Case Report

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Pathology Section

A Rare Case of Compound Heterozygous Sickle Cell Beta Thalassaemia with High HbF and Normal HbA2 Levels Detected on HPLC

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ABSTRACT

Compound heterozygous Sickle Haemoglobin (HbS) beta (β) thalassaemia arises from the mutations associated with sickle cell and β thalassaemia and significantly affects populations in low income countries like India. Elevated levels of Haemoglobin A2 (HbA2) represent the primary indicator for identifying carriers of β thalassaemia. However, it's worth noting that sometimes, High Performance Liquid Chromatography (HPLC) encounters challenges in confirming a final diagnosis when levels of HbA2 and HbS fall outside the diagnostic range. A nine-year-old male and his sister 11-year-old female patient presented with high-grade fever and jaundice since two weeks. Following admission, Complete Blood Count (CBC) and HPLC of both children were done. Hb of male and female child revealed 1.8 g/dL and 1.5 g/dL, respectively. HPLC of male child revealed Haemoglobin F (HbF) 27.4%, HbS 56.3%, HbA2 3.8% and of female child revealed HbF 39.2%, HbS 43.5%, HbA2 3%. HPLC reports of both children were suggestive of differential of compound heterozygous HbS β thalassaemia and HbS homozygous. Later, HPLC of their parents was also done. HPLC of father was suggestive of thalassaemia trait and of mother suggestive of sickle cell trait, following which final diagnosis of both children was given as compound heterozygous HbS β thalassaemia. Diagnosing these compound heterozygous haemoglobinopathies can be challenging. As their is resemblance in clinicopathological features of sickle cell anaemia and β thalassaemia disorders, it is important to carefully differentiate between them although prognosis is better than thalassaemia major or sickle cell anaemia.

Keywords: Compound heterozygous sickle cell β thalassaemia, Foetal Haemoglobin, Haemoglobin A2, Haemolytic anaemia, High performance liquid chromatography, Sickling

