Journal of Medicine in the Tropics (2015) 17:2:56-59

Journal of Medicine in the Tropics

Original Article

Acute-phase reactants, essential trace elements and some hematological parameters in Nigerian children with steady state sickle cell disorder

T. D. Ogunleye, E. A. Disu[1], A. A. Adelakun, O. F. Ajani, S. K. Rahamon, O. G. Arinola

Department of Chemical Pathology and Immunology, College of Medicine, University of Ibadan, Ibadan, [1]Department of Paediatrics, Lagos State University Teaching Hospital, Lagos, Nigeria

Abstract

Background: Sickle cell disease (SCD) is an inherited chronic hematological disorder, with inflammatory responses arising from different pathways. Information is scarce about the levels of acute-phase reactants and essentials trace elements in HbSS Nigerian children. Understanding of these will further elucidate the pathophysiology of SCD, which may assist in the proper management of this condition in the pediatric population.

Aim: To measure the levels of acute-phase reactants (C-reactive protein [CRP], C1q, C4, ferritin and transferrin), trace elements (Fe, Zn, Cu) and some hematological parameters in HbSS children < 5 years of age.

Materials and Methods: A total number of 26 consecutive steady state HbSS children below the age of 5 years was recruited for the study. The same number of HbAA children was recruited as a control. Trace elements were determined with atomic absorption spectrophotometer. C4, C1q and CRP were quantified using immunoplates, and full blood count analysis was done according to standard hematological procedures.

Results: There were no significant differences between serum mean levels of Zn, Cu, Ferritin, C4, C1q, albumin and CRP in steady state HbSS children compared to their HbAA counterparts. There was a significant increase in the level of serum iron in steady state HbSS children compared to HbAA children. There was also a significant reduction in the serum level of transferrin in steady state HbSS children compared to HbAA children. There were no significant differences between the white blood cell, red blood cell, mean corpuscular hemoglobin (Hb), mean corpuscular Hb concentration, platelets, lymphocytes, monocytes and neutrophils in steady state HbSS children compared to their HbAA counterparts. However, there was a significant reduction in the Hb concentration and hematocrit value in steady state HbSS children compared to HbAA children.

Conclusion: The study observes reduced inflammation in steady state HbSS children below the age of 5 years. It is recommended that consumption of diets or use of iron containing drug by HbSS children be monitored to prevent iron overload.

Key words: Complement factors, C-reactive protein, hemoglobin, iron, sickle cell disease

Introduction

Sickle cell disease (SCD) is an inherited chronic hematological disorder, which is due to a point mutation (GAG → GTG) in exon one of the β globin genes resulting in the substitution of glutamic acid by valine at position six of the β globin polypeptide chain,[1] and this leads to transformation of normal hemoglobin (Hb) HbAA (α2β2) to "sickle Hb" (HbS) (α2βS 2). Upon deoxygenation, HbS undergoes aggregation and polymerization, thus changing the discoidal erythrocyte into a crescent or sickle shape that eventually leads to hemolysis and short life span of red blood cell (RBC).[2] The hemolysis and the release of molecules associated with the Hb catabolism generate an oxidant environment with production of reactive oxygen and nitrogen species, which play very important roles

Access this article online	
Quick Response Code:	Website: www.jmedtropics.org
	DOI: 10.4103/2276-7096.162263

Address for correspondence:
Prof. O. G. Arinola, Department of Chemical Pathology and Immunology, College of Medicine, University of Ibadan, Ibadan, Nigeria.
E-mail: drarinolaog64@yahoo.com

in inflammation. Vascular dysfunction, activation of endothelial cells, with an expression of adhesion molecules and its ligands, and several receptors also participate in the inflammatory process.

Acute-phase reactants such as transferrin and C-reactive protein (CRP) are among plasma indicators of severity of disease in homozygous sickle cell individual.[3] In SCD, where inflammation is always present, levels of CRP have been observed to be elevated above normal values.[4] The acute-phase response to injury during inflammation was reported to be associated with changes in dynamics of many trace elements especially iron, zinc and copper,[5] thus it is expected that inflammation in SCD patients will affect the levels of trace elements. In 2008, Arinola *et al.* reported a significant variation in the levels of iron, zinc, manganese and total antioxidant status of adult HbSS patients compared to non-HbSS adults. But they found no significant differences in the levels of Mg, Cu, Cd, Se and albumin.[6] However, Kehinde *et al.*, reported significantly higher concentrations of Cu, Zn, and Mn in the plasma of adult SCD subjects with crisis relative to the non-SCD subjects or SCD subjects without crisis.[7] Durosinmi *et al.*, (1993) reported significantly higher concentration of Cu, K^+, and Fe in the plasma of adult SCD subjects relative to the non-SCD subjects.[8] There are many conflicting reports on the levels of acute-phase reactants and trace elements in stable-state HbSS adults.[5,6,9] Information is scarce about the levels of acute-phase reactants and essentials trace elements in HbSS Nigerian children. Understanding the levels of acute-phase reactants and trace elements in HbSS children will further elucidate the pathophysiology of SCD. This will assist in the proper management of this condition in the pediatric population.

