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# Evaluation of Nitric Oxide and Antioxidant Status of *Plasmodium falciparum* Infected Nigerian Children with Malaria.

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**ABSTRACT**

Malaria disease is the leading parasitic cause of mortality and morbidity worldwide, causing 458,000 deaths annually and the majority of the victims are children. This study evaluated the antioxidant status of Nigerian children with *falciparum*malaria infection. The result of this study showed a significant increase ($p < 0.05$) in the levels of MDA in the infected pre-adolescents (7.95 ± 0.56 µmol/ml) and adolescents (6.73 ± 1.83 µmol/ml) when compared to the control subjects (4.39± 0.32 µmol/mland 5.66 ± 0.45 µmol/ml respectively). The results obtained also showed that enzymic antioxidants had significant decrease ($p <0.05$) in serum activities of GPx, SOD and CAT in infected pre-adolescent (113.74 ± 6.31U/mg protein; 35.44 ± 2.74U/mgprotein and 28.94 ± 1.90U/mgprotein respectively) when compared to the control subjects (147.49 ± 0.93U/mgprotein; 42.37 ± 1.58 U/mgproteinand 49.72 ± 2.04U/mgprotein respectively) and in adolescents (116.82 ± 13.28 U/mgprotein;32.38 ± 2.74U/mgprotein and 32.68 ± 2.93U/mgprotein respectively) when compared to the control subjects (GPx163.47± 20.55 U/mgproteinand SOD 56.75 ± 0.46U/mgprotein respectively), but a significant increase ($p <0.05$) in CAT activity in the infected adolescent children (32.68 ± 2.93U/mgprotein) when compared to the control subjects (28. 91 ± 1.90U/mgprotein). Also, the results for molecular antioxidants showed a significant decrease ($p < 0.05$) in serum levels of GSH and VITC in both infected pre – adolescents (18.09± 1.90mg/ml and 0.79 ± 0.05 mg/dl; control: 24.71 ± 2. 10 mg/mland 1.14 ± 0.05 mg/dl respectively), and adolescent children(21.88± 6.55mg/mland 0.86 ± 0.29 mg/dl respectively) when compared to the control subjects (23.72 ± 4.21mg/dland 1.05±0.05 mg/dl). No significant changes($p > 0.05$) were observed in the levelsof vitamin E in the infected pre – adolescents, adolescents and the control subjects. There were significant increases ($p < 0.05$) in the levels of NO in the infected pre-adolescents (44.53 ± 3.50 µmol) and adolescents (54.61 ± 3.72 µmol/ml) when compared to the control (39.37 ± 1.78 µmol/ml and 45.53 ± 2.38 µmol/ml respectively). This study has shown that lipid peroxidation product malondialdehydeand nitric oxide were significantly increased and antioxidantstatus were significantly reduced and compromised especially in pre-adolescents children, ultimately exposing of the patients to free radical associated diseases.

Keywords: *Plasmodium falciparum*, children, antioxidant status, nitric oxide.

## INTRODUCTION

Malaria remains the major cause of mortality and morbidity in sub – Saharan Africa, with over 80% of deaths occurring in children [1, 2].The protozoan parasites *P.falciparum*,*P.vivax*, *P.malariae* and *P.ovale*(extremely rarely *P. knowlesi*),are transmitted by the bite of a sporozoite-bearing female anopheles mosquito [3, 4]. After a period of pre-erythrocytic development in the liver, the blood stage infection, which causes the disease, begins. Parasitic invasion of the erythrocyte consumes haemoglobin and alters the red cell membrane. This allows *P. Falciparum*infected erythrocytes to cytoadhere (or stick) inside the small blood vessels of brain, kidneys and other affected organs. Cytoadherence and rosetting (adherence of uninfected red blood cells) interfere with microcirculatory flow and metabolism of vital organs [5, 6]. Pathophysiological changes associated with malaria typically include fever, fatigue, vomiting, and headache [7].

