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Total Antioxidant Status, Vitamins A, C and β-carotene Levels of Children with *P. falciparum* Infection in University of Calabar Teaching Hospital (UCTH), Calabar

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Abstract: By using standard procedures, total antioxidant level, vitamins A, C, and β-carotene were assessed in 122 malaria infected children. The haemoglobin and parasite density status of the children were also measured. Sixty healthy children were used as controls. It was observed that all parameters measured were significantly lower in malaria infected children when compared with the respective control values. Malaria parasitemia correlated strongly and negatively with total antioxidant and haemoglobin levels (r=0.-432 and 0.-503, P<0.01) respectively but weakly with vitamin A level (r=0.-196, P<0.05). Reductions in the levels of total antioxidant and vitamin A were dependent on the severity of malaria. The more severe the malaria the lower the levels of total antioxidant and vitamin A. Ratios of vitamin A to vitamin C and beta-carotene to vitamin C were similar in both malaria-infected children and controls indicating a constant proportional relationship between vitamin A and beta-carotene to vitamin C. From this study it is observed that there is a general depression in antioxidant levels suggesting that antioxidant intervention may be crucial in the treatment of malaria infection. Furthermore lowered levels of antioxidants especially of vitamin C in malaria infection also suggest lowered immunity of host, which may be responsible for some of the complications of malaria infection

Key words: Antioxidant status, *P. falciparum* infection, malaria, β-carotene

Introduction

Malaria as well as being one of the most prevalent and dangerous diseases known to man, is also by far the world's most important tropical parasitic disease (Trigg and Kondrachin, 1993). Ninety percent of malaria cases and deaths occur in Africa, South of the Sahara, mostly among young children and fatality rate of 10-30% has been reported among patients with severe malaria referred to hospitals in tropical Africa (Sherman, 1988).

In children, micronutrient status has been shown to influence resistance to several infectious diseases, including measles and diarrhea and respiratory diseases (Hussey and Clement, 1996; Black, 1998). A randomized trial has shown that periodic vitamin A supplementation could reduce the incidence of febrile episodes and parasitemia due to plasmodium *Falciparum* in Papua New Guinea (Hussey and Clement, 1996). Vitamin A is essential for normal immune function and has been shown to influence both antibody response and cell mediated immunity (Semba, 1998). Vitamin A is a negative acute-phase reactant and has been reported to fall to a minimum of about 40% 48hrs after surgery (Louw *et al.*, 1992). The changes in Vitamin A concentration were accompanied by almost identical changes in serum Retinol Binding Protein (RBP) concentration (Labadarios *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 was attributed to inflammatory response, (Thurnham, 1996) and redistribution of vitamin A into extra vascular species to allow increased bioavailability to the tissues.

Strong antioxidants such as dietary carotenoids, vitamins E and C have been shown to modulate immune function in humans (Hughes, 1999; Meydani and Beharka, 1998). Low β-carotene concentration has been observed in children who had malaria in Ile-Ife, Nigeria (Cooper *et al.*, 2002). Vitamin C is a negative inflammatory reactant. Plasma vitamin C concentrations correlated inversely with white-cell count, alph-1-acid glycoprotein and IL-6, all of which are markers of inflammation (Winklehofor-Roob *et al.*, 1997). Vitamin C can rejuvenate vitamin E, making it an indirect contributor to the fight against free radical damage in the lipids (Das *et al.*, 1996). The synergistic combination of vitamins C and E may be further enhanced by the addition of vitamin A.

