Original Article

Assessment of Zinc Level and its Relationship with Some Hematological Parameters among Patients with Sickle Cell Anemia in Abakaliki, Nigeria

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Abstract

Background: Sickle cell anemia (SCA) is an inherited hemoglobin (Hb) disorder with susceptibility for oxidative damage due to chronic redox imbalance in red blood cells (RBCs) which often results in hemolysis, endothelial injury, recurrent vaso-occlusive episodes, and derangement in hematological parameters. Zinc is an antioxidant which helps to reduce oxidative damage. This study aimed to assess the serum level of zinc and its relationship with some hematological parameters in patients with SCA (HbSS). **Materials and Methods:** This was a cross-sectional comparative study which involved HbSS patients in steady state with sex- and age-matched HbAA control. Assay of Hb phenotype, serum zinc level, and some haematological parameters were done. Ethical approval was gotten from the institutional review board, and each participant gave informed written consent before recruitment into the study. Analysis of all data obtained was done using SPSS software, version 20. **Results:** Thirty adult patients with SCA and thirty sex- and age-matched controls with a mean age of 26.7 ± 7.6 years and 27.7± 5.3, respectively, were studied. There was a significant decrease in serum zinc level among patients with HbSS compared to those with HbAA (P = 0.038). Similarly, patients with HbSS had significantly lower Hb level, packed cell volume, and RBC count compared to HbAA control ($P < 0.05$). On the contrary, patients with HbSS had significantly higher white cell count and platelet count compared to HbAA individuals ($P < 0.05$). Correlation between serum zinc level and blood counts showed weak positive relationship between zinc level and Hb level (r = 0.04, P = 0.8) and weak negative relationship between serum zinc level and platelet count (r = −0.3, P = 0.1), as well as zinc and white blood cell (WBC) count (r = −0.2, P = 0.4). **Conclusion:** There was a significantly low level of zinc among patients with HbSS compared with HbAA controls. Patients with HbSS had zinc levels that weakly correlated with Hb level positively but weakly negatively correlated with platelet and WBC counts. Further studies are required on a wider scale to assess whether zinc supplementation may improve blood counts in patients with SCA.

Keywords: Hematological parameters, red blood cell, sickle cell anemia, zinc level

Introduction

Sickle cell anemia (SCA) is a genetic hemoglobin (Hb) disorder characterized by chronic hemolytic anemia and recurrent vaso-occlusive events.[1] It is a homozygous, monogenic autosomal recessive disorder that occurs as a result of a point mutation in the genetic code on chromosome 11, in which valine replaces glutamic acid at position six of the beta-globin amino acid chain.[2] In conditions of low oxygen tension, the abnormal Hb crystallizes and form tactoids which deforms the normal red cell shape from biconcave to sickle shape.[3] The abnormally shaped and rigid red cells are not able to pass through the microvasculature easily, resulting in occlusion of small blood vessels with associated ischemic-reperfusion injury, inflammation, and excessive production of reactive oxygen radicals including superoxide and hydrogen peroxide.[4] Even though patients with SCA have the same genetic abnormality, they usually present with

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unexplained variable clinical manifestations and disease severity.

It has been reported that patients with SCA have inefficient antioxidant defense system as well as low zinc level.[5,6] Antioxidant deficiency leads to chronic oxidative stress which play critical function in the development of microvascular dysfunction, damage to cellular macromolecules, and multiorgan dysfunction.[7] In addition, research has portrayed that SCA patients usually have high white blood cell (WBC) count and platelet count, which are known to be associated with disease severity.[8,9]

