

Hematological profile of children with sickle cell Disease in special reference to body iron stores

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Abstract

Introduction: Sickle cell anemia is a genetic disorder commonly seen in rural population of western and central India. Being an important cause of hemolytic anemia, repeated blood transfusions are frequently required for the sickle cell anemia cases. Few studies have exclusively addressed hematological profile of sickle cell disease patients. This study was planned to study the hematological profile of children with special reference to body iron stores. **Methods:** This was a prospective observational study carried out in department of pediatrics S.B.K.S MIRC. All the confirmed cases of children diagnosed with sickle cell disease or sickle β thalassemia were subjected to complete hematological analysis in special reference to body iron store by evaluation of serum iron, TIBC, % transferrin saturation and serum ferritin to find out total body iron store. **Results:** About 110 patients were included in the study. In our study low MCV values were seen in 60.4% of patients, low MCH values in 84.9% of patients while MCHC was normal in 94.3% of patients. Serum iron levels showed normal values in majority of patients (94.3%) while transferrin saturation was low in 62.3% patients thus showing the discrepancy on correlating both values for diagnosis of iron deficiency. **Conclusion:** Out of various parameters for diagnosis of iron status in SCD-SS patients, low MCV, MCH, Serum iron level and transferrin saturation more consistently associated with iron deficient state.

Key words- Sickle cell disease, Serum iron, % transferrin saturation, TIBC, Anemia

Introduction

Sickle Cell Disease (SCD) is one of the most common childhood-onset, single-gene disorder affecting approximately 80,000 people in India. Worldwide approximately 300,000 infants are born with Sickle Cell Disease. Sickle cell trait is most common in individuals originating from Africa, South America, Central America, the Caribbean islands, the Mediterranean, India, and Saudi Arabia [1].

Sickle cell disease results from an inherited hematological disorder involving defects in normal haemoglobin production. Sickle cell syndromes include sickle cell anaemia (SCD-SS),

Haemoglobin SC (SCD-SC), Haemoglobin S beta thalassemia (SCD- β -thal) and Sickle cell Trait (SCT-SA).

Sickle cell disease (SCD-SS) is the most common and severe variant. It results when an individual inherits a substitution of valine for the normal glutamic acid in the β - globin chain of haemoglobin from both parents [2]. This substitution alters the haemoglobin molecule so it crystallizes and deforms the red cell into a sickle shape when the haemoglobin loses oxygen. Both parents must be carriers of the defected gene in order for the transmission of the disease to occur. Children who only inherit one sickle cell gene have an SCD-AS pattern, the A representing a normal haemoglobin gene. This is also referred to

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as sickle cell trait. Overt symptoms rarely occur in this variant [3]. The clinical manifestations of sickle cell anemia result from increased blood viscosity and vascular obstruction by deformed sickled red cells. These clinical difficulties are related to the loss of oxygen to necessary tissue areas, in that the flexibility of the red blood cells is lessened causing a decrease in their ability to carry oxygen.

This causes vascular occlusions, hemorrhages, infarctions and ischemic necrosis of tissues and organs throughout the body causing several complications including recurrent vaso-occlusive crises and associated stroke, splenic sequestration crisis, aplastic crisis, infections, bone damage (i.e. hip necrosis), jaundice, leg ulcers, priapism, delayed growth, fatigue and painful episodes [2,4].

With the realization that patients with sickle cell anemia may lead relatively normal lives with Hb levels between 7.0 and 9.0 g/dl, regular blood transfusions are no longer used in the management of these patients. However, blood transfusions may be necessary for complications such as an 'aplastic' or 'sequestration' crisis. Among the hazards of repeated blood transfusion is accumulation of iron as the human body has no effective physiological mechanism for excreting excess iron [5]. Therefore, in conditions where transfusions are frequently indicated, exogenous iron can accumulate, circulate as non - transferrin bound iron, enter tissues, form reactive oxygen species and result in end organ damage.

It has been reported that iron deficiency anemia is uncommon in individuals with sickle cell disease because of availability of an adequate iron source potentially from increased red cell turnover and from blood transfusion [6,7]. It is believed that iron released by hemolysis is available for reutilization and that iron deficiency is uncommon in these conditions.