Materials and Methods

Before commencement of the study, ethical approval was obtained from Lagos State University Teaching Hospital Ethical Committee. A total number of 26 consecutive steady state HbSS children below the age of 5 years was recruited for the study. The same number of HbAA children was recruited as a control, after obtaining informed consent from their parents as children were too young to give assent. Both groups were recruited by Consultant Pediatrician from Lagos State University Teaching Hospital, Lagos State, Nigeria. HbSS Children were ensured to be in a steady state, and all subjects belonged to the same socioeconomic class of the society.

Blood sample (5 mL) was collected as follows; 1 mL was collected in ethylenediaminetetraacetic acid (EDTA) bottle to determine red cell indices. One ml was put in a plain bottle for genotype screening. The remaining 2 mL was spun for assessment of trace metals and acute-phase reactants. Serum samples were stored at −20°C and analyzed within 2 days of collection.

Estimation of Trace Elements

Trace elements were determined with atomic absorption spectrophotometer (AAS/AAS – Buck 210/211, USA model). The principle of atomic absorption spectrophotometry is that atoms of the element, when aspirated into the AAS, absorb light of the same wavelength as that emitted by the element when in the excited state. The intensity of light absorbed is proportional to the concentration of the trace element in the sample.

C4, C1q and C-reactive Protein Estimation

C4, C1q and CRP were quantified using immunoplates based on the principle of antigen-antibody precipitation reaction in agarose gel. 5 μL of each sample was applied into each well of the immunoplate. During incubation, this was allowed to diffuse through the agar and react with specific antisera already incorporated in the agar. The diameter of precipitin rings formed after antigen-antibody reaction in a buffered agarose gel is proportional to the concentration of the analyte being determined (C4, C1q or CRP). Serum albumin concentration was determined using bromocresol green method, adapted in RANDOX albumin kit (Manufactured by Randox Laboratories).

Full Blood Count

The samples for full blood count (FBC) were taken into EDTA bottles. Analysis was done according to standard hematological procedure using (Sysmex XT–2000i, 2011 model, Japan Technology) auto-analyzer. FBC evaluation included the hematocrit and white cell, differential and platelet counts.

Data Analysis

Data were presented as mean ± standard deviation. Student's *t*-test was used to test the significance of differences between mean values. The $P < 0.05$ was considered significant.

Results

As shown in the Table 1 above, there were no significant differences ($P > 0.05$) in white blood cell, RBC, mean corpuscular Hb, mean corpuscular Hb concentration, platelets, lymphocytes, monocytes and neutrophils in steady state HbSS children < 5 years of age compared to their HbAA counterparts. There were also significant ($P = 0.05$) reduction in the levels of Hb and hematopoietic cell transplantation in steady state HbSS children compared to HbAA children.

As shown in the Table 2 above, there were no significant differences ($P > 0.05$) between the serum mean levels of Zn, Cu, Ferritin, C4, C1q, albumin and CRP in

Table 1: Hematological values (mean±SD) in steady HbSS children compared with the control

Group	Mean±SD	*P*
WBC		
HbAA	12.08±3.29	0.12
HbSS	15.92±5.77	
RBC		
HbAA	4.63±0.32	0.27
HbSS	3.48±1.09	
Hb		
HbAA	9.24±1.08	0.000**
HbSS	6.91±1.59	
HCT		
HbAA	33.13±3.10	0.000**
HbSS	23.97±5.33	
MCV		
HbAA	72.43±6.69	1.00
HbSS	72.79±16.42	
MCH		
HbAA	21.03±3.07	1.00
HbSS	21.72±1.34	
MCHC		
HbAA	27.84±1.34	0.22
HbSS	28.77±1.21	
PLT		
HbAA	344.56±164.96	0.78
HbSS	282.42±119.81	
LYM		
HbAA	64.71±12.32	0.051
HbSS	52.80±12.85	
MONO		
HbAA	9.23±3.64	0.99
HbSS	10.00±3.15	
HbAA	26.06±10.16	
NEUT		
HbSS	37.20±13.47	0.052

