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Erythrocyte antioxidant enzymes and lipid peroxidation in patients with acute attack of *Plasmodium falciparum*

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Abstract

The host immune response to malaria involves phagocytosis as well as the production of free radicals that form part of the host defence system and also contribute to the pathology of the disease. The aim of our study is to assess oxidative stress and antioxidant status in uncomplicated *Plasmodium falciparum* infected individuals. A total of 114 subjects aged between 18 and 30 years were followed up for 16 days to fulfill the research criteria. Another 114 healthy individuals were included to the study as control subjects. Antioxidants were measured on day 1 (D1), day 4 (D4) after completion of Artemisinin-based Combination Therapy and day 16 (D16) when parasitemia is <100/µl of blood. Compared to controls, the erythrocyte malondialdehyde was significantly higher whereas superoxide dismutase and catalase were significantly lower on D1 and D4. Glutathione and glutathione peroxidase were found to be significantly less than control subjects on D1, D4 and D16. Total antioxidant status showed a significant reduction on D1 and D4. Direct correlation was obtained between MDA and parasitemia on D1 and D4, whereas inverse correlation was recorded between antoxidants and parasitemia. In conclusion, parasitemia depleted erythrocyte antioxidants and increased their susceptibility to damage.

INTRODUCTION

Reactive oxygen species (ROS) as well as reactive nitrogen species (RNS), collectively described as free radicals, are products of normal cellular metabolism. ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems [1]. Beneficial effects of ROS occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, as in defense against infectious agents and in the function of a number of cellular signaling systems. One further beneficial example of ROS at low/moderate concentrations is the induction of a mitogenic response. The harmful effect of free radicals causing potential biological damage is termed oxidative stress and

nitrosative stress. This occurs in biological systems when there is an overproduction of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other. In other words, oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of pro-oxidant/antioxidant reactions in living organisms [2].

African countries continue to bear the brunt of malaria infections, millions cases are reported annually; of these 2.7 million die each year, most of whom are children. Almost all deaths from malaria each year worldwide are attributable to *Plasmodium falciparum* [3]. Malaria has remained a major public health problem in Nigeria; children under the age of five and pregnant women are still the most affected. More than

60% outpatient visits in Nigeria is due to malaria. The disease has impacted negatively on the economy with about 132 billion Naira (approx. 0.8 billion US Dollar) lost to the disease as cost of treatment and loss in man-hours [4]. *Plasmodium falciparum* is the causative agent of the most virulent form of human malaria with up to 2.7 million deaths per year attributed to this pathogen [5]. Entry of the malaria parasite into its host red blood cell initiates the intra-erythrocytic asexual cycle, which is responsible for the clinical manifestations of malaria. It is at this stage, that 75% of the parasite's genes are transcribed [6]. Malaria parasite is capable of generating ROS within the erythrocytes and the ROS resulting from immune activation can further damage the uninfected erythrocytes [7]. Previous study also reveals that apart from the large amounts of ROS generated by the active metabolism of the growing and multiplying malaria parasite, the main source of oxidative stress is the degradation of host hemoglobin [8]. Hemoglobin digestion in the food vacuole is the main source of amino acids for parasite development, and the release of free heme (ferri/ferroprotoporphyrin IX; FP IX) on degradation promotes ROS production. Free FP IX is toxic owing both to its detergent-like properties, whereby it disrupts membrane integrity through its incorporation in the lipid bilayer, and to its ferric state (3+) bound iron, which catalyzes and undergoes redox reactions resulting in ROS production [9]. In *P.falciparum*, most heme aggregates into the insoluble crystalline FP IX dimer, known as malaria pigment or hemozoin [10]. The body defense mechanisms against free radicals are both intra- and extracellular; the intracellular defence mechanisms include the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and non enzymatic compounds such as glutathione (GSH), ubiquinol and metallothionein. Extracellular defense mechanisms include the enzyme SOD, the transition metal binding proteins ferritin, transferrin, lactoferrin, haptoglobin, ceruloplasmin, and the small molecule "sacrificial" antioxidants such as the protein thiols (glutathione and albumin), β-carotene, ubiquinol, bilirubin, urate, and the antioxidant vitamins C and E [7].

