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Evaluation of plasma lipid peroxidation and antioxidants activity in *Plasmodium falciparum* infected subjects

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Abstract
Plasmodium falciparum remains the deadliest among the malaria causing species of protozoan found mostly in female anopheles mosquito. Malaria is a serious health challenge in sub Saharan Africa. This protozoan destroy red blood cells causing anemia which complicate major tissues in the body. This research was done to evaluate the level of lipid peroxidation and antioxidant profile in the plasma of *plasmodium falciparum* infected subjects. The study includes 50 *plasmodium falciparum* infected malaria patients as test and 50 apparently healthy subjects without *plasmodium falciparum* infection as control group from the outpatient Department of Wesley Guide Hospital, Ilesa, Osun State. An amount of blood (5mls) was taken from both test and control and processed to obtain the plasma which was stored at room temperature before analysis. The activity of SOD, GSH, GPx and CAT and lipid peroxidation level were determined in the plasma of both test and control subjects using appropriate method. The study revealed no significant change (p<0.05) in the level of lipid peroxidation, while activities of GSH, SOD and CAT reduced significantly (p<0.05) in the plasma of *plasmodium falciparum* infected malaria patients. There was no significantly difference in the activity of GPx in both test and control subjects. The decreased activity of antioxidant enzymes in these patients as obtained in this research could be due to increased activity of oxidants as a result of the infection which may later expose the body to further stress if nothing is done to boost the activity of these enzymes and other relevant antioxidant system.

Keywords: *Plasmodium falciparum*, malaria, lipid peroxidation, antioxidants

Introduction
Malaria fever constitutes a serious health challenge in many developing countries, resulting in almost 584,000 deaths, 90% of which are found in sub-Saharan Africa (WHO, 2019) [7]. It is endemic in most nations and territories around Africa, Asia, Latin Africa, the Middle East, and South Pacific. It results from a female anopheles mosquito infected with protozoan parasite of the genus *Plasmodium* biting an individual and is the deadliest among all the malaria causing species responsible for around 50% of all malaria cases (Klonis *et al*, 2013) [3]. *P. falciparum* breaks red blood cells, which may result in acute anemia and complications in certain body organs, such as the lungs, kidneys and brain

The relationship between the host and the parasite in malaria infected individual can enhance the release of reactive oxygen species (ROS) through stimulation of the immune system; the raised level of ROS has the potential to induce oxidative damage and cell destruction, thus the erythrocytes of Plasmodium-infected patients are exposed to increased oxidative stress (Kremsner *et al*., 2000) [4].

The breakdown of these erythrocytes cells is the reason for most of the clinical symptoms of *falciparum* malaria infection and these include fever (>92% of cases), chills (79%), headaches (70%), sweating (64%). Others are dizziness, malaise, muscle pain, abdominal pain, nausea, vomiting, mild diarrhea, and dry cough. High heart rate, jaundice, pallor, orthostatic hypotension, enlarged liver, and enlarged spleen are also diagnosed (Dondorp *et al.*, 2004) [5].

Antioxidants are molecules that mitigate against cellular damage as a result of the highly reactive free radicals activity. They are a complex system of molecules which includes some micronutrients such as vitamin C, E and β-carotene which the body cannot produce but must

be supplemented in the diet as well as enzymes, such as catalase (CAT), glutathione peroxidase(GPx), superoxide dismutase (SOD), and various peroxidases (Hamid *et al.* 2010) [2].

Materials and Methods

Subjects

A total of 50 malaria patients who tested positive for *plasmodium falciparum* and admitted at outpatients department of Wesley Hospital in Ilesha were used. The same numbers of apparently healthy subjects with no malaria infection were used as control. This study and the use of all bodily fluids were approved by the Committee on the Use of Human Subjects in Research at the Wesley Hospital, Ilesha, Osun state, Nigeria.

Sample collection and processing

Venous blood samples were collected from each of the test and control subjects into heparinized bottles. The sample was centrifuged using a bucket centrifuge at 4000rpm for 3 minutes to obtain the plasma which was used for analysis.

Biochemical analysis

Evaluation of superoxide dismutase (SOD) activity in plasma: SOD activity was determined using the method of Ookawara *et al.* (1998) [9].

Assessment of catalase activity

Catalase activity in plasma was assessed using the method of Beers and Sizer, 1952 [11].

Evaluation of the activity of Glutathione peroxidase.

Glutathione peroxidase activity was evaluated by the method described by Rotruck et al. (1973) [8].

Estimation of the activity of reduced glutathione (GSH) in plasma

The activity of Reduced glutathione (GSH) was evaluated using the method described by Anderson (1985) [10].

