Tropical Journal of Pharmaceutical Research October 2024; 23 (10): 1717-1724
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i10.17

Original Research Article

Serum levels of copper and zinc and their relationship with iron status in sickle cell anemia patients

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Sent for review: 15 April 2024 *Revised accepted: 7 October 2024*

Abstract

Purpose: *To determine the serum levels of copper and zinc and their relationship with iron status in patients with sickle cell anemia (SCA).*
Methods: *Sixty-six (66) subjects comprising thirty-five (35) HbSS steady state (SS) and thirty-one (31) HbAA as control subjects receiving treatment in the Hematology Clinic, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria were recruited for the study. Atomic absorption spectrometer (AAS) was used to analyze the level of serum iron, copper, and zinc, while ferritin enzyme immunoassay was used to determine the serum ferritin.*
Results: *Serum levels of copper and zinc were significantly lower compared to control in SCA (SS) subjects ($p < 0.05$). Similarly, serum iron and ferritin in SCA (SS), compared to control subjects, were significantly lower ($p < 0.05$). A positive correlation was observed in serum levels of copper and iron ($r = 0.46$ and $p < 0.05$). Likewise, a positive correlation also was observed between zinc and iron serum levels ($r = 0.65$ and $p < 0.05$). Furthermore, a significantly positive correlation was observed between copper and serum ferritin, and between zinc and serum ferritin ($r = 0.44$ and $p < 0.05$; $r = 0.69$ and $p < 0.05$), respectively.*
Conclusion: *This study shows significantly lower levels of copper and zinc in subjects with SCA compared to control. Furthermore, low levels of copper and zinc correlate with iron deficiency in SCA patients. This may be due to the function of these trace elements in iron mobilization and utilization for hemoglobin and red blood cell synthesis and immune functions. This specific mechanism will require further investigation.*

Keywords: *Sickle cell anemia, Serum level, Copper, Zinc, Iron, Zaria*

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INTRODUCTION

Trace elements such as copper and zinc play important roles in the body [1]. They function as activators in several biological activities. Deficiency of trace elements remains a global health issue. Individuals suffering from sickle cell anemia (SCA) are often disposed to trace element deficiency due to the increased requirement for these nutrients [2]. Increase in erythropoiesis production in sickle cell anemia is due to chronic hemolysis, which is responsible

for high energy. A low level of trace elements is associated with increased susceptibility to infection, and stunted growth and development [3]. Furthermore, altered levels of these elements have been documented to contribute substantially to the development of anaemia [4].

Copper is largely stored in the liver, and present in all tissues of the body. In the absorption and utilization of iron, copper plays a vital role necessary for red blood cell production [5]. An enzyme containing copper, ceruloplasmin, is essential in mobilizing stored iron in the liver for haemoglobin synthesis [5]. Copper deficiency is known to cause anemia. Zinc is the second most abundant element in the body after iron and is found in all categories of enzymes. It is vital for normal functioning of the alpha-aminolevulinic acid dehydratase enzyme that is important in iron metabolism [6]. It is also an integral part of independent 1B (GFi-1B), a growth factor, which plays an important role in erythroid cells' growth by regulating specific gene expression of erythroid series during erythropoiesis. Deficiency of this element could lead to the development of anemia [4]. Copper and zinc promote body's immune function. They are vital components of the Cu–Zn–SOD antioxidant system that activates the breakdown of superoxide radicals to hydrogen peroxide and their subsequent clearance by glutathione peroxidase [7]. Deficiency of these elements has been associated with lower antioxidant activity increased susceptibility to infection and stunted growth.

In red blood cell production, iron is essential; three iron components in the body define the status of iron sufficiency, iron stores, transport iron (iron to meet cellular requirements), and functional iron (iron available to tissues). Depletion of each of these components may lead to different stages of iron deficiency. The level of serum ferritin in the body is a reflection of the concentration of stored iron [8]. Once stored iron is depleted, it will lead to the first stage of iron deficiency, known as iron depletion without erythropoietic implication. At this point, iron transport component provides only iron primarily for red blood cell (RBC) production, with a demand higher than for other tissues. A deficiency of iron for red blood cell production leads to the second stage of iron deficiency often referred to as erythropoiesis iron deficiency, devoid of any significant drop in hemoglobin level. Transferrin saturation (TSAT), erythrocyte protoporphyrin concentration (EP), and soluble transferrin receptors (sTfR) are mainly indicators of iron supply adequacy [7].

