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SERUM ANTIOXIDANT MINERALS STATUS IN CHILDREN WITH SICKLE CELL ANAEMIA

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ABSTRACT
Sickle cell anaemia is associated with elevated oxidative stress via increase generation of reactive oxygen species (ROS), and decline in antioxidant defences. Increased oxidative stress is thought to play a role in the development of sickle cell anaemia and its attendant complications. In the current study, chromium, zinc, manganese, copper and body mass index (BMI) levels were evaluated in 35 sickle cell anaemics attending the Paediatric medical clinic of the Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria and the results compared to those of apparently healthy non-sickle cell anaemic volunteers of comparable age and social status. Serum levels of chromium, zinc, manganese, copper and BMI were 12.43±2.92 µg/dL, 5.33±2.46 µg/dL, 11.36±3.08 µg/dL, 6.55±0.48 µg/dL, 15.16±0.64 kg/m^2 and 20.44±5.93 µg/dL, 9.32±2.67 µg/dL, 17.07±4.24 µg/dL, 6.86±0.57 µg/dL, and 17.67±0.53 kg/m^2 in sickle cell anaemics and non-sickle cell anaemic subjects, respectively. There was significantly ($P<0.05$) decreased levels of antioxidant minerals in sickle cell anaemic subjects. Age and gender did not have significant ($P>0.05$) effect. The results suggest that sickle cell anaemics in the study area have low serum levels of antioxidant minerals, an indication that the sickle cell anaemics are predisposed to increased oxidative onslaught.

KEYWORDS: Sickle cell anaemia, serum chromium, copper, manganese and zinc.

INTRODUCTION
Sickle cell anaemia (SCA) is a hereditary disorder caused by mutant haemoglobin (HbS) arising from the replacement of a hydrophilic amino acids residue-glutamic acid by a hydrophobic moiety valine, at the sixth (6) position of the β-chain of haemoglobin molecules with high potential for oxidative damage due to a chronic redox imbalance in red cells that often results in continuous generation of oxygen species and clinical manifestations of mild to severe haemolysis (Rank *et al.*, 1985; Cheesbrough, 2004; Kitadi *et al.*, 2015; Obeagu *et al.*, 2015). This substitution causes a drastic reduction in the solubility of sickle cell haemoglobin (HbS) when deoxygenated under this conditions, the HbS molecules polymerize to form intracellular fibres which are responsible for the deformation of the biconcave disc shaped erythrocyte into a sickle shape (Bisnu *et al.*, 2013). The ailment is characterized by premature breakdown of the red blood cells causing constant anaemia and occlusion of small blood vessels leading to excruciating body pains, increase susceptibility to infection and other manisfestations. The disease stems from inadequate oxygen transport by red blood cells. *In vivo*, sickled erythrocytes tend to block capillaries, causing stasis, and thereby starve organs of both nutrients and oxygen and eventually cause hypofunction or complete tissue destruction (Bunn, 1997; Imaga, 2013).

At low oxygen tension, the mutant haemoglobin polymerization within the red blood cells into a gel or further into fibres leads to a drastic decrease in the red cell flexibility, increase in fragility, decreasing its survival rate and subsequently causing haemolysis (Kitadi *et al.*, 2015). Polymerization of the sickled cells also causes peroxidation of membrane lipids that produces malondialdehyde which damages membrane structure, alter water permeability and decrease cell deformability. Phosphatidylserine is exposed on the outer cell surface from disruption of membrane phospholipids, enabling macrophages recognize these erythrocytes hence engulfing and degrading them(Nur *et al.*, 2011). The production of reactive oxygen species (ROS) can be grossly amplified in response to a variety of pathophysiological conditions such as hypoxia,

inflammation, infection, dehydration and deficiency in antioxidant vitamins and minerals (Bunn, 1997).