There are three classes of antioxidants viz Primary antioxidants, (Thurnham, 1996), Secondary antioxidants (Gaby and Singh, 1990; Weisburger, 1991) and Tertiary antioxidants (Miller *et al.*, 1993). All these antioxidants in the body together form the total antioxidant status of an individual. (Miller *et al.*, 1993) defined total antioxidant status as the sum total of endogenous and food derived antioxidants of the extra cellular fluid of an individual. Co-operation of all the different antioxidants provides greater protection against attack by reactive oxygen or nitrogen

Table 1: Plasma antioxidants level, haemoglobin and parasite counts in malaria infected children

Parameters	Control subjects n=60	Patients N =122	P-value
Total antioxidant Status mmol/l	1.26±0.2	0.63±0.31	P<0.001
Vitamin A µmol/l	0.77±0.30	0.55±0.11	P<0.001
Beta-Carotene µmol/l	1.16±0.57	0.77±0.10	P<0.001
Vitamine C µmol/l	59.0±28.0	38.6±18.8	P<0.001
Haemoglobin g/l	109.7±11.2	90.7±24.5	P<0.001
Parasite count dl	_____	5,391.3±2923.1	P<0.001

n= number of subjects studied

Table 2: Plasma antioxidants level, haemoglobin and parasite counts based on clinical and laboratory findings of malaria infected children

Parameters	Clinical/Laboratory Findings		P-value
	Mild/moderate parasite count <7000 n=79	Severe malaria parasite count >7,000 n=43	
Total antioxidant Level mmoL/L	0.74±0.27	0.45±0.27	<0.001
Vitamin A µmol/L	0.56±0.1	0.52±0.1	<0.05
Beta-Carotene µmoL/L	0.75±0.1	0.74±0.1	>0.05
Vitamin C µmol/L	39.7±17.0	36.6±20.9	>0.05
Haemoglobin g/L	101.3±20.4	72.1±18.7	<0.001
Parasite count dL	3698.5 ± 1210.8	8499.6 ± 2556.3	<0.001

n= number of subjects studied

radicals than any single compound alone. Thus, the overall antioxidant status will give more relevant biological information compared to that obtained by the measurement of individual components. In Caucasians, a reference range of 1.30-1.78 mmoL/L has been reported (Miller *et al*., 1993). So far no report has been made for the blacks and Nigeria in particular. This work may be the first to attempt to do so.

The role of antioxidants and oxidative stress in the pathogenesis of malaria in human is unclear. In murine models, pro-oxidants such as fish oil have been shown to protect against experimental cerebral malaria infection, especially in the presence of vitamin C and E (Levander *et al*., 1998). It has been argued that vitamin E deficiency might be protective against malaria in mice (Levander, 1992), but this relationship in humans is not well understood. On the other hand it has been reported that antioxidants such as carotenoids, vitamin C and E would provide protection against the oxidative stress induced by malaria infection (Adelekan *et al*., 1997). To gain insight into the relationship between *P. falciparum* malaria and antioxidants a hospital-based study among children with uncomplicated *Falciparum* malaria was conducted in Calabar, Cross-River State and the association of malaria parasitemia and vitamin A, C, β-carotene and total antioxidant status, was also examined.

Materials and Methods

The study Subjects consisted of one hundred and twenty two (122) children between the ages of 1-10 years, who were seen in emergency unit of university of Calabar Teaching hospital Calabar between march, 2004 and June 2005. The study subjects were children infected with *P. falciparum* malaria parasite, who reported ill with fever in the hospital. The children who did not meet these criteria were excluded from the study. Apparently healthy children, consisting of sixty (60) subjects who were found to be negative for *P. falciparum* in their peripheral blood were, used as controls. Both groups of subjects must have resided in the city of Calabar for at least one year before the study.

Five millitres of venous blood was obtained from patient and control subjects by venepuncture. Two millitres of the blood was placed into an EDTA bottle for the determination of haemoglobin, parasite count and plasma ascorbic acid concentration and the rest was discharged into a clean plain tube and allowed to clot at room temperature. The plasma or serum (as appropriate) was obtained by centrifugation for 10 minutes at 3000rmp. Total antioxidant was determined immediately by the method of (Miller *et al*., 1993) and the remaining samples were stored at-20^{o}C for vitamin A assay by the method of (Olson, 1979). Beta-carotene assay was carried out by the method of Caarrprice as modified by (Kaser and Stekol, 1943). Vitamin C assay was by the method of (Roe and Kuether, 1943).