Hemoglobin concentration in the body is a key indicator of any form of iron deficiency. The depletion of stored iron in the body will consequently lead to iron deficiency which is the third stage that causes low hemoglobin concentration and, invariably, anemia. Other causes of low hemoglobin concentration that are non-iron-related include blood loss, reduced RBC production, or destruction of RBCs. Studies have suggested that chronic intravascular haemolysis, inflammation, hypoxia, and urinary loss contribute to the development of iron deficiency in SCD. Several studies have reported iron deficiency among people with SCD [8]. Iron deficiency reduces the quality of life and has serious health implications irrespective of whether anaemia is present or not.

This study aimed to assess the levels of copper and zinc in serum and their relationship with some iron status in patients with SCA in Zaria, North-Western region of Nigeria.

METHODS

Study location and population

This study was conducted in the Hematology Clinic of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria. The recruitment of patients was based on the clinical diagnosis of SCA. A total of (66) sixty-six subjects consisting of 35 patients with sickle cell anemia (SCA) in steady state and 31 healthy individuals with HbAA genotype as controls (C) were recruited into the study. Ethical approval was obtained from the Ethics Committee of Ahmadu Bello University Hospital Zaria (approval no. ABUTHZ/HREC/L31/2014). The study was conducted by following guidelines in the Helsinki Declaration [9]. Written and verbal informed consents were obtained from the patients.

Inclusion criteria

All patients in the study group were from 18 to 46 years old and confirmed sickle cell anemia cases that have been treated and stabilized for more than 3 months, without any other pathology and not on any other treatment that may interfere with the outcome of this investigation were included in the study.

Exclusion criteria

Patients with hemoglobinopathies other than sickle cell anemia, subjects dependent on transfusions, and those who received transfusion in the last three months as well as subjects on

iron supplementation were excluded from the study.

Data collection

Questionnaires were administered to participants. The filled-out validated structured questionnaires included social demographic data (age, sex, weight, educational status, and marital status), blood transfusion history, history of any crises, date of last crises, cigarette and alcohol intake, and medication history.

Sample collection

Control samples were obtained from apparently healthy blood donors who visited the blood bank of the hospital. Under aseptic conditions, 6 mL of venous blood samples were taken from each patient. Approximately 2 mL of blood was collected in EDTA tubes containing two (2) drops of 10 % EDTA for hemoglobin electrophoresis. Four (4 mL) of blood were collected into plain tubes for analysis of trace elements (serum iron and serum ferritin). Blood sample was allowed to clot, retracted, and centrifuged at 3500 rpm for 5 min after which serum was separated and stored at -20 ^{o}C until ready for hemoglobin assay. Hemoglobin phenotypes were determined using cellulose acetate electrophoresis [10].

Laboratory analysis

Flame atomic absorption spectrophotometer (FAAS)

Serum iron, copper, and zinc were determined with a flame atomic absorption spectrophotometer (AAS) using the method described by Kaneko [11]. Serum ferritin was determined by ferritin enzyme immunoassay. Atomic absorption spectrophotometric measurement of serum trace elements concentrations was performed on a Buck 200 (AAS). The frozen serum samples were thawed and 1:25 dilution was made for magnesium (Mg) while samples for other trace elements were aspirated directly into AAS for analyses. Working standard solutions were prepared by diluting the stock standard with de-ionized water and the required part per million (PPM) used for standardization of corresponding trace elements. Flame was ignited and the lamp switched on. Instrument was allowed to warm up while aspirating de-ionized water into the flame and then adjusted to zero absorbance. Standard was aspirated to standardize the machine. This standardization was repeated periodically to assess any drift of sensitivity. Each sample was aspirated (diluted in case of Mg and undiluted in cases of Fe, Cu, and Zn) into the machine. The results of the assays were recorded.

Hemoglobin electrophoresis

Titan cellulose acetate membrane (76 x 60 mm) was soaked in distilled water, blotted then soaked in Tris-EDTA-borate. Hemolysate was prepared by adding 0.005 M EDTA in deionized water with 0.07 % potassium cyanide to whole blood and was applied to Titan III Cellulose Acetate Plate. Hemoglobin electrophoresis was performed at 350 volts for 25 min using alkaline buffer (pH 8.2 - 8.6) and acid and migration of hemoglobin were noted. Control samples containing hemoglobin A, S, and C were included with each electrophoresis run [10].

