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Original Article

Pathology Section

Variability of Iron Load in Patients of Sickle Cell Anaemia (HbSS): A study from Eastern India

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ABSTRACT

Introduction: Sickle Cell Anaemia (SCA) is one of the commonest haemoglobinopathies due to a point mutation (A→T) of the β-globin gene. Out of five haplotypes, the Arab-Indian haplotype present in India is one of the least severe phenotype and least studied also. It is characterized by lifelong haemolytic anaemia requiring red cell transfusion leading to iron overload. In contrast, there is very high incidence of deficiency of iron, folic acid and vitamin B12.

Aim: Our objective was to access the Iron status of SCA patients and to find its correlation with various parameters like red cell transfusion, haemolysis and serum hepcidin.

Materials and Methods: This was a cross-sectional study conducted on 208 patients for a period of five years. Complete Blood Count (CBC), iron profile, haemolytic parameters and transfusion requirement were studied and data compared with 52 healthy controls.

Results: Few patients (9.6%) revealed significant iron overload (Serum ferritin > 1000 ng/ml). In majority (80.8%) it was either normal or border line raised (300 to 1000 ng/ml) or iron deficiency was noted in a small fraction (9.6%). Frequency of transfusion is the principal factor which positively correlated with level of iron load ($p<0.001$) while parameters of haemolysis and serum hepcidin level play an insignificant role in this context ($p= 0.0634$).

Conclusion: This study supports the notion that the presentation of SCA patients in India is of "Viscosity – Vaso-Occlusive Crisis (VOC) phenotype" with high incidence of VOC, low haemolytic rate and transfusion requirement. Iron deficiency may be present in SCA patients requiring Iron supplementation. We suggest further studies to establish the role of hepcidin, ferroportin and other factors that control iron absorption in these patients.

Keywords: Arab- Indian haplotype, Hepcidin, Point mutation, Red cell transfusion, Serum ferritin

INTRODUCTION

SCA (HbSS) is one of the commonest autosomal recessive haemoglobinopathies which is due to a point mutation (A→T) of the β-globin gene located on chromosome 11 that results in the substitution of valine for glutamic acid at the sixth position of the β-chain of haemoglobin (Hb) [1]. It is characterized by life-long haemolytic anaemia, recurrent attacks of painful crisis and chronic organ damage. Chronic haemolysis, repeated blood transfusion, accompanied by increased iron absorption from the gut altogether contributes to increased availability of iron and there by predisposing to iron overload [2].

However, there are five different haplotypes of SCA like Bantu, Senegal, Benin, Cameroon and Arab-Indian (Asian) with variable phenotypes. The Bantu haplotype has the most severe clinical course while the Arab-Indian haplotype has the least severe one. This less severe haplotype is present in India. The incidence of Iron Deficiency Anaemia (IDA) is very high in India i.e., 50% of total population which can alter the body iron load in patients of SCA. Literature review reveals marked geographical variation in the serum iron level of SCA patients while it was reported to be significantly higher in young children with HbSS [3]. Furthermore, a prevalence of IDA has been reported as 12% [4] and 18% [5] in patients of SCA.

Our earlier prospective study in 60 females with SCA regarding perinatal outcome in planned pregnancy has documented normal serum ferritin in 29 (48.33%), low in 6 (10%) and high (more than 1000 ng/ml) in 25 (41.66%) cases [6]. The Asian haplotype, in relation to body iron store has been investigated least. The present study is the largest study from India designed to evaluate iron status of SCA and its correlation with other parameters which may influence it.

MATERIALS AND METHODS

This was a hospital based cross-sectional study conducted on 208 adult patients of SCA from January 2010 to December 2014. Confirmation of the diagnosis was done in the Department of Clinical Haematology SCB Medical College and Hospital, Cuttack, Odisha, India with the quantification of Sickle Hb (HbS) by a fully automated Capillary Zone Electrophoresis (Sebia Minicap, Sebia Lisses, France) and other relevant haematological investigations like CBC, peripheral smear examination, sickling test and reticulocyte count. All the patients were in stable condition and were receiving hydroxyurea (20 mg/kg/day), folic acid and other supportive therapy regularly. Inclusion criteria were adult patients (age >18 years), and no history of other comorbidities such as genetic/metabolic diseases that may alter the iron load, chronic infection, inflammatory/auto immune/liver and renal diseases. Patients with double heterozygous states or any other haemoglobinopathies were excluded from the study.

