J Parasit Dis (Oct-Dec 2017) 41(4):1093–1097
DOI 10.1007/s12639-017-0940-4

ORIGINAL ARTICLE

Malaria-induced anaemia and serum micronutrients in asymptomatic *Plasmodium falciparum* infected patients

Babamale O. Abdulkareem[1] · Abdulkareem O. Adam[2] · Akande O. Ahmed[1] · Afolayan A. Mariam[1] · Ugbomoiko U. Samuel[1]

Received: 28 April 2017 / Accepted: 5 July 2017 / Published online: 15 July 2017

Abstract Interaction between malaria, anaemia and malnutrition is poorly understood in asymptomatic malaria patients. This information is important in the management of malaria infection in many endemic regions in sub-Saharan Africa. Malaria parasitaemia, full blood counts and serum levels of essential micronutrients particularly iron (Fe), zinc (Zn) and copper (Cu) of the patients attending Health Centres in Ilorin, Kwara state were investigated using microscope, auto-haemanalyzer and atomic absorption spectrophotometer respectively. A total of 123 (55.2%) of our study population were positive of *Plasmodium falciparum*. Infection was age-specific ($p < 0.0001$), and a significant proportion (88.6%) of malaria infected patients were 28.5% mild, 45.5% moderate and 14.6% severely anaemic. The severity of anaemia increases as parasite density increases. Analysis of serum micronutrients revealed a significant low level of iron (3.72 mg/l), copper (2.05 mg/l) and zinc (3.67 mg/l) in infected patients ($p < 0.0001$); which further increased their anaemic condition. This study confirmed a significant relationship between severity of anaemia and nutritional deficiency in the pathogenesis of malaria infection. We therefore, recommend that immunomodulation potential of micronutrients may be essential in the management of malaria infection.

✉ Babamale O. Abdulkareem
Babamale.oa@unilorin.edu.ng; olas4nice2004@yahoo.co.uk

[1] Parasitology Unit of Department of Zoology, University of Ilorin, Ilorin, Nigeria

[2] Physiology Unit of Department of Zoology, University of Ilorin, Ilorin, Nigeria

Keywords Interaction · Microscopy · Micronutrient · Anaemia · Pathogenesis · Immunomodulation

Introduction

Malaria-induced anemia is an important contributing factor in the global burden of *Plasmodium falciparum* infection. Almost 83 million of children suffering from anaemia are in sub-Saharan Africa, where global burden of malaria infection is heavily concentrated (Dia and Ismael 2012; WHO 2015). Several reports showed that at least three in every five malaria-infected pregnant women and preschool children are anaemic worldwide (McLean et al. 2009; WHO 2008), and approximately 8–15% of malaria–related death amongst preschool children (<5 years) in endemic areas are due to severe anaemia (WHO 2003).

Study has shown that chronic anaemia alters individuals' cognitive function, impaired motor development and growth (Dinesh and Jagdish 2011). Most affected individuals are characterized by poor school performance, low immune function and increased susceptibility to infections (Dia and Ismael 2012). Other disease conditions such as thrombocytopenia, leucopenia and lymphopenia are common concomitant haematological alterations in anemic individuals (Babamale et al. 2017). These alterations vary with the density of parasitaemia, hemoglobinopathy, nutritional status, and host immunological status (Erhart et al. 2004; Price et al. 2001; Kotepui et al. 2015).

The mechanisms of malaria-induced anaemia and thrombocytopenia though complex, the destruction of parasitized and non-parasitized RBCs, poor erythropoiesis and unregulated stimulation of pro-inflammatory cytokines such as TNF and IL-10 were reported to be responsible for anemic condition (Kreil et al. 2000; Touze et al. 1990;

Beale et al. 1972). Similarly, immune-induced platelet destruction, low thrombopoietin synthesis, low medullar platelet production and palpable splenomegaly have been documented for thrombocytopenic condition in malaria patients (Kathryn et al. 2004; Kreil et al. 2000).