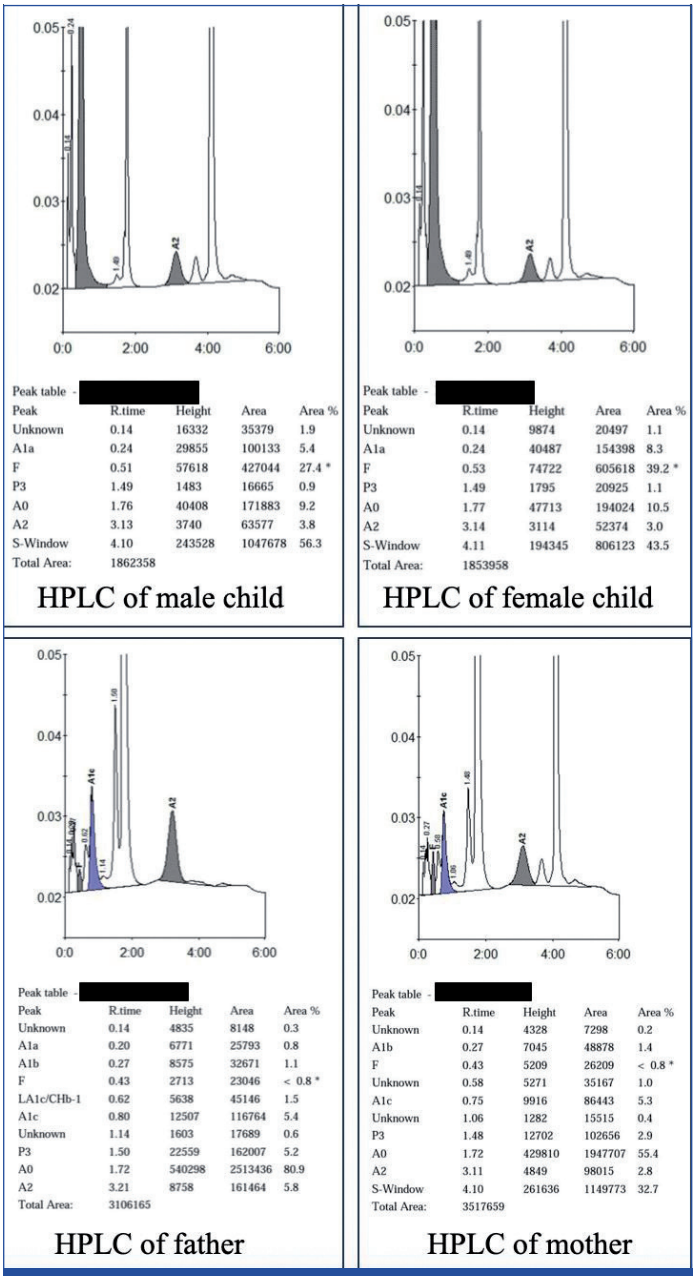
CASE REPORT

A nine-year-old male and his 11-year-old sister presented to Paediatric Outpatient Department (OPD) with complaints of fever and jaundice since two weeks. Fever was high-grade (102.5°F), continuous and relieved for some time after taking medication. They had past history of repeated episodes of respiratory infections and jaundice since birth. On general examination, both patients were thin built with presence of pallor and mild icterus. On systemic examination, mild splenomegaly was present in both patients. Haematological analysis (Horiba yumizen H2500), Peripheral Blood Smear (PBS) examination, sickling test and High Performance Liquid Chromatography (HPLC) (Biorad D10) of both patients [Table/Fig-1,2] were done. Biochemical investigations were suggestive of mild jaundice detected by increased serum bilirubin levels and increased iron loads (high serum ferritin levels of >800 ng/mL in male child and 777 ng/mL, in female child) in both patients. The differential diagnosis of Sickle Haemoglobin (HbS) homozygous and compound heterozygous HbS β thalassaemia were suggested as HbS level was <80% and HbA2 level was normal in both cases.

Haematological findings				
Variables	**Male child**	**Female child**	**Father**	**Mother**
Hb (12-15 g/dL)	1.8	1.5	9.1	10
RBC (4.5-5.5 million/cumm)	0.98	0.76	5.0	4.49
MCV (80-100 fl)	63	69	65.7	77.1
MCH (27-32 pg)	18.3	19.2	18.3	22.1
MCHC (31.5-34.5 g/dL)	28.9	27.6	27.8	28.8
RDW (11.6-14%)	18.2	24.1	18.3	16.3
TLC (4000-11000 cells/cumm)	8800	11500	6800	6500
Platelets (1.5-4.5 lacs/cumm)	1.03	2.35	3.40	2.22
PBS 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
Biochemical tests				
Total bilirubin (N- 0.8-1.2 mg/dL)	2.7	2.2	-	-
Conjugated bilirubin (N-0.0-0.2 mg/dL)	0.8	0.5	-	-
Unconjugated bilirubin (N-0.8-1.2 mg/dL)	1.9	1.7	-	-
Serum iron (N-45-158 µg/dL)	249	233	-	-
Ferritin (N- men- 20-350 ng/mL, women-10-200 ng/mL)	> 800	777	-	-
Total iron binding capacity (N-225-535 µg/dL)	374	360	-	-

Chest X-ray	Within normal limit	Within normal limit	-	-
Ultrasound-Abdomen	Within normal limit	Hepatomegaly	-	-
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

[Table/Fig-1]: Laboratory investigations of both the patients and their parents. Hb: Haemoglobin; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; MCV: Mean Corpuscular Volume; N: Normal value; PBS: Peripheral Blood Smear; RBC: Red Blood Cell; RDW: Red cell Distribution Width; TLC: Total Leucocyte Count



[Table/Fig-2]: Chromatograms of male child, female child, father and mother showing compound heterozygous HbS β thalassaemia in both children along with β thalassaemia trait and HbS heterozygous in father and mother, respectively.

Molecular studies were advised but due to financial constraints it couldn't be done.

Later on, HPLC of father and mother were performed and were suggestive of thalassaemia trait and sickle cell trait, respectively. Then final diagnosis of both children was given as compound heterozygous HbS β thalassaemia. Both patients were managed conservatively. Antipyretics were given to relieve fever and four units of packed RBCs were transfused. They were counselled for future precautions to prevent sickling crisis along with various genetic aspects related to the disease.

