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Evaluation of Antioxidant Levels and Trace Element Status in Nigerian Sickle Cell Disease Patients with *Plasmodium* Parasitaemia

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Abstract: Homozygous sickle cell disease (genotype HbSS) confers a certain degree of protection to human (especially Africans) *Plasmodium falciparum* malaria, yet the precise mechanism remains unknown. Recent observations suggest that the mechanism might also involve immune and non-immune components. In this study, the plasma levels of trace elements (Mg, Fe, Zn, Mn, Cu, Cr, Cd and Se) and antioxidants (urea, albumin and total antioxidants) were determined in Nigerians with sickle cell disease with (HbSS+M), or without (HbSS-M) *Plasmodium* parasitaemia, haemoglobin AA with *Plasmodium* parasitaemia (HbAA+M) compared with those having haemoglobin AA without *Plasmodium* parasitaemia (HbAA-M) using spectrophotometric method. The mean level of urea was significantly higher while the total antioxidants (TAS), Mg, Fe, Zn, Mn, Cu, Cr, Cd, Se and albumin were not significantly different in HbAA+M subjects when compared with HbAA-M subjects. In HbSS-M subjects, the levels of Fe, Zn, Mn and TAS were significantly lower while the level of urea was significantly higher when compared with HbSS+M subjects. There were no significant differences in the values of Mg, Cu, Cd, Se and albumin in HbSS-M subjects when compared with HbAA-M. In HbSS+M subjects, significantly lower levels of Fe, Zn, Mn, Cu, Cr, Cd, Se, total antioxidants, but significantly higher level of urea were observed when compared with the HbAA-M subjects. The result of this study revealed an aggravating effect of *Plasmodium* parasitaemia on deranged levels of trace elements in Nigerians with sickle cell disease.

Key words: Sickle cell disease, malaria, essential metals

Introduction

Several reasons had been attributed to the susceptibility or otherwise of HbSS subjects to *Plasmodium* infection (Ayi *et al.*, 2004; Ntoumi *et al.*, 2005; Arinola and Ezeh, 2007), but this is still a subject of intense research. The mutation giving rise to the sickle gene was reported to have occurred independently in several locations where malaria was prevalent (Read and Vichinsky, 1998). Sickle gene was formerly regarded as nature's compensatory way of protecting the inhabitants of regions with high incidence of malaria (Ayi *et al.*, 2004). There are several reports supporting the slow rate of multiplication of *Plasmodium* in erythrocytes of haemoglobin AS, SS and SC (Oriji 2005; Ntoumi *et al.*, 2005). But if the parasites survive the initial inhibition, it will eventually produce high parasitaemia, become pro-oxidant agents and cause cell death/tissue damage (Oriji, 2005). Like malaria parasites, sickle cell disease has been associated with abnormal renal function (Bayazit *et al.*, 2002), cellular stress syndrome (Klings *et al.*, 2005) and low immunity (Remero *et al.*, 1995). High serum levels of Cu (Oluwole *et al.*, 1990), uric acid, potassium (Bayazit *et al.*, 2002), but low levels of calcium (Nduka *et al.*, 1995) and Zn (Prasada, 1976) have been reported in sickle cell disease patients.

Malaria or sickle cell disease affect the erythrocyte cycle, induce micro-thrombosis (including vaso-occlusion), organ dysfunction and necrosis (Mariethoz *et al.*, 1999). The multiple inflammation in either malaria or HbSS/SC result in low local oxygen tension, sickling of erythrocytes, increased blood viscosity thrombosis with subsequent ischemic tissue breakdown. A previous findings show that co-existence of both malaria and HbSS could generate disastrous effect in the affected individuals (Arinola and Ezeh, 2007). To the knowledge of the authors, no information is available on the antioxidant status and trace elements in sickle cell disease patients harbouring malaria parasitaemia. This study is therefore designed to bridge this gap in knowledge and to suggest possible strategies for improved management of sickle cell disease patients having malaria parasitaemia.

Materials and Methods

Participants: A total number of ninety-four (94) volunteers were selected for this study. They included twenty sickle cell disease patients without malaria parasitaemia (HbSS-M), twenty-four sickle cell disease with malaria parasitaemia (Hb+SS). Both groups of sickle cell disease patients were recruited from

Haematology Day Care Unit of the University College Hospital, Ibadan, Nigeria), eighteen HbAA subjects with malaria parasiteamia (selected from Medical Out-patient Department, University College Hospital, Ibadan, Nigeria) and thirty-two apparently healthy students in School of Medical Laboratory Sciences, UCH, Ibadan, Nigeria served as controls. The controls have HbAA genotype and *Plasmodium* parasite was not detected in their blood samples. Ten (10) ml of blood sample was collected by venepuncture from each participant. Two (2) ml of which was put in bottle containing E.D.T.A (ethylene diamine tetra acid) for haemoglobin genotype and the detection of malaria parasites while the remaining eight (8) ml was put in bottle containing lithium heparin for the assessment of trace metals, albumin, total antioxidants and urea. The bottles were labeled appropriately and were spun at 3,000 revolutions per minute for 5 minutes to obtain the plasma, which was stored at -20°C until analyzed.

