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Journal Name:	Asian Journal of Research in Medical and Pharmaceutical Sciences
Manuscript Number:	Ms_AJRIMPS_44564
Title of the Manuscript:	Assessment of The Levels of Serum Zinc and Copper Among Sudanese Patients with Sickle Cell Anemia in Khartoum State
Type of the Article	

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Assessment of The Levels of Serum Zinc and Copper Among Sudanese Patients with Sickle Cell Anemia in Khartoum State Reviewer's Comments 1. Background: Sickle cell anemia (SCA) is associated with increased risks of multiple micronutrient deficiencies, zinc deficiency has been observed in patients with Sickle cell anemia, due to chronic hemolysis with subsequent loss of zinc from RBCs. Break into two sentences Background: Sickle cell anemia (SCA) is associated with increased risks of multiple micronutrient deficiencies. Zinc deficiency has been observed in patients with Sickle cell anemia, due to chronic hemolysis with subsequent loss of zinc from RBCs. 2. Objectives: The aim of this study was to assess the level of zinc and copper among Sudanese Patients with sickle cell anemia (SCA). Use lower case Objectives: The aim of this study was to assess the level of zinc and copper among Sudanese patients with sickle cell anemia (SCA). 3. Methods: Across sectional study was conducted during the period from March to April 2018, fourty samples from diagnosed patients with sickle cell anemia (SCA), admitted to Albuluk hospital in Khartoum state as cases and fourty samples from healthy individual With normal hemoglobin as controls, the level of zinc and copper was measured by using atomic absorption spectrophotometer, Data analysis was carried out by SPSS version 21. The highlighted words/phrases need to be corrected Methods: Across-sectional study was conducted from March to April 2018, involving forty subjects who had been diagnosed of sickle cell anemia (SCA), and had been admitted to Albuluk Hospital, in Khartoum State, as cases. Forty healthy individual with normal hemoglobin were recruited as controls. The levels of zinc and copper were measured, using atomic absorption spectrophotometer. Data analysis was carried out, using SPSS version, 21. 4. Indicate the biological sample that was collected for analysis 5. What were the ages of the patients? 6. What statistical parameters were determined? Results: There were a significant decreased in the levels of zinc and copper in patients with sickle cell anemia (SCA) with p-value = (0.000) (0.000) respectively, when compared to healthy individuals. The (Mean $\pm$SD; 0.137$\pm$0.079 versus 0.705$\pm$0.138ng/L) (0.512$\pm$0.290 versus 0.923$\pm$0.214ng/l) respectively. Also there was insignificant variation in the levels of zinc and copper in male	