An imbalance between the oxidative stress and the cell antioxidant system may produce cell membrane damage by oxidation of its lipid matrix, particularly polyunsaturated fatty acids (PUFAs), which are the main substrates for lipid peroxidation. Lipid peroxidation is a chain reaction, initiated with attack of free radical on phospholipids or polyunsaturated fatty acid of membrane of cellular or subcellular organelle resulting in the generation of complex mixture of aldehydes, ketones and polymerization products which

react and destroy the biomolecules, enzymes and nucleic acids resulting to the destruction of erythrocytes [8]. Malondialdehyde (MDA) is the most abundant and excellent marker of lipid peroxidation among reactive aldehydes [9]. The erythrocyte membrane is prone to lipid peroxidation under oxidative stress that involves cleavage of polyunsaturated fatty acids at their double bonds leading to the formation of MDA; high level of lipid peroxidation products can be detected in cell degradation after cell injury or disease [11, 12].

There is a dearth of data on investigated cases of oxidative stress during *P.falciparum* infection on the erythrocytes. However, most of these observations were made in experimental models that were usually subjected to a lower or higher parasite load than that observed in human malaria. Therefore the present study aimed to assess the extent and genesis of oxidative stress, and to examine the relationship between parasitemia and oxidative stress, in uncomplicated *P.falciparum* infected individuals.

MATERIALS AND METHOD

Subjects

The study took place at the Malaria research clinic and laboratory, Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital (LTH) and General Hospital Asubiaro, both in Osogbo Metropolis. Osogbo lies 100 km North of Ibadan in Southwest Nigeria, where malaria is hyper-endemic [13]. Malaria transmission in Southwest Nigeria is perennial but seasonal and peaks during the rainy season, which normally runs from April to November. The study received ethical approval from Joint College of Health Sciences, Ladoke Akitola University Teaching Hospital and Osun State Hospitals Management Board ethics review committees. Out of 450 volunteers that were screened, 237 were enrolled and 114 were successfully followed up for 16 days and these were the subjects that fulfill the research criteria. To be enrolled the subjects had to be between 18-30 years old, presenting with symptoms compatible with acute uncomplicated *P.falciparum* malaria with history of fever in the preceding 24-48 h or temperature ≥37.5ºC and *P.falciparum* parasitemia >2000 asexual forms/µl of blood [14]. Patients with concomitant illnesses, sickle cell anemia or severe complicated malaria were excluded. The control group consisted of 114 healthy adult, matched for age, sex and socioeconomic status with the study group and were recruited from the general population. Informed consent was obtained from each patient and control after explaining the rationale for the study. Before enrollment, a medical history of each patient was obtained, after which the

patients were examined. Body weight, height and axiliary temperature were recorded, body mass index (BMI; weight[kg]:height[m]^2) was calculated and the blood pressure (BP) was also obtained.

Sample collection

Thick smears were prepared from finger-prick blood samples on day 1 (D1) before the commencement of Artemisinin-based Combination Therapy (ACT). ACT (co-formulated Artemether-Lumefantrine tablet that contains 20 mg artemether and 120 mg lumefantrine). Since the weights of all the patients were >35 kg, 4 tabs twice daily x 3 days were administered. To counter the threat of resistance of *P.falciparum* and to improve treatment outcomes, ACTs against malaria are now recommended [15]; this is the reason why ACTs is therapy of choice for this research.

In order to ensure compliance, administration of drugs was done by the staffs of malaria research clinic and laboratory. Another thick smear was prepared from finger-prick blood samples on days 4 and 16 (D4 and D16) after completion of medication for parasite identification and quantification. The thick blood smear was examined and parasitemia was estimated by counting asexual parasites against 200 leucocytes with the assumption that each patient had 8,000 leucocytes/µl blood [16]. At any level any subject that showed failure to ACT was automatically withdrawn from the study.

After an overnight fast, 15 mls of venous blood samples were collected at the antecubital fossa in sitting position without stasis; 10 mls into lithium heparinized bottles and 5 mls into EDTA bottles on D1, D4 and D16. All sample collection procedures were conducted with minimum light exposure, and kept cold in dark until processed in the laboratory. Plasma was obtained from heparin-treated tubes by centrifugation at 1000g for 15 min at room temperature within one hour of collection. The red blood cells portion were washed three times with equal volumes of normal saline and centrifuged at 2000g for 15 min. The washed red blood cells were then hemolyzed in distilled water (1:4, v/v) and by freezing and thawing. The hemolysate was centrifuged and the supernatant and plasma was then stored at –20ºC until they were analyzed.

Analytical methods

Hemoglobin (Hb) was measured using the method of Schoen and Solomon [17] and determination of packed cell volume (PCV) by the microhematocrit method.

