

Full Length Research Paper

Lipid peroxidation and ascorbic acid levels in Nigeria children with acute falciparum malaria

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This study was undertaken to establish data on the roles of lipid peroxidation and ascorbic acid in the pathology of malaria in Nigeria children. We measured the levels of malondialdehyde (MDA), a marker of lipid peroxidation and ascorbic acid in the plasma of 406 parasitaemic and 212 non-parasitaemic Nigerian children. Lipid peroxidant levels were significantly higher in children with moderate and high parasitaemia (16.88 and 13.64 MDA μ M/ml, respectively), than in non-parasitaemic controls (8.71 MDA μ M/ml). Malaria infection resulted in significant reduction in ascorbic acid levels of children with moderate and high parasitaemia. The MDA and ascorbic acid levels of children with low parasitaemia were not significantly higher than the levels in non-parasitaemic controls. High levels of lipid peroxidation corresponded with low levels of ascorbic acid and this may be responsible for tissue damage associated with pathology of malaria in Nigerian children.

Key words: Lipid peroxidation, ascorbic, acid Nigerian children, *falciparum* malaria.

INTRODUCTION

Countries in Africa continue to bear the brunt of malaria, accounting for more than 90% of the estimated 300 million annual cases. Of these 2.7 million die each year, most of whom are children (Darwin, 2002). Almost all deaths from malaria each year worldwide are attributable to *Plasmodium falciparum*.

Falciparum malaria in children may result in prostration, fever, headache, nausea and vomiting, abdominal pain, convulsion and severe anaemia (WHO, 2000). Anaemia in children with falciparum malaria is mainly a result of haemolysis of both parasitised and non parasitised red blood cells. Most cells, even in normal metabolic rate, are

exposed to oxidative stress and are equipped with inherent protective antioxidative mechanisms (Saltman, 1989). *Plasmodium* – infected erythrocytes are under increased endogenous oxidative stress exerted by the malaria parasite (Eaton et al., 1976). Several studies have reported evidence showing that physicochemical changes in the membrane of the erythrocyte induced by oxidative stress is responsible for membrane lipid peroxidation and haemolysis seen in malaria (Clark and Hant, 199; Clark et al., 1984; Das and Nanda, 1999).

Reactive oxygen intermediates, including hydrogen peroxide, generated by macrophages and polymorphonuclear neutrophils (PMNS) play an important role in the host defence against malaria (Kharazmi, 1986). Golenser and Chevion (1989) reported an increase in the concentration of malondialdehyde, a lipid peroxidation product in the blood of mice infected with *Plasmodium berghei*. Also free radical generators caused a transient haemolysis in *Plasmodium vinckei* – infected mice (Clark et al., 1984). Ascorbic acid forms the first line

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Table 1. Occurrence of *falciparum* malaria in 406 parasitaemic children by intensity of infections.

Intensity of Infection (Parasitaemia)	Number of children (%)
No. of asexual forms/ml of blood <1000 (low)	75(18.4)
1000 – 10,000 (Moderate)	224 (55.17)
>10,000 (High)	117 (28.82)

of defence in human plasma exposed to a variety of oxidative attacks including aqueous free radicals and activated polymorphonuclear leucocytes (Frei et al., 1988).

There is a dearth of data on investigated cases of reactive oxygen species production during *P. falciparum* infection in Nigerian children. We therefore report a prospective study of 406 malarious children in Niger Delta area of Nigeria, and measured their plasma malondialdehyde and ascorbic acid levels.

MATERIALS AND METHODS

Subjects

The study group comprised 406 children (208 girls and 198 boys), aged 2-12 years (with mean age of 5.85 ± 1.8 years), presenting with clinical features of malaria. They had asexual parasites in peripheral blood smear and were admitted to Baptist Medical Centre, Eku, Delta State, Nigeria between April and October 2003. The control group was made up of 212 (109 girls and 103 boys) malaria free children matched for age, sex and socio-economic status with the study group. The control group was recruited from a nursery and primary school in the same town where the hospital is located.

Data were obtained on some clinical features of the subjects, such as body temperature, nausea, vomiting and abdominal pain.

