Nigerian Journal of Paediatrics, **3** (**2**), 45

Serum Calcium, Phosphate and Alkaline Phosphatase Levels in Protein-Energy Malnutrition

O. Olambiwonnu and A. Johnson
Department of Paediatrics, University College Hospital, Ibadan, Nigeria

Summary

Olambiwonnu, O. and Johnson, A. (**1976**). *Nigerian Journal of Paediatrics*, **3** (**2**), 45. **Serum Calcium, Phosphate and Alkaline Phosphatase Levels in Protein-Energy Malnutrition.** Serum calcium, inorganic phosphate, alkaline phosphatase, total protein and albumin were determined in 100 children with different types of protein-energy malnutrition (PEM). Twenty apparently healthy children selected from the general clinic population served as controls.

Hypocalcemia and hypophosphatemia were common in PEM, especially in kwashiorkor and nutritional oedema. Serum alkaline phosphatase was also low, even in those children with clinical and/or radiological rickets. It is concluded that "latent rickets" is common in all forms of PEM and that overt rickets occasionally occurs. There is however, no compelling need for routine calcium or Vitamin D therapy in these patients since the abnormal biochemical parameters return to normal with successful dietary management.

The mineral disturbances in patients with Protein-Energy Malnutrition (PEM) have been the subject of several studies and two recent reviews (McCance, 1971; Whitehead and Alleyne, 1972). In both reviews, only scanty comments were made on the clinical consequences of calcium and phosphate deficiencies in PEM. McCance, Rutishauser and Boozer (1970) conducted balance studies of several minerals, including calcium and phosphate, on six Ugandan children with kwashiorkor and found normal absorption and high retention of all the minerals. McCance (1971) also observed that hypocalcaemia and osseous lesions which are attributable to calcium deficiencies were rare in children with malnutrition. In contrast, Khan (1954) reported clinical and radiological rickets in two of eight infants with malnutrition. Similar predisposition of malnourished children to rickets were more recently observed by Nantulya and Okeahialam (1974) and Laditan and Adeniyi (personal communication).

The present study was undertaken to assess the serum calcium, inorganic phosphate and alkaline phosphatase status of children with different types of PEM, and to determine the need, if any, for supplementary calcium or vitamin D therapy in PEM.

Classification of PEM

The clinical and biochemical features of PEM present a wide spectrum which has led to numerous classifications. Perhaps the most

acceptable classification at present is that proposed in 1969 by an International Working Party at a Conference in Jamaica, West Indies. This classification, based upon the weight of the patient as a percentage of the expected average weight for age and the presence or absence of oedema, is limited in its use in our environment by the difficulty in obtaining the correct ages of many patients. We therefore elected to use in the present study the older but somewhat similar classification of Mukherjee (1967). By this classification, kwashiorkor is defined as PEM in which hypoproteinemia is marked, oedema is present along with skin and hair changes, with or without other evidence of multivitamin deficiency; while marasmus consists of severe growth retardation, and decreased subcutaneous fat, without oedema; and nutritional oedema patients have the clinical picture of marasmus except for the presence of oedema.

Materials and Methods

Blood was obtained via the jugular or femoral vein from 100 children with PEM. Twenty-three of these (11 boys and 12 girls) were classified as marasmic; 29 (20 boys and 9 girls) had nutritional oedema while 48 (18 boys and 30 girls) had kwashiorkor. These 100 children will be referred to as the study group. Twenty apparently healthy children (9 boys and 11 girls) aged 4 months to 10 years (mean age 2.3 years) served as controls. The age range of the children with kwashiorkor was 1.25-9.5 years (mean 3.8 years) whilst the age range of the children with nutritional oedema and marasmus were 9 months-8 years (mean 2.8 years) and 4 months–2.5 years (mean 1.3 years) respectively. Children with Hb genotype SS were excluded from the study. Seven children had Hb AS and the remaining 93 had Hb genotype AA.

Sera from the blood samples were extracted within a few hours of sampling and processed immediately or stored in a deep freeze until analysed. Serum calcium, phosphorus, and alkaline phosphatase were determined using the methods of Trinder (1960), Delsal and Manhouri (1958) and Kind and King (1954) respectively. Total protein and albumin were determined by the biuret and salting out methods respectively.

