ORIGINAL RESEARCH ARTICLE

Effect of Antimalarial Drugs and Malaria Infection on Oxidative Stress in Pregnant Women

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Abstract

This work studied the effect of malaria infection and antimalarial drugs on oxidative stress in 259 pregnant and non-pregnant women at Ade-Oyo hospital, Ibadan, Nigeria. Oxidative stress was determined by measuring serum lipid peroxidation, ascorbic acid, and reduced glutathione (GSH) levels using spectrophotometer. The results showed that mean lipid peroxidation was significantly higher ($p<0.05$) in malaria positive than malaria negative women, while GSH and ascorbic acid levels were significantly ($p<0.05$) reduced. The parasite density was significantly reduced in patients who had taken antimalarial drugs relative to those without. While mean ascorbic acid and GSH levels were significantly reduced in those who had taken drugs as compared with those without drugs, the lipid peroxidation level was significantly higher in them. The increase in lipid peroxidation and decrease in GSH and ascorbic acid levels in women who were malaria positive and in those who had taken drugs is indicative of oxidative stress. (*Afr. J. Reprod. Health* 2010; 14[3]: 209-212).

Résumé

Effet des médicaments antipaludiques et l'infection causée par le paludisme sur le stress oxydatif chez les femmes enceintes. Cette étude a examiné l'effet de l'infection causée par le paludisme et des médicaments antipaludiques sur le stress oxydatif chez 250 femmes enceintes et non enceintes à l'hôpital Adeoyo à Ibadan, Nigéria. Le stress oxydatif a été déterminé en mesurant la peroxydation du lipide sérique, l'acide ascorbique et les niveaux réduits du glutathion (GSH) à l'aide d'un spectrophotomètre. Les résultats ont montré que la peroxydation lipide moyen a été élevée de manière significative ($p<0,05$) chez les femmes dont l'analyse pour déterminer la présence du paludisme a été positive par rapport à celles dont l'analyse a été négative, alors que les niveaux du GSH et l'acide ascorbique ont été réduits de manière significative ($p<0,05$). La densité parasitaire a été réduite de manière significative chez les patientes qui avaient pris des médicaments antipaludiques par rapport à celles qui n'en avaient pas pris. Alors que les niveaux de l'acide ascorbique et du GSH ont été réduits de manière significative par rapport à celles qui n'avaient pas pris des médicaments, le niveau de la peroxydation a été beaucoup plus élevé chez elles. L'augmentation dans la peroxydation lipide et la réduction dans le GSH et dans les niveaux de l'acide ascorbique chez les femmes dont l'analyse du paludisme a été positive et chez celles qui avaient pris des médicaments est une indication du stress oxydatif (*Afr. J. Reprod. Health* 2010; 14[3]: 209-212).

Key words: Pregnant women, malaria, lipid peroxidation, reduced glutathione, Ascorbic acid.

Introduction

Pregnancy predisposes women living in malaria endemic areas to serious malaria infection that could lead to abortion, still birth, intra-uterine growth retardation and premature labour, and sometimes death of the mother[1]. Pregnancy also favours oxidative stress probably because of the mitochondria rich placenta during pregnancy[2]. Generally, plasma lipid peroxidation level is reportedly high during pregnancy while plasma reduced glutathione (GSH) level and superoxide dismutase (SOD) activity in erythrocytes are lowered during pregnancy, suggesting an oxidative environment and stress in pregnancy[3]. Apart from pregnancy, oxidative stress is common among malaria patients[4] as a result of the activation of the immune responses by malaria parasite; thereby, causing release of reactive oxygen species (ROS)[5,6]. The increase in lipid peroxidation level in malaria patients and decrease in ascorbic acid and GSH has been observed to be accountable for the development of oxidative stress in malaria

Table 1. The parasite density, lipid peroxidation, GSH and ascorbic acid status in malaria positive pregnant and non-pregnant women.

	n (%)	Parasite density	Lipid peroxidation	GSH	Ascorbic acid
Pregnant women	79(43)	4625±11	4.02±0.6	38.9±6.5	7.32±1.7
Non-pregnant	8(11)	2011±10	2.92±0.9	50.3±0.9	9.73±2.0
Total	87				

'n' stands for number of pregnant and non-pregnant women who were malaria positive. Figure in parenthesis are in percentage. Values are expressed as Mean ±S.D.

patients[7].

Malaria parasites are sensitive to oxidative damage and this is demonstrated by the efficacy of some antimalarial drugs that are known to act by generation of ROS when administered clinically or experimentally[8]. Antimalarial drugs such as chloroquine and amodiaquine have been reported to increase the lipid peroxidation level while decreasing plasma antioxidant levels such as GSH and vitamins C and β carotene[8]. There is little information about the role of antimalarial drugs and malaria infection on oxidative stress in pregnant women in southwestern Nigeria, therefore this work reports the effect of antimalarial drugs and malaria infection on oxidative stress in pregnancy in south-western Nigeria.

