

ORIGINAL ARTICLE

The impact of proteinuria on serum levels of trace elements in sickle cell disease patients

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Micronutrient deficiency has been recognized as a serious complication in sickle cell disease (SCD) patients and the impact of proteinuria on the levels of trace metals has not been evaluated in our setting. This study evaluates the impact of proteinuria on serum levels of copper, iron, zinc and magnesium in SCD patients. Serum Iron, Zinc, copper and magnesium were assayed by colorimetric methods. Urine protein was initially assayed using urinalysis dipstick method while those that were positive for macro-albuminuria were re-evaluated using sulphosalicylic acid colorimetric technique. Out of the 100 SCD patients, 29 had macro-albuminuria while 71 were negative for macro-albuminuria. Serum levels of copper in SCD patients was higher ($p < 0.001$) than that in controls, while serum levels of zinc, iron and magnesium were lower ($p < 0.001$) in SCD patients compared to that of the control group. The levels of copper ($p < 0.02$), iron ($p < 0.05$) and magnesium ($p < 0.05$) were significantly lower in SCD patients with proteinuria while Zinc ($p < 0.02$) was significantly higher in SCD patients with proteinuria compared to those without proteinuria. Proteinuria correlated negatively ($p < 0.001$) with copper while magnesium, iron and zinc correlated positively with proteinuria in SCD patients. Proteinuria in SCD patients impacted on the levels of measured trace metals and magnesium. The levels of these metals may be routinely determined in this group of patients.

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INTRODUCTION

Sickle cell disease (SCD) is known to interact with diverse gene and environmental factors to produce a multi-systemic disease with diverse clinical manifestations (Driss *et al.*, 2009). The red cells 'sickled' in reduced oxygen tensions, leading to the formation of insoluble polymers that aggregate into tubular fibers within the blood vessels (Platt *et al.*, 2004). This condition is characterized by chronic anaemia, pains, infections and multi-organ diseases due to irreversible sickling of the erythrocytes (Durosini *et al.*, 1993).

Inadequate nutrition has been recognized as a serious complication of the disease which must be treat-

ed as part of the required clinical management (Hyacinth *et al.*, 2010). Trace elements are essential micronutrients in humans and they act as cofactors for many enzymes, antioxidants and play important roles in human growth and development (Mahyar *et al.*, 2010). These trace elements include zinc, copper, selenium, manganese, chromium, fluorine, cobalt, iron and iodine.

Free radicals are generated in sickle cell disease; hence a balance between minerals and antioxidants is imperative in maintaining red cell membrane integrity and function (Okpuzor and Okochi, 2005). Protection of red cell membrane from free radical mediated oxidative stress is crucial to the management of sickle cell disease. Trace elements and magnesium levels have been evaluated in general population and sickle cell disease patients with conflicting findings (Okpuzor and Okochi, 2005; Bovio *et al.*, 2007). Some studies have addressed

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the association of SCD with a variety of micronutrient deficiencies (Reed *et al.*, 1987), but none to the best of our knowledge has associated proteinuria with micronutrients in SCD patients in Nigeria.

The concept of a relative shortage of nutrients for growth and development in SCD patients despite apparently adequate dietary intake has evolved. Anaemia is common in SCD leading to an elevated turnover of haemopoietic cells due to chronic hemolysis and cell death. This leads to hyper-metabolic rate and increases in micronutrient and energy demand. Proteinuria is an early manifestation of SCD related renal involvements and is considered to be a risk factor for developing renal impairment in future.

This study hypothesized that trace elements and magnesium may be further depleted in SCD patients with proteinuria. In the light of the above, there is need to evaluate the impact of proteinuria on levels of trace elements, which are necessary for the maintenance of membrane integrity and quality of life among SCD patients. Assessment of the urinary protein excretion is not only diagnostic but also has prognostic value in the monitoring of SCD severity. Prolonged glomerular hyperfiltration in SCD during childhood and early years may lead to glomerular injury resulting in glomerular sclerosis, proteinuria and progressive renal failure (Aleem, 2008; Emokpae *et al.*, 2010). The objective of this study was to evaluate the impact of proteinuria on serum levels of copper, iron, zinc and magnesium in SCD patients.

