Original Article

Total Antioxidant Status and other Antioxidant Agent Levels in Children with P. Falciparum Infection in Calabar, Nigeria

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Malaria infection and its treatment with antimalaria drugs have been reported to generate oxygen free radicals. These free radicals generated in the cause of infection and its treatment need to be removed from the body by reducing agents called antioxidants. This study is aimed at evaluating the impact of malaria infection on total antioxidant status of children. By using standard procedures and kits purchased from Randox laboratories, USA and Quimica Clinica Aplicada Spain, total antioxidant level, vitamin C, Total and HDL cholesterol and albumin 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 except the total cholesterol (which was similar in both malaria infected children and the control subjects), were significantly lower in malaria infected children when compared with the respective control values. Malaria parasitemia correlated strongly and negatively with total antioxidant levels (r= 0.-432).Reduction in the level of total antioxidant was dependent on the severity of malaria hence the more severe the malaria, the lower the level of total antioxidant. From this study it is observed that there is a general depression in total antioxidant levels as well as HDL Cholesterol, albumin and vitamin C levels suggesting increased consumption of antioxidants and its agents during malaria infection. Therefore, antioxidant intervention may be crucial in the treatment of malaria infection.

Key words*: *Antioxidant status, P. Falciparum infection, malaria, Vitamin C

Introduction

Malaria is one of the most devastating global public health problems. About 107 countries and territories involving about 3.2 billion people are still at risk of malaria attack as at 2004 (World Health Organization (1).Present estimates suggest that around 350–500 million clinical disease episodes occur annually (2) and around 60% of clinical cases and over 80% of the deaths due to malaria occur in Africa south of the Sahara (3). Malaria is responsible for an estimated average annual reduction of 1.3% in economic growth for those countries with the highest burden, Nigeria inclusive. Malaria also contributes to anaemia in children and undermines their growth and development. An estimated one million fatalities per year has been reported in which 85 % of deaths are children (4). Reactive oxygen species (ROS) are generated by inflammatory cells recruited during infection .The potentially damaging effects of oxidative stress are normally limited by antioxidants that scavenge ROS in the body (5). However, low dietary intakes of antioxidant vitamins or reduced synthesis of nondietary antioxidants such as albumin, bilirubin, glutathione peroxidase cholesterol and uric acid are likely to

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result in an oxidant-antioxidant imbalance that exacerbates inflammation and tissue damage (6). Total antioxidant status is defined as the sum total of endogenous and food derived antioxidants of the extra cellular fluid of an individual (7). Cooperation of all the different antioxidants provides greater protection against attack by reactive oxygen or nitrogen radicals than any single compound alone. Measurement of serum total antioxidant capacity (TAC) level was reported to provide an integrated index, as opposed to one based on simple summation of measurable antioxidants (8). It possibly could be used to assess the real change in antioxidant status in patients with severe infection and might lead to universally useful treatment (8).

Albumin represents a very abundant and important circulating antioxidant. Albumin has several important physiological and pharmacological functions. It transports metals, fatty acids, cholesterol, bile pigments, and drugs. It is a key element in the regulation of osmotic pressure and distribution of fluid between different compartments and its plasma concentration represents equilibrium not only between its synthesis in the liver and its catabolism, but also its transcapillary escape. It represents the major and predominant antioxidant in plasma, which is the body compartment known to be exposed to continuous oxidative stress. Previous work has shown that more than 70% of the free radical-trapping activity of serum was due to human serum albumin (HSA) as assayed using the free radical-induced hemolysis test (9). Cholesterol is carried around in the blood on carrier molecules called lipoproteins. HDL cholesterol's main function is to transport fat from the cell to the liver. When the HDL cholesterol level is high, it is more cardio protective (10). One theory advanced by some scientists is that the total serum cholesterol is really an indicator of the amount of free radical damage in the body (10). The higher the free radical level, the higher the body needs to produce cholesterol internally from the liver to act as an antioxidant and free radical scavenger. Plasma vitamin C is a good antioxidant (11). Vitamin C has also been reported to rejuvenate vitamin E, making it an indirect contributor to the fight against free radical damage in the lipids (12).

The relationship in humans as regards the role of antioxidants and oxidative stress in the pathogenesis of malaria is not well understood though reports have been made in murine models. On the other hand, reports have shown that antioxidants and antioxidant agents such as cholesterol, albumin and vitamin C would provide protection against the oxidative stress induced by malaria infection(11,13). To gain insight into the relationship between P. Falciparum malaria and these antioxidant agents, a hospital based study among children with uncomplicated Falciparum malaria infection was conducted in Calabar, Cross-River State, Nigeria and the association of malaria parasitemia and vitamin C, total and HDL cholesterol, and total antioxidant levels, was 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, Nigeria. 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 milliliters of venous blood was obtained from patient and control subjects by venepuncture. Two milliliters of the blood was placed into an EDTA bottle for the determination of haemoglobin using Avid 60 CT automated haematoloyg analyzer, and parasite count WHO, 1991 method (14) 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 3000rpm. Total antioxidant was determined immediately by the method of Miller *et al* 1993 (7) and the remaining samples were stored at -200C for vitamin C assay by the method of Roe and Kuether, 1943(15). Serum total cholesterol and high density cholesterol was determined by kit methods purchased from Quimica Clinica Aplicada Spain, while serum albumin was also determined by kit method purchased from Randox laboratories, USA. The data were analyzed by student's "t" test and Pearson's correlation. Unless otherwise stated the data were expressed as means +/- standard deviation. $P<0.05$ was considered significant in all statistical comparisons