They were given daily supplements of calcium, multivitamin and zinc upon discharge. Later, they could not be followed further.

DISCUSSION

Thalassaemia and sickle cell disease have been adversely impacting the population of low income countries, including India. The birth of a thalassaemic child places a great deal of physical, financial and emotional pressure on the child, family, society, and country as a whole [1].

According to World Health Organisation (WHO) reports, nearly 5% of global population carries an abnormal Haemoglobin (Hb) gene [2]. In India, the average frequency of sickle cell disorders and β thalassaemia is 4.3% and 3-4%, respectively [3]. There are various coinheritance disorders of sickle cell allele with β thalassaemia trait, Haemoglobin E (HbE) and Haemoglobin D (HbD) [4,5]. Compound heterozygous HbS β thalassaemia is a rare combinational haemoglobinopathy with a prevalence of 0.02% in India and only a few single case reports have been published in the literature [6-8]. It was first described by Silvestroni and Bianco in 1944 [7].

The clinical manifestations of compound heterozygous HbS β thalassaemia are quite similar to thalassaemia intermedia in addition to sickling features. On its PBS target cells, basophilic stippling, microcytic hypochromic Red Blood Cells (RBCs) and low levels of Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) and few sickle cells can be confused with sickle cell anaemia. It is difficult to distinguish between PBS of compound heterozygous HbS β thalassaemia and individuals with homozygous sickle cell disease, since they both exhibit anaemia with sickle cells in variable proportions. So, Hb identification using HPLC, Hb electrophoresis and isoelectric focussing help to reach definitive diagnosis [3]. Elevated HbA2 is the most significant parameter in the identification of β thalassaemia carriers [8]. However, diagnostic discrepancies arise if levels of Haemoglobin F (HbF), HbA2 and Haemoglobin A (HbA) are not present in the diagnostic ranges, making it difficult to distinguish homozygous HbS and compound heterozygous HbS β thalassaemia. Family study plays a pivotal role in resolution of such discrepancies as seen in the present case [9].

Shrestha B et al., reported a case of five year old male who was reported as sickle cell anaemia as HbS was 63.2% but considering his unusual presentation of apparently well thriving and with no history of blood transfusion since birth, his diagnosis was reconsidered. Both his parents were evaluated on HPLC and reported as β thalassaemia trait and sickle cell trait [6].

In a study by Pathak MS et al., a three-year-old female complained of weakness, joint pain and stomach pain. The HPLC report revealed that the patient was compound heterozygous for HbS β thalassaemia. The family study revealed β thalassaemia trait in father and sickle cell trait in mother. Further confirmation was done using molecular study revealing Intervening Sequence (IVS) 1-5 G→C mutation in the patient and father [10].

In another study by Kaur M et al., a 32-year-old male with complaints of fever with chills and rigour along with pain in legs and hip joint for seven days was reported. On PBS evaluation, there were many target cells, microcytes, normocytes and few sickle cells. To confirm the presence of sickle cells, sodium metabisuphite sickling test was done which showed immediate sickling. The HPLC report revealed that the patient was compound heterozygous for HbS β thalassaemia. Only after the diagnosis report, the patient became aware about the Hb variant carrier state [3].

Chandra S et al., reported a case of a 16-year-old female patient with complaints of generalised weakness with off and on mild jaundice

CBC findings							
Variables	Shrestha B et al., [6] (2011)	Pathak MS et al., [10] (2014)	Kaur M et al., [3] (2017)	Chandra S et al., [11] (2017)	Teli AB et al., [9] (2016)	Priya J et al., [8] (2020)	Meliti A et al., [12] (2023)
Hb (g/dL)	8.4	1.1	6.7	7.7	5.9	6.4	9.9
RBC (million/cumm)	3.38	1.17	-	-	2.56	2.85	-
MCV (fl)	72.5	91.5	68.4	-	75	66.1	-
MCH (pg)	-	29.1	32.2	-	23	21.1	-
MCHC (g/dL)	-	31.8	47.1	-	30.7	31.9	-
RDW (%)	-	-	-	-	18.3	-	-
TLC (/cumm)	-	16.4	7000	-	7000	-	
Platelets (lacs/cumm)	-	-	0.23	0.68	0.20	-	-
HPLC findings							
HbA (%)	6.5	5.9	28.6	42.1	6.3%	5.2	-
HbA2 (%)	4.9	4.7	4.4	3.8	5.6%	4.2	-
HbF (%)	25	30.5	23	15.7	13.5%	35.1	-
HbS (%)	63.2	49.2	44.7	38.4	69.8%	53.5	-