Ferritin enzyme immunoassay

Twenty microliters of standard, specimens, and controls were dispensed into appropriate wells. One hundred microliter (100 µL) of enzyme conjugate reagent was dispensed into each well and was gently mixed for 30 sec. It is very important to have a complete mixing in this setup. It was incubated at room temperature for 45 min. Incubation mixture was removed by flicking the plate contents into the sink. Microtiter wells were rinsed and flicked 5 times with distilled or deionized water. The wells were struck sharply onto absorbent paper or paper towels to remove all residual water droplets. One hundred microliters (100 µL) of 3,3',5,5' tetramethylbenzidine (TMB) reagent was dispensed into each well. It was gently mixed for 10 sec and incubated at room temperature in the dark for 20 min. The reaction was stopped by adding 100 µL of stop solution to each well. It was gently mixed for 30 sec. Absorbance was read at 450 nm with a microtiter plate reader [11].

Data analysis

Data obtained were analyzed using Student's *t*-test for significant differences, Pearson's correlation using SPSS version 20 for relationships, and a *p*-value of < 0.05 was considered statistically significant.

RESULTS

Demographic pattern

A total of sixty-six (66) patients recruited for this study consisted of 35 HbSS in steady state (SS) and 31 HbAA as controls (C). There were more women 34 (51.5 %) than men 32 (48.5 %). The overall mean age for study group was 23.94 ± 5.83 years and 25.55 ± 5.33 years for the control

subjects. Furthermore, the total mean weight of patients in study group was 53.49 ± 8.79 years and the control was 58.19 ± 7.55 years (Table 1).

Serum level of copper and zinc

Serum levels of copper and zinc were significantly lower in subjects with sickle cell anemia compared to the control. Furthermore, serum levels of iron and ferritin were significantly lower in subjects with sickle cell anemia compared to the control (Table 2).

Serum levels of copper and iron

A significant positive relationship was observed between the level of copper and serum iron (r = 0.457 and *p* = 0.00), and serum ferritin (r = 0.440 and *p* = 0.00) in the study subjects (Table 3, Figure 1 and Figure 2).

Serum-level of zinc and iron

Furthermore, there was a significant positive relationship between zinc level and serum iron (r = 0.645 and p = 0.00), and serum ferritin (r = 0.695 and p = 0.00) in study subjects (Figure 3, and Figure 4).

Table 1: Demographic pattern of study subjects

Group	Steady State (n=35)	Control (n=31)
Age (years)	23.94±5.87	25.55±5.33
Gender		
Male	11	21
Female	24	10
Weight (kg)	53.49±8.79	58.19±7.55

Note: **P* < 0.05

Table 3: Correlation of copper and zinc with serum iron and ferritin in study subjects

Parameter	Correlation coefficient (r)	*P*-value
Copper vs. serum iron	0.457	0.00*
Copper versus serum ferritin	0.440	0.00*
Zinc / Iron	0.645	0.00*
Zinc / Ferritin	0.690	0.00*

Note: r = Pearson correlation coefficient; **p* < 0.05

Table 2: The mean values of serum copper, zinc, iron, and ferritin of the SCA subjects compared to the control subjects

Group	Copper (µg/dL)	Zinc (µg/dL)	Iron (µg/dL)	Ferritin (µg/dL)
Study (n=35)	120.03±12.19	100.76±18.08	150.75±18.55	94.22±1.69
Control (n=31)	135.73±18.56	125.44±15.12	166.33±19.03	105.73±12.14
F (P) value	7.43 (0.00)*	18.02 (0.00)*	7.16 (0.00)*	11.71 (0.00)*
P-value	0.00*	0.00*	0.00*	0.00*

