Research Article

Discordant Hba_2 Expression In Hbs-B Thalassemia: A Case Series Of Rare HPLC Phenotypes With Elevated Hbf

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ABSTRACT

Introduction
Sickle cell disease results due to substitution of single amino acid at the sixth position in the gene encoding the globin subunit for glutamic acid instead of valine producing an abnormal hemoglobin (Hb). Clinical picture of compound heterozygous Hb S beta thalassemia resembles thalassemia intermedia in addition to sickling features. Prognosis is better than thalassemia major or sickle cell anemia.
Case Series:
We report three sibling pairs (six children) who presented with fever and varying combinations of jaundice, cough, vomiting, and loose stools. Clinical examination revealed pallor with or without mild icterus. Laboratory investigations demonstrated severe anemia, with hemoglobin levels ranging from 1.5 to 3.0 g/dL. HPLC in all patients showed elevated HbS (40.5–56.3%) and HbF (21.4–39.2%) with HbA2 levels ranging from 2.0% to 3.8%, raising the differential diagnosis of homozygous sickle cell disease and compound heterozygous HbS/β-thalassemia. HPLC analysis of the parents in each family identified one parent with β-thalassemia trait and the other with sickle cell trait, confirming the diagnosis of compound heterozygous HbS/β-thalassemia in all six children.
Conclusion:
This case series highlights the importance of integrating HPLC findings with parental screening to differentiate compound heterozygous HbS/β-thalassemia from homozygous sickle cell disease. Early recognition of this condition enables appropriate clinical management, family counseling, and informed genetic risk assessment

Key Words: Compound heterozygous sickle cell beta thalassemia, hemolytic anemia, high-performance liquid chromatography, sickling.

INTRODUCTION

Hemoglobin is a tetramer consisting of two pairs of alpha and beta globin chains, any abnormality in these chains cause hemoglobinoapthies.1 Sickle cell disease results from a single amino acid substitution at the sixth position in the gene encoding the globin subunit for glutamic acid instead of valine producing abnormal hemoglobin (Hb) called Hb S.2

According to WHO reports, nearly 5% of global population carries an abnormal Hb gene.3 In India, the average frequency of sickle cell disorders and beta thalassemia is 4.3% and 3-4% respectively.4 There are various coinheritance disorders of sickle cell allele with beta thalassemia trait, Hb E and Hb D.5,6 Compound heterozygous Hb S beta thalassemia is a rare combinational hemoglobinopathy with a prevalence of 0.02% in India and only few single case reports published in literature.1,7,8 It was first described by Silvestroni and Bianco in 1944.7 It is usually seen among black population in North and South America, West Indies and Africa. Additionally, it is also found in some areas of India, Middle East, Greece, Turkey and Italy.9

The clinical manifestations of compound heterozygous Hb S beta thalassemia is quite similar to thalassemia intermedia in addition to sickling features. Pallor, splenomegaly, mild growth retardation, leg ulcers, aseptic necrosis of the femoral head and vaso occlusive crises are some of its possible symptoms. On its peripheral blood smear (PBS) target cells, basophilic stippling, microcytic hypochromic red blood cells (RBCs) and low levels of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and few sickle cells can be confused with sickle cell anemia. Hb identification using high performance liquid chromatography (HPLC), Hb electrophoresis and isoelectric focussing help to reach definitive diagnosis.4 However, diagnostic discrepancies arise if levels of Hb F, Hb A2 and Hb A are not present in the diagnostic ranges making it difficult to distinguish

homozygous Hb S and compound heterozygous Hb S beta thalassemia. Family study plays a pivotal role in resolution of such discrepancies as seen in our case.10

Hemoglobin is a tetrameric protein composed of two α-globin and two β-globin chains. Mutations affecting the structure or synthesis of these globin chains result in a group of inherited disorders collectively known as hemoglobinopathies. Among these, sickle cell disease (SCD) is caused by a point mutation in the β-globin gene, leading to the substitution of valine for glutamic acid at the sixth amino acid position. This structural alteration produces hemoglobin S (HbS), which polymerizes under deoxygenated conditions, causing erythrocyte sickling, chronic hemolysis, and vaso-occlusive complications.[1,]