Data analysis

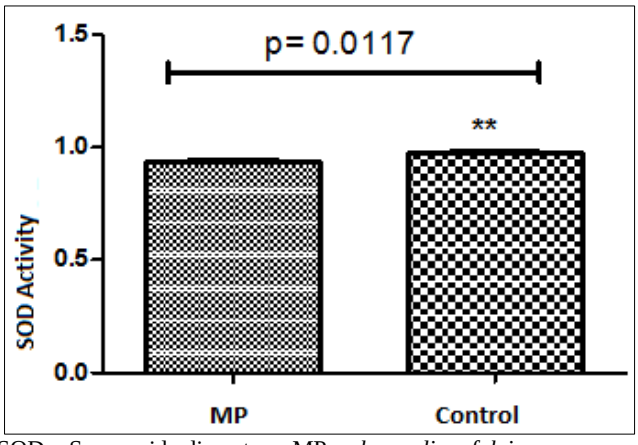
Values were presented as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA). P-value less than 0.05 ($p<0.05$) was considered to be statistically significant.

Results

Table 1: Present the result of the level of MDA in *plasmodium falciparum* infected malaria patients and control subjects.

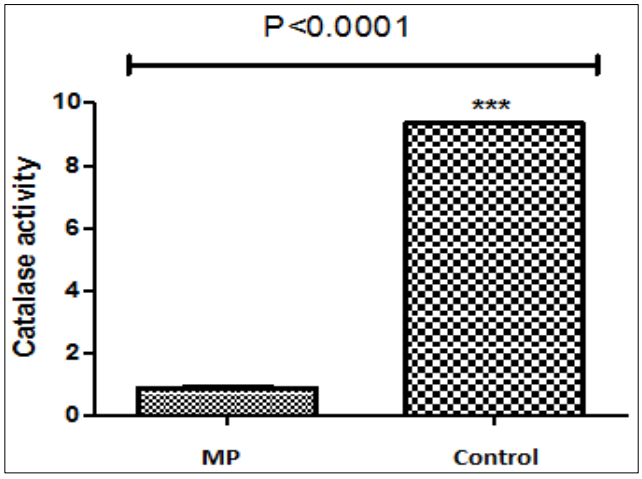
	Control	Malaria positive	p-value
MDA	$5.0392\times10^{-6} \pm 2.6\times10^{-6}$	$7.2157\times10^{-6} \pm 3.08\times10^{-6}$	0.112

MDA – Malonyl di aldehyde



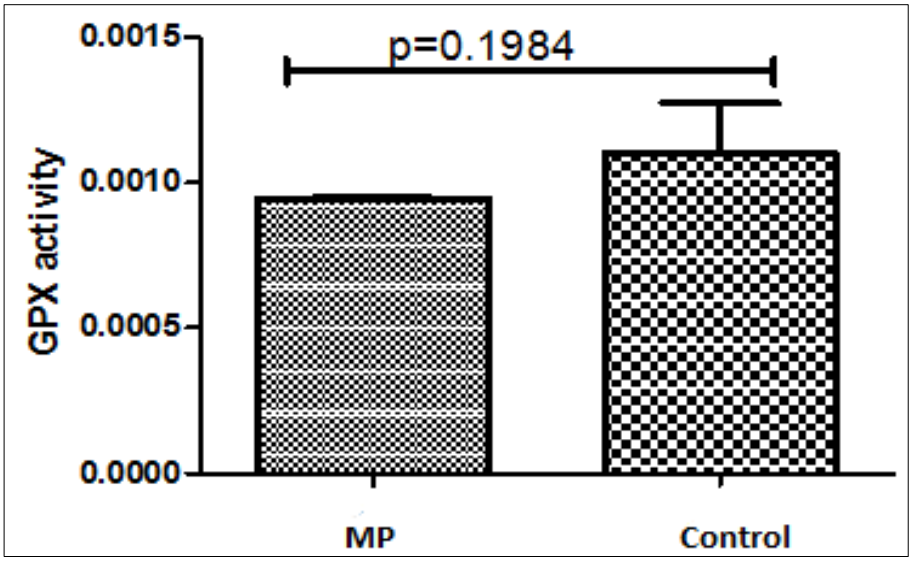
SOD – Superoxide dismutase, MP - *plasmodium falciparum* infected malaria positive patients
** shows significant difference at ($p<0.05$)

Fig 1: Showing the plasma activity of SOD in *plasmodium falciparum* infected malaria patients and control subjects.



