LIVER FUNCTION STATUS IN SOME NIGERIAN CHILDREN WITH PROTEIN ENERGY MALNUTRITION

By

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SUMMARY

Objective: To ascertain functional status of the liver in Nigeria Children with Protein energy malnutrition

Materials and Methods: Liver function tests were performed on a total of 88 children with protein energy malnutrition (PEM). These were compared with 22 apparently well-nourished children who served as controls. High performance enzymatic colorimetric commercial kits (RANDOX Laboratories Ltd. UK) were used for the assay. Anthropometry, haematocrit, liver enzymes and bilirubin levels of the patients and controls were measured.

Results: Serum total proteins and albumin fraction were significantly reduced ($p < 0.05$) in all the types of PEM compared with the controls. There was marked hypoproteinaemia and hypoalbuminaemia in kwashiorkor. Haemoglobin (Hb), packed cell volume (PCV) and alkaline phosphatase (ALP) levels were reduced ($p < 0.05$) while aspartate transaminase (AST), and alanine transaminase (ALT) were significantly elevated ($p < 0.05$). Although serum unconjugated bilirubin was reduced in the PEM cases, its value did not differ significantly in marasmic patients.

Conclusion: These findings suggest that abnormalities in serum levels of these parameters occur in any form of PEM and are related to the severity of the condition.

Key Words: *Liver Function status in PEM; Nigeria*

INTRODUCTION

Malnutrition is a global problem that has been recognized for centuries, which leads to retardation of growth and development in childhood[1,2]. A study of childhood mortality in Latin American countries cited malnutrition as the cause of death in over 1/3 of children less than five years of age[3].

The development of malnutrition depends on a complex of interactions between the host and his environment[4]. Altered biochemical parameters in protein energy malnutrition (PEM) have been related to inadequate nutrient intake encompassing micro and macro nutrients in graded proportions[1,5]. Prolonged dietary protein deficiency results in significant reduction in somatic and visceral protein levels[6]. Plasma proteins are reduced in kwashiorkor, the greatest reduction being the albumin fraction[7,8].

Albumin is the single most important substance that contributes to plasma colloidal osmotic pressure. Marked hypoproteinaemia and hypoalbuminaemia have been reported among kwashiorkor patients in South Africa, India, Jamaica and Nigeria[9,10,11]. Hypoalbuminaemia was believed to be the main cause of oedema in PEM. Plasma globulin levels appear unaffected by nutritional states; rather its synthesis appears increased in the presence of infection.

The liver is uniquely placed for handling dietary compounds because it receives most of the blood from the gut via the portal venous system and is responsible for the synthesis of many metabolically important compounds from diet-derived precursors and for their inter-conversion. Albumin and most other proteins including coagulating factors are synthesized in the liver parenchyma cells. Normal albumin synthesis is about 10g/24hours, and this represents about 30% of the total protein synthetic capacity of the liver.

When the liver is diseased, some but not necessarily all its functions are impaired. Some membrane bound enzymes of the biliary epithelial cells of the liver canaliculi such as alkaline phosphatase (ALP) show a rise in their plasma activity when there is cholestasis. Raised plasma ALP, particularly to more than 180IU/L (and usually in association with a raised plasma bilirubin), provides an indication of extrahepatic or intrahepatic biliary obstruction e.g. in primary

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biliary cirrhosis. Also active hepatocellular damage is reflected by increased plasma levels of aspartate transaminase (AST), reference range being 11 – 35 IU/L and alanine transaminase (ALT), reference range being 5 – 40IU/L. Plasma bilirubin have also been found to act as a determinant of the excretory ability of the liver[12].

The objective of this study is to investigate the liver status of children suffering from PEM using as markers serum proteins, ALP, AST, ALT and serum bilirubin.

MATERIALS AND METHODS

Blood sample and patients

The subjects used in this study were recruited from Massey Specialist hospital, Ikeja General Hospital, Lagos and Central Hospital Benin City. After applying minimal pressure to the forearm with an automatic tourniquet to avoid haemolysis, 10ml of whole blood samples were collected from each of 88 PEM children and 22 controls. The samples were allowed to stand for one hour at room temperature so that the clot can retract.

