J Med Allied Sci 2017; 7 (1): 29-33
www.jmas.in
Print ISSN: 2231 1696 Online ISSN: 2231 170X
Journal of Medical & Allied Sciences

Original article

Falciparum malaria associated changes in biochemical indices in children

Augusta Chinyere Nsonwu-Anyanwu[1], Edmund Richard Egbe[1], Uloma Opara Osuoha[2], Paul Columba Inyang-Etoh[1], Sunday Jeremiah Offor[1], Chinyere Adanna Opara Usoro[1]

[1]Department of Medical Laboratory Science, University of Calabar, Cross River State, Nigeria.
[2]College of Science and Technology, Temple University, Philadelphia, PA 19122, USA.

Article history

Received 22 December 2016
Revised 02 January 2017
Accepted 03 January 2017
Early online 24 January 2017
Print 31 January 2017

Corresponding author

Augusta Chinyere Nsonwu-Anyanwu
Department of Medical Laboratory Science,
University of Calabar,
PMB 1115 Calabar,
Cross River State, Nigeria.
Phone: +234-8033515095
Email: austadechic@yahoo.com

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 (HCO^{3-}), calcium (Ca^{2+}), magnesium (Mg^{2+})] 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^+, Mg^{2+}, AG and TP were significantly lower and Ca^{2+} and TB higher in children with malaria compared to children without malaria. The serum Na^+, K^+, AG, TP and albumin were significantly lower and Ca^{2+}, HCO^{3-} 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

DOI: 10.5455/jmas.253029

Malaria has been ranked among the five most common causes of death and accounts for over 200,000 deaths in children annually in Nigeria[1]. *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 women[2]. The clinical spectrum/outcome of severe malaria in African children encompasses a wide range of patho-physiological derangements that effect multiple organ systems[3], which are dependent on host factors (e.g. immunity, age), parasite factors[2], geographic and socio-cultural factors[4]. Severe falciparum malaria frequently presents with cerebral malaria, severe anemia and metabolic disturbances associated with fluid and electrolyte imbalance[2], 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 complications[4]. The most common electrolytes disturbances in malaria are hyperkalemia, hypokalemia and some cases of hyponatremia and hypernatremia[5]. 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 billirubin, 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_2EDTA) 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^oC in appropriate sample vials for assay of total proteins, albumin, total billirubin, sodium, potassium, chloride, bicarbonate, calcium and magnesium. Samples in the fluoride oxalate and K_2EDTA 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^-, $HC0_3^-$, 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 billirubin 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 billirubin, 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-

dren with severe malaria (Parasite Density; 11, 290.25±728.7) had significantly lower serum Na^+, K^+, anion gap, albumin and total protein and higher Ca^{2+}, HCO_3^- 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^-, $HC0_3$, Ca^{2+}, $Mg^{2+}$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
Ca^{2+} (mmol/l)	2.65 ±0.08	2.29 ±0.21	0.021*
Mg^{2+} (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
HCO_3^- (mmo/l)	26.50 ±2.89	26.1 0 ±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, HCO_3^- = Bicarbonate, Ca^{2+} = Calcium, Mg^{2+} = Magnesium, TB = total billirubin, TP = total proteins, AG = anion gap.

Table 2: Effect of parasite density on PCV, total billirubin, RBG, albumin, total proteins, Na^+, K^+, Cl^-, $HC0_3$, Ca^{2+}, $Mg^{2+}$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*
Ca^{2+} (mmol/l)	2.66 ±0.08	2.36±0.20	0.001*
Mg^{2+} (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
HCO_3^- (mmo/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, HCO_3^- = Bicarbonate, Ca^{2+} = Calcium, Mg^{2+} = 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 has shown that children with malaria parasite infection have lower PCV, RBG, serum Na^+, Mg^{2+}, anion gap, total protein and higher Ca^{2+} 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[4]. The relative hypoglycemia reported in children with malaria in this study is consistent with WHO criteria for the diagnosis of severe malaria[15]. Hypoglycemia has been described as a complication of many different pediatric illnesses and is usually associated with a poor outcome[16]. 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[17]. Heavy parasitemia have been associated with high glucose requirements by malaria parasites[18].

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[5]. The patho-physiology 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[4,23].

Increased serum calcium level was observed in children with malaria infection compared to those without malaria. Intra-erythrocytic 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[2]. 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[27]. 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 billirubin 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 hypoproteinemia 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 billirubin 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.

Acknowledgments: None

Financial disclosure: There was no financial support or funding for this research project.

