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Copper to Zinc Ratio in Pediatric Patients with Sickle Cell Disease (Steady-State and Crises)

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Abstract:

BACKGROUND: Inflammation has a pivotal role in acquiring acute and chronic complications in sickle cell disease (SCD), therapeutic approaches directed to inflammation pathways may have therapeutic and preventative benefits.

OBJECTIVES: The objective of the study was to evaluate the copper to zinc (Cu/Zn) ratio in SCD pediatric patients (in steady state and crises) and compare it with the control group and to look for its association with other inflammatory markers.

MATERIALS AND METHODS: Sixty patients known to have SCD and 60 healthy children and adolescents were evaluated, their age ranged from 2 to 14 years. Patients were evaluated while they were in crisis and followed until they were in a steady state. Complete blood count, reticulocyte count, lactate dehydrogenase (LDH), C-reactive protein (CRP), serum Cu, and Zn concentrations were assessed.

RESULTS: Patients with SCD during crises demonstrated a significantly reduced mean hemoglobin and serum Zn levels and significantly higher mean reticulocytes, white blood cells, neutrophils and platelet counts, LDH, CRP, and serum Cu compared to those without crises and healthy controls, $P < 0.05$. Cu/Zn ratio showed a significant increase during crises (1.98 ± 0.52) compared to that during steady state (1.35 ± 0.34) and healthy controls (1.05 ± 0.21), $P = 0.001$. A significant association between Cu/Zn ratio and CRP (R = 0.335, $P = 0.009$) was found.

CONCLUSION: The Cu/Zn ratio may function as a critical index of the inflammatory process in SCD patients, especially during vaso-occlusive crises, and also as an indicator of Zn status. Continued research is essential to examine the role of Zn intake in maintaining Cu-Zn homeostasis.

Keywords:

Copper to zinc ratio, pediatric patients, sickle cell disease

Introduction

Sickle cell disease (SCD) is the most widely spread genetic blood disorder affecting millions of individuals worldwide, with around 4.4 million affected individuals.[1] It is typically linked with numerous complications and increased risk of death, the mortality is higher in children, particularly in countries with the greatest burden of under-5 deaths.[1,2]

SCD arises from a mutation in the β-globin-gene (single base-pair point mutation), leading to the formation of sickle hemoglobin (HbS). Hemoglobin S undergoes polymerization following red blood cells (RBCs) deoxygenation, leading to vaso-occlusion, presenting as pain and organ damage.[3] In conjunction with vaso-occlusion, the chronic inflammatory process these patients experience and augmented oxidative stress contribute significantly to the pathogenesis of SCD. Patients with SCD have dysregulation in the balance between

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oxidative stress and antioxidant capacity. This imbalance functions as a critical element in vaso-occlusion, the initiation of endothelial cell dysfunction and inflammation, leading to chronic vasculopathy and various organ damage including the spleen (auto-splenectomy), the central nervous system (e.g., stroke), respiratory and cardiovascular systems as acute chest syndrome (ACS) and pulmonary hypertension, genitourinary system (e.g. nephropathy), and leg ulcers.[4-6] SCD is marked by a vicious cycle of altered erythrocyte rheology and ongoing inflammation that affects disease severity and clinical outcome of patients.[4]

Because of the significant role of inflammation in the initiation and progression of acute and chronic complications in SCD, pharmacologic approaches targeting the inflammatory pathways may offer both therapeutic and preventative advantages.[7]

Copper (Cu) and zinc (Zn) are essential components of the superoxide dismutase (SOD) antioxidant enzyme that has a key function in protecting the cells and tissues from oxidants through the elimination of reactive oxygen species generated during body metabolism.[8] Patients with SCD experience alterations in markers of oxidative stress, lower antioxidant enzyme levels including SOD, and changes in enzyme cofactors levels such as Zn, Cu, and iron.[9]