**P* < 0.05

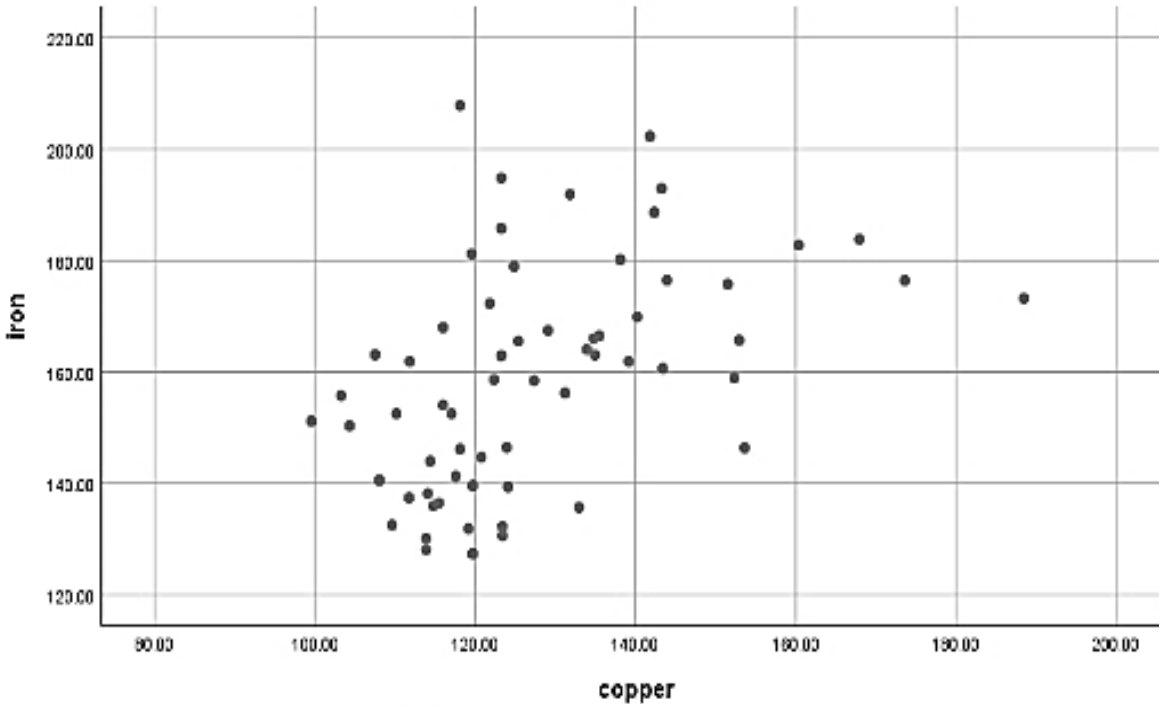

Figure 1: Correlation between copper and iron

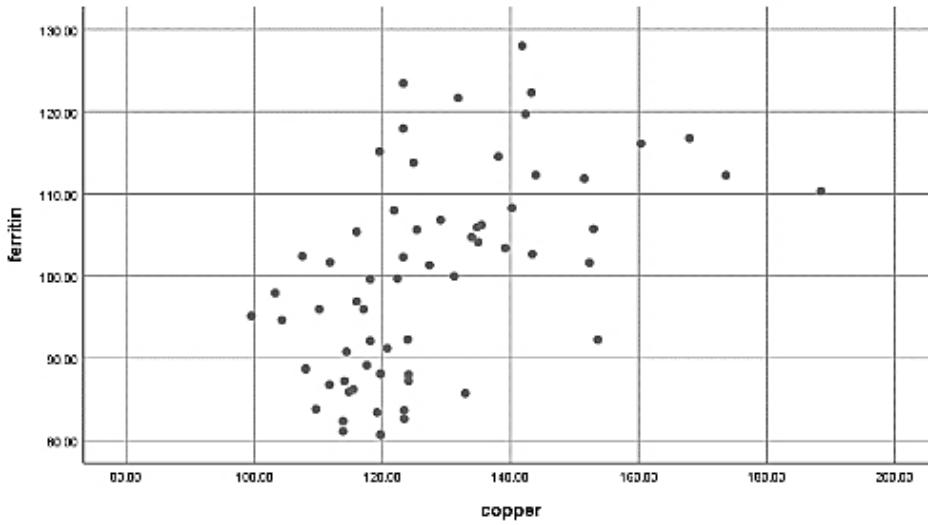

Figure 2: Correlation between copper and ferritin

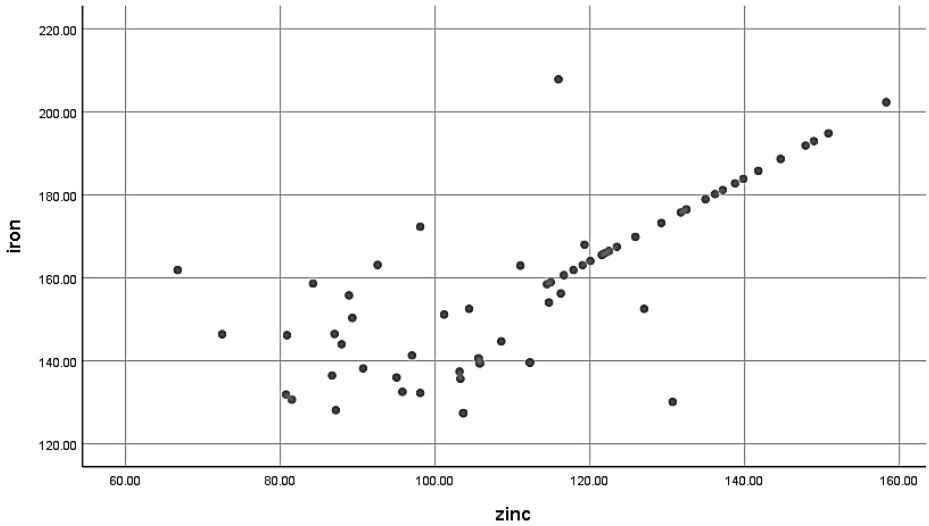

Figure 3: Correlation between zinc and iron

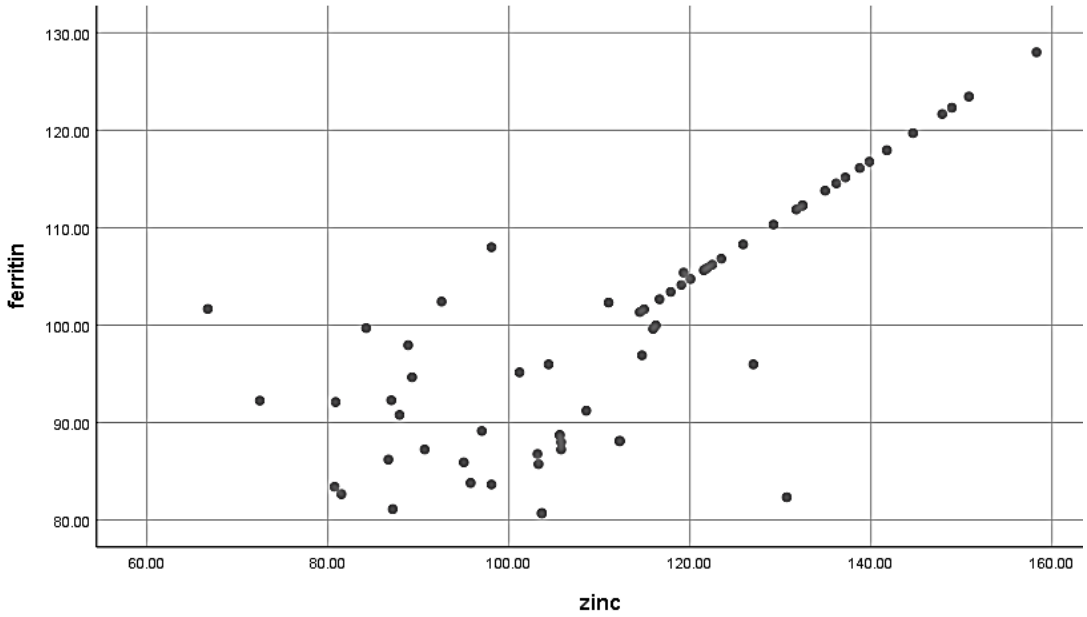

Figure 4: Correlation between zinc and ferritin

DISCUSSION

In this study, significantly lower copper levels were observed in subjects with SCA compared to control (hemoglobin AA individuals). The result of this study is consistent with reports from previous studies that individuals with iron deficiency anemia have lower serum levels of copper [12]. The low levels of copper in subjects with SCA are believed to affect the copper-containing enzyme, ceruloplasmin, that is involved in iron mobilization for hemoglobin synthesis even when there is an elevated level of iron in the liver. Copper deficiency is known to cause myelopathy. Copper and iron deficiency are associated with serious health implications in the body. There is no evidence of a compromised immune response in copper deficiency.

