



Changes in serum zinc, magnesium and copper in sickle cell patients: A case study in Jos, Nigeria

¹Asanga Edet E.*, ²Eseyin Olorunfemi A, ³Ekanem Aniebiet N, ¹Amaechi Dennis

¹Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria

²Department of medicinal and pharmaceutical chemistry, University of Uyo, Akwa Ibom State, Nigeria

³Federal School of Medical laboratory science, Jos, Plateau State, Nigeria.

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ABSTRACT

This research was designed to understudy trace elements (zinc, magnesium and copper) implications in 120 subjects (60 sickle cell anemia patients and 60 non-sickle cell individuals) attending clinic at Bingham University Teaching Hospital, Jos, Plateau State, Nigeria. Their blood samples were collected and their sera used for the estimation of zinc, magnesium and copper using inductively coupled Plasma-optical emission spectrometry technique. Their Mean \pm SEM values for zinc, magnesium and Copper in 60 sickle cell anemic patients are 64.5 ± 5.9 $\mu\text{g/dL}$, 5.7 ± 1.7 mg/L and 2.1 ± 0.1 mg/L , respectively. While those of normal or non-sickle cell patients i.e. control were 94.5 ± 4.8 $\mu\text{g/dL}$, 16.8 ± 2.9 mg/L and 1.9 ± 0.1 mg/L , for zinc, magnesium and copper, respectively). The result showed significant decrease ($p < 0.05$) in zinc and magnesium levels of sickle cell patients when compared with non-sickle cell subjects (control). Copper which have been implicated in antagonizing zinc for the same binding site showed no significant change in sickle cell patients when compared with the Hb-AA control group. Growth retardation factors (height and weight) significantly decreased ($p < 0.05$) in sickle cell patients when compared with control group. These results confirm that zinc and magnesium are deficient in sickle cell patients hence, trace element supplementation may be necessary in the management of sickle cell disease as it will arrest oxidative stress and per-oxidation with resultant stability of erythrocyte membranes as well as reduction in the rate of hemolysis and other symptoms often noticed in sickle cell anemic patients.

Key Words: zinc, copper, magnesium, sickle cell, Jos, anemia

INTRODUCTION

Sickle cell anemia is the most common inherited disorder of black race in the world. The sickle-cell trait is now known to be widespread, reaching its highest prevalence in parts of Africa as well as among people with origins in equatorial Africa, the Mediterranean basin and Saudi Arabia. Report by WHO [29] showed that, in Africa the highest prevalence of sickle-cell trait occurs between latitudes 15° North and 20° south, ranging between 10% and 40% of the population in some areas. Prevalence levels decrease to between 1% and 2% in North Africa and to less than 1% in southern Africa. In countries such as Nigeria, Cameroon, Republic of Congo, Ghana and Gabon, the prevalence is between 20% and 30% while in some parts of Uganda it is as high as 45%. In countries where the trait prevalence is above 20% the disease

affects about 2% of the population [29]. Moreover, countries around Nigeria also have an S gene carrier frequency of about 1 in 4 of their populations; Nigeria's large population has ensured that over 40 million Nigerians are healthy carriers of the S gene. This number of carriers far exceeds the total population of every other affected African country and indeed, of several of them put together. Consequently, about 150,000 Nigerian children are born each year with sickle cell anaemia (Hb-SS), the prevailing type of sickle cell disorder (SCD) in this Region [2]. Sickle cell diseases affects red blood cells resulting in chronic anemia of varying severity and in some patients, periodic painful crisis caused by occlusion of small blood vessels by spontaneous intravascular sickling with multi-organ affectation [25, 10]. Some of the complications are growth retardation, acute chest syndrome, impaired immune functions and

*Corresponding Author Address: Asanga Edet Effiong, Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Nigeria, E-mail: edyasangae1@yahoo.com

increased [9], oxidative stress and peroxidation as well as low anti-oxidant potential which predispose the patients to crisis [27]. The global use of micronutrient in health care delivery system has taken central stage due to the realization of their importance in disease management. People with sickle cell disease suffer from micronutrients deficiency [9] but preliminary research on dietary habits, shows that foods and nutrients intake by sickle cell patients meet or exceed recommendation and is not significantly different from healthy controls, this suggest that higher rates of nutrients deficiency may be due to increased needs of many nutrients in sickle cell patients [26]. Magnesium, zinc and copper are of great benefit towards the relief of oxidative stress associated with RBC cell membranes [24] as well as the fact that the later two are known to compete with each other for similar binding sites in the body [14]. This study was objectively designed to evaluate and determine the level of magnesium, zinc and copper in sickle cell patients as well as provide an insight whether these trace elements supplementation could contribute to reduction of the various symptoms suffered by sickle cell patients.