**P<0.01 (P value significant at <0.01). SD - Standard deviation, WBC - White blood cell, RBC - Red blood cell, Hb - Hemoglobin, MCV - Mean corpuscular volume, MCHC - Mean cell hemoglobin concentration, PLT - Platelet, LYM - Lymphocyte, MONO - Monocytes, NEUT - Neutrophils, HCT - Hematocrit, MCH - Mean cell hemoglobin

Table 2: Trace elements and acute-phase reactants in steady HbSS children compared with the control

Group	Mean±SD	*P*
Fe (µg/ml)		
HbAA	89.87±27.79	0.04*
HbSS	120.43±29.41	
Zn (µg/dl)		
HbAA	64.87±8.63	0.35
HbSS	70.79±8.19	
Cu (µg/dl)		
HbAA	115.57±27.05	0.93
HbSS	123.50±16.42	
Ferritin (ng/ml)		
HbAA	79.86±22.75	0.25
HbSS	95.80±17.39	
Transferrin (mg/dl)		
HbAA	122.58±37.37	0.024*
HbSS	78.22±39.29	
C4 (mg/dl)		
HbAA	21.00±8.92	1.00
HbSS	21.00±8.03	
C1q (mg/dl)		
HbAA	27.13±10.87	0.25
HbSS	22.98±15.99	
Albumin (g/dl)		
HbAA	4.83±0.456	1.00
HbSS	4.81±0.32	
CRP (mg/l)		
HbAA	3.53±0.90	0.59
HbSS	4.35±2.53	

**P*<0.05 (*P* value significant at <0.05). SD - Standard deviation, CRP - C-reactive protein

steady state HbSS children < 5 years of age compared to HbAA control. However, there was a significant ($P < 0.05$) increase in the level of serum iron in steady state HbSS children compared to HbAA children. There was also a significant ($P < 0.05$) reduction in the serum level of transferrin in steady state HbSS children compared to HbAA children.

Discussion

Sickle cell disease is a hereditary disorder with inflammatory responses arising from different pathways, such as vaso-occlusive phenomenon, tissue ischemia, surface ligand molecule activation from stressed reticulocytes, sickled erythrocytes, leukocytes and endothelial cells.[1] There is also increase in oxidative stress as a result of the hemolytic episodes and hem cytotoxicity, thus a shortened longevity of RBC. There is temporary suppression of erythropoiesis that results in anemia, and the rate of destruction of RBC does not match the rate of creation of new ones.[10] This explains the marked ($P < 0.001$) decrease in the hematocrit level and Hb concentration of HbSS children compared to the control observed in this study. However, there are no significant differences ($P = 0.05$) in other hematological parameters in steady state sickle cell children below the age of 5 years compared to control as observed in this study. This is contrary to reports of other studies carried out in older children and adults.[11-13]

Some studies suggested that circulating platelets in HbSS patients are chronically activated. Products released by these activated platelets are potent inflammatory and mitogenic substances resulting in increase of transmigration of leukocytes to the site of inflammation.[14] A significantly raised level of CRP in adults sickle cell patients compared to nonsickle cell adults has been reported. These findings suggested the use of serum concentration of CRP as an indicator of the severity of disease and possible onset of complications in adults SCD patients.[15] Their findings suggested the use of serum concentration of CRP as an indicator of the severity of disease and possible onset of complications in adults SCD patients.[15] However, the concentration of CRP, C4, albumin and ferritin considered in this study were not significantly different from nonsickle cell children. The result might suggest that sickle cell children below

the age of 5 years have no severe inflammation coupled with the fact that the SCD children were in their steady state and were apparently healthy. Moreover, it is likely that RBC sickling is not severe in these children due to relatively higher fetal hemoglobin (HbF) compared with the adults. HbF is known to decrease the onset and the severity of SCD and increase survival time of RBCs.[16]

There is a greater potential for values of trace elements to be deranged during SCD depending on the severity of the disease. From this study, there was a significant increase in the serum concentration of free iron in SCD children compared to nonsickle cell children. Also transferrin concentration in SCD children was significantly lower compared to nonsickle cell children. Increase iron in SCD could be due to increased hemolysis from short-lived sickled and fragile RBCs while decrease in transferrin could be due to the fact that it is being used-up by binding with and transportation of excess free iron. Saturation of transferrin by excess circulating iron results in increased nontranferrin bound iron (NTBI).[17] NTBI tends to enter tissues more readily and which causes the formation of reactive oxygen species. Therefore, excess free iron may be one of the factors responsible for oxidative stress in SCD patients. Apart from iron contributing to oxidative stress in sickle cell patients, free iron may be used by micro-organism to grow and therefore explaining why SCD patients are prone to infections.