According to the World Health Organization, approximately 5% of the world's population carries a clinically significant hemoglobin variant.[3] In India, the estimated prevalence of sickle cell disorders is about 4.3%, while β-thalassemia trait affects approximately 3–4% of the population.[4] Co-inheritance of the HbS gene with other abnormal hemoglobin variants, including β-thalassemia, hemoglobin E, and hemoglobin D, gives rise to compound heterozygous hemoglobinopathies with variable clinical severity.[5,6]

Compound heterozygous HbS/β-thalassemia is an uncommon disorder resulting from inheritance of the HbS mutation from one parent and a β-thalassemia allele from the other. The reported prevalence in India is approximately 0.02%, and only a limited number of cases have been described in the literature.[1,7,8] First reported by Silvestroni and Bianco in 1944, this disorder is more frequently encountered in populations from sub-Saharan Africa, the Mediterranean region, the Middle East, and parts of the Indian subcontinent.[7,9]

The clinical spectrum of HbS/β-thalassemia ranges from mild to severe depending on the type of βthalassemia mutation and the amount of residual β-globin production. Patients may present with features of both sickle cell disease and thalassemia intermedia, including chronic anemia, pallor, jaundice, splenomegaly, growth retardation, vaso-occlusive episodes, leg ulcers, and avascular necrosis. Peripheral blood smear findings often include microcytic hypochromic red cells, target cells, basophilic stippling, anisopoikilocytosis, and occasional sickled erythrocytes, while red cell indices typically demonstrate reduced mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). These overlapping clinical and hematological features can make differentiation from homozygous sickle cell disease challenging.

Definitive diagnosis relies on hemoglobin characterization using high-performance liquid chromatography (HPLC), hemoglobin electrophoresis, or isoelectric focusing. However, interpretation may be difficult in patients with atypical HbF, HbA_2, or HbA levels, leading to diagnostic uncertainty between homozygous HbS disease and compound heterozygous HbS/β-thalassemia. In such situations, parental hemoglobin analysis provides valuable confirmatory evidence. In the present case series, we describe three sibling pairs in whom family screening was instrumental in establishing the diagnosis of compound heterozygous HbS/β-thalassemia when HPLC findings alone were inconclusive.

CASE REPORT 1:

Case Report

A 9-year-old boy and his 11-year-old sister presented to the pediatric outpatient department with a two-week history of high-grade fever and jaundice. The fever was continuous in nature and showed only temporary relief with antipyretic medications. Both siblings had a history of recurrent respiratory tract infections and intermittent episodes of jaundice since early childhood.

On physical examination, both children were poorly built and exhibited marked pallor with mild scleral icterus. Abdominal examination revealed mild splenomegaly in each patient, while the remaining systemic examination was unremarkable.

Baseline investigations included complete blood count using the Horiba Yumizen H2500 hematology analyzer, peripheral blood smear (PBS), sickling test, and hemoglobin analysis by high-performance liquid chromatography (HPLC) using the Bio-Rad D-10 system (Table/Fig. 1 and 2). Biochemical evaluation demonstrated mild hyperbilirubinemia with evidence of increased body iron stores in both children. HPLC findings showed HbS levels below 80% with normal HbA_2 values, making it difficult to differentiate between homozygous sickle cell disease (HbSS) and compound heterozygous HbS/β-thalassemia. Although molecular genetic testing was recommended for definitive confirmation, it could not be performed because of financial limitations.

To resolve the diagnostic uncertainty, HPLC analysis was subsequently performed in both parents.

The father was found to be a carrier of β-thalassemia trait, whereas the mother had sickle cell trait. Correlation of the parental carrier status with the children's HPLC findings established the diagnosis of compound heterozygous HbS/β-thalassemia in both siblings.
The patients received supportive management with intravenous antibiotics for the febrile illness and transfusion of four units of packed red blood cells to correct the severe anemia. Before discharge, the family was counseled regarding disease progression, measures to prevent sickling crises, the importance of regular follow-up, and the genetic implications of the disorder, including the need for family screening and genetic counseling.