Zinc is an essential trace element and the most common mineral in the body after iron. It is the only metal that occurs in all enzyme classes found in every cell with an important role in wound healing, immune system, reproduction, growth, blood clotting, smell, vision, taste, proper insulin, and thyroid function and has antioxidant properties.[10,11] Zinc has been reported to inhibit calcium binding to the membrane of red blood cells (RBCs) which is responsible for the formation of irreversible sickled RBCs in addition to inhibition of lipid peroxidation of cells.[12] This helps to stabilize biomembranes, thereby protecting the body against oxidative stress. It is believed that the ability of zinc to reduce the vaso-occlusive crisis in SCA is consequent upon these effects.[13] Other reported effects of zinc supplementation in SCA include a reduction in the incidence of vaso-occlusive crises, reduced incidence of infection, enhanced wound healing, and growth.[14,15] Several studies have reported low zinc level in patients with SCA.[16,17] To the best of our knowledge, no published report in the literature had evaluated zinc level and its relationship with blood counts among patients with SCA in our locality. This study, therefore, aimed to determine zinc level in patients with HbSS (SCA) and compare it with that of persons with HbAA control as well as to establish the relationship between zinc level and some hematological parameters in patients with SCA.

Materials and Methods

Study area

The study area was Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria, between June 2019 and March 2020.

Study population

The study population was made up of patients with HbSS in steady state aged 18–47 years with age- and sex-matched HbAA control.

Study inclusion and exclusion criteria

The following inclusion criteria were used to recruit participants into the study – patients with HbSS (SCA) confirmed with Hb electrophoresis, patients who have not received blood transfusion in the preceding 3 months to the study, and those not on hydroxyurea or zinc supplementation, absence of febrile illness in the preceding 4 weeks.

While patients who failed to give consent, those with febrile illness, history of blood transfusion in the preceding 3 months into the study, those on hydroxyurea or zinc, pregnant women, and those with HIV or other comorbidities were excluded from the study.

Study design

This was a cross-sectional comparative study involving patients with HbSS in steady state and HbAA controls (confirmed with Hb electrophoresis in alkaline medium).

Steady state was defined as a period without any acute illness, pain, and infection for at least 4 weeks before recruitment and no blood transfusion in the preceding 3 months.

Data collection methods

Patients were recruited consecutively from the sickle cell clinic of Alex Ekwueme Federal University Teaching Hospital, Abakaliki. Age- and sex-matched controls with HbAA were recruited from among medical students and staff.

Information on sociodemographic characteristics (including age, sex, date of last crisis, blood transfusion history, and comorbidities) and clinical status were obtained using semi-structured interviewer-administered questionnaire and review of medical records.

The skin overlying any prominent vein at the antecubital region or dorsum of the hand was thoroughly circularly cleaned with methylated spirit and allowed to air dry. Four milliliters of blood was collected using size 21G needle with an attached syringe. Dry cotton wool was placed at the site of blood collection, and gentle pressure was applied until the bleeding stopped. 2.5 ml of blood was dispensed into ethylenediaminetetraacetic acid (EDTA) bottle and the remaining 1.5 ml of blood into a plain bottle and allowed to stand for 2 h at room temperature to clot and subsequently centrifuged at 3000 g for 5 min using the universal benchtop centrifuge model 80-2 (Gallenkomp, England). The serum extracted was transferred to another plain bottle and stored at −20°C freezer till thawed at room temperature and analyzed for zinc level using a spectrophotometer (Spectrumlab 752s, Gallenkomp, England) and zinc reagent kit supplied by Centronic GmbH Wartenberg, Germany. Blood in EDTA bottle was used for the analysis of Hb phenotypes using Hb electrophoresis by cellulose acetate method in an alkaline medium and for the analysis of full blood count using hematology analyzer BC-2800 (manufactured by Shenzhen Mindray Bio-Medical Electronic Co. Ltd, Germany). Each blood sample collected was well mixed, applied to the machine, and approximately, 20ul of blood was aspirated and analyzed.

Data analysis

Data collected were analyzed with SPSS software version 20 (SPSS Chicago Inc., IL, USA). Percentages, proportions, means, and standard deviation were computed with descriptive statistics. Association between the mean values of zinc and blood counts was explored using Pearson's linear

regression for bivariate correlation. Statistical significance was established when probability, $P < 0.05$.

