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ORIGINAL ARTICLE

Possible Biochemical Markers in *Plasmodium falciparum* Malaria Infected Children with or without Malnutrition at Webuye and Eldoret, Western Kenya

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ABSTRACT

Malaria and Protein Energy Malnutrition are major health problems of concern in Kenya especially during rainy season in March to May and during the dry season from December to February every year. Assay of selected biochemical factors was carried out on children affected by these two disease conditions. To determine possible biochemical markers in children suffering from P. Falciparum malaria and Protein Energy Malnutrition in a Hospital setting in Western Kenya. Spectrophotometric assays of selected biochemical parameters: Albumin, Total proteins, Glucose, Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and Bilirubin. The assays were done on serum samples obtained from children < 5 years of age admitted to the paediatric ward as well as outpatient clinics at Webuye District Hospital and MTRH in Western Kenya suffering from either or both of the two disease conditions. Plasma albumin levels showed 33% of the children to be below the normal range and 40% above normal, Mean total protein concentration was 56.0mg/l, Mean glucose concentration was 65 mmol/l, GOT and GPT concentrations were 9.0 and 5.9 µl/l respectively. Total bilirubin was 0.3 mg/dl while mean concentration for Creatinine was 0.75 mg/dl.The biochemical parameters studied did not show any unusual values at the time of the assays, but Serum glucose and Albumin levels showed potential as diagnostic markers for the two disease conditions.

INTRODUCTION

Most of the estimated one or two million malaria deaths occur every year in children aged 1-5 years who live in malaria hyperendemic areas [1]. The most common and important complications of *falciparum* malaria in children are cerebral malaria and severe anaemia. Other complications of *falciparum* malaria occur in children but are less common than in adults [2].

Plasma albumin levels have been shown to be lower in patients suffering from severe *Plasmodium falciparum* malaria as compared to non-infected individuals [3]. In malnutrition, plasma albumin has been reported to be useful in determination of Kwashiorkor . Of all the serum proteins, only albumin measurement is of proven value in the detection of sub-clinical malnutrition in kwashiorkor endemic areas. It was further shown [4] that albumin was one of the biochemical factors that were found to be low in serum of malnourished patients. It could thus be used as a predictor of nutritional status and was independently influenced by age and sex. Although use of albumin, cholesterol, prealbumin and transferrin could be useful in diagnosis of hospitalised patients, their use in older patients should be treated with caution [5]. Other studies have found no relation between albumin levels and the nutrition status [6]. Combining malaria infection and malnutrition, it has also been observed that malnutrition does not appear to increase susceptibility to severe *falciparum* malaria but evidence shows that well nourished children are more susceptible to severe disease than those who are malnourished [7-10,1].

During the study period there occurred unusually heavy rains in the history of Kenya. This gave rise to massive flooding leading to an upsurge in both malnutrition and epidemic malaria in non-endemic areas of the country. This study was carried out with the main objective of determining whether plasma albumin levels and other biochemical factors in children suffering from *P. falciparum* admitted to the paediatric wards of Moi National Teaching and Referral Hospital, within the specified period, showed a difference from the expected values. Plasma albumin levels and other biochemical factors were measured in children suffering from *P. falciparum* in relation to their nutritional status.

MATERIALS AND METHODS

Design: Descriptive cross-sectional.

Sampling: Convenient sampling.

Patients: Sample size for patients was entirely determined by the cases that presented themselves to the hospital during the, 15 children and later at Webuye Hospital, Bungoma District, Western Kenya, 20 children. No random selection of patients under study, nor prior statistical determination of the sample size was done. The sample size presented here was thus representative of the cases observed in hospital at that time.

Fifteen children suffering from *P. falciparum*, aged between 3-14 years, admitted to the paediatric wards of Moi National Teaching and Referral Hospital, Eldoret, and 20 children suffering from malnutrition at Webuye District Hospital were investigated in this study.

Controls: Clinically healthy children attending outpatient MCH clinic at Webuye District Hospital for routine immunisation, and the Moi National Teaching and Referral Hospital, Eldoret, Kenya were used as controls.

Consent: The parents or guardians of the children screened gave consent. Institutional consent was also obtained from the Ministry of Higher Education, Science and Technology in Nairobi as well as from the Medical Superintendent Bungoma District Hospital as well as the Medical officer of Health in charge of Webuye Sub-District Hospital.

Parasites: Giemsa stained thin blood smears from finger-prick blood samples of the children were used to confirm the presence of *P. falciparum* parasites in addition to clinical diagnosis of malaria.

