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Full Length Research Paper

Evaluation of the effect of riboflavin supplementation in malaria patients

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This study evaluated the effect of riboflavin supplementation in malaria patients. Fifteen patients (GpA) were given chloroquine tablets (25 mg/kg-body weight for three days, while 15 others (GpB) were given riboflavin tablets (300 mg per day for five days in addition to chloroquine). Twenty healthy individuals who were not given any drug served as control. Packed cell volume (PCV) and haemoglobin (Hb) levels in the patients were significantly lower ($p< 0.05$) while their serum lipid hydroperoxide (LHP) levels were significantly higher compared to the control subjects during the study period. Five days after commencement of treatment the PCV and Hb levels increased significantly by 4 and 6%, respectively, in GpA compared to14 and 13% in GpB. The LHP level reduced by 9% in GpA compared to 31% in the supplemented group. The negative correlation observed between PCV and LHP ($r= -0.648$, $p<0.05$, $n=30$) and also between Hb and LHP ($r=-0.825$, $p<0.05$, $n=30$), suggests anaemia due to destruction of erythrocytes. The results show that there is oxidative stress in malaria infection and that chloroquine supplementation with riboflavin could reduce lipid peroxidation of erythrocytes and improve the outcome of malaria.

Key words: Malaria, chloroquine, riboflavin, haemoglobin, packed cell volume, lipid hydroperoxide.

INTRODUCTION

About 40% of the world population is at risk of malarial infection (WHO, 2007). Malaria is an endemic parasitic infection in Africa and remains a contributing factor to morbidity and mortality to many populations in the world in general and Africa in particular. Almost all the estimated over one million deaths from malaria infection each year worldwide especially in sub-Saharan Africa is attributed to *Plasmodium falciparum* (Wadie, 2002). *P. falciparum* infection is becoming drug resistant and virulent causing malaria to be life threatening (Kirk, 2001; Mahajan et al., 2005) . Any infection, including malaria activates the immune system of the body causing the release of reactive oxygen species (ROS), which can attack the plasma membrane of the erythrocyte compromising its integrity (Kulkarni et al., 2003). Chloroquine treatment is reported to increase oxidative stress indices in experimental animals (Iyawe and Onigbinde, 2004), and also add to oxidative stress in metabolically active mammalian cells (Toler et al., 2006). However, it is the drug most commonly used in the treatment of malaria in malaria endemic zones (Farombi et al., 2003). Earlier studies had shown that there was increased lipid peroxidation during malarial infection and that the combined supplementation with vitamins C and E in malaria patients improved haemoglobin (Hb) and packed cell volume (PCV) levels (George and Nmoka, 2003). In addition, vitamin C also reduced the incidence and improved the outcome of pneumonia, malaria and diarrhoea infections (Wintergerst et al., 2006).

Malarial infection and micronutrient deficiencies coexist in most parts of Africa (Nussenblatt and Semba, 2002; Cusick et al., 2005). In Nigeria, there is an all the year round malarial parasite transmission, peaking during the rainy season (Salako, 1984). This present study on the effect of oral riboflavin supplementation on lipid peroxidation and haematological indices in malaria patients will provide further data on effects of micronutrients supple-mentation on the outcome of malaria and sustain the interest on the effect of vitamin supplementa-

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tion on the effect of malaria as a means of effective management of the disease.

MATERIALS AND METHODS

Participants

Subjects were recruited from outpatients attending Delta State University, Abraka health centre. A total of 50 adults aged between 18-28 years, after informed consent volunteered to participate in this study. Thirty of them had uncomplicated malaria (positive peripheral blood films for *P. falciparum*) and had not taken any malaria chemotherapy or vitamins before the commencement of this study. Fifteen of these patients were given only chloroquine tablets [25 mg/kg-body weight for three days (group A)], while fifteen were given riboflavin tablets (300 mg per day for five days) in addition to chloroquine (group B). Twenty age-matched apparently healthy individuals who served as the control group were not given any drug. The study was carried out from September 2003-January, 2004 during wet to dry season. The Ethics Committee of Delta State University Abraka Health Centre, where the study was carried out approved of the protocol.

Blood sampling

Venous blood sample (5 ml) was collected from participants. Part of the blood sample was dispensed into anticoagulant bottles and centrifuged (1500 g for 10 min) at room temperature for plasma and stored at -20°C until analysed. The remaining blood sample was used for the determination of malaria parasitemia, packed cell volume (PCV) and haemoglobin (Hb) concentration.

