

*Full Length Research paper*

# Serum Levels of micronutrients and antioxidants during malaria in pregnant women In Ado-Ekiti, Ekiti State, Nigeria

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The comparative effects of malaria on serum micronutrients and antioxidants were studied in two hundred and ninety five (295) pregnant women aged 20–43 years. The malaria cases were 195 while 100 served as controls. Serum levels of copper, calcium, magnesium, zinc, iron, vitamin A, folic acid, cobalamine and antioxidants such as vitamins C and E, carotenes, glutathione and superoxide dismutase were measured. Results indicate that malaria was associated with lower levels of both micronutrients and antioxidants in pregnant women. It may be concluded that malaria has a severe damageable effect on the micronutrients and antioxidants of pregnant Nigerian women with malaria.

**Key words:** Micronutrients, antioxidants, pregnant, superoxide dismutase, malaria.

## INTRODUCTION

Malaria is one of the most prevalent and dangerous diseases known to man most importantly in tropical and subtropical climates (Sachs and Malaney, 2002). Malaria affects more than three million pregnant women per year in developing countries, where it commonly causes poor birth outcomes, maternal anaemia and deaths (Menendez et al., 2000).

Malarious infections during pregnancy present a second source of maternal anaemia by increasing red blood cell destruction and decreasing erythropoiesis (Huddle et al., 1999). The risk of malaria is greater in nulliparous than in multiparous and it is more pronounced on the second trimester of pregnancy. However, the degree of parasitaemia falls with the advancing age of the expectant mother (WHO, 1997).

Micronutrients deficiency such as vitamin A, zinc, iron, calcium etc is more common in pregnant women in developing countries (Stephenson et al., 2000). These deficiencies may contribute to an increased risk of parasite infection such as malaria (Mahomed, 2000). Iron plays an important role in the production of haemo-globin, oxygenation of red blood cells and lymphocytes.

It improves the function of enzymes in protein metabolism and enhances the function of calcium and copper. It is also needed for the metabolism of vitamins B complexes. Pregnant women have increased needs because of the high demand and this account for the recommendation of iron supplement in pregnancy. Zinc is a micronutrient that acts as a cofactor for several enzymes most importantly those that regulate storage and metabolism of vitamin A (Macdonald, 2000). It also takes part in gene expression, cellular growth and differentiation. Zinc deficiency decreases the ability of the body to respond to infection, affecting both cell mediated immune responses and humoral responses (Okochi and Okpuzor, 2005). Calcium is an important nutrient that plays a major role in bone and teeth formation, impulse transmission, catalytic activation among others (Nordin, 1997). Deficiency of calcium may be associated with abnormal fetal development, pregnancy induced hypertension and preterm delivery (Ritchie and King, 2000). Vitamin A is an essential micronutrient that is responsible for vision, reproduction, normal immune function etc. Deficiency of vitamin A may result in growth retardation and may be conditioned by environmental factors such as malaria infections (Gouado et al., 2007). Vitamin C has been shown to take part in bone formation, folic acid metabolism, formation and maturation of red blood cells and immune response mechanisms (Garba et al., 2003). Deficiency of vitamin C may affect immune system as

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**Table 1.** Mean and standard error of means of serum micronutrients and antioxidants in pregnant women with malaria infection compared with healthy controls.

Parameters	Controls	Malaria cases
	n = 90	n = 70
Ca <sup>2+</sup> (mg/ml)	9.23 ± 0.39	5.82 ± 1.29
Fe <sup>2+</sup> (mol/l)	25.65 ± 2.19	14.38 ± 4.20
Zn (mol/l)	18.70 ± 2.18	12.55 ± 1.18
Mg (mmol/l)	0.94 ± 0.46	1.12 ± 0.16
Cu (mol/l)	38.15 ± 4.33	48.50 ± 6.11
Vit A (mol/l)	1.91 ± 0.30	1.21 ± 0.40
Vit C (mol/l)	63.50 ± 10.22	100.80 ± 14.19
Cobalamin (Pmol/l)	44.31 ± 5.08	62.11 ± 10.14
Folic Acid (nmol/l)	15.25 ± 5.11	25.32 ± 7.33
Vitamin E (mg/dl)	0.15 ± 0.14	0.46 ± 0.21
Carotenes (mg/dl)	30.59 ± 5.48	40.68 ± 8.17
Glutathione (mol/ml)	2.21 ± 0.25	1.90 ± 0.32
SOD (U/L)	2.9 ± 0.55	3.29 ± 0.76

well as causing anaemia which are involved in resistance to malaria.

Micronutrients are known to be an integral part of antioxidant. The role of antioxidants in malaria infection is currently receiving attention. However, antioxidants have been found to influence host cellular and humoral immunological functions (Spallhoiz et al., 1990). These essential factors are very important in the body in order for the immune system to cope with the challenges imposed by infectious agents (Sherman, 1990). Decrease of cell mediated immune responses to malaria parasite during pregnancy has been reported by Riche et al. (2000) and one of the consequences of oxidative stress resulting from antioxidant deficiency is the development of malarial anaemia (Kremsner et al., 2000). These antioxidants have been shown to provide protection against oxidative stress induced by malaria (Adelekan et al., 1997). To this end, the present study determined the serum levels of some micronutrients and antioxidants in pregnant women infected with malaria.

## MATERIALS AND METHODS

### Collection and preparation of blood samples

The scope, nature, aims and objectives of our study were thoroughly explained to the participants for their consent and all of them were made to sign an informed consent agreement. The age, weight and height of the participants were measured and recorded.

