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Copper-to-Zinc Ratio Correlates with an Inflammatory Marker in Patients with Sickle Cell Disease

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Abstract: Sickle cell disease (SCD) is an inherited disorder of major health challenge in Nigeria. Micronutrients deficiencies often associated with the disorder may cause inflammation and abnormal metabolisms in the body. The copper-to-zinc ratio is a more important assessment than the concentrations of either of the metals in clinical practice. This study seeks to evaluate serum levels of c-reactive protein (CRP), copper, zinc and copper-to-zinc ratio and to correlate copper-to-zinc ratio with CRP in adult subjects with SCD. Serum copper, zinc, CRP and plasma fibrinogen were assayed in 100 confirmed SCD patients in steady clinical state and 100 age and sex matched subjects with normal haemoglobin. Serum copper and zinc were assayed by colorimetric method using reagents supplied by Centronic, Germany while CRP and fibrinogen were assayed using reagents supplied by Sigma (St. Louis, MO, USA) and Anogen (Ontario, Canada), respectively. The copper to zinc ratio was calculated from serum levels of copper and zinc. The measured parameters were compared between the groups using Students t-test and Pearson correlation coefficient was used to relate CRP with the other parameters. Serum copper, CRP, fibrinogen and copper-to-zinc ratio were significantly higher ($p < 0.001$) while zinc level was lower in SCD patients than controls. Serum CRP concentration correlated with copper ($r = 0.10$; $p < 0.02$), zinc ($r = -0.199$; $p < 0.05$) and Copper-to-zinc ratio ($r = 0.312$; $p < 0.002$) but the correlation between CRP and fibrinogen was not significant. Inflammatory condition may modulate copper and zinc homeostasis and copper-to-zinc ratio may be used as marker of nutritional deficiency and inflammation in SCD patients.

Keywords: Copper; c-reactive protein; inflammation; sickle cell disease; zinc

1. Introduction

Sickle cell disease (SCD) is an inherited disorder of major health challenge in sub-Saharan African including Nigeria. The condition is characterized by hemolytic anemia and periodic painful crisis as a result of occlusion of small blood vessels due to spontaneous intravascular red blood cells polymerization at reduced oxygen tension [1]. The associated complications of SCD include growth retardation, impaired immune function, acute chest syndrome, abdominal pain [2], proteinuria [3], increased oxidative stress and damage to cell membranes [4]. Micronutrients deficiencies have been reported in patients with SCD [5]; a situation made worse by proteinuria [6]. Some authors have suggested that dietary habits of subjects with SCD met or even exceed the recommended dietary allowance (RDA) and not different from the general population [7]. The deficiency may be due to abnormal metabolisms of key trace elements in the body which are very important for the maintenance of red blood cell membrane, growth and development of the body [7].

The copper-to-zinc ratio is a more important assessment than the concentration of either of the metals in clinical practice. Zinc is the second most abundant transition element present in humans after iron and it is the only metal that occurs in all enzyme classes. Zinc is involved in several cellular metabolism, plays a role in immune function, wound healing, protein DNA synthesis as well as cell

division. It also plays a role in the maintenance of proper sense of taste and smell, supports growth and development. Zinc possesses antioxidant and antimicrobial properties and confers protection against accelerated ageing [8].

Copper is the third most abundant trace element in the body after iron and zinc. Even though copper is an important micro nutrient, only small amount is needed by the body to function properly. It is essential for maintaining the strength of the skin, blood vessel, epithelial and connective tissues. Copper plays a role in the production of hemoglobin, myelin, melanin and proper functioning of thyroid gland. It also acts as both antioxidant and pro-oxidant [9].

Immune activation, damaged endothelial cells and activation of adhesion molecules often lead to inflammation and the secretion of c-reactive protein (CRP) and other inflammatory mediators in the body. C-reactive protein is a known regulator of inflammation and is the most commonly assayed biomarker of acute and chronic inflammation in clinical practice. It is associated with SCD, and some authors have correlated CRP with other markers of inflammation in SCD patients [10,11].

