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# INFLUENCE OF MALARIA IN PREGNANCY ON OXIDATIVE STRESS AND ANTIOXIDANTS MICRONUTRIENTS

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## ABSTRACT

Oxidative stress is thought to be involved in the pathophysiology of malaria, especially in pregnancy where natural resistance is markedly reduced. The current study investigated parasite density, malondialdehyde and antioxidants micronutrients of patients with malaria in pregnancy across the trimesters of pregnancy. A total of 174 pregnant women out of which 87 had *plasmodium falciparum* malaria and 87 controls were grouped into six with twenty-nine participants in each of the trimester within the age range of 17 to 41 years. The mean ±SD of parasite density in women with malaria was obtained across the trimesters (5242±3579/µl of blood) ($p<0.05$) while in controls we had no parasites found in mRDT and thick smears. Levels of malondialdehyde (MDA) were significantly higher in patients with parasitaemia than in healthy control subjects.MDA observed to be higher in the first and the second trimester compared to the third trimester and also correlates well with parasite density ($p<0.001$) and also observed a higher level in primigravidea than in multigravidae. Superoxide dismutase (SOD) activity in women with malaria did not differ from that of controls even though there is decrease level in SOD. Glutathione peroxidase (GPx) activity of women with malaria was found to be significantly lower than that of controls. Our results suggest an imbalance between oxidants and antioxidants in pregnant women suffering from malaria, a condition which could lead to increasing morbidity and mortality of malaria in pregnancy.

**KEYWORDS:** Malaria in pregnancy, oxidative stress, antioxidants micronutrients.

## INTRODUCTION

Malaria infection during pregnancy is a significant public health problem and a major contributor to adverse maternal and perinatal outcome (Udomah *et al.*, 2015). In hyper endemic areas like Nigeria, it is a common cause of anaemia, stillbirth, low birth weight, maternal and foetal death during pregnancy. These problems are aggravated by poor socioeconomic circumstances (Udomah *et al.*, 2015; Bauserman *et al.*, 2019). The management of malaria in pregnancy requires a multi-displinary and multi-dimensional approach (Nadjm and Behrens, 2012). Globally, one hundred and twenty-five (125) million pregnant women resides in areas where they are at risk for contracting malaria, these are areas of the sub-Saharan African countries like Nigeria, south east Asia and western Pacific (Bauserman *et al.*, 2019). Over 50 million women are at risk of malaria in pregnancy worldwide every year, resulting in 2,500 to 10,000 maternal deaths annually, majority of whom live in sub-Saharan Africa (Steketee *et al*, 2001; Desai *et al.*, 2007).

Reactive oxygen species (ROS) are harmful in larger amounts and may overcome the body's natural antioxidant defense system, thereby causing various diseases (Ogbodo *et al.*, 2014). An increase in free radicals causes excessive production of malondialdehyde (MDA) which is commonly known as marker of oxidative stress. MDA is one of the products of lipid peroxidation in the cells especially polyunsaturated fatty acids (PUFA) (Cheignon *et al.*, 2018).

Antioxidants are widely used to detoxify the excess reactive oxygen species. These antioxidant compounds are either endogenous or exogenous molecules that when present in the body, significantly delays or inhibits the oxidation of these lipid molecules (Gorrini *et al.*, 2013). These antioxidants could be enzymes (such as superoxide dismutase, catalase and glutathione peroxidase), vitamins (such as A, C and E) or minerals (such as copper, zinc and manganese) (Gorrini *et al.*, 2013).

Malaria parasitization is thought to increase oxidative stress, by increasing the concentrations of ROS in patients (Ogbodo *et al.*, 2014). Hence the severity of

parasitic infestation and prognosis may depend largely on the patients' antioxidant capacities, which in turn is determined by the concentrations of antioxidant micronutrients.

The aim of this study was to evaluate the influence of malaria in pregnancy on oxidative stress and antioxidants micronutrients.

## MATERIALS AND METHODS

**Chemicals and reagents:** All the chemicals and reagents used were of analytical grade and used before their expiration date.

