ABSTRACT

Some biochemical and haematological changes have been reported in malaria parasitemic pregnant women in urban and rural malaria-endemic areas, indicating different oxidative status of symptomatic and asymptomatic patients. The aim of this study was to assess the levels of some antioxidant vitamins in symptomatic malaria parasitemic pregnant women to re-appraise the need and extent of vitamin supplementations in this condition. It is a cross-sectional study done between September, 2011 and March, 2012. The study involved 119 pregnant women aged between 24 and 36 years, who presented in antenatal clinics with full symptoms of malaria infection. They were within the second and third trimesters of pregnancy. Malaria density was determined by absolute malaria parasite count while the vitamins (A, C and E) were estimated by spectrophotometric methods. Our results showed that vitamin A concentrations in controls, mild and moderate malaria densities were 16.48 ± 0.75 µg/ml, 15.72 ± 0.58 µg/ml and 16.19 ± 1.40 µg/ml respectively, vitamin C were 31.31 ± 0.97 mg/dL; 38.33 ± 2.73 mg/dL and 52.17 mg/dL respectively while vitamin E were 0.89 ± 0.09 mg/dL; 2.05 ± 0.27 mg/dL and 3.32 ± 0.23 mg/dL respectively. The results indicated that there were no significant changes in vitamin A while vitamins C and E increased significantly as malaria density increased. The changes in these vitamins are indications of increased endogenous mobilization to fight oxidative stress. We opine that the results are suggestive of extra oxidative stress in symptomatic malaria parasitemia, and that inadequate concentrations of these antioxidants can potentiate the virulence of these parasites.

INTRODUCTION

Vitamins are essential micronutrients needed during early stages of life formation, necessitating increased demand and need for supplementations during pregnancy. Recently, there have been increased studies on vitamin supplements for pregnant mothers, aimed at avoiding the consequences of deficiencies on the neonates, and pregnancy complications that may affect both the mothers and the unborn babies (Trindade, 2007). These vitamins, especially the antioxidant vitamins (vitamins A, C and E), prevent the above conditions by preventing oxidative damage in the chorionic membranes, and premature membrane rupture (Romero et al. 2003). Their involvement in reducing oxidative damage implies that their deficiencies may potentiate malaria parasite virulence, especially in immuno-compromised pregnant women. Important functions of vitamin A in the body range from proper functioning of the retina, gene expression and transcription, reproduction and embryonic development to normal functioning of the immune system (Zhang and Omaye, 2001; Nelson and Cox, 2005; Russel, 2007; Trindade, 2007). Its deficiency is strongly associated with depressed immune function and higher maternal and infant morbidity and mortality, as well as increased mother-to-child transmission of HIV-1 (Azais-Breaesco and Pascal, 2000). Vitamin C, the only water-soluble vitamin that has antioxidant activity, is a highly effective antioxidant that can protect large amount of
indispensable molecules in the body such as proteins, lipids, carbohydrates and nucleic acids from damage by free radicals, and can regenerate other antioxidants, especially vitamin E (Carr and Frei, 1999; Bruno et al. 2006). A large number of studies have shown that increased consumption of fresh fruits and vegetables, that contains vitamin C in large amount, was associated with a reduced risk of most types of cancer (Steinmetz and Potter, 1996), gout (Choi et al. 2009) and coronary heart disease (Ye and Song, 2008). Vitamin C deficiency results in widespread pathological lesions in the bones and blood vessels leading to haemorrhagic manifestations of scurvy - a potentially fatal disease of vitamin C deficiency. The vitamin is involved in prevention of oxidative stress and protection against generated reactive oxygen species (ROS) occasioned by pregnancy (Guerin et al. 2001; Chappell et al. 2002), particularly during pre-implantation period. It changes the indices of oxidative stress and placenta functions (Chappell et al. 2002), thereby preventing recurrent abortion, intrauterine growth retardation, premature membrane rupture, preterm delivery and stillbirth. Vitamin E is a collective name for a family of eight antioxidants – four tocopherols (alpha, beta, gamma and delta) and four tocotrienols (alpha, beta, gamma and delta), that exhibit similar activities (Nelson and Cox, 2005; Traber, 2008). It is the most important lipophilic radical-chain breaking antioxidant in living tissues where it stabilizes biological membranes (Netto, 2001). Vitamin E appears to be the first line of defence against peroxidation of cellular and subcellular membrane phospholipids. It functions as nature's most potent fat-soluble antioxidant, and plays considerable role in selenium metabolism (Martin, 1981). It participates in enhancement of the expression and activities of molecules and enzymes in immune and inflammatory cells, inhibition of the activity of protein kinase C (an important cell-signalling molecule), vasodilatation, cell proliferation, platelet aggregation, monocyte adhesion and oxygen burst in neutrophils (Azzi et al. 2000; Traber, 2001).

