The Journal of Medical Research

Research Article

JMR 2017; 3(3): 110-113
May- June
ISSN: 2395-7565

www.medicinearticle.com
Received: 13-03-2017
Accepted: 30-05-2017

Indices of Iron status are nearer to normal levels in foetal haemoglobin persistent Sickle cell anaemia compared to the sickle cell anaemia with low foetal haemoglobin

Emokpae MA[1], Ogbebor MH[1], Olufemi BI[2]

1 *Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria*

2 *Sickle Cell Centre, Health Management Board, Benin City. Edo State, Nigeria*

Abstract

Background: Foetal haemoglobin (HbF) is a major regulator of the haematologic and clinical features of Sickle cell anaemia (SCA). This study examines the frequency of high HbF concentration and its effect on the levels of indices of iron status in adult SCA subjects on steady clinical state. **Materials and Methods:** Iron, total iron binding capacity (TIBC), percentage transferrin saturation (TS), HbF and full blood count were determined in adult sickle cell disease and control subjects using spectrophotometric method and haematology analyzer. **Results:** One hundred (100) adults with SCA on steady clinical state and 50 with normal haemoglobin were recruited for the study. Out of the 100 SCA subjects, 25(25%) had hereditary persistent HbF (HPFH) (≥5%). Serum iron and TS were significantly lower ($p<0.001$) in SCA subjects compared with controls, while mean TIBC and HbF were significantly higher ($p<0.001$) in SCA subjects than controls. The mean serum iron and TS were significantly higher ($p<0.001$) in HPFH SCA subjects compared to SCA subjects with low (≤4.9%) HbF concentrations, while TIBC was significantly lower ($p<0.001$) in HPFH SCA subjects than those with low HbF concentrations. **Conclusion:** The indices of iron status were near normal levels in HPFH SCA compared to the levels in SCA with low HbF which may due to increased haemoglobin, relative decrease in intravascular haemolysis and urinary loss of iron. The proportion of SCD subjects with HPFH as observed was 25%. It is suggested that iron status in SCA subjects should be done routinely.

Keywords: Sickle cell anaemia, Foetal haemoglobin, Serum iron, Total iron binding capacity, Percentage transferrin saturation.

INTRODUCTION

Sickle cell Disease (SCD) is a major health problem in Nigeria which affects more than 1 million persons while over 40 million individuals are carriers of the sickle cell genes [1]. Sickle cell anaemia (SCA) is an inherited disorder of haemoglobin caused by a simple nucleotide substitution of thymidine for adenine of the β-chain that leads to amino acid valine instead of glutamic acid. This substitution is responsible for the alterations in the properties of the haemoglobin tetramer, with the tendencies to polymerize in the deoxygenated state [2]. The SCD is often associated with life -long chronic haemolytic anaemia, recurrent painful crisis, organ damage and decreased life expectancy.

Iron deficiency in SCA may be common than envisage since more circulating iron is often lost through the urine [3,4]. Also, under nutrition has been recognized as a major complication of SCD that should be attended to as part of clinical care [5]. Because of chronic nature of SCD symptoms, some researchers have advocated low cost self administered oral therapy which may improve patient's outcome. Even though under nutrition has been identified as a critical feature of SCD, the problem has not been sufficiently addressed at an empirical level [5].

Studies have shown that iron deficiency may be beneficial and ameliorates sickling while overt iron deficiency may be associated with a marked reduction in the number of sickled erythrocytes in blood smears, decreased levels of serum unconjugated bilirubin and lactate dehydrogenase [6,7,8].

Hereditary persistent foetal haemoglobin (HPFH) occurs in some SCA subject in which there is sustained high level of foetal haemoglobin (HbF) in the circulation. During human embryonic and foetal development HbF($\gamma_2\beta_2$) become the dominant haemoglobin to aid metabolism in the developing foetus.

***Corresponding author:**
Dr. MA Emokpae
Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria
Email:
mathias.emokpae[at]uniben.edu

But after birth the synthesis of HbF is switched to normal adult haemoglobin ($\alpha_2\beta_2$). The failure or delay in globin gene switching from HbF gene to normal adult haemoglobin gene in some SCA occurs such that HbF levels remained above normal in some subjects [9,10]. Failure to switch may occur due to a deletion of both the beta and delta globin genes with the resultant persistence of HbF into adulthood [9,10]. Foetal haemoglobin is a major genetic regulator of the haematologic and clinical features of SCD, HbF gene are genetically modulated and levels of HbF among sickle erythrocytes may vary in SCD subjects. Iron status in adult SCD patients in relation to HbF concentrations has not been evaluated in this centre. This study therefore examines the frequency of HPFH and its effect on the levels of indices of iron status in SCA subjects on steady clinical state.

