosphatase activity of the different groups studied are presented in Table 2. Mean plasma zinc levels in the control group were  $73.15 \mu\text{g/dl}$ . In kwashiorkor and marasmic kwashiorkor, the levels were  $41.61 \mu\text{g/dl}$  and  $40.18 \mu\text{g/dl}$  respectively, while those for marasmus and undernutrition were  $46.7 \mu\text{g/dl}$  and  $47.9 \mu\text{g/dl}$  respectively. These values indicate that the mean plasma zinc concentrations were significantly lower in the malnourished groups than in the controls ( $P < 0.05$ ). No significant differences existed between the zinc levels of the various groups of malnourished children. Compared to the control group, the difference in mean zinc level of some of the malnourished groups was as high as 45%. Mean plasma zinc concentrations according to this grouping (Table 3) were as follows: 1st degree malnutrition  $53.08 \mu\text{g/dl}$ , 2nd degree malnutrition  $48.36 \mu\text{g/dl}$  and 3rd degree  $47.64 \mu\text{g/dl}$ . These values were significantly different when compared with the values for the control group. However, there was no significant difference between the three degrees of malnutrition.

Utilising Waterlow's classification for linear growth retardation (Waterlow, 1976), the malnourished children were reclassified into four grades of retardation. The mean plasma zinc concentrations were as shown in Table 3. Malnourished children with no linear growth retardation had a mean zinc level of  $56.27 \mu\text{g/dl}$ , children with mild moderate and severe retardation had plasma zinc levels of  $45.10 \mu\text{g/dl}$ ,  $43.27 \mu\text{g/dl}$  and  $41.95 \mu\text{g/dl}$  respectively. Plasma zinc levels for moderate and severe forms of retardation were significantly different

Table 1. Anthropometric data (mean  $\pm$  S.D.)

	Kwashiorkor (13)	Marasmic kwashiorkor (11)	Marasmus (10)	Undernutri- tion (10)	Control (10)	F*	P value
Age (months) . . . . .	20.25 $\pm$ 4.5	20.86 $\pm$ 5.6	20.9 $\pm$ 6.4	21.7 $\pm$ 9.1	21.1 $\pm$ 10.7	8.39	0.05
Weight (kg) . . . . .	8.06 $\pm$ 0.91	7.26 $\pm$ 0.84	5.72 $\pm$ 1.34	9.03 $\pm$ 2.08	10.76 $\pm$ 3.28	8.39	0.05
Height (cm) . . . . .	77.9 $\pm$ 4.2	74.9 $\pm$ 4.4	70.9 $\pm$ 3.9	75.5 $\pm$ 5.4	82.5 $\pm$ 15.7	—	—
MAC (cm) . . . . .	12.4 $\pm$ 1.1	11.4 $\pm$ 0.4	10.8 $\pm$ 0.9	12.9 $\pm$ 1.9	15.2 $\pm$ 0.8	13.18	0.01
TSFT (mm) . . . . .	5.5 $\pm$ 1.8	5.6 $\pm$ 1.1	4.7 $\pm$ 1.1	5.8 $\pm$ 1.8	7.4 $\pm$ 0.9	39.17	0.05
MA (cm <sup>2</sup> ) . . . . .	9.42 $\pm$ 2.10	7.49 $\pm$ 0.93	6.99 $\pm$ 1.24	10.11 $\pm$ 2.51	13.2 $\pm$ 1.90	12.74	0.01
FA (cm <sup>2</sup> ) . . . . .	3.17 $\pm$ 1.09	2.98 $\pm$ 0.99	2.4 $\pm$ 0.70	3.7 $\pm$ 1.6	5.2 $\pm$ 0.6	7.37	0.05

Number in parenthesis shows number of subjects.

\* F = one way analysis of variance

MAC = mid-arm circumference; TSFT = triceps skinfold thickness; MA = muscle area; FA = fat area

Table 2. Plasma zinc, albumin and alkaline phosphatase of different groups of malnourished children (mean  $\pm$  S.D.)

	Control (10)	Marasmus (10)	Marasmic kwashiorkor (11)	Kwashiorkor (13)	Undernutri- tion (10)	F*	P value
Plasma zinc ( $\mu$ g/dl) . . . . .	73.15 $\pm$ 12.9	46.7 $\pm$ 12.2 <sup>a</sup>	40.18 $\pm$ 19.5 <sup>a</sup>	41.61 $\pm$ 9.1 <sup>a</sup>	47.9 $\pm$ 16.80 <sup>a</sup>	7.35	.05
Albumin (g/dl) . . . . .	3.11 $\pm$ 0.31	2.41 $\pm$ 0.50 <sup>b</sup>	2.50 $\pm$ 0.60 <sup>b</sup>	2.29 $\pm$ 0.44 <sup>b</sup>	2.66 $\pm$ 0.45 <sup>b</sup>	8.81	.05
Alkaline phosphatase (iu/l) . . . . .	145.14 $\pm$ 28.65	89.65 $\pm$ 8.51 <sup>c</sup>	87.59 $\pm$ 14.64 <sup>c</sup>	85.52 $\pm$ 8.70 <sup>c</sup>	104.65 $\pm$ 30.26	19.4	.01

Number in parenthesis shows number of samples analysed in duplicate.

