

Original Research Article

Haematological profile of sickle cell patients attending tertiary health care centre of southern odisha: a cross sectional study

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ABSTRACT

Background: Sickle Cell Disorder (SCD) is a major health problem in India. After introduction of High-Performance Liquid Chromatography (HPLC) in MKCG Medical College, this study is first of its kind to describe haemoglobin variants of SCD. The aim of the study was to document haematological profile and pattern of haemoglobin variants in SCD patients.

Methods: A Hospital based cross sectional study was conducted in Pathology department, MKCG medical college from October 2018 to May 2019. Sickle cell patients were included and patients in Sickle cell crisis or transfused with blood in last 3 months were excluded. Hematological indices were measured by Sysmex XT 2000i blood analyzer. Quantification of hemoglobin variants was done by HPLC. All data were analyzed using SPSS and Independent t-test was applied.

Results: In this study 100 heterozygous and 116 homozygous cases were reported. In homozygous cases Hb were significantly low and MCV, MCH, RDW-CV were significantly high than heterozygous. Hb level was significantly lower in homozygous children. Hb F was significantly higher in children and homozygous cases. A significant positive correlation was seen between Hb and RBC in both cases.

Conclusions: In homozygous cases moderate anaemia (microcytic hypochromic to normocytic hypochromic) with High Hb F and in heterozygous cases mild anaemia (microcytic hypochromic) dominated the haematological profile. Children were significantly more anaemic than adults in homozygous cases. Anisocytosis was significantly more in homozygous cases and pediatric age group. Average fetal Haemoglobin variant (Hb F) was significantly more in homozygous cases and lower in adult group in both homo and heterozygous cases.

Keywords: Anaemia, Fetal hemoglobin, Hemoglobin variant, High-performance liquid chromatography, Sickle cell disease

INTRODUCTION

Sickle cell disorder is an inherited genetic disorder of blood caused by single-point mutation in gene in which thymine is substituted for adenine, thereby encoding valine instead of glutamine in the sixth position of the

beta globin chain of hemoglobin. The resultant Hb S formed has poor solubility in the deoxygenated state causing polymerization of hemoglobin and sickling of red blood cell in low oxygen tension. The red cells become distorted and assume a characteristic shape called as sickle shape. It leads to reduced life span of RBCs and blockage of blood vessels thus causing wide spectrum of

symptoms and signs including anemia, crisis and organ damages etc. This disorder is a broad-spectrum disease that includes sickle cell anemia- Homozygous state (Hb SS), Sickle cell trait- Heterozygous state (Hb AS), Sickle cell diseases- with beta thalassemia or Hb D/E/C.

Globally the Prevalence of Hb S is highest in sub-Saharan Africa, followed by the Arabian and Indian subcontinents. It is the 2nd most common hemoglobinopathy in India after Thalassemia.¹ This is not a disease of the present era rather the archeological findings from the site of Indus valley civilization reveal the existence of anemia (hereditary anemia like SCD or Beta-thal can't be ignored) in Indian subcontinent during the period of 2000-5000 BC. Herrick first described a case of SCD in 1910.² But in India it was Lehman and Catbush who first reported a SCD case from southern India in the year 1952.³ The incidence of the SCD in India is 1-44%.⁴⁻⁶ It is one of the major health problems with a considerable epidemiological, clinical and public health pertinence in developing country like India, mostly in sickle cell belt. Odisha is one of the states in this sickle cell belt area.⁷

Hematological profile of SCD patients is extremely variable. There is a paucity of data about this from India and that to very little data are available from southern Odisha. SCD remained neglected in field of research in southern Odisha. After introduction of High Performance Liquid Chromatography (HPLC) in M.K.C.G Medical College, this study is first of its kind to describe the pattern of hemoglobin variants of SCD patients attending the college, which is the tertiary health care center and referral center of southern Odisha. The aim of this study is to document the hematological profile findings of SCD patients. Adding to this, it also intended to know the pattern of hemoglobin variants in them.

