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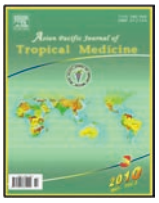

Document heading

Role of malaria induced oxidative stress on anaemia in pregnancy

Akanbi OM[1*], Odaibo AB[2], Olatoregun R[1], Ademowo AB[3]

[1]*Department of Environmental Biology and Fisheries, Faculty of Science, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria*
[2]*Department of Zoology, Parasitology Unit, University of Ibadan, Ibadan, Nigeria*
[3]*Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria*

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ABSTRACT

Objective: To assess the role of oxidative stress on anaemia in pregnancy. **Methods:** Blood samples were collected from pregnant and non-pregnant women who came for antenatal clinic and medical check at Comprehensive Health Center, Akungba-Akoko and Iwaro General Hospital in Akoko Area of Ondo State, Nigeria. Thick and thin blood films were prepared and used for malaria parasite counts. Haemoglobin level was determined by colorimetric method using Drabkin´s solution. Oxidative status was determined using malondiadelhyde level as an indicator of lipid peroxidation, while ascorbic acid and reduced glutathione levels were measured by standard spectrophotometric methods. **Results:** Mean parasite density was significantly higher in pregnant women than non-pregnant women ($P<0.05$). Haemoglobin level was significantly reduced in malaria positive pregnant and non-pregnant women than malaria negative (8.3-10.0 g/dL) ($P<0.05$). The oxidative status indicated that malondialdehyde (MDA) was significantly increased in pregnant [(2.5±0.7) nmol/mL] than non-pregnant women [(1.8±0.1) nmol/mL] ($P<0.05$), while Vit C and superoxide dismutase (SOD) levels were significantly reduced in pregnant than non-pregnant women($P<0.05$). There was an inverse correlation between Hb and MDA levels in pregnant women studied. Positive correlation was observed between the mean MDA level and parasite density ($r = 0.53$). The Hb level decreased as the parasite density and MDA level increased in pregnant women. **Conclusions:** This study shows that oxidative stress, caused by malaria infection could be part of the contributing factors responsible for anaemia in pregnancy.

1. Introduction

Anaemia in pregnancy continues to be a major public health problem in developing country[1]. The adverse effect of anaemia in pregnancy includes the risk of perinatal mortality, morbidity and low birth weight[2]. Malaria has been considered to be one of the factors responsible for anaemia during pregnancy, especially in the tropics[3]. In malaria endemic area pregnant women are likely to experience higher parasite density, anaemia and malaria-related death than their non-pregnant counterpart[4]. During the erythrocytic stage of parasite development, haemoglobin (Hb) is progressively digested and a concurrent release of high levels of iron-containing breakdown products take place in the red blood cells[4,5]. By degradation of haemoglobin, the malaria parasite fulfils its need of amino acids which contributed to anaemia in the hosts[6].

Anaemia during malaria infection is not caused by haemolysis of parasitized red blood cell alone, but it also involves haemolysis of non-parasitized red blood cell[7]. This could be as a result of non-specific effector function of reactive oxygen species (ROS) produced by the immune system in the presence of the parasite[8]. The major event in malaria infection during pregnancy is increased production of highly ROS and reactive nitrogen species produced by the immune system of the host[7]. Extensive haemolysis occurs in severe falciparum malaria releasing free haemoglobin into the circulation, however, haemoglobin and possibly other lytic products of blood might also be pro-oxidants in the overall lipid peroxidation process in malaria infection[9]. A number of studies have demonstrated the susceptibility of erythrocytes infected with the human malaria parasite *P. falciparum* to oxidant mediated damage[9]. The physicochemical changes in the membrane of the erythrocyte induced by oxidative stress are responsible for membrane lipid peroxidation and haemolysis seen in malaria which is common during pregnancy[9]. However, haemolysis and haemoglobin degradation by malaria parasite contribute to anaemia in malaria patients[10].

*Corresponding author: Akanbi OM, Department of Environmental Biology and Fisheries, Faculty of science, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria
Tel: 234-8077-895251
Email: s_akanbi@hotmail.com

The plasma antioxidants potential are able to counteract oxidative stress in normal pregnancy through enzymatic induction activity [such as super oxide-dismutase (SOD), glutathione peroxidase (G6PD)] as well as through non-enzymatic free radical protectors and scavengers [such as vitamin C, E, uric acid, and reduced glutathione (GSH)]. Though it is clear that ROS has a lot to do in the destruction of red blood cell during pregnancy, but its effect on anaemia in pregnancy has not been well studied in Akoko South of Ondo State of Nigeria. Therefore this work assessed the role of ROS and malaria infection on anaemia in pregnancy in Akoko South of Ondo State.

