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Full Length Research Paper

Biochemical alteration in Nigerian children with acute *falciparum* malaria

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This study was undertaken to establish data on the effect of acute *falciparum* malaria on plasma levels some biochemical parameters in the pathology of malaria in Nigeria children. We estimated the levels of Na^+, K^+, HCO_3, Ca^{++}, inorganic $PO_4^=$, bilirubin, total protein, albumin, urea, creatinine and glucose in the plasma of 250 parasitaemic and 150 non-parasitaemic Nigerian children. Inorganic $PO_4^=$, urea, creatinine and bilirubin levels were significantly elevated in the acute *falciparum* malarious children than in the non-parasitaemic controls. Acute *falciparum* malaria resulted in significant reduction of HCO_3^-, total protein, albumin and glucose levels in the malarious children. There was no significant difference in the mean values of the biochemical parameters between malarious children with relative parasite count of 1-10 asexual form of parasite in 100 high power field (hpf) of thick blood film (+) and those with 11-100 asexual form of parasite in 100 hpf of thick blood film(++) .

Key words: Biochemical parameters, Nigerian children, acute *falciparum* malaria.

INTRODUCTION

Malaria is a potentially life-threatening disease in the tropics as it affects over 400 million people yearly and is responsible for the deaths of an estimated 10,000 women of reproductive age and over 1 million infants and young children each year (Barbain, 1989; Mishra et al., 2003).

It is a disease caused by a protozoan parasite belonging to the genus *Plasmodium*.

Sagamu and Ile-Ife are towns located in the tropical rain forest in south-west Nigeria. Ogunledun et al. (1991) reported that *Plasmodium falciparum* accounts for 93.5% of malaria in Sagamu. Worldwide, majority of the deaths from malaria has been attributed to *P. falciparum* (Egwunyenga et al., 2004).

The diagnosis of malaria is made with certainty on identification of malaria parasite together with other symptoms associated with the disease. Oftentimes the diagnosis of malaria may be missed using these two criteria for major technical and seldom pathophysiological reasons. Technically, detection of malaria parasite may be missed due to low parasite density at sampling time and poor blood film preparation. Occasionally, malaria infection comes without any symptom (asymptomatic malaria); hence, diagnosis becomes difficult using associated signs and symptoms. It is therefore desirable to complement the existing methods/criteria of malaria diagnosis to achieve proper diagnosis and management of malaria disease and its associated complications.

Malaria pathogenesis is based mainly on extensive changes in biochemical and haematological parameters (Bidaki and Dalimi, 2003). The World health Organization`s (WHO) criteria acknowledges that some biochemical and haematological features should raise the suspicion of severe malaria (WHO, 2000). There are scientific publications on biochemical and haematological changes in acute *falciparum* malaria in different parts of the world including Nigeria (Mishra et al., 2003; Egwunyenga et al., 2004; Bidaki and Dalimi, 2003; Udosen, 2003) but non have been reported at Ile- Ife, Nigeria. Therefore, this present work was carried out to determine the effects of acute *falciparum* malaria on some biochemical parameters and to apply any changes

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observed to malaria diagnosis and management as well as understanding malaria pathogenesis.

MATERIALS AND METHODS

The study group comprised 250 children (130 girls and 120 boys) aged 1-10 years (with mean age 3.4 ± 2.5 years) presenting with clinical features of malaria. They were recruited from the children outpatient and emergency wards of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile-Ife, Nigeria. .The control group was made up of 150 children (78 boys and 72 girls) malaria-free children matched for age, sex and socio-economic status with the study group. The control group was recruited from a nursery and primary school in the same town where the hospital is located.

Collection of blood samples

6 ml of venous blood was collected from children at the wards or parasitology laboratory, and their names, age and gender recorded. Plasma was separated from the whole blood cells after centrifugation and stored at -4°C.

Parasitological examination

The presence and relative parasite count of *P. falciparum* in each blood sample was determined from Giemsa stained thin and thick films after staining for 30 min. The identification of the species of human parasites in the blood films was carried out according to WHO method (WHO, 1980). A slide was scored as negative when 100 high power fields (at 1000x magnification) had been examined for about 30 min without seeing any parasites.

The amount of relative parasite count in positive smears was done using a simple code from one to four crosses (+ - ++++) according to Brace-chwat (1980) as stated below:

+: 1-10 parasites per 100 thick film fields.
++: 11-100 parasites per 100 thick film fields.
+++: 1-10 parasites per 100 thick film fields.
++++: More than 10 parasites per one thick film field.

