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A CROSS SECTIONAL STUDY OF IRON STATUS IN CHILDREN WITH SICKLE CELL ANEMIA

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ABSTRACT **Back ground and objectives :** SCD is basically chronic hemolytic disease associated with release of heme and toxic iron molecules into circulation . There is disturbed iron homeostasis as the central pathogenic mechanism causing endothelial dysfunction resulting in vasculopathy , organ damage, and premature deaths in children. There are a very few studies on iron homeostasis in SCD that too with conflicting results. So we will be studying Iron homeostasis and handling in SCD through S.Iron , S.ferritin , Total Iron Binding capacity(TIBC), transferrin saturation as few markers for studying iron homeostasis in SCD. Our aim is to find the true nature of iron status in view of conflicting results of previous studies on this subject in SCD patients from endemic areas world over. Although sickle cell anemia is widely prevalent in the tribal areas in and around Vizianagaram district, no studies on iron status have been undertaken in this geographic area and hence the need for this study. This study is undertaken with a view to assess iron status in children with sickle cell anemia and compare it with age and sex matched controls with normal HbAA genotype. **Methods :** From June 2020 to January 2022 , children between 6 months to 15 years with sickle cell anemia were selected from hematology clinics at Pediatrics Out patient departments (OPD) and normal children were selected from well baby clinics, immunization clinics and schools. Both groups were age and sex matched. Participants underwent tests for serum iron, serum ferritin, Total Iron Binding Capacity, Transferrin saturation. Based on the above mentioned parameters we have compared both the study groups. **Results :** Of 100 cases, 38% have serum iron <70 microgram/dl , 20% has in between 70-100, 4% has in between 100-150 ,26% has in between 150-175 and 12% children has >175. Serum iron in cases ranged between 35-210 with a mean of 105.7 ± 55.31. In controls, serum iron ranged from 54-171 with a mean of 99.25 ±33.16. TIBC ranged between 172- 389 microgram/dl with a mean of 288.4 ± 50.76 in cases, of which 26% has TIBC 400. Among controls TIBC ranged in between 260-596, with a mean of 397.35 ± 81.75. Transferrin saturation among cases ranged between 10.3% -95.93% with a mean saturation of 38.16 ±21.63 %. Of 100 cases 30% has saturation 50%, 40% has in between 20-50%.6 of the cases have iron saturation > 75% of which 1 case has 95.93% . Of the 100 cases none had serum ferritin <50 ng/ml ,10% had in between 50- 100ng/ml , 26% had in between 100-150 , 24% cases ranged between 150- 200 , 24% cases ranged between 200-300 , 16% cases have serum ferritin >300ng/ml , of which 1 have a value of >500 and another has as high as 1000ng/ml. Mean serum ferritin of cases is 213.84 ± 147.57. Mean Serum ferritin of controls is 92.64 ± 53.63 ng/ml with range between 08 – 215ng/ml. **Conclusions :** Through our study we have observed that the children diagnosed with sickle cell anemia have low serum iron and TIBC levels and raised serum ferritin and transferrin saturation levels when compared to the normal children of same age and sex. The findings of the study are attributed to sickle cell anemia as it is a chronic hemolytic condition with ongoing inflammation and vasculopathy.

KEYWORDS : Sickle cell Anemia , Iron status

INTRODUCTION:

Sickle cell disease (SCD) is one of the common monogenic disorders associated with morbidity and mortality around the world with a very few available effective modalities of treatment. There are more than 15 different genotypes which are known to cause SCD, although three predominate in most populations: Homozygous HbSS (often called sickle cell anemia, SCA), compound heterozygous conditions which include HbSC, and HbS / thalassaemia, the latter is a variable condition depending on the severity of the co-inherited thalassaemia allele[1]. The molecular basis of the sickle cell hemoglobin is a point mutation (GAG →GTG) in exon 1 of the β globin gene resulting in the substitution of glutamic acid by valine at position 6 of the β globin polypeptide chain [2,3], which resulting in formation of sickle hemoglobin (HbS) instead of adult hemoglobin i.e., hemoglobin A (HbA). The abnormality of the gene results in sickle hemoglobin (HbS) when deoxygenated, to polymerize intracellularly and deform red blood cells into acharacteristic sickle shape, thereby producing clinical manifestation of a chronichemolytic anemia with potential iron overload . Sickle cell anemia is known to be prevalent in Africa for the past 5,000 years. In1910, Dr James Herrick first described SCA ,who noticed abnormally shaped RBCs under the microscope in a dental student from Grenada . These cells looked like sickle and so the name . The presence of sickle cell Hb was first described in India in 1952 in the tribal populations of Nilgiri hills in South India by Lehmann and Cutbush[6]. Prevalence of Sickle cell disease is observed in many parts of India, which has ranged between 9.4 to 22.2 % in more endemic areas . It's prevalence ranges from 0-20% in North east India, 22-44% in Central India , 0-33% in western India and 0- 40% in South India . Nearly 20 million people are effected in India .

