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Research Article
Erythrocyte Lipid and Antioxidant Changes in *Plasmodium falciparum*-infected Children Attending Mother and Child Hospital in Akure, Nigeria

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Abstract

Background and Objective: Understanding the molecular and cellular pathways activated in response to *Plasmodium falciparum* infection is crucial for the development of pharmacological intervention to malaria. The present study was designed to evaluate the lipid components and the oxidative status of erythrocyte obtained from children under 5 years infected with *Plasmodium falciparum*. **Materials and Methods:** Parasitemia was assessed prior and after treatment with antimalarial, erythrocyte lipid profile, levels of lipid peroxidation and antioxidant status (reduced glutathione, GSH; superoxide dismutase, SOD; catalase and glutathione peroxidase, GPx) were measured. **Results:** Results obtained showed that in Plasmodium infected erythrocyte, the total phospholipids, cholesterol and LDL-cholesterol concentrations were significantly elevated beyond the normal level. In addition, an upsurge in erythrocyte oxidant (lipid peroxide) status with a concomitant downregulation of the antioxidant status (GSH concentration and SOD, catalase and GPx activities) was observed in *P. falciparum*-infected children. However, following a three-day treatment with artemisinin combination drugs, there was a significant reduction in erythrocyte phospholipids, total cholesterol and LDL-cholesterol concentration as well as lipid peroxide levels. Significant augmentation in erythrocyte antioxidant status (GSH, SOD, catalase and GPx) were also observed after treatment with antimalarials. **Conclusion:** This study demonstrated that erythrocyte lipids and oxidative status are usually altered in *Plasmodium falciparum*-infected children. Thus, monitoring erythrocyte lipid profile and oxidative status could offer a viable diagnostic strategy in early detection of malaria in children.

Key words: Infected erythrocyte, membrane lipid, oxidative stress, *Plasmodium falciparum*, malaria

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Changes in erythrocyte lipid composition and the host oxidant-antioxidant status are two critical associated events involved in the pathogenesis of malaria[1]. However, the overriding mechanisms involved in lipid-antioxidant dynamics in malaria have not been fully elucidated. Within the erythrocytes in the human host, the malaria parasite undergoes cyclical development. During the intraerythrocytic stage of the parasite development, about 5-10 merozoites are produced every 24 h. A corresponding increase in metabolic activity with concomitantly membrane turn-over rate is required to sustain the growing parasite at this stage. Labaied *et al.*[2] have shown that *Plasmodium falciparum* infection induces a six-fold increase erythrocyte phospholipid content. In order to meet up with its high requirement for phospholipids, the infected erythrocytes contain phospholipid synthesizing enzymes[3]. Thus, potent inhibitors of plasmodial phospholipid synthesis was previously characterized as potential target for antimalarial chemotherapy due to its crucial role to the parasite survival[4].

It is not fully understood why children of African descent develop severe malaria neither has there been justifiable reasons for the high mortality rate among African children who develop malaria[5]. Therefore, the elucidation of genetic, biochemical and immunologic factors the pathogenesis of severe and fatal malaria is a key objective. Several scientific justifications have been advanced to implicate changes in redox metabolism in the process. First, oxidative changes are involved in the host response to malaria. Therefore, plasma lipid peroxides are usually elevated in malaria infected children, especially in riboflavin-deficient children[6,7]. It has also been established that lipid peroxidation is also increased with a corresponding decrease in antioxidant status in infected erythrocytes compared to non-infected control[6]. The involvement of oxygen radicals in the parasite clearance in both mice and humans have been reported[8,9]. Drugs, such as; paracetamol which decrease the production of oxygen radical have been shown to delay parasite clearance[10]. Mice with NADPH oxidase mutant gene unable to produce superoxide radical were reported to demonstrate a high susceptibility and severity to malarial parasite infection compared to their wild type counterpart[11]. Taken together, these observations among other suggested that derangement in erythrocyte ROS generation might exacerbate infection.

The existence of oxidative stress and changes in erythrocyte lipid profile during acute malarial infection have been demonstrated in some studies. It is hypothesized that the developing malarial parasites and the associated increase in metabolic activities within the erythrocytes coupled with the immunologic induction of ROS production by the host in response to malarial infection could possibly account for the changes in the erythrocyte lipid profile and the host antioxidant status[12,13]. This study was undertaken to evaluate the changes in erythrocyte lipids and the host oxidant/antioxidant status in *Plasmodium falciparum* infected children.