2. Materials and methods

Blood samples were collected from pregnant and non-pregnant women who came for antenatal clinic and medical check at Comprehensive Health Center, Akungba-Akoko and Iwaro General Hospital in Akoko Area of Ondo State (Southwest) in Nigeria. Verbal informed consent was obtained from the subjects. Those who have been transfused two months before the period of blood collection were excluded, and those who were malaria positive were treated according to WHO regulation. The work was carried out according to the rule and regulation guiding research involving human sample by Ondo State Ministry of Health. The study was reviewed and approved by the local Ethical Committee in Nigeria.

Thick and thin peripheral blood films were prepared from each sample, and slides were screened for malaria parasite using Giemsa stain. For the positive slides, the number of parasite counted per 200 white blood cells was recorded and used to calculate parasite density on the basis of 8 000 leucocytes/ μ L of blood as described by Nwagwu *et al*[11].

Hb level was determined by colorimetric method using Drabkin´s solution as described by Akanbi *et al*[12].

Lipid peroxidation in serum was assessed by measuring the thiobarbituric acid reactive substances (TBARS) and expressed in term of malondialdehyde (MDA) formed per mg protein according to the procedure described by Kulkarni *et al*[6].

SOD and Vit C level were measured by the methods described by Farombi *et al*[9].

The mean parasitaemia, Hb, SOD, Vit C, MDA levels were analyzed using student´s *t*- test and correlation. Linear correlation was used to ascertain whether there is a positive correlation or not. The levels of significance were estimated at $P < 0.05$. The software packages used were SPSS version 11.0.

3. Results

The mean Hb level was significantly lower in pregnant than non-pregnant women ($P<0.05$). Antioxidant (SOD and Vit C) levels were significantly lower ($P<0.05$) in pregnant than non-pregnant women, while the mean MDA level was higher in pregnant than non-pregnant women ($P<0.05$) (Figure 1).

The prevalence of malaria infection was higher in *P. falciparum* positive pregnant (70/210, 33%) than non-pregnant women (23/100, 23%). While the incidence in *P. falciparum* negative pregnant was lower (140/210, 67%) than non pregnant women (77/100, 77%). The parasite density was correspondingly higher in pregnant (2 300±101) than non-pregnant women (910±87).

The mean Hb level was lower in *P. falciparum* positive women than *P. falciparum* negative women. The mean SOD and Vit C levels were also lower in *P. falciparum* positive women than *P. falciparum* negative women, while the mean MDA level was higher in *P. falciparum* positive women than *P. falciparum* negative women (Figure 2).

There was a significant positive correlation between MDA level and parasite density as shown in Figure 3 (r=0.53). The mean Hb level was negatively correlated with parasite density. The Hb level decreased as the parasite density increased in both multigravidae and primigravidae (r= -0.33 and - 0.48) (Figure 4 and 5). Mean SOD and Vit C levels were significantly increased as the mean parasite density decreased (r =- 0.43 and 0.35, respectively) (Figure 6 and 7). There was a negative correlation between mean Hb level and MDA level in pregnant women studied (r = 0.64) (Figure 8).

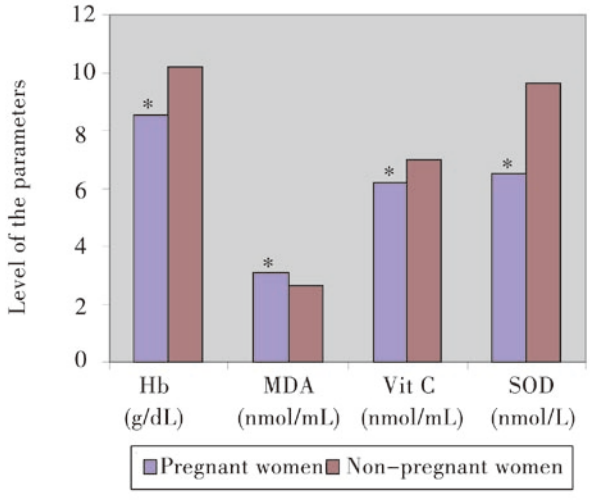

Figure 1. Mean Hb, MDA, Vit C and SOD level in *P. falciparum* pregnant and non-pregnant women.
*:$P<0.05$.

