Iron status in sickle cell disorders

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Abstract

Background: There is a general view that patients with sickle cell disease (SCD) reveal iron overload and that iron deficiency anemia is uncommon in them because of the availability of an adequate iron source from increased red cell turnover and blood transfusions. However, there are reports of iron deficiency in a proportion of these patients, which can often be missed because they exhibit already anemic condition.

Objective: To evaluate the iron status and to determine the incidence of iron deficiency in sickle cell disorders including subjects with SCD, sickle cell trait (SCT), and compound heterozygosity.

Materials and Methods: A total of 155 subjects were included in study; 138 of these showed homozygous sickle cell anemia (SS) and 17 sickle cell trait (AS). Presence of HbS was detected by solubility test, followed by hemoglobin phenotype determination by automated HPLC. The measurement of serum iron and total iron-binding capacity was done by ferrozine method, serum ferritin by radioimmunoassay, and zinc protoporphyrin (ZPP) by using ProtoFluor-Z Hemato-fluorimeter, while hematological parameters were determined using a Sysmex Kx21 autoanalyzer.

Result: Of the 155 patients studied, 7 (4.5%) showed iron deficiency and 33 (21.3%) showed iron overload. Iron and serum ferritin values in transfused patients were significantly higher than in nontransfused patients. The mean reticulocyte count was significantly lower in iron deficient group than that in iron sufficient group. The ZPP values were elevated in all iron deficient and the 39 of 148 iron sufficient/overloaded subjects; however, ZPP values were much higher in the iron deficiency group.

Conclusion: A state of iron deficiency may be present in SCD and SCT patients. Therefore, we recommend that patients with SCD and SCT should be screened for iron deficiency by conventional laboratory tests.

KEY WORDS: Sickle cell anemia, iron deficiency anemia, iron overload, zinc protoporphyrin

Introduction

Sickle cell disorders (SCDs) that are characterized by the presence of hemoglobin S (HbS) in red cells[1] refer to a group of disorders caused by autosomal recessive inheritance of a pair of abnormal hemoglobin genes, including the sickle cell gene.[2] In HbS (β6 glu→val), the glutamic acid in the sixth position on the β-chain is replaced by valine.[3–5] SCD comprises (1) sickle cell anemia (SCA)—this is the homozygous state (HbSS), one gene inherited from each of the parents; (2) sickle cell trait—this is the heterozygous state (HbAS), one gene from one parent is for HbS while other gene is for HbA; and (3) SCD (compound heterozygosity)—this term refers to all disease states in which at least one gene is of HbS that may be in combination with abnormal gene of β thalassemia, α thalassemia, HbD, HbE, HbC, HbQ, or any other hemoglobinopathy and includes SCA, sickle cell thalassemia, or HbS-D and HbS-E disease.[6]

Iron deficiency anemia (IDA) is not generally recognized as a medical problem in patients who have SCDs, as there is increased gastrointestinal absorption of iron associated with hemolysis and repeated blood transfusions, which are thought to provide a sufficient source of iron.[7,8] However, there are several reasons to believe that iron deficiency may occur in patients with SCDs as a SCA patient is not immune to environmental factors that precipitate IDA. Such factors,

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especially in the tropics, include poor nutrition and parasitic infestations and varying bacterial infections, which may disturb iron metabolism.[9] In addition, excessive urinary iron loss, poor absorption and metabolism of iron owing to multiple mucosal/submucosal infarcts, and progressive multiple organ damage/failure make SCA patients highly susceptible to IDA.[8,9]

In patients with SCD, conventional laboratory tests for iron deficiency such as mean corpuscular volume (MCV), transferrin saturation, serum ferritin, and free erythrocyte protoporphyrin (FEP) may be at abnormal levels as serum ferritin, serum iron, and transferrin are acute-phase reactants that are affected by both inflammation and infection in patients. Hence, interpretation of these parameter may be difficult in SCA patients whose pathology is underlined by a chronic inflammatory state and overlapping acute inflammatory events precipitated by infections.[8–10]

Although IDA is encountered in various hemoglobinopathies, there is controversy on status of iron in SCDs. This study has been undertaken to evaluate the usefulness of MCV, serum iron, serum total iron-binding capacity (TIBC), serum ferritin, and zinc protoporphyrin (ZPP) for detection of iron deficiency in patients of SCD, which may prove helpful to guide further investigation and treatment plan in patients with SCDs and prevent additional morbidity in them.