MATERIALS AND METHODS

Ethical approval: The study protocol was approved by the Mother and Child Hospital Akure (MCHA), Research and Ethics Committee and the Joseph Ayo Babalola University, Research and Ethics Committee (JABUHE002/2015) and was carried out in compliance with the tenets of the Helsinki Declaration.

Subjects: The study population consisted of children aged 0-5 years admitted in the acute care unit of the MCHA between April and June, 2015. The MCHA serves as a WHO accredited tertiary care referral center for pregnant women, nursing mothers and children under 5 years for Ondo state and neighbouring states like Ekiti, Edo, Osun and Kogi (all in Nigeria). A finger-prick blood sample was obtained from each subject to prepare thick and thin blood films used to determine the presence or absence of malaria parasites and the level of parasitemia. Subjects with a primary clinical diagnosis and positive peripheral blood smear for *Plasmodium falciparum* were enrolled into the study after a written signed informed consent form was obtained from respective parents or guardians as appropriate. The consent form was written in both English and Yoruba languages and read to the parents and guardians before appending their signatures or thumb prints. A signed or thumb printed copy of the consent form was given to each parent or guardian.

Subjects with one or more complications of severe malaria (prostration, coma or respiratory distress) as defined by Marsh *et al.*[14] were grouped under complicated malaria subjects while those with acute malaria and severe anaemia (haemoglobin <5 g dL^{-1}) devoid of other complications of complicated malaria were group as acute malaria subjects. The non-malaria control subjects consisted of asymptomatic children with no demonstrated *Plasmodium falciparum* in their peripheral blood smear. Prior to obtaining venous blood sample from each subject, a brief clinical history was taken to ascertain their health status. In addition, data on sex, age, weight and height were also obtained.

Exclusion criteria: The following categories of subjects were excluded in the study, those known to have received blood transfusion within 3 months prior to the study, subjects on any known antimalarial drug or on antioxidant or iron supplementation therapy.

Blood collection and plasma preparation: Venous blood sample (5 mL) was collected by venipuncture on two occasions (prior to the administration of any antimalarial drug at the time of hospitalization and after 3 days of antimalaria treatment) into lithium heparin tubes and centrifuged at 5000 rpm for 10 min to obtain plasma. The plasma was stored at -4°C until required for further analysis.

Assessment of blood parasitemia: Giemsa-stained thick and thin blood films obtained from each subject were analyzed to determine the number of parasites per 200 white blood cells. Slides were considered negative if no parasites were seen in 100 fields on the thick film.

Erythrocyte membrane preparation: Erythrocyte membrane 'ghosts' were prepared within 12 h of plasma separation, essentially as described by Burton *et al.*[15].

Biochemical analysis: Erythrocyte phospholipids content was estimated by using the method described by Stewart[16]. Triglyceride concentration was determined following the protocol described by Carr *et al.*[17]. Total cholesterol concentration was estimated according to the method of Allain *et al.*[18] and HDL-cholesterol according to Warmick *et al.*[19]. LDL-cholesterol was evaluated by using Friedewald's equation[20]. Lipid peroxidation was determined by the method described by Buege and Aust[21]. Superoxide dismutase (SOD) activity was determined following the method of McCord and Fridovich[22]. Catalase (CAT) and glutathione peroxidase activities were assayed according to the methods described by Aebi[23] and Flohé and Günzler[24], respectively. Reduced glutathione (GSH) level was estimated according to the method of Moron *et al.*[25].

Statistical analysis: The data obtained are presented as mean±SEM of 25 determinations. The mean values of control and test groups were compared by using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) performed by using GraphPad Prism software (version 6.05). The $p<0.05$ was considered to be significant.

RESULTS

Subject's characteristics: About 75 children categorized either as normal (25), acute (25) and complicated (25) were enrolled in the study. Subjects with complicated *Plasmodium falciparum* malaria were admitted placed on admission while those with acute malaria were asked to return after 3 days of antimalaria treatment. About 80 and 92% for complicated and normal subjects, respectively returned at day 4 for follow up. Characteristics of the subjects at baseline, including sex, age, weight and height were as shown in Table 1. At enrollment, subjects with acute and complicated malaria had significantly ($p<0.5$) lower body weight and height compared with normal subjects. Mean parasitemia before and after treatment in were significantly ($p<0.05$) higher in subjects with complicated malaria compared with non-complicated malaria subjects (Fig. 1).