Malaria parasitemia, PCV, Hb, plasma 5 -nucleotidase activity as well as lipid hydroperoxide levels were measured at enrolment, and 7 days after commencement of treatment (Figure 1).

Biochemical analysis

Malaria parasite test and haemoglobin levels (Dacie and Lewis, 1974), PCV (Biggs and MacMillan, 1978) were determined on an aliquot of the blood sample, while serum 5 -nucleotidase (Burtis and Ashwood, 1999) and serum lipid hydroperoxide (Beuge and Aust, 1978) analysis were also carried out on test and control samples.

Statistical evaluation

The parameters are presented as mean ± SD. Student's 't' test was used to test differences between means. Pearson's correlation coefficients were used to examine the relationship between parameters. Statistical significant differences were indicated by $p<0.05$.

RESULTS

The haematologic and lipid peroxidation indicators before therapy began

Blood samples from the malaria patients showed positive blood smear for the malaria parasites, while no parasites were found in the healthy control subjects. The effects of malarial infection on the parameters measured are compared in Table 1. Haematological indices, (Hb and PCV) were lower, while lipid hydroperoxide, an index of lipid peroxidation are significantly higher ($p<0.05$) in the malaria patients compared to the healthy control subjects. Thirty- three percent of the malaria patients had PCV levels < 30%. Malaria infection showed no measurable effect on 5' nucleotidase activity in this study.

Effect of riboflavin supplementation

In Table 2 the effect of riboflavin supplementation is measured by comparing the values at day 0 with the values at the end of day 7 in the malaria patients. The results show a significant increase ($p< 0.05$) in the Hb and PCV values in the malaria patients supplemented with riboflavin in addition to chloroquine. The lipid hydroperoxide value was also significantly reduced in the supplemented group. Despite these improvements in the supplemented group, PCV, and Hb values were still significantly higher in the healthy control subjects while lipid hydroperoxide level was lower (Hb, 12.49±1.13 mg/dl; PCV, 37.47±3.4%; LHP, 5.65±1.32 µmol/L for supplemented malaria subjects, and Hb, 13.4±0.83; PCV, 40.1±2.86%; LHP, 4.35 ±0.89 µmol/L for healthy control subjects). In general an association was observed between PCV and LHP ($r = -0.648$, $p< 0.05$ $n= 30$) and also between Hb and LHP ($r= -0.825$, $p<0.05$, $n= 30$).

DISCUSSION

This study was carried out to evaluate the effects of malaria on haematological indices (Hb and PCV) and lipid hydroperoxide (LHP), an indicator of membrane lipid peroxidation and also to compare the effect of riboflavin supplementation on these indicators in malarious patients. The measurement of lipid peroxidation is a convenient method for monitoring oxidative damage to cell membrane (Viani et al., 1991). The results from this study has confirmed that malarial infection is associated with destruction of erythrocytes as indicated by the significantly lower levels of Hb and PCV values as well as the significantly higher ($p< 0.05$) levels of LHP values in the malaria patients compared to the healthy control subjects at the beginning of the study period. Before therapy began, anaemia (PCV< 30%) was observed in 33% of the malaria patients. 50% of them had PCV values less than the group average of 32.6% at the beginning of the study. A similar observation of lower haematological values in malaria patients was reported in other studies (Kulkarni et al., 2003). The cause of anaemia in malaria has been attributed to various factors including lysis of parasitised erythrocytes and dyserythropioeitic changes (Cusick et al., 2005).

The concurrent significantly lower levels of Hb and PCV and higher levels in LHP in this study suggest that anaemia observed in the malaria patients could be due to lipid peroxidation of erythrocyte membrane. In similar stu-