Blood for assay of plasma: Approximately 2-5 mls of venous blood was drawn in EDTA coated vaccutainer tubes and left for about 1 hour at room temperature (23^0C). After centrifuging at 3,000 rpm plasma was taken off and stored at -20^0C and later at **-**70^0C, until required. Total protein was estimated in 35 of the samples collected.

Plasma Albumin and total protein: Albumin was estimated in the heparinised or EDTA plasma from all the patients and compared to the standard albumin ranges as specified by the manufacturer using the bromo-cresol blue method. A spectrophotometer was used in the assays. Bromocresol blue or green method was preferred because of the reproducibility of the results [11].

Glucose: Glucose was determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The H_2O_2 formed reacted under catalysis of peroxidase (POD) with phenol and 4-amino-antipyrine to form quinoneimine. The intensity of the colour was proportional to the glucose concentration in the sample

Bilirubin: Total bilirubin was determined using the Randox method as specified by the manufacturers. In brief, direct (conjugated) bilirubin reacted with diazotised sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin was determined in the presence caffeine, which released albumin bound bilirubin, by the reaction with diazotised sulphanilic acid.

GOT and GPT: Determination of serum aspartate transaminase (SGOT) and serum aspartate alanine transferase (SGPT) according to Boeringer Biochemicals, Germany, was carried out as specified by the manufacturers. Briefly, the amount of oxaloacetate and pyruvate formed by each of the two assays were measured by means of the 2,4—dinitrophenylhydrazone of pyruvic acid, the colour of which was read at 520 nm by spectrophotometer. The intensity of colour was proportional to the amount of enzyme in each sample.

Creatinine: The principle was as described by the manufacturers: After precipitating proteins in test serum, the creatinine was adsorbed on to Lloyd's reagent, a hydrated aluminium silicate, and the colour then developed with alkaline picrate. The intensity of colour was proportional to the amount of creatinine present.

Anthropometry: To ascertain the nutritional status of the patients, weight for age measurements were taken for 9 out of 15 patients and compared with the standard recommended scale. In Kenya (GOK, Ministry of health) a well-nourished child is between 2 Kgs and 3.5 Kgs at birth; between 7.5-10.2 Kgs at one year; between 9.5-12 Kgs at two years; between 11.4-14.7 Kgs at three years; between 12.7-16.6 Kgs at four years and between 14-18.9 Kgs at five years of age. A malnourished child falls above or below these ranges. Use of Z- scores (SD) [12] is normally employed to report the nutritional status of the children. This is based on measurements of weight, height, Age and mid upper arm circumference. Any value ≤ -2Z score WFA- underweight, WFH- wasted or HFA- stunted, is considered malnourished.

Statistics: The main statistical parameter determined was the mean concentration of Glucose, Bilirubin, GOT, GPT and Creatinine. Chi square test to determine the difference between malaria, malnourished and control children was also determined, using SPSS 10 computer programme.

RESULTS

Plasma Albumin

Five patients out of fifteen showed albumin levels below the normal range (3.5-5.5 g/dl) representing 33% while 6 patients out of fifteen showed albumin levels above the normal range, representing 40%. Only one patient showed Plasma albumin range within the normal range representing 6.6%. (Tables 1a). This particular patient showed a weight below the expected normal.

Table 1a. Plasma *albumin levels* in malnourished and control children. (Eldoret Hospital.)

Plasma-albumin Concentration (g/dl)	< 3.5	3.5-5.5	> 5.5
Well-nourished	5/12 (41.6**%**)	0/12 (**0%**)	6/12 (50**%**)
Mal-nourished	0/12 **(0%**)	1/12 (0.8**%**)	0/12 (**0%**)

NB. Statistical significance not determined due to small sample.

(b) Percent (%) Plasma albumin levels (g/dl) taken from children suffering from *Plasmodium falciparum* infections, at admission with or without malnutrition in Paediatric wards of Moi National Teaching & Referral Hospital, Eldoret, Kenya.

	Below normal	**Within normal**	**Above normal**	**Spoiled**	**Total**
Number of samples	5/15	1/15	6/15	3/15	15
Percentage (%)	**33.3**	**6.6**	**40**	**20**	**100**

Normal values (3.5-5.5 g/dl).

Total Protein

Using an established protocol, total protein was determined by use of a spectrophotometer in control samples (Table 2a). Only 37.5% of the clinically healthy children tested for total protein showed values within the normal range (6.6-8.7g/dl). The rest (50%) showed values below normal.

