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Antioxidant and oxidative stress status in human *Plasmodium* malaria

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ABSTRACT

The present study investigated the protective state of endogenous antioxidants against free radicals generated in human plasmodium malaria. Two hundred structured questionnaires were administered to the participants and blood samples were collected to assess the activities of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and reduced glutathione (GSH). Results showed that the 200 volunteered participants, 32% (73) and 68% (127) were males and females respectively. There was discrepancy in the number of male (12%) and female (31%) respondents in the low oxidative stress category as well as in the moderate oxidative stress category where 69% were females and 84% were males. However, in the high oxidative stress category 4% of the observed respondents were males. All participants tested were positive for parasitaemia and categorized as high and low parasitaemic patients. Qualitative examinations of the impact of malaria showed that 30%, 67% and 3% high parasitaemic patients exhibited low, medium and high oxidative stress respectively while 55%, 45% and 0% low parasitaemic patients demonstrated low, medium and high oxidative stress respectively. Furthermore, there were significant elevation ($P<0.05$) in the levels of plasma protein concentration, superoxide dismutase and glutathione S-transferase activities in the low parasitaemic patients compared to the high parasitaemic groups. However, there was no significant difference ($P>0.05$) in the level of glutathione and catalase activity between high and low parasitaemic patients. This study indicates that high parasitaemic patients are at greater risk of oxidative damage than low parasitaemic group, hence early diagnosis and treatment of malaria is highly encouraged.

Key words: *Plasmodium falciparum*, human, antioxidants and free radicals

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INTRODUCTION

Plasmodium is a protozoan parasite classified under the phylum Apicomplexa which also includes parasites like *Toxoplasma*, *Eimeria* and *Cryptosporidium*, all of which are endowed with a specialized apical complex for host cell invasion. Four species of *Plasmodium* infect

man – *P. vivax*, *P. ovale*, *P. malariae* and *P. falciparum*, among which *P. falciparum* is by far the most virulent (1). Malaria, the disease caused by *Plasmodium* continues to be the disease with the highest mortality rate, next only to tuberculosis. Approximately 500 million cases of malaria are reported every year, and around 3000 children die of malaria every day (1, 2). Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development (3).

In humans, *P. falciparum* metabolizes hemoglobin resulting in the formation of reactive oxygen species (ROS), the main cause of oxidative stress (4, 5). Reactive oxygen species are involved in various oxidative related diseases including aging (6), cancer (7) and atherosclerosis (8). The ROS can also be beneficial as they are used by the immune system to attack and kill pathogens (9).

In other to protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system (10). It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize reactive oxygen species (11). Oxidative stress occurs when there is an imbalance between these antioxidants and pro-oxidant in favor of pro-oxidant (4). In the present study, attempt was made to assess the status of oxidative stress in *Plasmodium* infected human erythrocytes using questionnaire and biomarkers such as superoxide dismutase (SOD) and catalase (CAT), glutathione *S*-transferase (GST) and glutathione (GSH).

MATERIALS AND METHODS

Subjects: A cross sectional study was conducted at Ilara-Remo in Remo-North Area of Ogun State, Nigeria from January 2009 Through March 2009. The inhabitants were mainly traders, farmers, fashion designers and motorcyclist. A total of 200 volunteers within the age range of 25 - 60 years participated in this study. All the volunteers signed an informed consent form prior the study and were adequately informed of the procedures, risks and benefits involved. Ethical approval was obtained through the Institutional Ethics Committee. All participants filled out questionnaire which include the following personal information: sex, age, occupation, education and qualitative assessment of status of oxidative stress grouped into low, moderate and high categories as adapted from Holly questionnaire with modification (12).

Collection of Blood Sample: Blood sample collection was carried out through the medical officers of the Babcock University Medical Centre. A 5 ml whole blood samples were drawn with 10 ml syringe from subjects by venipunture into EDTA vacutainer tubes to prevent blood clot. Randomization was performed through the selection of patients with the diagnosis of malaria by thick blood smearing.

Parasitological examination: The presence and density of *P. falciparum* in each blood sample was determined from Giemsa-stained thin and thick blood films. Slides were examined for malaria parasite under light microscope in 200 high power fields. Malaria parasites were counted against white blood cells, assuming a constant leucocytes count of 8000 per microlitre of blood. Parasitaemia was calculated using the formula:

$$\frac{\text{No. of parasite}}{\text{Count WBC}} \times \frac{8000}{200}$$

Positive smears were grouped into two:
Low parasitaemia, with parasite density of <1000 asexual forms per ml of blood.
High parasitaemia, with parasite density of > 10,000 asexual forms per ml of blood.

Antioxidant assay: The blood samples were centrifuged at 3000 rpm for 10 min to obtain plasma used for estimation of superoxide dismutase (SOD) and catalase (CAT), glutathione *S*-transferase (GST) and glutathione (GSH). The protein content of the plasma was determined by Gornall's 1949 method using Bovine Serum Albumin (BSA) as standard (13). Assessment of plasma antioxidant enzymes activities were done through determining the levels of reduced glutathione (GSH) (14, 15), glutathione *S*-transferase (GST) (16), catalase (17), and superoxide dismutase (SOD) (18).

Statistical analysis: Statistical significant difference between subjects was calculated using the student's t-test and chi-square (SPSS 14.0). A value of $P<0.05$ was considered significant. One way ANOVA was also performed.