Disturbances in Cu and Zn homeostasis can impact the body antioxidant defense mechanisms that eventually lead to oxidative injury and SCD-associated complications in these patients and Cu/Zn ratio may serve as a marker of oxidative stress in SCD, its complications, SCD severity, and indirect indicator for the increased Zn utilization in SCD patients with increasing disease severity.[10,11]

SCD represents a considerable health issue in Basrah (Southern Iraq) where 6.5% of the population are carriers of the sickle cell gene.[12] Children and adolescents with SCD represent 4.1% of all pediatric patients admitted to the emergency department of Basrah Maternity and Children Hospital, which is the main pediatric hospital in Basrah.[13] Several studies have evaluated the inflammatory and oxidant/antioxidant status of pediatric patients with SCD in Basrah.[9,14,15] However, the ratio of Cu to Zn was not evaluated; therefore, the current study aimed to evaluate Cu/Zn ratio in SCD pediatric patients during steady state and crises and compare it with the control group and also to look for its association with other inflammatory markers.

Materials and Methods

Patients

This prospective case–control study was conducted on children and adolescents diagnosed with SCD and registered at Hereditary Blood Diseases Center and healthy controls throughout the period from the first of December 2023 to August 2024.

Patients were evaluated while they were in crisis and followed until they were in a steady state. Sixty-four patients, aged 2–14 years were enrolled initially. Later on, four patients were excluded because they did not attend the center and it was not possible to contact them when they were in the steady state.

The data obtained included demographic data, type of SCD, previous disease-related complications as vaso-occlusive crises (VOC), ACS, acute splenic sequestration crises (ASSC), and stroke, past medical history, and drug history (hydroxyurea and Zn supplement).

Definition of variables

Steady state

A patient in a steady state needs to fulfill these requirements; the patient does not have a history of acute painful events necessitating hospital admission for ≥4 weeks following a prior painful episode, no recent infection during the past 4 weeks or medications use that could impact the blood count like antibiotics over the past 3 weeks, and no blood transfusion within the past 12 weeks.[16-18]

Severity of sickle cell disease

The patient was designated as having severe disease when the patient had developed painful VOC necessitating hospitalization ≥3 per year, had recieved blood transfusion ≥3 per year, and if the patient had developed at least one attack of the following SCD complications; ASSC, ACS, avascular bone necrosis (AVN), and/or stroke.[19,20]

The genotype of SCD and the level of fetal hemoglobin (HbF) also reflect disease severity, where patients with homozygos Hb S and HbS/β^0 thalassemia are considered as having severe disease, whereas those with other genotypes such as HbS/C and HbS/β^+ thalassemia have milder disease.[21] Patients with HbF level <10% were considered severe disease, while those with a level ≥10% were considered a less severe disease.[22]

Patients with other hemoglobinopathies other than SCD and those on Zn supplements were excluded.

Control group

Sixty age- and sex-matched children and adolescents, who had consulted the Outpatient Clinic of Basrah Maternity and Children Hospital and two Primary Health Care Centers for vaccination, or routine healthcare visits were recruited. Children and adolescents included in this

group were free from acute illnesses or fever and had no family history of hemoglobinopathies.

Ethical approval

Before enrollment in the study, the participants were provided and understood the necessary information required to make an informed decision about their participation.

The project proposal has been approved by the Scientific and Ethical Committee of Basrah College of Medicine (030401-043-2023 on 5/11/2023).

Methods

Laboratory tests comprised full blood count that was assessed utilizing Automated Hematology Analyzer (Sysmex XN-350, Japan) and the reticulocyte count that was evaluated manually by the laboratory hematologist using new methylene blue stain.

Both lactate dehydrogenase (LDH) and C-reactive protein (CRP) were measured through Cobas c111 Biochemistry Analyzer, Roche Diagnostics, Germany. Normal ranges for LDH were 0–150 U/L and for CRP <6 mg/dl.

Serum Cu and Zn levels were quantified by a spectrophotometric method (Abbott Architect plus C4000, Japan). The normal serum Zn and Cu levels in children are 63–110 and 80–190 µg/dl, respectively.