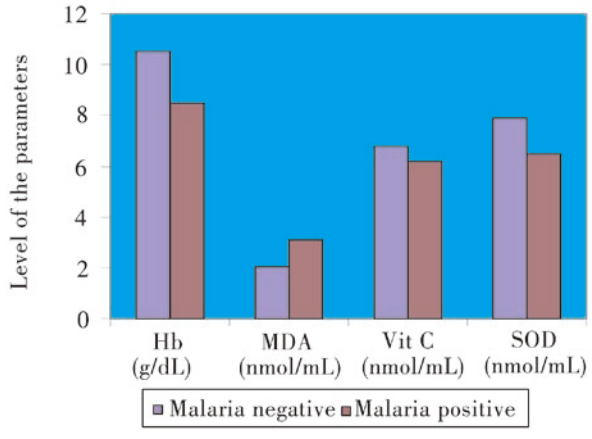

Figure 2. Mean Hb, MDA, Vit C and SOD level in *P. falciparum* negative and positive pregnant women.

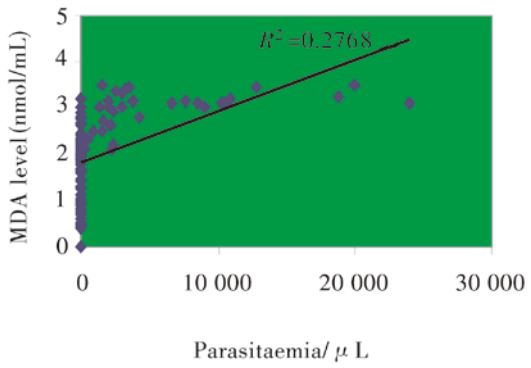

Figure 3. Correlation between mean MDA level and parasite density in malaria positive pregnant women.

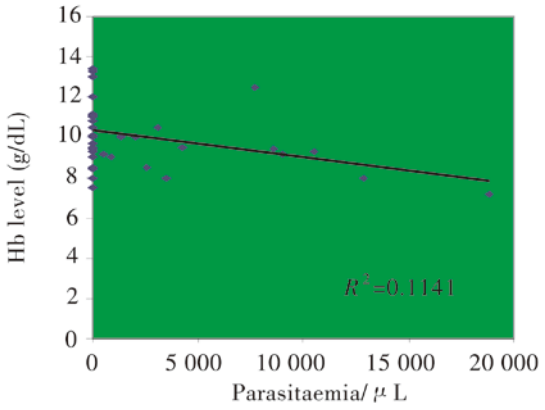

Figure 4. Correlation between mean Hb level and parasite density in *P. falciparum* positive multigravidae.

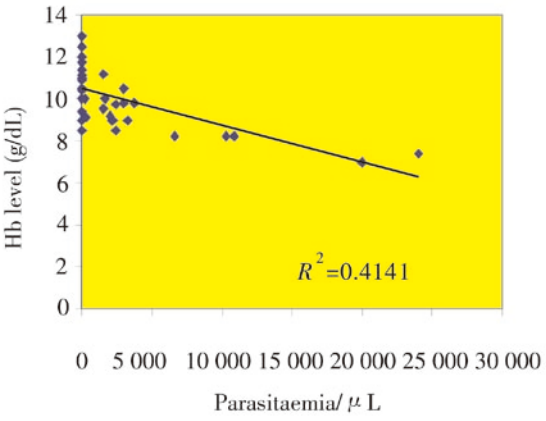

Figure 5. Correlation between mean Hb level and parasite density in *P. falciparum* positive primigravidae.

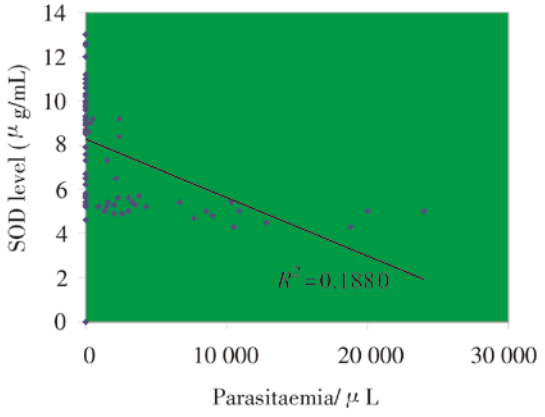

Figure 6. Correlation between mean SOD level and parasite density in *P. falciparum* positive pregnant women.

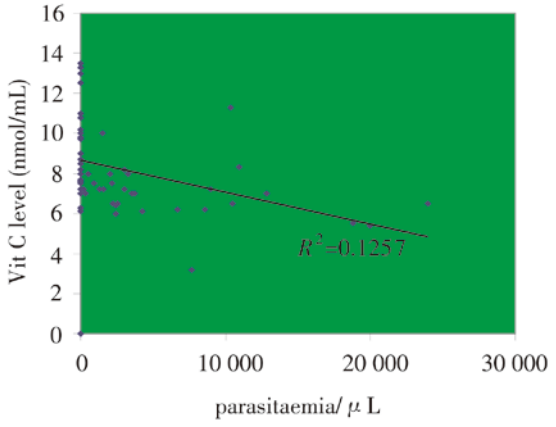

Figure 7. Correlation between mean Vit C level and parasite density in *P. falciparum* positive pregnant women.

