

# Antioxidant vitamin levels among preschool children with uncomplicated *Plasmodium falciparum* malaria in Sokoto, Nigeria

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**Objective:** To assess antioxidant vitamin levels among preschool children with plasmodium malarial infection.

**Methods:** We assessed antioxidant vitamin levels by using a standard procedure in 130 malaria-parasitized preschool children. Packed cell volume and parasite density were also evaluated. Forty healthy age- and gender-matched nonparasitized children were included as controls.

**Results:** *Plasmodium falciparum* was the causative species in all subjects. The mean malaria parasitemia was  $4529.45 \pm 1237.5/\mu\text{L}$ . The mean antioxidant concentrations for vitamins A, C, and E among plasmodium-parasitized subjects were  $33.15 \pm 1.79 \mu\text{g/dL}$ ,  $0.51 \pm 0.02 \text{ mg/dL}$ , and  $0.61 \pm 0.02 \text{ mg/dL}$ , respectively. The mean concentrations of vitamins A, C, and E among the non-malaria-parasitized controls were  $69.72 \pm 1.71 \mu\text{g/dL}$ ,  $1.25 \pm 0.04 \text{ mg/dL}$ , and  $1.31 \pm 0.04 \text{ mg/dL}$  respectively. We observed that the mean antioxidant concentrations of vitamins A, C, and E were significantly lower among plasmodium-parasitized subjects compared with non-parasitized controls ( $P = 0.01$ ). Malaria parasitemia correlated negatively with antioxidant concentrations and packed cell volume ( $r = -0.736$  and  $-0.723$ ,  $P = 0.001$ ). We observed that the higher the level of parasitemia, the lower the antioxidant concentration.

**Conclusion:** Our study has shown that the antioxidant levels in plasmodium-parasitized children in the North-West of Nigeria are low and that the more severe the malarial infection, the lower the antioxidant level and the packed cell volume. One key strategic intervention is the provision of early diagnosis and prompt effective treatment. We recommend that malaria-parasitized children, particularly those in the North-West of Nigeria, be placed routinely on antioxidant vitamins to manage the micronutrient deficiencies seen in these children. There is also the need for the promotion of insecticide-treated bed nets, intermittent preventive treatment, and effective case management of malarial illness among children.

**Keywords:** vitamin A, vitamin C, vitamin E, *P. falciparum*

## Introduction

Malaria poses an enormous public health burden, and greater than 75% of the global clinical episodes of malarial infection each year are concentrated in Africa. According to latest estimates from the World Health Organization, in 2009, there were 225 million cases of malaria and an estimated 781,000 deaths worldwide. Most of these deaths occurred among children living in the World Health Organization African Region (mainly sub-Saharan Africa).<sup>1</sup> Malaria was the eighth highest contributor to the world loss of disability-adjusted life years in 2001, and second in Africa.<sup>2,3</sup> It is one of the greatest and oldest health challenges affecting 40% of the world's population.<sup>4</sup>

The clinical and epidemiological disease burden of malaria morbidity and mortality is dependent largely on the complex pathogenesis of this parasite infection.<sup>5</sup> It is

