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Serum zinc, copper and magnesium in sickle cell disease at Ibadan, South Western Nigeria

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Summary
Serum zinc, copper and magnesium were studied in patients with sickle cell disease in the steady state. There was significantly lower serum zinc concentration ($P < 0.01$) and significantly higher serum copper and magnesium in haemoglobin S patients compared with controls (HbA). In haemoglobin SC patients, there was a significant reduction in serum zinc ($P < 0.01$) but no significant difference in serum copper and magnesium concentrations compared with the controls. There was no significant difference in serum zinc concentration between the HbS and HbS+C patients. However there were significantly higher serum copper and magnesium in HbS than HbS+C patients ($P < 0.01$) The level of serum zinc has no correlation with the steady state Haematocrit or severity index score in HbS and HbS+C patients.

Keywords: *Zinc, copper, and magnesium – sickle cell disease, steady state*

Résumé
Le taux serrique de zinc, cuiure et magnesium await ete etudie chez les drepanositaires etoant dans un etat stable. La concentration du zinc dans le serum etait tres basse ($P < 0.01$) et la quantite de cuivre et magnesium dons le serum etail forte chez les patients a hemoglobiness compare aux controls (HbA).
Chez les patients haemoglobine HbSC, il ya eu une reduction de zinc dans le serum ($P < 0.01$) mais pas de difference significative en concentration de cuivre et magnesium dans le urs serums compares aux controls. Il n'yawait pas dle difference significatives de concentration de zinc dans le serum entre les patients a HbS et HbSxC, mais il y await une quantite ssez elevee de cuivre et magnesium dans le serum des HbS que celui depatients HbSxC ($P<0.01$) Le taux serologioue de zinc n'est pas en relation arec l'etat stable de l'ematocrite ou l'index de severite chez les patients a lHbS et HbSxC

Introduction

Sickle cell anaemia is a chronic, commonly fatal hereditary haemolytic disease which is characterised by acute episodes of exacerbation of the disease often referred to as crises. Severe anaemia and often overwhelming infection are some of the clinical conditions responsible for early death in many of the patients [1,2]. Patients with the disorder are found worldwide, but there is a higher concentration in black Africa primarily in the sub-Saharan region. Migration of people from these regions to North and South America, the West Indies, the Mediterranean, Middle East and Far East is responsible for the geographical spread [1]. Patients with sickle cell disease have delayed sexual growth pattern and can have endocrinopathies [3]. The disease is usually complicated by growth retardation, chronic leg ulcers, and multiple end-organ damage [4].

Prasad reported the occurrence of clinical zinc deficiency in Iranian HbS boys with growth retardation and a failure to undergo sexual maturity. The signs were completely reversed by the addition of adequate zinc to the diet [7,8]. Flynn *et al.* [9] showed that some patients treated with cortico-steroids develop low serum zinc concentration and delayed wound healing and that wound healing was accelerated by adding zinc to the diet in those patients who have a low serum zinc concentration.

Furthermore, the role of copper and caeruloplasmin in iron mobilization is very important in chronic haemolytic anaemia. Copper deficiency could account for instances of resistant iron deficiency anaemia in milk-fed infants [5,6].
Considering the role of zinc in normal growth, wound healing, and the pathophysiology of sickle cell disease, it was decided that the level of zinc, copper and magnesium be estimated.

This is to determine the role trace elements might play in the pathogenesis of the disease in the steady state and also observe any correlation in the levels of zinc with the severity index score, haematocrit, transfusion requirements, bone pain crisis per year and organ enlargement.

Materials and methods

Thirty-five patients diagnosed to have sickle cell disease aged between 16 and 42 years were recruited into the study from the medical out-patients department of the University College Hospital, Ibadan. Twenty-five age and sex-matched subjects who are non-sickle cell anaemia patients, i.e., HbA, served as controls. The haemoglobin electrophoresis of each patient was re-confirmed using cellulose acetate paper electrophoresis at pH 8.4. The haematocrit, transfusion requirements, severity index score and the number of painful crisis per year were noted. The study was approved by the University College Hospital Ethical Committee and informed consent was obtained from all participants.

Blood sampling

Ten ml of venous blood was taken from each patient from the anterior cubital vein after the skin had been cleaned with methylated spirit. Blood specimens were divided into two sets of bottles for haematological and biochemical analyses.

a. Samples in Na EDTA bottles were used to determine full blood count and haemoglobin electrophoresis
b. Samples in non-anti-coagulant bottles (bijou) were used for estimation of serum zinc (Zn), copper (Cu) and magnesium (Mg) and were stored at – 20 °C before analysis.

Serum Zn, Cu, Mg estimation

Serum Zn, Cu, and Mg concentrations were measured using a pye-Unicam sp 90A series 2 atomic absorption Spectrophotometer (Pye Unicam Ltd. Cambridge, England) as described by the manufactures. Trace elements standards were obtained from SIGMA Chemical Company Ltd. (St. Louis, United States of America).

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Results

The mean serum zinc concentration in HbS patients was 9.6 nmol/L, 10.9 umol/L in HbS+C patients and 13.8 nmol/L in the control subjects. The results showed a significant difference in the mean serum zinc level ($P < 0.01$) between the HbS patients and controls. The mean serum zinc concentration in HbS+C patients was 10.9 nmol/L. There was also a significantly higher level of zinc in the controls compared with HbS+C. ($P < 0.1$) patients.

Table 1: Mean serum zinc, copper and magnesium levels in controls, HbS and HbS+C patients.

