Original article

Falciparum malaria associated changes in biochemical indices in children

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Abstract
Metabolic disturbances associated with fluid and electrolyte imbalance, and changes in the synthetic functions of the liver are common complications of malaria and are dependent on the degree of parasitemia. Packed cell volume (PCV), random blood glucose (RBG), total bilirubin (TB), total proteins (TP), albumin, serum electrolytes [sodium (Na⁺), potassium (K⁺), chloride (Cl⁻), bicarbonate (HCO₃⁻), calcium (Ca²⁺), magnesium (Mg²⁺)] and anion gap (AG) were determined in fifty children with malaria aged between 1-15 years and thirty age matched apparently healthy children without malaria, using colorimetric and flame photometric methods. Data was analyzed using t-test at p < 0.05. The PCV, RBG, Na⁺, Mg²⁺, AG and TP were significantly lower and Ca²⁺ and TB higher in children with malaria compared to children without malaria. The serum Na⁺, K⁺, AG, TP and albumin were significantly lower and Ca²⁺, HCO₃⁻ and TB higher in children with severe malaria compared to those with mild malaria. Malaria and high parasite density is associated with perturbations in homeostasis of proteins and electrolytes and these may be implicated in the deleterious consequences associated with malaria in children.

Key words: Billirubin, Children, Electrolytes, Malaria, Proteins

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Malaria has been ranked among the five most common causes of death and accounts for over 200,000 deaths in children annually in Nigeria¹. Plasmodium falciparum has been reported to be responsible for 90% of all infections being the species associated with most severe cases, especially in young children and pregnant women². The clinical spectrum/outcome of severe malaria in African children encompasses a wide range of patho-physiological derangements that effect multiple organ systems³, which are dependent on host factors (e.g. immunity, age), parasite factors², geographic and socio-cultural factors⁴. Severe falciparum malaria frequently presents with cerebral malaria, severe anemia and metabolic disturbances associated with fluid and electrolyte imbalance⁵, and changes in the synthetic functions of the liver. The cardinal symptoms of severe malaria including high fever, nausea, vomiting, diarrhea, abdominal pain and dehydration; wherein invasion of hepatocytes by sporozoites are implicated in the development of various malarial complications⁶. The most common electrolytes disturbances in malaria are hyperkalemia, hypokalemia and some cases of hyponatremia and hypernatremia⁷. Changes in total protein, albumin and glucose levels are also common findings in severe malaria. However, little or no attention has been
given to falciparum induced perturbations in calcium and magnesium metabolism in parallel with other electrolyte imbalances and protein synthesis; considering the fact that maintenance of their plasma concentration within a narrow physiological range is vital to the integrity of a variety of cellular metabolic processes.

Alterations in the homeostasis of these biomolecules associated with malaria, which are more pronounced in children, can therefore be used as indices for degree of parasitemia. This work therefore assesses the effects of falciparum malaria infection and parasite density on packed cell volume (PCV), random blood glucose (RBG), total proteins (TP), albumin, total bilirubin (TB), sodium (Na\(^+\)), potassium (K\(^+\)), chloride (Cl\(^-\)), bicarbonate (HCO\(_3^-\)), calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) in children with malaria infection.

Materials and methods

Selection of subjects

This case control study was conducted at Pediatric Unit of the University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria. A total of eighty (80) subjects were enrolled into the study. Fifty (50) children with microscopy confirmed malaria infection aged 1–15 years and thirty (30) age-matched apparently healthy children without malaria infection, were investigated for PCV, total bilirubin, total proteins, albumin and electrolytes. The purpose and nature of the research was explained to the parents/guardians of the participants and their consent sought and obtained before recruitment into the study. This study was carried out in accordance with the Ethical Principles for Medical Research involving Human Subjects as outlined in the Helsinki Declaration in 1975 and subsequent revisions. Subjects were selected based on the following criteria; children with microscopy confirmed cases of malaria in the last three days served as test subjects. The control subjects were apparently healthy children without malaria in the last three days. Children with history of hepatitis B virus (HBV), hepatitis C (HCV), human immunodeficiency virus (HIV) infections and any form of chronic organ or systemic illness and prolonged medication were excluded from the study.