Statistical analysis

The statistical analysis was achieved through the Statistical Package for the Social Sciences (SPSS), Version 26. Chi-square statistics were applied to examine the association for categorical data when the expected frequency in each cell is ≥5, and the Fisher's exact test was utilized if the expected frequency in one or more of cells is <5.

For quantitative data, the paired *t*-test was used for patients in the steady and crisis states, whereas independent sample *t*-test was applied to compare the means of two distinct parameters and ANOVA for more than two parameters. ANOVA *post hoc* range tests were done to determine the difference between multiple group means using the Scheffe test.

Pearson correlation was used to assess the correlation between continuous variables. A $P < 0.05$ denotes statistical significance.

Results

Demographic variables

The study recruited 120 children and adolescents in total; 60 have SCD and 60 healthy controls. Patient mean age was 7.38 ± 3.7 years, while the control group had a mean age of 7.13 ± 2.1 years, $P > 0.05$. Meanwhile, a significantly higher number of patients were from Basrah peripheries 24 (40%) as contrasted with healthy children 13 (21.7%), $P = 0.001$.

Sickle cell disease-related variables

The study revealed that most of the studied patients (76.7%) have sickle cell anemia (SCA), and the majority have high HbF (90%). Furthermore, more than half had a history of frequent (≥3) VOC that required hospitalization during the last year, and 21.7% required blood transfusion. The Cu/Zn ratio did not show significant changes among studied patients in relation to disease severity indicators, $P > 0.05$ [Table 1].

Hematological and biochemical parameters

Patients during crises had significantly lower mean Hb and serum Zn levels and significantly higher mean reticulocytes, WBC, neutrophils and platelets counts, LDH, CRP, and Cu/Zn ratio compared to those during steady state, $P < 0.05$, whereas serum Cu was not statistically significantly different. Further statistical analysis utilizing *post hoc* tests also showed similar significant differences between both patient groups (crises and steady) compared to the control group in all variables, where patients during crises and steady states had lower mean Hb and serum Zn levels and significantly higher mean reticulocytes, WBC, neutrophils and platelet counts, LDH, CRP, serum Cu, and Cu/Zn ratio compared to healthy controls, except for platelets count which was statistically not significant between patients during steady state and healthy children, $P > 0.05$ [Table 2].

Pearson correlation analysis between Cu/Zn ratio and various hematological and biochemical variables revealed a significantly marked association with CRP only ($R = 0.335$, $P = 0.009$) [Table 3 and Figure 1].

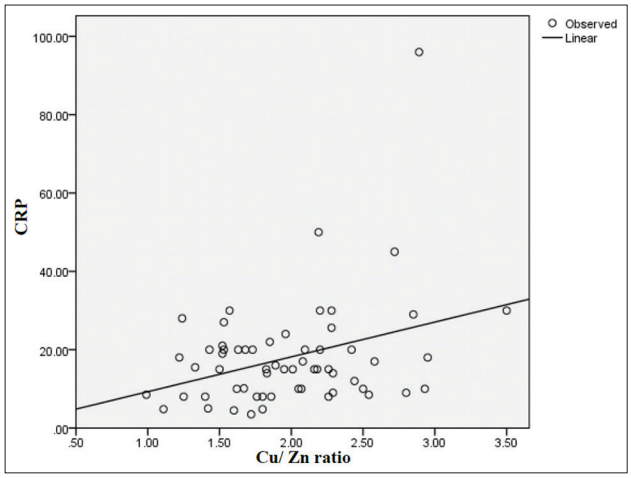

Figure 1: Correlation between copper-zinc ratio and C-reactive protein

Table 1: Copper/zinc ratio in relation to indicators of sickle cell disease severity