METHODS

The present hospital based cross sectional study was conducted in the Department of Pathology, MKCG medical college, Berhampur, Ganjam, Odisha from October 2018 to May 2019. Before commencement of this study, permission was obtained from the Institutional Ethical Committee (IEC). Inclusion criteria- sickle cell patients (Sickle cell anaemic and trait patients) who were diagnosed at Pathology department of MKCG medical college, after being referred from OPDs and IPDs or already diagnosed cases coming for routine evaluation. Patients diagnosed after screening of family members of sickling patients are also included. They were all in steady state. Exclusion criteria- patients who were in Sickle cell crisis or had blood transfusion in last 3 months or with diseases, which can affect the hematological parameters such as chronic kidney disease, chronic obstructive pulmonary diseases were excluded from the study. With the prior informed consent from the patients

a case format was filled containing information about name, age, sex, chief complains, family history, other relevant histories, sickle cell related symptoms, general examination findings. Under all aseptic precautions, 2 ml of venous blood from antecubital vein of each patient was collected in Ethylene Diamine Tetra Acetic acid (EDTA) vial and hematological indices were measured by Sysmex XT 2000i automated blood analyzer.

Sickling test was done by freshly prepared 2% sodium metabisulphite. Quantification of hemoglobin variants (Hb A, Hb A2, Hb F, Hb S etc.) was done by High Performance Liquid Chromatography (HPLC) with the help of Bio-Rad system using beta-thal short Programme. Then the data were recorded, and statistical analysis was done using SPSS (Version 16.0). Categorical values (age, sex) were expressed in number and percentage whereas continuous variables (Hb, MCV, MCH, MCHC, RDW-CV, Retic etc.) were expressed in mean and standard deviation. All hematological parameters were normally distributed according to homozygous and heterozygous cases, sex and age group. Hence, analysis between groups was done using independent t-test. In this study $p < 0.05$ was considered as statistically significant.

RESULTS

Total 216 cases were included in the study out of which 100 cases were sickle cell heterozygous and 116 were sickle cell homozygous. There were total 102 males (42 in heterozygous and 60 in homozygous) and 114 females (58 in heterozygous and 56 in homozygous). Age ranged from 6 month to 55 years. In heterozygous group, maximum number of males (54.8%) belonged to pediatric age group whereas maximum number of females (56.9%) belonged to reproductive age group. Similar things were observed in homozygous group i.e. 51.7% of males were in pediatric age group and 53.6% of females were in reproductive age group. Numbers of cases reported after 30 years in both sex and in both cases were less. The age and sex wise distribution of sickle cell cases were depicted in (Table 1).

Hematological parameters in homozygous and heterozygous cases were compared in (Table 2). Average Hb, average RBC and average Platelet value were significantly lower in homozygous cases than in heterozygous cases. However, there was no significant difference in average MCHC value between two groups. Similarly, average MCV, MCH, RDW-CV, WBC and Retic count were significantly high in homozygous cases. Percentage of Hb A variant was significantly very low in homozygous cases than in heterozygous cases whereas percentage of both Hb S and Hb F variants was significantly high in homozygous cases than heterozygous cases. However, there was no significant difference in average Hb A2 between two cases (Table 2).

Table 1: Age and sex wise distribution of sickle cell cases (n=216).

Age group	Homozygous cases (n = 116)			Heterozygous cases (n = 100)		
	Male (%)	Female (%)	Total	Male (%)	Female (%)	Total
0 - <14	31(51.7%)	23(41.1%)	54	23(54.8%)	22(37.9%)	45
14 - <30	19(31.7%)	25(44.6%)	44	10(23.9%)	26(44.8%)	36
30 - <45	07(11.7%)	05(8.9%)	12	08(19%)	07(12.1%)	15
45 - <60	03(5.0%)	03(5.4%)	06	01(2.3%)	03(5.2%)	04
All age groups	60	56	116	42	58	100

Table 2: Haematological parameters and Hb variants in homozygous and heterozygous cases.

Haematological parameters and Hb variants	Homozygous (mean±SD)	Heterozygous (mean±SD)	p value
Average HB (gm/dl)	7.3±2.4	10.2±2.6	0.000*
Average MCV (fl)	79.3±10.4	69.8±8.7	0.000*
Average MCH (pg)	25.5±3.3	22.5±3.9	0.000*
Average MCHC (gm/dl)	30.9±3.7	31.01±2.3	0.706
Average RDW-CV (%)	21.0±7.5	17.4±4.9	0.000*
Average RBC (million/cu.mm)	3.0±0.9	4.1±1.9	0.000*
Average WBC (thousand/cu.mm)	12.9±5.9	9.9±4.4	0.000*
Average platelets (lac/cu.mm)	1.9±1.4	2.5±1.2	0.001*
Average retic count (%)	7.6±8.9	1.7±2.4	0.000*
Average HB A (%)	9.5±12.9	57.2±5.6	0.000*
Average HB S (%)	68.6±13.5	32.2±5.7	0.000*
Average HB F (%)	16.9±6.4	2.2±4.9	0.000*
Average HB A ₂ (%)	3.5±3.2	3.2±0.43	0.400

* Statistically significant as p<0.05

Table 3: Hematological Parameters according to sex in both homozygous and heterozygous cases.