Sample collection

Five millilitres (5ml) of peripheral whole blood samples were collected aseptically from the subjects by venepuncture and dispensed 1ml each into appropriately labeled plain, lithium heparin, fluoride oxalate and dipotassium ethylene diamine tetra acetic acid (K\(_2\)EDTA) containers respectively. An aliquot of the sample from the syringe was used to prepare thick and thin blood films on appropriately labeled clean grease-free slides. The samples in plain containers were allowed to clot and retract after which there were spun at 5000 revolutions per minute for 5 minutes. The sera was collected and stored at 4\(^\circ\)C in appropriate sample vials for assay of total proteins, albumin, total bilirubin, sodium, potassium, chloride, bicarbonate, calcium and magnesium. Samples in the fluoride oxalate and K\(_2\)EDTA containers were used for determination of packed cell volume and random blood glucose respectively within one hour.

Laboratory methods

Identification of malaria parasite was done by Giemsa staining technique and calculation of parasite density by World Health Organization (WHO) criteria\(^6\). Packed cell volume (PCV) was determined using hematocrit method. Estimation of serum albumin was done by bromocresol green method\(^7\). Total protein was estimated by Biuret reaction method\(^8\). Glucose was estimated by glucose oxidase method\(^9\). Total bilirubin was estimated by modified Valley’s method\(^10\). Chloride and bicarbonate were determined using titrimetric methods\(^11,12\). Determination of sodium and potassium was done by flame photometry\(^13\), while magnesium was determined colorimetrically with lipid cleaning factor\(^14\).

Statistical analysis

Data analysis was done using the statistical package for social sciences (IBM SPSS version 20.0, International Business Machines Corporation, Armonk, New York, USA). Student’s t-test analysis was used to determine mean differences between variables. A probability value p < 0.05 was considered statistically significant.

Results

The mean PCV, TB, RBG, albumin, TP, Na\(^+\), K\(^+\), Cl\(^-\), HCO\(_3^-\), Ca\(^{2+}\), Mg\(^{2+}\) and anion gap in children with malaria parasite infection and those without malaria infection is shown in table 1. The PCV, RBG, Na\(^+\), Mg\(^{2+}\), anion gap and total protein were significantly lower and Ca\(^{2+}\) and bilirubin higher in children with malaria compared to children without malaria (p<0.05). No significant differences were seen in the levels of other indices in both groups (p>0.05).

The effect of parasite density on mean PCV, total bilirubin, RBG, albumin, total proteins, Na\(^+\), K\(^+\), Cl\(^-\), HCO\(_3^-\), Ca\(^{2+}\), Mg\(^{2+}\) and anion gap in children with malaria parasite infection is shown in table 2. Chil-
Children with severe malaria (Parasite Density; 11, 290.25±728.7) had significantly lower serum Na⁺, K⁺, anion gap, albumin and total protein and higher Ca²⁺, HCO₃⁻ and total billirubin compared to children with mild malaria (Parasite Density: 893.95±379.6) (p<0.05). No significant differences were seen in the levels of other indices in both groups (p>0.05).

Table 1: Mean PCV, total billirubin, RBG, albumin, total proteins, Na⁺, K⁺, Cl⁻, HCO₃⁻, Ca²⁺, Mg²⁺ and anion-gap in children with malaria and children without malaria