Case Report 2
A 8-year-old boy and his 6-year-old sister was evaluated in the pediatric outpatient department with complaints of persistent fever, cough, vomiting, and diarrhea of two weeks' duration. Clinical examination revealed pallor and mild scleral icterus in both children. Mild enlargement of the spleen was noted on abdominal examination, while the remaining systemic examination showed no significant abnormalities.
Laboratory workup included complete blood count (Mindray BC-2500), peripheral blood smear, sickling test, liver function tests, and hemoglobin fraction analysis by high-performance liquid chromatography (Bio-Rad D-20) (Table/Fig. 3 and 4). Both siblings had severe anemia, with hemoglobin values of 2 g/dL in the boy and 3 g/dL in the girl. Biochemical parameters indicated mild hemolysis with hyperbilirubinemia and evidence of increased iron stores.
HPLC demonstrated HbF 24.4%, HbS 53.3%, and HbA_2 3.1% in the male child, whereas the female child showed HbF 33.2%, HbS 40.5%, and HbA_2 2.0%. These findings were not sufficiently characteristic to distinguish between homozygous sickle cell disease and compound heterozygous HbS/β-thalassemia. Therefore, HPLC evaluation was extended to both parents. The mother was identified as a carrier of β-thalassemia trait, whereas the father had sickle cell trait. Considering the
inheritance pattern together with the children's HPLC findings, a diagnosis of compound heterozygous HbS/β-thalassemia was established.
The children received supportive treatment comprising broad-spectrum antibiotics and packed red blood cell transfusions. Prior to discharge, the family underwent counseling regarding disease inheritance, measures to minimize sickling episodes, and the importance of long-term follow-up and genetic screening.
Case Report 3
A 12-year-old girl and her 8-year-old younger sister presented with a history of fever, vomiting, and diarrhea lasting for two weeks. Physical examination demonstrated pallor and mild jaundice in both children. Mild splenomegaly was also evident on abdominal examination.
Investigations included complete blood count, peripheral blood smear, sickling test, liver function tests, and HPLC (Table/Fig. 5 and 6). The elder and younger siblings had hemoglobin concentrations of 3 g/dL and 2 g/dL, respectively. Laboratory evaluation further revealed biochemical evidence of mild hemolysis with elevated bilirubin levels and increased iron stores.
HPLC analysis showed HbF 21.4%, HbS 52.3%, and HbA_2 2.1% in one sibling, while the other demonstrated HbF 32.2%, HbS 41.5%, and HbA_2 3.0%. Because these results overlapped with the HPLC profile of homozygous sickle cell disease, differentiation based solely on hemoglobin fractions was inconclusive. Subsequent parental screening identified β-thalassemia trait in the father and sickle cell trait in the mother. These findings established the diagnosis of compound heterozygous HbS/β-thalassemia in both siblings.
The patients were managed conservatively with antibiotic therapy and packed red blood cell transfusions to correct severe anemia. Detailed counseling was provided regarding the hereditary nature of the disease, recognition and prevention of sickling crises, and the importance of family screening and genetic counseling.

Discussion

Inherited hemoglobin disorders, particularly β-thalassemia and sickle cell disease, continue to represent a major public health concern in many developing countries, including India. Besides contributing to significant morbidity and mortality, these disorders impose considerable psychological, social, and economic burdens on affected families and healthcare systems.[11] Early diagnosis is therefore essential to ensure appropriate treatment, genetic counseling, and prevention strategies.
Compound heterozygous HbS/β-thalassemia results from the inheritance of the HbS mutation from one parent and a β-thalassemia allele from the other. The disorder exhibits marked clinical heterogeneity depending on the type of β-thalassemia mutation and the level of residual β-globin synthesis. Because its clinical and hematological features overlap considerably with those of homozygous sickle cell disease, establishing the

correct diagnosis may be challenging. Peripheral blood smear findings, including microcytic hypochromic red cells, target cells, anisopoikilocytosis, and occasional sickled erythrocytes, are not sufficiently specific to differentiate the two conditions. Although an elevated HbA_2 level is considered an important indicator of β-thalassemia carrier status, normal or borderline HbA_2 values may occasionally be encountered, creating diagnostic uncertainty.[8,12]

High-performance liquid chromatography (HPLC) is widely used for the identification of hemoglobin variants; however, interpretation may become difficult when HbS, HbF, HbA_2, and HbA values do not fall within characteristic diagnostic ranges. In such situations, parental hemoglobin analysis provides valuable additional information and may help distinguish compound heterozygous HbS/βthalassemia from homozygous sickle cell disease, particularly in resource-limited settings where molecular testing is unavailable.