Results

The total antioxidant level and haemoglobin levels in our control subjects (1.26±0.20moL/L and 10 9.7±11.2g/L) were lower than the reference values (1.30-1.78 mmoL/L and 115-145g/L) for Caucasians. Statistical analysis showed that the differences were significant (P<0.05) for both total antioxidant status and haemoglobin respectively. Comparison of plasma antioxidant levels, hemoglobin and parasite counts between normal subjects or control and malaria infected children is shown in Table 1 Total antioxidant status, vitamin A, beta-carotene vitamin C, were observed to be

Table 3: Measured antioxidants as percentage of total antioxidant activity

	Malaria infected children (n =1 22)	Control subjects (n = 60)	P-value
Total antioxidant activity (mmoL/l)	0.63±0.31	1.26±0.2	<0.001
Vitamin A µmol/l	0.55±0.11	0.77±0.30	<0.001
Ratio of A to total (%)	0.12±0.11	0.10±0.1	>0.05
Vit. C µmol/l	38.6±18.1	59.0±28.0	<0.001
Ratio of C to total (%)	7.2±5.0	5.8±2.0	<0.001
β-carotene µmol/l	0.77±0.10	1.16±0.57	<0.001
Ratio of β Carotene to total (%)	0.16±0.1	0.13±0.1	>0.05

n= number of subjects studied

Table 4: Molar ratio (%) of vitamin A and beta-carotene to vitamin C

Parameters	Malaria infected children	Control subjects	P value
Vitamin C (umol/l)	38.6±18.8	59.0±28.0	<0.001
Vitamin A (umol/l)	0.55±0.11	0.77±0.30	<0.001
Beta-carotene (umol/l)	0.77±0.10	1.16±0.57	<0.001
% ratio of Vitamin A to Vitamin C	2.7±1.4	2.6±1.1	>0.05
% ratio of Beta-carotene to Vitamin C	2.2±1.1	2.2±1.0	>0.05
n	122	60	

n= number of subjects studied

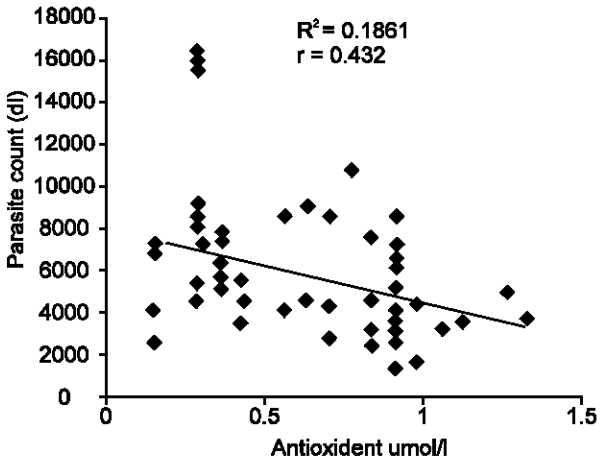

Fig. 1: Scatter plot of parasite count against total antioxidant status

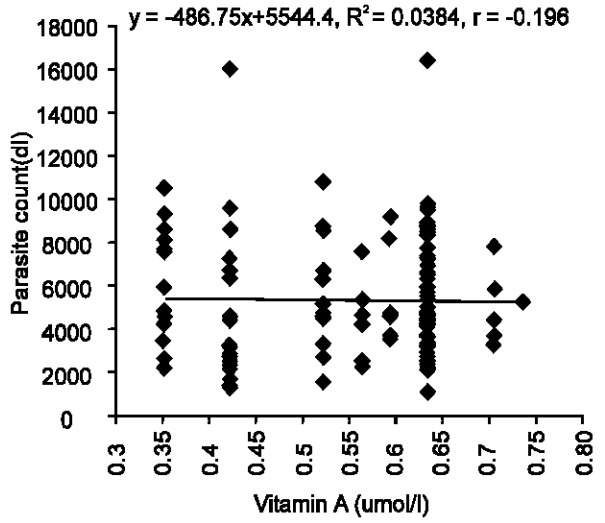

Fig. 2: Scatter plot of parasite count against vitamin A

significantly lower (p<0.001) in malaria-infected children when compared with the normal subjects. The haemoglobin levels in patients were significantly lower than control values (P<0.001).