Biochemical Parameters	Control (n = 25) (HbAA)	HbSS (n = 23)	HbSC (n = 12)
Zinc (umol/L	13.8 ± 2.8	9.6 ± 2.4	10.9 ± 2.4
Copper (umol/L)	15.9 ± 5.5	23.9 ± 5.3	18.9 ± 8.2
Magnesium (umol/L)	0.81 ± 0.16	0.89 ± 0.12	0.78 ± 0.07

There was no significant difference in the level of serum zinc between the HbS and HbS+C patients. The serum copper concentration in the HbS patients was 23.9 nmol/L while in the controls was 15.9 nmol ($P < 0.01$) the difference was significant. Mean serum copper in HbS+C patients was 18.9. There was a significant difference ($P < 0.1$) in mean serum levels of copper between the HbS+C patients and controls.

Table 2: Comparison of mean serum zinc, copper and magnesium levels in control and HbSS patients.

Biochemical parameter	Control (n = 25) (HbAA)	HbSS (n = 23)	P-value	Signi ficance
	(n = 25)	(n = 23)	T-value	
Zinc (umol/L)	13.8 ± 2.8	9.6 ± 2.4	$P<0.01$ (5.1)	S
Copper (umol/L)	15.9 ± 5.5	23.9 ± 5.3	$P<0.01$ (5.1)	S
Magnesium (umol/L)	0.81 ± 0.16	0.89 ± 0.12	$P<0.05$ (2.1)	S

Table 3: Comparison of mean serum zinc, copper and magnesium levels in control (HbAA) and HbSS patients.

Biochemical Parameters	Control (n=25) (n = 25)	HbS+C (n = 12)	P-value T-value	Significance
Zinc (umol/L)	13.8 ± 2.8	10.9 ± 2.4	$P<0.01$ (3.1)	S
Copper (umol/l)	15.9 ± 8.2	18.9 ± 8.2	$P>0.01$ (1.3)	NS
Magnesium (umol/L)	0.81 ± 0.16	0.89 ± 0.12	$P>0.2$ (0.02)	NS

However the mean serum magnesium in HbS patients was 0.89 nmol/L. In the controls it was 0.81 nmol/L. The difference was significant ($P<0.05$), while in HbS+C the mean serum was 0.78 nmol/L the difference was not significant ($P > 0.2$). There was no significant correlation between the serum levels of zinc and the steady state haematocrit, and the severity index score.

Discussion

Zinc is an essential element in plants, microorganisms, and animals. A deficiency of zinc has been associated with poor wound healing and failure of sexual maturity while an excess results in toxicity. Deficiency of zinc states were reversed quickly with the addition of zinc supplement to the diet[10].

Deficiency of zinc in sickle cell disease has been recognised by Prasad et al. [11, 12, 13]. It is known that zinc is an important constituent of erythrocyte and since patients with sickle cell disease undergo a chronic haemolytic state, this can explain the deficient zinc state and some clinical manifestations like chronic leg ulcers, wasting and infarctive crisis often observed in some of these patients. Zinc also plays a biochemical role as a metallo-enzyme and biochemical functions in which zinc has been implicated as necessary include enzyme function, protein synthesis and nucleic acid metabolism [11]. Zinc plays a role in amino-acid metabolism particularly cysteine and methionine. Deficiency of zinc has been associated with vesicular dermatitis, alopecia and diarrhoea. There is also the condition referred to as acrodermatitis entero-pathica caused by defective absorption of zinc from the gastro-intestinal tract [11].

Since zinc is an important constituent of erythrocytes [14], the chronic haemolysis in these patients can lead to a zinc deficient state and could account for some of the clinical manifestations like chronic leg ulcers, wasting and infarctive crisis observed [13]. Zinc therapy has been documented to result in weight gain, increased growth of public, facial and body hair and healing of leg ulcers in a few weeks [15]. Recent studies have demonstrated a potential beneficial effect of zinc on the sickling process while in one uncontrolled study, zinc appears to have been effective in decreasing symptoms and crises of sickle cell anaemia patients [15]. Although the results in this study indicate significantly reduced serum zinc in patients with sickle cell anaemia, the low levels bear no relationship with the different degrees of clinical severity of the disease in these patients.

As Phebus suggested, zinc deficiency might be due to renal loss and most sickle cell anaemia patients do infact have renal complications. It was also observed in this study, that the level of zinc in each patient has no correlation with the haematocrit, susceptibility to infection, blood transfusion requirement, frequency of bone pain crises and organ enlargement.

The higher mean serum copper (Cu) concentration in sickle cell anaemia patients compared to that of the control group confirms the findings of Olatunbosun et al. [16] in a survey carried out in Ibadan. The low zinc and high copper recorded in this present study could be as a result of the natural antagonism of these two elements. Copper is important in red cell metabolism and has a vital role in iron absorption and in haemoglobin synthesis. Increases in serum Copper levels have also been reported in aplastic anaemia, pernicious anaemia and in various other haemolytic disorders such as Thalassaemia [11].

In this study, the mean serum copper was significantly higher in HbS and HbS+C compared with the controls. There was however no correlation between the serum copper and serum haematocrit, frequency of painful crisis and transfusion requirement. The mean serum magnesium (Mg) concentration in sickle cell anaemia patients was significantly higher than in the controls in this study.

The raised serum magnesium (Mg) could be due to the release in a chronic haemolytic state. There is paucity of information regarding serum magnesium concentration in sickle cell anaemia.The findings in the present study suggests a need for an expanded investigation of trace elements in sickle cell

disease and to include other trace elements and more patients from other locations. This would help establish the possible role of these elements in the pathogenesis of sickle cell disease and help determine management strategies.

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