Materials and Methods

Study Design

This study was conducted in a tertiary-care hospital, Surat, India, from July 2011 to November 2013. A total of 155 patients were included mainly from: (1) subjects screened for presence of HbS by outdoor camps and positive for HbS and (2) outdoor and indoor patients of New Civil Hospital, Surat, with presence of HbS.

Of total 155 patients, 138 patients were of homozygous sickle cell anemia (SS) and 17 patients were of sickle cell trait (AS), who fulfilled inclusion and exclusion criteria as mentioned further. None of the included patients had been transfused during a 3-month interval before study.

Inclusion Criteria

Patients of all age groups and of either sex having presence of HbS who were ready to provide sample for hematological studies were included.

Exclusion Criteria

Subjects with absence of HbS and those with history blood transfusion in last 3 months were excluded.

An informed consent was obtained from individual subjects.

Blood Sample Collection

Approximately 6 mL of venous blood was collected from each of the subjects; 2 mL of the sample was collected into an EDTA vacutte and used for the analysis of hematologic parameters. The remaining 4 mL of the sample was collected in a plain vacutte for iron studies.

Diagnostic Method

Presence of HbS was detected by solubility test, followed by hemoglobin phenotype determination by automated HPLC using the Bio-Rad Hb variant testing system (Bio-Rad Laboratory, Hercules, CA). Serum iron and TIBC was measured by ferrozine method using the fully automated biochemistry auto-analyzer. Serum ferritin was measured by radioimmunoassay. Hematological parameters (complete blood count with blood indices) were determined using Sysmex Kx21 autoanalyzer. ZPP was measured on ProtoFluor-Z Hematofluorimeter (Helena Laboratories, UK).

The reticulocyte count was determined from blood smears after supravital staining with methylene blue and reported as a percentage of circulating RBCs. The corrected reticulocyte count was calculated as per the following formula:

Corrected reticulocyte count = (reticulocyte% × patient's HCT)/normal HCT.

The diagnosis of IDA was established based on the following criteria: (1) low serum iron <60 µg/dL[10]; (2) high TIBC ≥ 450 µg/dL[11]; (3) low serum ferritin < 15 ng/mL[6]; (4) low MCV for age: 0.5–2 years < 70 fL, 2–5 years < 73 fL, 5–8 years < 75 fL, 9–13 years < 76 fL, 14–17 years < 77 fL, 18 years or older < 80 fL[11]; (5) ZPP > 38 µg/dL.[10]

Statistical Analysis

The data were presented as mean ± standard deviation (±SD). *T*-test was used to compare the mean values. Statistical significance was accepted when *P* value was <0.05.

Results

Our study included a total of 155 patients from both genders (62 male and 93 female subjects) with age ranging between 5 and 65 years. Hematological and biochemical parameters of all 155 patients with SCA by transfusion history are shown in Table 1. There were no statistically significant differences in the mean hemoglobin level, mean MCV, mean serum TIBC, mean reticulocyte count, and mean ZPP (*P* > 0.05) between the two groups but the values of serum iron and serum ferritin were significantly higher in transfused patients than that in nontransfused patients (*P* < 0.0000001).

Of the 155 patients, 7 (4.5%; 4 male and 3 female subjects) met the criteria for iron deficiency. The age, hematological features, and biochemical data of these iron-deficient patients are shown in Table 2. Of these seven iron-deficient patients, six were from the nontransfused group, and only one was from the transfused group who was a female aged 40 years. The mean age for iron-deficient patients was 24.3 years. The first stage of iron deficiency was detected by a low serum ferritin level without reduced serum iron levels. In our study, one subject showed normal iron level with reduced ferritin level.