Results

Comparison of total antioxidant level, total Cholesterol, HDL-Cholesterol, vitamin C, albumin, haemoglo-

bin and parasite count of malaria infected children and normal subjects or control are shown on table 1. Total antioxidant status, vitamin C, albumin, haemoglobin and HDL-Cholesterol were observed to be significantly lower (p<0.001) in malaria-infected children when compared with the normal subjects. While total Cholesterol was observed to be similar in both patients of malaria infection and control subjects. Total plasma antioxidant level 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 40oC and above, with vomiting, and dehydration. Total plasma antioxidant, and haemoglobin levels were in severe malaria significantly lower than levels in Mild/moderate malaria. However no significant change was observed in the levels of vitamin C, albumin, and total cholesterol and HDL-cholesterol in both severe and mild/moderate malaria infection. Table 3 shows the relationship between age on one hand, and plasma total antioxidant, vitamin C, albumin, total cholesterol, HDL cholesterol, haemoglobin and parasite counts on the other hand in patients. The table shows that in control subjects and within the age bracket (1 to 10 years) plasma total antioxidant level, vitamin C, albumin, total cholesterol, HDL cholesterol, and haemoglobin do not change significantly (p>0.05) with age. However, during malaria infection, total plasma antioxidant level and albumin were observed to be significantly lower in children between 1 and 5 years when compared with those between 6 and10 years

Figure 1 shows the correlation graph of parasite count against total antioxidant level. The total antioxidant level correlated negatively with parasite count (r= -0.432, p <0.01).

Table 1 Total plasma antioxidant level, total and HDL-cholesterol, vitamin C, albumin, haemoglobin and parasite counts in malaria infected children.

Parameters	Control subjects n=60	Patients n=122	Critical t	Cal t	P-value
Total antioxidant Status mmol/l	1.26 ± 0.2	0.63 ± 0.31	3.29	9.5	P<0.001
Vitamin C µmol/l	59.0 ± 28.0	38.6 ± 18.8	3.29	5.8	P<0.001
Albumin g/l	39.6 ± 8.1	30.9 ± 4.7	3.29	7.7	P<0.001
Total cholesterol mmol /l	3.8 ± 0.7	3.7 ± 0.9	3.29	0.8	P>0.05
HDL-Cholesterol mmol/l	0.89 ± 0.5	0.56 ± 0.3	3.29	4.7	P<0.001
Haemoglobin g/l	109.7 ± 11.2	90.7 ± 24.5	3.29	25.9	P<0.001
Parasite count dl	_____	5,391.3 ±2923.1	-	nc	-

n=Number of subjects studied.

nc No comparison

Table 2 Total plasma antioxidant level, total and HDL-cholesterol, vitamin C, albumin, haemoglobin and parasite counts based on clinical and laboratory findings of malaria infected children.

Parameters	Clinical/Laboratory Findings		Critical t	Cal t	P-value
	Mild/moderate parasite count <7,000 n=79	Severe malaria parasite count >7,000 n=43			
Total antioxidant level mmoL/L	0.74 ± 0.27	0.45 ± 0.27	3.29	4.0	<0.001
Vitamin C µmol/L	39.7 ± 17.0	36.6 ± 20.9	3.29	0.8	>0.05
Albumin g/L	31.7 ± 5.2	29.8 ± 4.1	3.29	1.5	>0.05
Total cholesterol mmoL L/L	3.1 ± 1.0	3.0 ± 0.8	3.29	0.6	>0.05
HDL-Cholesterol mmoL/L	0.6 ± 0.3	0.6 ± 0.4	3.29	0	>0.05
Haemoglobin g/L	101.3 ± 20.4	72.1 ± 18.7	3.29	7.9	<0.001
Parasite count dL	3698.5 ± 1210.8	8499.6 ± 2556.3	3.29	11.6	<0.001

n=Number of subjects studied.

Table 3 Effect of age on plasma total antioxidant, vitamin C, albumin, total cholesterol, HDL cholesterol, haemoglobin and parasite counts in malaria infected children and Control Subjects.