The serum was removed with a Pasteur pipette into venojet tubes and centrifuged at 10,000g for about 5 minutes at room temperature to remove unwanted red cells. The serum was then kept in a sterile venojet tube, stoppered and stored at 4°C until analyzed.

All the reagents and chemicals were analytical grade.

Enzymic Assays

High performance enzymatic colorimetric commercial kits supplied by RANDOX Laboratories Ltd. UK were used for ALT and AST using the method described by Reitman and Frankel[13]. ALP was assayed using the method of Kind and King[14].

Protein Assays

Serum globulin and total protein were estimated using the methods of Goldenbery and Drewes[15] and Gornall et al[16] respectively. Whilst serum albumin was estimated using the method of Doumas and Biggs[17].

Bilirubin Assay

Total bilirubin, conjugated bilirubin and unconjugated bilirubin were estimated using the method of Winkelman et al[18].

Statistical Analysis

All data were expressed as means ± standard error of means (SEM). Analysis of variance (ANOVA) was used to detect any significant difference among the mean of groups while Duncan's multiple range test was used to test the significant difference among the means[19].

RESULTS

Table 1
Characteristics of the Different PEM and Control Groups

Criteria	Control	Kwashiorkor	Marasmus	Marasmic Kwashiorkor	Undernutrition
PCV %	43.4±0.70^a	22.5±0.70^b	17.70±0.80^c	21.7±0.60^c	19.50±0.80^c
HB g%	14.3±0.30^a	7.80±0.20^b	6.00±0.20^c	7.20±0.20^c	6.40±0.30^c
Age (months)	11.3±2.96	13.0±2.89	14.17±3.30	10.29±1.82	13.45±3.08
Height (cm)	71.59±1.40	70.97±3.12	68.65±1.64	70.00±0.72	69.00±0.40
Weight (kg)	7.65±1.18	5.86±0.94	4.92±0.97	5.77±0.19	6.99±2.03
Mid arm circumference (cm)	14.00±3.01	9.35±0.67	7.77±0.62	7.41±2.18	11.13±0.19

Values are expressed as mean± Standard Error of Mean (SEM)
Means of the same row followed by different letter differ significantly; b: $p<0.05$; c: $p<0.01$.

The characteristics of the different PEM and control groups are shown in table 1. The PCV and Hb content of red blood cells were significantly reduced in all PEM cases. The reductions were more significant in marasmus, marasmic kwashiorkor and under nutrition ($p < 0.01$) than in kwashiorkor patients ($p < 0.05$).

Table 2
Serum Enzyme Levels of PEM Children and Controls

	Enzyme(u/l)	Control	Kwashiorkor	Marasmus	Marasmic Kwashiorkor	Undernutrition
Male	**AST**	13.4 ± 0.9^{a}	30.5 ± 0.9^{b}	41.1 ± 2.8^{c}	42.0 ± 2.4^{c}	31.6 ± 1.1^{b}
	ALT	10.6 ± 0.6^{a}	28.3 ± 0.8^{b}	24.2 ± 2.7^{b}	34.6 ± 2.2^{c}	26.3 ± 1.8^{b}
	ALP	189.9 ± 0.4^{a}	102.5 ± 3.5^{d}	163.6 ± 3.3^{b}	127.7 ± 3.6^{d}	144.4 ± 7.5^{c}
Female	**AST**	12.7 ± 0.9^{a}	33.2 ± 1.2^{b}	38.8 ± 2.8^{c}	38.8 ± 2.0^{c}	32.6 ± 1.5^{b}
	ALT	9.8 ± 0.7^{a}	29.8 ± 0.8^{b}	22.9 ± 3.3^{c}	33.9 ± 1.8^{d}	24.0 ± 2.5^{b}
	ALP	194.54 ± 1.4^{a}	107.90 ± 5.2^{d}	162.5 ± 2.6^{c}	128.0 ± 3.1^{d}	135.0 ± 5.9^{d}

Values represent mean ± SEM
Means of the same row followed by different letters differ significantly; b: $p<0.05$; c: $p<0.01$; d: $p<0.005$.

Table 2 shows the enzyme levels of AST, ALT and ALP used to determine the functional status of the liver. Results obtained indicate that the levels of AST and ALT were significantly elevated in all the PEM cases including male and female patients, while ALP levels were significantly reduced.