Many biochemical and haematological changes have been reported in malaria parasitemic children and pregnant women from urban and rural malaria-endemic areas of South-Eastern Nigeria (Ogbodo et al. 2010; Nwagha et al. 2011; Ogbodo et al. 2012; Ogbodo et al. 2013; Ogbodo et al. 2014). Our earlier reports indicated that asymptomatic malaria parasitemia during pregnancy did not induce additional oxidative stress (Nwagha et al. 2009), while symptomatic parasitemia did (Ogbodo et al. 2014). Presently, some studies from different parts of our country have shown that antioxidant vitamins decreased significantly in malaria parasitemic children (Akpotuzor et al. 2007; Ekeanyanwu et al. 2009; Onyesom et al. 2010; Aghedo et al. 2013). However, there is dearth of information on their levels in parasitemic pregnant women. Based on this and the fact that symptomatic patients do experience different physiological and clinical changes from that of asymptomatic patients, we went further to determine the levels of some antioxidant vitamins in symptomatic malaria parasitemia during pregnancy as a measure of oxidative stress in such condition. This will also help to re-appraise the need, or otherwise, for vitamin supplementation in this condition and postulate the possible effect of the antioxidants status on the virulence of the parasites.

MATERIALS AND METHODS

Ethical clearance: Ethical clearance for this study was obtained from the Research Ethics Committee of University of Nigeria Teaching Hospital, Enugu, while additional consents of the patients were sought and obtained after counselling. The patients also got access to their results for treatment without paying for them.

Patients: Patients were drawn from the antenatal clinics of some hospitals in Enugu metropolis – area known to be malaria endemic (Nwagha et al. 2009). The number of patients, recruitment, preparation, classification and exclusion criteria were as earlier reported (Ogbodo et al. 2014).

Dietary index: The dietary indices of the subjects were assessed as earlier reported.
(Ogbodo et al. 2014). The results showed that both patients and controls have similar dietary indices.

**Laboratory analysis**: Patients' preparations, sample collections, preparations and determination of malaria densities by absolute count were as earlier reported (Ogbodo et al. 2014). The concentrations of the vitamins were determined by spectrophotometric methods as earlier reported (Pearson, 1980; Ene-Obong et al. 2003).

**Statistical analysis**: Statistical analyses were done using Graph Pad Prism 5.0 series. Values were expressed as means and standard deviations. Differences between means were calculated using one-way analysis of variance (ANOVA) and significance was taken at $P<0.05$.

**RESULTS**

**Figure 1** shows vitamin A concentrations in different malaria densities and aparasitemic controls. The figures obtained were $16.48 \pm 0.75 \mu g/ml$ for controls, $15.72 \pm 0.58 \mu g/ml$ for mild density and $16.19 \pm 1.40 \mu g/ml$ for moderate density, indicating no significant changes ($P=0.802$) as density increases.