Antioxidants are molecules stable enough to donate electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage cells and cellular components [8]. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property [9]. Antioxidants and antioxidant systems may be obtained from diets or synthesized in the body through various intracellular mechanisms. Those synthesized in the body include glutathione peroxidase, glutathione reductase, reduced glutathione, superoxide dismutase and catalase while those gotten from the diets include vitamins A, C, E etc[10].The body cannot manufacture these micronutrients, and as such they must be supplied in the diet [11]. In malaria infection, low levels of antioxidant vitamins have been reported [12, 13].

Nitric oxide(NO) as a biological mediator plays an important role in a variety of biological processes. It is produced *in vivo*by the enzymatic conversion of L-arginine and molecular oxygen to L – citrulline by members of the nitric oxide synthases (NOS) family of proteins [14, 15].Nitric oxide has been shown to possess various direct and indirect response leading to its effect on various biological systems. The aim of this study is to evaluatenitric oxide and antioxidant status of *Plasmodium falciparum* infected Nigerian children with malaria infection.

## MATERIALS AND METHODS

### Study location:

The study was conducted at the Central Hospital, Benin, Edo State, Nigeria. The study subjects are children suffering from malaria who satisfy the following criteria: Fever (auxiliary temperature ≥ 37°C) or has history of fever within the past 72 hours, headache, 6 months – 10years for pre – adolescents and 11 – 13years for adolescents. Healthy non - malaria children were also used as the control subjects. Informed consent was obtained from all the parents and guardians of the children. The study was approved by the Edo State Ministry of Health Ethical Committee before its commencement.

### Study subjects:

A total of seventy – five children were recruited and screened for malaria parasites. Thirty -four (45.33%) were positive for malaria parasite while forty - one (54.67%) were negative for malaria parasite. All the patients in this study were infected with the specie *P. falciparum.*The children were 6 months to 13years of age and were divided into pre – adolescents and adolescents. Subjects negative for malaria were used as the control.

### Blood collection and preparation:

10ml of venous blood was drawn by venipuncture from the children and8ml was immediately transferred into a plain sterile sample bottle and were allowed to clot before centrifuging for 10 minutes at 3000 r.p.m. The serum was used to determine the activities of glutathione peroxidase, superoxide dismutase, catalase and the levels of malondialdehyde, reduced glutathione, vitamin C, vitamin E and nitric oxide.

### Determination of parasitaemia:

The remaining 2ml of blood was transferred into EDTA tube, swirled gently and shielded from bright light. Parasitaemia was determined by the use of thick and thin blood films using Geimsa stain. The thick blood film was used to attain a qualitative diagnose for malarial infection and the thin blood film was used to identify the *Plasmodium* specie present. The thick and thin films were viewed for the number of parasites per 200 blood cells. Patients were labelled as malaria negative only if three consecutive smears were negative.The degree of parasitaemia was expressed as mild (+), moderate (++) and severe (+++).

### Biochemical assays:

Serum lipid peroxidation was quantified by the method of [15] also known as thiobarbituric acid method. Serum glutathione peroxidase activity was measured by [16];superoxide dismutase activity by the method of [17], and catalase activity by the method of [18]. Reduced glutathione was estimated by the method of [19], vitamin C by the

method of [20] ,vitamin E was estimated by the method of [21], while serum nitric oxide was estimated by the method of [22].

## STATISTICAL ANALYSIS

Results obtained were subjected to statistical analysis using automated package SPSS (version 21.0) and were expressed asmean±SEM. One way ANOVA and Duncan multiple range comparison tests were used for comparison of means and to test for levels of significance at 95% confidence level.

