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Oxidative stress in human malaria patients

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ABSTRACT: The intention of this study is to ascertain if there is significant oxidative stress in patients infected with *Plasmodium falciparum*, in Benin metropolis, South-South Nigeria. Serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-P) and catalase (CAT) were assayed in eighty (80) subjects of both sexes, composed of forty (40) test subjects and forty (40) control subjects. The 40 test subjects presenting in the clinic with features of malaria infection, were confirmed to be infected with *P. falciparum*. The results obtained revealed a statistically significant increase ($P<0.05$) in the level of malondialdehyde and a decreased level in superoxide dismutase, glutathione peroxidase and catalase activities $P<0.05$) in the malaria patients, when compared with the control subjects. These results suggest that malaria infection causes oxidative stress in patients within southern Nigeria. Administering antioxidants after anti-malaria treatment is therefore suggested.

Keywords: Oxidative Stress, Malaria, Malondialdehyde, Enzymic Antioxidants.

Introduction

During normal body physiologic processes the body produces free radicals, the inability of the biological system to detoxify the reactive intermediates or to repair the resulting damage in a timely manner results in the manifestation of one diseased state or the other. They are produced as a physiological response to specific noxia (Amer *et al*., 2006). Oxidative Stress represents an imbalance in production and clearance of reactive oxygen species/free radicals in biological systems (Lennon *et al*., 1991). Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including protein, lipid and DNA, hence in humans, oxidative stress has been identified as one of the causal factors in many diseases (Amer *et al*., 2006). Reactive oxygen species may be beneficial as they are used by the immune system as a way to attract and kill pathogens (Amer *et al*., 2006).

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Several studies have reported increased oxidative stress in isolated, infected red blood cells (IRBC) (Hunt *et al*., 1990). Hydrogen peroxide production is reported in *P-berghei*-IRBC and oxygen production in *P. falciparum*-IRBC (Hunt *et al*., 1990). It is suggested that reactive oxygen species are produced as a result of oxidation and degradation of ingested haemoglobin in the acid environment of the parasites food vacuole. Acidification of the lysate in *P. falciparum* infected erythrocyte (which also occur during the digestion of host cell cytosol in the acid food vacuole of the parasite) result in the generation of hydrogen peroxide (Atamna *et al*., 1993). This is probably produced during the oxidation of oxy-haemoglobin to met-haemoglobin (Atamna *et al*., 1993). During malaria infection, massively recruited activated monocyte and neutrophil produce increased levels of reactive oxygen species (oxidative stress), although other mechanism are involved as well (Golenser *et al*., 1991).

Although oxidative stress is beneficial in combating against intra-erythrocytic parasite, reactive oxygen species play a role in the pathology of malaria (Hunt *et al*., 1990). Excessive oxidative stress particularly at unwanted places (e.g vascular lining, blood brain barrier) will damage the defense system. Endogenous intra- and extracellular antioxidant systems are present to prevent damage but they may be unable to overcome the increased oxidant levels during disease conditions hence the need for treatment with antioxidants (Hunt *et al*., 1990). Chemically, oxidative stress is associated with increase production of oxidizing species or a significant decrease in the capability of antioxidant defenses such as glutathione to do their job (Lennon *et al*., 1991; Golenser *et al*., 1991).

To counteract oxidative stress, the body produces an armory of antioxidants to defend itself. It is the job of antioxidants to neutralize or mop up free radicals that can harm the cells of the body. The body's production of internal antioxidant is not enough to neutralize all the free radicals (Thumbwood *et al*., 1989). The formation and biotransformation (detoxification) of reactive oxygen species in biological systems start when phagocytic cells are activated by immune modulators. One of the consequences of activation of macrophages is the generation of respiratory burst. During the activation of respiratory burst, oxygen is taken up by membrane bound NADPH-oxidase complex and reduced to superoxide radical (Rice *et al*., 1995).