Erythrocyte MDA levels were determined using the method of Draper and Hadley [18] based on the reaction of MDA with thiobarbituric acid (TBA) at 95ºC. In the TBA test reaction, MDA and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2-3 at 95ºC for 15 min. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifigation and an aliquot of supernatant was reacted with 0.67% TBA in a boiling water-bath for 15 min. After cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxypropane).

Measurement of total antioxidant status (TAS) in the plasma was performed using a commercial kit from Randox Laboratories (Crumlin, County Antrim, Northern Ireland)[19]. The assay was calibrated using 6-hydroxy-2,5,8-tetrame-thylchroman-2-carboxylic acid (trolox). The results were expressed as mmol/L of trolox equivalent.

Measurement of erythrocyte GSH-Px (EC 1.11.1.9) activity was performed using the commercial kit RANSEL (Randox Laboratories Ltd, Diamond Road, Crumlin, County Antrim, Northern Ireland). GSH-Px catalyses the oxidation of GSH to glutathione disulphide (GSSG) by cumene hydroperoxide; in the presence of GR and NADPH, GSSG is immediately converted to GSH with a concomitant oxidation of NADPH to NADP$^+$ according to the method of Paglia and Valentine [20]. The concentration of GSH-Px activity is assessed from the decrease in absorption at 340 nm and at 37ºC using Humalyzer 2000 analyzer (Human Diagnostics Worldwide, Wiesbaden, Germany). A standard curve was prepared by using the standard provided in the kit, and the value for each sample was read from this curve.

SOD (EC 1.15.1.1) activity was estimated by employing the xanthine/xanthine oxidase assay commercial kit RANSOD (Randox Laboratories, Northern Ireland) [21]. The results of SOD activity were normalized to the hemoglobin content in the erythrocyte lysate and expressed as U/gHb.

The CAT (EC 1.11.1.6) peroxidative activity was measured by the reaction of formaldehyde produced from methanol with purpald to produce a chromophore according to the method of Johansson and Hakan Borg [22]. Quantitation was carried out by measuring the absorbance at 540 nm and comparing the results with those obtained with formaldehyde calibrators.

GSH concentration in erythrocytes was determined in the presence of low-molecular-mass free thiol groups, mercuric salt and sulfanilamide, in a highly acidic medium, biazonian salt was produced. The salt so obtained was conjugated with amine salt, producing a coloured complex, the absorbance of which was measured at 535 nm and calculations were made according to the model curve for GSH and expressed in µmoles GSH/gHb [23].

Statistical analysis

All values were expressed as the mean ± standard deviation (SD). Data was analyzed using one-way ANOVA followed by the post-hoc Duncan multiple range test for analysis of bio chemical data using SPSS version 11 (SPSS Inc Chicago, IIinois, USA). Method of correlation analysis (Pearson) was also used to determine the degree of association between variable of interest.Values were considered statistically significant at $p < 0.05$.

RESULTS

All the studied subjects and the controls were non-obese and normotensive. The anthropometric measurements and physical clinical parameters of the subjects and the controls at various days were depicted in Table 1. Table 2 shows the entire numeric outcome for the measured parameters.

The mean body temperature of 38.9 ± 0.7ºC for the subjects on D1 was significantly higher ($p < 0.05$) compared to 35.9 ± 0.4ºC for the controls. The PCV of $27.6 \pm 1.9\%$ for untreated subjects on D1 and $26.9 \pm 1.3\%$ for D4 at the end of ACT treatment were both significantly less ($p < 0.05$) than the controls. Likewise, the Hb concentration of 94.35 ± 4.7 g/L for D1 and 86.93 ± 3.5 g/L for D4 for the subjects were significantly lower ($p < 0.01$) compared to the controls.

On the other hand, the level of parasitemia of 32019.8 ± 390.7/µl of blood and 2156.9 ± 79.3/µl of blood for the subjects on D1 and D4, respectively, were significantly higher ($p < 0.001$) compared to the controls. However, in the present study we recorded 69.6 ± 2.9/µl of blood level of parasitemia for controls, this scanty parasitemia was detected on the blood smear of <4% of the controls; we included them on our study because they were healthy individual and it is not possible for every healthy individual to be blood smear free of malaria parasite because we live in malaria endemic region of the world.