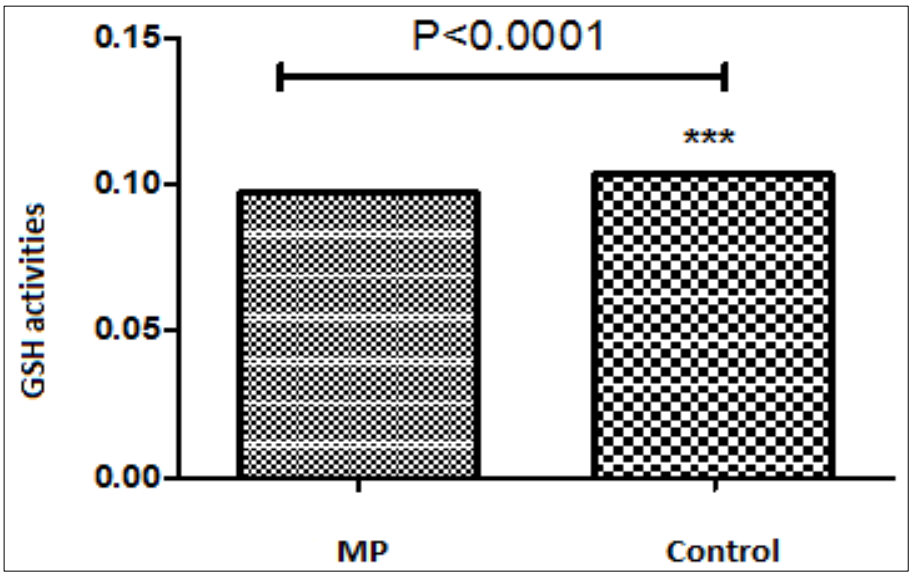
MP - *plasmodium falciparum* infected malaria positive patients
*** signifies significant difference ($p<0.005$).

Fig 2: Showing the plasma activity of Catalase in *plasmodium falciparum* infected malaria patients and control subjects.



GPx – Glutathione peroxidase, MP - *Plasmodium falciparum* infected malaria positive patients

Fig 3: Showing plasma activity of GPx in *plasmodium falciparum* infected malaria patients and control subjects.



GSH – Glutathione, MP - *Plasmodium falciparum* infected malaria positive patients
*** indicate significant difference (p<0.005).

Fig 4: Showing plasma activity of GSH in *plasmodium falciparum* infected malaria patients and control subjects.

Discussion

Malaria infection induced by *plasmodium falciparum* remains a significant health challenge in sub Saharan Africa characterize by physiologic and metabolic changes which could result in significant level of mortality and morbidity among the population. The result of the study in table 1 shows an increase but not statistically significant (p<0.05) in plasma level of MDA and hence lipid peroxidation levels in *plasmodium falciparum* infected malaria patients when compared with the control subjects. This raised level of lipid peroxidation might indicates high level of free radical productions and activities especially that of reactive oxygen species. There had been previous report of increased plasma MDA level in malaria patients. The increased level of free radicals arises from stimulating effect of the parasite on the host immune system (Kremsner *et al*., 2000) [4].

Furthermore, the outcome of the research shown in figures 1, 2 and 4 shows a significant decrease (p<0.05) in the activity of SOD, catalase and glutathione which are major antioxidant defense system of the body. The decrease activity of these antioxidants in these patients could be because of the increased level of reactive oxygen species which could mean they have been used up in neutralizing these oxidants. Similar outcome was previously reported about the activity of these antioxidants in *plasmodium falciparum* infected malaria patients, (Amit *et al.*, 2017) [1] reported decreased activity of catalase and GSH in *plasmodium falciparum* infected malaria patients as reported in this study. However the activity of GPx reported in these patients was not significantly different (p<0.05) from the control subjects which contradict the outcome of a previous study by (Mohammed *et al.*, 2014) [6] who reported a decrease activity of GPx in *plasmodium falciparum* infected malaria patients.

The outcome of this research indicate possible presence of oxidative stress with reduced level of antioxidant defense system in *plasmodium falciparum* infected malaria patients which might present serious threat to the overall health of these patients if not well managed.

Conclusion

The findings of this study underscore the importance of understanding the role of antioxidants in mitigating the cellular damage caused by malaria infection, particularly Plasmodium falciparum. Our results indicate that malaria patients exhibit alterations in antioxidant enzyme activities, including superoxide dismutase, catalase, and glutathione peroxidase, as well as reduced glutathione levels, suggesting a heightened oxidative stress state. This oxidative stress, induced by the interaction between the host and the parasite, likely contributes to the pathogenesis of malaria and the manifestation of clinical symptoms. These insights highlight the potential therapeutic value of antioxidants in adjunctive malaria management strategies. However, further research is warranted to elucidate the specific mechanisms underlying antioxidant dysregulation in malaria and to evaluate the efficacy of antioxidant supplementation as a complementary approach to malaria treatment.

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