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Plasma electrolytes, total cholesterol, liver enzymes, and selected anti-oxidant status in protein energy malnutrition

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Summary

Golden and Ramdath proposed the free radical theory of kwashiorkor, suggesting that the changes seen in kwashiorkor may be the result of an imbalance between the production and safe disposal of free radicals. In malnourished children, mineral metabolism and anti-oxidant status need renewed attention especially in relation to cause and functional significance of the changes in concentration of these substances. In the present study, the modified Wellcome classification was used to classify the protein energy malnourished children into kwashiorkor [32] marasmic-kwashiorkor [15], marasmus [12] and underweight [11]. Twenty-six healthy and normal children were used as controls. Standard procedures were used for the analyses of the biochemical parameters. Our results showed that plasma total cholesterol, sodium, potassium and bicarbonate, β-carotene, retinol and uric acid were significantly lower in the malnourished group than the control group ($P<0.05$), while transaminases were significantly increased in the malnourished group ($P<0.05$). These findings suggest an altered electrolyte and anti-oxidant status in protein energy malnutrition.

Keywords: *Cholesterol, Electrolytes, Enzymes, Anti-oxidant, Protein energy malnutrition*

Résumé

Golden et Ramdath ont proposes la theorie des radiocaux libres du Kwashiorkor, suggerant que les changements constates dans les cas de Kwashiorkor pourraient etre le resultat d'un d éséquilibre entre la production et la disposition saiene des radicaux libres. Chez les enfants malnourcis, le metabolism des mineraux et le status des antioxydant ont besoins d'un renouvellement d'attention surtout en relation avec la cause et la significance fonctionelle de les changements dans la concentration de ces substances. Dans la presente etude, la classification modifie Wellcome a ete utilise pour classifie l'energie les enfants malnourient ayant une deficience en proteine ayant le Kwashiorkor [32], le Kwashiorkor-marasmique [15], marasme [12] et en dessous du poids [11]. Vingt-six enfants en sante et bien portant ont été utilises comme controles. Les procedures standards ont été utilises pour analyser les parametres biochimiques. Nos resultat, ont montrés que le taux total de cholesterol, sodium, potassium. Et bicarbonate B-Carotene, retionol, et acide urique dans le plasma ont été trouvés significativement faible chez les enfants malnourrient par rapport aux controles (*P* 0.05). Les transaminases ont ete significativement eleves chez les malnourient (*P* 0.05). Ces resultats suggerent une alteration du status des electrolytes et des antioxydants dans la malnutrition due a la deficience en proteine.

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Introduction

Kwashiorkor with the characteristic sign of stunted growth, depigmentation of the hair, fatty liver, cracked skin and variable degree of oedema [1] first described by Williams in 1933 [1], was thought to be due to a deficiencv of protein rather than infection or vitamin deficiency. However, the exact metabolic factors responsible for this disorder remain largely unclear.

Recently, it was proposed that kwashiorkor results from an imbalance between the production of free radicals, which cause oxidative cellular damage [3-6], and their safe disposal [2] mediated by anti-oxidant system. These anti-oxidant systems consist of a group of enzymes; gluthathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase catalase, NADH-methaemoglobin reductase and superoxide dismutase as well as vitamins A,C,E, and β-carotene which prevent peroxidation of the structural membrane lipids [6,7].

While anti-oxidant deficiency results in ocular lesion, mineral metabolism in PEM requires attention especially in relation to the possible role of free radical imbalance in the observed changes [8-12]. Plasma uric acid as an anti-oxidant has not previously been studied in PEM and previous studies have shown that plasma concentrations of potassium and sodium may be normal [13] or reduced [14,15] in malnourished children. It has been suggested that the extent of hypokalaemia may reflect the degree of recovery from malnutrition and extent of alteration in plasma enzymes [16].

In the present study, the changes in plasma electrolytes, total cholesterol, liver enzymes as well as plasma retinol, β-carotene and uric acid were assayed in PEM in order to define the status in PEM.

Materials and method

Seventy malnourished children defined by Wellcome classification [17] into 32 cases of kwashiorkor, 15 cases of marasmic-kwashiorkor, 12 cases of marasmus and 11 cases of the underweight children were included in the study which was carried out in the Department of Paediatrics of the University College Hospital, Ibadan after approval of the Ethical Committee.