	Malaria infected children (n = 122)		Crit T	Cal t	P-value	Control Subject (n = 60)		Crit T	Cal t	P-Value
	Age1–5years n=93	6-10 years n=29				Age 1- 5 n=30	6-10years n=30			
Total antioxidant Status mmol/l	0.59 ± 0.3	0.76 ± 0.3	1.96	2.1	<0.05	1.23 ± 0.2	1.33 ± 0.2	1.96	1.9	P>0.05
VitaminC µmol/l	37.9 ± 18.3	41.6 ± 19.2	1.96	0.9	>0.05	66.7± 25.8	59.2 ± 24.6	1.96	1.1	P>0.05
Albumin g/L	21.3 ± 3.5	30.7 ± 4.7	3.29	11.3	<0.001	38.2 ± 7.8	40.3 ± 7.6	1.96	1.0	P>0.05
Total cholesterol mno l/l	3.1 ± 0.9	2.9 ± 0.9	1.96	1.2	>0.05	3.7 ± 0.7	3.9 ± 0.7	1.96	1.1	P>0.05
HDL-Cholesterol mnol/l	0.57 ± 0.3	0.51 ± 0.3	1.96	0.9	>0.05	0.89 ± 0.5	0.85 ± 0.4	1.96	0.3	P>0.05
Haemoglobin g/l	89.8 ± 24.0	85.3 ± 33.2	1.96	0.7	>0.05	111.6± 1.5	108.4± 10.6	1.96	1.1	P>0.05
Parasite count dl	3,387.0±2564.4	2,004.2±358.7	1.96	1.9	>0.05	-	-	-	-	-

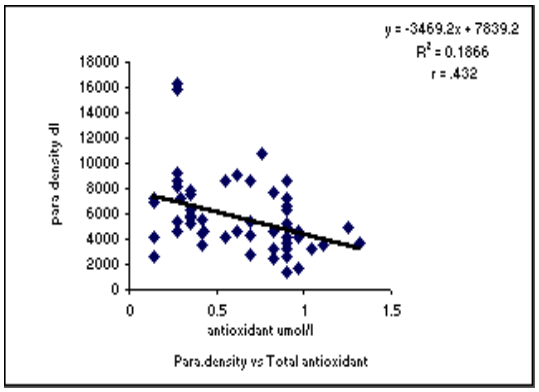

Fig.1 scatter plot of parasite count against total antioxidant.

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. Degradation of antioxidant enzymes as well as haemoglobin by malaria parasite to produce its own protein has been reported (16). This might thus be contributing to the decline in total antioxidant status. Furthermore a negative correlation was obtained between parasite count and total antioxidant level in malaria patients, which indicated that at higher parasitemia, there is increased consumption of total antioxidant thus lowering its level.

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. Furthermore, there was a significantly lower total antioxidant level in children 5 years and below as compared with those of 6 years and above. Previous observations (4, 17) on under fives have shown that they are the most vulnerable age group for malaria. In this age bracket, it could be seen that children had much reduced antioxidant level, which may be the consequence of frequent malaria infection or of severity of malaria.

Total cholesterol in normal subjects and patients were similar. The mean total cholesterol for control and patients were within our reference range. On the contrary, a significantly lower serum HDL-Cholesterol (0.56 + 0.3 mmol/l) was observed in malaria patients when compared with controls (0.89 + 0.5mmol/l). This finding agrees with the report of Metzger *et al* 2001 (18), which made similar observations in 273 children (aged 1-10 years), with acute malaria but different from the findings of Das *et al*, 1996 (12), who reported significant decrease in total cholesterol. The low HDL-Cholesterol observed in malaria infection is attributed to increased catabolism seen during inflammatory processes (19). Low HDL-Cholesterol in adults is associated with increased risk of coronary heart

disease (19). The implication of low plasma HDL-Cholesterol seen in our patients is that those in malaria endemic regions may be predisposed to future coronary diseases as a result of frequent malaria infection.

In our present work, vitamin C level was also significantly lower (p<0.05) in malaria patients when compared with control subjects. This was attributed to either, increased requirement or increased destruction during malaria infection. Age had no independent effect on the

levels of vitamin C in children. Albumin was significantly lower ($p<0.01$) in malaria-infected children particularly among patients 5years and below. Low albumin level in malaria may be due to a number of factors: these include inhibition of synthesis by increasing levels of cytokines such as TNF, ILI and IL6 (20), reduced food intake as a consequence of loss of appetite, and redistribution into extravascular spaces as a result of inflammation. The albumin in extravascular spaces enhances antioxidant activity at these locations (21).

There was a reduction in haemoglobin levels in malaria patients as 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 (17).

Malaria undermines the health and welfare of families. It endangers the survival of children. It debilitates the active population and strains the resources of the affected persons. Anaemia is a common occurrence in malaria infection particularly in severe cases as seen in this work. Parasitemia of red cells appears to be the major factor in development of anaemia in malaria infection. The major function of antioxidant is to combat oxidative challenges. Our findings have revealed significant reduction in total plasma antioxidant level, as well as Vitamin C, HDL-Cholesterol and albumin in malaria patients. Reduction in the activity of these analytes as antioxidant agents may lead to reduction in the body's capacity to mop-up free radicals generated. The accumulation of these free radicals in the body during malaria infections, cause stress on the cellular vitality ultimately leading to destructive effect on cells as well as exposure of the patient to free radical associated disease. To ameliorate these effects, we suggest the incorporation of antioxidant agents as part of the component drugs in the treatment and management of malaria infections particularly in children.

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