The findings in our case series are comparable with previously published reports. Shrestha et al. described a 5-year-old boy who was initially diagnosed with sickle cell anemia based on an HbS level of 63.2%. However, his relatively mild clinical course and absence of previous blood transfusions prompted further investigation. HPLC analysis of his parents revealed β-thalassemia trait in one parent and sickle cell trait in the other, confirming the diagnosis of compound heterozygous HbS/βthalassemia.[1]

Similarly, Jain et al. reported a 12-year-old boy presenting with fever and splenomegaly whose peripheral blood smear showed variable numbers of sickled erythrocytes, closely resembling sickle cell anemia. HPLC demonstrated HbS levels greater than 50% with markedly elevated HbF (35.1%), making differentiation between homozygous sickle cell disease and HbS/β-thalassemia difficult. The final diagnosis was established only after parental evaluation demonstrated β-thalassemia trait in one parent and sickle cell trait in the other.[8]

Chandra et al. reported a 16-year-old female with generalized weakness and recurrent episodes of jaundice. Hematological evaluation revealed microcytic hypochromic anemia with occasional sickled cells, while HPLC findings initially suggested sickle cell disease because of predominant HbS and normal HbA_2 levels. Family screening subsequently identified sickle cell trait in her brother and βthalassemia trait in her sister, thereby supporting the diagnosis of compound heterozygous HbS/βthalassemia.[13] Similar diagnostic challenges were encountered in our patients, where parental HPLC analysis proved crucial for confirming the diagnosis.

Likewise, Teli et al. described a 14-year-old girl presenting with anemia, generalized weakness, and joint pain. HPLC findings, together with parental evaluation, established the diagnosis of HbS/βthalassemia. Molecular analysis further demonstrated the IVS-I-5 (G→C) mutation in both the patient and her father. The authors emphasized that interpretation of HbS, HbF, and HbA_2 percentages in conjunction with hematological findings, family history, and parental screening improves diagnostic accuracy, particularly when molecular confirmation is not readily available.[10]

Our case series reinforces these observations by demonstrating that family-based HPLC evaluation is an invaluable diagnostic tool when hemoglobin fractions are inconclusive. In all three families, the affected children exhibited overlapping HPLC profiles that could not reliably distinguish HbS/βthalassemia from homozygous sickle cell disease. Identification of β-thalassemia trait in one parent and sickle cell trait in the other established the diagnosis in each case, highlighting the importance of parental screening in resource-constrained settings.

Conclusion

Compound heterozygous HbS/β-thalassemia should be considered in patients with severe anemia and HPLC findings that do not clearly fit the profile of homozygous sickle cell disease. Although HPLC remains the primary diagnostic modality, its interpretation may occasionally be inconclusive. In such circumstances, careful assessment of hematological parameters together with parental hemoglobin analysis can provide a reliable and cost-effective means of establishing the diagnosis, especially where molecular genetic testing is unavailable. Early recognition facilitates appropriate clinical management, genetic counseling, and screening of at-risk family members.

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Table no 1 - Laboratory investigations of both the patients and their parents.

a) Hematological findings and b) biochemical test c) HPLC findings

(a) Hematological Findings

Parameters	Male child	Female child	Father	Mother
Hb (gm/dl)	1.8	1.5	9.1	10
RBC (million/cumm)	0.98	0.76	5.0	4.49
MCV (fl)	63	69	65.7	77.1
MCH (pg)	18.3	19.2	18.3	22.1
MCHC (gm/dl)	28.9	27.6	27.8	28.8
RDW (%)	18.2	24.1	18.3	16.3
TLC (cells/cumm)	8800	11500	6800	6500

Platelets (lacs/cumm)	1.03	2.35	3.40	2.22
PBF findings	Microcytic hypochromic RBC, few sickle cells and few target cells	Microcytic hypochromic RBC, few sickle cells and few target cells	Microcytic hypochromic RBC, few target cells	Microcytic hypochromic RBC, occasional sickle cells
Sickling test using sodium metasulphite	Positive	Positive	Negative	Positive
(b) Biochemical tests				
Total bilirubin (mg/dl)	2.7	2.2	-	-
Conjugated bilirubin (mg/dl)	0.8	0.5	-	-
Unconjugated bilirubin (mg/dl)	1.9	1.7	-	-
Serum iron (µg/dl)	249	233	-	-
Ferritin (ng/dl)	> 800	777	-	-
Total iron binding capacity (µg/dl)	374	360	-	-
Chest X ray	Within normal limit	Within normal limit	-	-
Ultrasound - Abdomen	Within normal limit	Hepatomegaly	-	-
(c) HPLC findings				
HbA0	9.2	9.2	80.9	55.4
HbA2	3.8	3.0	5.8	2.8
HbF	27.4	39.2	<0.8	<0.8