Many infectious diseases including malaria-induced morbidities have been associated with micronutrients deficiency. *P. falciparum* competes for copper, zinc and iron for its proliferation and survival in the host (Hommel 2007; Wander et al. 2009), thus leads to malnutrition and other negative effects on host immunity. Previous reports showed that zinc deficiency results in diminished lymphocyte proliferation and impaired host defence, while iron deficient individuals are characterized by delayed-type and antibody-dependent cytotoxicity hypersensitivities, decreased secretion of IL-2 and IFN-γ, poor lymphocyte proliferation (Deshpande et al. 2013). However, copper is a cofactor in the mobilization of iron during haemoglobin synthesis, hence a deficiency of copper may contribute to iron deficiency anaemia (Gropper et al. 2005).

In Nigeria, continuous surveillance and monitoring of the relationship between *P. falciparum,* anaemia and malnutrition are essential for effective and evidence-based management and treatment of *falciparum* malaria. This study, therefore, investigates the induced anaemia and alterations in serum micronutrients in malaria infected patients in Kwara state, Nigeria.

Materials and method

The study was hospital-based, conducted in Ilorin, Kwara State, Nigeria. Pregnant women and individuals who were on antimalarial drugs were excluded in the study. Ethical approval was sought and obtained from the University of Ilorin Ethical Review Committee and Medical Directors of the randomly selected Hospitals. The age of participants and clinical history were obtained from the pre-tested questionnaire and validated from Hospital records.

Blood samples of 223 patients attending University of Ilorin Teaching Hospital (UITH), University of Ilorin Health Center (UIHC) and Children Specialist Hospital (CHS) were screened for malaria parasites by microscopy, and parasitaemia was calculated and classified as mild, moderate and severe based on WHO recommendation. Individuals were considered uninfected if no malaria parasites was observed in at least 200 field of stained blood smear slide. Full blood counts and serum levels of Fe, Zn and Cu of the patients were investigated using auto-haemanalyzer (Abacus junior hematology analyzer, Australia) and atomic absorption spectrophotometer respectively in accordance with manufacturer instruction. Classification of anaemia into mild (10.1–10.9 g/dl), moderate (7.0–10.0 g/dl) and severe (<7 g/dl) follows the recommendation of World Health Organisation.

Statistical analysis

Data was entered into spread sheets using Microsoft Excel and analyzed using International Business Machine IBM (SPSS) statistics package version 21. Data obtained were expressed as mean ± standard deviation in the assessment of descriptive statistics. The Chi square was used to compare means. Significant levels were measured at 95% confidence level (CI) with significant differences recorded at $p < 0.05$.

Results

Our result revealed that 55.2% of 223 (100 male and 123 female) were infected with *P. falciparum* and infection was not sex-dependent ($p > 0.05$) but age-specific ($p < 0.0001$). Prevalences of malaria infection were higher in individuals in the age groups of ≤10 and ≥21 years (Table 1). Infection was statistically correlated with anaemia ($p < 0.0001$). Of the 88.6% infected patients, 28.5, 45.5 and 14.6% had mild, moderate and severe anaemia respectively. Generally, the serum levels of iron, copper and zinc in infected patients were lower than the uninfected ones (Table 1), and severity of anaemia increases with increasing intensity of malaria infection ($p < 0.0001$). However, individuals with mild and severe anaemia had average parasitaemia load of 223.45 and 7889.67 µl of blood respectively. Blood parameters such as PCV and MCH follow similar pattern. Table 2 detailed the differential blood counts with respect to the degree of anaemic conditions. Serum iron, copper and zinc in anaemic and non-anaemic patients were comparable.

Discussion

Malaria and anaemia remain major health problems in many tropical countries (Adedotun et al. 2013; Tine et al. 2012). Approximately 55% point prevalence of malaria infection in this study population is relatively high when compare with previous studies in the same ecological zone (Anumudu et al. 2006; Abdullahi et al. 2009; Umeanaeto and Ekejindu 2006; Aribodor et al. 2003). This may be attributed to the period of the study (rainy season) which coincides with the peak period of mosquitoes breeding and parasite transmission (Erhabor et al. 2010). Age and gender patterns paralleled the observations in malaria-endemic regions (Babamale and Ugbomoiko 2016).