<sup>a</sup>, <sup>b</sup>, <sup>c</sup> = values not significantly different from each other

\* F = one way analysis of variance

Table 3. Mean plasma zinc levels according to degree of malnutrition<sup>a</sup>, linear retardation<sup>b</sup>

	Plasma zinc level ( $\mu\text{g/dl}$ ) mean + S.D.	F	P value
a) Degree of malnutrition:			
1st degree (9) <sup>c</sup> .....	53.08 $\pm$ 17.62	0.534	N.S.
2nd degree (13) .....	48.36 $\pm$ 16.89		
3rd degree (17) .....	47.64 $\pm$ 16.38		
b) Degree of retardation:			
normal (12) <sup>c</sup> (95% height for age) .....	56.27 $\pm$ 19.29 <sup>ef</sup>	2.729	0.05
mild (9) (95–90% height for age) .....	45.01 $\pm$ 16.31		
moderate (10) (90–85% height for age) .....	43.27 $\pm$ 15.71 <sup>f</sup>		
severe (13) (85% height for age) .....	41.95 $\pm$ 9.87 <sup>c</sup>		

<sup>a</sup> Degree of malnutrition classified according to Gomez et al. (1955) with Harvard Values as the Standard

<sup>b</sup> Linear growth retardation classification according to Waterlow (1976)

<sup>c</sup> Numbers in parenthesis show number of subjects in each classified group

<sup>e</sup> Values significantly different from each other ( $P < .02$ )

<sup>f</sup> Values significantly different from each other ( $P < .05$ )

F = one-way analysis of variance

( $P < 0.05$ ) from those of the normal groups. Correlation between plasma zinc and height was positive ( $r = 0.61$ ).

### *Plasma albumin*

Analysis of plasma for albumin levels gave results which correlated positively with zinc levels (Table 4). The correlation was significant ( $P < 0.05$ ) for kwashiorkor with an albumin level, of 2.29 g/dl. In marasmic kwashiorkor, undernutrition, marasmus and the control groups, the levels were 2.50 g/dl, 2.66 g/dl, 2.41 g/dl and 3.11 g/dl respectively (Table 2).

### *Alkaline phosphatase activity*

The activity of this enzyme was also lower in the four groups of malnourished children when compared with the control group (Table 2). The mean activity in the control group was 145.141 U/l. Activity was more than 41% lower in the kwashiorkor group, 40% in marasmic kwashiorkor, about 38% in marasmus and 28% in the under-nutrition group. There was a positive correlation between the enzyme activities and the zinc concentrations in the plasma in each of the groups (Table 4). The correlation was significant ( $r = 0.85$ ,  $P < 0.05$ ) for marasmic kwashiorkor.



Table 4. Correlation analysis between plasma zinc concentration and 1. albumin level<sup>a</sup>, 2. alkaline phosphatase activity<sup>b</sup>

Group	Plasma zinc concentration vs.	
	1. albumin level	2. alkaline phosphatase activity
Kwashiorkor (13) . . . . .	0.43	0.27
Marasmic kwashiorkor (11) . . . . .	0.61 <sup>a</sup>	0.85 <sup>a</sup>
Marasmus (10) . . . . .	0.12	0.59
Undernutrition (10) . . . . .	0.66 <sup>a</sup>	0.38
Control (10) . . . . .	0.73 <sup>a</sup>	0.53

Numbers in parenthesis show samples analysed.

<sup>a</sup> P < 0.05

## Discussion

The results of our study show that malnourished Nigerian children have a markedly low plasma zinc status. These low levels are in agreement with those of other studies carried out in Cairo (Sandstead et al., 1965), Cape Town (Hansen and Lehman, 1969) and Hyderabad (Kumar and Rao, 1973). Several factors might have contributed to these low levels. The incidence of measles and diarrhoea was a common precipitating factor of malnutrition in most of these children and it is likely that abnormal quantities of zinc might have been lost in their stools thus leading to non-availability of dietary zinc to the tissues. The feeding practices of the malnourished infants and children may also contribute to the levels of zinc. Breastmilk is a good source of zinc for infants and young children being breastfed, but usually PEM occurs when breastmilk is either insufficient or no longer given to the baby. Previous observations in Nigeria (Woodruff, 1951; UNICEF, 1963) showed that many pregnant women and nursing mothers consume inadequate and poor quality food and reports of milk yield showed that daily production is poor especially in the second year of lactation. Eighty-eight percent of mothers of the PEM subjects studied normally wean their children by the 18th month. The low production of breastmilk at this time coupled with the poor weaning foods such as watery maize gruel (Ogi), cassava flour (Amala), bread and some beans make zinc malnutrition inevitable in these children. These foods as earlier reported by Mbofung and Atinmo (1980) (Table 5) are poor sources of zinc.