We presumed that our patients belong to Arab-Indian (Asian) haplotype as their ancestry from three to four generations were traced and found them to be of Indian origin; none had migrated from Africa or other parts of the world where other haplotypes are prevalent. The approval from Institutional Ethics Committee was obtained and this study was conducted according to the provision of the Declaration of Helsinki 2008. All participants provided written informed consent before participating in this study.

Study Design, Treatment Evaluation

The study group was compared with controls. The controls were 52 healthy, age and sex matched subjects without any haemoglobinopathies and iron abnormality, who were randomly selected from the local population.

On their first visit, a detailed general examination and clinical evaluation of all systems was done and recorded. The following investigations were done in all the cases: CBC (Hb, differential and total leukocyte counts, platelet count, red cell indices, etc.,) were done by a five part fully automated cell counter (Sysmex XT 2000i, Sysmex, Kobe, Japan). Peripheral Blood Smear (PBS) (Leishman stain) examination, blood grouping & Rh typing, with viral markers study for Human Immunodeficiency Virus (HIV), Hepatitis C virus (HCV), Hepatitis B (HBs Ag) were also done.

Renal function tests like serum urea (urease UV method, N: 15-40 mg/dl), serum creatinine (enzymatic method, N: 0.7-1.5 mg/ dl), Liver Function Test (LFT) like serum bilirubin (TBA method, N: 0.2–1.2 mg/dl), Aspartate Transaminase (AST) (mod. IFCC method, N: 5-40 IU/L), Alanine Transaminase (ALT) (mod.IFCC method, N: 5-40 IU/L) and Alkaline Phosphatase (ALP) (DGKC Method, N: <310 IU/L), serum Lactate Dehydrogenase (LDH) (enzymatic method, N: 250-450 U/L) all were done by auto analyzer. Electrolytes like Na^+, K^+, Ca^{++} and other relevant parameters were done by electrolyte analyzer (ISE method, N: 135-155mEq/L, 3.5-5.0mEq/L, 1.1-1.4 m mol/l respectively).

Iron profile study including serum ferritin, serum iron and Total Iron Binding Capacity (TIBC) were determined in all patients of both the groups by Cobas E 411 (chemilumenescence method, N:20-300 ng/ml, 50-150 µg/dl, 310-340 µg/dl, respectively) and transferrin saturation (N: 30-40%) was calculated. Red cell transfusion requirement was recorded in all the cases.

Categorization was done as follows: Multiple red cell transfusion (regular) when it was ≥ 3 units/year, rare transfusion (<3 units/year) and nil transfusion (no transfusion) [7]. Serum hepcidin level was measured by ELISA for correlation with serum ferritin level. Liver biopsy was done randomly in six cases with raised serum ferritin level (500-1000 ng/ml).

All the patients were advised to continue hydroxyurea, folic acid and other supportive therapy regularly. Acute painful VOC was managed with standard therapy like I.V fluid, I.V opioids, Oxyzen inhalation etc. Packed red cell (250 ml each unit) administration was allowed when Hb decreased below 7 gm% or with features of hypoxia with Hb level in between 7 to 9 gm% (The upper limit of post-transfusion Hb% being ≤10 gm%).

STATISTICAL ANALYSIS

Primary end point was serum ferritin level. Secondary end points were red cell transfusion requirement and markers of haemolysis (LFT, LDH, haemolytic peripheral blood picture, reticulocyte count). Statistical analysis was done by chi-square test.

