

Full Length Research Paper

Antioxidant status of Nigerian children with *Plasmodium falciparum* malaria

Nmorsi O.P.G.^{1*}, Ukwandu N.C.D.² and Egwunyenga A.O.³

¹ Tropical Disease Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Nigeria.

² Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Nigeria.

³ Department of Zoology, Delta State University, Abraka, Nigeria.

Accepted 17 April, 2012

We evaluated the antioxidant status of 148 Nigerian children with *Plasmodium falciparum* malaria. The mean malarial parasitaemia was $4701.05 \pm 2160.53/\mu\text{L}$. The mean antioxidant concentrations of the infected children were determined for vitamin A ($12.16 \pm 1 - 16 \mu\text{g/dL}$), vitamin C ($0.43 \pm 0.03 \text{ mg/dL}$), 5 carotenes ($40.96 \pm 5.38 \mu\text{g/dL}$), and vitamin E ($0.45 \pm 0.03 \text{ mg/dL}$). The control subjects had higher mean concentrations of vitamin A ($72.12 \pm 3.12 \mu\text{g/dL}$), and of the 5 carotenes ($132.63 \pm 22.45 \mu\text{g/dL}$), and these differences were statistically significant ($X^2 = 42.86$, $P > 0.05$ and $X^2 = 50.64$, $P > 0.05$, respectively). The mean concentrations of vitamin C ($1.22 \pm 0.31 \text{ mg/dL}$) and vitamin E ($1.03 \pm 0.48 \text{ mg/dL}$) in the control children were not statistically significant when compared with their infected children ($X^2 = 0.34$, $P < 0.05$) and ($X^2 = 0.66$, $P < 0.05$), respectively. The relationship between malarial parasitaemia and the concentrations of vitamin E and the 5 carotenes were positively correlated ($r = 0.83$ and $r = 0.99$, respectively). The levels of plasma vitamin A and vitamin C were negatively correlated with the malarial parasitaemia ($r = -0.98$, and $r = -0.96$, respectively). Children within their first 5 years of age had higher malarial parasitaemia ($7628.42 \pm 3151.42/\mu\text{L}$) than those > 6 years ($1176.58 \pm 956/\mu\text{L}$). The children between 1 - 5 years old had lower concentrations of vitamin A ($8.89 \pm 3.74 \mu\text{g/dL}$) and vitamin C ($0.28 \pm 0.21 \mu\text{g/dL}$), while the concentration of the 5 carotenes ($44.54 \mu\text{g/dL}$) and of vitamin E ($0.50 \pm 0.16 \mu\text{g/dL}$) was higher in these children. In conclusion, the depressed levels of plasma antioxidants in the *P. falciparum*-infected children suggested lowered immunity of the children, which may contribute to the morbidity and mortality of malaria in our locality.

Key words: Vitamin A, vitamin C, vitamin E, antioxidants, Children, *Plasmodium falciparum*, Nigeria.

INTRODUCTION

Malaria, a disease caused by *Plasmodium* species, is one of the oldest and greatest health challenges affecting 40% of the world's population (Greenwood and Mutabingwa, 2002). It affects 300 - 500 million people and kills 1.5 - 2.7 million people annually (Philips, 2001). The majority of these cases are children where the disease can exist in a severe form, often with devastating consequences.

The wide spectrum of malaria morbidity and mortality is dependent largely on the complex pathogenesis of this parasitic infection. Micronutrients are known to influence the disease progression in man. For instance, a randomized trial has shown that periodic vitamin A supple-

mentation could reduce the incidence of febrile episodes and parasitaemia due to *Plasmodium falciparum* in Papua New Guinea (Hussey and Clement, 1996). Also, vitamin A is essential for normal immune function and has been shown to influence both antibody response and cell-mediated immunity (Semba, 1998). Vitamin C is a negative inflammatory reactant. Plasma vitamin C concentrations correlated with white cell count, alpha-1-acid glycoprotein and IL-6, all of which are markers of inflammation (Winklehofer-Roob et al., 1997). Vitamin C can also rejuvenate vitamin E, making it an indirect contributor to fighting free radical damage in membrane lipids (Das et al., 1996). These free radicals are products of oxidative stress that is aggravated in malarial infection to decrease the antioxidant defense system. One of the consequences of oxidative stress is the development of malarial anaemia (Kremsner et al., 2000; Clark and Hunt

*Corresponding author. E-mail: nmorsiopg@yahoo.com.

Table 1. The antioxidant profile and the intensity of infection (malarial parasitaemia) of the volunteers.