However, contrary to the previous belief few studies reported that iron deficiency was common than expected in untransfused sickle cell anemia cases [8,9]. Iron deficiency anemia often goes unnoticed because the sickle cell disease patients are already anemic. Iron deficiency in sickle cell patients may result in lowering the intracellular haemoglobin concentration and this may ameliorate

sickling [5]. The patients with sickle cell disease do not acquire excessive iron burden during the first two decades of life.

The gold standard for assessing liver iron stores in the absence of cirrhosis is the hepatic iron content determined by liver biopsy and quantification with atomic absorption spectrometry. Noninvasive methods include blood tests (Serum Iron, ferritin, Percentage Transferrin saturation and Total Iron binding Capacity) and imaging techniques (MRI based techniques). Sickle cell disorder has remained a neglected field of research in our country and magnitude of the problem has never been appreciated. This study was carried out with the main purpose to assess the hematological profile of children in special reference to body iron status of patients with sickle cell disease.

Materials and Methods

Study design– Prospective observational study

Setting– Department pediatrics S.B.K.S MIRC rural teaching hospital

Inclusion & exclusion criteria- children between the age of 6 months to 18 years were eligible for the study. All diagnosed and suspected cases of sickle cell disease reporting to the hospital were included in the study. Prior consent was taken from parents/ guardian before enrollment and ascent was sought from adolescents. Children with age less than 6 months, with concomitant chronic infective diseases (like chronic osteomyelitis, HIV etc.), with history of blood transfusion in the preceding 3 months or patients not consenting for participation were excluded from the study.

All the patients with positive solubility test and strongly suspected clinically but sickle negative were subjected to high performance liquid chromatography (HPLC) for confirmation and to differentiate sickle cell disease from other variants such as sickle cell trait, Sickle Beta thalassemia etc. The patients showing the presence of band representing HbS in the Hb electrophoresis were further categorized as Sickle Cell Disease, Sickle cell Disease with thalassemia and sickle cell trait depending upon HbS and HbA2 levels. All the patients having HbS more than 50% were enrolled in the study for further analysis. Patients with sickle cell disease and sickle cell disease with

thalassemia were further investigated for Serum Iron, Serum ferritin, Total iron Binding capacity (TIBC) and Percentage Transferrin Saturation (% TFR). All Children were investigated for complete blood picture (Hemoglobin, Total leucocyte count, Platelet Count, RBC Indices). All the tests were carried out in institution's laboratory. Institutional ethics committee's permission was taken before conducting the study.

Result

200 patients were screened in the study out of which 110 (55%) who tested positive for sickling by the solubility test were subjected to hemoglobin electrophoresis. Out of the total 200 patients, 26 patients tested positive for SCD-SS, 27 for SCD-β thalassaemia and 57 for sickle cell trait. Mean age of the enrolled patients were 10.2 years and 73.6% were males.

As patients with sickle cell trait remain asymptomatic most of the time or have minor manifestation only, patients with SCD-SS and SCD-β-thalassaemia only were included in further analysis. Distribution of patients was studied with respect to hematological profile. (Table-1) and no significant difference was observed between the two groups. In both group the MCV and MCH was low suggestive of probable underlying iron deficiency specially in SCD – SS group.

Table-1: Distribution according to hematological profile.

Hematological parameter	SCD-SS (26)	SCD- β- Thal (27)
Hemoglobin (gm%)	8.7±1.5	8.5±1.4
TLC(count/mm ³)	10,200±4160	11,800±4360
Platelet count(L/mm ³)	2.6±0.75	2.84±0.95
PCV(vf)	24.04±4.2	24.68±4.1
MCV(fl)	69.00±8.3	65.32±6.5
MCH(pg/cell)	23.45±2.2	22.13±1.9
MCHC(g/dl)	34.16±2.0	34.29±3.9
RDW(%)	20.48±3.0	19.68±2.8
Normal reticulocyte count	5(19.2%)	5(18.5%)
High reticulocyte count	21(80.8%)	22(81.4%)

Hb-Hemoglobin, HCT hematocrit, TLC- Total Leucocyte Count,, PCV Packed Cell Volume,, MCV mean cell volume, MCH mean cell hemoglobin, MCHC mean cell hemoglobin concentration, RDW Red Cell Distribution Width, HbF fetal hemoglobin,

Distribution of various hemoglobin variants in our study population is described in Table 2. No significant difference was observed in the levels of HbF and HbS amongst the two groups but the HbA2 level was significantly different with higher levels being observed in Sickle thalassaemia patients.