In iron-sufficient group [normal iron profile and iron overloaded subjects (*n* = 148)], mean reticulocyte was 6.60, with SD of 2.67. In the iron-deficient group (*n* = 7), mean was 1.64, with SD of 1.02.The mean reticulocyte count was significantly lower in iron-deficient group than that in iron-sufficient patients (*P* < 0.02079).

Table 1: Hematological and biochemical parameters of 155 patients with sickle cell anemia by transfusion history

Variable	Transfused patients (*n* = 37) (M = 17, F = 20), mean (SD)	Nontransfused patients (*n* = 118) (M = 45, F = 73), mean (SD)
Age (years)	24.8 (14.5)	22.08 (12.1)
Hemoglobin(g/dL)	8.6 (1.3)	9.09 (1.6)
MCV(fL)	71.4 (7.06)	69.9 (7.1)
Serum iron	335.1 (293.5)	172.6 (149.9)
Serum TIBC	387.9 (83.2)	410 (70)
Serum ferritin	402.7 (429.5)	146.4 (149.9)
RC	7.8 (3.1)	5.8 (2.5)
ZPP	33 (11.4)	33.8 (14.2)

Table 2: Hematological and biochemical parameter of 7 identified iron deficient patients with sickle cell disease

Patient No. (status of sickle cell disease)	Age (years)	Sex	Hb (g/dL)	MCV (fL)	Serum iron (mg/dL)	Serum TIBC (mg/dL)	Serum ferritin (ng/dL)	ZPP (mg/dL)	RC (%)
1 (AS)	15	M	3.4	50.2	41	453	14.5	103	1.1
2 (SS)	40	F	9.5	75	25	457	12.9	69	1
3 (SS)	45	F	6.2	59	42	484	12.4	98	1.5
4 (AS)	15	M	10.9	62	45	380	5.7	77	2
5 (SS)	15	M	7.6	72	60	476	12.4	80	0.9
6 (AS)	15	M	11.7	62	54	399	8.7	84	1
7 (SS)	25	F	10.2	69	30	491	12.8	93	4
Mean (SD)	24.3 (12.1)		8.5 (2.7)	64.2 (7.9)	42.4 (11.3)	448.6 (39.7)	11.3 (2.8)	86.28 (11.28)	1.64 (1.02)

Of 155 cases, ZPP values were elevated in 45 cases, which included all iron-deficient and 39 of 148 iron-sufficient patients. However, the ZPP values in the deficient group were higher.

In patients with SCD, serum ferritin was increased in 33 (23.9%) patients, at normal level in 101 (73.2%) patients, and decreased in 4 (2.9%) patients. However, in patients with sickle cell trait, serum iron was increased in 4 (23.5%) patients, at normal levels in 10 (58.8%) patients, and decreased in 3 (17.6%) patients.

Serum iron level was significantly decreased in higher number of patients with SCD and was normal in most sickle cell trait patients. In patients with SCD, serum iron was increased in 41 (29.7%) patients, at normal levels in 93 (67.4%) patients, and decreased in 4 (2.9%) patients. However, in patients with sickle cell trait, serum iron was increased in 6 (35.3%) patients, at normal levels in 8 (47.1%) patients, and decreased in 3 (17.6%) patients.

In patients with SCD, TIBC was increased in 47 (34.1%) patients, at normal levels in 85 (61.6%) patients, and decreased in 6 (4.3%) patients. However, in patients with sickle cell trait, TIBC was increased in 5 (29.4%) patients, at normal levels in 12 (70.6%) patients, and decreased in none.

Discussion

It is commonly observed that patients with SCA similar to other chronic hemolytic anemia patients are iron loaded because of enhanced hemolysis. But, the recent studies show that the patients with SCA may be iron deficient. In this study, seven patients (4.51%) met the criteria for iron deficiency. As six of these were from the nontransfused group and one from the transfused group, the incidence of iron deficiency among the nontransfused subjects (118) was 5.09%.