Biochemical analysis

Sodium, potassium and bicarbonate were estimated from lithium heparin bottles using the method reported by Korzum and Miller (1996) and Segal (1955) respectively. Calcium, total protein, creatinine and inorganic phosphate were also estimated from lithium heparin bottles with the methods reported by Kazmierczack (1996a). Plasma albumin was estimated by the method described by Cheung and Hickman (1996), while plasma bilirubin and random blood glucose was assayed by standard techniques reported by Kazmierczak (1996) and Gochman and Schmitz (1972), respectively.

RESULTS

Of the 250 children infected with *P. falciparum*. 130 (52%) had relative parasite count of one cross (+), 1-10 asexual form parasites in 100 high power field (HPF) of thick film. 120 (48%) had relative parasite count of two crosses (++), 11-100 asexual form of parasite in 100 hpf of thick blood film (Table 1)

Plasma inorganic phosphate, urea, creatinine, total and conjugated bilirubin levels were significantly elevated ($p<0.001$) in children with acute *falciparum* malaria than in the non-parasitaemic controls. Plasma bicarbonate, calcium, total protein, albumin, and random blood glucose levels in the parasitaemic children were significantly lower ($p<0.05$ and $p<0.001$) than in the non-parasitaemic controls. However, there was no significant difference in the mean plasma sodium, potassium and globulin between the malarious children and malaria free children (Table 2). The mean levels of biochemical parameters in parasitaemic children with relative parasite count of 1-10 asexual form of parasite /HPF (+) and that of parasitaemic children with relative parasite count of 11-100 asexual form of parasite /HPF (++) were compared with the non-parasitaemic controls. The pattern of result obtained was similar to the result reported in Table 2 (Tables 3 and 4). However, there was no significant difference in the mean plasma biochemical variables between parasitaemic children with relative parasite count of 1-10 asexual form of parasite /HPF (+) and parasitaemic children with relative parasite count of 11-100 asexual form of parasite /HPF (++) (Table 5), indicating that the biochemical alterations are not cumulative.

Table 1. Occurrence of *Plasmodium falciparum* in 250 Parasitaemic children by intensity of infection (relative parasite count).

No. of asexual forms/1000Hpf		Number of children
1 - 10	(+)	130 (52%)
11 - 100	(++)	120 (48%)

DISCUSSION

This study has shown the effect acute *falciparum* malaria on biochemical parameters. Eleven out of the thirteen parameters investigated were significantly altered ($p<0.05$ and $p<0.001$) in the parasitaemic children (Tables 2, 3 and 4). Ogunledun et al. (1991) reported that diagnosis of malaria parasite in blood film could be made with certainty on identification in blood films along with signs and symptoms associated with the disease. However, this claim has been faulted by another report that malaria diagnosis may be missed for purely technical reasons and limitation of evidence of signs and symptoms. Warrel et al. (1990), Mishra and Mohanty, (2004), WHO report/criteria (WHO, 2000) and many published works (Mishra et al., 2003; Udosen, 2003; Bidaki and Dalimi, 2003) have investigated biochemical alteration in malaria infection and reported significant

Table 2. Comparison of biochemical parameters of *P. falciparum* in malarious children and malaria-free controls.

Biochemical parameters	*P. falciparum* parasitaemic children (N=250)	Non parasitaemic controls (N=150)	Comparison
Age (years)	3.4±2.5	3.3±1.7	p>0.05
Sodium (mmol/L)	135±6.5	134±3.1	p>0.05
Potassium (mmol/L)	3.5 ±0.5	3.4±0.3	p>0.05
Bicarbonate (mmol/L)	22±2.6	24 ±1.6	P<0.05
Calcium (mmol/L)	1.9 ±0.4	2.5±0.2	P<0.001
Inorganic PO_4 (mmol/L)	1.44±0.20	1.23± 0.23	P<0.001
Urea (mmol/L)	5.9± 2.8	3.9±0.5	P<0.001
Creatinine (µmol/L)	98 ±16.6	82±12.6	P<0.001
Total protein (mmol/L)	55±11.3	65±6.1	P≤0.001
Albumin (mmol/L)	35 ±1 8.5	42±3.6	P<0.001
Globulin (mmol/L)	21± 7.0	24±3.0	P<0.05
Total bilirubin (mmol/L)	23 ± 5.0	11±2.0	P<0.001
Conjugated bilirubin (mmol/L)	12 ± 8.5	3 ± 1.0	P<0.001
Random glucose (mmol/L)	3.5± 0.9	5.0±1 0.2	P<0.001

Table 3. Comparison of biochemical parameters in parasitaemic children with relative parasite count of 1-10 HPF asexual forms of parasite (+) in thick blob film with non-parasitaemic controls.