Materials and Methods

Study group

Two hundred and fifty nine pregnant and non-pregnant women who gave informed consent were recruited for this study (16-35 years). This included 184 pregnant women who visited the antenatal clinic at Ade-Oyo hospital, Ibadan, Nigeria and 75 non-pregnant women who came for routine medical check-up at the same hospital. Both groups were of similar age group and socio-economic status. History of treatment of malaria in the previous week with any form of antimalarial chemotherapy and chemo prophylactic agents were obtained from the participants. Variables from the patients, such as: age, parity, number of still-births and last menstrual period were obtained using questionnaire. Gestational age was obtained from the last menstrual period. Those who have been transfused two months before the study commenced were excluded, and those who were malaria positive were treated according to WHO regulation. The study was reviewed and approved by the Joint Ethical Committee of the College of Medicine and University of Ibadan, Ibadan, Nigeria.

Blood collection

Five milliliters of blood was drawn by venipuncture from each woman. Two milliliters of blood was immediately transferred into a bottle containing ethylenediamine tetra acetic acid (EDTA), and 3 ml of blood was transferred into a serum bottle to obtain serum after clotting. The serum was used to determine GSH, lipid peroxidation and ascorbic acid levels.

Parasitological study

Thick and thin peripheral blood films were prepared from each sample, stained with Giemsa's stain and examined for the presence of parasites using routine microscopy to determine the parasitaemia. For the positive slides, the number of parasite counted per 200 white blood cells was recorded and used to calculate parasite density assuming 8000 leucocytes/µl of blood.

Determination of Lipid peroxidation

Lipid peroxidation in serum was assessed by measuring the thiobarbituric acid reactive substances and expressed in terms of MDA formed per mg protein according to the procedure described by Rice-Evans et al[9].

Determination of antioxidants

The level of GSH in the supernatant of serum was determined using the method described by Jollow et al [10]. Serum ascorbic acid was assayed according to the method of Thumham and Stephen[11].

Statistical analysis

Data was presented as percentage, means and standard deviation. Chi-square test was used to compare the prevalence which is in percentage. Student's paired and unpaired 't' test was used to compare the means. The level of significance was estimated at $p<0.05$. The software packages used were SPSS 11.0 and Excel.

Results

The prevalence of malaria infection was higher in pregnant (43%) than in non-pregnant women (11%) ($X^{2} = 13.1$, $P<0.05$) (Table 1). Mean parasite density and lipid peroxidation levels were significantly hig-

Table 2: Lipid peroxidation, GSH, and Ascorbic acid levels in malaria positive and malaria negative women.

	n (%)	lipid peroxidation	GSH	Ascorbic acid
Malaria positive	89(34)	3.03±6.9	38.3±7.2	7.32±1.7
Malaria negative	170(66)	2.33±0.5	49.1±8.9	8.23±1.4
Total	259			

'n' stands for number of participants who were malaria positive and number who were malaria negative. Figure in parenthesis are in percentage. Values are expressed as Mean ±S.D.

Table 3. The parasite density, lipid peroxidation, GSH, and ascorbic acid status among participants who had taken and who had not taken antimalarial drugs.

Drug taken	n (%)	Parasite density	Lipid peroxidation	GSH	Ascorbic acid
Yes	180 (69)	2465±12	3.07 ±0.3	36.3±6.4	7.32±1.6
No	79 (31)	5462±32	2.06±0.4	45±8.4	9.2±1.5
Total	259				

'n' stands for number of participants who had taken antimalarial drugs and those who had not taken antimalaria drugs. Figure in parenthesis are in percentage. Values are expressed as Mean ±S.D.

her (P<0.05) in pregnant than non-pregnant women, while mean GSH and ascorbic acid levels were significantly reduced (p<0.05) in pregnant than non pregnant women (Table 1). Lipid peroxidation was significantly higher (P<0.05) in both malaria positive pregnant and non-pregnant women when compared to malaria negative pregnant and non-pregnant women studied. GSH and ascorbic acid were significantly (P<0.05) reduced in malaria positive pregnant and non-pregnant women than in malaria negative pregnant and non-pregnant women (Table 2). The parasite density was significantly reduced (P<0.05) in women who had taken antimalarial drugs as compared to those with-out antimalarial drugs. Lipid peroxidation level was also significantly higher (P<0.05) in those who had taken antimalarial drugs than those without anti-malarial drugs, while GSH and ascorbic acid were reduced in those who had taken antimalarial drugs as compared to those who did not (Table 3).

Discussion

The prevalence of malaria infection was higher in pregnant than non-pregnant women in this study. Similar findings have been reported in parts of Africa[1,12]. It has been reported that pregnant women, especially primigravidae are more susceptible to malaria infection than non-pregnant women. The level of susceptibility of pregnant women to malaria infection decreases with increase in number of pregnancies[13]. The results also revealed an increase in lipid peroxidation and decrease in GSH and ascorbic acid levels in pregnant women when compared to non-pregnant women, which suggests an oxidative environment and stress in pregnant women. This could be because of dynamic changes in multiple body systems which result in increased basal oxygen consumption[14].