Results of current study were close to the study conducted by Patra et al.[11] and Okeahialam and Obi [12] who found iron deficiency in 6% and 7.76% cases, respectively. However, iron deficiency was diagnosed by Kassim et al.[13] using similar criteria as ours in Yemini patients in 13.3% and by Vichinsky et al.[8] in 16% of nontransfused patients. Mohanty et al.[14] using the single criteria of ZPP/heme ratio (ZPP/heme) for diagnosis in Indian subjects reported iron deficiency in 67.7% of SCDs patients and 26.2% of sickle cell trait patients, which is much higher than all other studies. This variation may be owing to different study populations or geographic variation and environmental factors and can also be explained on the basis of difference between Western and Indian dietary

habits. ZPP may be significantly elevated in many other conditions such as infection, inflammation, lead poisoning, and hemolytic anemia, thus mandating the use of additional tests such as serum iron, serum ferritin, and TIBC for accurate diagnosis of iron deficiency.[15] In our study, serum ferritin values in transfused patients were significantly higher than those in nontransfused patients, which was statistically significant ($P < 0.0000001$). In addition, 48.6% of subjects in our study who received blood transfusion and 16.1% of subjects who did not receive blood transfusion showed increased serum ferritin levels .This result is well correlated with the study by Patra et al.[14] in which 75% transfused patients and 16% nontransfused patients showed increased serum ferritin levels, and the study done by Ikusemoro et al.[2] showed a positive correlation between serum ferritin and number of units of blood transfused ($r = 0.719$, $P = 0.0000$) with a linear increase in serum ferritin levels seen in cumulative transfusions. However, in the study done by Akinbami et al.,[16] 2% of patients who revealed history of blood transfusion showed increased serum ferritin levels, while 90% of the patients showed serum ferritin within normal reference range. In addition, in our study, serum iron values in transfused patients were significantly higher than those in nontransfused patients, which was statistically significant ($P < 0.0000001$). This is well correlated with the study by Kassim et al.[13]. In our study, 59.5% of blood transfused patients and 21.2% of nontransfused patients showed increased serum iron values. However, in the study by Patra et al.,[11] 13.6% transfused patients and 14.2% nontransfused patients revealed increased serum iron and found this finding was statistically insignificant. In this study, 24.3% of blood transfused and 33.1% of nontransfused patients showed elevated serum TIBC level but was statistically not significant. This finding was close to that of Patra et al.[11] who noted 34% transfused patients and 23.2% nontransfused patients showed elevated TIBC levels. In this study, reticulocyte count was significantly lower in iron-deficient group than that in noniron-deficient group ($P < 0.02079$). Mean value of reticulocyte count in iron-deficient group was 1.64 and in noniron-deficient group was 6.60. This correlated well with the study done by Kassim et al.[13] in which mean value of reticulocyte in iron-deficient group was 2.5 and in noniron-deficient group was 9.7. This low reticulocyte count is a sign of impaired erythropoiesis, which is known to be associated with many conditions including iron, folate, and vitamin B12 deficiency, and when MCV is low for age, the reduced reticulocyte count/index supports the diagnosis of iron deficiency. In our study, ZPP values were elevated in all (7) the iron-deficient and in 39 of 148 noniron-deficient patients. This findings are well correlated with those in the study Vichinsky et al.[8] in which they noted elevated FEP in 38/61 noniron-deficient patients.

The limitation of our study is its cross-sectional design, which does not allow for a longitudinal evaluation of the laboratory data of the patients with SCD.

There is a great deal of debate on the most adequate method of diagnosing iron deficiency in individuals with SCA owing to characteristics that are inherent to the disease. As the majority of currently used tests for the diagnosis experience difficulties in their interpretation, be it a combined or isolated approach,[17] there is a pressing need for a standard diagnostic approach.

Conclusion

Contrary to the common assumption that patients with SCDs are usually iron sufficient, we conclude from this study that a state of iron deficiency may be present in them. Therefore, we recommend that patients with SCDs should be screened for iron deficiency by a comprehensive panel of laboratory tests that should include blood indices and ZPP, reticulocyte count, serum ferritin, serum iron, and serum TIBC. This would facilitate prompt diagnosis and prevent further morbidity in patients of SCDs.

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