10 ml of venous blood was collected from two hundred and ninety five pregnant women who came for consultation in the University of Ado Ekiti, Teaching Hospital, Ado Ekiti, Ekiti State, Nigeria. One hundred and ninety-five of these pregnant women have been infected with *Plasmodium falciparum* and served as test participants while the remaining one hundred aparasitaemia pregnant women served as control participants were. The blood sample was allowed to clot, retracted and centrifuged at 3,500 rpm for

5 min after which the serum was separated and stored at - 20°C until ready for assay. Serum levels of Ca, Fe, Zn, Mg and Cu were determined using atomic absorption spectrophotometer as previously described by Olaniyi and Arinola (2007).

Analysis of vitamin A was done by a method described by Bieri et al. (1979) while vitamin C was determined colorimetrically using the method described by Chittleborough (1980). Quantitation of cobalamin and folic acid were done using competitive protein binding (CPB) radioassays described by Virgil et al. (1986). Albumin was determined using Bromocresol Green (BCG) method of Peters et al. (1982). Glutathione concentration was estimated by the method described by Tietz (1986).

### Diagnosis of malaria

Malaria was diagnosed using the thick smear and thin smear methods respectively as described by Moretti and Mandoul (1977).

### Statistical analysis

Data obtained from this study were subjected to statistical analysis using means, standard error of means and student's-t-tests. Statistical significance was accepted at  $p < 0.05$

## RESULTS

A total of one hundred and ninety five (195) pregnant women with malaria infection and one hundred (100) healthy controls were recruited for the investigation. Table 1 shows the mean values of micronutrients and antioxidants in pregnant women with malaria infection compared with the controls. However, mean serum levels of virtually all the micronutrients and antioxidants studied were significantly reduced in pregnant women with malaria compared with the controls. The results obtained for vitamins show that the levels of vitamin C, cobalamin and folic acid were significantly higher in pregnant women

with malaria than in the healthy pregnant women whereas there was significant decrease in the value of vitamin A.

## DISCUSSION

Results obtained from the present study indicate that the concentration of virtually all the micronutrients studied was significantly ( $p < 0.05$ ) lowered in pregnant women with malaria infection than in aparasitaemia healthy pregnant women. The significant decreased in serum level of zinc in pregnant women infected with malaria parasite was in conformity with the report of Brown et al. (1993) where infection has been found to have an effect on the plasma level of zinc. This might be due to the redistribution of zinc from plasma to lymphocytes and liver during the acute phase response. Plasma zinc concentrations have been found to vary inversely with malaria parasitemia and may preferentially protect against more severe malaria with high levels of parasitemia (Gouado et al., 2007). Deficiency of zinc will therefore be beneficial for malaria cases as reduction of zinc in circulation reduces the zinc available for metabolism of microorganisms during infection creating the same advantage as in iron (Isaksen and Fagerhol, 2001).

The significant reduction in serum concentration of Ca observed in malaria cases ( $5.82 \pm 1.29$  mg/ml) when compared with that of control ( $9.23 \pm 0.39$ ) may be due to erythrocytes parasites cytoadhering in the glomerular capillaries which may lead to urinary excretion of Ca in malaria. The entry of Ca in the infected cells caused by changes in the form and fluidity of the erythrocytes membrane infected by *Plasmodium falciparum* could also cause the reduction of calcium in circulation (Kon et al., 2003). In this study, serum level of Cu showed a significant decrease in the malarous group when compared to controls. This might be due to the uptake of Cu from the blood by the malaria parasites.

The significant decrease in serum of vitamin A and iron in infected pregnant women observed in this study indicates that pregnant women infected with *P. falciparum* may be significantly more anemic than non infected pregnant control subjects. The non significant increase that was observed in magnesium in infected pregnant women might be due to liver malfunctioning that sometimes occur in malaria. There was a non significant increase in the folic acid level of infected pregnant women when compared with their non-infected counterpart. This might be due to increased permeability of erythrocytes cell membrane to a variety of nutrients (folic acid inclusive) during infections (Sherman et al., 1990).

Lower concentrations of Glutathione and superoxide dismutase were observed in the serum of *P. falciparum*-infected pregnant women than that of the control volunteers. This might be due to hemolysis that sometimes occur during malaria infection and it can also be attributed to the counter effects of these antioxidant on free

radicals that was generated in gestational malarial.

The concentration of vitamins E and C reported in the present study were lower in infected pregnant women than malaria-free volunteers. The low circulating vitamin E and C can be attributed in part to over utilization and total destruction during the malaria infection. Another contributing factor is their transfer to red blood cell membrane in order to counteract the increase oxidative stress that occurs during the acute phase of malaria by inhibiting membrane lipid peroxidation. Also, it was observed that pregnant women with parasitaemia had lower concentration of Carotenes. This agrees with investigation of Akpotuzor et al. (2007).

In general, the lower concentrations of antioxidants observed in our present study reveals the status of antioxidant in gestational malaria in Nigeria. This shows that there is invasion of human erythrocytes by the malaria parasite.

The present study shows the serum levels of micronutrients and antioxidants to be depressed in pregnant women with malaria in pregnancy. This may be an indication that micronutrients and antioxidants are associated with the pathogenesis of malaria in pregnancy. Therefore, concerted efforts as to the involvement of micronutrients and antioxidants in the management of gestational malaria may be considered.

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