Ethical consideration

Ethical approval for this study was gotten from the Research and Ethics Committee of Ebonyi State University as well as that of Alex Ekwueme Federal University Teaching Hospital, Abakaliki. Each enrolled participant gave informed written consent.

Results

Sixty participants were recruited and were made up of thirty SCA (HbSS) patients and thirty sex- and age-matched HbAA controls. Among the SCA patients were 17 (56.7%) females and 13 (43.3%) males with the mean age of 26.7 ± 7.6 years. Majority of them were single, students, and attained secondary educational level. The mean age of the control group was 27.8 ± 5.3 years [Table 1].

Patients with HbSS had significantly lower mean zinc level compared to HbAA individuals ($P = 0.038$). Similarly, patients with HbSS had significantly lower Hb level, packed cell volume, and RBC count compared to HbAA control ($P < 0.05$). On the contrary, patients with HbSS had significantly higher white cell count and platelet count compared to HbAA individuals [Table 2].

Result of this study also showed that patients with HbSS who had severe anemia with Hb value of <7 g/dl had lower zinc level than those with Hb value >7 g/dl, though this was not significant ($\chi^2 = 0.017$, $P = 0.8$).

Table 1: Sociodemographic features of the participants

Parameter	HbSS, *n* (%)	HbAA control, *n* (%)
Mean age	26.7±7.6	27.8±5.3
Gender		
Male	13 (43.3)	14 (46.7)
Female	17 (56.7)	16 (53.3)
Total	30 (100)	30 (100)
Marital status		
Single	27 (90)	21 (70)
Married	3 (10)	9 (30)
Total	30 (100)	30 (100)

HbSS: Hemoglobin SS, HbAA: Hemoglobin AA

Table 2: Zinc and hematological parameters among patients with hemoglobin SS and hemoglobin AA

Parameter	Mean±SD		*P*
	HbSS	HbAA	
Zinc level (ug/dl)	129.3±19.1	134.2±30.3	0.038
Hemoglobin level (g/dl)	7.7±1.8	14.4±1.4	<0.0001
Packed cell volume (%)	23.1±5.2	42.3±4.6	<0.0001
Red blood cell count ($\times 10^{12}$/l)	2.9±0.8	4.9±0.5	<0.0001
White blood cell count ($\times 10^9$/l)	13.3±7.3	6.3±1.6	<0.0001
Platelet count ($\times 10^9$/l)	355.1±131	277.2±83.5	0.008

HbSS: Hemoglobin SS, HbAA: Hemoglobin AA, SD: Standard deviation

Patients with HbSS who had thrombocytosis with platelet count of > 400 × 10^9/l had lower zinc level compared to those who do not have thrombocytosis with platelet count of ≤400 × 10^9/l. However, this was not significant ($\chi^2 = 1.655$, $P = 0.1$).

In the same way, a greater proportion of patients with HbSS who had leukocytosis with WBC count >11 × 10^9/l had lower zinc level than those with white cell count within the normal reference range of 4–11 × 10^9/l ($\chi^2 = 26.06$, $P = 0.000$).

Correlation between serum zinc level and blood counts showed weak positive relationship between zinc level and Hb level ($r = 0.04$, $P = 0.8$) [Figure 1]. In addition, there was weak negative relationship between serum zinc level and platelet count ($r = -0.3$, $P = 0.1$) [Figure 2], as well as zinc level and WBC count ($r = -0.2$, $P = 0.4$) [Figure 3].