Table 1 Prevalence of malaria infection with respect to serum micronutrient and anaemic level in the study population

Variable	Malaria status		Total (%)	*p* value
	Infected (%)	Uninfected (%)		
Overall	123 (55.2)	100 (44.8)	223	
Sex				
Male	55 (44.7)	45 (45.0)	100 (44.8)	0.940
Female	68 (55.3)	55 (44.7)	123 (55.2)	
Age (years)				
≤10	23(52.3)	21 (47.7)	44 (19.7)	<0.0001
10–20	25 (45.5)	30 (54.5)	55 (24.7)	
≥21	75 (60.5)	49 (39.5)	124 (55.6)	
Aneamia status				
No	14 (11.4)	82 (82.0)	96 (43.0)	<0.0001
Mild	35 (28.5)	8.0 (8.0)	43 (19.3)	
Moderate	56 (45.5)	0.0 (0.0)	56 (25.1)	
Severe	18 (14.6)	0.0 (0.0)	18 (8.1)	
Serum micronutrients (mg/dl)				
Iron (X ± SD)	2.61 ± 0.69	3.71 ± 0.30	2.84 ± 0.77	<0.0001
95% CI	2.48–2.73	3.61–3.81	2.72–2.96	
Copper (X ± SD)	1.99 ± 0.44	2.04 ± 0.30	2.00 ± 0.41	0.580
95% CI	1.91–2.07	1.93–2.15	1.93–2.07	
Zinc (X ± SD)	3.17 ± 0.80	3.69 ± 0.55	3.28 ± 0.79	0.001
95% CI	3.02 ± 3.31	3.50 ± 3.90	3.16 ± 3.40	

Table 2 Severity of anaemia stratified with hosts' parasitaemia, haematological indices and serum micronutrients

Parameters	Anaemia status				Overall	*p* value
	No	Mild	Moderate	Severe		
Parasite intensity (µl of blood)	223.45 ± 41.50	2224.63 ± 1740.0 0	4322.10 ± 2789.66	7889.67 ± 7351.11	4473.03 ± 2523.64	<0.0001
Anaemia indices						
RBC (10^3µ/l)	3.52 ± 0.69	3.42 ± 0.77	3.46 ± 0.89	3.47 ± 0.22	3.49 ± 0.69	0.005
PCV (%)	34.25 ± 5.28	28.50 ± 3.23	26.01 ± 4.67	24.90 ± 3.23	28.04 ± 5.24	<0.0001
MCHC (g/dl)	32.36 ± 2.52	31.46 ± 4.42	32.46 ± 6.25	33.36 ± 2.81	32.25 ± 4.91	0.528
MCV (pg)	91.99 ± 15.20	67.51 ± 9.16	75.46 ± 6.63	72.97 ± 6.17	75.69 ± 12.35	<0.0001
MCH (pg)	30.82 ± 4.62	27.42 ± 3.90	26.84 ± 2.58	26.80 ± 2.98	27.69 ± 3.72	<0.0001
Other blood indices						
Platelet (10^3µ/l)	80.59 ± 10.34	59.87 ± 22.97	40.42 ± 26.67	49.55 ± 25.60	54.16 ± 27.38	<0.0001
Neutrophil	2066.11 ± 438.99	1481.80 ± 474.26	989.93 ± 570.25	1559.00 ± 362.25	1386.89 ± 631.54	<0.0001
Basophil	22.74 ± 1.28	34.63 ± 10.21	47.37 ± 10.21	33.28 ± 21.29	37.73 ± 17.39	<0.0001
Serum micronutrients						
Iron (mg/dl)	3.72 ± 0.29	2.66 ± 0.66	2.59 ± 0.76	2.86 ± 0.66	2.84 ± 0.77	<0.0001
Copper (mg/dl)	2.05 ± 0.30	2.07 ± 0.49	2.01 ± 0.42	1.76 ± 0.22	2.00 ± 0.41	0.060
Zinc (mg/dl)	3.67 ± 0.55	3.63 ± 0.76	2.77 ± 0.68	3.63 ± 0.45	3.28 ± 0.79	<0.0001