There was an increase in the level of lipid peroxidation in malaria positive women when compared to malaria negative women. This might have occurred as a result of toxic effects of upsurge reactive oxygen species produced by immune system as well as synchronized released of O^{2-} during haemoglobin degradation by malaria parasite[15]. Ascorbic acid and GSH levels were significantly reduced in malaria positive participants in this study. These findings agree with the studies of Cassanueva[2] and Kulkarni et al[5]. The significant reduction in GSH and ascorbic acid levels in malaria positive patients could be because of a pivotal role played by them as antioxidants in protecting serum lipid from reactive oxygen species (ROS) attack[8]. Reduced antioxidant defense in the serum of malaria infected women may be responsible for the significant increase in lipid peroxidation and oxidative stress in malaria patients[2]. This shows that parasite enhances the production of large quantity of ROS purposely to kill the parasite[16].

The parasite density was significantly reduced among those who took anti-malarial drug than those who did not. The result showed a significant increase in lipid peroxidation among women who had taken anti-malarial drugs as compared to those who did not take anti-malarial drugs. It has been reported that malaria parasites are sensitive to oxidative damage and this has been confirmed by some of the anti-malarial drugs which act through generation of ROS when administered clinically or experimentally[8]. The GSH and ascorbic acid levels were signi-

ficantly reduced among those who took drugs as compared to those who did not. Studies have shown that the redox state of ascorbic acid is controlled by the level of GSH[8]. Therefore the apparent decrease in the level of GSH which could be altered as a result of oxidative stress in malaria patients treated with drugs may prevent the conversion of oxidized dehydroascorbate back to ascorbate.

This study therefore shows that malaria infection and antimalarial drugs could contribute to oxidative stress in pregnant women.

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References

1. Akanbi OM, Odaibo AB, Afolabi KA, Ademowo OG. Effect of self-medication with antimalarial drugs on malaria infection in pregnant women in south western Nigeria. Med Princ Pract 2005; 14 (1): 6-9.
2. Casanueua E, Viteri FE. Iron and oxidative stress in pregnancy. Metabolism 2003; 0022-3166/03:1700S-1708S.
3. Ilouno LE, Shu EN, Igbokwe GE. An improved technique for the assay of red blood cell superoxide dismutase (SOD) activity. Clinical of chemistry Acta 1996; 247: 1-6
4. Egwunyenga AO, Isamah G, Nmorsi OP. Lipid peroxidation and ascorbic acid levels in Nigeria children with acute falciparum malaria. Afri J Biotech 2004; 3: 560-563.
5. Kulkarni AG, Suryakar AN, Sardeshmukh AS, Rathi DB. Studies on biochemical changes with special reference to oxidant and antioxidants in malaria patients. Indian J Clin Biochem 2003; 18: 136-149.
6. Akanbi OM, Odaibo AB, Afolabi KA, Ademowo OG. Anti-MSP-1(19) antibody (IgG) and reactive oxygen species (ROS) response against malaria infection in pregnancy in southwestern Nigeria. Asian Pac J Trop Med 2009; 2: 9-15.
7. Loria P, Miller S, Foler M, and Tilley L. Inhibition of peroxidative degradation of heme as the basis of action of chloroquine and other quinoline anti-malarials. Biochem J 1999; 339: 363-370.
8. Farombi EO, Syntum YY, and Emerole GO. Influence of chloroquine treatment and *P. falciparum* malaria infection on some enzymatic and non-enzymatic antioxidant defense indices in humans. Drug and Chemical Toxicology 2003; 26: 59-71
9. Rice-Evans C, Omorphos SC, Baysal E. Sickle cell membranes and oxidative damage. Biochem J 1986; 237: 265-269.
10. Jollow DJ, Mitchell JR, Zampaglione N, Gillete JR. Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4-bromobenzene oxida as the hepatotoxic metabolite. Pharmacology 1974; 11: 151-169.
11. Thurham, D.I. and Stephen, J.M.L. Biochemical methods: appendix D. Nutrition and health in old age. Report on health and social subjects, No. 16. London: Her majesty's stationary office 1979; 191-197.
12. Weatherall DJ, Miller LH, Baruch DI, March K, Doumbo OK, Casals-Pascual, C, et al. Malaria and red cell. Haematology (America Society of Haematology Educational Programme.) 2002; 35-57.
13. Mockenhaupt FP, Rong B, Eggelte TA, Beck S, Gyasi-sarpong C, Till H et al. Submicroscopic *Plasmodium falciparum* infections in pregnancy in Ghana. Trop Med Int Health 2000; 5: 167-173.
14. Romero R. Interauterine infection, premature birth and the fetal inflammatory response syndrome. Journal of Nutrition 2003; 133: 1668S-1673S
15. Erel O, Kocyigit A, Avci S, Aktepe N, Bulut V. Oxidative stress and antioxidative status of plasma and erythrocytes in patients with malaria. Clin. Biochem 1997; 30: 631-639
16. Wisdom SJ, Wilson R, Mckillop JH, Walker JJ. Antioxidant systems in normal pregnancy and in pregnancy induced hypertension. American Journal of Obstetrics and Gynaecology 1991; 165: 170-174