MATERIALS & METHODS:

This study was conducted from June 2020 to January 2022 , at MIMS , VIZIANAGARAM, 100 children between 6 months to 15 years with sickle cell anemia HbSS were selected from hematology clinics at Pediatrics Out patient departments (OPD) and 100 children with age and sex were selected from well baby clinics, immunization clinics and schools. Children with Blood transfusion in previous 3 months and Child on long term transfusion or frequent transfusion therapy and on Iron supplementation 3 months prior to enrollment were excluded

METHODOLOGY:

PROCEDURE OF STUDY:

The study was prospective comparative study of iron status in sickle cell anemia with age and sex matched controls.

Consecutive sickle cell anemia patients who attended the haematology clinics for follow-up care and who satisfy the inclusion criteria were recruited in the study after obtaining informed consent from their parents. Venous blood was collected with aseptic precautions. Five millilitres of blood was drawn from a convenient peripheral vein into plain and EDTA tubes. They were transported to the laboratory of the Maharajah's institute of Medical sciences. Time of collection of samples was documented on samples of both groups for reference. Collected blood samples were analyzed for Hb, serum iron, ferritin , TIBC, Transferrin saturation was calculated. Serum iron, TIBC were measured by using Iron Ferrozine test kit. Serum ferritin, transferrin were measured by CLIA technique. Hb electrophoresis was measured by using electrophoretic technique was measured by Slide latex agglutination method. Age and sex matched controls were selected. Sick children were not included under the study as serum ferritin is an acute phase reactant. In controls who satisfy the inclusion criteria, blood samples were collected and were analyzed for Hb, Serum iron ferritin, TIBC, Transferrin saturation was calculated.

RESULTS AND OBSERVATIONS:

Table – 1 IRON STUDIES

S. Iron (microgram/dl)	Cases	Controls
<65	34%	20%
65-175	54%	80%
>175	12%	0

Of 100 cases , 38% have serum iron <70 microgram/dl , 20% has in between70-100, 4% has in between 100-150 ,26% has in between 150-175 and 12% children has >175.Serum iron in cases ranged between 35-210 with a mean of in105.7 ± 55.31.

In controls, serum iron ranged from 54-171 with a mean of 99.25 ±33.16. Of 100 controls 20% has iron concentration <65, 80% has in between 60-175 and none has iron > 175. Statistically significant difference is not observed between mean serum iron of cases and controls as calculated P value is 0.48

Figure – 1 IRON STUDIES

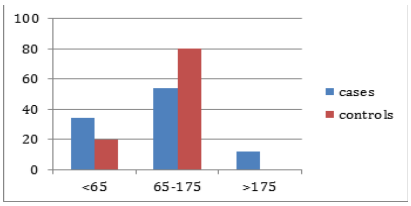

Table – 2 : TIBC (microgram/dl)

TIBC (microgram/dl)	Cases	Controls
<250	26%	0
250-450	74%	28%
>450	0	72%

TIBC ranged between 172- 389 microgram/dl with a mean of 288.4 ± 50.76 in cases, of which 26% has TIBC 400. Among controls TIBC ranged in between 260-596, with a mean of 397.35 ± 81.75. Of controls 28% have TIBC in between 250-450 microgram/dl and 72% have TIBC > 450. None of controls have TIBC < 250 microgram/dl. There is statistically significant difference observed between mean TIBC of cases and controls as P value calculated is found to be 0.002

FIGURE – 2 : TIBC (microgram/dl)