Table 1. Anthropometric and clinical parameters (mean±SD) of the controls and subjects on various days

| | Controls (n=114) | Subjects (n=114) | | | | | |
		Day 1	p value	Day 4	p value	Day 16	p value
Age (years)	25.7 ± 2.5	26.1 ± 3.1	ns	26.1 ± 3.1	ns	26.1 ± 3.1	ns
Height (cm)	171.2 ± 2.1	170.3 ± 1.2	ns	170.3 ± 1.2	ns	170.3 ± 1.2	ns
Weight (kg)	70.5 ± 1.4	69.4 ± 1.7	ns	70.1 ± 1.1	ns	70.4 ± 1.5	ns
BMI (kg/m^2)	24.1 ± 0.3	23.9 ± 0.5	ns	24.2 ± 0.2	ns	24.3 ± 0.1	ns
Systolic BP (mmHg)	109.9 ± 2.5	110.8 ± 3.8	ns	117.2 ± 2.7	ns	112.2 ± 4.1	ns
Diastolic BP (mmHg)	77.8 ± 1.7	79.3 ± 2.1	ns	78.7 ± 1.9	ns	76.5 ± 2.4	ns

BP, blood pressure; ns, not significant.

Table 2. Detailed numeric outcome for all measured parameters

| | Controls (n=114) | Subjects (n=114) | | | | | |
		Day 1	p	Day 4	p	Day 16	p
Body Temperature (ºC)	35.9 ± 0.4	38.9 ± 0.7	0.023*	37.9 ± 0.5	0.122	36.4 ± 0.2	0.941
PCV (hematocrit) (%)	39.2 ± 2.1	27.6 ± 1.9	0.017*	26.9 ± 1.3	0.012*	38.1 ± 2.7	0.310
Hemoglobin (g/L)	141.48 ± 6.7	94.35 ± 4.7	0.005**	86.93 ± 5.3	0.005**	119.47 ± 4.1	0.061
Parasitemia (/µl of blood)	69.6 ± 2.9	32020 ± 390.7	<0.001***	2156.9 ± 79.3	<0.001***	75.3 ± 7.4	0.108
Erythrocyte MDA (nmol/ml)	2.51 ± 0.39	4.19 ± 0.42	0.005**	3.97 ± 0.35	0.025*	2.97 ± 0.35	0.164
Plasma TAS (mmol/L)	1.84 ± 0.26	1.19 ± 0.13	0.022*	1.58 ± 0.09	0.043*	1.71 ± 0.07	0.109
Erythrocyte GSH-Px (U/gHb)	36.56 ± 5.13	21.13 ± 3.78	0.009**	20.47 ± 3.19	0.009**	27.49 ± 3.13	0.032*
Erythrocyte SOD (U/gHb)	904.6 ± 81.3	874.8 ± 72.6	0.039*	618.1 ± 43.3	0.007**	894.6 ± 61.9	0.107
Erythrocyte GSH (µmol/gHb)	5.10 ± 0.8	4.17 ± 0.5	0.047*	3.29 ± 0.6	0.009**	4.31 ± 0.7	0.045*
Erythrocyte CAT (U/gHb)	1416.1 ± 79.2	1370.5 ± 49.6	0.041*	997.9 ± 45.3	0.018*	1401.1 ± 84.3	0.117

Significance level at ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$.

Table 3. Correlation analysis between level of parasitemia and markers of oxidative stress

	Parasitemia					
	D1		D4		D16	
	r value	p value	r value	p value	r value	p value
MDA	+0.5314	0.0008	+0.4721	0.0013	+0.1217	0.1207
TAS	−0.3165	0.0347	−0.2993	0.0371	−0.1471	0.1069
GSH-Px	−0.4579	0.0056	−0.4385	0.0072	−0.2152	0.0451
SOD	−0.2983	0.0407	−0.3758	0.0098	−0.1775	0.1008
GSH	−0.3056	0.0311	−0.4747	0.0059	−0.1948	0.0815
CAT	−0.3361	0.0287	−0.2952	0.0439	−0.1836	0.0978

r, correlation coefficient

Table 4. Correlation analysis between lipid preoxidation level and hematological indices

	MDA					
	D1		D4		D16	
	r value	p value	r value	p value	r value	p value
Hemoglobin	−0.4851	0.0074	−0.4382	0.0051	−0.1057	−0.1843
Hematocrit	−0.2673	0.0418	−0.3015	0.0401	−0.1192	−0.1407