Anaemia is a common health complication with 88.6% cases among malaria infected patients in this study, and it agrees with many other reports worldwide (Bakhubaira 2013; Ifeanyichukwu and Esan 2014; Francis et al. 2014; Erhabor et al. 2010). This disease condition results from a combination of haemolysis of parasitized and non-

parasitized red blood cells, and a depression in erythropoiesis (Bashawri et al. 2002; WHO 2002). Our data also showed a significantly reduced haemoglobin concentration with proportional increase in parasitaemia amongst infected patients. This, according to Wanitchar and Mongkol (2012), suggests the upregulatory stimulation of TNF-α, interferon-gamma (INF-γ) and interleukin-1 (IL-1) in malaria-infected patients. This outcome is further validated by a decrease in RBC, MCV and MCH recorded in our infected individuals; indicating the inhibitory effects of *P. falciparum* on the incorporation of haemoglobin in red blood cells that leads to microcytic anaemia. Overall, our observation depicts an increased parasites' competition for hosts' daily metabolic demand, resulting in lowering of oxygen-carrying capacity below the body physiological needs in the infected patients. However, the occurrence of anaemia and thrombocytopenia in malaria infected patients is not uncommon in many malaria-endemic regions in sub-Saharan Africa (Grynberg et al. 2007; Erhart et al. 2004; Price et al. 2001; Kotepui et al. 2015; Babamale et al. 2017). Our report of 60% thrombocytopenic malaria-infected patients in this study confirms these reports. Many workers (Johnston et al. 2009; Weatherall et al. 2002) have attributed this disease condition to increase consumption of the platelets in the peripheral blood, which may follow absorption of immune complexes. However, Al-Omar et al. (2010) reported that stimulated cytokines, in response to parasitized RBCs, activate coagulation cascades, consumption of antithrombin III, increased concentration of fibrinogen degradation products (FDP) and increased splenic clearance of platelets result to coagulopathy and thrombocytopenia in malaria patients.

In this study, a depletion of some basic micronutrients (Fe, Cu, and Zn) in malaria-infected hosts was observed. This has a grave pathological implication in malaria. Roy (2013) ascribed reduction in serum iron during infection to reallocation of iron into liver and macrophage. This deficiency, in agreement with the reports of Akpotuzor et al. (2007) and Modaresinejad et al. (2016), affects the immunological status of the infected host since iron deficiency causes decrease in total T-lymphocyte count and reduction in the proportion of CD4+ and CD8+ cells, thus rendering the host less hostile for the proliferation of parasites.

Similarly, the significant concurrent reduction of Fe and Zn may result in the fragility of RBC and platelets which again cause anaemia. The serum level of Zinc and Copper were also lower in malaria-infected patients than non-infected, a reduction that increased concomitantly with the severity of infection (Melaine et al. 2010, Anuraj and Shankar 2000). In agreement with the reports of Steketee (2003), Gouado et al. (2007) and Deshpande et al. (2013), these observations may probably lower the spontaneous production of host immune response required for the elimination of parasites at a low cost of damage to the host, and ultimately increase host vulnerability to tissues and organs damage.

In this study, the Health Centers from where patients were recruited cater for patients of all socio-economic status. However, there were limitations in methodology as diagnosis of malaria infection was based solely on microscopy; therefore the prevalence of malaria infection may be underestimated. To reduce this limitation, three blood smears made from each patient at different times were re-examined independently by different investigators and confirmed by experienced parasitologist (USU).

Conclusion

Anaemia is indeed a serious pathological condition in both symptomatic and asymptomatic malaria patients and, its concurrence with other associated hematological alterations and micronutrient depletion will further complicate the disease condition in the infected hosts. Our study confirmed a significant relationship between severity of anaemia and nutritional immunodeficiency in malaria infection. However, we recommend further study on the immunomodulation potential of the micronutrients which will validate and establish nutrients that are effective in the management of malaria infection.

References

Abdullahi K, Abubakar U, Adamu T, Daneji AL, Aliyu RU, Jiya N, Ibraheem MTO, Nata'ala SU (2009) Malaria in Sokoto, North Western Nigeria. Afr J Biotech 24:7101–7105

Adedotun AA, Salawu OT, Morenikeji OA, Odaibo AB (2013) Plasmodial infection and haematological parameters in febrile patients in a hospital in Oyo town, South-western Nigeria. J Publ Health Epidemiol 5(3):144–148

Akpotuzor JO, Udoh AE, Etukudo MH (2007) Total antioxidant status, vitamin A, C and β-carotene levels of children with *P. falciparum* in University of Calabar Teaching Hospital (UCTH), Calabar. Pak J Nat 615:485–489