Discussion

Finding from this study showed lower zinc level among patients with HbSS (SCA) in steady state compared with HbAA control which was significant. This observation is consistent with previous reports of reduced zinc levels in SCA.[18,19] Despite normal diet, the low zinc level may be due to increased hemolysis often associated with SCA which releases a considerable amount of zinc into the plasma. This is because RBCs are important storage sites for zinc, resulting in increased demand and consumption. Increased hemolysis results in increased turnover of hemopoietic cells, leading to tremendous red marrow expansion. These conditions lead to hypermetabolic rate and increase in energy and nutrient demand including zinc.[20] Another explanation for low zinc level in patients with SCA is elevated zinc loss in urine which is likely due to impaired renal tubular reabsorption of zinc, presumably due to renal tubular damage due to repeated vaso-occlusive events.[21] Hyperzincuria resulting from increased hemolysis raises the daily zinc requirement significantly in patients with SCA which is not met by the usual dietary intake.[21] Furthermore, bone is the main storage site for zinc and increased bone degradation in SCA consequent upon recurrent bone ischemia, particularly during painful crisis may also add to the elevated loss of zinc in urine.[22] Thus, zinc may be lost by multiple mechanisms in people with SCA.

This study also found that there was significantly lower Hb concentration, packed cell volume, and red cell count among patients with SCA in steady state. This finding also agrees with the report of previous studies.[17,23] These results were not surprising because patients with SCA suffer from continuous hemolysis of red cells, with a short survival rate of the RBCs. Hence, the Hb values, packed cell volume, and RBC count are usually lower than normal healthy (HbAA) individuals. In addition, the rate of increase in Hb concentration in response to anemia is not proportional to the degree of anemia due to blunted response to erythropoietin secretion.[24] The reason may be because renal pathology occurs in SCA which may affect erythropoietin production.[25] It has been shown that Hb S releases oxygen more easily to the tissues compared to Hb A