Table-2: Mean of HbS, HbA2, Hb F in SCD-SS and SCD β- thal

Mean	SCD-SS(n=26)	SCD-β Thal(n=27)
HbS	75.36%	75.67%
HbF	20.28%	17.93%
HbA2	2.78%	4.34%

On comparing parameters of body iron store in the two groups, serum iron was marginally more in **SCD-SS** (61.9±31.9) as compared to SCD- β thal (58.7±40.1), whereas Serum ferritin was significant higher in **SCD-β-thal** (244.5) as compared to **SCD-SS** (61.9±31.9)

Table-3: Mean body iron stores in patients with SCD-SS and SCD- β thal.

	SCD-SS $\pm$ SD (range) [n=26]	SCD- β -thal $\pm$ SD (range) (n=27)
Serum Iron (μ g/dl)	61.9 $\pm$ 31.9	58.7 $\pm$ 24.3.
TIBC (μ g/dl)	268.8 $\pm$ 47	250 $\pm$ 47.9
Serum Ferritin(ng/ml)	195 $\pm$ 22	244.5 $\pm$ 34
% TFR	28.39 $\pm$.9	31.4 $\pm$ 12.8

TIBC –Total Iron Binding Capacity, TFR –Transferrin Saturation

Serum iron, TIBC were within normal limits while % Transferrin saturation were low. Few records of serum ferritin were too high probably it may be acting as acute inflammatory marker in patient admitted due to various reasons.

Discussion

Sickle cell disease is a chronic hemolytic disease that results from a single base pair change, thymine for adenine at the 6th codon of β -globin chain leading to polymerization of deoxygenated hemoglobin S and distorting the shape of the cell.

A total of 53 patients, majority of whom were males (73.6%) were included in the study. Similar findings were seen by Khan et al [10]. This could be explained by increased chances of severe sickness and crisis in males or gender bias in tribal community, resulting in health care seeking behavior favoring males.

Most of the patients were in the age group of 7-12 years as also seen in study by Sahu et al [11]. However Kar et al. found minimum age of presentation to be 6 months. Probable reason for patients reporting at a later stage in our study might be initial treatment at primary level before reporting to our tertiary care hospital [12].

In our study, out of total 110 patients screened positive for sickle cell, 57(51.8%) were heterozygous and 53(48.2%) were homozygous. Mandout et al in 2009, in a cross section analysis of tribal patients attending hospitals or mobile clinics in Rajasthan, showed that the prevalence of sickle cell anemia was 9.2% out of which 0.8% were homozygous and 8.4% were heterozygous [13]. Probable reason for this difference is that our study was hospital based.

In our study out of 53 patients, 26(49.1%) patients were diagnosed with SCD-SS while 27(50.9%) with SCD- β Thal. Our finding differs from Kar et al who found 91.9% cases of SCD-SS and 8.1 %

cases of SCD β - thal [12]. The mean fetal hemoglobin level in our study in patients with SCD-SS was 20.28% and in those with SCD- β Thal. was 17.93%. The mean HbF level in this study correlates with study done by Walke et al (16.79%) [14]. However, in a study done by Kaur et al, the mean HbF varied between 9.7-13.5%. the distribution of values could be attributed to inclusion of adult patients in their study [15].

All the patients were found to be anemic. The mean hemoglobin concentration was 8.2 gm% in patients with SCD-SS and 8.5gm% in patients with SCD- β -thal. The reticulocyte count was found to be high in majority of patients (81.1%) suggesting chronic response to hemolysis. Similar findings were seen in study done by Khan et al [10] and Juwah et al [16].