Collection of blood samples

A 5ml sample of venous blood was collected from each child with the aid of hospital laboratory staff, and their names, age, gender and body temperatures recorded. Plasma was separated from the blood cells after centrifugation, and stored at -4°C .

Parasitological examination

The presence and density of *P. falciparum* in each blood sample was determined from Giemsa-stained thin and thick blood films. A slide was scored as negative if 100 high power fields (at 1000x magnification) had been examined for about 30 min without seeing any parasites. The amount of parasites in positive smears was counted to determine the intensity of infection. Positive smears were grouped into three:

- Low parasitaemia, with parasite density of <1000 asexual forms per ml of blood.
- Moderate parasitaemia, with parasite density of 1000 – 10,000 asexual forms per ml of blood.

- High parasitaemia, with parasite density of > 10,000 asexual forms per ml of blood.

Determination of haemoglobin concentration

To investigate anaemia, the haemoglobin concentration of each blood sample was determined using the technique described by Dacie and Lewis (1975).

Assay of lipid peroxidation and ascorbic acid

Plasma of venous blood of subjects was assayed for lipid peroxidation by determining their malondialdehyde (MDA) levels according to the protocol outlined by Gutteridge and Wilkins (1982). The data obtained was quantified using a molar extinction coefficient of $1.56 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ and expressed as MDA μMml^{-1} (Buege and Aust, 1978). Plasma total ascorbate was estimated by the 2,4-dinitrophenylhydrazine method reported by Thurnham and Stephen (1979).

Statistical analysis

Data obtained on lipid peroxidation and ascorbic acid level for the three groups of malarious children and control group were tested for statistical difference using one-way analysis of variance (ANOVA).

RESULTS

Of the 406 children infected with *P. falciparum*, 75 (18.447%) had low intensity of infection (<1000 asexual forms per ml of blood), 224 (55.179%) had moderate intensity of infection (1000 – 10,000 asexual forms per ml of blood) and 117 (28.82%) had high intensity of infection (>10,000 asexual forms per ml of blood) (Table 1). The clinical features of the subjects in relation to the intensity of infection is shown in Table 2. Fevers (body temperature $>37.5^{\circ}\text{C}$), vomiting, nausea, abdominal pain and anaemia were more prevalent in malarious children with high intensity of infection. These clinical features (except vomiting) also occurred to a lesser degree among non malarious controls.

Lipid peroxidant levels were significantly more in children with moderate and high parasitaemia ($P<0.05$) than in non-parasitaemic controls. However there was no significant difference in lipid peroxidant levels between malarious children with low parasitaemia and malaria-free

Table 2. Clinical features recorded in parasitaemic children and controls.

Subjects	Parasitaemic children			Controls (non-parasitaemic) n = 212 Number (%)
	Low(n=75); Number (%)	Moderate (n=224); Number (%)	High(n=117); Number (%)	
Fever (BodyTemperature >37.5°C)	12 (16)	156 (69.64)	112 (95.73)	45 (21.23)
Vomiting	0 (0)	65 (29.02)	48 (41.03)	0 (0)
Nausea	15 (20)	122 (54.46)	69 (58.97)	28 (13.21)
Abdominal pain	18 (24)	103 (45.98)	75 (64.10)	51 (24.06)
Anaemia (Mean Hb. Conc.<11g/dl)	27 (36)	170 (75.89)	102 (87.18)	76 (35.85)
Males	44 (58.67)	109, (48.66)	55 (47.01)	103 (48.59)
Females	31 (41.33)	115 (51.34)	62 (52.99)	109 (51.41)

Table 3. Mean lipid peroxidant levels in parasitaemic children and controls.

Subjects	Lipid Peroxidant Level * (MDA $\mu\text{M/ml} \pm \text{SD}$)
<i>Parasitaemic</i>	
Low	9.34 \pm 0.74 ^a
Moderate	13.64 \pm 0.98 ^b
High	16.88 \pm 1.02 ^c
<i>Non-Parasitaemic</i>	
Control	8.71 \pm 0.62 ^{ad}

* Values followed by the same letter within a column are not significantly different at 5% level; LSD (0.05) = 0.69

controls ($P>0.05$) (Table 3). Malaria infections resulted in reduction in ascorbic levels among subjects (Table 4). Differences in plasma ascorbate levels were significantly different ($P<0.05$) among the three groups of parasitaemic children. Plasma ascorbate level in children with low parasitaemia was however not significantly ($P>0.05$) higher than what obtained in non-parasitaemic control group.