RESULTS

Details of the patients' characteristics at the time of initial presentation are given in [Table/Fig-1]. Majority of the patients (62.5%) presented with VOC followed by pallor in 22.5%, Avascular Necrosis (AVN) of femoral head in 13.9%, splenomegaly in 9.1%, leg ulcer in 5.8% and icterus in 3.8% of cases. The median value of ferritin was high (390 ng/ml) in patient group in comparison to control (86 ng/ml). It was high in 44.2% vs. 5.8%, normal in 46.2% vs 71.2% and even low in 9.6% vs 23.0% cases in both the groups respectively [Table/Fig-2]. Among total 92 patients (44.2%) showing high ferritin level, majority i.e., 40 (19.2%) had level less than 500 ng/ml, 32 (15.3%) had in between 500 to 1000 ng/ml while only 20 patients (9.6%) had more than 1000 ng/ml. Similarly Transferrin Saturation (TS) more than 50% was observed in 22 cases (10.8%); which were observed mostly in the group showing high ferritin level (>1000 mg/ml). Only two cases showed TS>50% in the group of patients showing ferritin 500 to 1000 ng/ml.

The correlation of secondary end points like red cell transfusion requirement and markers of haemolysis are depicted in the [Table/Fig-3]. All except one case in the group of serum ferritin >1000 ng/ml had received red cell transfusion support regularly i.e., three or more units per year in previous years while no history of transfusion or occasional transfusion (<3 units/year) in the past was observed in the patients showing normal ferritin level as well as patients showing ferritin < 500 ng/ml. Four cases in the group of 500 to 1000 ng/ml received red cell transfusion regularly at the rate of three units per year. The median values of parameters ongoing haemolysis like Absolute Reticulocyte Count (ARC), serum LDH, unconjugated serum bilirubin were proportionately higher in relation to the group of patients with increased serum ferritin levels. But only three cases revealed ARC more than 100x109 /l and one case with definite haemolytic peripheral blood picture (fragmented red cell, normoblasts, polychromasia etc.,); all came in the group of serum ferritin more than 1000 ng/ml. There was no statistical significance of other haemolytic parameters among the groups of different serum ferritin level. The median value of serum hepcidin was found to be reduced in patients group of serum ferritin more than 1000 ng/ml

Type	Patients	Control
Number	208	52
Age in years	18-48	20-45
VOC (≥ 3 / year)	90 (62.5%)	Nil
Pallor	46 (22.5%)	12
AVN of femoral head	32 (13.9%)	Nil
Splenomegaly	20 (9.1%)	3
Leg ulcer	12 (5.8%)	Nil
Icterus	8 (3.8%)	1

[Table/Fig-1]: Patients' characteristics.

Parameters	Patients, n (%) 208	Control, n (%) 52	p-value
Serum Ferritin (ng/ml)			
Range	12-2093	12- 240	
Median	390	86	
Normal (20-300)	96 (46.2%)	37 (71.2%)	Chi-square = 47.53 df = 4 p < 0.00001
300 - 500	40 (19.2%)	3 (5.8%)	
500-1000	32 (15.3%)	0(0%)	
>1000	20 (9.6%)	0(0%)	
Low (<20)	20 (9.6%)	12 (23.0%)	
Transferrin Saturation (%)			
Range	12-42	14-56	
Median	27	35	
<50%	186 (89.2%)	52 (100%)	Chi-square = 11.42 df = 1 p = 0.0007
≥50%	22 (10.8%)	0(0%)	
Serum Iron (µg/dl)			
Range	28-432	25-168	
Median	186	80	
High	95 (45.7%)	1 (1.9%)	p = 0.00001
Low	22 (10.6%)	18 (8.7%)	
TIBC (µg/dl)			
Range	308-400	280-420	
Median	322	395	
High	18 (8.7%)	14 (26.9%)	p = 0.00023
Low	32 (15.4%)	2 (3.8%)	

[Table/Fig-2]: Profile of serum ferritin (primary end point) and other parameters of iron kinetics.