Hematological parameters	Homozygous (mean±SD)			Heterozygous (mean±SD)		
	Male	Female	p value	Male	Female	p value
1. Avg HB (gm/dl)	7.5±2.8	7.2±2.0	0.472	10.8±2.8	9.8±2.3	0.057
2. Avg MCV (fl)	77.0±9.7	81.8±10.7	0.014*	69.0±7.2	70.5±9.7	0.379
3. Avg MCH (pg)	25.0±3.0	26.0±3.4	0.150	22.1±4.3	22.8±3.5	0.401
4. Avg MCHC (gm/dl)	31.0±2.9	31.0±4.4	0.841	30.8±1.6	31.2±2.7	0.391
5. Avg RDW-CV (%)	20.9±9.0	21.0±5.4	0.998	16.6±4.6	18.0±5.0	0.180
6. Avg RBC (million/cu.mm)	3.0±0.9	2.9±0.8	0.323	4.3±1.3	4.0±1.0	0.132
7. Avg WBC (thousand/cu.mm)	12.5±3.4	13.3±6.5	0.536	9.8±3.7	10.0±4.9	0.760
8. Avg platelets (lac/cu.mm)	2.0±1.3	1.7±1.4	0.164	2.9±1.5	2.3±0.9	0.017*
9. Avg retic count (%)	7.1±8.1	8.2±9.7	0.514	1.4±0.9	2.0±3.0	0.273

* Statistically significant as p<0.05

Table 3 described the distribution of average hematological parameters among male and female. Average platelet count was significantly high in male in heterozygous cases.

Though it was also high in male in homozygous cases but that was not significant. Average MCV value was significantly high female in homozygous cases but not in heterozygous cases. Though average Hb level and average RBC value were lower in female than male in both cases, these were not significant.

Table 4 depicted the distribution of hematological parameters in children and adult in both cases. Average Hb level was significantly lower in children than in adult in homozygous cases but not in heterozygous cases.

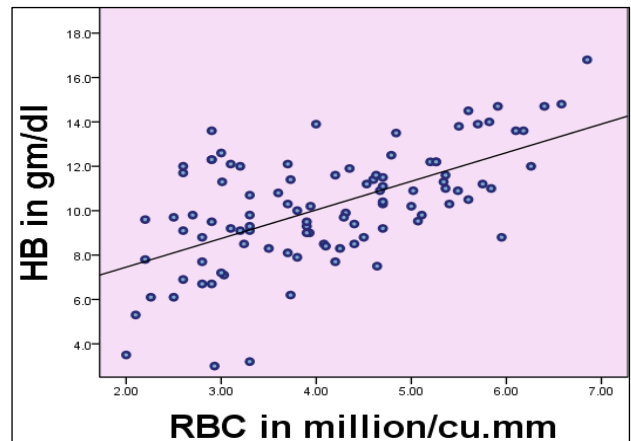
Average platelet count was significantly lower in children in homozygous cases whereas significantly higher in adult in heterozygous cases. Percentage of Hb F variant was significantly higher in children than in adults in both homozygous and heterozygous cases. Average MCH was significantly lower in both homozygous and heterozygous cases.

Table 4: Haematological parameters and Hb variants according to age in both homozygous and heterozygous cases.

