Case Report

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Pathology Section

Rare Compound Heterozygous HbE-β Thalassaemia Diagnosed using HPLC and Familial History: A Case Report

RUCHI AGARWAL[1], HEMLATA KAMRA[2], PARUL[3], KULWANT SINGH[4], SWARAN KAUR[5]

ABSTRACT

Haemoglobin E-beta thalassaemia (HbE/βT) is a compound heterozygous haemoglobinopathy caused by a mutation in the β-globin chain (glutamic acid → lysine at position 26) and co-inheritance of a β-thalassaemia allele. Diagnosis is difficult because to its wide range of phenotypic heterogeneity, which includes severe thalassaemia major-like disease and transfusion independence. An 11-year-old female presented with pallor, growing weakening and history of single blood transfusion. Anisopoikilocytosis revealing target cells and microcytic hypochromic red cells was observed in the peripheral smear. Significantly increased HbA2 (29.7%) and HbF (11.3%) were detected by High Performance Liquid Chromatography (HPLC). Homozygous HbE and HbE/βT were considered as differential diagnosis on the basis of various investigations carried out.The diagnosis of HbE/βT in the patient was supported by family HPLC investigations, which showed the mother was HbE heterozygous, the younger brother had thalassaemia trait and elder brother had HbE/βT. After three months, repeat HPLC or molecular testing was advised for confirmation. This case emphasises how crucial family studies are for clearing up diagnostic ambiguity, especially when recent history of blood transfusions can cloud laboratory test results. It underlines the importance of maintaining a high index of suspicion for compound heterozygous states and encourages a thorough diagnostic workup, including family study using HPLC. Through this report, we hope to contribute for improved awareness and earlier detection of this challenging and often misdiagnosed condition.

Keywords: Haemoglobin E, Haemoglobinopathies, High performance liquid chromatography

CASE REPORT

An 11-year-old female presented to pediatric Outpatient Department (OPD) with progressively increasing weakness and difficulty in performing daily activities. On general examination, patient was thin built with presence of pallor. In view of above symptoms, general examination findings and absence of organomegaly clinical diagnosis of nutritional anaemia and haemolytic anaemia were considered. Haematological analysis (Horiba yumizen H2500), Peripheral Blood Smear (PBS) examination, Retic stain, osmotic fragility testing, sickling test and HPLC (Biorad D10) [Table/Fig-1] were done. Biochemical investigations were suggestive of decreased iron levels. PBS examination revealed anisopoikilocytosis with microcytic hypochromic red blood cells along with target cells with neutrophilic leucocytosis and reduced platelets with normal morphology [Table/Fig-2]. HPLC report revealed Hb A2 (29.7%) with elevated Hb F (11.3%). Later on, past history of one unit transfusion of packed RBCs one month back was found. In view of microcytic hypochromic anaemia with raised Hb A2 (<50%) and Hb F(11.3%) along with history of blood transfusion one month back, final diagnosis was challenging however the differential diagnosis of HbE homozygous and compound HbE/βT were suggested. Genetic testing of patient and family study by HPLC was advised. HPLC of younger brother, elder brother and mother were performed and were suggestive of beta thalassaemia trait, HbE/βT and HbE heterozygous respectively [Table/Fig-3]. The diagnosis of HbE/βT was suggested for the patient on the basis of family study. The patient was given daily supplements of iron, multivitamin and zinc upon discharge. Later she could not be followed further.

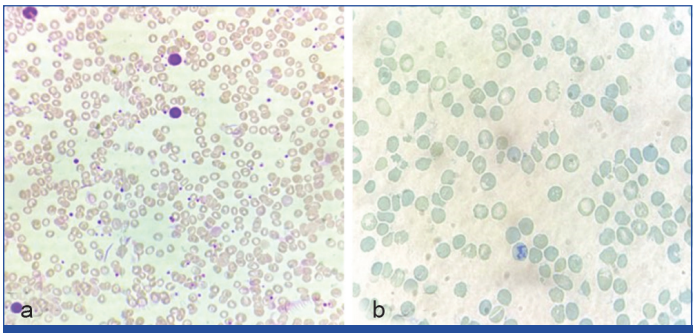
Parameters	Patient	Elder brother	Younger brother	Mother
(a) Haematological findings				
Hb (12-15 gm/dL)	9.6	5.5	5.5	10.8
RBC (4.5-5.5 million/cumm)	4.45	3.29	2.64	4.45
MCV (80-100fl)	69	56.8	71.2	75.3
MCH (27-32pg)	21.6	16.7	20.8	24.3
MCHC (31.5-34.5gm/dL)	31.3	29.4	29.3	32.2
RDW (11.6-14%)	33.9	31.6	32.2	15.1
TLC (4000-11000cells/cumm)	16,000	14,000	3,000	7,200
Platelets (1.5-4.5lacs/cumm)	1.80	1.50	0.80	1.50
PBF findings	Microcytic hypochromic RBC, few target cells	Microcytic hypochromic RBC, few target cells	Microcytic hypochromic RBC	Microcytic hypochromic RBC
Retic count	1.2%	1.1%	0.8%	1.0%
Osmotic fragility testing	Within normal limits	Within normal limits	Within normal limits	Within normal limits
Sickling test using sodium metasulphite	Negative	Negative	Negative	Negative
(b) Biochemical tests				
Total bilirubin (N - 0.8-1.2 mg/dL)	0.9	-	-	-
Conjugated bilirubin (N - 0.0-0.2mg/dL)	0.0	-	-	-
Unconjugated bilirubin (N - 0.8-1.2 mg/dL)	0.9	-	-	-
Serum iron (N - 45 - 158 g/dL)	40	-	-	-