MATERIALS AND METHODS

Blood specimen collection: This was a prospective case-control study of adult SCD patients. The study subjects were randomly selected from among the patients on routine visit to the Sickle cell centre, Benin City, Nigeria. Blood specimens were collected from all the SCD patients who met the inclusion criteria.

Inclusion criteria: All SCD patients diagnosed by cellulose acetate electrophoresis at pH 8.6 and in clinical steady state. Clinical steady state was defined by a steady haematocrit and haemoglobin values over a given period of 4-6 weeks without any symptoms suggestive of crisis which was established clinically by a careful history and physical examinations. The control subjects were ambulatory adults with HbAA selected from among the staff and students of university of Benin, Benin City. The haemoglobin status was confirmed by cellulose acetate electrophoresis at pH 8.6. Informed consent was obtained from both SCA and control subjects. None of the study participants was on any form of iron supplementation for at least two weeks before the sample collection and had not received blood transfusion within 3 months prior to blood collection. Neither were they on any special iron-rich diet.

Ethical Consideration: The protocol for the study was approved by the ethics committee of the Edo state Ministry of Health before the commencement of study. The individual subjects gave informed consent to participate in the study.

Preparation of samples: Five (5) milliliters (mL) of venous blood was collected from each subjects with 2.5mL aliquot dispensed into a plain and EDTA anticoagulated containers. The anticoagulated blood was used to determine the full blood count using haematology auto-analyzer while the excess was used to prepare lysate for HbF estimation using alkaline denaturation method. The non-anticoagulated blood was allowed to clot at room temperature and centrifuged at 2000rpm for 10minutes. The supernatant serum was separated into a clean test tube and was used for iron study. The serum was stored at -20^{o}C for about 2weeks before analyses was done. A control serum was included in the assays to ensure accuracy of determinations.

Methods

Serum iron and TIBC were determined spectrophotometrically using reagent test kits supplied by BioLabo SA. Serum iron bound to transferrin in an acid medium provided by citric acid dissociates, to release ferric ion which is reduced to ferrous form by ascorbic acid. The ferrous iron reacts with chromogen (ferene) to produce a colour complex which is measured at 600nm. The measured absorbance is directly proportional to the amount of iron present in the specimen.

The unsaturated site on apotransferrin is saturated by the addition of sufficient ferric iron, after which the excess is removed by adsorption with basic magnesium carbonate powder. After centrifugation, the bound iron remaining in supernatant is measured spectrophotometrically and the absorbance is directly proportional to the concentration of iron binding capacity present in the sample.

Total iron binding capacity (TIBC) was calculated as iron concentration multiply by 5, while percentage transferrin saturation (TS) was calculated as serum iron divided by TIBC expressed in percent.

Foetal haemoglobin concentration was determined using the method of Betke et al.,1959 [12]. The principle of test is based on the fact that HbF is more resistant to alkaline denaturation than any other haemoglobin. Denaturation is stopped by the addition of ammonium sulphate to lower the pH and precipitate the denatured haemoglobin. After precipitation, the amount of unaltered haemoglobin is measured and expressed as percentage of the total haemoglobin present.

Data Analysis: Data presented were normally distributed as assessed by W/S test for normality. Differences in the data were analyzed by the Student's t-test and p-value <0.05 was considered as significant. Values obtained were presented as mean± standard error of mean (SEM). The measured variables were compared in SCA subjects with HPFH and low HbF concentrations as well as HbAA controls.

RESULTS

One hundred (100) adults (male: female; 57:43) with SCA (HbSS) on steady clinical state and 50 (male : female; 29:21) with normal haemoglobin (HbAA) were recruited for the study. Out of the 100 SCA subjects, 25(25%) had HPFH with HBF ≥5%. The age of the SCA subjects ranged from 18-25 with a mean of 21±0.8years, while the age of the HbAA controls ranged from 18-25 years with a mean of 23±1.2years.

Table 1 shows the comparison of serum iron, TIBC, percentage transferrin saturation and foetal haemoglobin concentrations in SCA with control subjects.