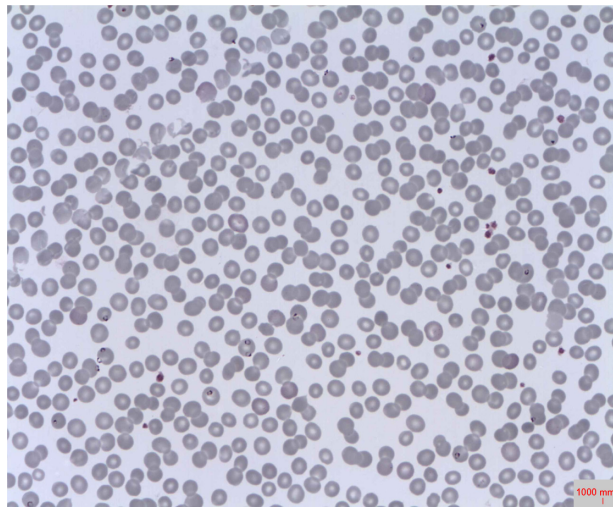
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characterized by a stable, perennial transmission in all parts of Nigeria, with transmission reaching its peak in the wet seasons. The disease can be contracted at least two times in a year, and 30%–40% of out-patient consultations and pediatric admissions are due to malaria.<sup>6,7</sup> The mechanism that might affect the pathogenesis and antioxidant status of children with malaria is thought to be the production of excess free radicals, which induces lipid peroxidation and cell damage. In children, micronutrient status has been shown to influence resistance to several infectious diseases, including measles, diarrhea, and respiratory diseases.<sup>8–11</sup> A randomized trial in Papua New Guinea has shown that periodic vitamin A supplementation could reduce the incidence of febrile episodes and parasitemia due to *Plasmodium falciparum*.<sup>12</sup> Vitamin A is essential for normal immune function and has been shown to influence both antibody response and cell-mediated immunity.<sup>13</sup> Vitamin C is a negative acute phase reactant. Plasma vitamin C concentration correlates inversely with white cell count, alpha-1-acid glycoprotein, and interleukin-6, all of which are markers of inflammation.<sup>14</sup> Vitamin C can rejuvenate vitamin E, making it an indirect contributor to fighting free-radical damage in membrane lipids.<sup>15</sup> These free radicals are products of oxidative stress that is aggravated in malarial infection to decrease the antioxidant defense system. One of the implications of oxidative stress is the development of malarial anemia.<sup>16</sup> It has been reported that antioxidants such as carotenoid and vitamins C and E could provide protection against oxidative stress induced by malaria.<sup>16–18</sup> There is paucity of literature on the antioxidant level in plasmodium-parasitized children in the North-West of Nigeria. The aim of this present study was to investigate the relationship between *P. falciparum* malaria and antioxidant concentration in a hospital-based study involving preschool children with uncomplicated *P. falciparum* malaria in Sokoto State in the North-West geopolitical zone of Nigeria.

## Materials and method

### Study site

The study was conducted in the Sokoto municipality between June 2009 and August 2010. Sokoto State is located in the extreme North-West of Nigeria, near to the confluence of the Sokoto River and the Rima River. With an annual average temperature of 28.3°C (82.9°F), Sokoto is, on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under 40°C (104.0°F). The warmest months are February to April, when daytime temperatures can exceed 45°C (113.0°F). The rainy season is from June to October, during which showers are a daily occurrence. There are two



**Figure 1** A *Plasmodium falciparum*-positive slide.

major seasons: wet and dry, which are distinct and characterized by high and low malarial transmission, respectively. The state has a population of 3.6 million.<sup>19</sup> In Sokoto, as in other cities in Northern Nigeria, malaria is hyper-endemic<sup>20</sup> and *P. falciparum* is the predominant species (Figure 1).<sup>21</sup>

### Subjects

The study subjects consisted of 130 children aged 2–60 months old who were recruited at the outpatient clinic of the Department of Paediatrics of Usmanu Danfodiyo University Teaching Hospital, Sokoto. Consenting subjects who presented with signs and symptoms suggestive of simple/uncomplicated malaria<sup>22</sup> (fever with axillary temperature >37.5°C, headache, vomiting, diarrhea, prostration, pallor, jaundice, respiratory distress, and other clinical signs and symptoms) were recruited into this study. Written informed consent was obtained from the parent/guardian of each child after thoroughly explaining the scope, nature, and objective of the study and offering counseling. Demographic data including age, weight, and height were measured and recorded. Exclusion criteria for subjects included age (less than 2 months or older than 60 months), children with sickle-cell disease, viral hepatitis B, tonsillitis, otitis media, respiratory distress, recent history of convulsion and history of HIV (human immunodeficiency virus)/AIDS (acquired immunodeficiency syndrome) infection. Ethical approval was obtained from the ethical committee of Usmanu Danfodiyo University Teaching Hospital.