A positive and significant correlation was obtained between plasma albumin and zinc levels in marasmic kwashiorkor, under-nutrition and in controls (Table 3). Zinc in plasma is distributed between two major fractions – albumin (60–70%) and zinc alpha macroglobulin (Parish and Vallee, 1970). Results of albumin determination suggest a state of hypo-albuminaemia in all the malnourished children. This has been shown to be the case in protein deficiency



Table 5. Mean zinc content of some foods commonly eaten by infants and young children<sup>a</sup>

Food	No analysis	Zinc mg/100 g dry matter (mean $\pm$ S.D.)
Ogi (maize gruel) . . . . .	10	2.06 $\pm$ .56
Beans . . . . .	10	2.97 $\pm$ .23
White bread . . . . .	5	0.78 $\pm$ .23
Amala (cassava flour) . . . . .	10	1.45 $\pm$ .10

<sup>a</sup> obtained from Mbofung and Atinmo (1980)

states in adults as well as in children (Holmes et al., 1951; Patel et al., 1957; Whitehead et al., 1971). It is likely that the occurrence of hypo-albuminaemia might have contributed to the low levels of plasma zinc.

A decrease in zinc status leads to a decrease in the activity of some zinc dependent enzymes (Kirchgessner, 1976). Earlier studies by Roth and Kirchgessner (1974) had shown that alkaline phosphatase activity decreases by as much as 48% in rats fed on a zinc deficient diet. In the present study we observed a decrease in activity (47% compared to control) in plasma alkaline phosphatase of all the groups. These low levels of activity are similar to those obtained by other investigators in this part of the country (Edozien, 1961; Olanbiwonnu and Johnson, 1976; Atinmo and Faoye, 1978) and in Ugandan children with kwashiorkor (Reinhold and Kfoury, 1969). In Nigeria mean dietary zinc intake by children has been reported to be low about (3.6 mg/day) (Mbofung and Atinmo, 1980). It is likely that this low intake of zinc coupled with the high incidence of low protein intake are contributory factors for the reported low enzyme activity.

According to Gomez et al. (1955) estimates of MA and FA in the upper arm indicate the relative importance of total protein and calorie reserves in the body. Assuming the control group as forming the pre-PEM nutritional status of the malnourished children, it is evident from calculations that the calorie reserves were most reduced in marasmus (54%) followed by marasmic kwashiorkor (43%), kwashiorkor (40%) and undernutrition (29%) groups. Protein reserves as estimated by MA were most affected in kwashiorkor. Muscle loss ranged from 23% in marasmus to 47% in kwashiorkor. These relative losses in muscle tissue must have been accompanied by large losses in body zinc content. In terms of zinc requirements, such losses will require very high intakes of dietary zinc for proper rehabilitation and restoration of the body zinc.

Our use of percentage height for age as an index of past nutritional status as suggested by Waterlow (1976) showed that more than 75% of the malnourished children were retarded in height and the mean plasma zinc levels of children in the moderate and severe grades of growth retardation were significantly lower ( $P < 0.05$ ) than those of the control group. Meanwhile, plasma zinc levels

correlated positively ( $r = .61$ ) with height for age and thus plasma zinc concentration tended to decrease with increasing severity of retardation. Malnourished children who had normal height for age were obviously acute cases of malnutrition. Their mean zinc levels were higher ( $56.27 \mu\text{g}\%$ ) than those of the retarded groups. Thus, the relative difference in zinc levels between this group and the retarded groups tend to suggest a long existing zinc deficiency among the latter group.

A good proportion of rehabilitated children and indeed thousands of Nigerian children are known to be in sub-clinical level of protein energy malnutrition. The control group plasma level of  $73.15 \text{ mg/dl}$  is just slightly above the reported lower range ( $65\text{--}70 \text{ mg/dl}$ ) of normal zinc concentration in plasma of children (Committee on Nutrition, 1978). There is thus a probable widespread incidence of marginal zinc nutrition. The exact extent of such a marginal state of zinc nutrition and its likely response to dietary zinc supplementation is currently being investigated.

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