Indicators of disease severity	SCD patients total (*n*=60)	Copper/ Zinc ratio, mean±SD	*P*
Frequency of VOC requiring hospitalization/year			
No	7 (11.7)	2.06±0.65	0.717*
<3	21 (35.0)	2.02±0.58	
≥3	32 (53.3)	1.92±0.46	
Frequency of BT/year			
No	32 (53.3)	2.03±0.54	0.765*
<3	15 (25.0)	1.92±0.45	
≥3	13 (21.7)	1.93±0.55	
ASSC			
Yes	7 (11.7)	1.98±0.56	0.997**
No	53 (88.3)	1.97±0.52	
ACS			
Yes	8 (13.3)	1.98±0.64	0.973**
No	52 (86.7)	1.98±0.50	
Stroke			
Yes	2 (3.3)	1.44±0.26	0.300**
No	58 (96.7)	1.99±0.52	
AVN			
Yes	4 (6.7)	2.32±0.35	0.174**
No	56 (93.3)	1.95±0.52	
Type of SCD			
SCA	46 (76.7)	2.03±0.52	0.138**
S/β° thalassemia	14 (23.3)	1.79±0.51	
HbF (%)			
<10	6 (10.0)	2.23±0.63	0.204**
≥10	54 (90.0)	1.95±0.51	

*ANOVA test was used, **Independent *t*-test was used. SCD=Sickle cell disease; VOC-Vaso-occlusive crises; BT=Blood transfusion; ASSC=Acute splenic sequestration crises; ACS=Acute chest syndrome; AVN=Avascular necrosis; SD=Standard deviation; HbF=Fetal hemoglobin; SCA=Sickle cell anemia

Discussion

The current study reported that children and adolescents affected by SCD had a marked elevation in Cu/Zn ratios than healthy participants. Furthermore, the Cu/Zn ratio was significantly higher during crises than steady state, and it was strongly correlated with CRP.

We also observed that serum Zn was significantly lower in patients during crises as opposed to those in steady state and the control group, On the other hand; significantly higher serum Cu was noticed in patients (during both steady and crises) as compared to the control group only.

Many researchers have examined the role of trace elements including serum Zn and Cu in patients with SCD. Our results align with those reported by Antwi-Boasiako *et al*., in Ghana,[10] Temiye *et al*.,[23] and Emokpae *et al*. both from Nigeria.[24] Furthermore, Elkhidir *et al*., in their meta-analysis, observed that SCD patients had significantly lower Zn and higher serum Cu than their controls.[25] An earlier study in Basrah, by Yousif *et al*.,[26] has revealed also a lower serum Zn and higher serum Cu in pediatric patients with SCA (steady state) compared to the control group.

The role of trace elements serum Zn and Cu in inflammation in patients with SCD has been studied thoroughly. In SCD, serum Cu level is inversely proportional to Zn level and this inverse relationship enhances free oxygen radicals generation, therefore worsening SCD complications. Zn supplementation promotes the immune status and can prevent infections.[27] Datta *et al*. in their randomized, placebo-controlled, double-blind clinical trial on Ugandan children with SCA observed a reduction in the incidence of severe/invasive infections and concluded that Zn supplementation can reduce infection-related morbidity and mortality and hence improve the health status of these patients.[28] Providing Zn supplement to SCD patients was found to increase RBCs, Hb, hematocrit, and antioxidant properties, where plasma nitrite, lipid peroxidation products, DNA oxidation products, soluble vascular cell adhesion molecule-1 levels, lipopolysaccharide-induced tumor necrosis factor-alpha (TNF-alpha), and IL-1 became lower after Zn supplementation.[29]

Disturbances in Cu and Zn levels and their ratio (Cu/Zn ratio) were found to be associated with the pathophysiology of SCD.[10] In SCD, chronic hemolysis shortens the RBCs life span and releases microparticles and free Hb in the circulation, therefore increasing the oxidative stress and Zn demand and consumption.[30] The lower serum Zn in SCD patients can also be due to chronic pain, reduced appetite, and increased urinary excretion.[31]