Hematological parameters and HB variants	Homozygous (mean±SD)			Heterozygous (mean±SD)		
	0-14 years	>14 years	p value	0-14 years	>14 years	p value
Avg HB (gm/dl)	6.5±2.2	8.0±2.5	0.001*	9.8±2.2	10.5±2.8	0.125
Avg MCV (fl)	77.9±11.9	80.5±10.5	0.109	68.4±7.8	71.0±9.3	0.214
Avg MCH (pg)	24.9±3.2	26.0±3.6	0.044*	21.5±4.6	23.3±3.0	0.027*
Avg MCHC (gm/dl)	31.2±1.8	30.6±3.7	0.434	30.3±2.6	31.6±1.9	0.007*
Avg RDW-CV (%)	23.1±9.8	19.0±7.5	0.002*	17.4±5.0	17.4±4.9	0.831
Avg RBC (million/cu.mm)	2.8±0.9	3.1±0.9	0.085	3.9±1.0	4.3±1.3	0.190
Avg WBC (thousand/cu.mm)	13.4±4.8	12.5±6.8	0.391	11.3±4.9	8.8±3.7	0.005*
Avg platelets (lac/cu.mm)	1.5±0.8	2.6±1.7	0.011*	2.9±1.5	2.2±0.9	0.011*
Avg retic count (%)	9.3±10.2	6.2±7.5	0.096	2.1±3.4	1.4±0.8	0.176
Avg HB S (%)	70.1±13.2	67.1±13.7	0.229	32.8±5.6	31.5±5.8	0.247
Avg HB F (%)	18.5±6.1	15.2±6.3	0.005*	3.2±6.5	1.3±1.8	0.050*
Avg. HB A ₂ (%)	2.9±0.71	3.9±4.4	0.080	3.2±0.51	3.2±0.33	0.982

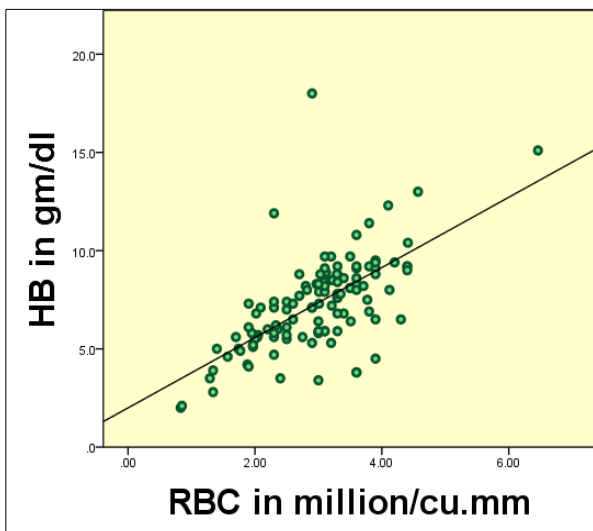
* Statistically significant as p<0.05

Correlation between different variables in homo and heterozygous cases were shown in figure 1 to figure 6. (Figure 1 and 2) showed a significant positive correlation (with correlation coefficients as 0.6) between Hb and RBC in homozygous and heterozygous cases respectively. There was a negative correlation (correlation coefficient was -0.133) between Hb and MCV in homozygous cases (Figure 3) whereas positive correlation (r=0.016) existed between Hb and MCV in heterozygous cases (Figure 4). However, these correlations were not statistically significant. There was a significant negative correlation (r=-0.431) between Hb F and Hb A₂ in heterozygous cases (Figure 6) but this correlation was not significant in homozygous cases (Figure 5).



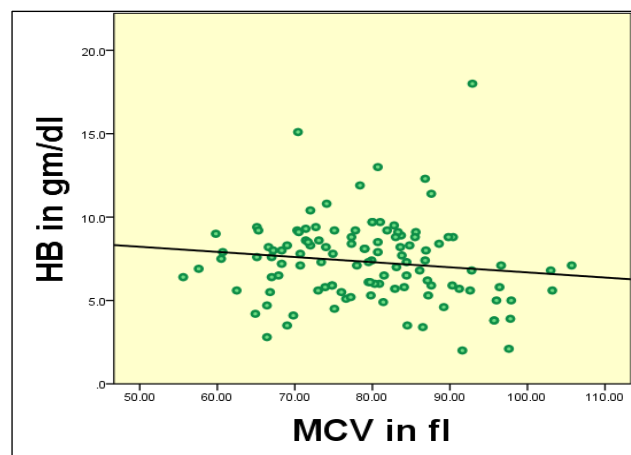
Heterozygous cases (r=0.60, p=0.000)

Figure 2: Correlation of HB with RBC in heterozygous cases.