Table 1: Serum Iron, total iron binding capacity, percentage transferrin saturation and foetal haemoglobin levels in Sickle cell anaemia and control subjects (Mean±SEM)

Measured Variables	SCA subjects (HbSS) n=100	Controls (HbAA) n=50	P-value
Number of Males	57(57%)	29(58%)	-
Number of Females	43(43%)	21(42%)	-
Age (Years)	21.4±0.8	23.2±1.2	-
Serum Iron(µg/dL)	58.9±3.15	119.0±5.52	0.001
TIBC (µg/dL)	270.4±3.36	245.7±4.59	0.001
Percentage transferrin saturation (%)	21.8±1.39	48.7±2.41	0.001
HPFH (Foetal haemoglobin) (%)	4.9±0.53	0.9±0.19	0.001

TIBC=total iron binding capacity.

Table 2: Serum Iron, TIBC, percentage transferrin saturation levels in foetal haemoglobin in Hereditary Persistence Foetal Haemoglobin SCA Subjects (Mean±SEM)

Measured Parameters	SCA subjects with HPFH (≥5%) n=25	SCA subjects with Foetal haemoglobin(≤4.9%) n=75	P-value
Serum Iron (µg/dL)	71.9±2.1	53.6±3.2	0.001
TIBC (µg/dL)	258.9±2.5	278.8±3.6	0.001
Percentage transferrin saturation (%)	27.6±1.0	19.3±4.6	0.001

TIBC=total Iron binding capacity, HPFH= Hereditary persistence foetal haemoglobin

Table 3: Mean±SEM of haematological Indices of SCA and Control Subjects

Measured Parameters	SCA subjects (HbSS) n=100	Control subjects (HbAA) n=50	P-value
White Blood Cell ($x10^3$/L)	15.5±1.13	5.2±0.17	0.001
Red Blood Cell (10^3/L)	2.6±0.11	5.2±0.08	0.001
Haemoglobin (g/dL)	7.0±0.26	13.0±0.22	0.001
Haematocrit (%)	23.1±0.82	42.2±0.49	0.001
Mean corpuscular volume (fl)	87.8±1.79	81.4±1.10	0.002
Mean corpuscular haemoglobin (pg)	26.2±0.49	25.1±0.48	0.05
Mean corpuscular haemoglobin conc(g/dL)	29.7±0.25	30.7±0.28	0.001

Table 4: Haematological parameters of Hereditary Persistence Foetal Haemoglobin SCA subjects and SCA subjects with low foetal haemoglobin (Mean±SEM)

Measured Parameters	SCA subjects with HPFH (≥5%)	SCA subjects with low Foetal haemoglobin (≤4.9%)	p-value
Numbers of subjects	24	75	-
Age (Years)	22±0.9	21±0.3	>0.05
White Blood Cell ($x10^3$/L)	14.8±1.10	15.3±0.90	>0.05
Red Blood Cell (10^3/L)	2.7±0.11	2.5±0.12	>0.05
Haemoglobin (g/dL)	7.7±0.20	6.7±0.21	0.001
Haematocrit (%)	24.4±0.52	22.3±0.41	0.001
Mean corpuscular volume (fl)	87.3±1.21	82.5±2.11	0.001
Mean corpuscular haemoglobin (pg)	25.6±4.81	24.2±0.50	0.05
Mean corpuscular haemoglobin concentration (g/dL)	29.8±0.22	28.5±0.31	0.001

Table 2 shows the comparison of measured parameters in HPFH SCA with SCA subjects with HbF levels ≤4.9%.

Table 3 shows the comparison of red blood cell indicators of iron status in SCA and controls while table 4 compares the red blood cell parameters between HPFH SCA and those with low HbF.

DISCUSSION

Studies have shown that HPFH with high HbF have been observed to be important in influencing the clinical course of SCD patients and the frequency of HPFH vary from place to place. This study evaluates the frequency HPFH and its influence on the indices of iron status in SCA subjects from our centre. The proportion of SCA subjects with HPFH observed in this study was 25% and lower than 29% reported in Sokoto, Nigeria and elsewhere [13,14]. Even much higher prevalence of 37% was reported in SCA subjects in Kampala, Uganda [15]. Our findings was similar to 25% reported in Dakar, Senegal [16], but higher than 17% observed in 1992 by Fatunde and Scott-Emuakpor in Western Nigeria[17]. The overall mean HbF level observed in our study was 4.9±0.53% which was lower than the overall means of 8.8% in Congo [18], 9.0% in Uganda [15] and 9.1% in Saudi Arabia [19] even though the previous studies were conducted in Children. The presence of HbF within each erythrocyte in SCA subject tends to impair sickling at reduced oxygen tension and has been reported to be clinically protective against SCA associated complications [13,20,21]. Foetal haemoglobin levels were associated with a reduced rate of painful crisis episodes, fewer leg ulcers, less acute chest syndromes, osteonecrosis and disease severity [15]. Delay in globin gene switching from haemoglobin delta to haemoglobin beta expression occurs in SCA, such that HbF levels remain high in some subjects [10]. The exact mechanism is not clear but might reflect the slower centripetal regression of red or haematopoietic marrow to the axial skeleton in the presence of expanded erythropoiesis that is the result of sustained hemolysis [10].This may explain why there are different levels of HbF in SCD patients.

