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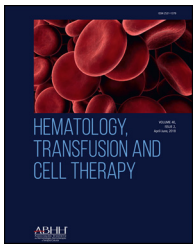

Original article

Iron deficiency, still a rarity in children with sickle cell anemia in Ile-Ife, Nigeria

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ABSTRACT

In this study, children with sickle cell anemia were evaluated for iron deficiency. Serum ferritin and free erythrocyte protoporphyrin free erythrocyte protoporphyrin (FEP) levels, mean corpuscular volume mean corpuscular volume (MCV) and mean corpuscular hemoglobin mean corpuscular hemoglobin (MCH) were used in determining their iron status. The study was done at Pediatric Hematology Outpatient Clinic of the Obafemi Awolowo University Teaching Hospitals' Complex, Ile-Ife. Forty-eight HbSS subjects in steady state and 48 apparently well age and sex matched HbAA controls were evaluated. Serum ferritin less than 25 ng/dL FEP greater than cut off for age, mean corpuscular volume MCV and mean corpuscular hemoglobin MCH less than cut off for age were regarded as indicating iron deficiency. Serum ferritin values ranged from 34.2 to 3282.9 μg/L, with a mean of 381.2 (1.0), median 180 μg/L; which was significantly higher than the controls ($p = 0.000$). FEP was lower in the subjects but none was iron deficient compared with the controls. The mean corpuscular hemoglobin MCH of subjects was significantly lower than the controls. Subjects had lower mean corpuscular volume MCV compared with controls. Iron deficiency was not detected in any of the subjects with sickle cell anemia in comparison to a prevalence of 43.75% in the controls. Iron deficiency anemia (IDA) was found in 16.7% of the controls, using the WHO cut off for anemia which is hemoglobin concentration of <11 g/dl. While a high prevalence of iron deficiency was noted in the control group, patients with sickle cell anemia were largely iron sufficient, despite their anemia. Iron supplementation remains unnecessary as part of routine management of children with sickle cell anemia in our practice.

Abbreviations: EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; IDA, iron deficiency anemia; SCA, sickle cell anemia; SCD, sickle cell disease.

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Introduction

Sickle cell disease (SCD) is the most common genetic hemoglobin disorder in which there is an inheritance of mutant hemoglobin genes from both parents.[1] About 25% of adults in Nigeria have the sickle cell trait.[2] In Nigeria, sickle cell anemia disease (Hb SS) is fairly distributed across the regions including the State of Osun in South-West Nigeria, where this study was done. Sickle cell anemia (SCA) affects about 3% of the Nigerian population.[2]

The iron status of sickle cell anemia patients has previously been reported as indicating either sufficiency or iron overload.[3,4] Some authors have also reported iron deficiency among children with sickle cell anemia.[5,6] Evaluating for iron deficiency in SCA is important, as it could contribute to worsening anemia and impairment in growth and neurocognitive development.

Iron is an important component of hemoglobin in the red blood cells. Iron is needed for oxygen transport. While the human body tightly regulates iron absorption and recycling,[7] there is no physiological regulatory mechanism for iron excretion. Iron overload is prevented only by regulating iron absorption. The common causes of iron deficiency in children, including dietary deficiency, infections, malabsorption and blood loss through hookworm infestation, are prevalent in Nigeria.[5] Iron deficiency is mainly prevented by maintaining a balance between iron intake, iron absorption and excretion.

Individuals with sickle cell disease have an adequate iron source, potentially from increased red cell turnover and from repeated blood transfusions.[8] An increase in gut iron absorption also contributes significantly to the iron pool in subjects with SCA. However, children with SCA may have a similar predisposition to nutritional inadequacies, similar to others without SCA, and excessive urinary iron loss could additionally result in iron deficiency.[9]

In this study, iron deficiency was evaluated using serum ferritin, free erythrocyte protoporphyrin, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). The use of a battery of tests to define iron status in a population has been suggested to improve precision in diagnosis of iron deficiency anemia (IDA).[10] A low mean corpuscular volume for age and serum ferritin less than 25 ng/mL are each 100 percent sensitive to IDA in SCD.[11,12] While serum ferritin less than 25 ng/mL is 100 percent specific to IDA in SCD, low MCV for age has a specificity of 97 percent.[11,12]

Iron deficiency occurs more frequently during periods of rapid growth, as in infancy and adolescence, hence the inclusion of children aged up to fifteen years in this study. The study aimed to evaluate for iron deficiency among children with sickle cell anemia in an environment with a high prevalence of iron deficiency.

Method

This was a cross-sectional study of children homozygous for sickle cell disease who are attended to at the Consultant Outpatient Pediatric Hematology Clinic of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, State of Osun, Nigeria. The OAUTHC is a tertiary hospital which also serves as a referral center for the neighboring states in Southwest Nigeria.