r, correlation coefficient

The mean plasma TAS of 1.19 ± 0.13 mmol/L on D1 and 1.58 ± 0.09 mmol/L on D4 for the subjects were significantly decreased ($p < 0.05$) compared to 1.84 ± 0.26 mmol/L for the controls. Erythrocyte MDA concentration of 4.19 ± 0.42 nmol/gHb for subjects on D1 was significantly increased ($p < 0.01$) and 3.97 ± 0.35 nmol/gHb on D4 was also significantly increased ($p < 0.05$) when both were compared to 2.51 ± 0.39 nmol/ml for the controls. Contrarily, erythrocyte antioxidant GSH-Px activity of 21.13 ± 3.78 U/gHb for the subjects on D1 and 20.47 ± 3.19 U/gHb on D4 were significantly decreased ($p < 0.01$) when compared to 36.56 ± 5.13 U/gHb for the controls; GSH-Px activity of 27.49 ± 3.13 U/gHb for D16 also show a significant reduction ($p < 0.05$) compared to the controls. Similarly, erythrocytes SOD activity of 874.82 ± 72.63 U/gHb for subjects on D1 was significantly reduced ($p < 0.05$) compared to 904.58 ± 81.27 U/gHb for the controls and 618.13 ± 43.28 U/gHg for D4 was also significantly reduced ($p < 0.01$) compared to the controls. In a similar trend, CAT activity of 1370.51 ± 49.6 U/gHb for subjects on D1 and 997.93 ± 45.31 U/gHb for D4 were significantly reduced ($p < 0.05$) compared to 1416.12 ± 79.2 U/gHb for the controls. Erythrocyte GSH concentration of 4.17 ± 0.5 μmol/gHb for subjects on D1 and 4.31 ± 0.7 μmol/gHb for D16 were significantly decreased ($p < 0.05$) compared to 5.1 ± 0.8 μmol/gHb for the controls; GSH concentration of 3.29 ± 0.6 μmol/gHb for subjects on D4 also show a significant reduction ($p < 0.01$) compared with the controls.

Table 3 shows the correlation coefficient analysis between the levels of parasitemia and the markers of oxidative stress of the subjects on D1, D4 and D16. A strong positive correlation was obtained between the level of parasitemia and MDA on D1($r = +0.5314$; $p = 0.0008$) and ($r = + 0.4721$; $p = 0.0013$) for D4. However, a moderate inverse correlation was obtained between level of parasitemia and TAS on D1 ($r = -0.3165$; $p = 0.0347$) and a weak inverse correlation on D4 ($r = -0.2993$; $p = 0.0439$). A strong inverse correlation was recorded between level of parasitemia and GSH-Px activity on D1 ($r = -0.4579$; $p = 0.0056$) and ($r = -0.4385$; $p = 0.0072$) for D4; but a weak inverse correlation on D16 ($r = -0.2152$; $p = 0.0451$). Similarly, a weak inverse correlation was obtained between level of parasitemia and SOD activity on D1 ($r = -0.2983$; $p = 0.0371$), but a moderate inverse correlation was recorded for D4 ($r = -0.3758$, $p = 0.0098$). Moderate inverse correlation was recorded between the level of parasitemia and GSH on D1 ($r = -0.3056$; $p = 0.0311$); however, a strong inverse correlation on D4 ($r = -0.4747$; $p = 0.0059$). Similarly, a moderate inverse correlation was recorded between the level of parasitemia and CAT activity on D1 ($r = -0.3361$; $p = 0.0287$), but a weak inverse correlation on D4 ($r = -0.2952$; $p = 0.0407$).

Table 4 shows the correlation coefficient analysis between lipid peroxidation and hemotogical indices; an inverse correlation was recorded between MDA and Hb on D1 ($r = -0.4851$, $p = 0.0074$) and D4 ($r = -0.4382$, $p = 0.0051$. An inverse correlation was also obtained between MDA and PCV on D1 ($r = -0.2673$, $p = 0.0418$) and D4 ($r = -0.3015$, $p = 0.0401$).

Figure 1 shows the comparison of the measured parameters between D1 and D4 for the subjects with