Erythrocyte lipid profile: Prior to treatment, erythrocyte total phospholipid concentration was significantly ($p<0.01$) higher in complicated subjects compared to non-complicated subjects (Fig. 2a). However, after treatment with antimalarials, significant ($p<0.05$) decrease in erythrocyte total phospholipid content in subjects with complicated malaria compared to the

Table 1: Subjects baseline characteristics

Characteristics	Normal	Acute	Complicated
Male (%)	58.0	52.00	56.00
Female (%)	42.0	48.00	44.00
Age (months)[α]	36.0±5.3	38.00±5.0	42.00±7.1
Weight (kg)[α]	14.8±3.9	13.61±3.3*	12.73±2.5*
Height (cm)[α]	104.0±38.2	91.40±25.6*	76.20±20.3*

[α]Values are mean±SEM of 25 determinations. *Significantly different from normal ($p<0.05$)

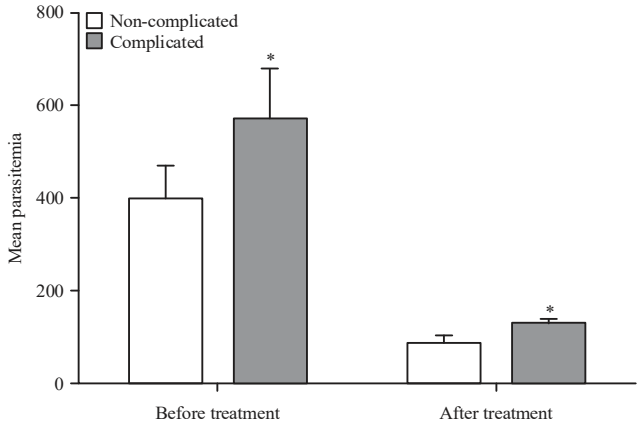

Fig. 1: Mean parasitemia level at baseline
Values are mean±SEM of 25 determinations. *Significantly different from non-complicated ($p<0.05$)