Total antioxidants: It was estimated by the method of Koracevic *et al.* (2001). A standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton -type reaction, leading to the formation of hydroxyl radicals (OH). These reactive oxygen species degrade be grade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS). The rate of inhibition of colour development is proportional to the concentration of antioxidative activity.

Urea estimation: The urea concentration was determined by a commercially prepared reagent supplied by Dialab Production and Vertrieb Vonchemisch-Technischen. Urease hydrolyses urea to ammonia and carbon dioxide. The ammonia reacts with alkaline hypochlorite and sodium salicylate in the presence of sodium nitroprusside to produce a colour complex which is directly proportional to the concentration of urea in the sample.

Estimation of albumin: The albumin concentration was determined by the brilliant cresol green solution supplied by Dialab Production and Vertrieb Vonchemisch-Technischen. Bromocresol green at a pH below the isoelectric point of albumin reacts with albumin to cause a change in colour, the colour; the colour which is proportional to the amount of albumin present was measure spectrophotometrically at 540nm.

Determination of trace elements: Trace elements were determined with atomic absorption spectrophotometer (AAS) using a direct method as described by previously described (Arinola *et al.*, 2004). The atoms of the element, when aspirated into the AAS vaporized and absorbed light of the same wavelength as that emitted by the element when in the excited state. The intensity of

light absorbed is proportional to the concentration of the trace element in the sample.

Malaria parasite detection: Thin blood film stained with Giemsa stain was observed with microscope using x 100 (oil immersion) objectives for the detection of malaria parasites.

Statistical analysis: The quantitative data were presented in the form of mean and standard deviation (mean \pm SD). Student (t) test was used for comparison between groups. Significance was considered when p-values are less than 0.05.

Results

As shown in Table 1, the mean values of Mg, Fe, Zn, Mn, Cu, Cr, Cd and Se were not statistically significantly different in subjects HbAA+M subjects compared with HbAA-M subjects. Significantly low levels of Fe, Zn, Mn, Cu, Cr, Cd and Se were observed in HbSS+M compared with the controls (HbAA-M). Significantly low levels of Fe, Zn and Mn were observed in HbSS-M subjects when compared with the HbAA-M subjects. In As shown in Table 2, the levels of albumin were not significantly different HbAA+M, HbSS-M and HbSS + M subjects when compared with HbAA-M subjects. The levels of urea were significantly higher in HbAA+M, HbSS-M and HbSS+M subjects compared with HbAA-M subjects. The levels of TAS were significantly lower in HbAA+M, HbSS -M and HbSS +M subjects compared with the HbAA-M subjects.

Discussion

Although there is evidence that *Plasmodium* parasitaemia is limited in HbSS red cells, yet malaria is a common cause of morbidity and mortality in HbSS subjects. Peculiar attributes of HbSS RBCs were put forward to explain these observations but a recent study (Arinola and Ezech, 2007) added an immunological based reason. Since there is a direct association between antioxidants, trace elements and immunity, it could be conjecture that the nature of *Plasmodium* malaria in HbSS may involve both immune and non-immune components. Till date none of these studies determined the levels of trace elements and total antioxidants in sickle cell disease patients.

In HbSS-M subjects, low levels of Fe, Zn, Mn and total antioxidants but significantly high level of urea was observed. The low level of Fe, Zn and Mn observed in our HbSS-M subjects could be linked with increase body demand for haemopoiesis and tissue repair. The sickle cell disease is pro-oxidant in nature with continuous generation of free radicals that requires antioxidants for mopping (Remero *et al.*, 1995). Thus low level of antioxidant as observed in HbSS-M subjects might be due to its consumption by pro-oxidant phenomenon in HbSS-M subjects.