Serum Ferritin (ng/ml) : Chi- square = 47.53, df = 4, p < 0.001
Transferrin Saturation (%) : Chi- square = 11.42, df = 1, p = 0.0007
Serum Iron (µg/dl) : p < 0.001
TIBC (µg/dl) : p = 0.00023

Parameters	Normal S.Ferritin (n = 96)	S.Ferritin <500 (n =40)	S.Ferritin 500-1000 (n=32)	S.Ferritin >1000 (n=20)	p-value
Red Cell Transfusion Requirement (n = 208)					
Regular(≥ 3 units/yr)	Nil	Nil	4 (2.0%)	19 (9.1%)	Chi- square = 31.46 df = 3 p<0.001
Occasional (< 3 units/yr)	2 (1.0%)	9 (4.3%)	20 (9.6%)	1 (0.5%)	
Haemolytic Parameters					
ARC (10^9/l)					
Range	32-86	43-92	55-100	68-145	
Median	58	62	68	89	
Sr.LDH (U/L)					
Range	228-350	250-450	308-480	376- 622	
Median	275	287	342	415	
Sr. Bilirubin (unconjugated)					
Range (mg/dl)	0.1-0.6	0.1-0.8	0.2 -1.0	0.5 -5.2	
Median	0.3	0.4	0.7	1.1	
AST (IU/L)					
Range	6-28	5-38	9-51	15-61	
Median	16	20	33	40	
ALT (IU/L)					
Range	5-26	7-30	10-48	13-63	
Median	15	18	30	42	
ALP (IU/L)					
Range	112-146	108-208	124-218	175-240	
Median	124	143	168	210	
PBS with haemolytic picture	Nil	Nil	Nil	1 (0.5%)	
Sr. Hepcidin (ng/ml)					P= 0.0634
Range	17-240	22-200	28-150	24-80	
Median	92	68	56	35	

[Table/Fig-3]: Correlation of secondary end points (red cell transfusion requirement and parameters of haemolysis) with different serum ferritin (ng/ml) groups.
Red Cell transfusion requirement (n = 208): Chi- square= 31.46, df= 3, p < 0.001

and was normal in cases of normal ferritin group. However, not a single case was reported to have the level below normal value (17 to 240 ng/ml).

DISCUSSION

The present study is the largest cross-sectional one from India documenting the profile of iron load and its correlation with other variables among SCA (HbSS) patients. All possibilities of primary end point (serum ferritin) were observed as follows: 46.2% with normal range, 34.5% with high levels (300 to 1000 ng/ml), 9.6% with highest level (more than 1000 ng /ml), 9.6% with low level. All cases belonging to the group of ferritin level more than 1000 ng/ml had T.S > 50% and relatively high level of serum iron and low TIBC which correlates with the iron overload condition. Majority of the patients (80.7%) had either normal or borderline raised serum ferritin level. Low serum ferritin and iron with increased TIBC in 9.6% cases observed by us is not surprising because of very high incidence of iron deficiency (50% of general population), helminthiasis, associated with *H-pylori* infection and cereal based nature of principal meals of our population.

Liver biopsy followed by estimation of Liver Iron Concentration (LIC) in six cases randomly selected in the group of serum ferritin 500 to 1000 ng/ml revealed LIC level lesser than 5 mg of iron/gram of dry weight in all cases. This well corroborates that this group of patients are not iron overloaded to the extent requiring iron chelation. Our previous prospective study among 60 SCA patients with pregnancy documented a relative high incidence of high serum ferritin level (>1000 ng/ml) i.e., in 25 cases (41.66%) [6]. This is because of the fact that 28 females (46.6%) received regular red cell transfusion during gestation in order to maintain a higher level of haemotocrit which will be beneficial for both mother and foetus.

Iron load in SCA has been studied by other investigators. Ikusemoro AI et al., 2014 from Benin city, Nigeria reported high level of serum ferritin (396.4±130.8) in patients of SCA receiving multiple red cell transfusion (≥3 units/ year) in comparison to patients with history of rare red cell transfusion (124.9±67.2) [7]. Akinbami AA et al., 2013 from Lagos, Nigeria studied 103 adult patients of SCA (HbSS) and reported normal ferritin level in 90% cases, low in 7.76% and high (>300 ng/ml) in two cases (1.94%) [8]. However, smaller Indian studies revealed very high incidence of iron deficiency i.e., 42 cases (67.7%) among 62 cases of HbSS [9] and low serum ferritin level in 100 cases of HbSS in comparison to control group (19.96±4.737 Vs 106.2±55.47) [10]. Ray D et al., 2014 like our present study also reported different serum ferritin levels in 42 paediatric SCA (HbSS) patients (3-18-year-old): normal level in 25 cases (59.52%), low level in five (11.9%) and high level in 12 cases (28.57%) [11]. Vichinsky E et al., 1981 reported 42% of SCA patients having serum ferritin level below normal while 58% have within normal level [5].