In healthy individuals, the body has the capacity to manage and control the amount of essential trace elements circulating in the blood and store in the tissue. The dietary essential metals are incorporated into blood if their blood levels are low, transported into the cells when their cellular levels are depleted or eliminated when blood and cellular levels are adequate or excess [12]. When this system fails to function optimally, abnormal levels and imbalance in their ratios occur. This study seeks to evaluate the levels of serum CRP, copper, zinc and copper-to-zinc ratio. It also correlates CRP with copper-to-zinc ratio in adult SCD patients.

2. Patients and Methods

The study participants were 100 confirmed SCD patients with mean age 18.8 ± 0.9 years in steady clinical state (55 males and 45 females) and 100 apparently healthy subjects with mean age 19.2 ± 0.9 years.

2.1. Ethical Consideration

The study protocol was reviewed and approved by the ethics committee of Edo State Hospital Management Board, Benin City (SCC34/2/45 dated 9 September 2014) and all participants gave informed consent before blood samples were collected.

2.2. Sample Collection and Preparation

Five mL of venous blood were obtained aseptically and 2 mL dispensed into a tube containing 3.8% sodium citrate for fibrinogen assay while 3 mL was dispensed into a plain container, which was allowed to clot at room temperature. Both were centrifuged at 3000 rpm for 10 min using a Compact II centrifuge (Pittsburg, PA, USA). The plasma and serum were stored at -20°C until analyzed.

2.3. Analytical Methods

Serum copper and zinc were assayed by colorimetric method using kits supplied by Centronic, Germany while serum CRP and plasma fibrinogen were assayed using reagents supplied by Sigma (St.Louis, MO, USA) and Anogen (Ontario, Canada) respectively. Commercially available control sera were included in the assay to ensure accuracy of analyses.

2.4. Statistical Analysis

The results obtained were presented as mean \pm standard error of the mean (SEM) and were analyzed using a statistical software package (SPSS version 20, IBM, Chicago, IL, USA). Students' t-test was used to compare the levels of copper, zinc and CRP in SCD patients and controls. Correlation analysis was carried out using GraphPad Prism 6 (Cal, USA) to test the relationship between copper-to-zinc ratio with measured inflammatory marker (CRP).

3. Results

The results obtained in this study are as presented in Tables 1 and 2. Serum copper, copper-to-zinc ratio, CRP and plasma fibrinogen were significantly higher ($p < 0.001$) in SCD patients than controls. On the other hand, serum zinc was significantly lower ($p < 0.001$) in SCD patients than controls. Table 2 indicated that copper-to-zinc ratio ($r = 0.312$; $p < 0.002$) and copper ($r = 0.210$; $p < 0.02$) correlated positively with CRP while zinc ($r = -0.199$; $p < 0.05$) correlated negatively with CRP in SCD patients in steady clinical state. No significant positive correlation ($r = 0.048$; $p = 0.08$) was observed between plasma fibrinogen and CRP.

Table 1. Comparison of measured variables in sickle cell disease patients and controls.

Parameters	Sickle Cell Disease Patients (n = 100)	Controls (n = 100)	P-value
Gender			
Number of males	55	58	
Number of females	45	42	
Age (years)	18.8 ± 0.9	19.2 ± 0.9	0.80
Serum copper (μmol/L)	28.92 ± 0.55	16.8 ± 0.5	0.001
Serum Zinc (μmol/L)	9.06 ± 0.38	13.54 ± 0.22	0.001
Copper-to-zinc ratio	3.16 ± 0.1	1.23 ± 0.09	0.001
Serum CRP (μg/mL)	1.14 ± 0.02	0.83 ± 0.82	0.001
Plasma fibrinogen (mg/dL)	295 ± 14.8	290 ± 16.1	0.001

Table 2. Correlation of copper-to-zinc ratio with C-reactive proteins in SCD patients.

Correlation	R-value	p-value
Copper/c-reactive protein	0.210	0.02
Zinc/c-reactive protein	-0.199	0.05
Copper-to-zinc ratio/c-reactive protein	0.312	0.002
Copper-to-zinc ratio/fibrinogen	0.048	0.08