The clinic has one hundred and two children with regular clinic attendance. Forty-eight study participants who were in steady state, aged between one and fifteen years and having fulfilled the inclusion criteria, were consecutively recruited into the study. Apparently healthy age and sex-matched children, with a confirmed hemoglobin AA (Hb AA) genotype, who were attending the Pediatric General Outpatient Clinic for follow-up after recovery for simple ailments, as well as those attending for a routine school entrance medical check-up, were recruited as controls.

Since the study involved the comparison of the proportions of two independent groups, the number (*N*) in each group was calculated using the formula by Taylor.[13]

The sample size for each group was forty-four and this was increased to forty-eight to address attrition. The subjects with SCA were on routine daily folic acid, vitamin C and multivitamin supplementation, as well as proguanil for malaria prophylaxis.

Written informed consent was obtained from parents/caregivers of the participants who had satisfied the eligibility criteria for the study. The study protocol was approved by the Research and Ethics Committee of the OAUTHC, Ile-Ife, Nigeria.

Inclusion criteria for subjects

1. Children aged one to fifteen years.
2. Confirmed Hb SS by electrophoresis.
3. Subjects must be in steady state, i.e., the absence of symptoms and signs attributable to acute illness for at least four weeks prior to recruitment.[14]

Exclusion criteria for subjects

1. Children with clinically suspected hepatic or malignant disease.
2. Children who had received blood transfusion within three months prior to recruitment.
3. Children who had received iron supplements within three months prior to recruitment.

This study did not consider thalassemia while determining the Hb genotype status of the study participants. Even though SCA is widely reported in Nigeria, the presence of thalassemic co-inheritance has not been widely reported.

The inclusion and exclusion criteria for the controls were the same (when appropriate) as for the patients, except for those confirmed to be Hb AA.

The medical history of each participant, including demographic and socioeconomic data, was obtained from the parent/guardian and the participants when appropriate and entered into a study-specific proforma. Each child was thoroughly examined. The weight in kilograms and height/length in centimeters of each child were recorded in the proforma.

Five milliliters of blood were drawn from a peripheral vein. The first 3 mL and then another 2 mL of blood were put into plain specimen and ethylenediaminetetraacetic acid (EDTA)

bottles, respectively. Blood specimens in plain bottles were centrifuged at 1000 revolutions per minute for 15 min. Serum was separated and kept frozen at −20 °C Lake until the sample was analyzed for ferritin and FEP.

Serum ferritn and free erythrocyte protoporphyrin were analyzed using the Enzyme-linked immunosorbent assay (ELISA) kit, Accubind Elisa Microwells (Monobind Inc. Forest, CA 92630, USA). The analysis of the samples was performed at the Department of Biochemistry Laboratory of the Obafemi Awolowo University, Ile-Ife. The Full Blood Count was determined using an auto analyzer (SYSMEX® UK) at the Department of Hematology, Obafemi Awolowo University, Ile-Ife. For this study, the cutoff values for iron deficiency were defined as serum ferritin less than 25 ng/dL,[11,12] for free erythrocyte protoporphyrin (FEP), greater than 70 μg/dL in children under the age of 5 years and greater than 80 μg/dL in children 5 years and above,[15] for mean corpuscular volume, less than 70 fL in children 0.6–2 years, less than 75 fL in 2–6 years, less than 77 fL in 6–12 years, and less than 81 fL in 12–15 years,[16] for mean corpuscular hemoglobin, less than 23 pg in children less than 2 years, less than 24 in 2–6 years, and less than 25 pg in 6–15 years,[16] and for serum ferritin levels, and for iron overload, greater than 300 μg/L.

The data was analyzed using the Statistical Package for Social Sciences (SPSS for Windows Version 17.0). Tests of statistical significance were done using the independent 't' test and the Chi-square (X^2), as applicable. The statistically significant level was set at a 'p' value less than 0.05.

Results

Forty-eight patients with Hb SS and 48 controls with Hb AA genotypes participated in the study. The age, sex and social class distribution of the participants is presented in Table 1.

The mean age (SD) was 8.0 (4.0) for both groups. The subject and control groups each comprised 22 males and 26 females, giving a male:female ratio of 1:1.2. Only the social classes 1–4 were represented. When the socioeconomic class was grouped into higher social classes (1, 2 and 3) and lower classes (4 and 5), 87.5% of the Hb SS was in the higher class, compared to 83.3% of the controls, while 12.5% of the Hb SS were in the lower class, compared to 16.7% of the controls. Hence, the social class distribution of both patients and controls was essentially similar ($p = 0.9$).

Table 1 – Showing the distribution of age, sex and social class of the children studied.