Intensity of infection/ μL	No. Infected (148)	Vitamin A $\mu\text{g/dL}$	Vitamin C mg/dL	Vitamin E mg/dL	5 carotenes $\mu\text{g/dL}$
Mild (521.15 \pm 95.01)	28	13.55 \pm 4.67	0.55 \pm 0.21	0.41 \pm 0.17	35.54 \pm 4.56
Moderate (3432.0 \pm 1051.71)	68	12.55 \pm 3.06	0.45 \pm 0.26	0.46 \pm 0.16	39.05 \pm 2.45
Severe (10,150.0 \pm 51.71)	52	10.70 \pm 1.75	0.29 \pm 0.98	0.47 \pm 0.13	48.30 \pm 4.0
Mean infected volunteers (4701.05 \pm 2160.53)	-	12.16 \pm 1.16	0.43 \pm 0.03	0.45 \pm 0.03	40.96 \pm 5.38
Mean control volunteers	40	72.12 \pm 3.12	1.22 \pm 0.31	1.63 \pm 0.48	132.63 \pm 22.45

Table 2. The antioxidant profile and malaria parasitaemia according to the age of the infected volunteers.

Age groups In years	Vitamin A $\mu\text{g/dL}$	Vitamin C mg/dL	Vitamin E mg/dL	Carotene $\mu\text{g/dL}$	Infection Rate (No %)	Malaria parasitaemia
1 - 5	8.89 \pm 3.74	0.28 \pm 0.21	0.50 \pm 0.16	44.54 \pm 3.41	80	7628.42 \pm 3151.43
>6	15.49 \pm 3.58	0.59 \pm 0.54	0.38 \pm 0.19	37.43 \pm 2.68	68	1776.58 \pm 956
Mean	12.19 \pm 3.6	0.44 \pm 0.1	0.44 \pm 0.01	40.99 \pm 3.56	-	4701.80 \pm 4032.1

1983).

It has been documented that antioxidants such as carotenoids and vitamins C and E could provide protection against oxidative stress induced by malaria (Adelekan et al., 1997). Despite the role that these antioxidants could play in malarial pathogenesis, there is limited information on the antioxidant profile amongst Nigerian children with *P. falciparum* malaria (Akpotuzor, 2007). To bridge this gap, we investigated the antioxidant profile among Nigerian children diagnosed with *P. falciparum* malaria and studied the correlation between antioxidant concentrations and malarial parasitaemia

MATERIALS AND METHODS

Our study was conducted in the town of Ekpoma, Edo State, Nigeria, between April 2006 and March 2007. Ekpoma lies on latitude 6°N and longitude 6°E and is located in a rainforest belt of the state that is endemic for *P. falciparum* malaria. It has a rainy period from April to October, which is followed by dry season from November to March. The transmission of malaria is perennial, but highest during the rainy season. Ekpoma has a population comprising civil servants, students of a university located within the town, traders, university workers and natives. The study population in this investigation comprised 148 malarious children who attended the pediatric clinic of Faithdome Medical Centre, Ekpoma, Nigeria. In addition, 40 control volunteers, comprising 22 children that were 1 - 5 years old and 18 children that were > 6 years old, were included in this study. The control participants were non-malarious children without malarial parasitaemia, while their counterparts who participated in the study had malaria attack based on *P. falciparum* parasitaemia and clinical symptoms and signs. These included fever (axillary temperature $> 37.5^{\circ}\text{C}$), headache, vomiting, diarrhea, prostration, pallor, jaundice, respiratory distress and other clinical signs and symptoms, as documented earlier (WHO, 2000). The age, weight and height of these children were measured and recorded. The children with other clinical conditions and diseases such as HIV / AIDS, sickle cell anaemia, viral hepatitis B and mal-nutrition, as diagnosed by standard procedures and kits, were not included in

this investigation. Ethical permission was obtained from Faithdome Medical Centre and the State Ministry of Health, Benin City, Nigeria. The scope, nature and objective of our investigation were thoroughly explained to the parents/guardians of the children for their consent.

P. falciparum parasitaemia was determined in peripheral blood smears stained by Giemsa stain. The parasitaemia was graded as low (1-999/ μL), moderate (1000-9999/ μL) and severe ($>10,000/\mu\text{L}$). The concentration of plasma vitamin A, 5 carotenes (α -carotene, β -carotene, γ -cryptoxanthin, lycopene, lutein/zeaxanthin which was not separated) and vitamin E was determined by high-performance liquid chromatography (Sowell et al., 1994). The concentration of plasma vitamin C was measured by the 2-4 dinitro phenyl hydrazine method (Teiz, 1986). The data obtained in this investigation were subjected to statistical analysis, namely correlation and chi-square test, using the Microsoft Excel statistical package.