MATERIALS AND METHOD

Study Population and Area: Blood samples were obtained from 60 sickle cell patients and 60 non sickle cell individuals aged between 1 and 35 years. The blood samples were obtained from patients attending clinic at Bingham University Teaching Hospital, Jos, Plateau State, Nigeria. Their consents were sought and samples were obtained only from subjects that gave their consents.

Ethical Approval: Ethical approval was sought from the management of Bingham University Teaching Hospital, Jos, Nigeria and it was granted prior to collection of blood samples from patients.

Collection of Samples: After obtaining the consents of patients and caregivers, demographic and anthropometric data and history of the disease and treatment were collected by physical examination, medical records and laboratory measurements. Weight and height were measured by using standard methods by corresponding health workers. Weight measurement was performed using Seca scale with a precision rate of 100gr with no shoes and minimum covering. The height, while in standing position, was measured from head to heel using metal scale with a precision rate of one cm. The validity and reliability of instruments were checked on a regular basis. Since there are standard International serum values for zinc, magnesium and copper in human beings, the control group was no more needed [13]. Other information collected

were: number of blood transfusion per months, drugs taken for management of their bodies health, treatment with hydroxyurea, use of vitamins and trace element supplements other than folic acid. Blood (5ml) was collected from each subject by venous puncture and transferred into ion free non-heparinized bottles previously washed clean of all contamination by rinsing each bottle three times with deionized water and air dried before use. The clotted blood sample was centrifuged at 3,500 rates per minute for 10 minutes and the serum removed with ion free Pasteur pipette prepared same as the blood bottles above. The samples were then stored at -20°C pending analysis and were kept at assumed room temperature prior to analysis.

Determination of magnesium, zinc and copper levels: The determination of magnesium, zinc and copper levels was performed using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) technique as described by Nixon and Moyer [15].

Statistical analysis: The data obtained in this study were analyzed using Statistical Package for Social Sciences (SPSS) version 15, Student t-test was used to compare their means for significance at $p < 0.05$ confidence level. Results are presented as Mean \pm Standard Error of Mean (Mean \pm SEM) in milligram/liter (mg/L) and microgram /deciliter ($\mu\text{g/dL}$).

RESULTS AND DISCUSSION

The Mean \pm SEM values for zinc, copper and magnesium in 60 sickle cell anemic patients are $64.5 \pm 5.9 \mu\text{g/dL}$, $2.1 \pm 0.1 \text{ mg/L}$ and $5.7 \pm 1.7 \text{ mg/L}$ respectively. When compared to those of normal, non-sickle cell patients ($94.5 \pm 4.8 \mu\text{g/dL}$, $1.9 \pm 0.1 \text{ mg/L}$ and $16.8 \pm 2.9 \text{ mg/L}$, for zinc, copper and magnesium respectively), the values of zinc and magnesium was significantly different when compared with those from the control subjects.

Sickle cell disease has been known to cause the deficiency of essential elements, some of which are vital in red cell maintenance, body growth and development [8, 17]. The present study showed that 68.3% of sickle cell patients had hypozincemia when compared with the control subjects (Table 1). The observed result may be attributed to several factors including the chronic haemolysis that characterizes sickle cell anaemia leading to loss of zinc from red blood cells which is an important storage site for zinc, insufficient amount of zinc in daily meals, abnormality in urinary absorption of zinc, kidney dysfunction, urinary secretion of zinc, disturbance in zinc metabolism and higher level of

zinc excretion in sweat [4]. The result was consistent with earlier reports by Prasad *et al* [23]; Leonard *et al* [12]; Hasanato [9] who related zinc deficiency in sickle cell disease to manifestations such as growth retardation, hypogonadism in males and cell mediated immune disorders. Similarly, Idonije [32] reported some biochemical evidence for zinc deficiency in patients with sickle cell disease to include low zinc concentrations in plasma, erythrocytes and granulocytes. In another study by Prasad *et al* [22], low activities of zinc dependent enzymes such as carbonic anhydrase, alkaline phosphatase and thymidine kinase have also been reported.