Plasma antioxidants and haemoglobin levels decreased with the severity of malaria (Table 2). Malaria patients were divided into two groups, viz mild/moderate infection and severe infection. Patients classified as mild/moderate infection had parasite count of <7000/ dl, moderate temperature (<40°C) with no other severe symptoms. Those classified as having severe malaria had parasite counts of>7000/dl, temperature of 40°C and above, vomiting, and dehydration. Total antioxidant, status, vitamin A, and haemoglobin levels were in severe malaria significantly lower then levels in mild/moderate malaria. Table 3 shows measured antioxidants as percentage of total antioxidant activity. The table shows that in malaria the molar (%) ratio of vitamins A and beta-carotene to total antioxidant activity were similar to those of controls but the ratio of vitamin C to total antioxidant was significantly higher than that of controls. However, the percentage ratio of vitamin C to total antioxidant activity in both controls and malaria patients show's that vitamin C accounts for about 5% of the total antioxidant level.

Table 4 shows the molar concentration ratios of vitamin A and beta-carotene to Vitamin C. The table shows that the molar (%) ratios of Vitamin A to vitamin C and beta-carotene to vitamin C of malaria-infected children were similar to those of control subjects. Fig. 1 shows the correlation graph of parasite count against total antioxidant level. The total antioxidant level correlated negatively with parasite count (r= -0.43, p<0.01). Vitamin A (Fig. 2) also correlated negatively with parasite count (r= -196, p<0.05) as did haemoglobin (Fig. 3), r= -0.503, p<0.001).

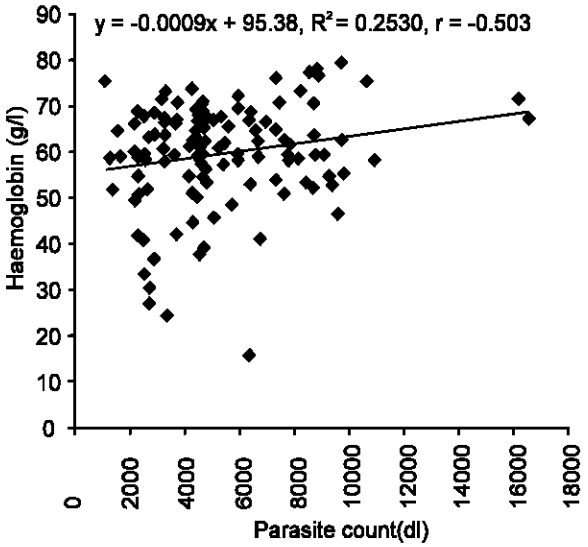

Fig. 3: Scatter plot of Haemoglobin against parasite count

Discussion

Total antioxidant level in malaria patients was lower than the level for control ($p<0.001$). The lower values observed in total antioxidant levels in malaria may be attributed to increased utilization of the host's plasma antioxidants by the malaria parasites to counteract oxidative damages. The total antioxidant level in our normal subjects (1.26+0.21 mmol/l), (range 0.84-1.68 mmol/l) is lower than that found in Caucasians (1.30-1.78 mmol/l), (Miller *et al.*, 1993). This may depict regional or genetic variations. Also the total antioxidant level varies inversely with the severity of malaria. Patients with severe malaria had significantly lower total antioxidant levels than those with mild/moderate malaria. Antioxidants are used up to counteract the effects of free radicals generated in the cause of malaria infection. This explains why reduction in antioxidant level is dependent on the severity of malaria. Plasma vitamin A concentrations were lower in malaria patient when compared with the control subjects. This follows the same pattern as total antioxidant level. The finding agrees with previous observations that redistribution of vitamin A takes place in the extravascular spaces to allow for increase bioavailability to the tissues (Galloway *et al.*, 2000; Adelekan *et al.*, 1997) Significantly lower vitamin A was observed in this work in severe malaria when compared with levels in mild/moderate malaria. This finding implies that more vitamin A is utilized in the face of increased parasitemia seen in severe cases of malaria infection.