**Figure 2** shows vitamin C concentrations at different malaria densities. Values obtained were $31.31 \pm 0.97 \text{mg/dL}$; $38.33 \pm 2.73 \text{mg/dL}$ and $52.17 \text{mg/dL}$ for controls, mild and moderate malaria densities. The values show that vitamin C increased significantly ($P<0.001$) as malaria density increased.

**Figure 3** shows the means ± standard deviations of vitamin E concentrations in different malaria densities. These values were $0.89 \pm 0.09 \text{mg/dL}$; $2.05 \pm 0.27 \text{mg/dL}$ and $3.32 \pm 0.23 \text{mg/dL}$ for controls, mild and moderate malaria densities, indicating that vitamin E increased significantly ($P<0.001$) as malaria density increased.
DISCUSSION

Plasmodium parasitization increases oxidative stress on patients, and ROS have since been identified as the main mediators in this reaction (Eze et al. 1990). Likewise, certain antimalarial drugs like primaquine bring about their curative effects by inducing ROS production against their respective target pathogens (Summerfield and Tudhope, 1978; Kelman et al. 1982). Therefore, the cause of ROS production in malaria infection could be the malaria infection or the therapeutic agents administered or both. The degree of effects of the activities of parasites on the red blood cells of patients seem to depend largely on the patients' antioxidant capacities, making antioxidant micronutrients levels in malaria-infected person possible determinants of the virulence of the parasites and prognosis of the disease. Naturally, oxidative stress influences the entire reproductive lifespan of a woman and even thereafter ie menopause (Agarwal et al. 2005). Moreover, because of the mitochondria-rich placenta, pregnancy is a condition that favours increased production of ROS which peaks by the second trimester (Casanueva and Viteriy, 2003). During this gestational period, significant increase in oxidative stress markers and decrease in antioxidants like vitamin C, vitamin E and lycopenes are indications of pregnancy-induced hypertension (PIH) – preeclampsia (Moretti et al. 2004; Shama et al. 2006; Mohanty et al. 2006; Kamath et al. 2011). The situation worsens in the presence of other oxidative stress causing factors like malaria and other infections that cause phagocytic cells and macrophages to engage in respiratory burst as a host cell-mediated immune response (Forman and Thomas, 1986; Taverne et al. 1987; Kharazini et al. 1987).

Vitamin A is commonly known as anti-infective vitamin because it is required for routine maintenance of the integrity and functions of the skin and mucosal cells – the body's first line defence against infection (Russel, 2007). This is in addition to other functions in developing foetuses (Lynch, 1997; Russel, 2000; Trindade, 2007; Russel, 2007). Though vitamin A is sometimes not regarded as an out-right antioxidant, its rich sources – carotenoids, are known to trap ROS from sunlight, break the free radical chain reaction and prevent oxidative damage in the same fashion as vitamin E (Zhang and Omaye, 2001), hence some of its functions may be attributable to the antioxidant properties of the vitamin (Ogbodo, 2012). These functions are indications of the indispensability of vitamin A during pregnancy. Though this study showed no significant differences in the values of vitamin A as malaria density increased, the values were significantly lower than the reference values earlier reported (McGormick and Klee, 2006). However, the values did not differ significantly from the value obtained from apparently normal pregnant women from the same environment (Ogbodo, 2012), indicating that the low levels of vitamin A obtained from this study were due to the effect of pregnancy, not malaria parasitemia. Therefore, since the values obtained from this study are similar to that obtained in uncomplicated pregnancy, and which are significantly lower than the reference value, we strongly advocate for its supplementation during pregnancy as a routine, even in the absence of malaria infection.