[Table/Fig-3]: Complete Blood Count (CBC) and High Performance Liquid Chromatography (HPLC) findings of published case reports [3,6,8-12].

for two years. Complete blood examination revealed moderate microcytic hypochromic anaemia, few sickle cells, anisocytosis and late normoblasts. HPLC diagnosis of sickle cell anaemia was offered as HbS >50% and HbA2 were normal. It was later confirmed to be compound heterozygous HbS β thalassaemia by HPLC evaluation of siblings as brother was reported sickle cell trait and sister as β thalassaemia trait. These observations were similar to the authors' study as the diagnostic dilemma was also resolved after family study [11].

In another case report by Teli AB et al., a 14-year-old female patient with complaints of anaemia, weakness and joint pain was reported. The Hb typing results revealed that the patient was compound heterozygous for HbS β thalassaemia along with the father, who had β thalassaemia trait and the mother had sickle cell trait. Molecular analysis was done to further identify the mutation associated and it was found that both the child and father had IVS 1-5 (G→C) mutation. They concluded that when HbS variant is present, percentage of HbA2 and HbF, family history, clinical data and haematological parameters help in distinguishing between HbS homozygous and compound heterozygous HbS β thalassaemia [9].

Priya J et al., reported a case of a 12-year-old boy who was evaluated following a history of low-grade fever and splenomegaly. The PBS was similar to that of sickle cell anaemia showing sickle cells in varying proportions, making it difficult to differentiate from compound heterozygous HbS β thalassaemia on PBS. As HPLC revealed HbS >50%, with high Hb F value (35.1%), diagnosis of HbS homozygous and compound heterozygous HbS β thalassaemia were considered as possible differentials. The final diagnosis was made only after evaluation of parents and both of whom were carriers of thalassaemia trait and sickle cell trait, respectively [8].

In a study by Meliti A et al., an eight-year-old boy presented to the hospital for evaluation of short stature. The parents provided a clinical history detailing episodes of joint pain spanning the past four years, typically triggered by moderate physical activities. The X-ray was done to evaluate recurrent joint pain revealing acystic lesion of the left upper humerus which was diagnosed clinically to be an aneurysmal bone cyst. A fluid sample from the lesion revealed many sickle cells lymphocyte rich background admixed with few histiocytes. The cytological features indicated the presence of sickle cell disease. Since these cytological findings were unexpected in the clinical context, immediate communication was established with the orthopaedic surgeon, and the haematology team was notified and involved promptly. Additional clinical investigations, including molecular and laboratory tests, confirmed the diagnosis of compound heterogeneous HbS β thalassaemia. The patient has been under the care of paediatric haematology and endocrinology for nearly four years, and his progress has been satisfactory [12]. [Table/Fig-3] shows the CBC and HPLC findings of published case reports.

In all these cases discussed, the diagnostic dilemma was relieved only after either family study or molecular analysis. The authors concluded that detailed examination of PBS and HPLC findings might help to raise suspicion of rare cases like compound heterozygous HbS β thalassaemia. Family study is a reliable and easily available mode for confirmation of diagnosis where molecular studies are not available.

CONCLUSION(S)

For a clinician, haemoglobinopathies might pose a diagnostic dilemma. In absence of a correct diagnosis, the patients are given iron supplements which further increase the iron overload. An early and accurate diagnosis would significantly improve the prognosis and quality of life for those affected. It is crucial to emphasise the value of prompt follow-up, care, education, genetic counselling and proactive management of complications.

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