| Index          | Children with malaria (n = 50) | Children without malaria (n = 30) | p value |
|----------------|--------------------------------|----------------------------------|---------|
| PCV (%)        | 31.13±5.17                     | 35.93±6.19                       | 0.002*  |
| TB (µmol/l)    | 24.47±11.91                    | 8.64±2.12                        | 0.001*  |
| RBG (mmol/l)   | 3.10 ±0.46                     | 5.34±1.22                        | 0.003*  |
| Albumin (g/l)  | 28.64±8.90                     | 41.65±3.89                       | 0.609   |
| TP (g/l)       | 36.41±10.39                    | 66.83±4.25                       | 0.036*  |
| Na⁺ (mmol/l)   | 132.03±4.33                    | 139.87±3.22                      | 0.028*  |
| K⁺ (mmol/l)    | 4.02±0.89                      | 4.22±0.59                        | 0.404   |
| Ca²⁺ (mmol/l)  | 2.65±0.08                      | 2.29±0.21                        | 0.021*  |
| Mg²⁺ (mmol/l)  | 0.54±0.11                      | 0.86±0.09                        | 0.002*  |
| Cl⁻ (mmol/l)   | 99.64±2.19                     | 99.17±1.32                       | 0.541   |
| HCO₃⁻ (mmol/l) | 26.50±2.89                     | 26.10±1.93                       | 0.740   |
| AG (mmol/l)    | 13.94±6.38                     | 18.07±3.75                       | 0.000*  |

* = significant at p<0.05, PCV=packed cell volume, RBG = random blood glucose, Na⁺ = sodium, K⁺ = potassium, Cl⁻ = chloride, HCO₃⁻ = Bicarbonate, Ca²⁺ = Calcium, Mg²⁺ = Magnesium, TP = total proteins, AG = anion gap.

Table 2: Effect of parasite density on PCV, total billirubin, RBG, albumin, total proteins, Na⁺, K⁺, Cl⁻, HCO₃⁻, Ca²⁺, Mg²⁺ and anion-gap in children with malaria

| Index          | Severe malaria (PD=11, 290.25±728.7, n = 7) | Mild malaria (PD=893.95±379.6, n=43) | p value |
|----------------|---------------------------------------------|--------------------------------------|---------|
| PCV (%)        | 26.45±4.72                                  | 31.98±5.60                          | 0.005*  |
| Billirubin (µmol/l) | 26.33±17.68                      | 7.31±6.64                           | 0.001*  |
| RBG (mmol/l)   | 3.01±0.16                                   | 3.33±0.11                           | 0.861   |
| Albumin (g/l)  | 26.18±6.46                                  | 39.62±18.72                         | 0.020*  |
| TP (g/l)       | 33.10±12.30                                 | 44.04±13.25                         | 0.011*  |
| Na⁺ (mmol/l)   | 130.91±1.93                                 | 136.58±1.38                         | 0.002*  |
| K⁺ (mmol/l)    | 2.36±0.17                                   | 4.33±0.58                           | 0.000*  |
| Ca²⁺ (mmol/l)  | 2.66±0.08                                   | 2.36±0.20                           | 0.001*  |
| Mg²⁺ (mmol/l)  | 0.46±0.09                                   | 0.52±0.11                           | 0.674   |
| Cl⁻ (mmol/l)   | 98.18±1.85                                  | 99.88±2.09                          | 0.941   |
| HCO₃⁻ (mmol/l) | 31.27±1.60                                  | 25.63±2.08                          | 0.000*  |
| AG (mmol/l)    | 5.67±3.89                                   | 15.48±5.50                          | 0.000*  |

* = significant at p<0.05, PCV=packed cell volume, RBG = random blood glucose, TP = total protein, Na⁺ = sodium, K⁺ = potassium, Cl⁻ = chloride, HCO₃⁻ = Bicarbonate, Ca²⁺ = Calcium, Mg²⁺ = Magnesium, AG = anion gap, PD = parasite density.
Discussion

The effects of malaria infection and parasite density on some biochemical indices in children with malaria infection were investigated. Results from our study have shown that children with malaria parasite infection have lower PCV, RBG, serum Na\(^+\), Mg\(^2+\), anion gap, total protein and higher Ca\(^++\) and TB when compared to children without malaria. The lower PCV seen in children with malaria parasite infection has been attributed to severe premature erythrocyte destruction and ineffective erythropoiesis resulting in life threatening anemia seen in all forms of malaria infection especially in *Plasmodium falciparum* infections\(^4\). In the worst cases, disseminated intravascular coagulation and intravascular hemolysis is marked while hemoglobinuria is usually observed\(^5\). The relative hypoglycemia reported in children with malaria in this study is consistent with WHO criteria for the diagnosis of severe malaria\(^6\). Hypoglycemia has been described as a complication of many different pediatric illnesses and is usually associated with a poor outcome\(^7\). Malaria infection has been reported to be associated with pleiotropic changes in glucose metabolism, such as decrease in glycogenolysis, decrease in glucose uptake, and increase in insulin resistance\(^8\). Heavy parasitemia have been associated with high glucose requirements by malaria parasites\(^9\).