Biochemical parameters	Parasitaemic children with 1- 10 asexual form of *P. falciparum* in 100 HPF (+) (N=130)	Control (N=150)	Comparison
Age (years)	3.6±2.8	3.0±17	P > 0.05
Sodium (mmol/L)	135±7.3	134±3.1	P > 0.05
Potassium (mmol/L	3.5±0.5	3.4±0.2	P > 0.05
Bicarbonate (mmol/L	23±2.5	24±1.6	P > 0.05
Calcium (mmol/L)	1.9±0.4	2.5 ± 0.2	P < 0.001
Inorganic po_4 (mmol/L)	1.4 ± 0.2	1.2±0.2	P < 0.001
Urea (mmol/L)	5.1± 2.0	3.9±0.5	P < 0.001
Creatinine (µmol/L)	102±16.7	82±2.6	P < 0.001
Total protein (g/L)	53±11.6	65±6.1	P < 0.001
Albumin (g/l)	33 ± 8.5	42±3.6	P < 0.001
Globulin (g/L)	20± 9.7	24 ± 4.7	P >0.05
Total bilirubin (mmol/L	27±10.4	11 ± 0.9	P < 0.001
Conjugated bilirubin (mmol/L	13 ±5.7	3.0 ± 0.5	P < 0.001
Random glucose (mmol/L)	3.5 ±0.9	5.0±0.2	P < 0.001

alteration in both biochemical and haematological variables. The need for inclusion of biochemical and haematological investigation at malaria infection diagnosis cannot be over emphasized, as this is necessary for early recognition of complication associated with acute malaria infection that may require most urgent intensive care to avoid mortality due to complications (Mishra and Mohanty, 2003). `

The result of our study shows that acute *falciparum* malaria infection resulted in fluctuation or alteration of some biochemical parameters. The values obtained for plasma inorganic phosphate, urea, creatinine, total and conjugated bilirubin was significantly higher (p<0.001) in malarious children than in non-parasitaemic children. The significantly higher concentration of these biochemical parameters in malarious children suggests that enhanced concentration of these biochemical parameters is a marker for acute malaria severity. It has been reported that renal failure (serum creatinine of >3 mg/dl and hyperbilirubinaemia, serum bilirubin>3 mg/dl) are indicators of severe manifestation of severe *falciparum* malaria (Mishra et al., 2003; Trang et al., 1992; Rajpurkar, 1994). Our study confirms the two reports; we observed relative (compared with controls) hypercreatinifnaemia (plasma ceratinin 98 umol/l), uremia (plasma urea 5.2 mmol/L) and hyperbilirubinaemia (plasma total bilirubin 23 umol/L, conjugated bilirubin 12 mmol/L) in malarious children in Nigeria compared with the controls

Table 4. Comparison of biochemical parameters in parasitaemic children with relative parasite count of 11-100 HPF asexual forms of parasite (++) in thick blob film with non-parasitaemic control.

Biochemical parameters	Parasitaemic children with 11-100 asexual form of P.*falciparum* in 100 HPF (++) (N=120)	Control (N=150)	Comparison
Age (years)	3.6±2.6	3.0 ± 1.7	P> 0.05
Sodium (mmol/L)	136 ± 5.5	134 ± 3.1	P>0.05
Potassium (mmol/L)	3.6 ± 0.5	3.4 ± 0.2	P> 005
Bicarbonate (mmol/L)	22 ± 2.8	24 ±1.6	P< 0.05
Calcium (mmol/L)	2.0 ± 0.4	2.5 ± 0.2	P< 0.001
Inorganic po_4 (mmol/L)	1.4 ±0.2	1.2 ±0.2	P < 0.001
Urea (mmol/L)	6.4 ± 3.2	3.9 ± 0.5	P < 0.001
Creatinine (µmol/L)	93 ±15.5	82 ± 12.6	P < 0.001
Total protein (g/L)	57 ± 10.7	65 ± 6.1	P < 0.001
Albumin (g/l)	35 ± 7.3	42 ± 3.6	P < 0.001
Globulin (g/L)	22 ±5.7	24 ± 6.7	P > 0.05
Total bilirubin (mmol/L)	18.4 ± 9.7	11 ± 0.09	P <0.001
Conjugated bilirubin (mmol/L)	10.0 ±5.1	3.0 ± 0.5	P <0.001
Random glucose (mmol/L)	3.5 ±0.9	5.0 ± 0.2	P < 0.001

Table 5. Comparison of biochemical parameters in parasitaemic children with different levels of relative parasite count (parasitaemia).