4. Discussion

Some authors have reported that elevated serum copper level in the presence of low zinc (high copper-to-zinc ratio) may be a contributing factor to several diseases including hypertension, schizophrenia, autism, fatigue, headaches, childhood hyperactivity, depression, insomnia, senility, premenstrual syndrome, muscle and joint pain [12]. In this study, we observed significantly higher ($p < 0.001$) levels of copper-to-zinc ratio, serum copper, zinc, CRP and plasma fibrinogen in SCD patients than control subjects with normal hemoglobin. The finding also indicates that copper-to-zinc ratio correlated positively with CRP. Our finding is consistent with previous study [13]. Some authors reported significant association between inflammation and copper-to-zinc ratio in children while other observed no association [14]. Others reported significant association between inflammation and serum copper levels in hospitalized patients and adult volunteers. It was suggested that inflammation may influence micronutrients concentrations at CRP level as low as 0.6mg/L. Experimental and clinical studies have demonstrated that inflammatory cytokines disturb trace element homeostasis leading to elevated copper-to-zinc ratio in some disease conditions [15]. Wisniewska et al. [16] reported elevated copper-to-zinc ratio in neonates with early congenital infections and also observed that deficiencies of these micronutrients impaired the immune defense and make the patients to be susceptible to infectious diseases [17–19]. These micronutrients disturbances in response to inflammation may be contributing factors to a vicious cycle of impaired immune defense and higher risk of infection in susceptible individuals [16]. In a study which evaluated copper-to-zinc ratio and nutritional status in colorectal cancer patients during the perioperative period, it was observed that elevated copper-to-zinc ratio may be due to systemic inflammatory response to cancer [20]. Elevated copper-to-zinc ratio was considered as marker for asthma severity [21], renal disease among patients with peritoneal dialysis [22] and prostate cancer

[23]. In the elderly, high copper-to-zinc ratio was used as a predictive marker for mortality and associated with elevated serum CRP and erythrocyte sedimentation rate [24]. Elevated levels of inflammatory biomarkers have been associated with raised level of plasma copper. Over 90% of circulating copper is bound to caeruloplasmin and concentration of caeruloplasmin is elevated in response to inflammation, infection, and proinflammatory cytokines and also stimulates the production of acute phase proteins in the liver. Elevated copper-to-zinc ratio was used as a diagnostic and prognostic marker of inflammation in lymphoma and leukemia, gastric and breast cancer. Higher copper-to-zinc ratio was significantly correlated with under-nutrition, oxidative stress, inflammation and depressed immune function [20,22]. An elevated copper-to-zinc ratio also correlated with elevated level of prostate specific antigen in subjects with prostate cancer [23] and lipid peroxidation in patients with asthma [21].

The observed elevated level of CRP in SCD patients is consistent with previous study [25]. The authors suggested that the elevated levels of CRP in SCD patients may be due to repeated occlusion of blood vessels and eventual reperfusion of necrotized tissues resulting in the formation of increased generation of reactive oxygen species. The biologic role of CRP in inflammation is not completely understood, but it may stimulate leukocytes migration [26], reaction with bacterial surfaces to induce phagocytosis [27], enhance immune response [28] and facilitates lymphocyte blast transformation [25]. Other authors suggested that elevated CRP may have protective effect on endothelium derived nitric oxide bioavailability [7]. The high level of CRP in SCD patients may enable their immune system to respond better to numerous challenges associated with SCD [25].

The low level of zinc observed in SCD patients is consistent with previous studies [1,29]. Asanga et al. [1] reported that 68.3% of SCD patients had hypozincemia and it was attributed to several factors, such as chronic hemolysis, leading to loss of zinc from red blood cells which are vital storage sites for zinc, low dietary intake, increased urinary loss, kidney impairment, disturbance of zinc metabolism and high excretion in sweat [1]. The biochemical sequelae of low serum zinc in SCD patients may be reflected in low activities of some zinc-dependent-enzymes such as carbonic anhydrase in red blood cells, alkaline phosphatase in neutrophils and thymidine kinase. The effect of low zinc levels was also reported to affect urea cycle enzymes. Decreased activities of ornithine carbamoyl transferase, increased activities of glutamate dehydrogenase and carbamoyl-phosphate synthase 1 were reported in the liver of zinc deficient rats [29].

The observed high serum copper was however inconsistent with that reported by some authors [1], who observed no significant change in the level of copper in SCD patients compared with controls.

5. Conclusion

Copper-to-zinc ratio correlated positively with c-reactive protein in patients with SCD. The levels of copper and copper-to-zinc ratio were significantly higher while zinc was lower in SCD patients than control subjects. Inflammation may modulate copper and zinc homeostasis in SCD patients. It is suggested that copper-to-zinc ratio may be used as marker of nutritional deficiency and inflammation in patients with SCD.