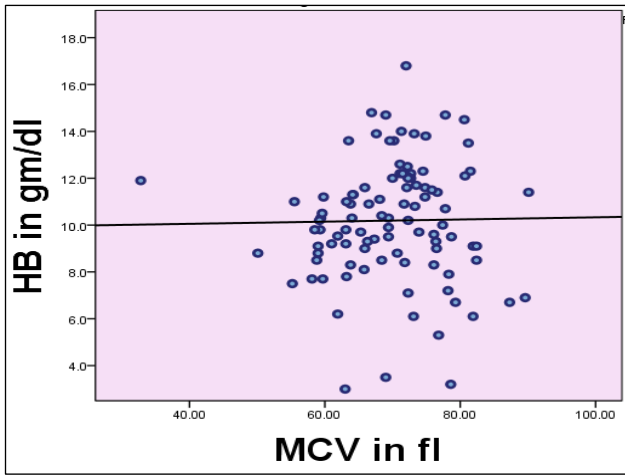
Homozygous cases (r= 0.65, p=0.000)

Figure 1: Correlation of HB with RBC in homozygous cases.



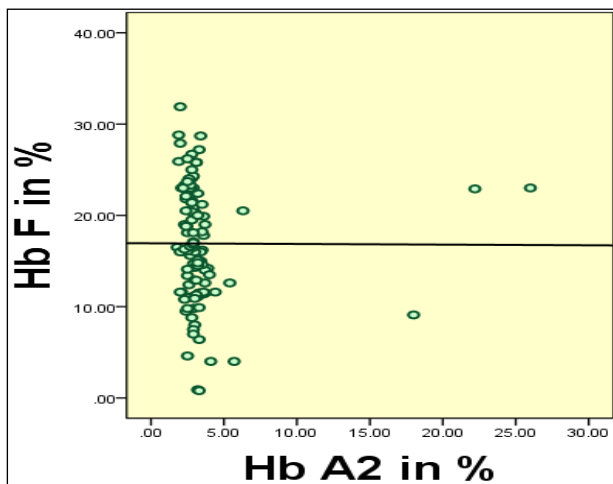
Homozygous cases (r= -0.133, p=0.15)

Figure 3: Correlation of HB with mcv in homozygous cases.



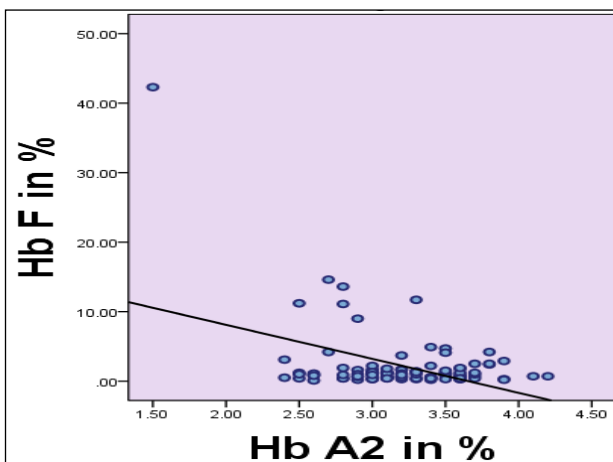
Heterozygous cases ($r=0.016$, $p=0.87$)

Figure 4: Correlation of HB with mcv in heterozygous cases.



Homozygous cases ($r=-0.004$, $p=0.97$)

Figure 5: Correlation of HB f with HBA2 in homozygous cases.



Heterozygous cases ($r=-0.431$, $p=0.000$)

Figure 6: Correlation of HB f with HBA2 in heterozygous cases.

Correlation between different variables in homo and heterozygous cases were shown in figure 1 to figure 6. (Figure 1 and 2) showed a significant positive correlation (with correlation coefficients as 0.6) between Hb and RBC in homozygous and heterozygous cases respectively. There was a negative correlation (correlation coefficient was -0.133) between Hb and MCV in homozygous cases (Figure 3) whereas positive correlation ($r=0.016$) existed between Hb and MCV in heterozygous cases (Figure 4). However, these correlations were not statistically significant. There was a significant negative correlation ($r=-0.431$) between Hb F and Hb A2 in heterozygous cases (Figure 6) but this correlation was not significant in homozygous cases (Figure 5).