Sickle cell anaemia is one of the most common severe monogenic disorders in the world and it affects millions of people globally. It was estimated that 8% of black population in America and 40% of the population in certain countries of tropical Africa have the sickle cell gene (Emechebe *et al.*, 2017). Nigeria has the largest population of people living with sickle cell disorder, with about 150,000 births annually (Annie *et al.*, 2010). It is also responsible for death of 25% of children under the age of 5 years in Africa (Brabin *et al*., 2001). Development of cerebrovascular disease and cognitive impairments are main problems of SCA in children. Recurrent episodes of vaso-occlusion and inflammation result in progressive damage to most organs including: brain, kidneys, lungs, bone and cardiovascular system, which become apparent with increasing age (Mueller *et al.*, 2007). SCA primarily affects Africans and African Americans and their descendents (Mueller *et al*., 2007).

The deleterious effects of the excessive generation of free radicals or ROS can be prevented by the body's antioxidant defence mechanism which may include antioxidant minerals (copper, chromium, manganese and zinc) and enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Essien, 1995). Antioxidant minerals help in neutralizing the effect of free radicals by mopping them off which further limit the sickle cell disease and its attendant complications (Sies, 1997).

Sickle cell anaemics are under chronic oxidative stress induced by excessive production of free radicals from sickle cell haemoglobin (Adam *et al.*, 2001). This may overwhelm the normal antioxidant defences and deplete antioxidants including copper, chromium, manganese and zinc, and increases the frequency of sickling crises accompanied by tissue or organ damage (Adam *et al.*, 2001). Sickle cell anaemia crises (haemolytic anaemia, jaundice, painful swelling of hands and feets and skeletal changes due to erythroid hyperplasia) is becoming rampant and is highly life threatening most especially in paediatric patients. It is expected that this study will stimulate interests, discussion and further studies on the role of antioxidant minerals vis-à-vis complications of sickle cell anaemia. In this current study serum chromium, copper, manganese, zinc and body mass index (BMI) were evaluated in sickle cell anaemics and the results compared to those of apparently healthy non-sickle cell anaemics of comparable socio-economic status.

MATERIALS AND METHODS

Participants: The subjects employed for this study were 35 sickle cell anaemic patients of both sexes who were attending the Paediatric medical clinic of the Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. Also 25 apparently healthy participants of both sexes were recruited to serve as control. The consents of all the subjects were sought for and obtained. Ethical Committee approval was also obtained for the study.

Blood samples: Blood samples were collected by venipuncture and delivered into clean dry tubes and allowed to clot at room temperature. The samples were centrifuged at 3000 rpm for 5 minutes using benchtop centrifuge and the serum separated and kept in labeled sample bottles at (-20^0C) until required.

Reagents: All chemicals and reagents were of analytical grade and purchased from Sigma Chemical Company, USA.

Analytical methods: Serum levels of copper, chromium, manganese and zinc were analysed using AA 6300 SHIMADZU Atomic Absorption Spectrophotometer by method of Kaneko, 1999. BMI was calculated from the ratio of weight in kilogram and height in metre square (m^2) (Hagan *et al.*, 2008).

Data analysis: Data are presented as mean±standard error of mean (SEM) and separated on the basis of gender and age. Significant differences in mean at 5% level were determined using Student t-test. Significant difference was considered at $P<0.05$.

RESULTS

The results of the current work showed significant decrease *(P<0.05)* of antioxidant minerals in sickle cell anaemia compared to non-sickle cell anaemic subjects (Table 1). Gender and age appear not to have significant (*P<0.05*) effect on serum antioxidant minerals Table 1 and Table 2 respectively.

Table 1: Serum Antioxidant Minerals and BMI of Sickle Cell Anaemics (SCA) Subjects.

Parameter	Sickle cell anaemia (n=35)			Non-sickle cell anaemia (n=25)		
	Male (n=18)	Female (n=17)	Total (n=35)	Male (n=13)	Female (n=12)	Total (n=25)
Cr (µg/dL)	15.35±5.65[a]	10.21±2.87[a]	12.43±2.92[a]	28.23±9.43[b]	13.35±7.13[b]	20.44±5.93[b]
Zn (µg/dL)	7.69±4.75[a]	2.81±0.44[a]	5.33±2.46[a]	7.52±3.18[b]	11.29±4.43[b]	9.32±2.66[b]
Mn (µg/dL)	14.75±5.54[a]	7.71±2.22[a]	11.36±3.07[a]	12.25±5.24[b]	21.88±6.59[b]	17.07±4.24[b]
Cu (µg/dL)	7.21±0.74[a]	5.86±0.58[a]	6.55±0.48[a]	6.16±0.52[b]	7.63±1.02[b]	6.86±2.84[b]
BMI(kg/m^2)	14.63±0.43[a]	15.72±0.43[a]	15.12±0.64[a]	17.35±0.68[b]	18.01±0.83[b]	17.67±0.53[b]