Table 2 a. Plasma Total protein levels in control Children: (Eldoret Hospital)

Concentration (g/dl)	<6.6	6.6-8.7	>8.7	ND (sample too small)
Clinically healthy	4/8 (**50%**)	3/8 (**37.5%**)	0/8 (**0%**)	6/15 (40%)
Clinical malaria	1/8 (**12.5%**)	0/8 (**0%**)	0/8 (**0%**)	1/8 (12.5%)
TOTAL	5/8 (**62.5%**)	3/8 (**37.5%**)	0/8 (**0%**)	7/15 (46.6%)

Normal values (6.6-8.7g/dl)

Glucose

The mean glucose concentration in all the samples was 6.5mmol/L (Table 2b, Fig. 1). The normal serum values of glucose fall in the range 76-110 mg/dl (4.2-6.1 mmol/L). The mean value was above the normal range. The children diagnosed with malaria showed elevated glucose levels (Table 2b). A small percentage (27.7%) showed values below normal. Glucose could also be used as an indicator of malnutrition in this study

Bilirubin

The mean bilirubin concentration was 0.28mg/dl (Table 2b, Fig 1). The normal range lies between 0-1mg/dl. The mean values obtained lie within the normal range. Almost all samples studied fell within the normal range.

Table 2 b. *Mean concentrations and percentages of Glucose, Bilirubin, GOT, GPT and Creatinine*

	Glucose	**Bilirubin**	**SGOT**	**SGPT**	**Creatinine**
Normal Range	(4.2-6.1 mmol/dl)	(0-1mg/dl)	(4-17u/l)	(3-16u/l)	(0.6-1mg/dl)
Mean concentration	**6.47**	**0.28**	**9.0**	**5.9**	**0.75**
Below normal	5/18 (**27.7%**)	0/18 (**0%**)	0/18 (**0%**)	0/18 (**0%**)	10/18 (**55.6%**)
Normal	10/18 (**55.6%**)	15/18 (**83.3%**)	17/18 (**94.4%**)	18/18 (**100%**)	8/18 (**44.4%**)
Above normal	3/18 (**16.6%**)	0/18 (**0%**)	1/18 (**5.5%**)	0/18 (**0%**)	0/18 (**0%**)

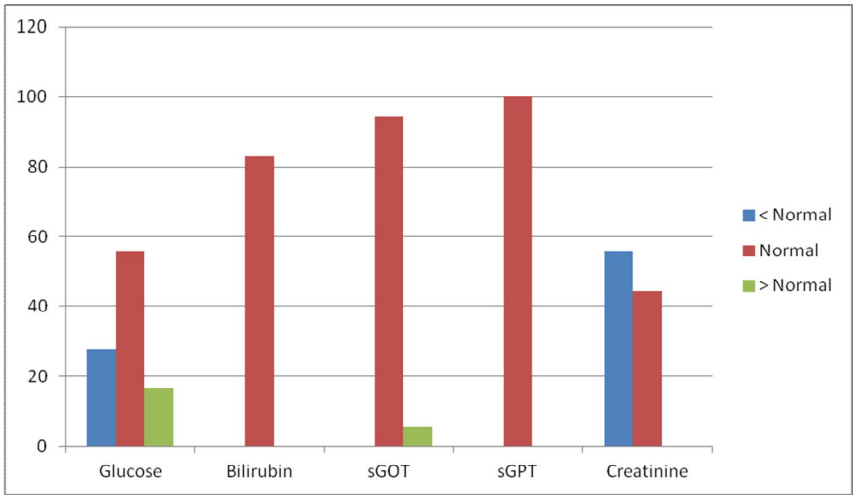

Figure 1. ***Percentages(y-axis) of Glucose, Bilirubin, GOT, GPT and Creatinine***

GOT & GPT

The mean concentrations of GOT and GPT was 9.0 and 5.9 µ/l respectively (Table 2b, Fig 1) The normal range in serum for SGOT and SGPT are 4-17 and 3-16 u/l respectively. Almost all samples assayed fell within the normal range.

Creatinine

The mean creatinine concentration was 0.75 mg/dl, (Table 2b, Fig 1) the normal blood creatinine values are between 1 and 2 mg/ 100ml (or 0.6 to 1 mg/dl in males and 0.5 to 1 mg/dl in females). The percentage of children with values below normal were 55.5% making it the best indicator of malnutrition in this study.