The height and weight of each child were measured at the first clinic visit and repeated at subsequent visits. Radiographs of the hands and wrists of each patient were obtained for assessment of skeletal maturation by the method of Greulich and Pyle (1959) and for evidence of rickets.

A majority of the children were successfully managed as out-patients with the cooperation of the Dieticians and Health Sisters, and the diligence of the mothers who were advised to give diets high in protein and calorie supplemented with multivitamins. The patients were reviewed weekly initially and subsequently at longer intervals. Those cases with severe PEM were admitted for inpatient management.

Results

Heights and Weights

Meaningful comparisons of heights and weights between the control and study groups could be drawn only in the 0–4-year groups because the number of patients in the older age group was inadequate. The mean height(70.3 cm) and weight (5.6 kg) of the children with marasmus were markedly lower than those of the control group (75.7 cm and 9.5 kg respectively). The mean weights of the children with nutritional oedema and kwashiorkor (8.4 and 9.1 kg respectively), despite varying degrees of fluid retention were less than that of the control children (9.5 kg), but the mean heights in these two types of PEM (80.7 and 83.3 cm respectively) were greater than that of control children (75.7 cm).

Serum Calcium

Seventeen (59 per cent) of 29 patients with nutritional oedema and 41 (85.4 per cent) of 48 with kwashiorkor had low serum calcium, while only 3 (13 per cent) of 23 marasmic

TABLE I

SERUM CALCIUM, INORGANIC PHOSPHATE CONCENTRATIONS (mg/100 ml) AND ALKALINE PHOSPHATASE (K.A. UNITS/100 ml) IN CHILDREN WITH PEM AND IN CONTROL CHILDREN

	Control	*Marasmus*	*Nutritional Oedema*	*Kwashiorkor*
Calicum:				
No. of Cases	20	23	29	48
Range	9.5–13.8	6.5–11.4	6.9–10.5	5.6–10.4
Mean	11.02	9.81[1]	8.74[1,2]	8.16[1,2,3]
SD	0.89	1.14	1.18	0.92
Phosphorus:				
No. of Cases	19	20	27	46
Range	3.7–6.7	1.7–6.2	1.9–4.7	1.5–5.0
Mean	5.30	4.30[1]	3.70[1]	3.47[1,2]
SD	0.70	1.10	0.74	0.94
Alkaline Phosphatase:				
No. of Cases	17	21	28	41
Range	6 –29	6 –27	6 –25	5 –24
Mean	18.30	13.76[1]	11.68[1]	10.77[1,4]
SD	6.66	5.05	4.80	4.40

1. $p < 0.001$ for differences from the mean for control
2. $p < 0.001$ for differences from the mean for marasmic children.
3. $p < 0.025$ for differences from the mean for nutritional oedema children.
4. $p < 0.05$ for differences from the mean for marasmic children.

children had low value (Table I). The mean calcium concentrations for patients with nutritional oedema and kwashiorkor were each significantly lower ($p<0.001$) than the mean for the control group. The mean for marasmic children, although within normal limits, was significantly lower ($p<0.001$) than the mean for the control group. Similarly, there was a significant difference between the mean concentrations for the children with nutritional oedema and kwashiorkor compared with the marasmic children ($p<0.001$). The difference between the concentrations in children with nutritional oedema and kwashiorkor was also significant ($p<0.025$).

Serum Inorganic Phosphate

This was uniformly lower in patients in the study group compared to the control group; it was most marked in children with nutritional oedema and kwashiorkor, among whom values as low as 1.9 and 1.5 mg/100 ml respectively were obtained. The mean concentrations for marasmic, nutritional oedema and kwashiorkor children respectively were significantly lower ($p<0.001$) than the mean concentration for the controls. The difference between the mean concentrations for marasmic and kwashiorkor children was also significant ($p<0.001$), but there was no significant difference between the concentrations in children with nutritional oedema and kwashiorkor. A less significant difference ($p<0.05$) was also observed between the mean concentrations for marasmic and nutritional oedema children (Table I).

Serum Alkaline Phosphatase

The mean concentrations of alkaline phosphatase for marasmic, nutritional oedema and kwashiorkor children respectively were lower than the values for control children. The values for patients with nutritional oedema and kwashiorkor respectively were significantly lower than those for controls ($p<0.001$). A less significant difference ($p<0.05$) was observed between the values for kwashiorkor and marasmus but the differences between the values for nutritional oedema and marasmus and nutritional oedema and kwashiorkor were not significant (Table I).

