

Abubakar et al., *Afr. J. Infect. Dis.* (2016) 10 (1): 17 – 20

<http://dx.doi.org/10.4314/ajid.v10i1.3>

OXIDANT STATUS OF CHILDREN INFECTED WITH *PLASMODIUM FALCIPARUM*
MALARIA IN KATSINA METROPOLIS, NORTHWESTERN NIGERIA

Abubakar, M. G., Usman, S. M. and Dandare, S.U.

Department of Biochemistry, Usmanu Danfodiyo University,
PMB 2346 Sokoto, Nigeria.

E-mail: magusau@hotmail.com

Abstract

Background: Malaria is a global menace caused by the transfer of a *plasmodium* parasite to a host by an infected anopheles mosquito. Upon infection, the overwhelmed host releases free radicals which have the capacity to induce oxidative damage by lipid peroxidation. This study was undertaken to assess the effect of malaria caused by *Plasmodium falciparum* on some antioxidant markers and lipid peroxidation levels in children attending hospitals in Katsina State, Nigeria.

Materials and Methods: Blood samples were collected from untreated subjects upon confirmation of *Plasmodium falciparum* parasitaemia using the Giemsa stain technique. One hundred and sixty (160) consenting individuals (80 infected patients and 80 uninfected subjects) comprising of both sexes were randomly selected. The levels of antioxidant markers and malondialdehyde (MDA) - a lipid peroxidation marker were determined. Descriptive analysis was employed using SPSS version 16.0 and significance between groups was ascertained using students' T-test.

Results: *P. falciparum* malarial infection significantly ($p < 0.05$) reduced the antioxidant markers [vitamins A, C, & E; and reduced glutathione (GSH)] by 65.4%, 29.7%, 48.1%, 40.4% respectively in males and by 54.2%, 36.6%, 55.7%, 36.6% in females when compared with values obtained from uninfected, healthy children. Conversely, lipid peroxidation levels were significantly ($p < 0.05$) higher in children with parasitaemia than in non-parasitaemic controls. Males showed greater than 200% increase, while it increased by 138% in females.

Conclusion: Our findings indicate a reciprocal relationship, where high levels of lipid peroxidation correspond to low levels of antioxidants, which may be due to over utilization of the antioxidants in order to counteract the effect of free radicals. This may be responsible for oxidative stress and consequently, tissue damage associated with pathology of malaria in Nigerian children.

Key words: Antioxidant markers, *Plasmodium falciparum*, lipid peroxidation and children.

Introduction

Malaria is a major health problem in developing countries, accounting for approximately 584,000 deaths, 90% of which occur in sub-Saharan Africa, predominantly in children less than 5 years of age (WHO, 2015). It is endemic to over 100 nations and territories in Africa, Asia, Latin America, the Middle East, and South Pacific. The disease is caused by the bite of an infected protozoan parasite of the genus *Plasmodium* - mainly female anopheles mosquito (Klonis et al., 2013).

Plasmodium falciparum is by far the deadliest of the four human malarial species (*Plasmodium falciparum*, *malariae*, *ovale* and *vivax*). In addition to being the deadliest form of malaria, *P. falciparum* destroys red blood cells, which can cause acute anaemia. Adherence of the parasite to cells in certain tissues may cause problems within those organs, such as the lungs, kidneys and brain. A major complication of *P. falciparum*, cerebral malaria, can lead to coma, transient or permanent neurological effects, and death. *P. vivax* is the most widespread. *P. malariae* and *P. ovale*, although also significant, cause fewer cases and less severe forms of the disease (Snow, 2000). Compared to *P. vivax*, *P. falciparum* is less widespread, but more likely to result in severe complications and be fatal (Murphy and Breman, 2001).

Host-parasite relationship in malarial infection has the capacity to cause the release of reactive oxygen species (ROS) by stimulating the immune system; this has the potency of inducing oxidative damage and causing cell destruction (Kremsner et al., 2000). Thus, Plasmodium-infected erythrocytes are under increased oxidative stress exerted by the malaria parasite. The body however, has numerous in-built mechanisms to minimise or counteract the cellular effects of ROS. Prominent amongst these defence mechanisms is the production of antioxidants (Onyesom et al., 2012).