Table 3
Serum Protein Levels of PEM Children and Controls

	Serum Protein g/L	Control	Kwashiorkor	Marasmus	Marasmic Kwashiorkor	Undernutrition
Male	**Total protein**	69.8 ± 0.9^{a}	51.9 ± 1.3^{b}	57.6 ± 2.4^{b}	53.7 ± 1.9^{b}	56.8 ± 1.5^{b}
	Albumin	39.6 ± 1.1^{a}	24.4 ± 0.8^{b}	26.9 ± 0.8^{b}	28.5 ± 0.7^{b}	30.5 ± 0.7^{b}
	Globulin	30.2 ± 0.7^{a}	27.5 ± 1.7^{a}	26.8 ± 1.3^{a}	29.0 ± 2.1^{a}	26.3 ± 1.5^{a}
	Albumin/Globulin Ratio	1:1.31	1:0.89	1:1.0	1.:0.98	1:1.2
Female	**Total Protein**	70.3 ± 1.1^{a}	52.5 ± 1.1^{b}	48.9 ± 0.9^{c}	52.1 ± 1.3^{b}	55.8 ± 2.0^{b}
	Albumin	38.5 ± 0.6^{a}	25.9 ± 1.0^{b}	25.0 ± 0.5^{b}	25.7 ± 0.8^{b}	28.9 ± 1.3^{b}
	Globulin	34.0 ± 1.3^{a}	26.6 ± 0.8^{a}	23.0 ± 0.8^{b}	26.0 ± 1.4^{a}	28.2 ± 1.6^{a}
	Albumin/Globulin ratio	1:1.31	1:0.97	1:1.08	1:0.98	1:1.02

Values represent mean ± SEM
Means of the same row followed by different letter differ significantly; b: $p<0.05$; c $p<0.01$

The serum protein levels of PEM children are shown in table 3. Total protein and albumin levels were significantly reduced in all PEM cases ($p < 0.05$) with the least level being recorded in kwashiorkor patients in males (51.91 ± 1.3; 24.4 ± 0.8 respectively) and marasmus in females (48.9 ± 0.9; 25.0 ± 0.5 respectively). However, there was no significant difference in the globulin level of all the PEM cases ($p < 0.05$) in both male and female patients except in female marasmic children ($p < 0.05$) with albumin/globulin ratio of 1:1.08. The albumin/globulin ratio was least in kwashiorkor patients (1.0: 0.89 for males; 1.0:0.97 for females) indicating that the greatest protein depletion occurs in kwashiorkor.

Table 4
Serum Bilirubin of Children with Protein Energy Malnutrition

	Analyte mg%	Control	Kwashiorkor	Marasmus	Marasmic Kwashiorkor	Undernutrition
Male	**Total Bilirubin**	0.72 ± 0.07^a	0.63 ± 0.03^b	0.86 ± 0.05^b	0.73 ± 0.06^a	0.46 ± 0.05^c
	Conjugate Bilirubin	0.17 ± 0.01^a	0.16 ± 0.01^a	0.21 ± 0.03^a	0.19 ± 0.01^a	0.15 ± 0.01^a
	Unconjugated Bilirubin	0.64 ± 0.04^a	0.48 ± 0.03^b	0.64 ± 0.05^a	0.54 ± 0.05^b	0.33 ± 0.04^c
Female	**Total Bilirubin**	0.79 ± 0.02^a	0.60 ± 0.01^b	0.77 ± 0.05^b	0.71 ± 0.05^a	0.54 ± 0.03^c
	Conjugated Bilirubin	0.18 ± 0.02^a	0.19 ± 0.01^a	0.18 ± 0.01^a	0.19 ± 0.01^a	0.16 ± 0.16^a
	Unconjugated Bilirubin	0.64 ± 0.04^a	0.48 ± 0.03^b	0.64 ± 0.05^a	0.54 ± 0.05^b	0.33 ± 0.04^c

Values represent mean ± SEM
Means of the same row followed by different letter differ significantly; b: $p<0.05$; c $p<0.01$

The serum bilirubin levels of PEM children compared with that of control group are presented in table 4. The total and unconjugated bilirubin values were significantly reduced in kwashiorkor ($p<0.05$): males (0.63 ± 0.03; 0.48± 0.03mg% respectively); females (0.60 ± 0.01; 0.48 ± 0.03mg% respectively); undernutrition ($p < 0.01$): males (0.46± 0.5; 0.33± 0.04mg% respectively); females (0.54 ± 0.3; 0.33 ± 0.04mg% respectively); and unconjugated bilirubin only in marasmic kwashiorkor ($p < 0.05$): males (0.54 ± 0.05mg%); females (0.54 ± 0.5mg%). The total bilirubin was significantly elevated in marasmic males only ($p < 0.05$; 0.86 ± 0.05mg%). There was no significant difference ($p > 0.05$) in conjugated bilirubin for all the PEM classes.