**Study Site:** The study was conducted at Women and Children Welfare Hospital Sokoto.

**Inclusion Criteria:** Pregnant women with malaria in pregnancy evidenced by positive parasitaemia on microscopy at each of the three trimesters.

Pregnant women without malaria in pregnancy at each of the three trimesters.

**Exclusion Criteria:** Women with malaria in pregnancy with other co-morbidities, those who received blood transfusion or women with multiple gestation and those on anti-malaria drugs will be excluded.

**Participants:** A total of one hundred and seventy four (174) pregnant women attending antenatal clinic at Women and Children Welfare Clinic (WCWC) of Sokoto State, were recruited for this study.

**Experimental Design:**

**Group 1:** Non- malaria in pregnancy in the first trimester (n = 29)
**Group 2:** Malaria in pregnancy in the first trimester (n = 29)
**Group 3:** Non- malaria in pregnancy in the second trimester (n = 29)
**Group 4:** Malaria in pregnancy in the second trimester (n = 29)
**Group 5:** Non- malaria in pregnancy in the third trimester (n = 29)
**Group 6:** Malaria in pregnancy in the third trimester (n = 29)

**Informed consent:** Written informed consent was obtained from the participants (through semi-structured interviewer administered questionnaire) and Ethical approval for the study was obtained from Ethical and Research committee of Sokoto State Ministry of Health and Hospital Services Management Board.

**Sample Collection:** Four (4 mLs) of blood was collected by venipuncture from each participating pregnant woman. Thick peripheral blood film was prepared immediately on a clean grease free slide for malaria parasite detection. While the remaining was transferred to a sterile plain container and centrifuged at 4000 revolution per minutes (rpm) for 5 minutes to obtain the serum which was stored at $4^{o}$C until required for analysis.

**Parasitological Study:** Thick peripheral blood films were prepared from each positive sample, by adding a drop of blood (5µL) on a clean grease free slide and allowed to air dry. The slides were stained using Giemsa stain. The stain was diluted using buffered water or saline as required (10% for 10 minutes) before the staining and was screened for the presence of malaria parasite using routine microscopy to determine the level of parasitaemia. For the positive slides, the number of parasite counted per 100 white blood cells (wbc) was recorded and used to calculate parasite density on the basis of 4000 wbc/µL of blood (Nwagwu *et al.*, 2005).

**Biochemical Analysis:** MDA concentration was determined using method of Hartman, 1983, SOD activity using Cayman's assay kit by the method of Maier and Chan, 2002, CAT activity using method of Apple *et al*., 1999, GPx activity by Paglia and Valentine, 1967. Vitamin A was determined using method of Bassey *et al*., 1946, Vitamin C using method of Natelson, 1971, and vitamin E by method of Hashim and Schuttringer, 1966. Copper, zinc and manganese were determined using method of Bhatti and Musarat, 2006.

**Data Analysis:** Data was collected and entered into excel spread sheet. Data analysis was conducted using SPSS IBM version 21. Comparison of means were done using one way analysis of variance (ANOVA). P value of $<0.05$ was considered statistically significant.

## RESULTS

Table 1 represents parasite density (PD), malondialdehyde (MDA) and antioxidants enzymes of malaria in pregnancy. There was significant increase in PD in first and second trimester compared to third trimester of malaria in pregnancy ($p < 0.05$). There was also significantly increased level of MDA across the trimesters, but the concentration was more in the first and second trimester as compared to controls ($p<0.05$) than in the third trimester. Decreased level of antioxidant enzyme of malaria in pregnancy compare to non malaria in pregnancy was also observed.'

Antioxidant vitamins concentrations of malaria in pregnancy is presented in Table 2. There was significant difference in the level of antioxidant vitamins as compared to the control group but a significant decrease was observed in the level of vitamin A ($p<0.05$).

Table 3 represents the antioxidant minerals concentrations of malaria in pregnancy. There was no significant difference in the level of antioxidant minerals especially in the level of copper across the trimesters ($p>0.05$). Manganese decreased significantly ($P<0.05$) in malaria-parasitized pregnant women. Serum zinc

concentration decreased, but non-significantly (P>0.01), in parasitemic pregnant women.