Total Serum Proteins

Mean total proteins of children with nutritional oedema and kwashiorkor were significantly lower than the mean concentrations for marasmic and control children (Table II). The differences between the mean concentrations for marasmic, nutritional oedema and kwashiorkor children were significantly less than the mean concentration for control children ($p<0.005$, $p<0.001$ and $p<0.001$ respectively) The mean concentration for marasmic children was significantly higher than the mean concentrations for nutritional oedema and kwashiorkor children ($p<0.001$) respectively; also the mean concentration for nutritional oedema children was significantly higher than the mean concentration for kwashiorkor children ($p>0.5$)

Serum Albumin

Table II also shows that only one (5 per cent) of 20 children with marasmus had albumin value below 2 gram/100 ml, whereas 14 (50 per cent) of the 28 children with nutritional oedema and 39 (81 per cent) of 48 children with kwashiorkor did. Albumin as low as 0.8 gram/100 ml was observed in one child with kwashiorkor. The differences between the mean concentrations for the control and the study groups were significant ($p<0.001$) respectively. Similarly, the differences among the mean concentrations for the study groups were also significant ($p<0.001$).

TABLE II

TOTAL SERUM PROTEIN AND ALBUMIN (GRAM/100 ml) IN CHILDREN WITH PEM AND IN CONTROL CHILDREN

	Control	*Marasmus*	*Nutritional Oedma*	*Kwashiorkor*
Total Serum Protein				
No. of Cases	19	21	28	47
Range	5.7–7.7	4.0–7.4	3.2–5.8	2.7–6.8
Mean	6.77	5.94[1]	4.66[2,3]	4.11[2,3,4]
SD	0.50	0.93	0.80	0.74
Serum Albumin				
No. of Cases	19	20	28	47
Range	3.0–4.5	1.7–4.1	0.9–3.3	0.8–2.7
Mean	3.57	2.94[2,3]	2.03[2,3]	1.55[2,3,5]
SD	0.44	0.62	0.52	0.42

1. $p<0.005$ for difference from the mean for the control.
2. $p<0.001$ for difference from the mean for control
3. $p<0.001$ for difference from the mean for marasmic children.
4. $p<0.05$ for difference from the mean for nutritional oedema
5. $p<0.001$ for difference from the mean for nutritional oedema.

Radiographic Findings

Bone age retardation was observed in 21 (80.8 per cent) of 26 children with known chronological ages in the study groups. This was most marked in children with kwashiorkor in whom bone age retardation of 6 months to 3.6 years occurred in 12 (85.7 per cent) of 14 children. Only four (80 per cent) of 5 children with nutritional oedema and 5 (71 per cent) of 7 children with marasmus showed a similar degree of bone age retardation. Subjective evaluation of the X-rays of the hands and wrists suggested diminished bone density and thin cortices in 15 children (kwashiorkor 6, nutritional oedema 6 and marasmus 3). Two patients with marasmus had clinical signs of rickets and showed typical radiological changes at the long bone metaphyses before treatment, while one patient with nutritional oedema developed clinical rickets during treatment.

All patients with kwashiorkor and nutritional oedema and some with marasmus exhibited generalized weakness and apathy; few patients had tremors of the extremities but no overt tetany was observed. A trend towards normalization of all chemical parameters after only two to six weeks of dietary management was observed in some of the malnourished children. Table III shows the data in those children whose serum calcium, phosphate and alkaline phosphatase had returned to normal within that period.

TABLE III

EFFECT OF DIETARY MANAGEMENT ON SERUM CALCIUM, PHOSPHATE, ALKALINE PHOSPHATASE, TOTAL PROTEIN AND ALBUMIN IN 11 CHILDREN WITH PEM