## RESULTS

Table 1 shows the antioxidant indices of Nigerian children infected with *Plasmodium falciparum*. Results obtained showed significant increase ($p < 0.05$) in MDA levels in the infected pre-adolescents (7.95 ± 0.56µmol/ml) and adolescents(6.73 ± 1.83µmol/ml) when compared to the control subjects (4.39 ± 0.32µmol/ml). In the infected pre – adolescents and adolescents, glutathione peroxidase (113.74 ± 6.31 U/mg protein and 116.82 ± 13.28U/mgprotein) and SOD (35.44 ± 2.74U/mgprotein and 32.38 ± 2.74U/mgprotein) activities, GSH(18.99 ± 1.90mg/ml and 21.88 ± 6.55mg/ml) and vitamin C (0.79 ± 0.05mg/dl and 0.86 ± 0.29mg/dl) levels were significantly decreased ($p < 0.05$) when compared to the control subjects (GPx 147.49 ± 0.93U/mgprotein, SOD 42.37 ± 1.58U/mgprotein and vitamin C 1.14 ± 0.05mg/dl respectively) but there was no significant change in the levels of vitamin E ($p >0.05$). CAT activity was only significantly elevated in infected adolescents (32.68 ± 2.93U/mgprotein) when compared to the control subjects (28.91 ± 1.90U/mgprotein). Also, there were significant increases ($p < 0.05$) in nitric oxide levels in the infected pre – adolescents (44.53 ± 3.50µmol/ml) and adolescents (54.61 ± 3.72µmol/ml) when compared to the control subjects (39.37 ± 1.78µmol/ml) (Table 1).

There was a significant decrease ($p <0.05$) in the levels of MDA in severe (5.79 ± 0.64µmol/ml) parasitaemia when compared to mild (7.83 ± 4.64µmol/ml) and moderate (7.59 ± 1.30µmol/ml) parasitaemia in the infected pre – adolescents (Table 2),while in the infected adolescents, no significant changewas observed in the levels of MDA. There were significant reductions ($p < 0.05$) in the activities of glutathione peroxidase (122.41 ± 7.20U/mgprotein; 112.28 ± 8.89U/mgprotein and 101.43 ±31.4U/mgprotein),SOD (35.76 ± 2.60U/mgprotein, 32.23 ± 4.52U/mgprotein and 31.51 ± 4.65U/mgprotein) and catalase (30.56 ± 1.20U/mg protein;29.83 ± 1.92U/mgprotein and 30.58 ± 1.66U/mgprotein respectively) with increase in parasitaemia (mild→ moderate → severe).There were no

significant changes(p >0.05) observed in the levels of GSH, vitamins C and E in the various degree of parasitaemia in both the infected pre – adolescents and adolescents (Table 2).There were reductions in NO levels in the infected pre – adolescents (mild 56.11 ± 2.49µmol/ml, moderate 55.27 ± 3.45µmol/ml and severe 53.19 ± 7.00µmol/ml respectively) in the various degree of parasitaemiaand in adolescents (mild 44.71 ± 4.64µmol/ml and moderate 40.04 ± 7.92µmol/ml).

**Table 1: Antioxidant status of *Plasmodium falciparum* infected Nigerian children with malaria.**

| | UIPA (Control) | IPA | UIA (Control) | IA |
|---|---|---|---|---|
| MDA (µmol/ml) | 4.39 ± 0.32$^{a}$ | 7.95 ± 0.56$^{b}$ | 5.66 ± 0.45$^{ab}$ | 6.73 ± 1.83$^{ab}$ |
| GPx (U/mg protein) | 147.49 ± 0.93$^{ab}$ | 113.74 ± 6.31$^{a}$ | 163.47 ± 20.55$^{b}$ | 116.82 ± 13.28$^{a}$ |
| SOD(U/mg protein) | 42.37 ± 1.58$^{ab}$ | 35.44 ± 2.74$^{a}$ | 56.75 ± 0.46$^{b}$ | 32.38 ± 2.74$^{a}$ |
| CAT(U/mg protein) | 49.72 ± 2.04$^{b}$ | 28.94 ± 1.90$^{a}$ | 28.91 ± 1.90$^{a}$ | 32.68 ± 2.93$^{ab}$ |
| GSH (mg/ml) | 24.71 ± 2.10$^{b}$ | 18.99 ± 1.90$^{a}$ | 23.72 ± 4.21$^{b}$ | 21.88 ± 6.55$^{ab}$ |
| VIT C (mg/dl) | 1.14 ± 0.05$^{b}$ | 0.79 ± 0.05$^{a}$ | 1.05 ± 0.05$^{ab}$ | 0.86 ± 0.29$^{a}$ |
| VIT E (mg/dl) | 0.83 ± 0.06$^{a}$ | 0.78 ± 0.60$^{a}$ | 0.79 ± 0.09$^{a}$ | 0.83 ± 0.19$^{a}$ |
| NO (µmol/ml) | 39.37 ± 1.78$^{a}$ | 44.53 ± 3.50$^{ab}$ | 45.53 ± 2.38$^{ab}$ | 54.61 ± 3.72$^{b}$ |