Antioxidants may be synthesized endogenously in the body through various mechanisms and can be food derived, however, the sum of these gives a measure of the total antioxidant status of the extracellular fluid of an individual (Akpotuzor et al., 2012). These antioxidants could be preventive or chain breaking. Preventive antioxidants (Vitamin C, glutathione peroxidase, and catalase) inhibit the initial production of free radicals including ROS, while chain breaking antioxidants (vitamin E, superoxide dismutase, uric acid) inhibit the damaging phase of ROS (Valko et al., 2007). It has been reported that low dietary intakes of antioxidant vitamins or reduced synthesis of nondietary antioxidants are likely to result in an oxidant-antioxidant imbalance that exacerbates inflammation and tissue damage (Cross, 2003). Therefore, cooperation of all the different antioxidants provides greater protection against attack by reactive oxygen or nitrogen radicals than any single compound.

Some biochemical changes regarding the oxidant and antioxidant levels in malaria infected patients have been observed (Kulkarni et al., 2003). A correlation between *Plasmodium falciparum* load and serum antioxidant vitamins (A, C and E) has been demonstrated among malarial infected children (Adelekan et al., 1997; Onyesom et al., 2010). For several reasons, the prevalence of malaria has increased in North-western Nigeria amongst children; yet, reports on antioxidant levels of patients are scarce in this region. Therefore, in addition to the levels of serum antioxidant vitamins, this study also reports the levels of a significant endogenous antioxidant - reduced glutathione and lipid peroxidation in children infected with malaria in Katsina metropolis, a community in North western Nigeria.

Materials and Methods

Subjects and Study Area

The study was carried out in Katsina, Katsina State, Nigeria. The city is located between latitude 11°00'N and 13°50'N and longitude 7°15'E and 9°00'E. One hundred and sixty (160) children, (males/females) between the ages of 1-10 infected with *Plasmodium falciparum* who attended different clinics in Katsina metropolis were selected for this study (from Mar 2014 to July 2014). 80 malaria-free children (males and females) within the same age range as the infected children were used as controls. The presence or absence of *Plasmodium falciparum* parasitaemia was confirmed by the Giemsa stain technique using both thin and thick peripheral blood smears.

Serum Sample Collection and Treatment

Prior to any treatment, blood samples were collected from each subject using sterile disposable hypodermic needle and syringe into clean, sterile plastic centrifuge tubes. One microlitre of the blood sample collected was transferred into sterile tubes containing ethylenediaminetetraacetic acid (EDTA), mixed with 7.0ml of distilled water and haemolysis was completed. A 2.0ml of 25% metaphosphoric acid was then added, mixed and centrifuged for 2000g for 10 minutes at room temperature, and the remaining blood samples were centrifuged at 2000g for 10 minutes after clotting. Sera were collected by Pasteur pipette for assay within 24 hours while the fresh blood in the syringe was used to make smear on a slide for thick film. *P. falciparum* parasitaemia was determined in peripheral blood smears by Giemsa staining.

Assay

In this study, the antioxidant markers; vitamins (A, C, and E), reduced glutathione and lipid peroxidation levels were estimated in non-infected and children infected with *Plasmodium falciparum*:

Vitamin A: Serum vitamin A was estimated using colorimetric method (Pett, 1940).

Vitamin C: Serum vitamin C was determined using 2, 4-dinitrophenylhydrazine (Roe and Kuether, 1943).

Vitamin E: Serum vitamin E was determined by Emmerie-Engel method (Quiafe *et al.*, 1949).

Reduced glutathione: Reduced glutathione was measured in blood using the method of Patterson and Lazarow (1955).

Lipid peroxidation: Malondialdehyde (MDA) was measured using the Thiobarbituric Acid reactions (TBARS) (Abubakar *et al.*, 2002).

Statistical Analysis

The data are presented as Mean \pm SEM. Statistical significance between groups was assessed by students' T-test and statistical significance was established at $p < 0.05$. The SPSS package version 16 was used for the analysis as described (Ogeibu, 2005).

Results

Study results (Table 1) indicate that malarial infection significantly ($p < 0.05$) decreases serum levels of antioxidant vitamins (A, C and E) and reduced glutathione by 65.4%, 29.7%, 48.1% and 40.4% respectively when compared with the non-infected male subjects. The infected females also showed significantly ($p < 0.05$) reduced (54.2%, 36.6%, 55.7% and 36.6%) mean concentrations of vitamins A, C and E and reduced glutathione respectively. Conversely, lipid peroxidation levels increased significantly ($p < 0.05$) in infected children; males having 228.3% and females 138.8% increase when compared with non-infected children respectively.

Table 1: Serum Levels of Vitamins A, C, E, Reduced Glutathione and Lipid Peroxidation in malaria infected and uninfected subjects.