Patient and Hospital No.	*Calcium*		*Phosphate*		*Alk. Phos.*		*Total Protein*		*Albumin*	
	Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Nutritional Oedema										
KJ 341791	7.6	10.8	3.9	5.2	6	11	5	6.4	0.2	2.7
RI 345761	8.7	11.5	2.6	4.2	11	17	4.2	5.0	1.8	1.6
SR 344040	7.8	9.3	4.6	4.1	10	8	3.9	4.3	2.5	3.1
MR 349023	8.0	8.8	1.9	3.8	18	22	–	5.2	–	2.0
AL 352627	8.9	10.2	5.6	4.4	–	12	6.2	6.4	1.8	2.4
Kwashiorkor										
ST 342008	7.1	8.2	2.8	4.3	12	12	3.5	7.1	1.0	2.2
DS 342942	6.4	10.1	4.3	5.2	–	11	4.4	6.3	2.1	2.8
BO 345814	8.3	11.7	4.0	5.0	5	14	4.2	6.5	2.1	3.6
AY 274434	7.0	9.7	4.0	6.4	10	–	3.9	6.4	1.1	2.5
MS 348177	7.6	10.8	4.9	5.6	9	19	4.2	–	1.6	–
JO 350558	8.5	9.4	–	–	9	7	3.7	6.3	1.3	2.5

Discussion

Of the several mineral disturbances in PEM one of the least emphasized is disturbance of calcium metabolism and its clinical consequences. Walker (1958) reported that overt signs of calcium deficiency were rare in protein deficient children. More recently, McCance (1971) also observed that osseous lesions due to calcium deficiencies were rare and that low serum calcium concentrations were exceptional in children with malnutrition. However, the present study has clearly shown that hypocalcaemia, hypophosphataemia and low serum alkaline phosphatase are common in children with kwashiorkor and nutritional oedema. This finding may be ascribed to any of several factors.

Widdowson and McCance (1970) found that urinary excretion of calcium and phosphorus in a group of African children was much less than in British children of comparable age. These authors inferred that this was due to deficiency of these minerals in the diet of the African children particularly after the first year of life. Although breast milk and most local Nigerian foodstuffs contain adequate amounts of Vitamin D and calcium (Antia 1970), it is known that pap (maize porridge), the commonest weanling diet among the Yoruba in Western Nigeria, is deficient in calcium (Winthrop Products, 1965). Other potential causes of hypocalcaemia are malabsorption and diarrhoea (Bowie, Brinkman and Hansen, 1965; Wharton, Jelliffe and Stanfield, 1968) but the Ugandan children with PEM, studied by McCance, Rutishauser and Boozer (1970) had normal mineral absorption and high mineral retention. Hypocalcaemia may also be ascribed to the low serum albumin found in both kwashiorkor and nutritional oedema. However, in the present study, not all patients with low serum albumin had hypocalcaemia. Serum calcium was greater than 9 mg/100 ml in six patients with nutritional oedema and in six others with kwashiorkor. The serum albumin level was less than 2 gm/100 ml in all these patients which suggests that low albumin *per se* is not the only factor causing hypocalcaemia in them.

Low serum phosphorus could be a reflection of gastrointestinal or urinary losses. Vitamin D deficiency and vitamin D resistance, as well as secondary hyperparathyroidism due to the hypocalcaemic state (Arnaud, Glorieux and Scriver, 1972) are other possible causes.

Whether or not the low alkaline phosphatase is a reflection of hepatic abnormality leading to failure of the normal contribution by the liver remains to be elucidated. The low alkaline phosphatase was certainly not due to age factor since clear differences were found among children of the same age groups. It is however quite conceivable that our finding is a result of the relative or absolute cessation of growth in these children, with decreased osteoclastic and osteoblastic activities. Unfortunately, urinary hydroxyproline which reflects the turnover of skeletal collage and is closely related to growth rate (Tanner, Whitehouse and Takaishi 1966) could not be determined in our patients. Our findings are in agreement with that of Nantulya and Okeahialam (1974) who found low or normal alkaline phosphatase in kwashiorkor and marasmus patients with clinical and radiological rickets. It also confirms the findings by Salimpour (1975) of low phosphorus and low alkaline phosphatase in malnourished children with rickets.

The present study thus shows that overt rickets in children with PEM may not be as rare as has been suggested by other workers (Walker, 1958; McCance, 1971). Overt rickets (clinical and/or radiological) was present in three of our patients and biochemical rickets (hypocalcaemia and hypophosphataemia) in a majority of them. Similar findings of either overt or subclinical rickets in malnutrition have been reported from South Africa (Kahn, 1954; Wayburne, 1968), from West Africa (Antia, 1970) and from India (Ghosh *et al.*, 1966).

It is noteworthy that the affected children responded to proper diet and vitamin supplements. This response to dietary management suggests that lack of adequate intake of calcium and/or Vitamin D was the major cause of overt and subclinical rickets in the present study. Further studies such as calcium balance, bone densitometry, assays of Vitamin D or its metabolites are likely to result in a better understanding of the significance of hypocalcaemia and hypophosphataemia in PEM.