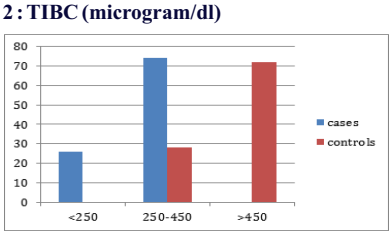

Table – 3 : % IRON SATURATION

% Iron saturation	Cases	Controls
<20%	30%	28%
20-50%	40%	72%
>50%	30%	0

Transferrin saturation in this study among cases ranged between 10.3% -95.93% with a mean saturation of 38.16 ±21.63 %. Of 100 cases 30% has saturation 50%, 40% has in between 20-50%.6 of the cases have iron saturation > 75% of which 1 case has is high as 95.93%. Among controls % of iron saturation is < 20% in 28% children and 20-50% is seen in 72% controls. No controls have iron saturation >50%. Mean iron saturation of controls is 25.9% ± 9.01 with a range between 9-41%. There is statistically significant difference observed between mean Transferrin saturation of cases and controls as P value calculated is found to be 0.00

Figure – 3 : % IRON SATURATION

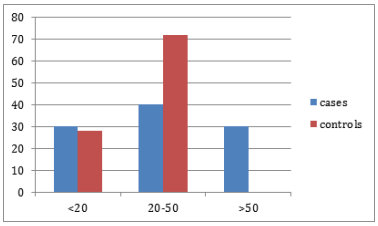

Table – 4 : S.FERRITIN (ng/ml)

S. Ferritin	Cases	Controls
<70	10%	30%
70-140	24%	50%
>140	66%	20%

Of the 100 cases none had serum ferritin <50 ng/ml ,10% had in between 50- 100ng/ml , 26% had in between 100-150 , 24% cases ranged between 150- 200 , 24% cases ranged between 200-300 , 16% cases have serum ferritin >300ng/ml , of which 1 have a value of >500 and another has as high as 1000ng/ml. Mean serum ferritin of cases is 213.84 ± 147.57.

Of 100 controls Serum Ferritin is in between 70-140 for 50%, in 30% serum ferritin is < 70 ng/ml and in other 20% it is > 140ng/ml. Mean Serum ferritin of controls is 92.64 ± 53.63 ng/ml with range between 08 - 215ng/ml. There is statistically significant difference observed between mean Serum Ferritin of cases and controls as P value calculated is found to be 0.003

Figure – 4 : S. Ferritin

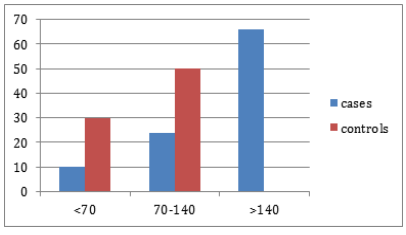

Table – 5 : Blood transfusions per year

Blood transfusions per year	Cases frequency
No transfusions	6%
1 / year	42%
2-3 / year	38%
>3 / year	14%

Discussion :

Multiple studies were conducted on Iron status in sickle cell anemia which showed contradictory results. Some observed that number of blood transfusions significantly correlated with iron load on the body while some other concluded that due to regular loss of iron through damaged sickle cells , there is no net iron load on the body. Our study observed there is significantly good relation between frequency of blood transfusions and Serum IRON and serum FERRITIN values. Hussain MA et al [9], in their study of serum Ferritin among sickle cell children observed that mean serum ferritin in sickle cell anemia children was significantly higher than control group and high serum ferritin in non transfused patients was correlated with age but serum ferritin in no child of sickle cell group correlated with number of units of transfusion.

As per study of B. S. Mahony et al [10],serum ferritin and serum transferrin values in sickle cell disease are dependent of total amount of blood transfused. Serum ferritin correlates with serum iron and transferrin saturations and inversely correlates with TIBC.

In the present study, Of the 100 cases 6 % had no previous transfusions, 42 % had 1 transfusion /year ,38% had 2-3 transfusions/year, and remaining 14% had greater than 3 (4-5) transfusions /year due to various symptoms and crises described below. Age of 1st blood transfusion is as early as 7 months in 1 child with majority 38% having their 1st transfusion in less than 2 years in this study. Mean transfusion in present study is 32.91 ± 21.91 ml/kg/year. Giulia et al [11] in their study reported that the median number of transfusions per year was almost identical in the two groups: a mean of 2.3 transfusions/year was reported for males and 1.9 transfusions/year for females. Our study showed blood transfusions frequency significantly correlated with serum iron and serum ferritin levels. Periodic monitoring of the children and therapeutic blood transfusions resulting to keep Hb S levels <30% also helped cognitive development and reduced school absenteeism in these children.