MATERIALS AND METHODS

This cross sectional case-control study was conducted in 150 participants of whom 100 were SCD patients in steady state aged 24.4 ± 1.4 years and 50 apparently healthy individuals with normal haemoglobin (Hb AA) aged 24.6 ± 0.4 years. The protocol was approved by the Ethical committee of the Edo state Ministry of Health, Benin City. All subjects who gave informed consent and met the inclusion criteria were enlisted into the study. Blood and early morning urine specimens were collected randomly from

confirmed sickle cell anaemia subjects attending the Sickle cell center, Benin City. Five milliliters of venous blood was collected aseptically by venepuncture with minimum stasis, from each subject into a dried plain container and was allowed to clot at room temperature.

The labeled samples were spun in a bucket centrifuge at a speed of 2500 rpm for 10 minutes to separate serum from red cells. The sera obtained were stored in a chest freezer at a temperature of -20°C until they were analyzed. Serum Iron, Zinc, copper and magnesium were assayed by colorimetric method using kits supplied by Centronic, Germany and Agape diagnostics, Switzerland. Commercially available control sera were included in the assay to ensure accuracy of analyses. Urine protein was initially assayed using urinalysis dipstick method while those that were positive for macro-albuminuria were re-evaluated using sulphosalicylic acid colorimetric technique.

Data analysis

Data were presented as mean \pm SEM and the mean of the study group and controls as well as those with and without proteinuria were compared using unpaired *t*-test. Pearson correlation coefficient was calculated between measured variables to assess the relationship between them. P-value less than 0.05 was considered statistically significant.

RESULTS

Out of the 100 SCD patients, 29 had macro-albuminuria while 71 were negative for macro-albuminuria. The mean \pm SEM of the measured variables in SCD patients and control subjects are shown in Table 1. Serum levels of copper in SCD patients was higher ($p < 0.001$) than that in controls, while serum levels of zinc, iron and magnesium were lower ($p < 0.001$) in SCD patients compared to that of the control group.

Table 2 shows the comparison of measured variables in 29 SCD patients with proteinuria and 71 SCD patients without proteinuria. The levels of Copper ($p < 0.02$) and Iron ($p < 0.05$) were significantly lower in SCD patients with proteinuria while

Zinc ($p < 0.02$) was significantly higher in SCD patients with proteinuria compared to those without proteinuria. Although magnesium level was lower in SCD patients with proteinuria, the difference was not however significant ($p > 0.05$).

Table 1: Serum trace element levels in SCD patients and Hb AA control subjects

Variable	SCD	Controls	P-value
Cu ($\mu\text{g dL}^{-1}$)	154.7 \pm 23.1	98.2 \pm 7.2	<0.001
Zn ($\mu\text{g dL}^{-1}$)	47.3 \pm 7.1	99.3 \pm 4.8	<0.001
Fe ($\mu\text{g dL}^{-1}$)	49.3 \pm 1.3	104.1 \pm 4.8	<0.001
Mg (mg dL $^{-1}$)	0.5 \pm 0.1	1.3 \pm 0.1	<0.001

Table 2: Comparison of measured variables in SCD patients with Proteinuria and SCD patients without proteinuria

Variables	Proteinuria	No Proteinuria	P-Value
Cu ($\mu\text{g dL}^{-1}$)	149.0 \pm 0.9	155.0 \pm 2.8	<0.02
Zn ($\mu\text{g dL}^{-1}$)	50.1 \pm 43.8	45.7 \pm 1.6	<0.02
Fe ($\mu\text{g dL}^{-1}$)	55.6 \pm 2.7	61.8 \pm 1.7	<0.05
Mg (mg dL $^{-1}$)	0.5 \pm 0.6	0.5 \pm 0.5	>0.05

Table 3 indicates that proteinuria correlated negatively ($p < 0.001$) with copper, while magnesium, iron and zinc correlated positively with proteinuria in SCD patients.