Another process in which the oxygen is formed is the reduction of molecular oxygen in the cell (Imlay, 2003). The oxygen is reduced by superoxide dismutase (SOD) to hydrogen peroxide and this in turn is reduced to water by glutathione peroxidase (GSH-P) which is in turn rapidly reduced by glutathione reductase (GSH-R) utilizing NADPH generated by various intracellular reactions including the hexose monophosphate shunt (HMS) (Amer *et al*., 2006). SOD, catalase and GSH-P/GSH-R are important intracellular antioxidant enzymes; they are however much less permanently present in extracellular fluid. Catalase is present in liver cell erythrocyte at high concentrations (Imlay, 2003). Oxidative stress is therefore the result of a disturbance in the balance of the naturally generated oxidants and antioxidants. This can be caused by increase in production of reactive oxygen species or decrease in activity of antioxidant systems.

The objective of this study is to assess the level of oxidative stress in malaria patients, by estimating the levels of serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-P) and catalase (CAT).

Materials and Methods

Subjects for the study

The subjects were made up of a total of 80 volunteers of both sexes. The test subjects were made up of 40 patients of both sexes who reported ill with signs and symptoms suggestive of malaria and who had not been placed on any anti-malaria drug. They were confirmed to have *Plasmodium falciparum* malaria. They were recruited from the God's Care Hospital, God's Time Hospital, Cicigide Hospital and Mount Gilead Hospital all in Benin metropolis. Informed consent was obtained from all the subjects and ethical approval was given by the personnel in charge of ethics in the health institutions before the study commenced. The patients were all Nigerians. They were divided into four groups with respect to their ages: 40-49, 50-59, 60-69, and 70-79years. Forty apparently healthy age-matched individuals, who also did not test positive to *P. falciparum* parasite, were used as control.

Sample collection and preparation

Two specimen bottles were used for each subject. Blood samples (5ml) were collected by clean venipuncture from the ante-cubital fossa of the subjects.1ml of such samples was put into already labeled EDTA bottles for malaria parasite examination, the remaining 4ml was put into a plain bottle. The blood was collected without undue pressure to either the arm or the plunger of the syringe. The samples were mixed by gentle inversion. The samples in the EDTA bottles were immediately checked for malaria parasite, after staining their thick films with Giemsa stain. Giemsa stained thin films confirmed the malaria parasite to be *Plasmodium falciparum.* The samples in the plane bottles were centrifuged at 3000r/min for 5 minutes to obtain serum, which was stored in a refrigerator at 2-8^{o}C when not needed for immediate analysis.

Assays

Malondialdehyde (MDA),was estimated according to the method described by Varshney and Kale (1990). Superoxide dismutase (SOD) was determined according to the method of Misra and Fridovich (1972) while glutathione peroxidase was determined by the method described by Sato *et al.* (1978). Catalase was estimated by the method of Cohen *et al.* (1970).

Malaria parasite density determination

The malaria parasite density was determined and graded following the method described by Cheesbrough (1998) by examining a thick blood film stained by Giemsa method.

Statistical analysis

All results were expressed as mean ± standard error of mean (SEM). The Data was analyzed using the student's t – test from the statistical package for social sciences (SPSS). Means of the same row or column with different superscript letters differ significantly at 95% level of confidence ($P<0.05$).

Results and Discussion

The results of this study reveal a statistically significant increase ($P<0.05$) in the MDA levels (6.01± 0.13 mmol/mg protein)x10^{-1}) and a decrease in the activity of the antioxidant enzymes SOD, GSH-P, and catalase (43.53±0.37; 6.98±0.16; 0.69±0.19 U/ml, respectively) when the values obtained for the test subjects are compared with those of the control subjects (1.45±0.04 mmol/mg protein x10^{-1}; 49.5±0.14; 21.09±1.16; 1.09±0.05 U/ml, respectively) (Table 1). These changes are of the same pattern irrespective of the sex and age of the patients and the degree of parasitaemia (Tables 2, 4 and 5). The degree of increase in MDA and decreased activities in the enzymic antioxidants are generally similar in both males and females as there was no statistically significant difference ($P>0.05$) in them, except for GSH-P where the decreased activity is significantly more ($P<0.05$) in the females (Tables 2 and 3).