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The twenty-six well nourished children (age range 6 months to 5 years) attending the Children's Outpatient Department, of the University College Hospital, Ibadan, for routine check up served as controls. About 10 ml of blood was collected by venepuncutre into lithium heparin bottles in each case. The plasma was separated into clean plastic containers after centrifuging at 3 g for five minutes. Plasma retinol and β carotene were assayed by the procedure described by Tietz [18].

The plasma sodium and potassium concentrations were determined by the flame photometry method using Corning 410C-flame photometer, while plasma chloride, bicarbonate and haematocrit were assayed by standard methods. Total cholesterol was determined by the modified of Liebermann Buchard reaction [19]. Aspartate transaminase (AST), alanine transaminase (ALT) and uric acid were assayed using the multi-channel Hitachi auto-analyser (BM-Hitahci 704, Mannheim, Germany). Results are expressed as mean ± SD. Group comparisons were made using one-way analysis of variance (ANOVA); paired comparisons were carried out using the Student's t test and $P < 0.05$ was regarded as significant.

Results

Plasma sodium and potassium concentrations were significantly ($P < 0.05$) decreased in the malnourished children compared with the control group, but the slight inter-group differences for both cations within the malnourished groups were not statistically significant ($P > 0.05$). Plasma chloride concentrations were similar ($P < 0.05$) in all the groups while bicarbonate concentration was significantly ($P < 0.05$) lowest in the marasmic-kwashiorkor group with significant inter-group differences (Table 1).

The mean plasma total cholesterol was significantly ($P < 0.05$) lower in the marasamus and marasmic-kwashiorkor groups than in the control group ($P < 0.05$), with the lowest cholesterol in the marasmic group (Table 1).

Table 1: Prevalence of associated Infections among the malnourished children.

	Kwashiorkor	Marasmus	Marasmic-kwashiorkor
Total number	32	12	15
No. without infection (s)	10	4	3
Fever	15	5	8
Cough	1	-	2
Diarrhoea	2	1	-
Malaria	3	-	-
URTI	1	-	-
Measles	-	-	1
Angular stomatitis	-	-	1
Bleeding gum	-	1	-
Worms	-	1	-

URTI = Upper Respiratory Tract Infection

Significant ($P < 0.05$) elevations of ALT and AST activities were found in all the malnourished groups with significantly higher AST activity in the marasmic-kwashiorkor patients. Compared with healthy children, the mean plasma retinol, β-carotene, haematocrit and uric acid values were significantly reduced in the different malnourished groups ($P < 0.05$) with differences in the β-carotene levels between kwashiorkor and underweight children being statistically significant ($P < 0.05$). There were also significant differences in mean plasma retinol values between the marasmic-kwashiorkor children as well as between kwashiorkor and underweight children ($P < 0.05$).

The haematocrit showed a significant reduction in all the malnourished groups when compared with the control group ($P<0.05$) with inter-group variations within the malnourished groups (Table 2). The mean plasma uric acid levels were significantly lower in the marasmic-kwashiorkor and kwashiorkor groups when compared with the corresponding control values ($P<0.05$). Also the difference in plasma uric acid concentration between the marasimc-kwashiorkor and underweight children was statistically significant ($P<0.05$). Evidence of associated diseases was sought in the various groups with no significant differences in the presence of pyrexia on admission. (Table 1).

Table 2: Plasma electrolyte, total cholesterol and liver enzyme values.