### Sample collection

The study population in this case-control study comprised 130 malaria-infected children who attended the pediatric

clinic of Usmanu Danfodiyo University Teaching Hospital, Sokoto. Forty healthy age- and gender-matched nonparasitized children were included as controls. The number of control participants is adequate for the power of the study. Five milliliters of venous blood was obtained from each participant by venepuncture. Two milliliters of the blood was collected into an EDTA (ethylenediaminetetraacetic acid) bottle for the determination of packed cell volume, confirmation of malaria parasitemia, speciation, and parasite count. Blood smears (thin and thick films) were prepared for all malaria-positive subjects and non-parasitized controls. Thick and thin films were stained with Field's stain (for confirmation, speciation, and parasite load determination) and were read for 200 fields. Parasite counts were reported per 500 white blood cells and for counts above 1,000 parasites. Malaria parasitemia was defined by the presence of asexual forms of *P. falciparum*, confirmed by microscopic examination of the peripheral blood. The parasitemia was graded as low (<500 parasites/ $\mu$ L), moderate (3,000 parasites/ $\mu$ L), and severe (>10,000 parasites/ $\mu$ L). Three milliliters of venous blood was collected into a clean plain tube without anticoagulant and allowed to clot at room temperature. The serum was obtained by centrifugation for 10 minutes at 3000 rpm. Vitamin A was assayed by the method of Bassey et al,<sup>23</sup> vitamin C was assayed by the method of Roe and Kuether,<sup>24</sup> and Vitamin E was assayed by the method of Neild and Person.<sup>25</sup> The data obtained from this investigation were subjected to statistical analysis.

## Statistical analysis

Data were entered and analyzed using statistical package SPSS version 9 (SPSS Inc, Chicago, IL, USA). Statistical analyses included descriptive analysis of mean, standard deviation, and chi-square analysis. A *P*-value of >0.05 was considered to be statistically significant in all statistical analyses. Correlation was compared using a version of linear regression analysis.

## Results

The plasma antioxidant levels, packed cell volume, and parasite counts of control subjects and malaria-infected children are shown in Table 1. The antioxidant vitamin A, C, and E levels and packed cell volume were lower in malaria-infected children when compared with control subjects. *P. falciparum* was the causative species in all the subjects studied. The values of the antioxidant vitamins A, C, and E ( $33.15 \pm 1.79$   $\mu$ g/dL,  $0.51 \pm 0.02$  mg/dL, and  $0.61 \pm 0.02$  mg/dL, respectively) and packed cell volume

**Table 1** Plasma antioxidant vitamin levels, packed cell volume and parasite count in malaria-infected children

| Parameters                | Control participants (N = 40) | Plasmodium parasitized subjects (N = 130) |
|---------------------------|-------------------------------|---|
| Vitamin A ( $\mu$ g/dL)   | $69.72 \pm 1.71$              | $33.15 \pm 1.79$                          |
| Vitamin C (mg/dL)         | $1.25 \pm 0.04$               | $0.51 \pm 0.02$                           |
| Vitamin E (mg/dL)         | $1.31 \pm 0.04$               | $0.61 \pm 0.02$                           |
| Packed cell volume (%)    | $38.55 \pm 0.63$              | $34.57 \pm 0.64$                          |
| Parasite count ( $\mu$ L) | Nil                           | $4529.45 \pm 1237.50$                     |

**Notes:** Mean  $\pm$  SD.

**Abbreviations:** N, number; SD, standard deviation.

( $34.57\% \pm 0.64\%$ ) were observed to be significantly lower in malaria-infected children compared with control subjects ( $P = 0.01$ ).