An increased level of serum iron and TS and decreased level of TIBC observed in SCA subjects with HPFH compared to those with low HbF concentration was observed in this study. The increased levels of iron status in those patients with high HbF may be due to increased levels

of haemoglobin, decreased intravascular haemolysis and probably decrease in urinary iron loss even though the latter was not determined in this study. Because of increased intravascular haemolysis occurring in SCD patients, most of the circulating iron is lost through the urine [22-23] which may be contributing factors to low levels of serum iron in SCD patients with low HbF. Increased HbF in SCD patients protects against intravascular haemolysis. Fetal haemoglobin does not only retard sickle cell polymerization but reduce sickle cell concentration, both HbF and its mixed HbS cannot enter deoxy sickle haemoglobin polymer phase. The anti-polymerization effect of HbF resides in the haemoglobin delta. The low levels of iron and TS and increased TIBC is an indication of decreased iron status in SCD subjects with low HbF levels. This is consistent with other studies [22,23]. They reported that iron deficiency was associated with a decrease in HbF concentration.

The indices of iron status in SCD were lowered than those observed in controls. This observation is not in agreement with others which reported that serum iron and TS were normal or modestly elevated in patients with SCA [24,25]. Some researchers have argued that low serum iron levels may be ameliorating sickling in SCA subjects and prolong sickle cell survival. It was demonstrated that ^{51}Cr-labeled autologous red cell survival in SCA patient with low indices of iron status showed a half-life of 15.9 days which was decreased to 5.2days upon iron supplementation [11]. The lower red cell production due of lack of iron appeared to have compensated by the improvement in red cell survival. The indices of iron status observed in our study were lower than that reported in multiple transfused SCD patients [26]. They reported that increased levels of iron, TS, and decreased TIBC in multiple transfused SCD patients suggests an increase in iron stores and the patients may be at risk of developing iron overload [26]. None of our studied subjects had transfusion within the last 3months prior to blood collection. Ray et al[27]reported that patients with sickle cell trait (SCT) had more chances to have decreased iron status than SCA. It was also observed that malnutrition was common in SCA than SCT even though malnutrition may be the major risk factor of iron deficiency anaemia. No patient with SCT was enlisted in this study.

The observed decreased levels of mean corpuscular haemoglobin concentration (MCHC) in SCA subjects compared to controls is consistent with that previously reported [11,28]. The decrease in mean corpuscular sickle haemoglobin concentration (MCHC-S) in SCD patients may cause a substantial delay in sickle haemoglobin polymerization. Prolongation of gelation time delay in excess of the capillary transit time would allow the sickle cells to transverse the capillary bed to escape to the arterial side before the occurrence of sickle haemoglobin polymerization, rheologic impairment and subsequent vascular occlusion [11,27].

CONCLUSION

The increased levels of indices of iron status observed in HPFH SCD compared to the levels in SCD with low HbF may be due to increased haemoglobin, relative decrease in intravascular haemolysis and probably reduced urinary loss of iron. The proportion of SCD subjects with HPFH as observed was 25%. Further study of urinary iron loss in SCD patients is suggested.

Conflicts of Interest

The authors do not state any conflict of interest.