DISCUSSION

The present hospital based cross sectional study was conducted in the Department of Pathology, MKCG medical college, Berhampur, Ganjam, Odisha to document the hematological profile findings of SCD patients and to know the pattern of hemoglobin variants in them. In this study a greater number of homozygous cases was registered than heterozygous cases. This may be due to fact that homozygous cases are more symptomatic and report to the hospital more than heterozygous cases. This was similar to the results found by Kamble M et al, and Chavda et al, in their study.^{8,9} Pattern of distribution of cases according to age is same in both heterozygous and homozygous case like most of the patients registered were in pediatric age group in both cases. This may be due to the fact that SCD patients become symptomatic and report to the hospital in early period of life. Maximum numbers of males were reported in childhood whereas maximum numbers of females were in reproductive age group. Similar results were also found in studies by Pathak et al, Khan et al, and Shrikhande et al.¹⁰⁻¹² Number of patients decreased after the age of 30 years in both cases.

Total number of male patients was more than female in homozygous cases, but the reverse was true for the heterozygous cases. In both homo and heterozygous cases, male patients outnumbered female in pediatric age group, but female patients outnumbered male in reproductive age group. The reason behind this may be due to more attentions are given to males than females in childhood period in society. In reproductive age group females are more reported may be due to nutritional deficiency, complications related to pregnancy, childbirth or reproductive morbidity. This type results were also found in Srikhande et al, and Rao et al, in their study.^{12,13}

Average Hb was low in both cases and it was significantly much lower in homozygous cases than heterozygous cases. This may be explained by hemolysis, blood loss, hematuria, repeated infections and nutritional

deficiency which are more in homozygous cases. Female were more anemic (low Hb value) than male in both cases. However, the findings were not significant. This finding was also similarly to findings by Srikhande et al.¹² Children were significantly more anemic than adults in homozygous cases. Srikhande et al, in their study also found total hemoglobin was significantly low till 14 years of age in both sexes and then raised with increase of age.¹² This could be explained by repeated infection and increased requirement of hemoglobin for growth in pediatric age group.

Average MCV was significantly lower (microcytosis) in heterozygous than homozygous cases. It may be due to deficiency of Vitamin B12 and folic acid as a result of increased erythropoiesis as chronic hemolysis occurs in homozygous cases. There was a significant increase occurrence of microcytosis in male as compared to female in homozygous cases. This findings was contradictory to the findings by Srikhande et al, where they found no significant difference in MCV value in males and females.¹² Low MCV value was found in this study as compared to other studies by Chavda et al, Srikhande et al, Kar et al, and Hayes et al.^{9,12,14,15} This may be due to co-existing of iron deficiency anemia and other unknown factors such as α -thalassemia or hemoglobinopathy in this study population.

Average RDW-CV was significantly more in homozygous than heterozygous cases. More anisocytosis (more RDW-CV value) was significantly found in pediatric age group in homozygous cases. However, there was no significant difference in RDW-CV value in male and female in both cases. Average RBC value was significantly lower in homozygous cases. There was no significant difference in average RBC among male, female and in pediatric, adult patients. Average fetal hemoglobin variant (Hb F) was significantly lower heterozygous cases and lower in adult group in both homo and heterozygous cases. But Srikhande et al, in their study found no age and sex related difference in HbF levels in patients.¹²

A significant positive correlation was found between Hb and RBC in both cases. There was a negative correlation between Hb and MCV in homozygous cases whereas positive correlation existed between Hb and MCV in heterozygous cases. However, these correlations were not statistically significant. A significant correlation was found between Hb F and Hb A2 in heterozygous cases, but this correlation was not significant in homozygous cases. That means inverse correlation existed between Hb F and Hb A2 in heterozygous cases. Significant negative correlation between Hb F and Hb A2 was also found by Srikhande et al, and Kar et al, in their study.^{12,14}

CONCLUSION

From the present study it can be concluded that in homozygous cases moderate anemia (microcytic

hypochromic to normocytic hypochromic) and High Hb F dominated the hematological profile whereas in heterozygous cases mild anaemia (microcytic hypochromic) dominated the hematological profile. In this study, children were significantly more anemic than adults in homozygous cases and more microcytosis was seen in heterozygous than homozygous cases. Average RDW-CV was significantly more in homozygous cases and more anisocytosis was found in pediatric age group in homozygous cases. Average fetal hemoglobin variant (Hb F) was significantly more in homozygous cases and lower in adult group in both homo and heterozygous cases.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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