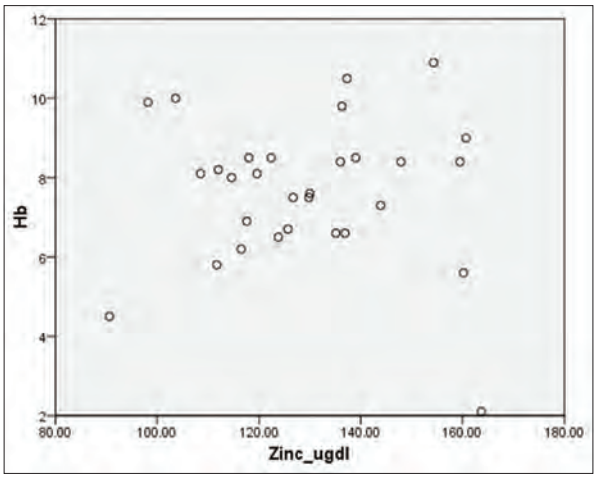

Figure 1: Correlation between zinc and hemoglobin level

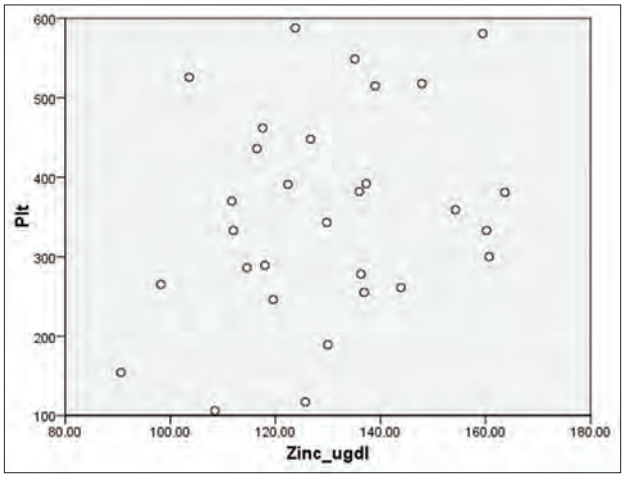

Figure 2: Correlation between zinc and platelet count

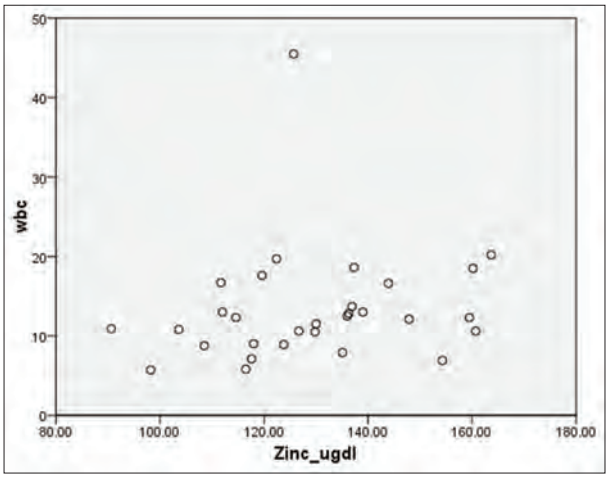

Figure 3: Correlation between zinc and white blood cell count

and that may explain the reason why individuals with SCA are usually stable at lower Hb concentrations compared to those with HbAA. On the other hand, raising the packed cell volume to over 30% could increase blood viscosity with resultant risk of vaso-occlusive crisis and other adverse consequences.

The mean total WBC count among patients with SCA was significantly higher than that of HbAA individuals. This is similar to findings from previous studies which also reported an increase in total white cell count in individuals with SCA.[8,9] This may be due to redistribution of WBCs between the marginal and circulating pools, which has been reported to be associated with factors such as pain, anxiety, and inflammation in the absence of infection.[8] Leukocytosis in SCA may also be due to autosplenectomy resulting from recurrent splenic vessel occlusion.[26]

This study also found that the overall mean platelet count was significantly higher in HbSS participants compared to those of HbAA individuals. Reports from previous studies gave similar findings.[17,27] In adult sickle cell patients, loss of splenic platelet pool consequent upon autosplenectomy may have contributed to higher mean platelet count in SCA patients compared to HbAA individuals. Besides, thrombocytosis observed in SCA may be consequent upon the negative feedback effect of erythropoietin production in SCA in response to anemia. About half of thrombopoietin structure has similarity to erythropoietin at the N-terminal end. Thrombocytosis is therefore known to be associated with certain chronic anemias.[28]

This study showed a positive correlation between the serum zinc and Hb concentration among patients with SCA, though not statistically significant. This conforms with the findings of Atasoy and Bugdayci,[29] who reported that low zinc level contributed to most of the anemia in children. This observation may be due to the chronic hemolytic state associated with SCA with resultant loss of zinc from the RBC which is one of the storage sites for zinc and its subsequent loss in urine. It has been proposed that the role of zinc in the care of patients with SCA is through its calcium antagonism. Zinc hinders the action of calmodulin which stimulates the calcium-ATPase that regulates the calcium pump system of the RBCs. An influx of calcium into the RBCs occurs during sickling and this may be as a result of overactivation of calmodulin with consequent membrane derangement. Therefore, zinc therapy produces antisickling effect through its action on calmodulin with consequent calcium antagonism.[30]

Furthermore, patients with SCA who had normal platelet count had a higher zinc level compared to those who had thrombocytosis though not significant. It has been reported that increased serum zinc level significantly increases platelet reactivity.[31]

Patients with SCA with leukocytosis had lower zinc level compared to those with white cell count within the normal reference range. This is supported by findings from a previous study which reported that dietary zinc deficiency increases the number and distribution of WBCs.[32]

Conclusion

Patients with SCA have lower serum zinc level compared to HbAA individuals. Low zinc level is associated with lower Hb level, higher white cell count as well as higher platelet count.

Recommendations

Periodic evaluation and supplementation of zinc should be considered as part of intervention strategies in the management of SCA to reduce complications such as irreversible red cell damage, hemolysis, and anemia.

Further studies on a wider scale are needed to confirm the effects of zinc supplementation on hematological parameters in SCA patients.

Study limitation

The power of this study to detect significant results could have been affected by the small sample size.

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Conflicts of interest

There are no conflicts of interest.

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