	Marasmus N = 12	M-kwashiorkor n =15	Kwashiorkor n = 32	Underweight n = 11	Control n = 26	F values	*P* value
Sodium (mEq/L)							
Mean ± SD	132 ± 7 3	128 ± 8 5	132 ± 5 7	132 ± 5 4	137 ± 3 9	5.6	<0.003
Range	116 – 140	112 – 140	113 – 140	124 – 143	130 – 144		
Potassium (m/Eq/L)							
Mean ± SD	3 2 ± 1 1	3.1 ± 1.1	3 2 ± 0 7	3 5 ± 1 1	4 0 ± 0 6	3 6	<0.01
Range	1 9 – 6 2	1 1 – 4 7	1 5 – 4 6	1 8 – 5 9	2 9 – 5 8		
Bicarbonate (mEq/L)							
Mean ± SD	19.0 ± 3.2	18 ± 3.6	20 ± 3 3	22 ± 2.7	22 ± 2 7	3 5	<0 01
Range	15 0 – 26 0	10 – 26	14 – 28	19 – 27	19 – 27		
Chloride (mEq/L)							
Mean ± SD	104 ± 7 7	101 ± 6.1	104 ± 3 3	101 ± 6.8	104 ± 6.9	1.2	NS
Range	90 – 123	83 – 111	95 – 112	90 – 110	90 – 110	90 - 110	
Total Cholesterol (mg/L)							
Mean ± SD	84 ± 2 1	101 ± 20	101 ± 30	137 ± 33	136 ± 3.9	9.2	<0.001
Range	43 – 119	70 – 130	54 – 141	59 – 195	84 – 227		
ALT (IU/L)							
Mean ± SD	26 8 ± 17 6	58 7 ± 55.2	47 7 ± 54 3	35 8 ± 9 1	7 2 ± 5 8	5 4	<0 005
Range	10 0 – 63 0	1 0 – 225 0	1 0 – 287 5	1 0 – 93.0	1 0 – 28 0		
AST (IU/L)							
Mean ± SD	73 3 ± 34.9	85.1 ± 54.2	57 3 ± 29 2	69 3 ± 43 4	30 7 ± 10.7	7 6	<0.001
Range	31 0 – 152 0	1.0 – 207 0	1 0 – 149 0	1.0 – 172 0	16.0 - 54		

N = No. of subjects *NS = Not Significant* *M-kwashiorkor = Marasmic-kwashiorkor*

Table 3: β-Carotene, Vitamin A, haematocrit and uric acid values in malnutrition

	Marasmus n = 12	M-kwashiorkor n = 15	Kwashiorkor n = 32	Underweight n = 11	Control n = 26	F value	*P* value
β-Carotene (μg/dl)							
Mean ± SD	38.1 ± 20.1	44.9 ± 26.3	49.7 ± 18.1	31.0 ± 18.1	96.5 ± 57.3	9 3	< 0.001
Range	10.1 – 80.4	20.1 – 115.6	10.1 – 120.6	15.1 – 17 4	10.1 – 211.0		
Vitamin A (μg/dl)							
Mean ± SD	52.2 ± 25.2	43.4 ± 10.2	56.3 ± 31.6	56.6 ± 28.6	73.0 ± 25.1	3.4	< 0.01
Range	32.5 – 198.1	35.5 – 119.2	10.8 – 141.8	21.6 – 173.3	10.8 – 135.4		
Haematocrit (%)							
Mean ± SD	28.7 ± 4.8	26.3 ± 5.0	28.5 ± 4.1	31.4 ± 6.9	36.7 ± 3.1	17.6	< 0.001
Range	20.0 – 38.0	15.0 – 33.0	20.0 – 36.0	20.0 – 43.0	20.0 – 41.0		
Uric acid (μg/dl)							
Mean ± SD	3 8 ± 1 5	2.7 ± 1.4	3.4 ± 1 5	4 0 ± 1.8	4 1 ± 0.9	3.0	< 0.05
Range	1.5 – 5.8	1.0 – 4.5	1.0 – 7.4	1 0 – 7 2	2.3 – 6.0		

Discussion

Deficiency of macro- and micro-nutrients are associated with malnutrition with stress factors associated with malnutrition resulting in the generation of free radicals and subsequent reduction in plasma anti-oxidants in these children [20] as confirmed by the low anti-oxidant status in our study. Seve *et al.* [21] demonstrated that red cell anti-oxidant enzymes such as selenium-dependent glutathione peroxidase and superoxide dismutase are low in kwashiorkor though not studied in our patients. However, our study confirmed low plasma anti-oxidants in malnutrition. The malnourished children presented with infections and these can cause further increase in free radical production.