Authors contributions: Mathias Abiodun Emokpae conceived, designed the experiments, performed the analysis and wrote the manuscript; Bamidele Emmanuel Fatimehin performed the data gathering, analysis and assisted in the writing of the manuscript.

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Conflicts of interest: The authors declare no conflict of interest.

References

1. Asanga, E.E.; Eseyin, O.A.; Ekanem, A.N.; Amaechi, D. Changes in serum zinc, magnesium and copper in sickle cell patients: A case study in Jos, Nigeria. *World. J. Pharm. Sci.* **2016**, *4*, 195–199.

2. Emokpae, M.A.; Ehioghase, I. Increased levels of pancreatic enzymes in sickle cell anemia and the effect of proteinuria. *J. Med. Invest. Pract.* **2015**, *10*, 13–15.
3. Emokpae, M.A.; Uadia, P.O.; Kuliya-Gwarzo, A. Antioxidant Enzymes and Acute phase Proteins correlate with markers of Lipid peroxide in adult Nigerian Sickle Cell Disease Patients. *Iran. J. Basic Med. Sci.* **2010**, *13*, 177–182.
4. Emokpae, M.A.; Uadia, P.O.; Gadzama, A.A. Correlation of Oxidative Stress and Inflammatory markers with the Severity of Sickle Cell Nephropathy. *Ann. Afr. Med.* **2010**, *9*, 141–146.
5. Emokpae, M.A.; Musa, M.O. Impact of fetal hemoglobin on micronutrients in sickle cell anemia. *J. Appl. Hematol* **2015**, *6*, 74–78.
6. Emokpae, M.A.; Tijani, A.D. The impact of proteinuria on serum levels of trace elements in sickle cell disease patients. *J. Med. Biomed. Sci.* **2014**, *3*, 16–20.
7. Bot, Y.S.; Benjamin, A.; Nyango, D.Y.; Ventmack, D.T.; Eunice, C.B.; Etukudu, N.S.; Obeta, M.U. Analysis of copper and Zinc in serum of sickle cell disease patients in Jos. *Int. J. Med. Sci.* **2015**, *3*, 207–209.
8. Maret, W.; Sandstead, H.H. Zinc requirements and the risks and benefits of zinc supplementation. *J. Trace Elem. Med. Biol.* **2006**, *20*, 3–18.
9. Classen, H.G.; Gröber, U.; Löw, D.; Schmidt, J.; Stracke, H. Zinc deficiency: Symptoms, causes, diagnosis and therapy. *Med. Monatsschr Pharm.* **2011**, *34*, 87–95.
10. Krishnan S, Setty Y, Betal SG, Vijender, V., Rao, K., Dampier, C. Increased levels of the inflammatory biomarker C-reactive protein at baseline are associated with childhood sickle cell vaso-occlusive crises. *Br. J. Haematol.* **2010**, *148*, 797–804.
11. Emokpae, M.A.; Aruomaren, A.; Osime, E. Relationship between neutrophil-to-lymphocyte ratio and inflammatory markers in Sickle cell anaemia patients with proteinuria. *Med. Sci.* **2016**, *4*, 11.
12. Osredkar, J.; Sustar, N.J. Copper and zinc, Biological role and significance of copper/zinc imbalance. *J. Clin Toxicol* **2011**, doi:10.4172/2161-0495.S3-001.
13. Bui, V.Q.; Stein, A.D.; Digirolamo, A.M.; Ramakrishnan, U.; Flores-Ayala, R.C.; Ramirez-Zea, M.; Grant, F.K.; Villalpando, S.; Martoreu, R. Associations between serum C-reactive protein and serum zinc, ferritin and copper in Guatemalan School children. *Biol. Trace Elem. Res.* **2012**, *148*, 154–160.
14. Galloway, P.; McMillan, D.C.; Sattar, N. Effect of the inflammatory response on trace element and vitamin status. *Ann. Clin. Biochem.* **2000**, *37*, 289–297.
15. DiGirolamo, A.M.; Ramirez-Zea, M.; Wang, M.; Flores-Ayala, R.; Martorell, R.; Neufeld, L.M.; Ramakrishnan, U.; Sellen, D.; Black, M.M.; Stein, A.D. Randomized trial of the effect of zinc supplementation on the mental health of school-age children in Guatemala. *Am. J. Clin. Nutr.* **2010**, *92*, 1241–1250.
16. Wisniewska, M.; Cremer, M.; Wiehe, L.; Beckerm, N.P.; Rijntjes, E.; Martitz, J.; Renko, K.; Bühner, C.; Schomburg, L. Copper to Zinc Ratio as Disease Biomarker in Neonates with Early-Onset Congenital Infections. *Nutrients* **2017**, *9*, 343.
17. Bonaventura, P.; Benedetti, G.; Albareda, F.; Miossec, P. Zinc and its role in immunity and inflammation. *Autoimmun. Rev.* **2015**, *14*, 277–285.
18. De Romana, D.L.; Olivares, M.; Uauy, R.; Araya, M. Risks and benefits of copper in light of new insights of copper homeostasis. *J. Trace Elem. Med. Biol.* **2011**, *25*, 3–13.
19. Krebs, N.F.; Miller, L.V.; Hambidge, K.M. Zinc deficiency in infants and children: A review of its complex and synergistic interactions. *Paediatr. Int. Child Health* **2014**, *34*, 279–288.
20. RibeiroI, S.M.F.; Moya, A.M.T.M.; Braga, C.B.M.; Domenici, F.A.; Feitosa, M.R.; Feres, O.; da Rocha, J.R.; da Cunha, S.F.C. Copper-Zinc ratio and nutritional status in colorectal cancer patients during the perioperative period. *Acta Cir. Bras.* **2016**, *31*, 24–28.
21. Guo, C.H.; Liu, P.J.; Hsia, S.; Chuang, C.J.; Chen, P.C. Role of certain trace minerals in oxidative stress, inflammation, CD4/CD8 lymphocyte ratios and lung function in asthmatic patients. *Ann. Clin. Biochem.* **2011**, *48*, 344–351.
22. Guo, C.H.; Chen, P.C.; Yeh, M.S.; Hsiung, D.Y.; Wang, C.L. Cu/Zn ratios are associated with nutritional status, oxidative stress, inflammation, and immune abnormalities in patients on peritoneal dialysis. *Clin. Biochem.* **2011**, *44*, 275–280.
23. Adaramoye, O.A.; Akinloye, O.; Olatunji, I.K. Trace elements and Vitamin E status in Nigerian patients with prostate cancer. *Afr. Health Sci.* **2010**, *10*, 2–8.