Al-Omar IA, Eligail AM, Al-Ashban RM, Shah AH (2010) Effect of falciparum malaria infection on blood cholesterol and platelets. J Saudi Chem Soc 14:83–89

Anumudu CI, Adepoju A, Adeniran M, Adeoye O, Kassim A, Oyewole I, Nwuba RI (2006) Malaria prevalence and treatment seeking behavior of young Nigerian adults. Ann Afr Med 15:82–88

Anuraj H, Shankar HA (2000) Nutritional modulation of malaria morbidity and mortality. J Infect Dis 182(1):S37–S53

Aribodor DN, Njoku OO, Eneanya CI, Onyido AE (2003) Studies on the prevalence of malaria and management practices of the Azia community in Ihiala L.G.A, Anambra state. Nigerian J Parasitol 24:33–38

Babamale OA, Ugbomoiko US (2016) Status of malaria infection in peri-urban community of north central region of Nigeria. J Bacteriol Parasito l 7:256. doi:10.4172/2155-9597.1000256

Babamale OA, Abdulkareem AO, Opeyemi OA (2017) Alterations in T-helper cell type 1 and blood cell parameters in malaria-infected patients. Egypt J Basic Appl Sci. doi: 10.1016/j.ejbas.2017.05.002

Bakhubaira S (2013) Hematological parameters in severe complicated *Plasmodium falciparum* malaria among adults in Aden. Turk J Haematol 30:394–399

Bashawri LA, Mandil AA, Bahnassy AA, Ahmed MA (2002) Malaria: hematological aspects. Ann Saudi Med 22:372–376

Beale PJ, Cormack JD, Oldrey TB (1972) Thrombocytopenia in malaria with immunoglobulin (IgM) changes. Br Med J 1:345–349

Deshpande JD, Joshi MM, Giri PA (2013) Zinc: the trace element of major importance in human nutrition and health. Int J Med Sci Publ Health 2:1–6

Dia S, Ismael N (2012) Risk factors for anemia in preschool children in Sub-Saharan Africa, Anemia, Dr. Donald Silverberg (Ed.), ISBN: 978-953-51-0138-3, InTech. http://www.intechopen.com/books/anemia/risk-factors-for-anemia-in-preschool-children-in-sub-saharan-africa

Dinesh Y, Jagdish C (2011) Iron deficiency: beyond anemia. Indian J Pediatr 78:65–72

Erhabor O, Adias TC, Hart ML (2010) Effects of falciparum malaria on the indices of anaemia among pregnant women in the Niger Delta of Nigeria. J Clin Med Res 2(3):035–041

Erhart LM, Yingyuen K, Chuanak N, Buathong N, Laoboonchai A, Miller RS et al (2004) Hematologic and clinical indices of malaria in a semi-immune population of western Thailand. Am J Trop Med Hyg 70:8–14 **(PMID: 14971691)**

Francis U, Isaac Z, Yakubu A, Enosakhare A, Felix E (2014) Haematological parameters of malaria infected patients in the University of Calabar Teaching Hospital, Calaba, Nigeriar. J Hematol Thromb Dis 2:171. doi:10.4172/2329-8790.1000171

Gouado I, Lehman LG, Some IT, Mbouyap YP, Mfonkeu JB, Ejoh AR, Tchouanguep MF (2007) Influence of malaria on the serum levels of vitamin A, zinc and calcium of children in Douala–Cameroon. Afr J Biotech 6(7):871–876

Gropper SS, Smith JL, Groff JL (2005) Advanced nutrition and human metabolism, 14th edn. Thomson Wadsworth, Belmont

Grynberg P, Fernandes Fontes CJ, Braga EM (2007) Association between particular polymorphic residues on apical membrane antigen 1 (AMA-1) and platelet levels in patients with vivax malaria. Clin Microbiol Infect 13:1089–1094

Hommel M (2007) Morphology, biology and life cycle of plasmodium parasites BullAcad. Natl Med 191(7):1235–1245