DISCUSSION

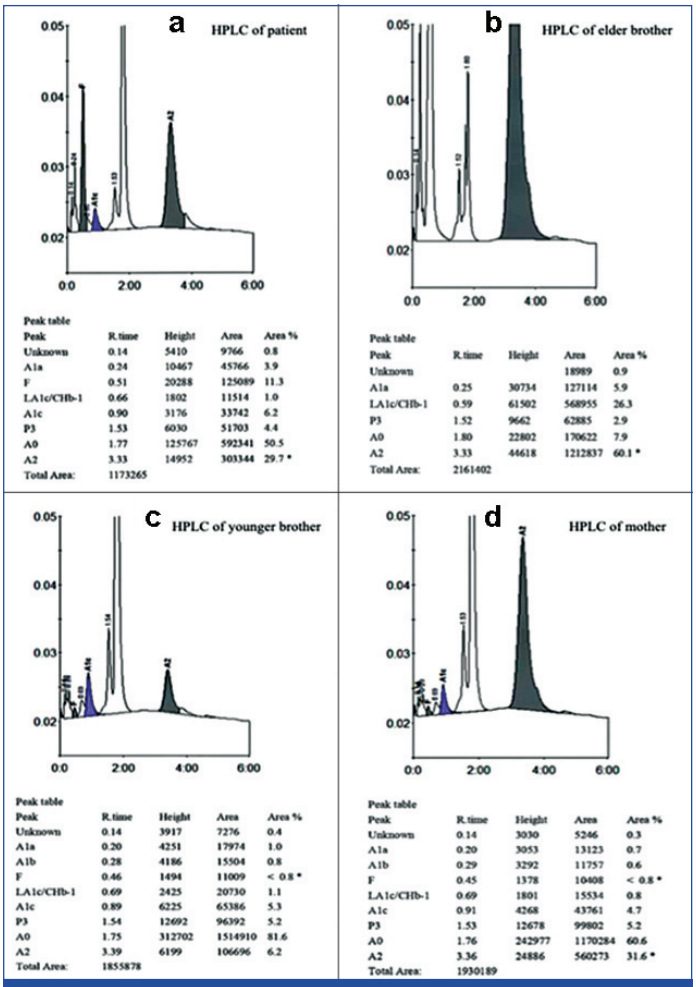
The HbE-beta thalassaemia (HbE/βT) is a rare haemoglobinopathy resulting from a mutation in the beta globin chain where at the 26th position, glutamic acid is replaced by lysine [1]. It has been estimated that in India, 0.37 per 1,000 foetuses have haemoglobinopathy. A large scale study analysing 119,336 cases

(c) HPLC findings				
HbA0 (%)	50.5	7.9	81.6	60.6
HbA2 (%)	29.7	60.1	6.2	31.6
HbF (%)	11.3	32.2	<0.8	<0.8

[Table/Fig-1]: Laboratory investigations of both the patient and the parents.



[Table/Fig-2]: a) Microphotograph of peripheral blood film of a case of patient showing anisopikilocytosis with microcytic hypochromic blood picture with target cells. (Leishman stain, 400X); b) Retic smear of patient (Retic stain, 1000X).



[Table/Fig-3]: Chromatograms of patient, elder brother, younger brother and mother: a,b) Compound heterozygous HbE-beta thalassaemia in patient and elder brother; c) Beta thalassaemia trait in younger brother; d) HbE of the mother.

over a decade reported the incidence of HbE/βT in the general population to be approximately 1.16% [2]. Despite its regional occurrence, the clinical variability and diagnostic challenges of compound heterozygous states like HbE/βT make it an under recognised and often misdiagnosed condition [3].

The HbE/βT accounts for approximately one half of all severe β thalassaemia cases worldwide [4]. The carrier frequency being highest in Southeast Asia and fall across the Indian subcontinent. In the Indian subcontinent, HbE is mostly restricted to North-Eastern states, i.e., West Bengal, Assam, Andhra Pradesh, Nagaland, Manipur, Tripura and Meghalaya with an average allele frequency of 10.9%. Increasing migration of population from high prevalence areas is resulting in rising prevalence in South and other parts of India [1]. Environmental and genetic variables both affect the severity and clinical course of anaemia in cases of HbE/βT. Its uniqueness lies in its phenotypic variability, ranging from a transfusion independent state to a severe, thalassaemia major like illness. This spectrum often misleads clinicians, delaying definitive diagnosis and hence appropriate management [5]. If unrecognised, this can lead to grave complications including iron overload, thrombotic episodes post splenectomy, hepatosplenomegaly and even heart failure [6]. This case report aims to spotlight an often overlooked yet clinically significant HbE/βT, a rare entity known for its diagnostic complexity and unpredictable clinical course.

