Assessment of ~~T~~the Levels of Serum Zinc and Copper Among Sudanese Patients with Sickle Cell Anaemia in Khartoum State

Abstract:

Background: Sickle cell anaemia (SCA) is associated with increased risks of multiple micronutrient deficiencies. Zinc deficiency has been observed in patients with Sickle cell anaemia, due to chronic haemolysis with subsequent loss of zinc from RBCs.

Objectives: The aim of this study was to assess the levels of zinc and copper among Sudanese patients with sickle cell anaemia (SCA).

Methods: Across-sectional study was conducted from March to April 2018, involving forty subjects who had been diagnosed of sickle cell anaemia (SCA), and had been admitted to Albuluk Hospital, in Khartoum State, as cases. Forty age-matched healthy individual with normal haemoglobin (HbA) were recruited as controls. The ~~(~~ages ~~was matched in the groups~~ ranged from 4 months to 16 years~~)~~. Blood samples were collected~~, and~~ serum was separated~~,~~ and then~~n~~ the levels of zinc and copper were measured, using atomic absorption spectrophotometer. Data analysis was carried out, using SPSS version, 21. ~~(~~Independent t-test was used to compare mean values in case versus control groups. Pearson's ~~C~~correlation was done to study the relationship between zinc and copper and age).

Results: There was significant decrease~~d~~ in the mean levels of zinc and copper in patients with sickle cell anaemia~~, when~~ compared to control groups. The ~~(M~~mean ±SD values were; 0.137±0.079 versus0.705±0.138ng/L, p, value= 0.00) for zinc; ~~(~~0.512±0.290 versus 0.923±0.214ng/l, p, value =0.00;~~)~~ for copper. There was no significant difference between males and females in the mean levels of zinc (p = 0.345) and copper (p = 0.656). The (Mean ±SD; 0.144±0.079 versus0.127±0.080 ng/L) for zinc (0.502±0.271ng/L versus 0.525±0.321 ng/L) for copper, ~~t~~There was no correlation between levels of zinc~~,~~ copper and ages,(r=0.052, p, value=0.750) for zinc~~,~~ (r=0.122, p, value=0.452) for copper.

Conclusion: The levels of zinc and copper were decreased in patients with sickle cell anaemia, compared to healthy individuals. There was also negative correlation between the levels of zinc and copper and age of patients.

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KEYWORDS: Sickle cell anaemia, zinc, copper, micronutrient and Sudanese.

Introduction:

Sickle cells anaemia is a haemoglobinopathy, characterized by chronic haemolysis, chronic inflammation, immune deficiency, a heterogeneous clinical picture and organ damage.[1] SCA is associated with increased risks of multiple micronutrient deficiencies These deficiencies may have a significant impact on SCA severity indices including growth retardation, cell-mediated immune dysfunction, and cognitive impairment with a negative impact on morbidity and mortality.[2] In addition, these nutrients have a major role in the protection of the red cell membrane against damage through free radical-mediated oxidation in SCA.[3] Sickle cell disease is common especially in Africa and among Negroid race. In sickle cell disease, there are deficiencies of some essential elements which are vital in maintenance of red cell integrity, body growth and development. [4] The sickle haemoglobin is known to interact with diverse genes and environmental factors, producing a multi-systemic disease with several phenotypes. [5]

Minerals are inorganic substances, present in all body tissues and fluids and they are necessary for the maintenance of certain physicochemical processes which are essential to life.[6,7] They are important for human,[8,9] so deficiencies or disturbances in the nutrition can cause a variety of diseases, which can arise in several ways.[10] Two most common trace metal imbalances are elevated copper and depressed zinc in SCA. Therefore, the aim of this study was to assess the level of serum zinc and copper among Sudanese patients with sickle cell anaemia.

Materials and methods:

- Study design: This was a cross-sectional case-control study.

-Study area and period of study: Blood samples were collected from Albuluk Hospital, in Khartoum State, from March to April 2018.

- Study population: Forty sickle cell anaemic patients, aged between 4 months and 16 years, were recruited as cases and forty normal children with normal haemoglobin were enrolled as controls. The cases and controls were age-matched; 17 of sickle cell anaemic patients were females and 23 were males, and 16 of controls were females and 24 of them were males.

-Inclusion criteria: The subjects were ~~Albuluk Hospital~~ in-patients with sickle cell anaemia, who were being treated with hydroxyurea~~, were included~~.

-Exclusion criteria: Any patients taking long term zinc supplements and patients with other chronic diseases (liver disease, renal disease and heart diseases), were excluded.