Ifeanyichukwu MO, Esan AJ (2014) Evaluation of hemoglobin concentration, packed cell volume and red cell indices in pre- and post-anti-malaria drug treatment in *plasmodium falciparum* malaria infected and control individuals. Glob Adv Res J Microbiol 3(2):012–017

Johnston V, Stockley JM, Dockrell D, Warrell D, Bailey R, Pasvol G, Klein J, Ustianowski A, Jones M, Beeching NJ, Brown Chapman AL, Sanderson F, Whitty C (2009) Fever in returned travelers presenting in the United Kingdom: recommendations for investigation and initial management. J Infect 59(1):1–18

Kathryn NS, Kevin C, Jay SK (2004) Malaria. C M A J 170(11):1503–1518

Kotepui M, Piwkham D, PhunPhuech B, Phiwklam N, Chupeerach C, Duangmano S (2015) Effects of Malaria Parasite Density on Blood Cell Parameters. PLoS ONE 10(3):e0121057. doi: 10.1371/journal.pone.0121057

Kreil A, Wenisch C, Brittenham G, Looareesuwan S, Peck-Radosavljevic M (2000) Thrombopoietin in plasmodium falciparum malaria. Br J Haematol 109:534–536

McLean E, Coqswell M, Egli I, Wojdyla D, de Benoist B (2009) Worldwide prevalence of anemia, WHO vitamin and mineral nutrition information system, 1993–2005. Publ Health Nutr 12(4):444–454

Melaine GM, Félix HY, Hugues TA, Yapo A, Brice KB, Joseph AD (2010) The effect of *falciparum* malaria infection on the quantity of trace elements (iron, copper, zinc) in the blood in children of Côte d'Ivoire. Agric Biol J N Am 1(4):565–570

Moderesinejad M, Nahrevanian H, Khatami S, Bagheri (2016) Biochemical association between essential trace elements and susceptibility to malaria in outbred mice after inhibition with Dexamethasone or induction with Lipopolysaccharide. Adv Stud Biol 8(2):91–99

Price RN, Simpson JA, Nosten F, Luxemburger C, Hkirjaroen L, ter Kuile F et al (2001) Factors contributing to anemia after uncomplicated falciparum malaria. Am J Trop Med Hyg 65:614–622 **(PMID: 11716124)**

Roy CN (2013) An update on iron homeostasis: make new friends, but keep the old. Am J Med Sci 346:413–419. doi: 10.1097/MAJ.0000000000000190

Steketee (2003) Pregnancy, nutrition and parasitic diseases. J Nutr 133:1667S

Tine RCK, Ndiaye M, Hansson H, Ndour C et al (2012) The Association between malaria, parasitaemia, erythrocytepolymorphism and anemia in children less than 10 years' in Senegal: a case control study. BMC Res Notes 5:565

Touze JE, Mercier P, Rogier C, Hovette P, Schmoor P, Dabanian C (1990) Platelet antibody activity in malaria thrombocytopenia. Pathol Biol (Paris) 38:678–681

Umeanaeto PU, Ekejindu IM (2006) Prevalence and intensity of malaria in blood donors at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nwewi, Anambra State, Nigeria. Nig J Parasitol 27:11–15

Wander K, Shell-Duncan B, McDade TW (2009) Evaluation of iron deficiency as a nutritional adaptation to infectious disease: an evolutionary medicine perspective. Am J Hum Biol 21:2–172

Wanitchar W, Mongkol B (2012) Presence of anemia and poor prognostic factors in patients with endometrial carcinoma. Asian Pacific J Cancer Prev 13:3187–3190

Weatherall DJ, Miller Louis H, Baruch Dror I, Marsh Kevin, Doumbo Ogobara K, Casals-Pascual Climent, Roberts David J (2002) Malaria and the red cell. The American Society of Hematology, Washington

World Health Organization (2002) The global malaria situation: current tools for prevention and control. Global Fund to fight AIDS, Tuberculosis, and Malaria. 55 World Assembly, WHO, Document, A551

World Health Organization (2003) SAMC Information for Action leaflet. WHO Harare Country/Inter-country Office for Southern Africa. Malaria: a case management update: special edition: Anaemia

World Health Organization (2008) World malaria report. WHO, Switzerland, pp 99–101

World Health Organization (2015) World malaria report. Who global malaria programme, Geneva, pp 2–243