The degree of increase in MDA and decreased activities in SOD, GSH-P and catalase activities are however generally similar in all the study age groups (Table 4). The observed changes in oxidative stress parameters increases with increase in the degree of parasitaemia as evidenced by the level of MDA (6.41±0.24 mmol/mg protein x10^{-1}) being highest, and the activity of SOD (43.21±0.74 U/ml) and catalase (0.66±0.03 U/ml) being lowest in severe malaria (+++) patients (Table 5); these changes with increasing parasitaemia are however not statistically significant ($P>0.05$).

Table 1: Oxidative Stress Parameters in Malaria Patients

	Control	Malaria Patients
MDA(mmol/mg protein)x10^{-1}	1.45±0.04 [a]	6.01±0.13[b]
SOD (U/ml)	49.5±0.14 [a]	43.53±0.37 [b]
GSH-P (U/ml)	21.09±1.16 [a]	6.98±0.16 [b]
CATALASE (U/ml)	1.09±0.05 [a]	0.69±0.19 [b]

Values are expressed as Mean ± SEM. Means of the same row with different superscript letters (a or b) differ significantly (P<0.05).

Table 2: Level of Oxidative Stress Parameters According to the Sex of the Malaria Patients

Sex	MDA (mmol/mg protein)x10^{-1}		SOD (U/ml)		GSH-P (U/ml)		CAT (U/ml)	
	Control	Malaria Patients	Control	Malaria Patients	Control	Malaria Patients	Control	Malaria Patients
M	1.43±0.05[a]	5.97± 1.80[b]	49.28±0.1[a]	43.17±0.4[b]	21.47±1.82[a]	7.15±0.25 [b]	1.02±0.07 [a]	0.70±0.00 [b]
F	1.50±0.0[a]	6.05±1.86[b]	49.80±0.2[a]	43.89±0.50[b]	20.59±1.37[a]	0.80±0.90 [b]	1.18±0.06 [a]	0.67±0.03 [b]

Values are expressed as Mean ± SEM. Means of the same row in the same column with different superscript letters differ significantly (P<0.05).

Table 3: Comparison of the level of Oxidative Stress Parameters Between the Sexes of the Malaria Patients

Sex	MDA (mmol/mg protein)x10^{-1}	SOD (U/ml)	GSH-P (U/ml)	CAT (U/ml)
	Malaria Patients	Malaria Patients	Malaria Patients	Malaria Patients
M	5.97± 1.80[a]	43.17±0.4[a]	7.15±0.25[a]	0.70±0.00[a]
F	6.05±1.86[a]	43.89±0.50[a]	0.80±0.90[b]	0.67±0.03[a]

Values are expressed as Mean ± SEM. Means of the same column with different superscript letters differ significantly (P<0.05).

Table 4: Level of Oxidative Stress Parameters According to the Age of the Malaria Patients

AGE (Years)	MDA(mmol/mg protein)x10^{-1}		SOD (U/ml)		GSH-P (U/ml)		CAT (U/ml)	
	Control	Malaria Px	Control	Malaria Px	Control	Malaria Px	Control	Malaria Px
40-49	1.40±0.05[b]	5.95±0.20[a]	49.60±0.23[b]	44.07±0.63[a]	20.56±2.02[a]	7.01±0.18[b]	1.11±0.89[a]	0.68± .30[b]
50-59	1.39±0.06[b]	6.11±0.26[a]	49.57±0.4[b]	43.18±0.87[a]	20.87±1.55[a]	7.20±0.31[b]	1.18±0.08[a]	0.60±0.20[b]
60-69	1.62±0.09[b]	5.83±0.25[a]	49.48±0.17[b]	44.09±0.71[a]	18.16±1.72[a]	7.10±0.44[b]	1.05±0.70[a]	0.75±0.30[b]
70-79	1.39±0.13[b]	6.34±0.43[a]	47.33±0.03[b]	43.53±0.40a	26.48±4.33[a]	6.50±0.37[b]	0.97±0.65[a]	0.70±0.40[b]

Values are expressed as Mean ± SEM. Means of the same row in the same column with different superscript letters differ significantly (P<0.05).