Of 100 cases, 38% have serum iron <70 microgram/dl , 20% has in between 70-100, 4% has in between 100-150 ,26% has in between 150-175 and 12% children has >175. Serum iron in cases ranged between 35-210 with a mean of 105.7 ± 55.31. However the study conducted by Mohanty et al [12] and Debkumar Roy et al [13] was 28-432 and 112.8 microgram/dl respectively.

In controls, serum iron ranged from 54-171 with a mean of 99.25 ±33.16. Of 100 controls 20% has iron concentration <65, 80% has in between 60-175 and none has iron > 175. Statistically significant difference is not observed between mean serum iron of cases and controls as calculated P value is 0.48

TIBC ranged between 172- 389 microgram/dl with a mean of 288.4 ± 50.76 in cases, of which 26% has TIBC <250, 74% has TIBC in between 250-400. None of the cases have TIBC >400. However the study conducted by Anjali et al [14] and Vanaja et al [15] was 354.62 and 271.9 ± 39.2

Among controls TIBC ranged in between 260-596, with a mean of 397.35± 81.75. Of 100 controls 28% have TIBC in between 250-450 microgram/dl and 72% have TIBC > 450. None of controls have TIBC < 250 microgram/dl. There is statistically significant difference observed between mean TIBC of cases and controls as P value calculated is found to be 0.002

Transferrin saturation or percentage of Iron saturation in this study among cases ranged between 10.3% -95.93% with a mean saturation of 38.16 ±21.63 %. Of 100 cases 30% has saturation <20% , another 30% has > 50%, 40% has in between 20-50%. 6 of the cases have iron saturation > 75% of which 1 case has is high as 95.93%. However the study conducted by Debkumar Roy et al [13] was 140.2 ng/ml.

Among controls % of iron saturation is < 20% in 28% children and 20-50% is seen in 72% controls. No controls have iron saturation >50%. Mean iron saturation of controls is 25.9% ± 9.01 with a range between 9-41%. There is statistically significant difference observed between mean Transferrin saturation of cases and controls as P value calculated is found to be 0.00. This high iron saturation in cases indicates sickle cell disease is a risk factor for iron overload especially in children on frequent transfusions.

In the present study, Of the 100 cases none had serum ferritin <50 ng/ml , 10% had in between 50-100ng/ml ,26% had in between 100-150, 24% cases ranged between 150-200, 24% cases ranged between 200-300, 16% cases have serum ferritin > 300ng/ml ,of which 1 have a value of >500 and another has as high as 1000ng/ml. Mean serum ferritin of cases is 213.84 ±147.57. Of 100 controls Serum Ferritin is in between 70-140 for 50%, in 30% serum ferritin is < 70 ng/ml and in other 20% it is > 140ng/ml. Mean Serum ferritin of controls is 92.64 ±53.63 ng/ml with range between 08-215ng/ml. There is statistically significant difference observed between mean Serum Ferritin of cases and controls as P value calculated is found to be 0.003

Mishra et al [16] in their study reported that 60% patients with sickle cell disease were found to have increased serum ferritin, 36.6% had normal serum ferritin and 3.3% patients had decreased ferritin. 12.5% patients with sickle cell trait had increased serum ferritin, 77.5% patients had normal ferritin level and 10% patients had decreased serum ferritin level. The difference was statistically significant ($p<0.0001$). As compared to sickle cell trait patients, more number of sickle cell disease patients had increased serum ferritin level.

CONCLUSION:

1. Low mean levels of Serum Iron, low TIBC levels observed in our study reflects the picture of Anemia of chronic disease.

2. Serum Ferritin levels , a marker as acute phase reactant have been observed in our study as remarkably high in SCD cases when compared to controls ,indicating that sickle cell disease is a condition associated with chronic inflammation. This is also supported by persistently high leukocytosis.

3. Higher values of Transferrin saturation reflects the presence of iron overload, especially in children who went frequent transfusions.

4. Hence sickle cell disease is said to be chronic hemolytic disorder associated with phenomenon of chronic inflammation, vasculopathy leading to spectrum of clinical manifestations & complications which influence the morbidity and mortality.

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