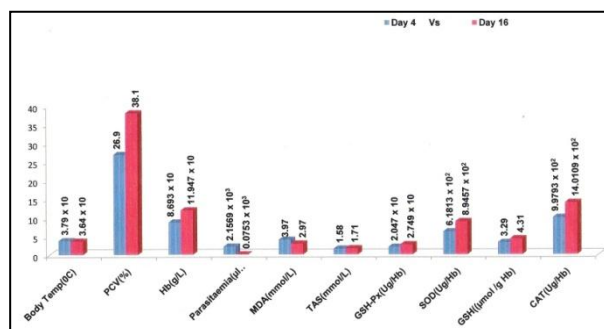acute malaria; Hb, MDA and GSH represented significant difference at $p < 0.05$, SOD and CAT at $p < 0.01$ and parasitemia at $p < 0.001$. Figure 2 shows the comparison of the measured parameters between D1 and D16 for the subjects with acute malaria; Hb, TAS and GSH-Px represented significant difference at $p < 0.05$; MDA at $p < 0.01$ and parasitemia at $p < 0.001$. Figure 3 shows the comparison of the measured parameters between D1 and D16 for the subjects with acute malaria; PCV, MDA and GSH represented significant difference at $p < 0.05$; Hb, SOD and CAT at $p < 0.01$ and parasitemia at $p < 0.001$.
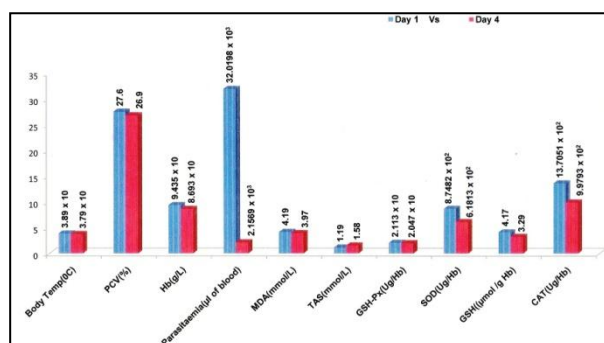
Figure 1. Changes in measured parameters in subjects with acute malaria on D1 vs D4. Hb, MDA and GSH showed significant difference at $p < 0.05$; SOD and CAT at $p < 0.01$ and parasitaemia at $p < 0.001$.
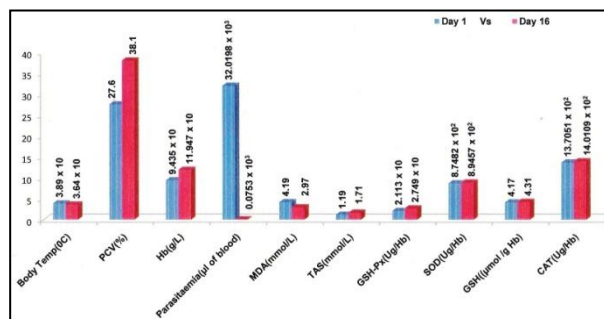
Figure 2. Changes in measured parameters in subjects with acute malaria on D1 vs D16. Hb, TAS and GSH-Px showed significant difference at $p < 0.05$; MDA at $p < 0.01$ and parasitaemia at $p < 0.001$.

Figure 3. Changes in measured parameters in subjects with acute malaria on D4 *vs* D16. PCV, MDA and GSH showed significant difference at $p < 0.05$; Hb, SOD and CAT at $p < 0.01$ and parasitaemia at $p < 0.001$.

DISCUSSION

In the present study we observed a significant reduction in hemoglobin concentration in all untreated *P.falciparum* infected patients as compared to the controls; significant reduction was also obtained on D4 but on D16 when the level of parasitemia is returning to normal no significant value was recorded. Hemoglobin serve as the major source of amino acids for *Plasmodium*, but its degradation in an acidic food vacuole results in the production of toxic FP IX and ROS [9].

PCV followed the same trend with significant reduction in untreated subjects and after treatment. In acute *P.falciparium* infection, the parasitized RBC is being removed by the reticuloendothelial system (RES), the body response to the reduced RBC by stimulating erythropoesis; the normal bone marrow is capable of increasing its RBC production about three to five times of the normal level within a week or two following maximal stimulations [24]. In addition, it is impossible that all circulatory RBCs were parasitized during acute infection and this may be one of the reason why we have a reduced PCV that is not proportional to the level of parasitemia recorded before and after treatment. A similar observation of lower hematological values in malaria patients was reported in a study conducted by Kulkarni *et al* [25].

We recorded an increased plasma concentration of MDA (marker of lipid peroxidation) among subjects on D1 before the commencement of ACT and on D4 after the completion of ACT but returned to normal in D16 of the study. The significant increase of MDA in malaria patients recorded in this study reflects increased peroxidation of membrane lipids of the infected erythrocytes. The inverse correlation between the level of hemoglobin and MDA on D1 and D4 is another pointer to hemolytic indices related to membrane lipid peroxidation .This finding is similar to the study conducted by Das *et al* [26] on evidence for erythrocyte lipid peroxidation in acute falciparum malaria.