Prevalence of oxidative stress (OS) among pregnant women with malaria by trimester is presented in Table 4. The prevalence of OS was obtained across the trimesters and the overall prevalence was found to be 86.2%.

**Table 1: Parasite Density (PD), Malondialdehyde (MDA) and Antioxidant Enzymes of Malaria in Pregnancy.**

| Parameter | Malaria in pregnancy (trimester) | | | Non-malaria in pregnancy (trimester) | | |
|---|---|---|---|---|---|---|
| | First (n=29) | Second (n=29) | Third (n=29) | First (n=29) | Second (n=29) | Third (n=29) |
| PD/µl of blood) | 5881.00±3889.30[a] | 5245.10±3269.20[a] | 3634.40±1844.90[b] | - | - | - |
| MDA (pmol/L) | 2.51 ±0.23[a] | 2.66 ±0.52[a] | 2.33 ±0.51[a] | 1.77±0.35[b] | 1.54±0.19[b] | 2.38±0.68[a] |
| SOD (u/ml) | 5.31± 0.78[a] | 5.27 ±0.84[a] | 5.39 ±0.91[a] | 5.44±0.86[a] | 5.35±0.82[a] | 5.36±0.78[a] |
| GPx (µmol/L) | 43.23 ±17.53[a] | 50.071±16.55[a] | 41.76 ±21.91[a] | 71.326±9.01[b] | 76.94±11.91[b] | 73.33±11.19[b] |
| CAT (µmol//ml) | 0.02 ±0.01[a] | 0.04±0.02[a] | 0.03 ±0.01[a] | 0.06±0.01[b] | 0.06±0.01[b] | 0.08±0.02[b] |

Values are represented in mean ± SD. Values bearing different superscript per row differ significantly (P<0.05) from respective groups using ANOVA. PD:Parasite density, MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT:Catalase

**Table 2: Antioxidant Vitamins Concentrations of Malaria in Pregnancy.**

| Parameter | Malaria in pregnancy (trimester) | | | Non-malaria in pregnancy (trimester) | | |
|---|---|---|---|---|---|---|
| | First (n=29) | Second (n=29) | Third (n=29) | First (n=29) | Second (n=29) | Third (n=29) |
| Vit A (µg/dL) | 39.79 ±11.21[a] | 38.10±8.87[a] | 38.17 ±10.62[a] | 44.24±12.44[b] | 47.72± 9.47[b] | 42.21±10.50[a] |
| Vit C (mg/dL) | 0.74 ±0.29[a] | 0.78± 0.22[a] | 0.66± 0.28[a] | 1.02 ±0.28[a] | 1.75 ±0.94[a] | 0.89±0.27[a] |
| Vit E (mg/dL) | 0.64±0.21[a] | 0.64±0.32[a] | 0.89±0.45[a] | 0.88 ±0.32[a] | 0.84 ±0.29[a] | 0.94 ±0.14[a] |

Values are presented as mean ± SD. Values bearing different superscript per row differ significantly (P<0.05) using ANOVA.

**Table 3: Antioxidant Minerals Concentrations of Malaria in Pregnancy.**

| Parameter | Malariainpregnancy(trimester) | | | Nonmalariainpregnancy(trimester) | | |
|---|---|---|---|---|---|---|
| | First (n=29) | Second(n=29) | Third (n=29) | First (n=29) | Second(n=29) | Third (n=29) |
| Cu (mg/L) | 0.26 ± 0.09[a] | 0.11 ± 0.09[a] | 0.23 ± 0.11[a] | 0.45 ± 0.17[b] | 0.16 ± 0.04[a] | 0.47 ± 0.13[a] |
| Zn (mg/L) | 0.13 ± 0.05[ab] | 0.13 ± 0.06[ab] | 0.14 ± 0.06[a] | 0.17 ± 0.05[b] | 0.19 ± 0.06[a] | 0.20 ± 0.08[a] |
| Mn (mg/L) | 0.48 ± 0.02[a] | 0.29 ± 0.02[a] | 0.22 ± 0.19[a] | 0.72 ± 0.09[a] | 0.58 ± 0.17[a] | 1.10 ± 0.39[b] |

Values are presented as mean ± SD. Values bearing different superscript horizontally differ significantly (P<0.05) from respective groups using ANOVA. **Cu :** Cupper; **Zn:** Zinc and **Mn:** Manganese.