Acknowledgements

Professors S. P. Bohrer and A. U. Antia gave valuable suggestions. We are also grateful to Dr O. Ayeni, Medical Statistician, Department of Preventive and Social Medicine, University of Ibadan for statistical analysis, and Mrs S. A. Sobulo, Department of Chemical Pathology who did the chemical determinations.

REFERENCES

Antia, A. U. (1970). Observations on nutritional rickets in childhood. *West Afr. Med. J.* **19,** 169–172.

Arnaud, C., Glorieux, F. and Scriver, C. R. (1972). Serum parathyroid hormone levels in acquired vitamin D deficiency of infancy. *Pediatrics,* **49,** 837–840.

Bowie, M. D., Brinkman, G. L. and Hansen, J. D. L. (1965). Acquired disaccharide intolerance in malnutrition. *J. Ped.* **66,** 1083–1091.

Delsal, J. L. and Manhouri, H. (1958). Comparative study of colorimetric determinations of phosphorus. IV Determination of orthophosphate in the presence of phosphoric acid esters; new methods. *Bull. Soc. Chim. Biol.* **40,** 1623–1636.

Ghosh, S., Bhardaway, O. P., Saini, L. and Narma, K. P. S. (1966). A survey of rickets. *Indian Pediat.* **3,** 9–14.

Greulich, W. W. and Pyle, S. I. (1959). A radiographic atlas of skeletal development of the hand and wrists, 2nd edition Stanford University Press, Stanford.

Kahn, E. (1954). A neurologic syndrome in infants recovering from malnutrition. *Arch. Dis. Childh.* **29,** 256–261.

Kind, P. R. N. and King, E. J. (1954). Estimation of plasma phosphate by determination of hydrolysed phenol with amino-antipyrine. *J. Clin. Path.* **7,** 322–326.

McCance, R. A., Rutishauser, I. H. E. and Boozer, C. N. (1970). The effect of kwashiorkor on the absorption and excretion of N, fat and minerals. *Arch. Dis. Childh.* **45,** 410–416.

McCance, R. (1971). Malnutrition in the children of under-developed countries. In "Recent Advances in Paediatrics" Ed. Gardner, D. & Hull, D. pp. 479–525. J. & A Churchill, London.

Mukherjee, K. L. (1967). Classification of protein-calorie undernutrition in children. *Arch. Dis. Childh.* **42,** 647–651.

Nantulya, V. M. and Okeahialam, T. C. (1974). Serum protein and alkaline phosphatase levels in protein calorie malnutrition with special reference to the diagnosis of rickets. *E. African J. Med. Res.* **1,** 253–263.

Salimpour, R. (1975). Rickets in Tehran: Study of 200 cases. *Arch. Dis. Childh.* **50,** 63–66.

Seminar on Protein Malnutrition, Jamaica (1969). *Lancet* (1970). **2,** 302–303.

Tanner, J. M., Whitehouse, R. H., Takaishi, M. (1966). Standards from Birth to Maturity for Height, Weight, Height Velocity and Weight Velocity: British Children, 1965. *Arch. Dis. Childh.* **41,** 454–471, 613–635.

Trinder, P. (1960). Colorimetric micro-determination of calcium in serum. *Analyst.* **85,** 889–894.

Walker, A. R. P. (1958). Certain biochemical findings in man in relation to diet. *Ann. N. Y. Acad. Sci.* **69,** 989–1008.

Wharton, B. A., Jelliffe, D. B. and Stanfield, J. P. (1968). Do We Know How to Treat Kwashiorkor? *J. Ped.* **72,** 721–726.

Wayburne, S. (1968). Malnutrition in Johannesburg. In "Calorie Deficiencies and Protein Deficiencies" 1st Ed. McCance, R. A. and Widdowson, E. M. pp. 7–20, Churchill, London.

Winthrop Products, "Nutrition in Retirement Chart", 1965.

Whitehead, R. G. and Alleyne, G. A. D. (1972). Pathophysiological factors of importance in protein-calorie malnutrition. *Brit. Med. Bull.* **28,** 72–79.

Widdowson, E. M. and McCance, R. A. (1970). Use of random specimens of urine to compare dietary intakes of African and British children. *Arch. Dis. Childh.* **45,** 547–552.