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Table 1: Levels of trace metals in HbAA or HbSS with or without Plasmodium malaria parasitaemia

	Mg (mg/l)	Fe (µg/l)	Zn (mg/l)	Mn (µg/l)	Cu (mg/l)	Cr (µg/l)	Cd (mg/l)	Se (mg/l)
HbAA-M	6.2±2.2	68.7±21.0	17.2±5.0	70.3±23.6	60.8±16.0	71.5±23.8	78.0±29.1	67.9±22.0
HbAA+M	5.1±2.4	54.7±23.8	13.7±5.8	52.9±23.5	51.4±20.7	56.2±27.6	58.2±27.6	52.7±23.8
HbSS-M	5.1±1.3	48.2±18.0	11.8±5.2	51.2±20.7	52.1±15.6	45.3±15.8	52.3±29.8	58.8±20.1
HbSS+M	4.7±2.7	44.0±24.6	10.8±6.1	43.8±24.8	39.0±22.2	43.0±27.1	45.4±26.6	42.0±24.6
t-, p (HbAA+M)	1.13,0.27	1.53, 0.14	1.55, 0.13	1.77,0.09	1.26, 0.22	1.46, 0.16	1.66, 0.11	1.62,0.12
t-, p (HbSS-M)	1.30,0.24	2.24,0.01*	2.35,0.01*	3.04,0.02*	1.30,0.27	1.42,0.12	1.53,0.18	1.74,0.18
t-, p (HbSS+M)	1.54,0.14	2.73,0.01*	2.90,0.01*	2.73, 0.01*	2.91,0.01*	2.82,0.01*	2.87,0.01*	2.80,0.01*

*Significantly different from the controls (HbAA-M).

Table 2: Levels of albumin, urea and total antioxidants in HbAA or HbSS with or without Plasmodium malaria parasitaemia

	Albumin (g/dl)	Urea (mg/dl)	TAS (mMol/L)
HbAA-M	4.1±0.7	30.1±14.1	1.8±0.4
HbAA+M	4.1±0.6	43.6±13.3	1.6±0.4
HbSS-M	3.8±1.0	40.8±9.6	1.3±0.2
HbSS+M	4.6±0.7	51.6±12.2	1.4±0.4
t-, p (HbAA+M)	0.07,0.94	2.35,0.03*	1.52,0.19*
t-, p (HbSS-M)	1.46,0.07	2.43,0.02*	2.63,0.01*
t-, p (HbSS+M)	1.70,0.1	3.99,0.01*	2.42,0.02*

*Significantly different from the controls (HbAA-M).

In HbSS+M, the levels of Fe, Zn, Mn, Cu, Cr, Cd, Se and total antioxidant were significantly low. Low Zn level was reported in pregnant women with malaria (Gibson and Nuddle, 1998; Arinola and Akiibinu, 2005) and in sickle cell disease (Prasad, 1976; McClain *et al.*, 1985) and HIV patients (Arinola *et al.*, 2004). Fleming (1982) also reported that severe infections from malaria parasite, bacteria and viruses were the major causes of iron deficiency anaemia. Trace metals are known to play important roles in the catalytic activities of major antioxidant enzymes. Cu and Zn are an integral part of Cu-ZnSOD (superoxide dismutase), Fe is an integral part of catalase, Mn is an integral part of Mn-SOD while Se is an integral part of Se-GPX (glutathione peroxidase) which is a major scavenger of H₂O₂. The SOD catalyses the dismutation of superoxide to H₂O₂, which must be removed by catalase or glutathione peroxidase. Deficiency of Se, Zn and Cu decreased activities of superoxide dismutase and glutathione peroxidase (El-Beahry *et al.*, 1997). The present study supports that micronutrients deficiency exists in subjects with HbSS or *Plasmodium* parasitaemia, thus leading to combined detrimental effects in HbSS subjects harbouring malaria parasites. This observation in HbSS+M could be a result of high demand of antioxidants for tissue repair. Total antioxidant is low in HbSS+M subjects and this may be related to free radical generation and high oxidative stress induced by combination of HbSS genotype and the presence of *Plasmodium* parasite which produce free radical.

Kumar and Bandyopadhyav (2005) stated that severe haemolysis in sickle cell disease could result in high levels of free haem causing undesirable toxicity, organ/tissue damage, cellular injury and oxidative

stress. To avoid organ damage, free haem is converted to excretable product such as urea. In HbAA+M and HbSS-M subjects, significantly high level of urea was observed when compared with HbAA-M subjects. High urea level in HbAA+M or HbSS-M subjects could be associated with haemolysis caused by malaria parasites and stickled RBCs. Urea is an antioxidant and its high level as observed in HbAA+M may be a mechanism for the neutralization of the pro-oxidant effect of malaria parasite.

Low levels of total antioxidants and trace elements in HbAA+M, HbSS-M and HbSS + subjects as observed in this study agreed with Remero *et al.* (1995) who stated that malaria and other pathogenic organisms are pro-oxidant agents causing cell death, tissue damage and reduction in total antioxidant levels. Das and Nanda (1999) also reported low plasma and erythrocyte antioxidants in *Plasmodium falciparum* malaria.

Based on the results of this study, significantly lower levels of total antioxidant and certain trace elements are common in sickle cell disease patients with malaria parasitaemia. It is therefore advisable to include trace metal supplements to the therapies used for the management of sickle cell disease patients in malaria endemic regions.

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