Anthropometry

Only one patient showed weight below the expected for age, < -2ZWFA. Weight could only be taken for 9 out of the 15 patients.

DISCUSSION

Although plasma albumin and total protein were the initial biochemical variables measured in this study, they were supplemented with other variables such as glucose, bilirubin, SGOT, SGPT and creatinine. It has been shown in this study that creatinine and glucose could be useful biochemical indicators of malnutrition in this study population. Bilirubin, SGOT and SGPT gave values within the normal range showing that they may not be useful biochemical factors to determine malnutrition status in the population studied.

Data on physical measurements of the children as well as clinical and parasitological diagnosis of malaria were also included. Clinical malaria was defined as temperature ≥ 37.5 ^{0}C as measured by a digital clinical thermometer. The varied plasma albumin levels in this brief study tend to agree with the unpredictable *P.falciparum* pattern during this period of varied weather pattern [13,14Khan, *et al*., 1991; Esamai, 2002). From previous studies [15Waterlow, 1992) the albumin and Ferretin levels are expected to be low in sera of malaria patients, especially in areas of unstable malaria. The results in this study partially agree with this findings (33%). Plasma albumin levels have also been reported to be low in such patients [3,11]. In a subsequent study [16] it was found that plasma

albumin level was significantly lower in both severe and mild malaria. This is because plasma albumin is a negative acute phase protein [17], the level of which falls as a result of malaria infection, probably because of an increase in its trans-capillary escape rate . Evidence for the influence of malnutrition to the serum albumin levels was shown by results of only one patient out of fifteen, whose value fell within the normal range (3.5-5.5g/dl). Abnormal plasma albumin levels were those that fell below 3.0g/dl. In a recent study [18]. Serum concentrations of total protein, albumin and C-reactive protein were determined in clinically distinct manifestations of severe malnutrition (Marasmus and kwashiorkor). The concentration of globulin (total protein minus albumin) was found to be higher in marasmus than Kwashiorkor. Since total protein in control samples was not above the normal limits, (6.6-8.7g/dl), (Table 2b), the results obtained in the children studied agree with this observation. What remains unexplained from the results is the percentage of patients (40%) with plasma albumin levels above normal, yet they were confirmed to be suffering from plasmodium infection. It is possible that some of the patients did not have complicated malaria but on the other hand it is possible to have been an anomaly since plasma albumin levels have been shown to be low in both types of malaria [16]. Unusual rains did not seem to have an effect on the albumin and total protein levels in both the test samples and the controls

Further study was carried out on samples obtained from malnourished children and those malnourished and suffering from malaria from Webuye sub-district hospital, Bungoma, Kenya. Apart from total protein, other biochemical variables mentioned in the introductory paragraph of the discussion section were determined. For creatinine, the mean concentration in samples assayed was within the normal range (1 and 2 mg/100ml). Pathological conditions have been observed in starvation and fevers where there is considerable muscular wasting. Malnutrition and malaria would be expected to produce such pathological levels but this was not observed in our study.

The mean SGOT and SGPT concentrations were also within the normal range. These two enzymes are well known for their diagnostic value in conditions such as myocardial infarction (SGOT) and liver conditions like cirrhosis (SGPT). The enzymes did not seem to be of diagnostic value in this study of malnutrition and malaria, although due to the fact that considerable muscle wasting (PEM) and anaemia due to malaria occur and could be a cause of pathological values.

The mean bilirubin concentration was within the normal range. Bilirubin is known for its diagnostic use in jaundice, where very high values are observed in obstructive jaundice as compared to haemolytic jaundice. From the results of this study, bilirubin did not seem to be of diagnostic value for PEM and malaria.

The mean glucose concentration was above the normal range. Glucose could be a useful diagnostic tool for PEM and malaria. This is true given the energy loss from muscle tissue in PEM giving rise to the term Protein-energy malnutrition.

From previous work [1] it has also been noted that contrary to the hypothesis above, malnourished children still have a higher risk of mortality and morbidity than well nourished ones. In the control samples, total protein was used as variable rather than albumin levels due to easy availability of the test kits. Total protein has however not been found to be useful for indication of malnutrition [11] as compared to serum albumin. The results obtained in this study reflect the previous findings although in this study, the weather phenomena were unique in this region.

In conclusion, serum albumin and glucose were found to be better biochemical markers of malnutrition in the study population, than creatinine, SGOT, SGPT, total proteins and bilirubin.

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