DISCUSSION

Nutritional deficiencies constitute major public heath problems in tropical and sub-tropical regions of the world. Though the deficiency diseases are primarily due to inadequate diets, they are closely related to poor socio=economic and environmental conditions prevailing in these areas. Among these, PEM is most widespread. Respiratory infection and diarrhoea are the common diseases that precipitate severe PEM and death[20]. The liver stands out unique in the human body and plays an intricate and diverse role in metabolism, it appears that a change it the physiological function of the liver could affect the general metabolism.

When the liver is diseased, some but not necessarily all of its functions are impaired. Disruption of the normal liver architecture results in increased pressure in the portal system. Many so called liver function tests in fact reflect damage to hepatocytes or biliary epithelial cells rather than their function. Thus, a combination of the levels of the enzymes ALP, ALT and AST, bilirubin (total and conjugated) and the protein have always been analyzed to ascertain the effectiveness of the liver[21]. PEM has recently been documented as one of the common causes of morbidity and mortality in Nigerian children[8].

Earlier studies on its aetiology have shown evidence of lack of protein, energy, vitamins and minerals particularly iron resulting in iron deficiency anaemia[22]. Observations from this study showed that the PCV level and Hb concentrations were significantly reduced ($p < 0.05$) in all PEM cases. Marasmus and undernutrition has the lowest level compared to control groups. The total serum protein and albumin were significantly lower in all the PEM patients with marked hypoproteinaemia amongst those cases with kwashiorkor. Serum albumin reduction is partly contributable to oedema seen in kwashiorkor and marasmic kwashiorkor and may be valuable in estimating the incidence of moridity[23]. Serum albumin concentration also correlates with body cell mass[24]. Albumin is the most important substance that contributes to plasma colloidal osmotic pressure[9]. Hypoalbuminaemia thus predisposes to oedema.

Golden et al[6] demonstrated a significant correlation of hypoproteinaemia with the degree of oedema. The low level of total serum proteins and albumin in all cases of PEM can be attributed to low protein intake in the diet leading to marked hypoproteinaemia earlier reported by Atinmo et a[18] or as a result of reduced synthesis by an ailing liver. However, the cause of reduced total body protein appears to be multifactorial. A combination of reduced gastric acid output, atrophic intestines, with marked reduction in pancreatic enzymes production, intercurrent infection, severe diarrhoea and the overriding effect of reduced dietary protein intake have been suggested[25,26].

ALP is an enzyme that mediates some of the complex reactions of bone formation. It is most abundant in the liver and bones with smaller quantities found in the small intestine and kidneys. A significant decrease was observed in all PEM cases studied ($p < 0.05$). This decrease was accompanied by lack of any change in serum conjugated bilirubin which therefore negates any physiological distress of the liver.

Elevated levels of AST and ALT were observed in this study and thus collaborates earlier findings of Kumari et al[21]. Transaminases catalyze the transfer of amino and keto groups between α-amino acid and α-keto groups. AST is found mainly in the tissue while AST is more abundant in the liver and is a more indicative parameter of liver disease. The observed increase recorded in these enzymes is a strong indication of the increased protein breakdown from the tissues since intake is limited. Dependence on tissue protein breakdown rather than dietary supply therefore correlates the hypoalbuminaemia and hypoproteinaemia observed in the PEM patients.

There is apparently no pathological manifestation of liver as evidence by the normal conjugated bilirubin levels obtained, but the additional metabolic stress placed on this organ is confirmed by the relative increase in the levels of AST and ALT. The levels of these enzymes were appreciably elevated when compared to controls. In fact, the clinical manifestations of malnutrition are well understood but considerable physiological and biochemical abnormalities still remain obscure.

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