Biochemical parameters	Parasitaemic children with 1- 10 asexual form of P.*falciparum* in 100HPF(+) (N=130)	Parasitaemic children with 11-100 asexual form of P.*falciparum* in 100 HPF (++) (N=120)	Comparison
Age (years)	3.6±2.8	3.6±2.6	P>0.05
Sodium (mmol/L)	135± 7.3	136± 5.5	P>0.05
Potassium (mmol/L	3.5 ± 0.5	3.6 ±0.5	P>0.05
Bicarbonate (mmol/L	23 ± 2.5	22 ±2.8	P>0.05
Calcium (mmol/L)	1.9 ± 0.4	2.0 ±0.4	P>0.05
Inorganic po_4 (mmol/L)	1.4 ± 0.2	1.4 ± 0.2	P>0.05
Urea (mmol/L)	5.1 ± 2.0	6.4 ± 3.2	P>0.05
Creatinine (µmol/L)	102 ± 16.7	93 ± 15.5	P>0.05
Total protein (g/L)	53 ±11.6	57 ±10.7	P>0.05
Albumin (g/l)	33 ± 8.5	35 ±7.3	P>0.05
Globulin (g/L)	20 ±9.7	22 ± 5.7	P>0.05
Total bilirubin (mmol/L)	27 ±10.4	18.4 ± 9.7	P>0.05
Conjugated bilirubin (mmol/L)	13 ±5.7	10.0± 5.1	P>0.05
Random glucose(mmol/L)	3.5 ±0.9	3.5 ±0.9	P>0.05

(Tables 2, 3 and 4). Rajpurkar (1994) and Balcerak (1972) attributed the causes of acute renal failure in *falciparum* malaria infection to direct effect of the parasite on the erythrocyte (RBC); drug induced oxidative stress in patients having glucose-6-phosphate dehydrogenase deficiency (G6PD), changes in RBC membrane among others. The causes of jaundice (hyperbilirubinaemia) are multifactorial, these include, intravascular haemolysis of PRbcs, haemolysis of non-parasitized Rbcs, G6PD amongs others (Mishra et al., 2003) .The various forms of haemolysis associated with malaria infection may result to elevated plasma inorganic phosphate (an intracellular element).

We have also reported significant reduction in plasma bicarbonate, calcium, total protein, albumin and random blood glucose. The relative hypoglyceamia reported in malarious children supports WHO criteria for suspecting severe malaria and Rajpurkar`s report of hypoglycemia as one of the biochemical changes in malaria infection (WHO report 2000; Rajpurkar, 1994). During heavy parasiteamia, secretion of insulin is induced by quinine and often leads to severe hypoglycemia, coupled with this, is the large glucose requirements of malaria parasite (Rajpurkar, 1994). The reduced level of plasma bicarbonate may be caused by the exhaustion of bicarbonate reserve to revert the metabolic acidosis caused by the

elevated plasma urea and creatinine. Rajpurkar (1994) also reported that hypervoleamia and replacement with only glucose containing intravascular solution might also cause low bicarbonate level. Hypoproteinemia and hypoalbuminemia reported in Nigerian children with acute *falciparum* malaria may have resulted from occasional transitory acute glomerolnephritis microscopic heamaturia and mild proteinuria of less than 1 g/24 h seen in as many as 25-50% patients (Rajpurkar, 1994; Sitprija, 1988). Impairment of hepatic function associated with severe malaria may also be responsible for the hypoproteinemia and hypoalbuminemia reported in this study. Our finding of hypocalcaemia among the young growing malarious children is astonishing. We cannot explain the mechanism behind this observation. However, we suspect leakage of calcium from the kidney tubules that may have resulted from the malaria nephropathy presenting as nephrotic syndrome in malaria patients (Rajpurkar, 1994). Other biochemical parameters investingated in this work did not show statistical significant difference between the malarious children and the controls.

In conclusion, acute *falciparum* malaria has been shown to significantly alter some biochemical parameters. Hence, those altered biochemical parameters are associated with acute *falciparum* malaria complications could be estimated in addition to malaria parasite identifyication. This will enhance the determination of severity of malaria disease at diagnosis and as well reduce delay in medical intervention required to avoid mortality arising from malaria complications.

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