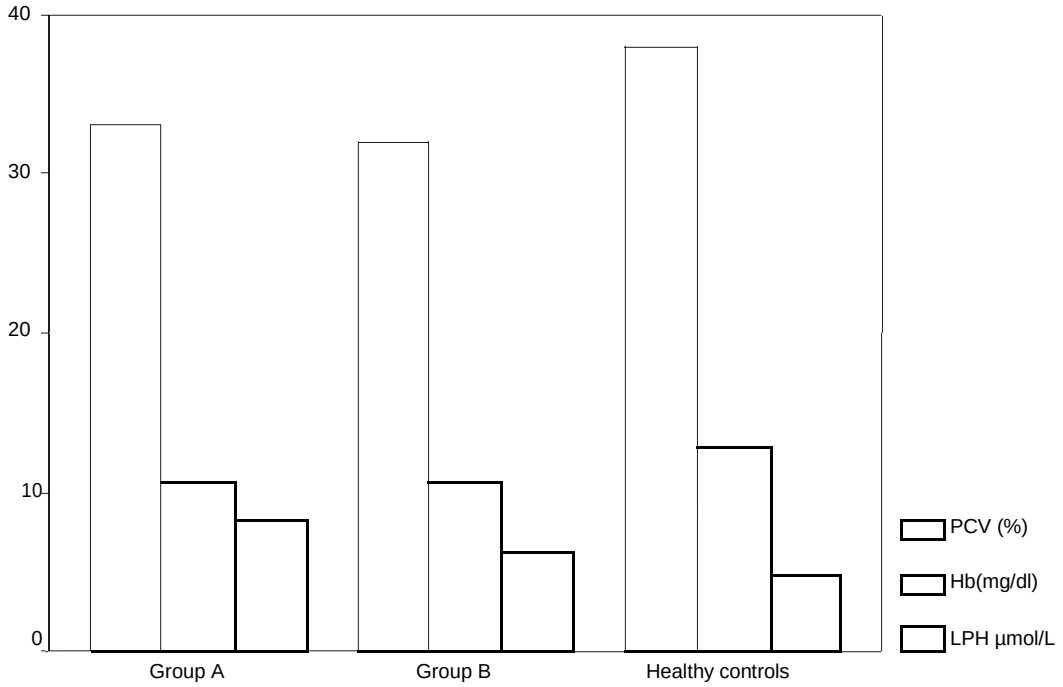

Figure 1. Initial haemoglobin, PCV, and plasma lipid hydroperoxide levels in the three group of participants. Group A, Malarial patients given chloroquine only; and Group B, malarial patients given riboflavin in addition to chloroquine.

Table 1. Comparison of haematological and lipid hydroperoxide levels in malaria patients and healthy control subjects at the beginning of the study.

Parameter	Malaria patients (n=30)	Healthy controls (n=20)	p-value
Haemoglobin (gm/dl)	10.83 ± 1.42	12.73 ± 1.06	p<0.05
Packed cell volume (%)	32.6 ± 3.62	37.62 ± 3.62	p< 0. 05
Lipid hydroperoxide (µmol/l)	7.2 ± 1.71	4.76 ± 0.67	p<0.05
5 -nucleotidase (units/l)	13.50 ± 3.64	13.27± 3.07	ns

Ns= no significant difference.

Table 2. Changes in haemoglobin, PCV, lipid hydroperoxide, and 5'-nucleoside activity 7 days after supplementation with riboflavin in malaria patients.

Group	Hb (gm/dl)	PCV (%)	LHP (µmol/l)	5 -nucleotidase (units/l)
Group A (before treatment)	10.66 ± 1.37^{ac}	32.00 ± 4.12^{ac}	6.19 ± 1.55^{cb}	13.27 ± 3.61^{a}
Group A (after treatment)	11.45 ± 1.29^{ac}	34.47 ± 4.03^{ac}	6.56 ± 1.32^{db}	13.35 ± 3.46^{a}
Group B (before treatment)	11.00 ± 1.53^{a}	33.00 ± 4.6^{a}	8.22 ± 1.30^{a}	13.72 ± 3.09^{a}
Group B (after treatment)	12.49 ± 1.13^{b}	37.47 ± 3.40^{b}	5.65 ± 1.32^{b}	13.31 ± 3.61^{a}

Group A, Malarial patients given chloroquine only; and Group B, malarial patients given riboflavin in addition to chloroquine.
Figures in a column with different alphabets differ significantly.

dy, higher levels of malonyldialdehyde (MDA) (another index of lipid peroxidation) was reported in malaria patients (Das et al., 1993). In falciparum malaria, both infected and uninfected red cells have structural and functional defects as a result of an interaction between the membrane cytoskeleton proteins (Omodeo-Sale et al., 2005).

Oxidative stress observed during acute non-complicated malaria is reported to result in lipid peroxidation of membrane lipids of red blood cells (RBCs) due to

increased production of reactive oxygen species (ROS). This is as a result of increased levels of reactive oxygen species ROS, released from haemoglobin breakdown during malarial infection (Mohan et al., 1994; Huy et al., 2003; Kulkarni et al., 2003). Reactive oxygen species are also generated during respiratory burst and in inflammation response. These mechanisms are employed by the infested RBCs to destroy the parasite leading to destruction of the RBC (Bozdech and Ginsberg, 2004). It has also been demonstrated that changes in erythrocyte membrane fluidity probably due to alterations in of the red cell membrane lipid composition (Omodeo-Sale et al., 2003) lead to severe damage (Parker et al., 2004), resulting in anaemia (Kulkarni et al., 2003).