Throughout gestational period, vitamin C plays crucial roles in the body including maintenance of collagen, synthesis of norepinephrine and carnitine, metabolism of cholesterol and intestinal absorption of dietary iron (Simon and Hudes, 2000; Shu and Ogbodo, 2005). In addition, it is already known to have profound effects on several components of the human
immune system, particularly stimulation of both the production and functions of leukocytes (Vallance, 1977; Anderson et al. 1980), and a well-known powerful antioxidant. Our results showed that vitamin C increased significantly (P<0.0001) as malaria density increased. However, previous studies within the environment and elsewhere (Shu and Ogbodo, 2005; Nwagha and Ejiezie, 2005; Suhail and Faizul-Suhail, 2009; Ghate et al. 2011) had indicated progressive reduction of vitamin C over gestation in uncomplicated pregnancy. Therefore, increased concentration of this vitamin in malaria parasitemia may imply increased endogenous mobilization of the vitamin in response to oxidative challenges. This is definitely a protective/compensatory mechanism that helps to reduce the adverse effects of parasitemia on the patients, supporting earlier finding that increased consumption of antioxidants during pregnancy can reduce many adverse conditions, including cerebral malaria in mice (Thumwood et al. 1989). Incidentally, even the highest value obtained from this study is lower than reference values obtained from the environment and elsewhere (McGormick and Klee, 2006; Ogbodo, 2012). This goes further to re-emphasise the need for inclusion of vitamin C as part of regimen for malaria treatment, especially during pregnancy.

Like vitamin C, this study showed that vitamin E increased significantly as malaria density increased. Though it is assumed that adequate vitamin E is consumed in the diet in well-nourished women, about 90% of Americans are said to take less than daily dietary recommendations for the vitamin (Ahuja et al. 2004; Maras et al. 2004). Therefore, its dietary intake in rural areas, especially among pregnant women is expected to be poor. This may be the actual cause of its significant decrease in uncomplicated pregnancy, with further decrease as pregnancy progresses (Ogbodo, 2012). Vitamin E is the most important lipophilic radical-chain breaking antioxidant in living tissues and nature's most potent fat-soluble antioxidant (Netto, 2001). It also affects the expression and activities of molecules and enzymes in immune and inflammatory cells, implying that it has serious immune and anti-oxidative functions. From the foregoing, increase in the concentration of vitamin E as malaria density increases is a positive development for patient's immunological and anti-oxidative activity against parasitic invasion. Furthermore, though the value increased as malaria density increased, the highest value is still less than the reference value expected for pregnant women. Therefore, it is recommended that like vitamin C, vitamin E should always be part of malaria treatment regimen, especially during pregnancy.

Though previous studies (Akpotuzor et al. 2007; Ekeanyanwu et al. 2009; Onyesom et al. 2010; Aghedo et al. 2013) showed decreased concentrations of these vitamins in malaria parasitemic children as against increased concentrations obtained from the present study, we are of the opinion that the increase may be due to increased endogenous mobilization of the vitamins to fight against oxidative stress caused by the parasitisation. Moreover, our subjects might have been already immuno-compromised because of pregnancy and therefore have been employing internal compensatory mechanism to correct the deficits already created by their conditions. From the foregoing, the results of this study support our earlier opinion (Ogbodo et al. 2014) that symptomatic malaria parasitemia induces extra oxidative stress. Already, pregnant women are known to have high oxidative stress resulting from increased oxygen requirement and low immunity, especially in primigravidae and secundigravidae (Scott and Shannon, 1999; Greenwood, 2004). Therefore, with extra oxidative stress from malaria parasitemia, we are of the opinion that the deficiencies of these antioxidants – which have both immune and anti-oxidant functions, might potentiate the virulence of malaria parasites in this class of patients, given that the patients will be incapable of offering strong resistance to the infection. This incapability may explain while malaria is considered a major contributor to adverse maternal and perinatal outcome (Ogbodo et al. 2009), in addition to its devastating effects on unprotected infants
(Faucher et al. 2000; Ogbodo et al. 2008). Therefore, we advocate for supervised supplementation of these vitamins in pregnancy, especially as part of malaria treatment or intermittent preventive treatment (IPT) in pregnant women and infants. We are also of the opinion that longitudinal studies may explain these effects better, and suggest that such studies be carried out on pregnant women, infants and children with malaria infections to further encourage the use of these vitamins as part of malaria treatment.

REFERENCES