Secondary end points like frequency of red cell transfusion (regular i.e., ≥ 3 units/year vs. occasional i.e., <3 units/year) and haemolytic parameters like ARC (>100 x 109/L vs < 100 x 109/L), serum LDH, liver function tests (unconjugated bilirubin, AST, ALT) and peripheral blood picture were correlated among various groups of different serum ferritin level. Red cell unit transfusion was the only parameter which showed a positive correlation between serum ferritin level (p<0.001) as seen in [Table/Fig-3]. Only 23 cases (11.1%) required regular red cell transfusion (≥3 units /year) while 88.9 % cases required occasional or no red cell transfusion. All 20 cases except one belonging to highest ferritin level (≥1000 ng/ml) required regular transfusion.

Though ARC more than 100 x 109/L and classical haemolytic blood picture could be detected in three cases and one case respectively in the group of highest serum ferritin level (>1000 ng/ml), there were no statistical significance of any haemolytic parameters among different groups of serum ferritin. This observation supports the notion that haemolysis is not a major component and iron overload requiring iron chelation is not an issue in majority (90.4%) of our SCA patients (Arab-Indian haplotype). In patients with symptomatic disproportionate anaemia, the possibility of nutritional deficiencies like iron, vitamin B12, folic acid and worm infestation should be looked for and treated whenever indicated which may improve the Hb level.

Iron status in patients with SCA is a matter of continuing investigation. It is presumed that these patients like other chronic haemolytic anaemia are iron loaded because of regular blood transfusion and increased iron absorption in the gastrointestinal tract due to ineffective erythropoiesis [10]. The pathophysiology and phenotypes of the Arab-Indian haplotype and other haplotypes (Benin, Bantu, Senegal) are different. In the former, the commonest presentation is that of "Viscosity – VOC phenotype" with lower haemolytic rate and lower transfusion requirement thus lesser Iron loads, while other parameters of haemolysis and serum hepcidin level play a significant role in the context. While in the latter group, it is of "haemolysis- endothelial dysfunction' phenotype with higher

haemolytic rate, anaemia, transfusion requirement and thus high iron overload [6].

Hepcidin, a cysteine rich 25- amino acid peptide synthesised in the liver from an 84- amino acid prepropeptide, plays a major role in iron haemostasis. This acts primarily as ferroportin controlling iron egress from enterocytes and macrophages, and impair dietary iron absorption as well as erythropoisis. Numerous studies have documented decreased hepcidin expression which is responsible for increased iron absorption in β-thalassaemia [12-14]. This is the principal mechanism of iron overload in non transfusion dependent–thalassaemia (β-thalassaemia intermedia, HbE β- thalassaemia, HbH disease, sickle β-thalassaemia, HbC-thalassaemia [15,16]. There is paucity of studies to establish the role of hepcidin among the patients of SCA (HbSS) in contributing to iron status. No large studies are available among Arab-Indian haplotypes in this context. The present study corroborated the serum hepcidin level in SCA with ferritin level. Though the range and median values showed negative correlation with serum ferritin level among different groups, there is no statistical significance (p= 0.0634).

LIMITATION

The present study did not corroborate the role of ferroportin and other parameters controlling the iron absorption and validate their contributions in the iron status among SCA (HbSS) of Arab-Indian haplotypes vis-a-vis with other haplotypes. The number of controls should have been increased in the present study (cases:control ration 4:1) which is acknowledged here.

CONCLUSION

Iron overload is an uncommon feature in SCA patients in Eastern India. Rather iron deficiency may be present in these patients requiring iron supplementation. VOC is the chief presentation with a low haemolytic rate and transfusion requirement. The major factor that controls the iron status is transfusion requirement while haemolytic parameters and hepcidin don't have a significant impact. Further studies requiring iron absorption, role of hepcidin and ferroportin are suggested.

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