Although sub-optimal nutritional status in malarial subjects has been reported to affect the survival of the parasites and hence expected to provide a sort of immunity from malarial infection, and normal riboflavin status has been reported in malaria patients in Gabon (Traunmuller et al., 2003), malaria is still one of the causes of debilitating conditions in sub-Saharan Africa where malaria and nutrient deficiencies co-exist (Nussenblatt and Sembe, 2002). Riboflavin-deficient patients reported to have lower haemoglobin concentration than non-deficient patients (Das et al., 1988).

In addition, there are other reports of associations of micronutrient deficiencies with the severity of malaria, (Villamor et al., 2007). Riboflavin plays an important role in protecting cells against oxidative stress because it is a component of FAD/$FADH_2$ system and FAD is a cofactor of glutathione reductase the major enzyme responsible for generating GSH from GSSG. GSH is used in the removal of H_2O_2 from tissues (Fang et al., 2002). Although chloroquine has been reported to induce oxidative stress in uninfected red blood cells (Iyawe and Onibginde, 2004), it remains the drug of choice (Farombi et al., 2003). The results from this study suggest that co-administration of riboflavin and chloroquine tablets could play an important role in the reduction of anaemia associated with malarial infection. Antioxidant vitamins contribute to maintaining the redox integrity of cells protecting them against reactive oxidant species generated during respiretory bout and in inflammatory response (Wintergerest et al., 2006) . Therefore the increase in Hb could be due to reduction in lipid peroxidation as indicated by the negative correlation between PCV and LHP ($r = -0.648$, $p< 0.05$ $n= 30$) and also between Hb and LHP ($r= -0.825$, $p<0.05$ $n=30$) and the reduced level of LHP in the supplemented group. Riboflavin supplementation (5 mg twice daily for 8 weeks) in sickle cell anaemia patients improved haematological indices (Ajayi et al., 1993). In this study the group on riboflavin showed significant increase in Hb and PCV and a reduction in LHP levels.

This improvement in haematological indices in the supplemented group suggests that riboflavin could enhance erythropoisis. Riboflavin improves anaemia and would reduce blood transfusion in severe malaria as a management in reducing anaemia due to malarial infection. Riboflavin deficiency is proposed to restrict regeneration of reduced glutathione (GSH) needed to regenerate GSSG by glutathione peroxidase in reducing H_2O_2 to water making *P. falciparum* parasitized erythrocytes more vulnerable to destructive lipid peroxidation (Das et al., 1990). Riboflavin is also reported to reduce the level of met haemoglobin in erythrocytes infested with *P. falciparum.* Treatment with riboflavin also resulted in decrease in methaemoglobin content, inhibition of food vacuole development and function and inhibition of asexual parasite growth in erythrocytes (Akompong et al., 2000). The malaria parasite *Plasmodium falciparum* is reported to be adapted to cope with the oxidative stress to which it is exposed during the erythrocytic stages of its lifecycle (Muller et al., 2004). However, the oxidative stress is partly responsible for the damage of the red blood cell that ultimately culminates in anaemia in the infected individual. The use of a combination of drugs such a chloroquine and antioxidant vitamins may be of primary importance in reducing the debilitating effect of anaemia resulting from extensive destruction of erythrocytes membrane by increased oxidative stress generation due to malarial infection. 5 -nucleotidase is localised in the plasma membrane. Its physiological function is not clearly understood, but it is believed to be an enzyme marker of hepatobiliary toxicity for plasma membrane of erythrocytes (Burtis and Ashwood, 1999). Its activity was not affected by malaria as suggested by the same level of activity in the subjects.

Conclusion

There is increased level of LHP in malaria patients, with a negative association between LHP and PCV and Hb levels suggesting oxidative stress that culminates in anaemia in infected individuals. The significant improvement in PCV and Hb in riboflavin supplemented malaria group suggests that riboflavin supplementation could reduce anaemia and improve the outcome of malaria infection. About 40% of the world population are at risk of malaria. Early and effective treatment of the disease will shorten its duration and prevent the development of complication, one of which is anaemia.

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