HbS	56.3	43.5	00	32.7

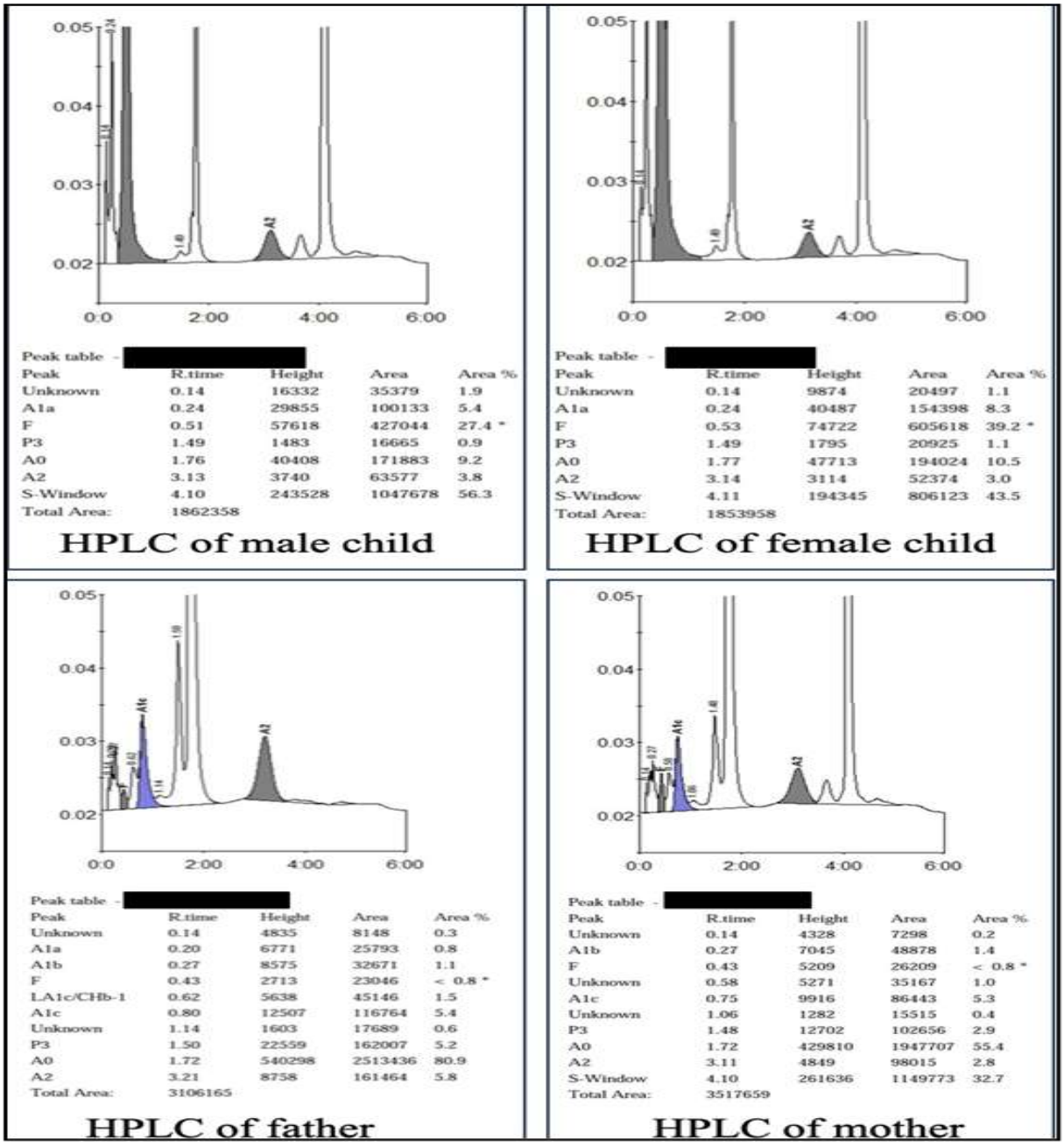

Fig no :1 Chromatograms of male child, female child, father and mother showing compound heterozygous Hb S beta thalassemia in both children along with beta thalassemia trait and Hb S heterozygous in father and mother respectively

Table 2 : CBC,Biochemistry and HPLC findings of case report 2 of 2 siblings.