Parameters	SCA subjects (N = 48) n (%)	Hb AA controls (N = 48) n (%)
Age [Completed years]		
1–5	17 (35.4)	17 (35.4)
6–10	17 (35.4)	17 (35.4)
11–15	14 (29.1)	14 (29.1)
Sex		
Male	22 (45.8)	22 (45.8)
Female	26 (54.2)	26 (54.2)
Social class		
I	9 (18.8)	14 (29.2)
II	17 (35.4)	11 (22.9)
III	16 (33.3)	15 (31.3)
IV	6 (12.5)	8 (16.7)
V	0 (0.00)	0 (0.00)

$X^2 = 2.691$; df = 3; $p = 0.442$.

Table 2 compares the mean values of the hematological parameters of the patients and the controls. The mean hemoglobin concentration and mean corpuscular volume were higher in the controls than in the patients, while the mean corpuscular hemoglobin was higher in the patients than in the controls. All the differences, except for the mean corpuscular volume, were statistically significant. Eight of the controls had anemia (Hb concentration less than 11 g/dL), while all subjects with SCA were anemic.

Table 3 shows the mean values for serum ferritin and free erythrocyte protoporphyrin for the patient and control groups. Mean serum ferritin was considerably higher (eight-fold) in the SCA subjects than in the Hb AA controls. This was statistically significant ($p < 0.001$). Mean log transformation of 5.31 (1.0) was obtained for the patients, as the serum ferritin levels were skewed. The mean FEP in the SCA patients was lower than the mean value for the controls. The difference was also statistically significant, ($p < 0.001$).

Table 4 shows the comparison of iron deficiency frequencies in patients with SCA and controls. None of the subjects with SCA had iron deficiency while 21 (43.75%) controls had iron deficiency ($p < 0.001$). Iron deficiency anemia (IDA) was found in 16.7% of the controls, using the WHO cut off for anemia, which is a hemoglobin concentration of <11 g/dl.[17]

Discussion

This study did not detect iron deficiency among Nigerian children with sickle cell anemia attending our clinic. Serum ferritin reflects the reticuloendothelial store and is considered to be a sensitive indicator of iron stores.[18] In steady state, the serum ferritin level correlates with total body iron stores and thus, is the most convenient laboratory test to estimate iron stores.[19]

A significantly higher serum ferritin level was found in patients, compared to their Hb AA counterparts. Raised serum ferritin level has been reported in earlier studies among sickle cell anemia patients in steady state.[4,20,21] None of the patients in this study had serum ferritin levels below the cutoff for iron deficiency. This contrasts with the findings among the controls.

A rise in the concentration of protoporphyrin is one of the first indicators of insufficient iron in the bone marrow.[22] The mean free erythrocyte protoporphyrin level was normal in both patients and controls. It was, however, higher in the controls, compared to the patients, and the difference was statistically significant. Free erythrocyte protoporphyrin was lower in the patients in this study, among whom none was iron deficient, compared to the controls, among whom 21 (43.75%) were iron deficient. Vichinsky[11] found elevated FEP levels in fifty Hb SS patients, of whom six were confirmed to have iron deficiency. In this study, four of the subjects with

Table 2 – Comparison of hematological indices of subjects and controls.

Hematological parameters	SCA subjects $n=48$ Mean (SD) Range	Controls $n=48$ Mean (SD) Range	t	p
Hemoglobin [g/dL]	6.91 (1.8) 4.3–9.6	11.1 (1.7) 7.9–14.6	−14 .5	<0 .001*
MCV [fL]	74.5 (14.3) 33.4–104.5	78.6 (6.6) 59.9–90.0	−1 .6	0 .112
MCH [pg]	25.5 (2.7) 18.9–30.5	23.6 (2.1) 17.7–28.5	3 .9	<0 .001*

*p< 0.05.

Table 3 – Comparison of serum ferritin and free erythrocyte protoporphyrin levels of the subjects and controls.

Variables	SCA subjects $n=48$	Hb AA control $n=48$	T	p
Serum ferritin [ng/dL]				
Mean	381.2	46.1 ± 46.0	−4.14	<0.001
Range	(34.2–3282.9)	(3.7–264.6)		
Mean log transformation	5.4 (1.0)	3.4 (0.9)		
Median	180	33.9		
Serum free erythrocyte protoporphyrin [μg/dL]				
Mean	23.1 ± 22.1	71.7 ± 27.7	−9.51	<0.001
Range	(5.8–106.6)	(59.9–90)		
Median	16.0	70.0		

Table 4 – Comparison of distribution of iron deficiency, iron sufficiency and iron overload in patients and controls.

Iron status	Hb SS subjects no (%)	Hb AA controls no (%)
Iron deficiency (SFER < 25 ng/dl + [a]FEP/MCV/MCH)	0	21 (43.8)
Iron-sufficient (SFER: 25–300 ng/dl)	32 (66.7)	27 (56.3)
Iron overload (SFER: >300 ng/dl)	16 (33.3)	0
Total	48 (100.0)	48 (100.0)

$X^2 = 37.42$; df = 2; $p < 0.001$ (Fischer's exact).