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- **Ethical consideration:** The study was approved by the ~~e~~Ethical ~~c~~Committee of Medical Laboratory Science, Clinical Chemistry Department-Alneelain University. A verbal informed consent was obtained from the parents of each minor participant.

-**Data collection:** A demographic data was collected by using a questionnaire.

- **Sampling:** About 2.5 ml of venous blood was collected from each participant, into a plain sample tube. ~~a~~After formation of clot at~~in~~ ~~the~~ room temperature,~~:~~ the samples were centrifuged for 10 minutes at~~in~~ 3000 rpm. ~~t~~Then, the serum samples were~~as~~ obtained and analyzed.

- **Method of assay of zinc and copper:** The levels of serum zinc and copper were~~as~~ measured by using atomic absorption spectrophotometer (BUCK SCINTIFIC 210/211 VGP VER3.94C, country?).

- **Quality Control:** Pathological and normal control sera were also used for the measurement of the metals, to assure accuracy and precision of results.

-Data analysis: Data was analyzed using SPSS, version 21.The results were expressed as percentages, mean and SD. Independent t-test was used to compare mean values in case versus control groups. Pearson's correlation test was done to study the relationship between ~~study~~ zinc and copper and age.~~,~~ p-~~v~~Values less than 0.05 were considered significant.

Results:

Eighty participants were enrolled in this study; 40 patients (17 females and 23 males) with mean age ~~mean~~ ± SD 6.67 ± 4.16 years and 40 controls (16 female and 24 males) with ~~age~~ mean age ± SD 6.37 ± 4.16 years, ~~age was matched in both groups and~~and the ages ranged from 4 months to 16 years. Statistical analysis showed a significant decrease in the levels of zinc and copper among patients with sickle cell anaemia (SCA), when compared to healthy individuals (table1).~~,~~ There was ~~also statistical analysis showed~~ ~~i~~non-significant difference~~variation~~ in the mean levels of zinc and copper among patients with sickle cell anaemia, based on ~~when compared according to~~ gender (table 2).~~,~~ There was non-significantly low~~and also statistical analysis showed~~ ~~no~~ correlation between the level of zinc and copper with ages of~~among~~ patients with sickle cell anaemia, (figure 1 and figure 2).

Table ~~(~~1~~)~~: Comparison the levels of zinc and copper in case versus control groups~~:~~

Parameters	Case (Mean±SD)	Control (Mean±SD)	*P-value*
Copper mg/l	0.512±0.290 ~~(0.03-0.372)*~~	0.923±0.214 ~~(0.474-1.01)*~~	0.000
Zinc mg/l	0.137±0.079 ~~(0.122-1.154)*~~	0.705±0.138 ~~(0.704-1.346)*~~	0.000

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P - values less than 0.05 were considered as significant. ~~* indicates the range of values (lowest and highest) of copper and zinc.~~

Table (2): Comparison the mean levels of copper and zinc in case group according to gender.

Parameters	Male (Mean±SD)	Female (Mean±SD)	*P-value*
Copper mg/l	0.502±0.271	0.525±0.321	0.656
Zinc mg/l	0.144±0.079	0.127±0.080	0.345

P - values less than 0.05 were considered as significant.