SUBJECTS (n=160)	VITAMINS			PARAMETERS	
	A ($\mu\text{g/dl}$)	C (mg/dl)	E (mg/dl)	GSH (mg/dl/100ml)	MDA (n moles/ml)
Uninfected (M) (n=48)	8.74 \pm 0.52	31.86 \pm 3.60	4.63 \pm 0.16	27.59 \pm 1.60	2.34 \pm 0.70
Infected (M) (n=48)	3.04 \pm 0.20*	21.22 \pm 1.36*	2.47 \pm 0.16*	16.16 \pm 0.66*	5.57 \pm 0.18*
Uninfected (F) (n=32)	7.84 \pm 0.60	34.86 \pm 5.88	4.85 \pm 0.21	26.46 \pm 3.28	2.63 \pm 0.33
Infected (F) (n=32)	3.61 \pm 0.30*	20.32 \pm 1.96*	2.21 \pm 0.16*	15.31 \pm 0.62*	5.71 \pm 0.26*

GSH= Reduced Glutathione; MDA= Malondialdehyde; M= Males; F= Females. Values are expressed as Mean \pm SEM; Values with (*) differ significantly ($p < 0.05$) in comparison with non-infected subjects. n= No. of subjects.

Discussion

In this study, antioxidant determinants (Vitamins A, C and E; and GSH) in serum of *P. falciparum* malaria infected patients were assayed and values were compared with figures obtained from uninfected, healthy individuals. Evidence shows that *P. falciparum* malarial infection significantly ($p < 0.05$) reduced the serum antioxidant levels (Table 1), with vitamins A and E showing very high percentage decreases in both males and females. Vitamin A is an essential micronutrient for normal immune function, which influences antibody response and all mediated immunity (Semba *et al.*, 1998). Our observation is consistent with earlier studies (Onyesom *et al.*, 2012) who reported a decrease in total antioxidant capacity (TAC); (Guha *et al.*, 2006 and Kulkarni *et al.*, 2003) reported an imbalance in the oxidant and antioxidant levels in the serum of malarial infected patients.

P. falciparum infection has been observed to immuno-compromise the host, consequently leading to increased free radicals production, especially reactive oxygen species, ROS (Kremsner, 2000). Excess free radicals in turn induce lipid peroxidation and cell damage (Chapelle, 1997). However, the body has a number of defense mechanisms which include the synthesis and utilization of antioxidants (Valko *et al.*, 2007). Thus, counteracting the effect of free radicals could induce decrease of antioxidant levels as observed in this study. Furthermore, this strengthens the crucial role antioxidants play in the defense of ROS induced damages. GSH is an extremely important cell protectant. It directly quenches reactive hydroxyl free radicals, other oxygen centered free radicals, and radical centers on DNA and other biomolecules (Kidd, 1997).

Malondialdehyde (MDA), which is a bioactive marker for the quantification of lipid peroxidation (Marnett, 1999), was assayed in this study. The significantly higher MDA concentration in infected subjects than non-parasitaemic controls suggest that enhanced MDA levels is a marker of malaria infection. Furthermore, increase in MDA directly reflects an increase in peroxidation of membrane lipids (Egwunyenga *et al.*, 2004). More than a 200% and 100% increase in lipid peroxidation was observed in male and female parasitaemic subjects respectively. This indicates that an inverse relationship exists between antioxidant levels and lipid peroxidation. This finding is in line with the study of Golenser and Chevion, (1989); they showed that reduced antioxidants in red blood cells of *P. falciparum* infected patients may be responsible for the significantly higher levels of lipid peroxidation and oxidative stress in children with parasitaemia. Egwunyenga *et al.*, (2004) also showed that a significant decrease in ascorbic acid (vitamin C) level by *P. falciparum* infection coincided with enhanced level of MDA. Ascorbate plays a pivotal role in protecting plasma lipids from reactive oxygen attack. However, once it is used up, or oxidized by oxidants released from activated polymorphonuclear neutrophils, initiation of lipid peroxidation proceeds (Frei, 1994). Thus, reduction in antioxidant levels may be a consequence of their hyper consumption by over produced oxidants during malaria infection.

Overall, the depressed antioxidant concentrations of vitamins A, C, E and reduced glutathione may be in part due to their increased utilization by the body in an attempt to counteract the effects of free radicals generated by parasitaemia induced oxidative stress or increased destruction (Das *et al.*, 1996), while the increased level of peroxidation is as a result of overwhelming reactive oxygen species produced in the body. Therefore, micronutrient supplementation with antioxidant vitamins (A, C and E) may be incorporated into the management of *P. falciparum* infections, regardless of sex.

Finally, although these changes in antioxidants and lipid peroxidation in association with malaria infection are not novel, our findings have added more information to the sparse reports (if any) on changes in antioxidant profile of malaria infected children living in Katsina metropolis, North-western Nigeria.

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