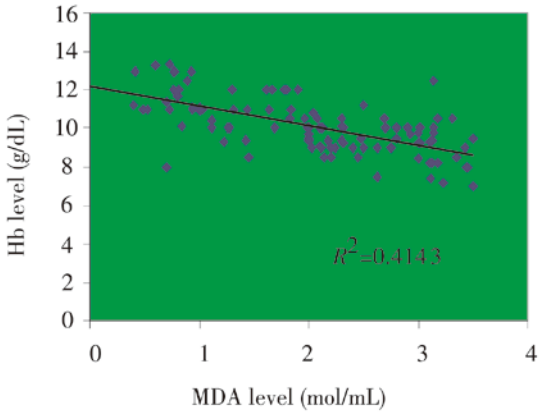

Figure 8. Correlation between mean Hb and MDA levels in pregnant women.

4. Discussion

Pregnant women, especially those who were malaria positive in the tropics are more anaemic than their non-pregnant counterpart living in the same malaria endemic area. Anaemia in pregnant women in the tropics is generally presumed to be due to the effect of malaria infection[13] and oxidative stress[6]. In this study, 33% of pregnant women and 23% of non-pregnant women were malaria positive. This supports the previous study, which shows that pregnant women were more susceptible to malaria infection than their non-pregnant women counterpart[1]. All malaria positive subjects of pregnant and non-pregnant women were anaemic. The factor responsible for this could be due to the destruction of red blood cell by the malaria parasite while feeding on the haemoglobin[5]. The level of anaemia was severe in pregnant than non-pregnant women. This could be because of the level of *P. falciparum* infection, which was higher in pregnant than non-pregnant women.

Pregnancy is characterized by dynamic changes in

multiple body systems resulting in increasing basal oxygen consumption[14]. It is a condition that favours oxidative stress. This could be because of the mitochondria-rich placenta[14]. Plasma reduced glutathione level and superoxide dismutase activity in erythrocytes were reduced during pregnancy suggesting an oxidative environment and stress[15]. In this study, the mean MDA level was higher in pregnant women than non-pregnant women. This could be due to the increase in ROS production during pregnancy as discussed by Akanbi OM *et al*[16] that lipid peroxidation products such as thiobarbituric acid-reactive substances increase in normal pregnant women[16]. The mean SOD and Vit C levels were reduced in pregnant than non-pregnant women. This indicates an oxidative stress in pregnant women as compared to non-pregnant women. This could be as a result of increase in oxygen demand during pregnancy[14]. The increase in oxidative stress in pregnant women could be one of the contributing factors to the state of anaemia in them.

Mean Hb, Vit C and SOD levels were higher in malaria positive non-pregnant women than malaria positive pregnant women. The reduction in Hb, Vit C and SOD levels in pregnant women as compared with non-pregnant women could be due to the physiological changes that occurred during pregnancy. Oxidative stress and malaria infection have been considered to be part of the factors responsible for this situation. All pregnant women studied were anaemic but those who were malaria positive were more anaemic than those who were malaria negative.

Oxidative tissue damage may occur under conditions of increased radical production and when antioxidant defenses are compromised[9]. Malaria infection is associated with an increased production of ROS by phagocyte, this change may play a vital role in host defense against malaria and it could also render host tissues such as erythrocytes more susceptible to oxidative damage[9]. MDA level was higher in malaria positive pregnant women as compared with malaria negative pregnant women, while SOD and Vit C levels were lower in malaria positive pregnant than malaria negative pregnant women. The increased in MDA levels may be responsible for the reduction in Hb level in malaria positive pregnant women as compared with malaria negative pregnant women. This could be due to the destruction of both parasitized and non-parasitized erythrocyte by ROS produced by phagocyte.

As the parasite density increases the MDA levels also increases, while Hb, SOD and Vit C levels were decreasing. This shows an increase in oxidative stress in malaria positive subjects.

Hb level was negatively correlated with MDA levels. This could be explained by the haemoglobin degradation by malaria parasite and also by the toxic effect of ROS produced by immune system on both the parasites and non-parasitized red blood cell[6]. This shows that increase in MDA level produced by phagocyte could also be one of the factors responsible for anaemia in pregnant women especially in malaria positive women.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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