The erythrocyte antioxidant enzymes SOD, GSH-Px, GR and CAT among other were the main endogenous enzymatic defence systems of all aerobic cells [7]. In the present study with parasitized erythrocytes by *P.falciparum*, we recorded a significant decrease in these enzymes activities, we also recorded an inverse correlation between the antioxidant enzymes' activities and the level of parasitemia. The presence of SOD in various compartments of our body enables it to dismutate superoxide radicals immediately they are produced and protects the cells from oxidative damage. Reduction in SOD activity observed in this study may be due to an increased endogenous production of ROS

as evidenced by increased MDA. Since malarial parasites derive their amino acids from the parasitized cells which cannot be replenished by red blood cells due to lack of protein synthesis ability, this might be one of the reasons behind overall decrease in erythrocyte SOD activity in malaria patients as compared to the healthy individuals. Our finding was in agreement with the study of Kulkarni *et al* [25] in their study on biochemical changes with special reference to oxidants and antioxidants in malaria patients. CAT activity is one of the most important mechanisms by which RBC dispose of H_2O_2 produced by dismutation reaction of O_2 in a cell. We recorded a significant reduction in the activity of CAT in the present study in subjects infected with *P.falciparium* in D1 and on completion of anti-malaria therapy in D4. This finding is in agreement with previous finding of Ittarat *et al* [27] on their study on the effect of dihydroartemisinin on the antioxidant capacity of *P.falciparum*-infected erythrocytes. On D16 when level of parasitemia of subjects were returning to normal with significant increase in unparasitized cells, no significant difference was observed in SOD activity compared to the controls; CAT activity follows the same trend. GSH-Px neutralizes hydrogen peroxide by taking hydrogen from two GSH molecules resulting in two H_2O and one GSSG. The enzyme GSH-Px then regenerates GSH from GSSG with NADPH as a source of hydrogen. In the present study GSH and the activity of GSH-Px were significantly reduced in subjects infected with *P.falciparium* on D1 and D4. However, a significant reduction was also recorded on D16 for GSH-Px. Our observation was in agreement with the study of Sohail *et al* [28] who reported that increased parasitemia accompanied decrease in the activities of enzymes of the GSH system, namely GSH-Px, GR and GST in the RBC lysates. Lower GSH values in this study can be explained by a drastic fall in GR activity in the face of significant decrease of GSH-Px. Decreased activity of GR hampers regeneration of GSH from GSSG leading to lower GSH concentration. In a similar study conducted by Das *et al* [26], a significant reduction in erythrocytes' GSH and GSH-Px activity in untreated patients with acute malaria was reported.

In patients with falciparum malaria, ROS appear to be generated both within the parasitized erythrocytes and in host phagocytes; they can cause damage to malaria parasites as well as to 'innocent bystanders' such as non-parasitized erythrocytes. Plasma is in close proximity to the phagocytes and therefore bears the footprints of phagocyte-generated ROS; this also explained why we recorded low level of plasma TAS concentration among the subjects on D1 and D4 of this study.

When we compared measured parameters of untreated subjects in D1 with that of D4 immediately after treatment we still recorded reduction in erythrocytes antioxidant activities. The level of parasitemia on D4 was significantly reduced compared to D1 which can be attributed to ACT administered to the subjects to clear the parasitized cells. The decline in plasma total lipid peroxides was observed on D4 compared to pre-treatment in malaria patients which might be due to reduction in ROS generation by malarial parasite due to parasitic clearance. Since the mechanism of action of ACT in the clearance of malaria parasites is by iron-mediated cleavage of the peroxide bridge and generation of organic free radicals [29], which may further have negative effect on the erythrocytes antioxidant enzymes activities immediately after treatment. At D16 after antimalarial treatment, the levels of all biochemical parameters were restoring back to normal levels.

In conclusion, it become obvious that with increase loads of parasite, the erythrocyte antioxidants are being depleted, the increased vulnerability of erythrocytes to damage and decreased antioxidant system emphasizes the need for early treatment of *P.falciparum*-infected patients to minimize the red cell destruction and subsequent sequel. Indeed, a number of currently used drugs, especially the endoperoxide antimalarials, that may increase oxidant stress may be discouraged, and novel drugs such as peroxidic compounds should be further studied in order to reduce the morbidity related to *P.falciparium* infection as a result of free radicals production.

REFERENCES

1. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006; 160:1-40.

2. Ridnour LA, Isenberg JS, Espey MG, Thomas DD, Roberts DD, Wink DA. Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1. Proc Natl Acad Sci USA 2005; 102:13147-52.

3. Darwin K. Malaria at a glance. In: Das BS, Nanda NK (eds) Research Directions in Malaria, Wellcome Trust, London, UK, pp 2-3, 2002.