**Table 4: Prevalence of Oxidative Stress Among Malaria in Pregnancy.**

| Trimester | Number of subjects | Number with OS | % prevalence |
|---|---|---|---|
| First trimester | 29 | 27 | 93.1% |
| Second trimester | 29 | 26 | 89.7% |
| Third trimester | 29 | 22 | 75.9% |
| Total | 87 | 75 | 86.2% |

Key: **OS:** Oxidative Stress. Reference range: 0.71- 2.03 (pmol/L) (Atiba *et al.,* 2014).

## DISCUSSION

This study revealed that there was significant increase in parasite density (PD) in first and second trimester compared to third trimester of malaria in pregnancy (Table 1). Women in endemic areas become highly susceptible to malaria during first and second trimester, despite immunity acquired after years of exposure to malaria infection but they acquire a strong immunity with an increasing number of pregnancies.

There was also increased level of MDA across the trimesters, but the concentration was more in the first and second trimester as compared to controls (p<0.05) (Table1) than in the third trimester.The increase level of the oxidative stress marker (MDA) was an ultimate toxic effect of raised reactive oxygen species production by the immune system of the body, as well as synchronized release of superoxide (O2•–) during haemoglobin degradation by malarial parasites (Cheignon *et al.,* 2018).

Decreased level of antioxidant enzyme of malaria in pregnancy compared to non malaria in pregnancy was also observed (Table 1). There was significant decrease in the level of glutathione peroxidase across the trimesters compared to the controls. This was in agreement with several studies that reported reduction of GPx activities of erythrocytes in patients with malaria (Tyagi *et al.*, 2013), which confirms results obtained.

There was significant difference in the level of antioxidant vitamins as compared to the control group but a significant decrease was observed in the level of vitamin A ($p<0.05$). The low level observed may probably be attributed to increased utilzation of the host's serum antioxidants by malaria parasite to counteract the oxidative damage.

There was no significant difference in the level of antioxidant minerals especially in the level of copper across the trimesters ($p>0.05$). The difference may be as a result of the effect of pregnancy on the trace element, since many studies have reported non-significant increased of copper concentration in pregnancy, especially in multiparous pregnant women.

In this study, the prevalence of malaria infection among pregnant women was found to be 86.2%. This finding is higher than in Osogbo where a prevalence of 63.6% was reported by Akinboro *et al.* (2010). Huddle *et al.* (2009) had reported a similar prevalence of 83% in rural Malawi. The high prevalence of malaria parasites can be traced to the fact that sample collection was made during the rainy season in Sokoto, Northwestern Nigeria, which is a good environment for mosquito breeding especially during the rainy season when this study was conducted.

There was positive correlation between parasite density and lipid peroxidation marker, malondialdehyde (MDA) in the first and second trimester as compared to the third trimester. As the parasite density increases the MDA levels also increases. This shows an increase in oxidative stress in malaria positive subjects. This was in agreement with Akanbi *et al.* (2010). The increase in MDA levels may be responsible for the reduction in haemoglobin level in malaria positive pregnant women as compared with malaria negative pregnant women which could be due to the destruction of both parasitized and non-parasitized erythrocyte by ROS produced by phagocyte (Akanbi *et al.*, 2010).

## CONCLUSSION

It is concluded that, malaria in pregnancy play vital role in inducing oxidative stress via increasing malondialdehyde and decreasing antioxidant enzymes and of course their coenzymes/cofactors. It was observed that women at their first pregnancy are more susceptible to the morbidity of *P. falciparum* malaria than those with multiple pregnancies. The prevalence of the oxidative stress among malaria in pregnancy was found to be 87.5%.