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1 Nmadu PM, Peter E, Alexander P, Koggie AZ, Maikenti JI. The prevalence of malaria in children between the ages 2-15 visiting Gwarinpa General Hospital Life-Camp, Abuja, Nigeria. J Health Sci. 2015; 5(3):47-51.
2 Zaki HY, Abdalla BE, Babkier HE. Biochemical profiles of children with severe *Plasmodium falciparum* malaria in Central Sudan: a case-control study. Al Neelain Med J. 2013; 3(8):1858-627.
3 Maitland K, Pamba A, Fegan G, Njuguna P, Nael S, Newton CR, et al. Pertubation in electrolyte levels in Kenyan children with severe malaria complicated by acidosis. Clin Infect Dis. 2005; 40:9-16.
4 Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. Nature 2002 Feb 7; 415(6872):673-9.
5 Das K, Sastry AS, Sahoo AK, Mahapatra SC. Acid-base imbalance and dyselectrolytemia in falciparum malaria. Ind Med Gaz 2014; 147(8):283-87.
6 World Health Organization (WHO). Fact sheet No. 94. W.H.O., Media Centre. 2010. Available from http://whqlibdoc.who.int/publications/2010/9789241547826_eng.pdf. [Last accessed November 28, 2016]
7 Rodkey FL. Binding of bromocresol green by human serum albumin. Arch Biochem Biophys 1964 Dec; 108:510-3.
8 George RK. The determination of serum total protein, albumin, and globulin by the biuret reaction. J Biol Chem. 1939; 131:197-200.
9 Trivelli LA, Ranney PH, Lai HT. Column chromatography method with cation exchange resin for glycated haemoglobin separation. N Eng J Med 1971; 284:353.
10 Watson D. Analytic methods for bilirubin in blood plasma. Clin Chem. 1961 Dec; 7:603-25.
11 Schales O, Schales SS. A simple and accurate method for the determination of chloride in biological fluids. J Biol Chem. 1941; 140:879-84.
12 Segal MA. A rapid electrotitrimetric method for determining CO2 combining power in plasma or serum. Am J Clin Pathol. 1955 Oct; 25(10):1212-6.
13 Everson ME. Spectrophotometric techniques. Burtis CA, Ashwood ER (eds). Tietz textbook of Clinical Chemistry, 3rd ed. Philadelphia: W. B. Saunders Co. pp.75-93, 1999.
14 Mann CK, Yoe JH. Photometric colorimetric test for magnesium with Lipid Clearing Factor (LCF). Anal Chem.1956; 28(2):202-5.
15 World Health Organization (WHO): Report. Severe falciparum malaria. Transac Roy Soc Trop Med Hyg. 2000; 94(1):1-90.
16 Kochar DK, Thanvi I, Kumawat BL, Shubhakaran K, Agarwal N. Importance of blood glucose level at the time of admission in severe and complicated malaria. J Assoc Physicians India 1998 Nov; 46(11):923-5.
17 Sethi JK, Xu H, Uysal KT, Wiesbrock SM, Scheja L, Hotamisligil GS. Characterisation of receptor-specific TNFalpha functions in adipocyte cell lines lacking type 1 and 2 TNF receptors. FEBS Lett. 2000 Mar 3; 469(1):77-82.
18 Adeosun OG, Oduola T, Akanji BO, Sunday AM, Udoh SJ, Bello IS. Biochemical alteration in Nigerian children with acute falciparum malaria. Afr J Biotechnol. 2007; 6(7):881-5.
19 Jasani JH, Sancheti SM, Gheewala BS, Bhuva KV, Varsha SD, Vacchani AB, et al. Association of the electrolyte disturbances (Na+, K+) with the type and severity of the malarial parasitic infection. J Clin Diag Res. 2012; 6(4)(Suppl-2):678-68.
20 Sowunmi A, Newton CR, Waruiru C, Lightman S, Dunger DB. Arginine and vasopressin secretion in Kenyan children with severe malaria. J Trop Pediatr. 2000 Aug; 46(4):195-9.
21 English M, Waruiru C, Amukoye E, Murphy S, Crawley J, Mwangi I, Peshu N, Marsh K. Deep breathing in children with severe malaria: indicator of metabolic acidosis and poor outcome. Am J Trop Med Hyg. 1996 Nov; 55(5):521-4.
22 Singh PS, Singh N. Tetany with Plasmodium falciparum Infection. J Assoc Physicians India. 2012 Jul; 60:57-8.
23 Cheesbrough M. Discrete laboratory practice in tropical countries Part 1, 2nd ed. Cambridge: Press Syndicate of the University of Cambridge, pp.247-258, 2005.
24 Krishna S, Ng LL. Cation metabolism in malaria-infected red cells. Exp Parasitol.1989 Nov; 69(4):402–6.
25 Tietz N, Pruden LE, Andersen S. Electrolytes: Burtis CA, Ashwood ER (eds). Tietz Fundamentals of Clinical Chemistry, 5th ed. Philadelphia, USA: WB Saunders Company, pp.546-467, 2001.
26 Ikekpeazu EJ, Neboh EE, Aguchime CN, Maduka IC, Aronu AE. A study on malaria parasitemia: effect on the sodium and potassium levels. J Biol Med. 2010; 2(2):20-5.
27 Heindrickse RG, Hasan AH, Olumide LO, Akinkunmi A. Malaria in early childhood. An investigation of five hundred seriously ill children in whom a "clinical" diagnosis of malaria was made on admission to the children's emergency room at University College Hospital, Ibadan. Ann Trop Med Parasitol. 1971 Mar; 65(1):1-20.
28 Kayode OT, Kayode AA, Awonuga OO. Status of selected haematological and biochemical parameters in malaria and malaria-typhoid co-infections. J Biol Sci. 2011; 11:367-73.
29 Kwena AM, Wakhisi J, Mambo FA. Possible biochemical markers in protein-energy malnutrition and malaria in children in Western Kenya. East Cent Afr J Pharmaceut Sci. 2012; 15:18-23.