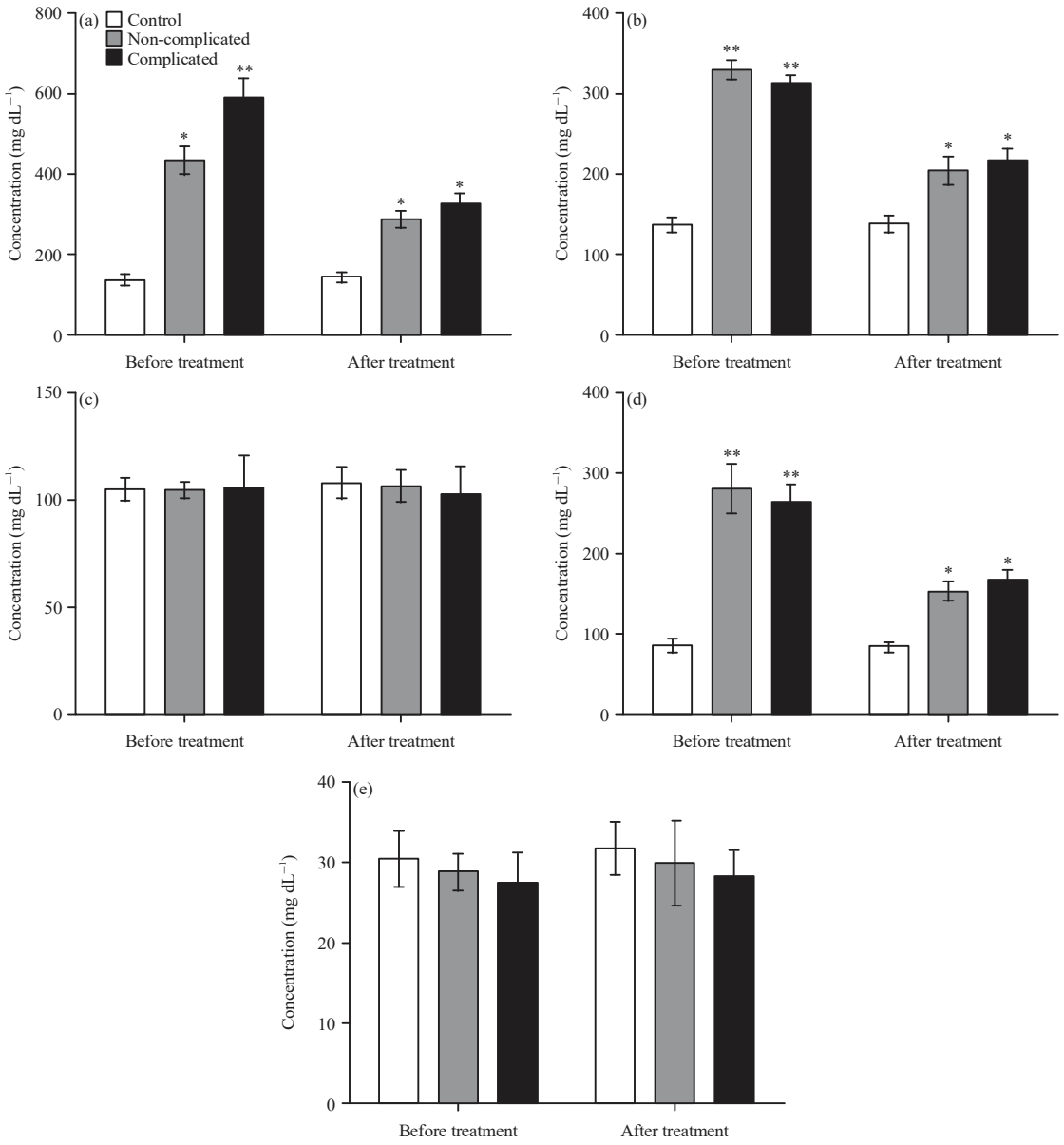

Fig. 2(a-e): (a) Erythrocyte total phospholipids, (b) Total cholesterol, (c) Triglycerides, (d) Low-density lipoprotein (LDL)-cholesterol and (e) High-density lipoprotein (HDL)-cholesterol concentrations in *Plasmodium falciparum* infected children before and after treatment with antimalarial drugs

Results are mean±SEM of 25 determinations. *Significantly different from normal ($p<0.05$). **Significantly different from normal ($p<0.01$)

baseline level. Baseline total cholesterol concentration in both non-complicated and complicated malaria subjects was significantly ($p<0.01$) higher compared to non-malaria control subjects (Fig. 2b). Significant decreases were observed in erythrocyte total cholesterol concentration in both non-complicated and complicated subjects compared to their respective baseline levels by following treatment with antimalaria drug (Fig. 2b). Erythrocyte triglycerides concentration was not significantly different across the different subject groups before and after treatment (Fig. 2c). LDL-cholesterol concentration in both non-complicated and complicated malaria subjects was significantly ($p<0.01$) higher compared to non-malaria control subjects before treatment. After treatment with antimalarials, erythrocyte LDL-cholesterol concentration in both non-complicated and complicated subjects showed significant decrease ($p<0.05$) compared to their respective baseline levels (Fig. 2d). HDL-cholesterol (Fig. 2e) was not significantly different in non-complicated, complicated and non-malaria control subjects before and after treatment.