Table 2 shows the antioxidant vitamin levels and intensity of malaria parasitemia. Plasma antioxidants and packed cell volume decreased with increasing malaria severity. Parasitemia was classified as mild (<500 parasites/ $\mu$ L), moderate (<3000 parasites/ $\mu$ L), and severe (>10,000/ $\mu$ L) infection. Antioxidant vitamins and packed cell volume in subjects with severe malaria were significantly lower than those with mild and moderate malarial infections. We observed a significant negative correlation between the parasite load, the antioxidant vitamin status, and packed cell volume ( $r = -0.736$  and  $-0.723$ ,  $P = 0.001$ ).

Table 3 shows the antioxidant profiles according to the age groups of the plasmodium-parasitized subjects. Children between the ages of 13 and 60 months old had the highest *P. falciparum* load of  $3979.0 \pm 1173.4/\mu$ L in their peripheral blood. Also, the younger children <12 months old had higher antioxidant vitamin values compared with those above 12 months old.

## Discussion

Malaria is a serious parasitic disease and is a major health problem in much of the tropics and subtropics. The justification for this study includes the fact that malaria is endemic in Sokoto and is responsible for mortality and morbidity particularly in the pediatric population, yet no study has been done in Sokoto and indeed the North-West geopolitical zone of Nigeria to investigate the effect of malaria on the antioxidant vitamin levels among preschool children with uncomplicated malaria. In the present study, investigating the antioxidant levels in preschool children with uncomplicated plasmodium malaria, we observed lower antioxidant vitamin concentrations in plasmodium-parasitized children than in the control subjects. The reduction of these antioxidants in the face of malarial infection may predispose the children to free-radical attack. This finding is in agreement with

**Table 2** The antioxidant vitamin status and intensity of malaria parasitemia

| Intensity of infection or parasite load ( $\mu\text{L}$ )       | Number of participants | Vitamin A ( $\mu\text{g/dL}$ ) | Vitamin C (mg/dL) | Vitamin E (mg/dL) |
|---|------------------------|--------------------------------|-------------------|-------------------|
| Mild (469.35 $\pm$ 33.34)                                       | 62                     | 45.00 $\pm$ 1.51               | 0.60 $\pm$ 0.03   | 0.72 $\pm$ 0.03   |
| Moderate (2646.0 $\pm$ 146.0)                                   | 40                     | 26.20 $\pm$ 1.32               | 0.47 $\pm$ 0.02   | 0.60 $\pm$ 0.02   |
| Severe (10473.00 $\pm$ 207.25)                                  | 28                     | 14.12 $\pm$ 0.87               | 0.31 $\pm$ 0.02   | 0.32 $\pm$ 0.02   |
| Mean parasite load of infected subjects (4529.45 $\pm$ 1237.50) | 130                    | 28.44 $\pm$ 4.13               | 0.46 $\pm$ 0.04   | 0.55 $\pm$ 0.05   |
| Mean parasite load of noninfected controls                      | 40                     | 69.72 $\pm$ 1.71               | 1.25 $\pm$ 0.04   | 1.31 $\pm$ 0.04   |

previous reports.<sup>9–12</sup> The low antioxidant vitamin levels observed in this study may be as a result of increased utilization of the host's plasma antioxidants by the malaria parasite to counteract the malarial infection-associated oxidative damages.<sup>26</sup> Previous reports among children in Papua New Guinea<sup>13</sup> suggest that vitamin A supplementation could reduce the incidence of plasmodium malaria. Antioxidants have been shown to influence both humoral and cell-mediated immunity as well as counteracting the effect of free-radicals and other products of oxidative stress associated with malarial infection. A significantly lower level of vitamin A was seen in severe malaria-parasitized children compared with the levels of both mild and moderate malarial infections.<sup>14,16,17</sup> The low concentration of vitamin A seen in malaria-infected children in this study may be attributable to inflammatory response<sup>27</sup> and redistribution of vitamin A into extra vascular spaces to allow for increased bioavailability to the tissues.<sup>28</sup>