Values are expressed as mean±SEM. Values bearing different superscripts differ significantly (P<0.05). BMI: body mass index; Cr: chromium; Zn: zinc; Mn: manganese; Cu: copper: n: number of participants.

Table 2: Age Distribution of Serum Antioxidant Minerals and BMI in Sickle Cell Anaemic Subjects.

Parameter	Sickle Cell Anaemic Subjects		
	1 - 5 Yrs (n=11)	6 – 10 Yrs (n=9)	11 – 15 Yrs (n=15)
Chromium (µg/dl)	10.94±2.56[a]	14.94±5.93[b]	11.62±5.33[b]
Zinc (µg/dl)	2.93±0.59[a]	12.70±9.44[a]	2.66±0.45[a]
Manganese (µg/dl)	4.14±1.04[a]	10.97±6.73[a]	16.55±4.36[b]
Copper (µg/dl)	6.58±0.99[a]	6.32±0.73[a]	6.67±0.78[a]
BMI (kg/m^2)	15.16±0.48[a]	13.88±0.73[a]	15.93±1.39[a]

Values are expressed as mean±SEM. Values show no significant different (P>0.05) using Student t-test.

DISCUSSION

Sickle cell anaemia is characterized by elevated oxidative stress via increased generation of ROS, and decline in antioxidant defences (Ayyub *et al.*, 2003). Increased oxidative stress is thought to play a role in the development of the disease and its complications (Ayyub *et al*., 2003). Antioxidant minerals (chromium, copper, manganese and zinc) are thought to be effective in increasing the activities of antioxidant defence enzymes, scavenging free radicals, preventing oxidative damage and thereby sparing lipid components of the cells against lipid peroxidation. Oxidative stress and hereditary disorder are suggested to be a potential contributor to the development of SCA and its attendant complications (Zingg *et al*., 2000). This might be connected to the fact that the antioxidant status may be inadequate in SCA subjects. The metabolic significance of the determination of antioxidant in SCA is therefore of paramount importance.

The current results indicate that serum copper, chromium, manganese and zinc of the sickle cell anaemic (SCA) subjects, in the study area were significantly (*P<0.05*) lower than the values obtained for non-sickle cell anaemic (NSCA) subjects. The results further reveal no significant *(P>0.05*) difference in levels of antioxidant minerals between male and female as well as age of SCA subjects.

The lowered levels of antioxidant minerals in SCA compared to control subjects are connected to increased oxidative stress in SCA patients, resulting in higher utilization of these minerals and consequently leading to their deficiencies. Thus, increased intake of synthetic or natural antioxidant minerals could help to reverse sickle cell anaemia and its complications (Bunn, 1997).

The decreased body mass index (BMI) in SCA could be due to poor socioeconomic and nutritional status in sickle cell anaemic subjects (Animasahun *et al.*, 2011). It could also be due to growth retardation and consequently lower BMI in SCA subjects (Prasad, 2002). And zinc supplementation in SCA was reported to reverse growth retardation and BMI (Prasad, 2002).

The non significant difference in levels of antioxidant minerals between male and female SCA subjects is in line of reports of Idonije *et al*., 2011; Bot *et al*., 2013; Digban *et al*., 2016 who reported similar to our findings. This is connected to the fact that similar pattern of antioxidant minerals were observed in both gender and age distribution (Digban *et al*., 2016).

CONCLUSION

In conclusion, there is significant low levels in serum antioxidant minerals and BMI in sickle cell anaemic subjects. Gender and age had no significant effect on antioxidant minerals.

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