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	patients compared to female patients, p-value (0.345) (0.656) respectively. The (Mean $\pm$SD; 0.144$\pm$0.079 versus 0.127$\pm$0.080 ng/L) (0.502$\pm$0.271ng/L versus 0.525$\pm$0.321 ng/L) in respectively, also there was negative correlation between levels of (zinc and copper) with duration time of disease, with (R= -0.603, p-value= 0.000) and (R= -0.443 p-value= 0.004) respectively, But there was no correlation between levels of (zinc and copper) with ages. 7. The results presentation is confusing; present it in a more clear way. 8. Do present the mean $\pm$SD first before the level of significance 9. Avoid the monotonous use of 'respectively' 10. Also there was insignificant variation in the levels of zinc and copper in male patients compared to female patients, p-value (0.345) (0.656) respectively. Modify as follows; 11. There was no significant difference between males and females in the mean levels of zinc (p = 0.345) and copper (p = 0.656) 12. In Conclusion: the levels of zinc and copper were decreased in patients with sickle cell anemia when compared to healthy individuals, and also there was negative correlation between the levels of (zinc and copper) and duration time of disease. Conclusion: The levels of zinc and copper were decreased in patients with sickle cell anemia, compared to healthy individuals. There was also negative correlation between the levels of zinc and copper and duration time of disease. 13. Use of duration is inappropriate. Sickle cell disease begins from infancy, so it should depend on the age of subjects. Introduction: 14. Sickle cells anemia (SCA) is a hemoglobinopathy characterized by chronic hemolysis, Chronic inflammation, immune deficiency, a heterogeneous Clinical picture and organ Damage Modify as follows; Sickle cells anemia (SCA) is a hemoglobinopathy, characterized by chronic hemolysis, chronic inflammation, immune deficiency, a heterogeneous clinical picture and organ damage 15. In addition, these nutrients have a major role in the protection of the red cell membrane against stress and free radical mediated by oxidation in SCA. 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. 16. In sickle cell disease; the deficiencies of essential elements some of which are vital in red cell maintenance, body growth and development have been observed.(4) Modify as follows; In sickle cell disease, there are deficiencies of some essential elements which are vital in maintenance of red cell integrity, body growth and	
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	development.(4) 17. The sickle hemoglobin is known to interact with diverse gene and environmental factors producing a multisystemic disease with several phenotypes. (5) The sickle hemoglobin is known to interact with diverse genes and environmental factors, producing a multi-systemic disease with several phenotypes. (5) 18. Minerals are inorganic substances, with chemical constituent 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) Modify as follows; 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) 19. They are important for human,(8,9) so deficiencies or disturbances in the nutrition can cause a variety of diseases and can arise in several ways.(10) Modify as follows; 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) Nutrient metals from diet are incorporated into blood if blood levels are depleted, transported into cells if cellular levels are inadequate, or excreted if blood and cell levels are sufficient or overloaded. 20. This highlighted statement is not clear 21. One of the most common trace-metal imbalances is elevated copper and depressed zinc Modify as follows; Two most common trace metal imbalances are elevated copper and depressed zinc in SCA 22. however the aim of this study was to assess the level of serum zinc and copper among Sudanese patients with sickle cell anemia. Re-frame to; Therefore, the aim of this study was to assess the level of serum zinc and copper among Sudanese patients with sickle cell anemia. 23. Study design: this was a Cross sectional case control study Study design: This was a cross-sectional case-control study 24. Study area and period: Blood samples were collected from Albuluk hospital, in Khartoum state, during the period from March to April 2018. Study area and period of study: Blood samples were collected from Albuluk Hospital, in Khartoum State, from March to April 2018. 25. Study population: forty Patients with sickle cell anemia (SCA) as a	
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	case and forty sample from normal individual with normal hemoglobin as control, gender and ages was matched Study population: Forty sickle cell anemia (SCA) patients, aged between... were recruited as cases and forty normal children with normal hemoglobin were enrolled as controls. The cases and control were gender and age-matched 26. Provide the range of ages for the cases and control. The mean ages and SD should be stated in the results. 27. Specify the clinical state of the cases 28. Provide information on the control 29. Provide the number of subjects, not their percentages 30. The inclusion criteria for the SCD patients is vague. Were they in-patients or out-patients? 31. Were the SCD patients in steady state or crisis? Were they taking drugs? 32. Exclusion criteria: any patients take long term zinc supplements Exclusion criteria: Any patients taking long term zinc supplements Subjects involved in this study were informed by the aims of the study and its importance, and verbal informed consent was obtained from each participant. 33.'informed by the aims of the study' is a wrong phrase 34. The study subjects were children below seventeen years. It is very odd to seek informed consent from children. Authors should take note of anomaly and report on the right procedure Data collection: by using questionnaire. 35. A complete statement should be made. 36. What was the questionnaire used for? Sampling: blood samples were collected and serum was separated. 37. The authors are too casual in the write-up. Each step should be described with some detail that will bring out what was exactly done, to allow the assessment of whether the proper procedure was carried out. This will also allow the study to be repeated by other scientists, to check the validity of the findings of the study. 38. What type of blood samples were collected; fasting or random? 39. From which part of the body was the blood sample taken? 40. What volume of blood was taken? 41. How was serum separated? Method: The levels of serum zinc and copper was measured by using atomic absorption spectrophotometer. 42. The full title of sub-section should be 'Method of assay of zinc and copper' 43. What was the model of AAS used? 44. Which company is the manufacturer and country of origin of the AAS? Quality Control: Pathological and Normal control sera were measured, to assure accuracy and precision of results.	