The further decline in serum Zn during crises was also documented by Temiye *et al*.[23] and Kudirat *et al*.,[32] and they attributed this decline to the increased Zn utilization, poor intake, and increased consumption due to increased oxidative stress during VOC.[24]

The Cu can function as an antioxidant and a promotor of oxidative stress in SCD patients. As an antioxidant, Cu eliminates reactive oxygen species and therefore can decrease the damage of free radicals and even prevent it. However, when Cu acts as a pro-oxidant agent, especially in higher concentrations, it enhances free oxygen radicals tissue damage.[33] The cause of higher serum Cu level during crises compared to steady state could be due to chronic hemolysis that is increased by vaso-occlusion during crises.[10] An imbalance in homeostasis of Cu, Zn, and their ratio (Cu/Zn ratio) was found to be associated with the pathophysiology of SCD.[10]

Table 2: Selected hematological and biochemical variables of sickle cell disease patients (steady state and crises) and control group

Variable	SCD patients total (*n*=60)		Control group total (*n*=60), mean±SD	*P**
	Steady state, mean±SD	Crisis state, mean±SD		
Hb (g/dL)	9.03±1.77	8.278±1.34	11.95±1.21	<0.001
MCV (fl)	76.49±8.61	80.22±10.1	81.62±4.24	0.002
MCH (pg)	25.23±1.61	26.25±3.18	30.54±1.87	<0.001
Total reticulocytes (%)	5.99±4.70	8.16±5.50	1.58±0.45	<0.001
WBC (×10³/µL)	9.82±3.81	12.59±6.86	6.51±1.91	0.001
Neutrophils (×10³/µL)	4.96±2.15	7.64±4.70	3.00±0.64	<0.001
Platelets (×10³/µL)	188.40±0.18	637.80±0.20	194.8±0.18	0.05
LDH (U/L)				
Mean±SD	133.67±79.61	372.22±188.10	90.18±27.19	<0.001
IQR (Q1–Q3)	73.5–153.0	262.0–436.5	72.0–105.75	
CRP (mg/L)				
Mean±SD	3.20±0.28	17.98±1.78	1.40±0.21	<0.001
IQR (Q1–Q3)	1.2–5.0	9.25–20.0	0.1–2.0	
Serum Zn (µg/dL)				
Mean±SD	90.14±18.64	69.60±24.08	103.10±6.31	<0.001
IQR (Q1–Q3)	68.28–102.0	52.25–81.75	74.8–131.0	
Serum Cu (µg/dL)				
Mean±SD	125.44±44.29	132.37±42.21	93.22±20.74	<0.001
IQR (Q1–Q3)	93.0–161.25	105.0–156.5	80.25–102.0	
Copper/zinc ratio				
Mean±SD	1.35±0.34	1.98±0.52	1.05±0.21	<0.001
IQR (Q1–Q3)	1.12–1.54	1.57–2.28	0.95–1.175	

*ANOVA test was used. SCD=Sickle cell disease; Hb=Hemoglobin; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; WBC=White blood cells count; LDH=Lactate dehydrogenase; CRP=C-reactive protein; SD=Standard deviation; IQR=Interquartile range

Table 3: The correlation between the copper/zinc ratio and other laboratory investigations in a crisis state

Variable	Copper/Zinc ratio	
	R	*P*
Hb (g/dL)	0.001	0.992
MCV (fl)	0.086	0.516
MCH (pg)	−0.015	0.909
Total reticulocytes (%)	−0.146	0.265
WBC (×10³/µL)	−0.147	0.264
Neutrophils (×10³/µL)	−0.106	0.418
Platelets (×10³/µL)	0.233	0.073
HbF (%)	−0.091	0.490
LDH (U/L)	−0.236	0.068
CRP (mg/L)	0.335	0.009

R=Pearson correlation. HbF=Fetal Hemoglobin; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; WBC=White blood cells count; LDH=Lactate dehydrogenase; CRP=C-reactive protein