In our study low MCV values were seen in 60.4% of patients, low MCH values in 84.9% of patients while MCHC was normal in 94.3% of patients. Haddy et al. [17] Showed low Hb, decreased MCV, MCH and MCHC values in patients with sickle cell disease who showed response to iron supplements. Davis et al [18] found low MCV and MCH in his patients and raised the suspicion of iron deficiency anemia. Walke et al [14] found low MCV, MCH, MCHC and raised reticulocyte count.

Contrary to expectation despite sickle cell disease being hemolytic anemia, serum iron levels showed normal values in majority of patients (94.3%) while transferrin saturation was low in 62.3% patients thus showing the discrepancy on correlating both values for diagnosis of iron deficiency. However, Lulla et al showed that low transferrin saturation

was most indicative of iron deficiency anemia and an increase in transferrin saturation represented the most consistent indicator of response to iron supplementation [19]. The discrepancy between values of transferrin saturation and iron levels in our study could be explained from the fact that serum iron as a measure of iron deficiency has several limitations including wide normal of variations, errors from ingestion, diurnal variation and falls during mild or transient infections.

TIBC levels were found to be normal in 28(52.8%) patients and low in 25(47.2%) patients. Similarly ferritin was found to be normal in 19 (35.8%) patients and high in 34 (64.2%) patients. Olbuyde et al [20] found significantly higher levels of ferritin when sickle cell anemia was complicated by chronic osteomyelitis.

High ferritin levels and low TIBC levels do not correlate with iron deficient state as both are affected during acute illnesses. TIBC decreases while serum ferritin increases during acute illnesses (as ferritin is an acute phase reactant). In our study most of the patients reported to hospital with some or other acute illness, thus explaining higher serum ferritin values. Thus, serum ferritin cannot be used as a sensitive marker for analysis of estimation of body iron stores in sickle cell anemia or sickle cell disease unless the patient is in steady state.

Vichinsky et al [12] reported that serum ferritin levels below 25 ng/ml and low MCV to be the most useful screening tests. Rao et al [27] found an excellent inverse correlation between serum ferritin and serum transferrin and a significant positive correlation between serum ferritin and transferrin saturation, bone marrow iron and a history of blood transfusion. Guyatt et al [28] found serum ferritin radioimmunoassay to be the most sensitive test for same purpose. Khan et al [14] concluded that serum ferritin was more sensitive indicator than serum iron, TIBC, and transferrin saturation.

Thus, based on present and previous studies available, out of various parameters for diagnosis of iron status in SCD-SS patients, low MCV and MCH are more consistently associated with iron deficient state. Such patients need to be further evaluated on long term basis after giving trial of iron therapy. The limitation of the study was the small study group which was not enough regarding

the actual status of body iron among patients with sickle cell disease. The confounding effect of underlying illness of serum ferritin used to assess iron stores also added to problems.

We propose further studies with larger sample population in this area. More specific tests and serial estimations of iron status are required for better definition of iron deficiency status in sickle cell disease. Response to therapeutic iron trial can be used as a diagnostic criterion for iron deficiency in sickle cell disease.

Abbreviations

SCD- Sickle cell disease, **SCD-SS** Sickle cell Anemia, **SCD- β thal-** Sickle Beta Thalassemia, **SCD-AS-** Sickle Cell Trait, **Hb-** Hemoglobin, **HPLC-** High Performance Liquid Chromatography, **HbS-** Sickle Hemoglobin, **HbA2-** Hemoglobin A2 Level, **TIBC-** Total Iron Binding Capacity, **%TFR-** % Transferrin Saturation, **TLC-** Total Leucocyte count, **RBC-** Red blood cell, **MCV-** Mean corpuscular volume, **MCH-** Mean corpuscular hemoglobin, **MCHC-** Mean corpuscular hemoglobin concentration, **PCV-** Packed cell Volume, **SD-** Standard Deviation, **RDW-** Red cell Distribution width., **HbF-** Fetal hemoglobin

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