Table 5: Level of Oxidative Stress Parameters according to the Degree of Parasitaemia in the Malaria Patients

Degree of Parasitaemia	MDA (mmol/mg protein)x10^{-1}	SOD (U/ml)	GSH-P (U/ml)	CAT (U/ml)
+	5.96±0.13[a]	43.37±0.55[a]	6.88±0.28[a]	0.71±0.03[a]
++	5.61±0.23[a]	44.33±0.57[a]	6.92±0.23[a]	0.68±0.03[a]
+++	6.41±0.24[a]	43.21±0.74[a]	7.12±0.32[a]	0.66±0.03[a]

Values are expressed as Mean ± SEM. Means of the same column with different superscript letters differ significantly (P<0.05).

The observations from this study indicating an increase in oxidative stress with increasing parasitaemia is in tandem with the findings of Descamp *et al.* (1987) and Golenser *et al.* (1991) who showed from several in-vivo studies that during *P. falciparum* infection, reactive oxygen species production by phagocytic cells was strongly increased in more complicated infections than in milder cases and that during acute *P. knowlesi* infection in rhesus monkey, the OH production by monocytes was considerably elevated. Besides enhancing intra-erythrocytic oxidative stress, increase in production of reactive oxygen species has also been observed outside the parasitized erythrocyte (Descamp *et al*., 1987).

Descamp *et al*. (1987) demonstrated the capability of *P. falciparum* merozoites and soluble parasite antigen to generate reactive oxygen species from blood monocytes and neutrophils of the host. The elevated malondialdehyde (MDA) as seen in this study corroborates the findings of Imlay (2003) and James (1991) whose research revealed an elevation in MDA levels and a decrease enzymic antioxidants activities, in the serum of malaria patients. It also corroborates the conclusion reached by Nanda, *et. al.* (2004) that falciparum malaria caused oxidative stress in infected human malaria patients. They came to this conclusion while studying 61 falciparum malaria cases which included those with severe malaria complicated by renal failure; they discovered malondialdehyde levels was significantly elevated (P<0.05), especially in the patients with severe malaria. The malondialdehyde (MDA) level

increases in-vivo as the level of oxidants causing the lipid peroxidation increases in patients with malaria. In *P. falciparum*-IRBC, *P. berghei* and *P. chabaudi*-IRBC increased level of lipid peroxidation evidenced by increased levels of malondialdehyde (MDA) - a product of lipid peroxidation - have been demonstrated (Golenser *et al.*, 1991).

The reduction in the activity of the enzymic antioxidants namely superoxide dismutase (SOD), glutathione peroxidase (GSH-P) and catalase as observed in this study may be as a result of the consumption of the enzymic antioxidants as they tried to mop up the reactive oxygen species increasing in the system of the malaria subjects during the infection; and this is in keeping with the earlier results of James (1991) who demonstrated a decrease enzymic antioxidants activities, in the serum of malaria patients. This also aligns with the study carried out by Nasaria *et. al.* (2011) on patients with severe malaria where they came to the conclusion that the significantly elevated malondialdehyde levels reflect an increased oxidative stress, while the decreased levels of glutathione and superoxide dismutase points towards utilization of these antioxidants in severe malaria. The study by Pabon *et. al.* (2003) while admitting the existence of high oxidative stress in acute non-complicated *P. falciparum* infected patients as evidenced by elevated levels of MDA and reduced catalase activity in these patients, which aligns with our observations in this study, however differ from ours in not finding elevated glutathione peroxidase and superoxide dismutase activities in the patients used in their study.

This research has demonstrated that *P. falciparum* malaria causes oxidative stress in infected patients in Benin metropolis Southern Nigeria. It is therefore suggested that attention should be paid by attending physicians to the need for supplementing antimalaria treatment with antioxidants.

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