The study by Laditan and Reeds (1976) has emphasized the importance of inadequate diet and infection in the aetiology of kwashiorkor and marasmus [22]. It is therefore difficult to separate the effects of these two factors on the observed levels of anti-oxidants in children with PEM. It is possible that the low level of circulating anti-oxidants is due to either increased utilisation of micro-nutrient anti-oxidants or decreased synthesis of such anti-oxidants in protein malnourished children. However, this study did not demonstrate any differences related to the extent or presence of infections within the groups. The deficiency of these micro-nutrients, which scavenge free radicals, leads to the build up of excess free radicals, which would cause oxidative damage to the lens of the eye and also the skin lesions.

In the present study, there were no obvious ocular lesions in the malnourished children, probably because none of the malnourished children has a plasma vitamin A level below 10 ug/dl. This is consistent with earlier findings indicating that vitamin A supplementation reduces morbidity and mortality significantly in malnourished children [23]. Also vitamin A levels returned to normal within 8 days of vitamin A supplementation, suggesting dietary deficiency of vitamin A in malnourished children.

Anaemia is a common feature in the malnourished children [24] and this is more pronounced in the marasmic-kwashiorkor patients. Among the major factors responsible for the anemia in different groups are changes in the red blood cell, associated infections as well as deficiencies of iron, protein and vitamins in their diet [24]. These changes in the haematological system are consistent with earlier findings in malnourished children [24]. The excess production of free radicals in malnourished children [2] may cause substantial oxidation of protein and sulphydryl groups in the red blood cells leading to possible dissociation of hemoglobin into free units [25] which can cause a substantial loss of iron and protein from the red blood cells and may explain the reason for the relatively low haematocrit obtained in this study as experimental protein deformation has been shown to cause anaemia in animal models [26]. Another possibility is the preventable destruction of erythrocytes resulting from membrane damage caused by incomplete detoxification of superoxide radical and hydrogen peroxide in erythrocytes. The activities of superoxide dismutase and glutathione peroxidase; the two enzymes involved in the detoxification process are low in PEM due to the well-established deficiency of both copper and selenium in the condition [27]. As a result, the red blood cells become very vulnerable to membrane damage caused by exposure to superoxide radicals or hydrogen peroxide [25], which are known to be generated in excess in malnourished children [2].

Though there are no previous reports on uric acid status in PEM, the present study showed significantly decreased concentration of uric acid, which is a known anti-oxidant [28] and an endogenous free radical scanvenger [29]. This low plasma concentration of uric acid suggests that the ability of children suffering from marasmic-kwashiorkor and kwashiorkor to cope with higher radicals using uric acid as an anti-oxidant will be reduced. Alternatively, it could also mean a higher free radical concentration that has depleted the available uric acid by oxidation. This appears to be a more plausible explanation since uricase is non-existent in man.

The mean plasma sodium and potassium were significantly decreased in the malnourished children, though the chloride concentrations were not deranged. The measurement of electrolytes in this present study was to ascertain whether or not the cellular compartments are well maintained in malnourished patients. The plasma bicarbonate is of particular importance as it was found to be particularly low in our marasmic-kwashiorkor patients. The results of the present study suggest alteration in the electrolyte balance in the different malnourished groups and this may be due to destructive actions of excess free radicals at the plasma membrane [30] in malnourished children. Varying results as regards electrolyte composition have been obtained in the past in malnourished children [31]. These inconsistent findings have been attributed to the type and degree of malnutrition as well as the presence or absence of infection and differences in the electrolyte composition of the different diets in the various parts of the world [31].

Significant elevations of AST and ALT were demonstrated in this study as in other previous studies [32]. This could be suggestive of liver injury due to hepatotoxins [32] in these malnourished children as well as destructive effects of excess free radicals [33] which may cause lipid peroxidative changes, tissue damage and changes in membrane structure and function [33] of the liver. These damaging effects may be more pronounced in malnourished children with fatty liver.

The reduced total cholesterol found in the malnourished children in the present study could be due partly to their poor diet as well as to increased oxidation of membrane lipids and transmembrane proteins in the malnourished individuals, since free radicals are known to attack such molecules [34]. Previous reports show that plasma cholesterol could be low, normal or increased in marasmic patients [35].

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