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Full Length Research Paper

Correlation between moderate *Plasmodium falciparum* malarial parasitaemia and antioxidant vitamins in serum of infected children in South Eastern Nigeria

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The levels of antioxidant vitamins were estimated in *Plasmodium falciparum* malarial infected children aged 0 to 12 years. 113 children with *P. falciparum* infection were selected based on laboratory evidence and clinical symptoms. 87 apparently healthy children with no malarial parasitaemia were included as the control subjects. *P. falciparum* parasitaemia and serum levels of antioxidant vitamins (A, C and E) were determined using standard procedures. The results obtained showed that the mean malarial parasitaemia was 6203.01 ± 1216.79/µl and the mean serum antioxidant vitamin concentrations were 23.23±8.40 µl/dl for vitamin A, 0.49±0.18 mg/dL for vitamin C and 0.78±0.32mg/dL for vitamin E for the *P. falciparum* malarial infected children. The control children had higher concentrations of vitamins A (51.80±12.41 mg/dL, X^2 = 60.713, *P*<0.05), C (1.01±0.16 mg/dl, X^2 =0.031, *P*>0.05) and E (0.96±0.21 mg/dl, X^2 =0.039, *P*>0.05). The degree of malarial parasitaemia and serum concentration of vitamin E were positively correlated (r=0.42) but vitamins A (r= -0.05) and C (-0.06) were negatively correlated. Children within 0-5 years of age had higher malarial parasitaemia (7379.82± 918.99/µl), and these children had lower concentrations of vitamins A (21.27±8.68 µg/dL) and C (0.45±0.19 mg/dL) when compared with children between 6 to 12 years (vitamin A= 25.19±8.12 µg/dl and vitamin C = 0.53±0.16 mg/dL). Results suggest that the degree of malarial parasitaemia in especially children between 0 to 5 years could compromise immunity (as judged by the correlation with and reduction in vitamin E). Malarial infection among children (0 to 12 years) decreased the serum antioxidant vitamin levels, and this could lower free radical defense and contribute to the morbidity and mortality of malaria among children in this region. Health care providers should recognize these effects in planning malarial treatment and control programmes. Changes in serum antioxidant levels during post-treatment period should be investigated and documented.

Key words: *Plasmodium falciparum,* malaria, antioxidant vitamins, Owerri, fever.

INTRODUCTION

Malaria is a vector-borne infectious disease caused by protozoan parasites. It is widespread in tropical and sub-tropical regions, including parts of America, Asia and Africa. Each year, there are approximately 350-500 million cases of malaria (WHO, 2008) killing between one and three million people, the majority of whom are young children in Sub-Saharan Africa (Snow et al., 2005). 90% of malaria-related deaths occur in Sub-Saharan Africa (Greenwood et al., 2005). Usually, people get malaria by being bitten by infective female *Anopheles* mosquito. *Anopheles* mosquito can transmit malaria and they must have been infected through a previous blood meal taken on an infected person. When a mosquito bites an infected person, a small amount of blood is taken which contains microscopic malarial parasites. The disease, malaria is caused by protozoan parasites of the genus *Plasmodium* (Yoshida et al., 2007). Antioxidant vitamins (A, C and E)

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have various identified roles in cells. Vitamin A is essential for normal immune function and has been shown to influence both anti-body response and cell mediated immunity (Semba, 1998). The changes in vitamin A concentration were accomplished by almost identical changes in serum retinal binding protein (RBP) concentration (Lapidaries et al., 1987). In malaria infected persons, low vitamin A concentration has been reported (Galloway et al., 2000; Adelekan et al., 1997; Metzger et al., 2001). The low concentration of vitamin A seen in malaria sufferers were attributed to inflammatory response (Thurnham, 1996) and redistribution of vitamin A into extra vascular spaces to allow increased bioavailability to tissues.

Strong antioxidants such as dietary caroteniods, vitamins E and C have been shown to modulate immune function in humans (Hughes, 1999; Meydam and Berharka, 1998). Vitamin C is a negative inflammatory reactant. Plasma vitamin C concentrations correlated inversely with white cell count, alpha-1-acid glycoproteins and lL-6, all of which are markers of inflammation (Winkerloofer- Roob et al., 1996). The synergistic combination of vitamins C and E may be further enhanced by the addition of vitamin A.