Data are represented as mean ± SEM. Values in the same row with different alphabets differ significantly ($p < 0.05$). UIPA: Uninfected pre -adolescents; IPA: Infected pre - adolescents; UIA: Uninfected adolescents; IA: Infected adolescents; MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase: CAT: Catalase; GSH: Reduced glutathione; Vit C: Vitamin C; Vit E: Vitamin E; NO: Nitric oxide.

**Table 2:Effect of parasitaemia on antioxidant status of *Plasmodium falciparum* infected Nigerian children with malaria.**

| | | INFECTED PRE – ADOLESCENTS (6MONTHS – 10YRS) | | | INFECTED ADOLESCENTS (11 – 13 YRS) | | | |
|---|---|---|---|---|---|---|---|---|
| | | DEGREE OF PARASITAEMIA | | | DEGREE OF PARASITAEMIA | | | |
| | CONT. | MILD(+) | MODE(++) | SEV(+++) | CONT. | MILD(+) | MODE(++) | SEV(+++) |
| MDA(µmol/ml) | 4.39 ± 0.32[a] | 7.83 ± 4.64[b] | 7.59 ± 1.30[b] | 5.79 ± 0.64[a] | 5.66±0.45 | 8.09 ± 1.09[a] | 7.40 ± 0.71[a] | - |
| GPx(U/mg protein) | 147.49 ± 0.93[ab] | 122.41 ± 7.20[c] | 112.28 ± 8.89[b] | 101.43 ±31.4[a] | 163.47±2. | 119.54± 2.39[b] | 115.20 ± 0.21[a] | - |
| SOD(U/mg protein) | 42.37 ± 1.58[a] | 35.76 ± 2.60[b] | 32.23 ± 4.52[a] | 31.51 ± 4.65[a] | 56.75±0.4 | 41.70 ± 4.74[b] | 32.09 ± 11.42[a] | - |
| CAT(U/mg protein) | 49.72 ± 2.04[b] | 30.56 ± 1.20[a] | 29.83 ± 1.92[a] | 30.58 ± 1.66[a] | 28.91±1.9 | 30.49 ± 3.27[a] | 28.99 ± 0.99[a] | - |
| GSH ((mg/ml) | 24.71 ± 2.10[a] | 21.24 ± 2.04[a] | 21.23 ± 4.79[a] | 22.33 ± 1.83[a] | 23.72±4.2 | 21.69 ± 3.65[a] | 22.70 ± 6.70[a] | - |
| VITC (mg/dl) | 24.71 ± 2.10[a] | 0.84 ± 0.05[a] | 0.80 ± 0.08[a] | 1.00 ± 0.16[b] | 1.045±0.1 | 0.93 ± 0.05[a] | 0.88 ± 0.12[a] | - |
| VITE (mg/dl) | 1.14 ± 0.05[b] | 0.81 ± 0.05[a] | 0.95 ± 0.12[a] | 0.80 ± 0.09[a] | 0.79±0.1 | 0.80 ± 0.09[a] | 0.99 ± 0.05[a] | - |
| NO (µmol/ml) | 39.37 ± 1.78[a] | 56.11± 2.46[b] | 55.27± 3.45[b] | 53.19± 7.00[a] | 45.53±2.3 | 44.71± 4.64[b] | 40.04±7.92[a] | - |

All values are represented as mean ± SEM. Values in the same row with different alphabets differ significantly ($p < 0.05$).

Key: CONT: Control; MODE: Moderate; SEV: Severe; MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced glutathione; Vit C: Vitamin C; Vit E: Vitamin E; NO: Nitric oxide; (+): Mild; (++): Moderate; (+++): Severe parasitaemia.