Table 3: Pearson correlation coefficient between Proteinuria and measured trace elements in SCD patients

Variables	n	R-value	p-value
Cu ($\mu\text{g dL}^{-1}$)	29	-0.142	<0.001
Mg (mg dL $^{-1}$)	29	0.210	<0.001
Fe ($\mu\text{g dL}^{-1}$)	29	0.115	<0.001
Zn ($\mu\text{g dL}^{-1}$)	29	0.194	<0.001

DISCUSSION

This data indicate that SCD patients with proteinuria generally had lower levels of measured trace elements (Cu and Fe) as compared to those without proteinuria apart from Zn which was significantly higher. Serum Zn, Fe and Mg correlated positively with proteinuria while Cu correlated negatively with

proteinuria in SCD patients. This observation is contrary to previous study in patients with normal haemoglobin who had proteinuria (Bovio *et al.*, 2007). Bovio *et al.*, (2007) reported that proteinuric patients had normal concentrations of measured trace elements, which were unrelated to renal function, total protein or albumin. However, positive correlation was reported between Zn and proteinuria (Bovio *et al.*, 2007).

Proteinuria is a better and sensitive marker of glomerular injury and has been observed to be an early indicator of sickle cell nephropathy (Abdu *et al.*, 2008). SCD with proteinuria may adversely affect micronutrients metabolism in a number of ways including, oxidative stress (Emokpae *et al.*, 2010), intestinal malabsorption and increased catabolism. SCD patients may develop glomerulopathy with proteinuria and could progress to End Stage Renal Disease (ESRD) if not well managed (Guasch *et al.*, 2006). The severity of which is higher in SCD as compared those with other sickling haemoglobinopathies (Guasch *et al.*, 2006). In order to compensate for this renal insufficiency, copper is released from tissues into the blood leading to the hypercupremia in SCD patients (Pfeiffer and Mailloux, 1987).

Proteinuria correlated negatively with copper in SCD patients. Hypercupremia may have negative impact on the proximal tubule of the nephron as the body attempts to eliminate excess copper (Pfeiffer and Mailloux, 1987). Hypozincemia as well as hypercupremia in SCD patients as previously been reported (Hasanato, 2006). Hypercupremia may be contributing to free radical production and oxidative stress in SCD patient, a condition that was previously observed to be high in SCD patients with proteinuria (Emokpae *et al.*, 2010). The significance of high level of serum Cu may also occur in response to decreased Zn level (Hyacinth *et al.*, 2010). The observed low Zn level in SCD patients from this study is consistent with earlier report (Kaur *et al.*, 2013). Zn is not only involve in the activation of over 300 metalloenzymes and it also plays important roles in growth, development, acts as an antioxidant, insulin secretion, immunity and

thyroid metabolism (Chausmer, 1998; Fraker *et al.*, 2000; Powell, 2000; Reily, 2006).

Despite the fact that, the reduced Mg level among SCD patients with proteinuria did not reach a significant level, serum Mg was significantly reduced among SCD patients compared to the control group. Reports on the levels of serum Mg in SCD patients have not been consistent. Whereas some studies reported normal circulating levels (Akenami *et al.*, 1999; Oladipo *et al.*, 2005), others observed decreased levels (Zehtabchi *et al.*, 2004). Low levels of Mg in SCD patients may result in cellular dehydration because of abnormally high red cell permeability and loss of potassium via K-Cl co-transport pathway (Zehtabchi *et al.*, 2004). This pathway is reported to be abnormally activated in low Mg levels (de Franceschi *et al.*, 1997). The irreversible loss of potassium and chloride ions and water which follows osmosis results in dehydration. Reduced levels of Cu, Fe and Mg accompanied with proteinuria may be due to excessive urinary excretion of these trace metals due to damaged glomerulus and loss of their carrier proteins in SCD (de Franceschi *et al.*, 1997). Repeated ill-health and frequency of hospitalization may affect eating habit and reduce feeding time in SCD (Singhal *et al.*, 2002). The observed decreased level of serum Fe from this study is consistent with previous studies (Durosinmi *et al.*, 1993; Okpuzor and Okochi, 2005). Low Fe status reported in SCD patients in developing vs. developed countries was attributed to differences in environment and socio-economic status (Hyacinth *et al.*, 2010).

CONCLUSION

Proteinuria in SCD patients impacted on the levels of measured trace metals and magnesium. Generally, the levels of these trace metals and magnesium were significantly lower in SCD patients compared with controls. The levels of these metals may be routinely determined in the management of patients to improve clinical outcome.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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