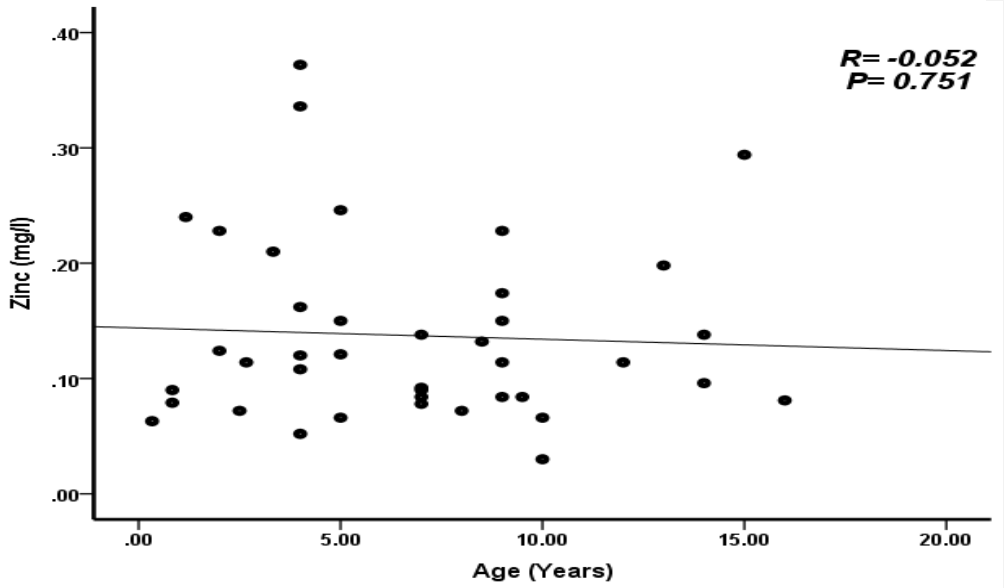

Figure (1): Correlation between zinc levels and ages.

P-value less than 0.05 is considered as significant:

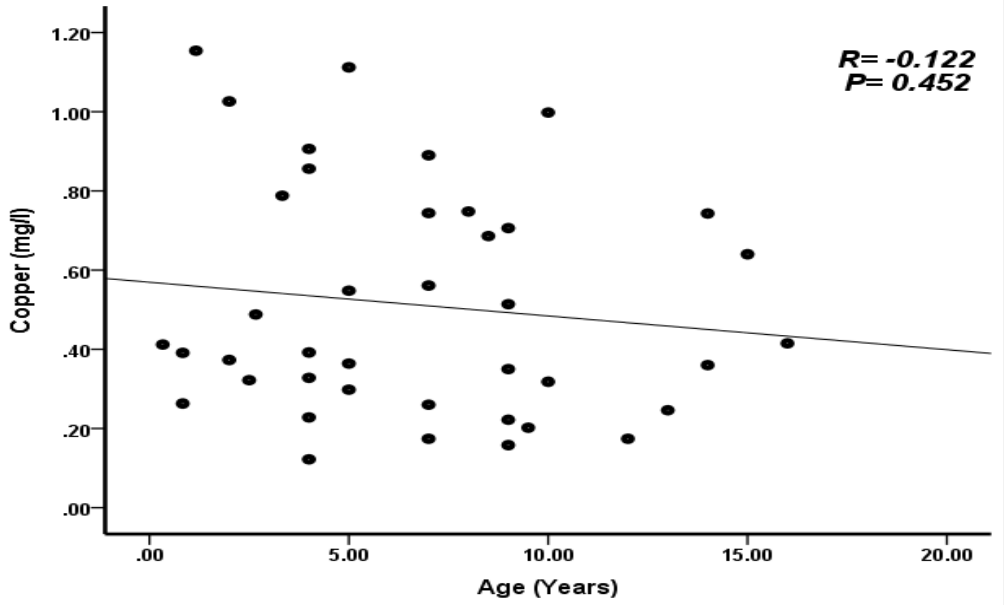

Figure (2): Correlation between copper levels and ages.

P-value less than 0.05 is considered as significant:

Discussion:

In sickle cell anaemia, there are deficiencies of some essential elements which are vital in maintenance of red cell integrity, body growth and development. In the current study, the levels of zinc and copper showed a significant decrease in patients with sickle cell anaemia, compared to healthy individuals (p value 0.000). This might have occurred due to chronic haemolysis with subsequent loss of zinc from RBCs. Zinc deficiency can also be the result of the adverse effect of hydroxyurea which increases zinc excretion. [11]

This finding was in agreement with results of some previous studies [12,13,14] in Central Africa and Nigeria,, which related zinc deficiency in sickle cell disease to manifestations such as growth retardation, hypogonadism in males, hyperammonaemia, abnormal dark adaptation and cell-mediated immune disorder. Similarly, the biochemical evidence for zinc deficiency in patients with SCA include low zinc concentrations in plasma, erythrocytes, hair, lymphocytes and granulocytes.[15] This biochemical difference appears to be due to various mechanisms such as chronic haemolysis, renal lossst due to repeated sickling, leading to abnormal renal tubular reabsorption of zinc,

abnormal binding of zinc to tissue proteins, disturbed metabolism of zinc metalloenzymes. [14] Also, the results revealed that there was non-significant difference between males and females, in the levels of zinc and copper in patients with sickle cell anaemia. Furthermore, there was negative correlation between levels of zinc and copper and age of the patients (r= -0.603, p-value= 0.000) and (r= -0.443 p- value= 0.004) respectively.

Conclusion:

The levels of blood zinc and copper were decreased in patients with sickle cell anaemia, compared to healthy individuals. There was also negative correlation between the levels of zinc and copper and age~~zinc~~.

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