REFERENCES

1. Steinberg M and Rodgers G. Pharmacologic modulation of foetal haemoglobin. Medicine 2001;80:328-344.
2. Akinyanju O. The National Burden of Sickle Cell Disorder and the way forward. Sickle Cell Foundation, Nigeria. 2010; pp 1-17.
3. Davies S, Henthorn J, Brozovic M. Iron deficiency in Sickle cell anaemia. J Clin Pathol 1983;36:1012-1015.
4. Rao KRP, Patel AR, McGinnis P, Patel MK. Iron stores in adults with Sickle cell anaemia. J Lab Clin Med 1984;103:792-797.
5. Hyacinth HI, Gee BE, Hibbert JM. The Role of Nutrition in Sickle Cell Disease. Nutr Metab Insight 2010;3:57-67.
6. Rao KRP, Ray VH, Patel AR. Serum Ferritin and sequestered stores of body iron. Am J Clin Pathol 1983;80:743-745.
7. Haddy TB, Castro O. Overt iron deficiency in sickle cell disease. Arch Intern Med 1982;142:1621-1624.
8. Rao KRP, Patel AR, McGinnis P. Iron deficiency anaemia in an adult with sickle cell disease. Blood 1978;52(Suppl):87.
9. Little JA, Demsey NJ,Tuchman M, Ginger GD. Metabolic persistence of foetal haemoglobin. Blood 1995; 85:1712-1718.
10. Akinsheye I, Alsultan A, Solovieff N, Ngo D, BaldwinCT, Sebastiani P, Chui DHK, Steinberg MH. Foetal Haemoglobin in Sickle Cell Anaemia. Blood 2011; 118(1):19-27.
11. Koduri PR. Iron in Sickle cell Disease: A Review Why Less is better. Am J Hematol 2003; 73:59-63.
12. Betke K,Marti HR, Schlicht I. Estimation of small percentages of foetal haemoglobin. Nature (Lond), 1959;184:1877-1878.
13. Jiya NM, Matazu LS, Bilbis, Shehu RA. Serum levels of Antioxidant vitamins in foetal persistent Sickle cell anaemia children in Sokoto, Nigeria, Ann Afri Med 2005;4(4):168-171.
14. Zago MA, Wood WG, Clegg JB, Weatherall DJ, O'Sullivan M, Gunson H. Genetic control of F-cells in human adults. Blood. 1979;53:977
15. Mpalamba L, Ndugwu CM, Ddungu H, Idro R. Foetal haemoglobin and disease severity in Sickle cell anaemia patients in Kampala,Uganda. BMC Blood Disorders 2012;12:11.
16. Diop S, Thiam D, Cisse M, Toure-Fall AO, Fall K, Diakhate L. New results in clinical severity of homozygous sickle cell anaemia in Dakar, Senegal. Haematol Cell Ther 1999;41:2217-2221.
17. Fatunde CJ,Scott-Emuakpor AB. Foetal haemoglobin in Nigerian children with sickle cell anaemia: Effect on haematological parameters and clinical severity. Trop Geogr Med 1992; 44:264-266.
18. Mouele R, Galacteros F, Feingold J. Haemoglobin F (HbF) levels in sickle cell anaemia patients homozygous for the Bantu haplotype. Eur J Haematol 1999;63:136-137.
19. El-Hazmi MAF. Clinical and haematological diversity of sickle cell disease in Saudi children. J Trop Pediatr 1992;38:106-112.
20. Noguchi CT, Rodgers GP, Serjeant G, Schechter A. A level of foetal haemoglobin necessary for treatment of sickle cell disease. N Engl J Med 1988;318:96.
21. Nathan DG, Linch D, Beardsly P, Platt O, Letvin N. Foetal haemoglobin (HbF) production is enhanced by hydroxyurea (HU). Blood 1983;62:74.
22. Castro O, Poillon WN, Finke H, Massac E. Improvement of sickle cell anaemia by iron-limited erythropoiesis. Am J Hematol 1994;47:74-81.
23. Bouchair N, Manigne P, Kanfer A, Raphalen P, be Montalembert M, Hagege L, Verschuur A, Maier-Redelsperger M, Girot R. Prevention of sickle cell crisis with multiple phlebotomies. Arch Pediatr 2000;7:249-255.
24. Rao KRP, Patel AR, McGinnis P, Patel MK. Iron stores in adults with sickle cell anaemia. J Lab Clin Med 1984;103:792-797.
25. Russo-Mancuso G, Samperi P, Gangarossa S, Consalvo C, Schiliro G. Iron status in white sickle cell disease patients. Eur J Haematol 1992;48:232-233.
26. Ikusemoro AI, Halim NKD, Awodu OA, Ehiaghe FA, Isoa EM, Ehiaghe IJ. Iron status of multiple transfused sickle cell anaemia patients attending a sickle cell clinic in Benin City, Nigeria. Open J Pathol 2014;4:25-31.
27. Ray D, Mondal R, Chakravarty UK, Burman DR. Assessment of Iron status in patient of sickle cell disease and trait and its relationship with the frequency of blood transfusion in Paediatric patients attending B.S Medical College and Hospital, Bankura, West Bengal, India. Int J Sc Stud 2014;2(4):37-39.
28. Eaton WA, Hofrichter J, Ross PD. Delay time of gelation: a possible determinant of clinical severity in sickle cell disease. Blood 1976;47: 621-627.