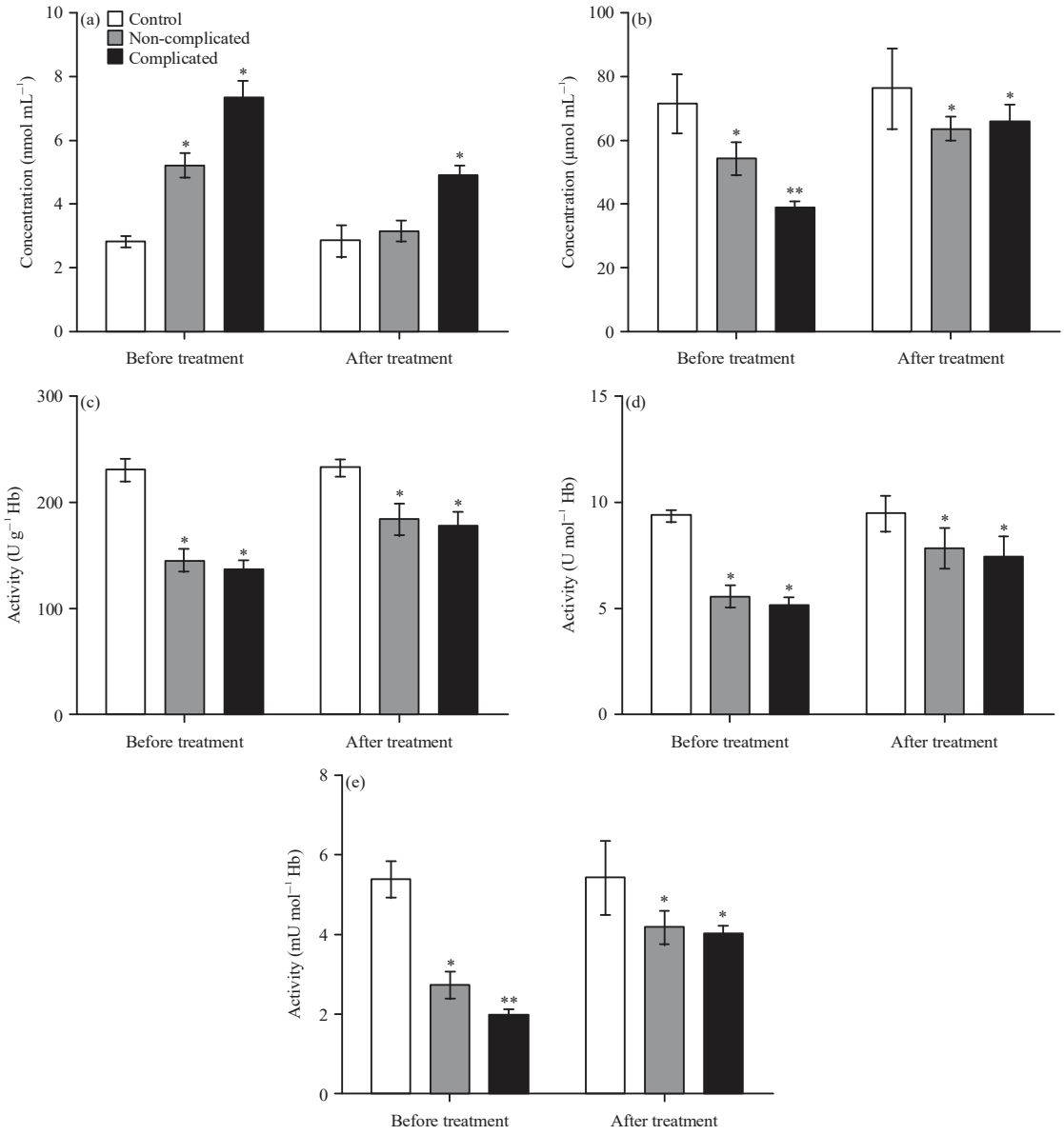

Fig. 3(a-e): (a) Erythrocyte lipid peroxide level, (b) Reduced glutathione concentration, (c) Superoxide dismutase, (d) Catalase and (e) Glutathione peroxidase activities in *Plasmodium falciparum* infected children before and after treatment with antimalarial drugs

Results are mean±SEM of 25 determinations. *Significantly different from normal ($p<0.05$). **Significantly different from normal ($p<0.01$)

Antioxidant status: Baseline erythrocyte lipid peroxide concentration was significantly higher in subjects with non-complicated ($p<0.05$) and complicated ($p<0.01$) subjects compared with non-malaria control subjects (Fig. 3a). However, after 3 days of medication, erythrocyte lipid peroxide level in the non-complicated malaria subjects was restored to the same level as non-malaria control subjects. On the other hand, lipid peroxide concentration in subjects with complicated malaria infection remained significantly ($p<0.05$) higher compared to non-malaria control subjects. Prior to medication, reduced glutathione (GSH) concentration was significantly lower in subjects with non-complicated ($p<0.05$) and complicated ($p<0.01$) malaria compared with non-malaria control subjects (Fig. 3b). However, following treatment with antimalaria drugs, GSH concentration in subjects with complicated malaria infection was restored to the same level observed for non-complicated malaria, but remained significantly ($p<0.05$) lower compared to that recorded for

non-malaria control subjects. Erythrocyte antioxidant enzymes, superoxide dismutase (SOD) (Fig. 3c) and catalase (Fig. 3d) activities prior to medication and after treatment with antimalarials were significantly lower ($p<0.05$) in both non-complicated and complicated malaria subjects compared to non-malaria control subjects. The SOD and catalase activities were not significantly different in subjects with non-complicated malaria compared with those with complicated malaria infection. Prior to medication, glutathione peroxidase (GPx) activity was significantly lower in subjects with non-complicated ($p<0.05$) and complicated ($p<0.01$) malaria compared with non-malaria control subjects (Fig. 3e) prior to medication. However, following antimalarial administration, GPx activity in subjects with complicated malaria infection was restored to the same level observed for non-complicated malaria, but remained significantly ($p<0.05$) lower compared to that recorded for non-malaria control subjects (Fig. 3e).