## REFERENCES


1. Akanbi, O. M., Odaibo, A. B., Olatoregun, R. and Ademowo, A. B. (2010). The role of malaria induced oxidative stress on anaemia in pregnancy. *Asian pacific journal of tropical medicine*, 6: 211-214.
2. Apple, F. S., Christenson, R.H., Valdes, R. J., Andriak, A. B., Duh, S., Feng, Y. J., Saeed, A. J., Johnson, N. J., Koplen, B., Mascotti, K. and Wu, A. (1999). Simultaneous Rapid Measurement of Whole Blood Myoglobin, Creatine kinase MB and Cardiac Troponin I by the Triage Cardiac Panel for Detection of Cardiac Infarction.*Clinical Chemistry*, 45: 199-205.
3. Bauserman, M., Andrea, L., Conroy, K. N., Jackie, P., Carl, B. and Steve, M. (2019). An Overview of Malaria in Pregnancy. *Seminars in Perinatology*, 43(5): 282-290.
4. Bessey, O.A., Lowry, O.H., and Brock M.J. (1946). The determination of vitamin A and β-carotene in small quantities of blood serum. *Journal of Biological Chemistry, 166: 177.*
5. Bhatti, A. U. and Musarat, M. (2006). Heavy Metal Hazard in Water, Soils and Plants in the Vicinity of Peshawar city: Effect of City and Industrial Effluents. *National Seminar on Soil Care for Sustainable Environment*, 15-17.
6. Cheignon, C., Tomas, M. and Collin, F. (2018).Oxidative stress and beta peptide in Alzeheimer's disease. *Redox Biology*, 14: 450-464.
7. Desai, M., Kuile, F., Nosten, F., McGready, R., Asamoa, K., Brabin, B., and Newman, R. (2007). Epidemeology and burden of malaria in pregnancy. *Lancet of infectious diseases*, 7(2): 93- 104.
8. Gorrini, C., Harris, I. S. and Mak, T. W. (2013).Modulation of oxidative stress as an anticancer strategy. *National Review for Drug Discovery*, 12(12): 931–947.
9. Hartman, P.E. (1983). Assay of malondialdehyde in serum. *Science*, 5(4): 603-607.
10. Hashim, S. A. and Schuttinger, G. R. (1966). Rapid determination of tocopherol in micro and macro quantities of plasma. Result obtained in various nutrition and metabolic studies. *American journal of clinical nutrition*, 19(2): 137-140.
11. Maier, C. M. and Chan, P. H. (2002). Role of superoxide dismutase in Oxidative damage and neurodegenerative disorders. *The neuroscientist*, 8(4): 323-334.
12. Udomah F. P., Isaac I. Z., Lukman I., Nwobodo D., Erhabor O., Abdulrahaman Y. and John R. T. (2015). Plasmodium Parasitaemia Among Pregnant Women Attending Antenatal Clinic in Sokoto, North Western Nigeria. *Journal of Nursing Science*, 1(1): 9-14.

13. Nadjm, B. and Behrens, R. H. (2012). Malaria; an update for physitians, *infectious Disease Clinics of North America*, 26(2): 243-259.
14. Natelson, S. (1971). Techniques of clinical chemistry, third edition, *Thomas, C. C. USA*, Pp 288.
15. Nwagwu, M., Anumudv, C. I., Adoro, S., Odaibo, A. B., Sodeinde, O. and Omosun, Y. O. (2005). Variation in the relationship between anti-MSP1(19) antibody response and age in children infected with Plasmodium falciparum during the dry and rainy seasons. *Acta Tropica*, 95(3): 233-47.
16. Ogbodo, S.O., Okaka, A.N.C. and Nwagha, U.I. (2014). Parity may determine levels of some antioxidant minerals in pregnancy, an experience from rural South-Eastern Nigeria. *Journal of Basic Clinical of Reproductive Science*, 3: 27–31.
17. Paglia, D. E., and Valentine, W. N. (1967). Studies on the Quantitative and Qualitative Characterisation of Erythrocyte Glutathione Peroxidase. *Journal of Laboratory and Clinical Medicine*, 70: 158-169.
18. Steketee, R. W., Nahlen, B. L., Parise, M. E. and Menendez, C. (2001). The burden of malaria in pregnancy in malaria endemic areas. *American Journal of Tropical Medical Hygiene*, 64(1-2): 28-35.