This study revealed that *P. falciparum*-infected children have lower levels of antioxidant vitamins C and E compared with control subjects. This finding is consistent with a previous report.<sup>16</sup> The low concentration of antioxidant vitamins E and C may be due to increased utilization of plasma antioxidant or increased destruction during malarial infection.<sup>10</sup> We observed that the antioxidant levels correlated inversely with the severity of malaria parasitemia and the packed cell volume. This finding is in agreement with the suggestion in a previous report that the higher the level of plasmodium parasitemia, the lower the antioxidant level.<sup>29</sup>

We observed that the predominant plasmodium species responsible for all malarial infections among the children

studied was *P. falciparum*. This finding is consistent with previous reports in Nigeria which found *P. falciparum* as the predominant species responsible for malarial infection in Nigeria.<sup>10,11,30,31</sup> Mapping the global distribution of malaria motivated by a need to define populations at risk for appropriate resource allocation, and to provide a robust framework for evaluating its global economic impact, has shown that malarial infection particularly caused by *P. falciparum* has been geographically restricted and remains entrenched in poor areas of the world, particularly in sub-Saharan Africa.<sup>32</sup>

Our study has shown that the antioxidant levels in plasmodium-parasitized children in the North-West of Nigeria are low and that the more severe the malarial infection the lower the antioxidant level and the packed cell volume. There are several daunting challenges associated with effective management of malaria among pediatric populations in developing countries: development of drug-resistant strains, late diagnosis, poor mosquito-infested environments, lack of access to health care services, high incidence of self-medication, and patronage of quack chemists and traditional healers for the treatment of malaria. One key strategic intervention is the provision of early diagnosis and prompt effective treatment. A major setback in most settings in sub-Saharan Africa has been the development of drug resistance to commonly used antimalarials. Resistance of *P. falciparum* parasites to antimalarial drugs has fuelled an increase in malaria prevalence and malaria-specific mortality, particularly among children during the past decade in sub-Saharan Africa. Promising efficacy results from field trials of artemisinin combination therapies are a source of optimism, and numerous African countries have changed their first-line malaria drug policy to artemisinin combination therapies to counter high levels of resistance.<sup>33</sup> There is also the need to routinely place malaria-parasitized children, particularly those in the North-West of Nigeria, on antioxidants to manage the micronutrient deficiencies seen in these children. There is also the need for the promotion of insecticide-treated bed nets, intermittent preventive treatment, and effective case management of malarial illness among children.

**Table 3** The antioxidant level and malaria parasitemia according to the age of infected subjects

| Age groups in years | Vitamin A ( $\mu\text{g/dL}$ ) | Vitamin C (mg/dL) | Vitamin E (mg/dL) | Malaria parasite ( $\mu\text{L}$ ) |
|---------------------|--------------------------------|-------------------|-------------------|------------------------------------|
| <1                  | 37.40 $\pm$ 2.20               | 0.57 $\pm$ 0.04   | 0.67 $\pm$ 0.04   | 1589.2 $\pm$ 421.47                |
| 1–5                 | 30.84 $\pm$ 4.53               | 0.48 $\pm$ 0.04   | 0.57 $\pm$ 0.04   | 3979.0 $\pm$ 1173.4                |
| Mean                | 34.12 $\pm$ 1.82               | 0.53 $\pm$ 0.04   | 0.62 $\pm$ 0.03   | 2784.1 $\pm$ 1148.2                |

## Limitations of study

The limitations of this study include the fact that we did not control for other factors that may be confounders that may affect the association between malaria parasitemia and low antioxidant vitamin levels among the preschool children studied. Also we did not include other antioxidant vitamins, particularly ascorbic acid, selenium, zinc, copper, and magnesium. We hope to study these antioxidant vitamins in a subsequent follow-up study.

## Disclosure

The authors report no conflicts of interest in this work.

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