In a retrospective study by Mondal SK and Mandal S et al., done over a period of twenty five years on 378 patients of HbE/βT revealed marked clinical variability ranging from mild asymptomatic anaemia to life threatening disease. At presentation, haemoglobin levels range from 3 to 13 gm/dL, with an average of 7.7 gm/dL. Mild form patients detected incidentally and do not need blood transfusions. A large number of individuals require frequent or sporadic blood transfusions due to moderate to severe anaemia, hepatosplenomegaly and growth retardation. Other causes of morbidity in these patients include infections, pericarditis, neurological problems, bone discomfort and iron overload that results in endocrinopathies [2].

In a 28-month prospective study conducted by Garje M et al., in 37 families, including 114 members who presented with anaemia, hepatosplenomegaly, positive sickling tests and/or elevated fetal haemoglobin, along with their family members. The study identified 11 (9.64%) cases of β-thalassaemia major, 16 cases (14.03%) sickle-β-thalassaemia, 7 (6.14%) cases each of sickle cell disease and Hb-E trait, four cases (3.50%) Hb-E β-thalassemia, two cases (1.75%) Hb lepore trait, 41 cases (35.96%) β thalassaemia trait, 20 cases (17.54%) sickle cell trait, and one case (0.87%) β-thalassaemia intermedia. They concluded that family studies play a crucial role in detecting asymptomatic carriers, enabling early identification of haemoglobin variants that may manifest later, and accurately diagnosing complex heterozygous cases in the absence of molecular testing. They are also essential for preventing the transmission of haemoglobinopathies through informed premarital and prenatal counselling. When paired with HPLC, community focused family screening can significantly contribute to reducing the overall disease burden [7].

In another retrospective study by Trivedi DP et al., in Jamnagar, 34 families were selected for study of haemoglobinopathies which comprises of total 120 family members. Out of 120 members, 100 members were found to have haemoglobinopathies. HPLC study and family study cases were classifed as: Group A Normal, Group B Sickle cell anaemia, Group C Sickle cell trait, Group D Sickle cell disease, Group E Beta thalassaemia major, Group F Beta thalassaemia trait, Group G Sickle beta thalassaemia, Group -H Beta thalassaemia major with Hb Variant HBH, Group -I Delta beta thalassaemia, Group J Hb D Trait, Group K HbF High for Age, Group L Beta thalassaemia major with Hb Lepore. Lowest Hb was found in Group E i.e. Beta thalassaemia major and highest level of Hb was found in Group C i.e., Sickle cell trait. The study highlight that HPLC based family studies are cost-effective, widely accessible, and reliable alternatives to genetic testing. They are crucial for identifying asymptomatic carriers, diagnosing late-presenting variants and complex heterozygotes and play a vital role in preventing the transmission of haemoglobinopathies through informed premarital and prenatal interventions [8].

In a study by Shekhar S et al., in Armed forces medical college, Pune, India, done on 300 blood samples analysed using HPLC, 158 (52.67%) showed variant haemoglobins while 142 (43.33%) were normal. Among the variants, the most common were S-β

thalassaemia (67 cases), sickle cell trait (39), and β-thalassaemia major (27) including two cases of HbE-βT. Notably, 59 of these variant cases had a history of consanguinity. Family studies were conducted in 94 cases involving parents or siblings, yielding conclusive diagnoses in 86, reinforcing the value of extended genetic evaluation [9].

Furthermore, in studies conducted by Agarwal R et al., and Brahmantyo M et al., cases of compound heterozygous haemoglobinopathies-Hb S/β-thalassaemia and HbE/β-thalassaemia respectively were reported. Interpretation in both studies were confounded [10,11]. In the former study there were borderline or near-normal haemoglobin variant values on HPLC and in the latter study there was a history of prior blood transfusion. In such scenarios, family studies proved invaluable, enabling a definitive diagnosis without the need for genetic testing.

CONCLUSION(S)

This case highlights the critical role of family studies in resolving diagnostic uncertainty, particularly when recent blood transfusions may interfere with laboratory results. It emphasizes the need for vigilance regarding compound heterozygous conditions and advocates for comprehensive diagnostic evaluations, including family analysis through HPLC. This report aims to enhance awareness and facilitate earlier identification of this complex and frequently misdiagnosed disorder.

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PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Pathology, BPS GMC(W), Sonepat, Haryana, India.
2. Professor and Head, Department of Pathology, KCGMC, Karnal, Haryana, India.
3. Senior Resident, Department of Pathology, KCGMC, Karnal, Haryana, India.
4. Professor, Department of Pathology, BPS GMC(W), Sonepat, Haryana, India.
5. Professor and Head, Department of Pathology, BPS GMC(W), Sonepat, Haryana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Parul,
Department of Pathology, KCGMC, Karnal, Haryana, India.
E-mail: parul.smgs@gmail.com

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