Conclusion

This study suggests that there is no severe inflammation in HbSS children below the age of 5 years. It is also recommended that consumption of diets or use of iron containing drug by HbSS children be monitored to prevent iron overload.

In conclusion, this study suggests that there is no severe inflammation in prevaccinated HbSS children below the age of 5 years, and pneumococcal vaccination of both HbSS and HbAA children can result in derangement of trace element. It is also recommended that consumption of diets or use of iron containing drug by HbSS children be monitored to prevent iron overload.

References

1. Steinberg MH. Sickle cell anemia, the first molecular disease: Overview of molecular etiology, pathophysiology, and therapeutic approaches. Scientific World Journal 2008;8:1295-324.
2. Kate SL, Lingojwar DP. The epidemiology of sickle cell disorder in the state of Maharashtra. Int J Hum Genet 2002;2:161-7.
3. Hedo CC, Aken'ova YA, Okpala IE, Durojaiye AO, Salimonu LS. Acute phase reactants and severity of homozygous sickle cell disease. J Intern Med 1993;233:467-70.
4. Okocha CE, Manafa PO, Ozomba JO, Ulasi TO, Chukwuma GO, Aneke JC. C-reactive protein and disease outcome in Nigerian sickle cell disease patients. Annals of Medical and Health Sciences Research 2014;4:701-705.
5. Shenkin A. Trace elements and inflammatory response: Implications for nutritional support. Nutrition 1995;11:100-5.
6. Arinola OG, Olaniyi JA, Akiibinu MO. Evaluation of antioxidant levels and trace element status in Nigerian sickle cell disease patients with *Plasmodium* parasitaemia. Pak J Nutr 2008;7:766-9.
7. Kehinde MO, Jaja SI, Adewumi OM, Adeniyi IM, Nezianya MO, Ayinla EO. Liver enzymes and trace elements in the acute phase of sickle cell anaemia. West Afr J Med 2010;29:244-8.
8. Durosinmi MA, Ojo JO, Oluwole AF, Akanle OA, Arshed W, Spyrou NM, *et al*. Trace elements in sickle cell disease. J Radioanalytical Nucl Chem 1993;168:233-42.
9. Okochi VI, Okpuzor J. Micronutrients as therapeutic tools in the management of sickle cell disease, malaria and diabetes. Afr J Biotechnol 2005;4:1568-79.
10. Glassberg J. Evidence-based management of sickle cell disease in the emergency department. Emerg Med Pract 2011;13:1-20.
11. Rao SS, Goyal JP, Raghunath SV, Shah VB. Hematological profile of sickle cell disease from South Gujarat, India. Hematol Rep 2012;4:e8.
12. Sanya JO. Comparative analysis of haematological Indices of haemoglobin genotypes Aa, Ac, As, Sc And Ss – A retrospective study. J Pharm Biol Sci 2012;2:2278-3008.
13. Fasola F, Adedapo K, Anetor J, Kuti M. Total antioxidants status and some hematological values in sickle cell disease patients in steady state. J Natl Med Assoc 2007;99:891-4.
14. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest 2005;115:3378-84.
15. Olaniyi JA, Arinola GO. Humoral immunity and haemoglobin F (HbF) status in steady state adult Nigerian sickle cell disease patients with asymptomatic malaria. Turk J Med Sci 2009;39:953-7.
16. Steinberg MH. Predicting clinical severity in sickle cell anaemia. Br J Haematol 2005;129:465-81.
17. Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol 2005;18:277-87.

How to cite this article: Ogunleye TD, Disu EA, Adelakun AA, Ajani OF, Rahamon SK, Arinola OG. Acute-phase reactants, essential trace elements and some hematological parameters in Nigerian children with steady state sickle cell disorder. J Med Trop 2015;17:56-9.

Source of Support: Nil, **Conflict of Interest:** None declared.