Low levels of copper in the body lead to diseases such as chronic toxicity, liver damage loss of collagen stability, osteoporosis, and increased susceptibility to infections [13]. Similarly, significantly lower zinc levels were observed in SCA subjects compared to the control. This result is consistent with previous studies of lower zinc levels in SCA. Zinc deficiency is associated with growth retardation and the formation of irreversible sickled red blood cells in people living with SCA [14]. Modified levels of copper and zinc have been reported to compromise iron absorption, leading to increased susceptibility to infection and various degrees of anemia [4,14]. The results of this study show that serum iron and ferritin levels were significantly lower in subjects with SCA compared to controls. This is consistent with previous studies that reported low serum iron and ferritin levels in subjects with sickle cell [14]. Serum ferritin and sustainable iron in tissue stores decrease even in the early stages of iron deficiency as iron stores become depleted. It progresses slowly without clear clinical symptoms until anemia becomes critical. Iron plays a significant role in haemoglobin synthesis [15]. It has been reported that sickle cell anemia, being a state of chronic inflammation [13], would be associated with a lower serum iron level. Inflammation reduces the body's ability to adequately use iron sequestered in tissues. The major role of iron in the immune system is confirmed by increased susceptibility to infections in children affected by iron-deficiency-related anemia where the immune response is decreased [15]. In this study, there was a significant correlation of serum copper with serum iron. Similarly, there was a significant correlation between serum copper and serum ferritin. This may be due to the critical role of ceruloplasmin ferroxidase in the oxidation of ferrous to ferric ions that influence iron stores and ferritin formation. It has been suggested that the copper-containing enzyme, ceruloplasmin, may play a specific role, probably related to its function in the mobilization of stored iron in the liver that makes iron available for hemoglobin synthesis [16]. Most of the copper in the blood is linked to ceruloplasmin, and therefore copper reduction may affect iron pharmacokinetics [2]. Hephaestin is a ceruloplasmin homolog and plays a role in iron absorption from the diet. It has been reported that in copper deficiency-induced iron deficiency, the rate of hemoglobin synthesis remains significantly reduced even when there is an elevated level of iron in the liver [16].

Studies suggest that copper deficiency containing the enzymes hephaestin and ceruloplasmin alters iron homeostasis, contributing to the development of systemic iron deficiency. Severe copper deficiency in humans causes iron accumulation in the liver, due to ferroxidase activity deficiency [17]. The current study revealed a significant correlation between serum zinc level with serum iron. Additionally, the study showed a significant correlation between serum zinc and serum ferritin. Zinc plays an important role in red blood cell production, iron absorption, and immune function. Zinc plays a central role in all aspects of cellular and humeral immunity, neutrophils, and natural killer cells. Furthermore, zinc is one of the essential trace elements required for the growth, development, and differentiation of all types of life and plays an important role in iron metabolism. Zinc is located in the structure of the growth factor independent 1B (Gfi-1B) zinc finger protein, which serves as a controller in erythroid cell growth by regulating gene expression specific to the erythroid series [18]. Zinc deficiency could reduce iron absorption leading to the development of anemia [6]. Individuals with sickle cell disease have growth and development-related problems.

Trace elements and iron status have been reported to have a significant correlation with growth and development in children. The absorption of trace elements and iron is upregulated in SCA, resulting in altered serum levels of these parameters. A serum trace element and iron deficiency state are responsible for anemia which is a major complication of sickle cell anemia [19]. This may be due to the critical roles trace elements and serum iron plays in hematopoiesis [5].

Limitations of this study

In this study, some of the constraints encountered were due to the inability to determine dietary intake and its effect on the levels of these trace elements, which could have affected the findings. Blood pressure and sugar levels may also have a consequential influence on trace elements such as copper and zinc which were not determined.

CONCLUSION

Serum levels of copper and zinc in subjects with SCA are significantly related to serum iron and ferritin. This relationship may be due to the roles of these trace elements in iron mobilization and absorption for hemoglobin and red blood cell synthesis, as well as immune function. Going forward, it would be necessary to determine the effect of food intake or other comorbidities on the levels of these trace elements to ensure that the results obtained are reflective of the patient's conditions.

DECLARATIONS

Acknowledgment

The Authors acknowledged the support of the technical staff of the Laboratory unit of the Department of Hematology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria.

Funding

None provided.

Ethical approval

Ethical approval was obtained from the Ethics Committee of Ahmadu Bello University Hospital Zaria (approval no. ABUTHZ/HREC/L31/2014).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors contributed to the review, editing, and final correction of the manuscript.

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