RESULTS

The antioxidant profile and intensity of infection (malarial parasitaemia) of the volunteers are presented in Table 1. The mean malarial parasitaemia was 4701.05 \pm 2160.53 / μL . The mean antioxidant concentrations of the infected children were 12.16 \pm 1.16 $\mu\text{g/dL}$ for vitamin A and 40.96 \pm 5.38 $\mu\text{g/dL}$ for the 5 carotenes. The control children had higher mean concentrations of vitamin A (72.12 \pm 3.12 $\mu\text{g/dL}$) and the 5 carotenes (132.63 \pm 22.45 $\mu\text{g/dL}$) than the infected children, and these differences were statistically significant with $P < 0.05$ for both. The mean concentrations of vitamin C (1.22 \pm 0.31 mg/dL) and vitamin E (1.63 \pm 0.48 mg/dL) in the control children were not statistically significant when compared with their infected counterparts. The relationship between malarial parasitaemia, vitamin E and the 5 carotenes were positively correlated with $r = 0.83$ and $r = 0.99$, respectively. The level of vitamin A and vitamin C was negatively correlated with the malarial parasitaemia ($r = -0.98$ and $r = -0.96$, respectively).

Table 2 shows the antioxidant profile according to the age groups of the infected volunteers. Children between 1 - 5 years old had the highest *P. falciparum* load of 7628.42 ± 3151.43 in their peripheral blood. Also, these children had a higher vitamin E concentration (0.50 ± 0.16 mg/dL) than those above 5 years of age (0.38 ± 0.19 mg/dL). The vitamin A (8.89 ± 3.74 µg/dL) and vitamin C (0.28 ± 0.21 mg/dL) were lower in the younger children within 1 - 5 years but carotenes (44.54 ± 3.41 µg/dL) and vitamin E (0.50 ± 0.16 µg/dL) concentrations were higher in these children when compared to children > 6 years old.

DISCUSSION

We observed a comparatively lower vitamin A concentration in *P. falciparum*-infected children than in the control subjects (Table 1). This observation had been documented earlier (Thurnham and Singkamani, 1991). This implicates vitamin A-deficiency as an important component of *P. falciparum* pathogenesis in our region. This assertion is considered valid considering the role of vitamin A as an essential micronutrient for normal immune function, which influences antibody response and cell-mediated immunity (Semba, 1998). The significantly lower concentration of vitamins A and C among the children with severe malaria than those with mild and moderate malaria (Table 1) suggests that these micronutrients may be utilized in the face of increased malaria parasitaemia. This observation is in agreement with a previous investigation performed in Pakistan, indicating that the lower concentrations of vitamin A and vitamin C observed among 1 - 5 year old children correlated with higher malarial parasitaemia (Akpotuzor et al., 2007).

We observed a lower concentration of vitamin E in the plasma of *P. falciparum*-infected children than that of the control volunteers (Table 1). This agrees with the investigation of Das et al. (1996), performed in India, where they documented that children with both severe and mild malaria had significant lower plasma vitamin E concentrations than the control children without malaria. The low concentration of antioxidant vitamins E and C in the infected children may be in part due to increased utilization of plasma antioxidants or increased destruction during the malaria. Their transfer to red blood cell membrane to counteract the increase oxidative stress during acute phase of the disease by inhibiting membrane lipid peroxidation, may be a contributing factor (Prasannachandra et al., 2006).

The concentration of the 5 carotenes reported in our present study was lower in infected children than the malaria-free volunteers (Table 1). This is in agreement with a previous report (Akpotuzor et al., 2007). The low circulating carotene concentration can be attributed in part to increased consumption of these antioxidants in the face of enhanced free radical activity (Galloway et al., 2000).

Also, we observed that the children within the first 5 years of age had higher concentrations of vitamin E and the 5 carotenes than those older than 5 years who had lower parasitaemia (Table 2). Therefore, we concluded that these micronutrients, which increased with higher malarial parasitaemia, may have protective effects against malaria in our region. This lends support to an earlier hypothesis that antioxidants such as carotenoids or vitamin E may offer protection against the oxidative stress induced by malaria infection (Clerc, 1992).

Overall, the depressed antioxidant concentrations in the children who had malaria indicated the impact of *P. falciparum* infection on the antioxidant status of children in our locality. This observation had been documented earlier (Metzser et al., 2001; Cooper et al., 2002; Galloway et al., 2000).

This observation can be further proved valid by the deduction of Akpotuzor et al. (2007) who indicated that antioxidants are used to counteract the effects of free radicals generated in the presence of malaria. This also explained the negative correlation we reported between the intensity of infection and the antioxidant concentrations among the infected children in our study area.