DISCUSSION

This study has shown that fevers and anaemia are highly associated with high infection levels of falciparum malaria since about 90% of all children with parasite count of $>10,000/\text{ml}$ of blood were febrile and anaemic. The occurrence of some clinical features such as nausea, abdominal pain and anaemia in non-malarious control children (Table 2) can be attributed to other causes, particularly intestinal helminth infections. Hookworm (*Necator americanus*), and roundworms (*Ascaris lumbricoides*) in earlier epidemiological studies have been found to infect over 50% of children in this part of Nigeria (Niger Delta), leading to anaemia, abdominal discomfort and adverse socio-economic effects (Obiamiwe and Nmorsi, 1991; Ogbe et al., 2002).

We have also reported lipid peroxidant and plasma ascorbate fluctuations in malarious children in Nigeria. The significantly higher MDA concentration of children with moderate and high infection levels than non-

parasitaemic controls suggest that enhanced plasma MDA levels is a marker of malaria severity. In addition, increase in MDA in malarious children reflects an increase in peroxidation of membrane lipids. The average MDA level reported in this study (13.2 ± 1.02) is greatly in excess when compared to the result obtained with Indian children suffering from acute falciparum malaria (Rath et al., 1991). Thus high oxidant insult could partially explain the enormity of the impact of malaria on the quality of life of Nigerian children.

During the development of malaria parasite, haemoglobin is progressively digested and a concurrent release of high levels of iron-containing breakdown products take place in the red blood cells (Golenser and Chevion, 1989). Free iron can stimulate free-radicals reactions to produce hydroxyl radical (OH^\cdot). Membrane lipids are known to succumb easily to the devastating actions of hydroxyl radicals (Slater et al., 1987).

Plasmodia cells accumulate protective enzymes (catalase, glutathione peroxidase and superoxide dismutase) which are depleted in the red blood cells of the host. Increased production of hydrogen peroxide and free oxygen radicals (Etkin and Eaton, 1975), and a decrease in antioxidant enzymes (Nair et al., 1984; Stocker et al., 1985; Mohan et al., 1992), have been observed in parasitised erythrocytes. Reduced antioxidant enzymes defence in the red blood cells of *P. falciparum* infected patients (Golenser and Chevion, 1989) may be responsible for the significantly higher levels of lipid peroxidation and oxidative stress in children with

Table 4. Mean ascorbic acid levels in parasitaemic children.

Subjects	Ascorbic acid Level *($\mu\text{M/ml} \pm \text{SD}$)
<i>Parasitaemic</i>	
Low	116.38 \pm 5.26 ^a
Moderate	89.53 \pm 3.43 ^b
High	67.41 \pm 2.63 ^c
<i>Non-Parasitaemic</i>	
Control	122.07 \pm 6.37 ^{ad}

* Values followed by the same letter within a column are not significantly different at 5% level; LSD (0.05) = 0.92.

moderate and high parasitaemia. This implies that the enhanced oxidative stress to erythrocytes in children with moderate and high parasitaemia was endogenously induced by malaria parasites during their consumption of haemoglobin and further leading to anaemia.

Plasma ascorbate plays a pivotal role in protecting plasma lipids from reactive oxygen attack. However, it is rapidly oxidised when challenged by oxidants released from activated polymorphonuclear neutrophils (Frei et al., 1988). Ascorbic acid level (Table 3) was significantly ($P < 0.05$) reduced by *P. falciparum* infection and this coincided with enhanced level of MDA (Table 3). Once ascorbic acid has been used up, there is initiation of lipid peroxidation (Frei, 1994).

The values of ascorbic acid obtained in this study (Table 3) is within the range (0-127.5 μMml^{-1}) reported for malarious malnourished children by another study in Nigeria (Thurnham, 1990). Our data demonstrate that a high level of lipid peroxidation corresponds with low level of ascorbic acid in malaria, and this may be responsible for the tissue damage associated with pathology of malaria.

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