4. National Guidelines for Diagnosis and Treatment of Malaria. Federal Ministry of Health, National Malaria and Vector Control Division, Abuja, Nigeria, March 2011.

5. Breman JG. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. Am J Trop Med Hyg 2001; 64:1-11.

6. Bozdech Z, Llinas M, Pulliam BL, Wong ED, Zhu J, DeRisi JL. The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. PLoS Biol 2003;1:E5.

7. Rath RN, Panigrahi N, Das BK, Das PK. Lipid peroxidation in acute falciparum malaria. Ind J Med Res 1991; 93:303-5.

8. Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H. Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. Int J Parasitol 2004; 34:163-89.

9. Muller S. Redox and antioxidant systems of the malaria parasite *Plasmodium falciparum*. Mol Microbiol 2004; 53:1291-305.

10. Egan TJ, Combrinck JM, Egan J, Hearne GR, Marques HM, Ntenteni S, Sewell BT, Smith PJ, Taylor D, van Schalkwyk DA, Walden JC. Fate of haemiron in the malaria parasite *Plasmodium falciparum*. Biochem J 2002; 365:343-7.

11. Pandey KB, Mishra N, Rizvi SI. Protective role of myricetin on markers of oxidative stress in human erythrocytes subjected to oxidative stress. Nat Prod Commun 2009; 4:221-6.

12. Kingsley M, Cunningham D, Mason L, Kilduff LP, McEneny J. Role of creatine supplementation on exercise-induced cardiovascular function and oxidative stress. Oxid Med Cell Longev 2009; 2:247-54.

13. Salako LA, Ajayi FO, Sowunmi A, Walker O. Malaria in Nigeria: a revisit. Ann Trop Med Parasitol 1990; 84:435-45.

14. Severe and complicated malaria. World Health Organization, Division of Control of Tropical Diseases. Trans R Soc Trop Med Hyg 1990; 84(Suppl 2):1-65.

15. World Health Organization. Guidelines for the treatment of malaria, WHO Press, Geneva, Switzerland, p 266, 2006.

16. White NJ. The treatment of malaria. N Engl J Med 1996; 335:800-6.

17. Schoen I, Solomon M. Control of blood haemoglobin determination by a simple effective method. J Clin Pathol1962; 15:44-5.

18. Draper H, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; 186:421-31.

19. McLemore JL, Beeley P, Thorton K, Morrisroe K, Blackwell W, Dasgupta A. Rapid automated determination of lipid hydroperoxide concentrations and total antioxidant status of serum samples from patients infected with HIV. Am J Clin Pathol 1998; 109:268-73.

20. Paglia DE, Valentine UN. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70:158-69.

21. Woollians JA, Wiener G, Anderson PH, McMurray CH. Variation in the activities of glutathione peroxidise and superoxide dismutase and in the concentration of copper in the blood in various breed crosses of sheep. Res Vet Sci 1983; 34:253-56.

22. Johansson LH, Borg LA. A spectrophotometric method fordetermination of catalase activity in small tissue samples. Anal Biochem 1988; 174:331-6.

23. Saville B. A scheme for the colorimetric determination of microgram amounts of thiols. Analyst 1958; 33:670-2.

24. Bunn HF. Hematologic alterations. In: Petersdorf RG, Adams RD, Braunwald E, Isselbacher KJ, Martin JB, Wilson JD (eds) Harrison's Principle of Internal Medicine, 10th edition, McGraw Hill, New York, NY, USA, pp 282-283, 1983.

25. Kulkarni AG., Suryakar AN, Sardeshmukh AS, Rathi DB. Studies on biochemical changes with special reference to oxidant and antioxidants in malaria patients. Indian J Clin Biochem 2003; 18:136-49.

26. Das BS, Nanda NK. Evidence for erythrocyte lipid peroxidation in acute falciparum malaria. Trans Roy Soc Trop Med Hyg 1999; 93:58-62.

27. Ittarat W, Sreepian A, Srisarin A, Pathepchotivong K. Effect of dihydroartemisinin on the antioxidant capacity of *P.falciparum*-infected erythrocytes. Southeast Asian J Trop Med Public Health 2003; 34:744-50.

28. Sohail M, Kaul A, Raziuddin M, Adak T. Decreased glutathione S-transferase activity: Diagnostic and protective role in vivax malaria. Clin Biochem 2007; 40:377-82.

29. Meshnick SR, Thomas A, Ran A, Xy CM, Pan HZ. Artemisinin (qinghaosu): role of intracellular hem in its mechanism of antimalarial action. Mol Biochem Parasitol 1999; 49:181-9.