This pattern of antioxidant status is a reflection of the malarial pathogenesis, which involves the invasion of human erythrocytes by the malarial parasite. This brings about metabolic changes in the host cell. The host cells may then become vulnerable to damage due to toxic metabolites derived from both host and parasites. Reactive oxygen species generated in the host-parasite interaction cause the lysis of erythrocytes and alteration of antioxidants (Prasannachandra et al., 2006; Ganguly et al., 1977; Allison and Engui, 1983; Erel et al., 1997), thus leading to the development of malarial anemia (Kremsner et al., 2000; Clark and Hunt, 1983).

Based on the results obtained in our study, there is a need to manage micronutrient deficiencies in people suffering from malaria. Currently, malaria is treated with no or little emphasis on antioxidant prescription. Notably, Shankar et al. (2000) documented that high doses of vitamin A supplementation reduced the morbidity due to *P. falciparum*. Considering the fact that antioxidant status were depressed in the malarious children when compared to the status in the normal volunteers in our study area, such results furthermore support our recommendation that micronutrient supplementation with vitamin A, C and E should be incorporated into the management of *P. falciparum* infections.

REFERENCES

- Adelekan DA, Adeodu OO, Thurnham (1997). Comparative effect of malaria and malnutrition on plasma antioxidant vitamins in children. *Ann. F. Trop. Paediat.* 17: 223-227.
- Akpotuzor JO, Udoh AE, Etukudo MH (2007). Total antioxidant status, vitamin A, C and -carotene levels of children with *P. falciparum* in University of Calabar Teaching Hospital (UCTH), Calabar. *Pakistan J. Nat.* 615: 485-489.

- Allison AC, Euguii (1983). The role of cell mediated immune responses in resistance to malaria with special reference to oxidant stress. *Ann. Rev. Immunol.* 1: 361-392.
- Clark IA, Hunt NH (1983). Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria. *Infect. Immun.* 39(1): 1-6.
- Clerc M (1992). Observations sur les vitamines anti-oxydantes et/ou anti-radicalaires en médecine tropicale. *Bull Acad. Nat. Med.* 176: 1393-1406.
- Cooper R, Labadarias D, Louw ME (2002). Serum vitamin A and E concentrations in acute *Falciparum malaria* modulators or markers of severity. *Am. J. Clin. Nutr.* 53: 84-96.
- Das BS, Thurnham DI, Das DB (1996). Plasma alpha-tocopherol, retinol and carotenoids in children with *Falciparum malaria*. *Am. J. Clin Nutr.* 64: 94-100.
- Erel KA, Aveli S, Aktepe N, Bulut V (1997). Oxidative stress and antioxidant status of plasma and erythrocytes in patients with *Vivax malaria*. *Clin. Biochem.* 30: 631-639.
- Galloway PO, McMillan C, Scatter N (2000). Effect of the inflammatory response on trace element and vitamin status. *Ann. Clin. Biochem.* 32: 289-291.
- Greenwood B, Mutabingwa T (2002). *Malaria in 2002*. *Nat.* 415: 670-672.
- Hussey DC, Clement CS (1996). Clinical problem in measles and management. *Ann. Trop. Paediat.* 16: 307-317.
- Kremsner PG, Greve B, Lell B, Luckner D, Schmid D (2000). Malarial anaemia in African children associated with high oxygen radical production. *Lancet.* 355: 40-41. The influence of zinc supplementation on morbidity due to *Plasmodium falciparum*: a randomized trial in preschool children in Papua New Guinea. *Am. J. Trop. Med. Hyg.* 62(6): 663-669.
- Sowell AL, Huff DL, Yeager PR, Caudill SP, Gunter EW (1994). Simultaneous determination of retinol, -tocopherol, lutein/zeaxanthin, -cryptoxanthin, lycopene, trans - -carotene and four retinyl esters in serum by reverse-phase high performance liquid chromatography with multi-wavelength detection. *Clin. Chem.* 40: 411-416.
- Teitz NW (1986). Methods of determination of ascorbic acid. In: *Text book of Clinical Chemistry*. Teitz NW ed). WB Saunders Company. Philadelphia. London. Toronto. pp. 960-962.
- Thurnham DI, Singkamani R (1991). The acute phase response and vitamin A status in malaria. *Trans. R. Soc. Trop. Med. Hyg.* 85(2): 194-199.
- Winkelhofer-Roob BM, Ellemunter H, Fruhwirth M, Schlese-Haueter SE, Kloschorui G, Vant MA (1997). Plasma vitamin C concentrations in patients with cystic fibrosis, evidence of association with lung inflammation. *Am. J. Clin. Nutr.* 65: 1858-1866.
- World Health Organization (2000). *Severe Falciparum malaria*. *Tran. R. Soc. Trop. Med. Hyg.* 94 (Suppl 1): 51-590.