The international acceptable normal value for zinc is 60 – 150 microgram/dL [5]. The results obtained from the subjects based in Jos, Nigeria were within the acceptable range for both the sickle cell patients and the control groups. This was inconsistent with the report by Akinkugbe and Ete [1] on low zinc levels common in Nigerian children. Although, the values were closer to the lower limit of the standard reference range, it may be due to high consumption of grains and cassava based diets in Jos which have been reported to contain limited zinc contents due to high phosphate and phytate contents. Zinc may form an unavailable complex with these compounds [5, 18].

Zinc deficiency is associated with poor growth among other clinical manifestations [7, 28]. In this study, the control group had higher weight and height than the sickle cell anemia group. This may be attributed to growth retardation associated with zinc deficiency. Hence, in sickle cell anemia patients, due to painful crisis with concomitant oxidative stress, more zinc is utilized.

Moreover, copper level in the sickle cell group showed insignificant increase ($p > 0.05$) when compared with the control group. This was inconsistent with the reports by Hasanato [9] on significant higher copper level in sickle cell patients when compared with the control group but was in agreement with the report by Alayasha [3]. The serum concentration of copper in Patients with

sickle cell disease depend on several factors including the amount of Copper taken in their daily diets, intestinal uptake of copper, iron accumulation, kidney function, copper to zinc ratio [6].

Furthermore, the present study showed that 33.9% of sickle cell patients had hypomagnesiumemia when compared with their control subjects. Significantly low serum magnesium in sickle cell subjects had been reported in previous studies [31, 32]. They proposed that the low level could contribute to red blood cell dehydration and concomitant increase in the symptoms of sickle cell disease.

For emphasis, the results in this study are seen to be higher than that those obtained by Okochi and Okpuzor [16]; Temiye [28] in Nigeria. This may be as result of the difference in the sensitivity of the instruments used for the analysis. In the past, it was Atomic Absorption Spectrophotometer (AAS) that was being used but in this study Inductively Coupled Plasma-Optical Emission Spectrometer (ICP - OES) which is seen to be more sensitive was used. Thus, it measures almost up to the least fraction of the analyte present in the sample.

CONCLUSION

The observed results in this study are in tandem with earlier reports by several researchers, hence, trace elements metabolism are implicated in sickle cell disease. Therefore, the deficiency of trace elements may in turn affect the stability of red blood cell membrane which is evidenced as hemolysis in sickle cell patients. It is recommended that changes in the levels of other trace elements in sickle cell subjects be investigated. Also, other body fluids such as urine, cerebrospinal fluid, sweat, human hair are used for further studies.

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Table I: serum zinc ($\mu\text{g/dL}$), copper (mg/L) and magnesium (mg/dL) levels in various age range for patients with and without sickle cell anemia.

PARAMETERS	TEST (PATIENTS WITH SICKLE CELL)			CONTROL (PATIENTS WITH NO SICKLE CELL)		
	AGE RANGE (YEARS)	NO. OF SAMPLES	RESULT	AGE RANGE (YEARS)	NO. OF SAMPLES	RESULT
ZINC	1 – 10	24	80.6 ± 9.6	1 - 10	15	98.3 ± 9.3
	10 – 20	14	53.4 ± 12.4	10 – 20	19	$96.2 \pm 8.6^*$
	20 and above	22	53.8 ± 8.6	20 and above	26	$91.1 \pm 7.4^*$
COPPER	1 – 10	24	2.1 ± 0.1	1-10	15	1.8 ± 0.1
	10 – 20	14	2.2 ± 0.2	10-20	19	1.9 ± 0.1
	20 and above	22	1.9 ± 0.1	20 and above	26	1.9 ± 0.1
MAGNESIUM	1 – 10	24	4.8 ± 3.6	1 – 10	15	$14.8 \pm 2.1^*$
	10 – 20	14	6.5 ± 0.9	10 - 20	19	$18.1 \pm 4.4^*$
	20 and above	22	5.7 ± 0.5	20 and above	26	$17.6 \pm 2.3^*$

Mean \pm SEM, *P < 0.05

Table 2: The relationship between mean serum zinc concentrations and different risk factors in subjects with sickle cell anemia

Risk factors	Zinc $\leq 60 \mu\text{g/dL}$ Mean \pm SEM (N=45)	Zinc $> 60 \mu\text{g/dL}$ Mean \pm SEM (N=15)	P value
Weight (Kg)	34.88 ± 6.17	43.66 ± 10.07	0.3
Height (m)	1.40 ± 0.63	1.42 ± 0.77	0.8
Body mass index (Kg/m ²)	17.80 ± 6.43	21.61 ± 7.78	0.4
Number of blood transfusion (per month)	1.93 ± 0.61	1.84 ± 0.56	0.7

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