It has been reported that antioxidants such as vitamins A, C and E would provide protection against the oxidative stress induced by malaria infection (Adelekan et al., 1996). To gain insight into this, the antioxidant profile among children in Owerri, Imo State, Nigeria, diagnosed with *P. falciparum* malaria was investigated, and the correlation between antioxidant concentrations and moderate malarial parasitaemia was studied.

MATERIALS AND METHODS

Study location

The study was conducted in Owerri, Imo State, Nigeria, between April and September 2009. Owerri town lies on latitude 5.485°N and longitude 7.035°E and is located in the rainforest belt of Imo State; endemic for *P. falciparum* malaria parasite which is transmitted by the female *Anopheles* mosquito. It has a rainy period of April to November which is when the mosquito bites are more rampant. The rainforest belt where the state is located is also a very good habitat for mosquito.

Subjects

The study subjects consisted of 113 children who attended the Paediatric Clinic of the Federal Medical Centre (FMC), Owerri, Nigeria. These subjects were children between 0 to 12 years, infected with *P. falciparum* malaria parasite who were reported ill with fever (temperature >37.5°C), headache, vomiting, diarrhea, respiratory distress and other clinical signs and symptoms of malaria as previously documented (WHO, 2000). The children who did not meet these criteria were excluded from the study. Apparently healthy children (87) who were symptomatic and negative for *P. falciparum* in their peripheral blood were used as control individuals. The scope, nature and objectives of the investigation were thoroughly explained to the parents or guardians of the children for their consent which was sought and obtained.

Collection of blood specimen

Venous blood samples were obtained from the subjects using a syringe. 5 ml of blood was obtained from patients and control subjects by venepuncture and placed into a clean blood container. Serum was obtained by centrifuging the whole blood sample at 3000 rpm for 10 min at room temperature (29 to 31°C). The serum was decanted into Bijou bottle and stored frozen until required for analyses.

Determination of serum antioxidant vitamins

The concentrations of serum vitamin A were determined using Carr-Price reagent (Kaser and Sketol, 1943). Serum vitamin C level was estimated using 2,4-dinitrophenylhydrazine method (Roe and Kuether, 1943). Serum levels of vitamin E was analysed using a micro-method (Quaife et al., 1949.)

Malaria parasite count

P. falciparum parasitaemia level was determined in blood films by Giemsa stain. The level of parasitaemia was graded as low (1 to 999/ µl), moderate (1000 to 9999/ µl) and severe (>10,000/ µl).

RESULTS

The results obtained are shown in Table 1 which shows a summary of the changes in serum antioxidant vitamins (A, C and E) levels induced by moderate *P. falciparum* malarial infection. The mean malarial parasitaemia was 6203.01 ± 1216.79/ µl. The mean serum antioxidant concentration of vitamin A for the malarial infected children was 23.23 ± 8 to 40 µg/dL but the control children had higher mean levels of vitamin A (51.80 ± 12.41 µg/dL). This difference was statistically significant ($P<0.05$). The mean concentration of vitamins C (1.01 ± 0.16 mg/dl) and E (0.96 ± 0.2 mg/dL) for the non-malarial infected (control) children were not statistically significant when compared with values (0.49±0.18mg/dL and 0.78 ± 0.32 mg/dL) for the infected children. Malarial parasitaemia and vitamin A or C were negatively correlated (r= -0.05 and -0.06, respectively). The level of vitamin E was positively correlated with malarial parasitaemia (r= 0.42).

Table 1 also shows that children between 0 to 5 years had a higher *P. falciparum* load (7379.82 ± 918.99/ µl) in their peripheral blood smear. These children also had lower vitamins A (21.27 ± 8.68 µg/dL, $P<0.05$), C (0.45±0.19 mg/dL) and E (0.69±0.22 mg/dL) when compared with the patients between 6 to 12 years.

DISCUSSION

The antioxidant vitamin levels in malarial patients were lower than the levels for the control children. The lower values observed in antioxidant vitamin levels in

Table 1. Serum antioxidant vitamin profile and malarial parasitaemia for children in different age groups.