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	45. Modify the above as follows; 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 percentage, Mean and SD. Independent T-test was obtained to compare the study parameters in case versus control group. Correlation was done to study the relationship between study parameters and study variables, p-value less than 0.05 considered significant. 46. Modify as follows; 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 group. Correlation was done to study the relationship between study parameters and study variables, p-value less than 0.05 considered significant. 47. What type of correlation analysis was done? 48. State the variables for which the correlation was carried out. Results 49. The range of values (lowest and highest) of ages and all the parameters measured, should have been stated for the cases and controls Tables 1&2 50. For the parameters column, the units of Zn and Cu should be shown in parentheses. P-value less than 0.05 consider as significant 51. p-values less than 0.05 were considered as significant Figures 52. Why is that for Figures 1&2, the x-axes have duration of disease, but for Figures 3&4, the same axes have ages? 53. Authors ought to have been consistent in using ages. 54. There is a serious omission; the correlation analyses for the controls. 55. Are the authors suggesting there was no correlation analyses for the controls? Discussion 56. From literature of previous work, what are the levels of zinc and copper in sickle cell subjects and healthy controls? 57. How does your result compare with literature values? In sickle cell disease, the deficiencies of essential elements some of which are vital in red cell maintenance, body growth and development have been observed, in the current study the levels of zinc and copper showed a significant decrease in patients with sickle cell anemia (SCA) when compared to healthy individuals with p135 value (0.000), that might be occur due to chronic hemolysis with subsequent loss of zinc from RBCs, 58. Modify as follows; In sickle cell disease, 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 anemia (SCA), compared to healthy individuals (p value 0.000). This might have occurred due to chronic hemolysis with subsequent loss of zinc from RBCs.	
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	Zinc deficiency can also be the result of the adverse effect of hydrourea which increase zinc excretion.(11) 59. Modify as follows; Zinc deficiency can also be the result of the adverse effect of hydroxyurea which increase zinc excretion.(11) 60. Where from the hydroxyurea? 61. In the method section, nothing was said about hydroxyurea, so why the speculation? This finding was agreement with results of previous study done by (tagney and Philips, 1993; Parad, 2002; Idonij et al., 2011), which related zinc deficiency in sickle cell disease to manifestations such as growth retardation, hypogonadism in males, hyperammonemia, abnormal dark adaptation and cell mediated immune disorder. 62. Modify as follows; This finding was in agreement with results of some previous studies (Tagney and Philips, 1993; Parad, 2002; Idonij et al., 2011), which related zinc deficiency in sickle cell disease to manifestations such as growth retardation, hypogonadism in males, hyperammonemia, abnormal dark adaptation and cell mediated immune disorder. 63. Why alter the pattern of referencing? Numbering is being used, but there is switch to use of surnames. Be consistent Similarly, the biochemical evidence for zinc deficiency in patients with SCD includes low zinc concentrations in plasma, erythrocytes, hair lymphocytes and granulocytes,(12) 64. Modify as follows; Similarly, the biochemical evidence for zinc deficiency in patients with SCD include low zinc concentrations in plasma, erythrocytes, hair, lymphocytes and granulocytes,(12) also there was insignificantvariation in the levels of zinc and copper in patients with sickle cell anemia when compared according to gender the p-value was (0.345 and 0.656) respectively, and also there was negative correlation between levels of (zinc and copper) with duration time of disease, with (R= -0.603, p-value= 0.000) and (R= -0.443 p- value= 0.004) respectively, 65. Modify as follows; Also, there was non-significant difference between males and females, in the levels of zinc and copper in patients with sickle cell anemia. 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. 66. What is the implication of the inverse relation? 67. Is there any comment in the difference in r for the two metals? But there was no correlation between levels of (zinc and copper) with ages. 68. Why is this statement being made? Conclusion The levels of zinc and copper were decreased in patients with sickle cell anemia when compared to healthy individuals, and also there was negative correlation	
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	between the levels of (zinc and copper) and duration time of disease. 69. Modify as follows; The levels of zinc and copper were decreased in patients with sickle cell anemia when compared to healthy individuals, and also there was negative correlation between the levels of zinc and copper and age of the SCA patients. Conclusions: The levels of zinc and copper were decreased in patients with sickle cell anemia when compared to healthy individuals, and also there was negative correlation between the levels of (zinc and copper) and duration time of disease. 70. Modify as follows; Conclusion: The levels of blood zinc and copper were decreased in patients with sickle cell anemia, compared to healthy individuals. There was also negative correlation between the levels of zinc and copper and zinc. References 3. Hierso, R., Waltz, X., Mora, P. Effects of oxidative stress on red blood cell heology in sickle cell patients. Br J Haematol. 2014; 166:601–606. 3. Hierso, R., Waltz, X., Mora, P. Effects of oxidative stress on red blood cell rheology in sickle cell patients. Br J Haematol. 2014; 166:601–606. 8. Arinola, O.G., Olaniyi, J.A ., Akiibnu, M.O. Evaluation of antioxidant level and trace element status in Nigerian sickle cell disease patients with plasmodium parasitaemia. Pakistan journal of Nutrition . 2008 ; 7: 766-769. 8. Arinola, O.G., Olaniyi, J.A., Akiibnu, M.O. Evaluation of antioxidant level and trace element status in Nigerian sickle cell disease patients with Plasmodium parasitaemia. Pakistan Journal of Nutrition . 2008 ; 7: 766-769. 12. Garba, N., Ifeanyichukwu., O.M., Amilo, G.I., Audu, I. Evaluation of Trace Elements in Adult Sickle Cell Anaemia Patients in Zaria, North Western Nigeria. J Blood Disord Transfus . 2016 ; 7: 347. 12. Garba, N., Ifeanyichukwu., O.M., Amilo, G.I., Audu, I. Evaluation of trace elements in adult sickle cell anaemia patients in Zaria, North Western Nigeria. J Blood Disord Transfus . 2016 ; 7: 347. 71. Provide numbers for Tagney and Philips, 1993; Parad, 2002; Idonij et al., 2011. Then include them in the list of references, while providing the other details of each. OVERALL RECOMMENDATION the authors can address the raised issues convincingly, the paper could be recommended for acceptance	
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Minor REVISION comments		
Optional/General comments		

PART 2:

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Are there ethical issues in this manuscript?	(If yes, Kindly please write down the ethical issues here in details)	

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