24. Malavolta, M.; Giacconi, R.; Piacenza, F.; Santarelli, L.; Cipriano, C.; Costarelli, L.; Tesei, S.; Pierpaoli, S.; Basso, A.; Galeazzi, R.; et al. Plasma copper/zinc ratio: An inflammatory/nutritional biomarker as predictor of all-cause mortality in elderly population. *Biogerontology* **2010**, *11*, 309–319.
25. Okocha, C.E.; Manafa, P.O.; Ozomba, J.O.; Ulasi, T.O.; Chukwuma, G.O.; Aneke, J.C. C-reactive protein and Disease outcome in Nigerian Sickle cell disease patients. *Ann. Med. Health Sci. Res.* **2014**, *4*, 701–705.
26. El Kebir, D.; Zhang, Y.; Potempa, L.A.; Wu, Y.; Fournier, A.; Filep, J.G. C-reactive protein-derived peptide 201-206 inhibits neutrophil adhesion to endothelial cells and platelets through CD32. *J. Leukoc. Biol.* **2011**, *90*, 1167–1175.
27. Lu, J.; Marjon, K.D.; Marnell, L.L.; Wang, R.; Mold, C.; Du Clos, T.W.; Sun, P. Recognition and functional activation of the human IgA receptor (FcαRI) by C-reactive protein. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 4974–4979.
28. Holmes, M.V.; Jiang, B.; McNeill, K.; Wong, M.; Oakley, S.P.; Kirkham, B.; Chowienzyk, P.J. Paradoxical association of C-reactive protein with endothelial function in rheumatoid arthritis. *PLoS One* **2010**, *5*, e10242.
29. Prasad, A.S. Zinc is an antioxidant and anti-inflammatory agent: Its role in human health. *Front. Nutr.* **2014**, *1*, 14.



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