The higher Cu/Zn ratio reported in this study in SCD patients in contrast to the control group and in SCD patients during crises compared to steady state, indicates its role as an inflammatory marker in patients with SCD. This result is similar to what was observed by Antwi-Boasiako *et al*., in Ghana,[10] Emokpae *et al*., in Nigeria,[24,34] and Bassenea *et al*. in Senegal.[35]

Both Zn and Cu are essential micronutrients playing a pivotal role in many cellular processes and different diseases. It has been stated that elevated serum Cu in the presence of low Zn (high Cu/Zn ratio) might play a role in the development of several childhood diseases as acute infections, obesity, asthma schizophrenia, autism, hyperactivity, thalassemia, and SCD.[11] The lower Zn and the higher Cu levels and Cu/Zn ratio reported in SCD patients than the controls and in patients during VOC than in steady state signifies the role of Cu/Zn ratio as a pro-oxidant and inflammatory biomarker and also as an indicator of SCD-disease severity and implies that the requirement for Zn consumption in SCD patients intensifies as disease severity increases as evidenced by VOC.[11,33,35] Serum Cu level is affected by Zn bioavailability, as low Zn in the body increases the absorption of Cu from the gastrointestinal tract.[25]

The role of biomarkers has been identified in different mechanisms related to SCD, as hemolysis, hypercoagulability, vasculopathy, inflammation, oxidative stress, and ischemia-reperfusion injury. Numerous inflammatory markers are elevated in SCD and could serve as clinical biomarkers. Evaluation of hemoglobin and HbF levels, reticulocytes, WBC counts, and CRP are among the principal diagnostic methods commonly used in the evaluation of SCD patients.[36]

Different inflammatory markers were evaluated in our study, a significantly lower mean Hb and higher

mean reticulocytes, WBC and neutrophils and platelets counts, LDH, and CRP were found in SCD during crises compared to those in steady state and the control group. These findings are similar to that found by Najim *et al*.[14] and Abdul-Hussein *et al*.[15] in Basrah.

The majority of studied patients had a HbF level >10%, this is in agreement with a previous study in Basrah which reported that 90.7% had a HbF level >10%.[37] Although higher HbF was associated with lower SCD-complications and disease severity,[22,37] we did not report a significant association between Cu/Zn ratio HbF or with other indicators of SCD severity. Wasnik *et al*., in India, did not report an association between Hb F and both Cu and Zn, although they reported a significant association between Cu/Zn ratio and disease severity and that Zn the requirement is higher in those with severe disease and they recommended the evaluation of Cu/Zn ratio during the treatment course.[38]

A positive correlation between HbF with serum Zn and a negative correlation with Cu has been reported by other researchers, suggesting that HbF may have a role in the interaction between oxidative stress and micronutrients such as Zn and Cu in SCD.[39]

The study also confirmed that only Cu/Zn ratio was significantly associated with CRP, a result consistent with that observed by Bassenea *et al*.[35] and Emokpae *et al*.[34] The CRP is a key parameter in the acute inflammatory process that is widely used in clinical practice as a marker of acute and chronic inflammatory conditions including SCD. It has an important regulatory role in the inflammatory response and host defense mechanisms against infections such as phagocytosis, the complement pathway, apoptosis, nitric oxide generation, and cytokines synthesis and release, especially interleukin-6 and tumor necrosis factor-α.[34,40]

Many limitations have been identified in this study, one limitation is that it did not study the dietary pattern of patients and also did not look for the nutritional status of studied patients. The other limitation is the relatively small participant's size.

Conclusion

Our findings reveal that Cu/Zn ratio can be recognized as a crucial inflammatory marker in SCD patients, especially during VOC, and also as an indicator of Zn status. We need further studies to examine the role of Zn fortification on Cu/Zn homeostasis.

Informed consent

A written informed consent was acquired from participants before their enrollment in the study.

Declaration of Helsinki

The research was performed in accordance with the principles of the Helsinki Declaration.

Availability of research data

Authors are prepared to provide data through the corresponding author upon request.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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