Moderately reduced Na\(^+\) levels were seen in children with malaria compared to controls. Mild and severe hyponatremia have been described in *P. falciparum* malaria infection\(^5,19\). This has been attributed to hypovolemia following vomiting and diarrhea and decreased oral fluid and food intake typical of malaria infection\(^10\). The pathophysiology of the hyponatremia in malaria remains unclear, but several studies have suggested that an increased secretion of vasopressin, either appropriately or inappropriately plays an important role\(^20\).

The findings of lower serum Mg\(^2+\) and anion gap in children with malaria infection compared to control subjects are consistent with previous findings\(^21\). Mild asymptomatic hypomagnesemia is known to occur in malaria\(^22\). Diarrhea and vomiting which are the features of malaria infection has been described as the common causes of loss of water and electrolytes\(^14,23\).

Increased serum calcium level was observed in children with malaria infection compared to those without malaria. Intra-erythrocyotic calcium levels have been reported to be substantially increased in parasitized red blood cells\(^24\), this may suggest that the increase in serum calcium levels may result from the intracellular release of calcium secondary to the predictable erythrocyte lysis due to malaria infection. This is supported by our observation of hypercalcemia in severe malaria compared to mild infection. Metabolic acidosis which is one of the complications of severe malaria leads to resorption of calcium from the bone leading to high serum calcium levels\(^3,25\). Contrary to our findings, hypocalcemia has been demonstrated in severe malaria infection\(^22\).

Lower K\(^+\) and higher serum HCO\(_3^-\) was seen in the children with severe malaria compared to those with mild malaria. Reduced K\(^+\) levels have also been reported in malaria infection\(^19,26\). The intraerythrocytic amplification of malaria parasites induces new pathways of solute permeability in the host cell's membrane, which might be deleterious to erythrocytic membrane cation transport\(^7\). Reduction in K\(^+\) levels has also been attributed to loss of about 75 to 80 % of host cell potassium content during the course of malaria infection\(^22\). Metabolic alkalosis is also a complication of malaria wherein K\(^+\) is transferred from extracellular fluid (ECF) to the cell. Vomiting and diarrhea associated with malaria infection are also causes of hypokalemia\(^25\). However, associations between potassium levels and severity of parasitemia were not established by a previous study\(^22\).

Significant increase in the total bilirubin (TB) of children with malaria infection was observed compared to their control counterparts. Increased total bilirubin levels have also been reported in mild malaria infection\(^28\). The causes of hyperbilirubinemia in malaria infection have been attributed to increased intravascular hemolysis of parasitized and non-parasitized red blood cells\(^28\).

We also report significant reduction in total proteins levels in both severe and mild malaria; and mild hypoalbuminemia only in severe malaria. Impairment of hepatic function associated with severe malaria may be responsible for the hypoproteinaemia and hypoalbuminemia reported in this study. Moreover, plasma albumin is a negative acute phase protein, the level of which falls as a result of malaria infection probably because of an increase in its trans-capillary escape rate\(^29\). Significant decrease in the levels of serum total protein and albumin was also observed in children with malaria compared with the control group\(^2,18,22\).

Conclusion

The findings of this study suggests that malaria parasite infection and high parasite density in children is associated with lower packed cell volume,
moderate increases in sodium, magnesium, potassium, anion gap, total proteins and albumin and higher calcium, bicarbonate and total bilirubin compared to children without malaria. The assessment of these biochemical parameters may therefore be useful clues in monitoring the efficacy of treatment regimen in children with malaria infection.

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