Parameter	Male child	Female child
Total bilirubin (mg/dL)	2.7	2.2

Direct bilirubin (mg/dL)	0.8	0.5
Indirect bilirubin (mg/dL)	1.9	1.7
Serum iron (µg/dL)	249	233
Ferritin (ng/mL)	>800	777
TIBC (µg/dL)	374	360
Chest X-ray	Normal	Normal
USG abdomen	Normal	Hepatomegaly

Table 3: CBC ,Biochemistry and HPLC findings of patient and parents of case report 2.

Parameter	Male child (8 years)	Female child (6 years)	Father	Mother
Hemoglobin (g/dL)	2.0	3.0	9.1	10.0
RBC (million/cumm)	—	—	5.0	4.49
MCV (fL)	63	69	65.7	77.1
MCH (pg)	18.3	19.2	18.3	22.1
MCHC (g/dL)	28.9	27.6	27.8	28.8
RDW (%)	18.2	24.1	18.3	16.3
TLC (cells/cumm)	8800	11500	6800	6500
Platelets (lacs/cumm)	1.03	2.35	3.40	2.22
Peripheral blood smear	Microcytic hypochro mic RBCs with few sickle and target cells	Microcytic hypochromic RBCs with few sickle and target cells	Microcytic hypochromic RBCs with few target cells	Microcytic hypochromic RBCs with occasional sickle cells
Sickling test	Positive	Positive	Negative	Positive

Table 4 : CBC ,Biochemical test and HPLC findings.Of case report Case Report 3

Parameter	Elder sister (12 years)	Younger sister (8 years)	Father	Mother

Hemoglobin (g/dL)	3.0	2.0	3.0	3.2
RBC (million/cumm)	0.63	0.83	1.2	1.2
MCV (fL)	53	63	78	72
MCH (pg)	19	18	19	19
MCHC (g/dL)	28.3	27.8	29	28
TLC (cells/cumm)	4500	4784	5800	4800
Platelets (lacs/cumm)	1.53	1.58	1.70	1.80
Peripheral blood smear	Microcytic hypochromic RBCs with occasional sickle cells	Microcytic hypochromic RBCs with occasional sickle cells,acanthocytes	Microcytic Hypochromic RBCs	Microcytic Hypochromic RBCs
Sickling test	Positive	Positive	Positive	Positive

Table 5: HPLC Findings (Case Report 3)

Hemoglobin fraction (%)	Elder sister (12 years)	Younger sister (8 years)	Father	Mother
HbA (HbA0)	7.2	7.6	7.8	7.5
HbA2	2.1	3.0	—	—
HbF	21.4	32.2	<0.8	<0.8
HbS	52.3	41.5	0	32.7

Table 6: Comparision of Hematological Findings of Case Report 2 and Case Report 3

Parameter	Case 2: Male child (8y)	Case 2: Female child (6y)	Case 3: Elder sister (12y)	Case 3: Younger sister (8y)
Hemoglobin (g/dL)	2.0	3.0	3.0	2.0
RBC (million/cumm)	0.83	1.0	1.2	1.3
MCV (fL)	63	69	73	— 72
MCH (pg)	18.3	19.2	17.9	18.2
MCHC (g/dL)	28.9	27.6	29.3	28.3
RDW (%)	18.2	24.1	19.2	18.2

TLC (cells/cumm)	8800	11500	7500	8600
Platelets (lacs/cumm)	1.03	2.35	1.67	1.72
Peripheral blood smear	Microcytic hypochromic RBCs with few sickle cells and target cells	Microcytic hypochromic RBCs with few sickle cells and target cells	Microcytic hypochromic RBCs with occasional sickle cells	Microcytic hypochromic RBCs with occasional sickle cells
Sickling test	Positive	Positive	Positive	Positive