DISCUSSION

This study demonstrated that in children infected with *Plasmodium falciparum*, erythrocyte total phospholipids, total cholesterol and LDL-cholesterol concentrations were significantly elevated beyond normal level. This could be attributed to the high rate of parasite multiplication in infected erythrocytes. The positive association between parasitemia and erythrocyte total cholesterol and phospholipid levels further buttresses this claim. According to a report by Sherman[26] infected erythrocytes was demonstrated to contain 3-5 times more phospholipids than uninfected cells during the late stage of the parasite development. Vial and Ben Momoun[3] have also shown that the eventual survival of the malaria parasite in the human host is dependent on the availability of cholesterol and phospholipids to support its growth and development. Thus, potent inhibitors of plasmodial phospholipid synthesis were previously characterized as potential target for antimalarial chemotherapy due to its crucial role to the parasite survival[4]. This study also showed an upsurge in erythrocyte oxidant (lipid peroxide) status with a concomitant downregulation of the red blood cell antioxidant status (GSH concentration and SOD, catalase and GPx activities) in response to *P. falciparum* infection.

The increased production of free radical in response to *Plasmodium falciparum* infection as observed in this study could be a pathogenic response triggered by the parasite[8] or an adaptive response by the host to abate the infection[27]. Thus, malaria infection could be viewed as a pathological stressor. An important mechanism of action of stress is the production of free radicals and or reactive oxygen species, which most often react with cellular components such as; DNA, membrane lipids, proteins and carbohydrates. These reactive oxygen species in the cells are neutralized by antioxidant defense system including GSH, SOD, catalase and GPx. Thus, oxidative stress is the result of an imbalance between oxidants and antioxidants[7]. Several studies have shown that alteration of antioxidant enzyme activities in different kinds of stress were associated with a depletion of GSH and an increase of lipid peroxidation, all of which can lead to oxidative stress and finally cell death[1,28].

Data obtained from this study also showed that following a three-day treatment with artemisinin combination drugs, there was significant reduction in erythrocyte phospholipids, total cholesterol and LDL-cholesterol concentration as well as lipid peroxide levels. Significant augmentation in erythrocyte antioxidant status (GSH, SOD, catalase and GPx) were also observed after treatment with antimalarials. It has been shown that therapeutic agents targeted at this stage of the parasite life cycle will go a long way in preventing clinical episodes of malaria[4]. The observed decrease in erythrocyte phospholipids, total cholesterol and LDL-cholesterol following treatment with antimalarial drugs in this study could be attributed to the suppression of parasite growth by the drugs thus less requirements for these lipids in membrane formation.

In the context of the present study, it is envisaged that hyperlipidemia, which is one of the indicators of malaria infection played a significant role in the observed depletion of both non-enzymic (GSH) and enzymic (SOD, catalase and GPx) in the *Plasmodium falciparum*-infected erythrocyte[29-31]. Lipoproteins are major lipid components in plasma and certainly the targets for oxidative stress, thus enhancement in the production of lipid peroxides as demonstrated by the high MDA concentration in infected erythrocyte in this study. Thus, findings from this study showed that *Plasmodium falciparum* malarial infection lead to the creation of a pro-oxidant state (as observed in high level of MDA) and depletion of the overall redox status in the infected red blood cell, as evident by reduced GSH concentration and low cellular SOD, catalase and GPx activities. This pro-oxidant environment is thought to contribute to the destruction or clearance of the parasite. Some antimalarials such as; chloroquine acting via this mechanism are reported be to produce toxic metabolites or free radicals as intermediates when metabolized[32]. This observation agrees concur with the report of Oluba *et al.*[33] which showed a significant increase in plasma MDA concentration with a concomitant decrease in GSH level in rabbits following chloroquine administration.

CONCLUSION

The metabolic machineries for the synthesis of phospholipids and fatty acids have stimulated great interest as potential targets for the development of novel antimalarial drugs, largely due to their importance for the growth, proliferation and pathogenesis of plasmodium parasites.

SIGNIFICANCE STATEMENT

The manuscript provides information on associated lipid changes in *Plasmodium falciparum* infected erythrocytes and how this modifies the antioxidant status of infected red blood cells. It is my view that the information provided in this study could be exploited in targeting the malaria parasite in drug design and malaria eradication and control.

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