RESULTS

Table 1 showed the data on oxidative stress status based on gender and parasitaemia level of the patients examined. Two hundred questionnaires were retrieved out of which 32% (73) and 68% (127) were males and females respectively. There was an observed difference in the number of male (12%) and female (31%) respondents in the low oxidative stress category as well as in the moderate oxidative stress category where 69% were females and 84% were males. However, in the high oxidative stress category 4% of the observed respondents were males. All participants examined were positive for parasitaemia and categorized as high and low parasitaemic patients. Qualitative examinations of the impact of malaria showed that 30%, 67% and 3% high parasitaemic patients exhibited low, medium and high oxidative stress respectively while 55%, 45% and 0% low parasitaemic patients demonstrated low, medium and high oxidative stress respectively (Table 1).

The study showed that plasma protein concentrations, superoxide dismutase and glutathione *S*-transferase activities of low parasitaemic patients were significantly elevated ($P<0.05$) compared to high parasitaemic patients. However, plasma catalase and glutathione levels were lower in patients with high parasitaemia than in low parasitaemic patients, although the difference was not statistically significant ($P>0.05$) (Table 2).

DISCUSSION

Previous reports have implicated *Plasmodium* parasites in the induction of oxidative stress in addition to its ability to cope with the oxidative stress generated during its erythrocytic stages (19, 20). The host builds up defense against the oxidative insults arising from the parasite's metabolism of hemoglobin which results in the formation of reactive oxygen species (ROS). Under normal circumstances, ROS are cleared from the host cell by the action of detoxification enzymes including SOD, GSH, and catalase (19). The ROS generated in host-parasite interactions can cause several biochemical changes including lyses of erythrocytes and alteration in major erythrocytic antioxidants. In view of these facts, this

study evaluated the oxidative stress status in patients infected with *Plasmodium* through administration of questionnaire and biochemical assays.

The analysis of the questionnaire revealed that patients with high parasitaemia count are more predispose to oxidative stress than those with low parasitaemia count. This is in agreement with previous report that *Plasmodium* parasites produces active redox products, free haem, and H_2O_2 leading to oxidative stress in infected cells (21). Other associated conditions such as environmental factors, gender, poverty and life styles including diet types were observed to influence the level of oxidative stress as been stated by the previous reports (1, 22, 23).

In this present study, plasma protein concentration, superoxide dismutase (SOD), and glutathione *S*-transferase activity were significantly higher in patients with low parasitaemia count than those with high parasitaemia counts. This implies a higher oxidative stress status in high parasitaemic patients.

The elevated plasma protein concentrations (0.90±0.02 mg/ml) in patients with low parasitaemia counts might have resulted from response of the host defensive system to the increase in reactive oxygen species generated by *P. falciparum* or its metabolites (24). Plasmodia erythrocyte cells accumulate protective antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase which are depleted in the infected host (25). This might be for the purpose of utilizing erythrocyte proteins for metabolic needs as the parasite matures (26). Previous studies have also reported that in parasitized erythrocytes, increase in the production of hydrogen peroxide and free oxygen radicals leads to a decrease in antioxidant enzymes (21, 27, 28). Thus, the significant reduction of plasma antioxidant enzymes in high parasitaemia patients might be the predisposing factor to higher oxidative stress causing damage to erythrocyte membranes (29). This also leads to reduction in the deformability of the cells, signaling the removal and massive destruction of erythrocytes by macrophages. These bring about the consequential increase in severe anemia, occlusion of peripheral microvasculature, cerebral pathology (hypoxia), and cardiac injury observed in severe malaria (25).

Table 1: Oxidative stress status based on patients' gender and parasitaemia level

Oxidative stress status	Gender		Parasitaemia level	
	Male N(%)	Female N (%)	Low (N)	High (N)
Low	10(5)	42(21)	55	30
Medium	60(30)	85(42.5)	45	67
High	3(1.5)	nil	nil	3

Table 2: Mean antioxidant levels in patients with low and high parasitaemia

Antioxidants biomarkers	Parasitaemia level	
	Low	High
Protein (mg/ml)	0.90±0.02*‡	0.05±0.03
Superoxide dismutase(unity of enzyme activity)	1.18±2.01‡	0.50±1.08
Catalase (Katf)	0.02±0.05	0.02±0.01
Glutathione S-transferase (µmol/min/mg protein)	39.48±1.03‡	26.86±0.05
Reduced glutathione (µg/ml)	0.07±0.01	0.06±0.21

**indicates mean ± standard deviation; ‡ indicates significant value at P<0.05*

There was an observed reduction in the activity of GST in high parasitaemic patients. It has also been reported that decreased or impaired GST activity would increase oxidative stress

thereby enhancing the severity of malaria pathology (20). From the results in Table 2, plasma catalase and glutathione levels were lower in patients with high parasitaemia than in low parasitaemic patients, although the difference was not statistically significant (P>0.05). This might have been due to high parasite load in the red blood cells resulting in reduction of plasma catalase and glutathione level as a survival strategy. This is in consonant with the previous findings that catalase and glutathione are required by *Plasmodium* parasites for growth as well as for protection against free radical attack (25, 30). This study further substantiate the opinion that high parasitaemia predisposes humans to oxidative stress and consequent risk factors such as anemia, occlusion of peripheral microvasculature, cerebral pathology (hypoxia), and cardiac injury observed in severe malaria. This is an implication for early diagnosis and treatment of malaria to prevent endogenous damage of erythrocyte.

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