## DISCUSSION

There are four *plasmodium* species capable of infecting man, of these,*P.falciparum*is the most pathogenic [1];[2][3] and the one capable of producing several clinical manifestations. The present study shows significant ($p < 0.05$) increase in lipid peroxidation in the infected pre – adolescents and adolescents when compared to the control subjects. Severe malaria infection results in inflammation with its associated increase in reactive oxygen species production and an imbalance in the removal of free radicals [4] .Malaria infection has been found to be associated with lipid peroxidation accompanying reduction in antioxidant status of the infected patients, especially in *Plasmodium falciparum* infection [5][6]. The elevated ($p < 0.05$)levels of MDA obtained in this study in malaria positive pre-adolescents and adolescents is an indication of increased production of reactive oxygen species which may lead to oxidative stress. Our results revealed that the infected pre-adolescents and adolescents have enhanced lipid peroxidation products in their sera and this indicatecompromised antioxidant defense system, possibly because of

*Plasmodium falciparum* infection. The pre- adolescents seem to respond more to ROS than the adolescents.

Several antioxidant systems occur in the bodywhich maintain the redox balance [7]. While enzymic and non-enzymicsystems preserve the antioxidant status, these defense systems become overwhelmed during oxidative stress, leading to a diminished antioxidant capacity [8]. Reactive oxygen species generated in host – parasite interactions causes the lysis of erythrocyte membrane and alteration of antioxidant systems [9][10]. Antioxidants are used up to counteract the effects of free radicals generated by the immune system of the host, in the cause of malaria infection[11][12]. This explains why reduction in antioxidantlevels is dependent on the severity of malaria[13]. The findings in this study has revealed significant ($p < 0.05$) reduction in the activities of serum antioxidant enzymes (GPx, SOD and CAT), as well as reduced levels of GSH, vitamins C and E in the infected pre – adolescents and adolescents when compared to the controls. This was in accordance with the findings of [14], who also reported reduced ($p < 0.05$) levels of vitamin C and E. The reductions in enzyme activities and the levels of antioxidant molecules were more pronounced in pre – adolescents than adolescents. These reductions are also correlated with increasing parasitaemia.

Reduction in the activities of these antioxidants may lead to reduction in the body's capacity to mop up free radicals generated. Vitamin C is reduced in malaria patients following its use to regenerate vitamin E from alpha tocopherol's radical at water lipid interface [15]. Therefore loss of ascorbate may interfere in tocopherol regeneration and may lead to impaired membrane function [16]. More so, the significant ($p < 0.05$) reduction in vitamin E levels in malaria may also probably be due to enhanced lipid peroxidation by the *P.falciparum*interaction. The decrease ($p< 0.05$)in antioxidant vitamins C and E in the infected pre – adolescents and adolescents may also be due to their transfer to red blood cell membrane to counteract the increased oxidative stress during acute phase of the disease by inhibiting membrane lipid peroxidation or due to their increased utilization as plasma antioxidants.

Nitric oxide (NO) levels were elevated ($p < 0.05$)in pre – adolescent and adolescent patients during malaria infection when compared to the controls. These levels were seen to decrease with degree of parasiteamia in the infected pre – adolescents and adolescents when compared to the control subjects. The reduction ($p < 0.05$)in NO levels with degree of parasitaemia is probably due to their involvement in immune response associated with *P.falciparum* infection. This agree with the observations that

individuals infected by*Plasmodium falciparum* produce significantly higher levels of nitric oxide [16].Excessive ROS produced by the parasites during haemoglobin digestion can react with NO to produce potently toxic peroxynitrite, which contributes to vascular oxidative stress [17].

## CONCLUSION

This study has shown that lipid peroxidation products,malondialdehyde and nitric oxide levels were significantly increased ($p < 0.05$) and the other antioxidant indices were significantly reduced($p < 0.05$) in children with malaria infection and this probably implies that oxidative stress plays a rolein the pathophysiology of malaria. Pre – adolescents tend to respond more to this oxidative damage than the adolescents.Vitamins supplementation and foods rich in antioxidants through exogenous ingestion should be encouraged in children with *falciparum* malaria.

## CONFLICT OF INTEREST

The authors declare no conflict of interest exits.


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