It has been demonstrated in this study that beta-carotene levels, along with levels of other antioxidants in children with malaria infection are depressed. A similar observation was also made in other reports (Cooper *et al.*, 2002; Galloway *et al.*, 2000; Adelekan *et al.*, 1997). The low circulating beta-carotene is attributed in part to increased consumption in the face of enhanced free radical activity (Galloway *et al.*, 2000). In our present work, vitamin C level was also significantly lower in malaria patients when compared with control subjects. This was attributed to either, increased requirement or increased destruction during malaria infection. The molar ratio (%) of vitamin A, and beta-carotene to total antioxidant concentration were similar in malaria patient and controls. However the ratio of vitamin C to total antioxidant concentration was significantly higher in malaria infected children when compared to control subjects. This finding is the consequence of significant reduction in the total antioxidant level of malaria-infected children. Of the three vitamins and provitamin determined in this study, vitamin C was the most abundant in both malaria patients (7.2%) and controls (5.8%). Vitamin A and beta-carotene constituted only about 0.1% and 0.2% respectively of the total plasma antioxidant level in malaria patients and 0.1% and 0.1% respectively in controls.

There is a constant ratio of vitamin A and beta-carotene to vitamin C in malaria infected children and controls despite wide differences in the actual concentration of these vitamins in each groups.

Reduction in vitamin A and beta-carotene levels in malaria-infected children appears to be proportional to reduction in Vitamin C level as shown by the constant ratio of the Vitamins to Vitamin C in both patients and controls. This suggests some dependency between these vitamins. It may mean that vitamin C is required to rejuvenate active vitamin A and beta-carotene as reported for Vitamin E (Das *et al.*, 1996). This may thus make Vitamin C an indirect factor in the repair of free radical damage even in lipid medium as reported for Vitamin E (Das *et al.*, 1996). Common practices in the treatment of malaria do not always involve the use of antioxidants and when prescribed usually it is as a minor drug with little or no importance attached. The implication of this finding may then mean that inclusion of antioxidant agents as one of the regimen drugs particularly Vitamin C because of its ability to act even in lipid and water medium as well as its availability and affordability may be quite beneficial in treatment of malaria patients. There was a reduction in haemoglobin levels in malaria patients when compared to controls. This is a standard feature in malaria infection. The reduction in haemoglobin is understandably due to haemolytic destruction especially of parasitized red blood cells, suppression of bone marrow activity and ineffective erythropoiesis (Adriana *et al.*, 2003). The antioxidant systems of living organism include enzymes, macromolecules, small molecules and trace elements and the sum of the products of these antioxidants represents the total antioxidant status of the

extra cellular fluid (Miller *et al*., 1993). Vitamins A, C, and beta-carotene, which are contributors to antioxidants activities, have been measured. From our observations in this study, these antioxidants are reduced in malaria infection and reduction of these antioxidants in turn caused the reduction of the total antioxidant levels of malaria-infected children. The more severe the malaria infection, the lower the antioxidant levels. It would be noted however that antioxidant levels in our controls were lower than values observed for normal subjects in other population (Miller *et al*., 1993). These suggest a reflection of relative deficiency even among apparently healthy subjects. This being so, the administration of antioxidant agents to malaria patients becomes more pertinent. Moreover, reduction of vitamin A and beta-carotene levels were proportional to reduction of vitamin C in malaria patients as indicated by a constant ratio of the vitamins to vitamin C. This implies dependency between the vitamins. Furthermore lowered levels of antioxidants, especially of vitamin C in malaria infection also suggest lowered immunity of host which may be responsible for some of the complications of malaria infection. The reduction of these antioxidants in the face of malaria infection may pre-dispose the children to free radical attack. To avert this consequence we recommend that antioxidant agent (particularly Vitamin C) be made one of the component drug regimens for the treatment of malaria infection. Government should therefore make the necessary policies to ensure that processed foods are fortified with antioxidant as obtained in technologically advanced countries.

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