Age group (yr)	0-5	6-12	Average	0-5	6-12	Average
Degree of *P. falciparum* malarial parasitaemia (/µL)	Moderate (7379.82 ± 918.99)	Moderate (5026.19 ±1514.58)		Nil (Control)	Nil (Control)	
Number of subject (n)	51	62		44	43	
Antioxidant vitamin						
Vitamin A (µg/dL)	21.27 ± 8.68*	25.19 ± 8.12*	23.23 ± 8.40*	51.60 ± 12.51	52.00 ±12.30	51.80± 12.41
Vitamin C (mg/dL)	0.45 ± 0.19	0.53 ±0.16	0.49 ± 0.18	0.93 ± 0.16	1.08 ±0.16	1.01± 0.16
Vitamin E (mg/dL)	0.69 ± 0.22	0.86 ±0.41	0.78 ± 0.32	0.91 ± 0.30	1.01 ±0.11	0.96± 0.21

Values are expressed as mean ± SD for 'n' subjects. *Significantly different from comparable control value ($p<0.05$).

malaria may be attributed to increased utilization of the host's serum antioxidants by the malaria parasites to counteract oxidative damages (Akpotuzor et al., 2007). It was observed that vitamin A concentration in *P. falciparum* infected children was significantly lower ($P<0.05$) than that of the control subjects (Table 1). This observation confirms earlier reports by Nmorsi et al. (2007) and Thurnham and Singkamani (1991). This study further implicates *P. falciparum* infection as an important component of vitamin A deficiency. This observation is regarded valid considering the role of vitamin A in immune function which influences antibody responses and cell mediated immunity (Nmorsi et al., 2007; Semba, 1998). The lower concentrations of vitamins A and C observed among the younger (0 to 5 years) infected children correlated with higher malarial parasitaemia.

From the present study, the level of serum vitamin E for *P. falciparum* malarial infected children was observed to be lower when compared with the non-infected children. This agrees with the investigation of Nmorsi et al. (2007) conducted in Ekpoma, Edo State, Nigeria. The low concentration of antioxidant vitamins E and C in infected children may be in part due to increased utilization of serum antioxidant or increased destruction during the malarial infection. Their transfer to the red blood cell membrane to counteract the increased oxidative stress during the acute phase of the disease by inhibiting membrane lipid peroxidation may be a contributing factor (Nmorsi et al., 2007).

Also, it was observed that the children within the first five years of age had lower concentrations of vitamin E than those older than 5 years who had lower parasitaemia (Table 1). However, previous studies by Nmorsi et al. (2007) showed that children within the first five years of age had lower parasitaemia. Therefore, they concluded that these micronutrients which increase with higher malarial parasitaemia may have protective effects against malaria in the Ekpoma region. This lends support to an earlier hypothesis that antioxidants (such as vitamin E) could offer protection against the oxidative stress induced by malarial infection (Clerc, 1992). However, Metzger et al. (2001) observed that low concentrations of vitamin E in serum were not associated with parasite clearance, which is not consistent with the hypothesis that low vitamin E status is protective against malaria (Levander, 1992). Overall, the depressed antioxidant concentrations in the children who had malaria indicated the impact of *P. falciparum* infection in the antioxidant vitamin status of children in Owerri, Imo State, Nigeria. This observation adds to accumulating reports (Nmorsi et al., 2007; Cooper et al., 2002; Metzger et al., 2001; Galloway et al., 2000). This observation can be further proven valid by the deduction of Akpotuzer et al. (2007) who indicated that antioxidants are used to counteract the effects of free radicals generated in the presence of malaria. This may also explain the negative correlation reported between moderate parasitaemia and antioxidant vitamins (A and C) concentrations among the uninfected children.

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 cells which 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 causes the lysis of erythrocytes and alteration of antioxidants (Nmorsi et al., 2007; Erel et al., 1997; Allison and Enguii, 1983) leading to the development of malarial anaemia (Nmorsi et al., 2007; Kremsner, et al., 2000; Clark and Hunt, 1983).

From the observations in this study, the serum concentrations of antioxidant vitamins were reduced in malarial infection and this may contribute to overall reduction in total antioxidant levels and capacity of malaria infected children. The reduction of these vitamin antioxidants in (the stages of) malaria infection may expose children to free radical attack. To reverse this condition and reduce morbidity due to *P. falciparum*, it is necessary to recommend antioxidant agents (particularly vitamins) as a component drug for the treatment of malarial infection. Dietary modifications, including foods rich in antioxidants (such as vegetables, fruits etc) should be encouraged. The outcome of such recommended supplementation and diet manipulation should be investigated, so that health care providers can be better advised.

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