[a] Free erythrocyte protoporphyrin, MCV, MCH less than cutoff for age.

SCA had elevated FEP without being iron deficient. It has been reported that elevated FEP may occur in patients with SCA in the absence of iron deficiency because of the increase in the rate of erythropoiesis occurring in the bone marrow.[23]

Patients with sickle cell anemia had lower mean corpuscular volume, compared to controls. However, the difference was not statistically significant. Akinbami[24] found lower mean corpuscular volume in a study on children with sickle cell anemia in steady state, in comparison to controls. Akodu et al.[23] in a study on children aged 1–5 years in Lagos, however, found higher mean corpuscular volume in the patients with SCA, compared to controls. The mean corpuscular volume is expected to be higher in children of an older age group, compared to the younger age group.[24] The contrasting values of the mean corpuscular volumes in their study and this present study seems unusual, as the MCV is expected to be higher in the older age group of patients, as noted in this study.

Mohanty et al.[6] and Hayes[26] have earlier reported higher mean corpuscular hemoglobin among Hb SS patients, compared to AA controls. Their findings were similar to those obtained in this study, as the mean corpuscular hemoglobin concentration was higher in our subjects than the controls, and the difference was statistically significant. In the SCD patient, vitamin B-12 and folic acid are maintained in a critically balanced state. An increase in the demand of erythropoesis resulting from chronic hemolysis may cause a deficiency state and macrocytosis.[24]

The use of a battery of tests has been assessed as being useful in the diagnosis of iron deficiency. The combination of serum ferritin, FEP, MCV and MCH was used to determine the iron deficiency status in this study. Based on the criteria used in this study, iron deficiency was not detected in subjects with sickle cell anemia, compared to their Hb AA counterparts, of whom 43.75% were iron deficient.

The rarity of iron deficiency in children with sickle cell anemia in northern Nigeria has been reported by Isah et al.[27] Other studies have also demonstrated elevated levels of serum ferritin in children who have sickle cell anemia.[4,25,28] This suggests that children with sickle cell anemia may not be prone to iron deficiency because of their chronic hemolytic state, as the iron from the breakdown of red blood cells is usually retained in the body. It is instructive that 21 Hb AA (52.4% of whom were 1–5 years old) were iron deficient, using the earlier stated criteria to determine iron status. Eight (16.7%) of the controls had IDA, using Hb concentration <11.0 g/dl.[17] Seven of the controls that had IDA were 1–5 years old. This shows the high prevalence of IDA among healthy Nigerian children, as reported in earlier studies,[29,30] in spite of their higher social economic class. The social economic class of the groups studied (patients and controls) reflects the urban setting of the study, which is mainly characterized by the middle- to upper-income class. In addition, they represent the class most likely to present for medical care at a tertiary institution because of the challenge of out-of-pocket expenses. Most of the subjects with SCA were iron sufficient (66.7%), in comparison to the controls (56.25%). About 33.3% of the subjects had iron overload, this not being unusual, as chronic hemolysis and multiple blood transfusions have been known to contribute to iron overload.[31]

Akodu et al.[25] studied children in a younger age group, compared to this study. Ninety-seven pre-school children with sickle cell anemia participated in their study and three models were used to define IDA. The model that employed use of low Hb plus low MCV and low serum ferritin, which is similar to this study, did not detect IDA. Furthermore, the model using low Hb plus low serum ferritin and low transferrin saturation also did not detect IDA among the SCA group, compared to the controls. However, the third model using low Hb plus low MCV and low transferrin detected IDA in three of the ninety-seven study participants with sickle cell anemia. Other studies have also detected iron deficiency among children with SCA,[5,6,32] using different models for their study. There might be a need for a standard model for screening for iron deficiency among subjects with SCA. Rodriguez et al.[33] also reported iron deficiency among infants with SCD. This was attributed to the rapid growth and consequent increased iron demand characteristic in infancy. Our study did not include children in this age group. At our practice, children with SCA are usually diagnosed in late infancy or after infancy, due to the lack of facilities for newborn screening.

In conclusion, children with SCA at our practice do not have iron deficiency, even though the latter is prevalent among their Hb AA counterparts. Despite their anemia, these children with sickle cell anemia do not need iron supplementation.

Limitation of study

The Hb SS patients were not differentiated from sickle cell-beta0 thalassemia (Sbeta0 thalassemia) patients. It probably does not bias the results because it is conceivable that the prevalence of beta thalassemia alleles would be similar in the Hb AA control group, and so the percentage of children with a decrease in MCV and MCH would be similar in both groups.

Conflicts of interest

The authors declare no conflicts of interest.

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