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COMPOUND HETEROZYGOUS HAEMOGLOBIN LEPORE/BETA THALASSEMIA PRESENTING AS NON-TRANSFUSION DEPENDENT THALASSEMIA: A BRIEF REPORT AND REVIEW OF LITERATURE WITH INDIAN PERSPECTIVE

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ABSTRACT

Haemoglobin Lepore is a rare structurally abnormal haemoglobin (Hb) resulting from δβ rearrangements. The compound heterozygous state of Hb Lepore/β-thalassemia may present either as β-thalassemia major or non-transfusion dependent thalassemia depending on the severity of mutation. A 2 years old boy presented to our center with fever, cough, coryza and easy fatigability for 3 months. His complete blood count revealed mild anemia and peripheral blood smear examination revealed microcytic hypochromic red blood cells, with moderate anisopoikilocytosis and nucleated RBCs, and left shift of leukocytes. High performance liquid chromatography suggested a possibility of compound heterozygous Hb Lepore/β thalassemia. Molecular studies showed father to be a carrier of beta thalassemia, and mother to be a carrier of Hb Lepore. Parents were advised for regular follow up. Compound heterozygous state of Hb Lepore/β-thalassemia in the Indian population seems to be rare due to few available case reports. If presenting as β-thalassemia major, the management guidelines can easily be followed. However, patients may present with non-transfusion dependent thalassemia as the present case, the management of which can be challenging, especially in the Indian set up. Regular follow up, early recognition of complications and avoidance of overtreatment are important in such patients.

KEYWORDS: Haemoglobin Lepore; β-thalassemia; Compound heterozygous Hb Lepore/β-thalassemia; Non-transfusion dependent thalassemia.

INTRODUCTION

Haemoglobin Lepore ($\alpha_2\delta\beta_2$) is abnormal haemoglobin resulting from a hybrid globin chain gene containing amino acid sequence similar to that in the carboxy-terminal end of human β globin chain and amino-terminal sequence of δ globin chain. This gene is resultant of unequal crossing over during meiosis.[1] This hybrid gene causes mild microcytic hypochromic anemia, however, homozygosity or compound heterozygosity in combination with β thalassemia gene gives rise to clinical features of thalassemia major or intermedia.[2] Analyzing the high performance liquid chromatography (HPLC) in a case having compound heterozygous Lepore haemoglobin can be challenging as well as is important. With this aim, we present a rare case of compound heterozygous Lepore haemoglobin/β thalassemia to understand the molecular, clinical and diagnostic aspects.

CASE REPORT

A 2 years old boy presented to our center with fever, cough, coryza and easy fatigability for 3 months. His birth and developmental history were unremarkable. On general examination, pallor was present with no lymphadenopathy.Systemic examination was also unremarkable with no evidence of hepatosplenomegaly. He was on hematinics therapy for 4 months prior to current presentation. His complete blood count (CBC) revealed mild anemia. Other parameters are enumerated in Table 1. Peripheral smear examination revealed microcytic hypochromic red blood cells (RBCs) and moderate to marked anisopoikilocytosis with the presence of leptocytes, elliptocytes, few tear drop cells and polychromatic cells (Fig 1). Also noted was leukoerythroblastosis with nucleated RBCs (20 nRBCs/100 leukocytes) and left shift of leukocytes. Taking into consideration of CBC and peripheral smear findings, HPLC was performed. HPLC revealed raised HbF levels (94.8%) and HbA_2 levels (6.9%) with the retention time of HbA_2 being 3.40 min (Fig 2). With this

shorter elution time of HbA_2 along with raised HbF levels compound heterozygous Hb Lepore/β thalassemia was the provisional diagnosis and hematological assessment of the boy's parents was advised. The hematological parameters of the parents are presented in Table 1. Further analysis by HPLC showed the father to be having β thalassemia trait and mother a case of heterozygous Hb Lepore (Fig 2). Molecular study was also performed to confirm the diagnosis. HBB gene sequencing of father revealed him to be a carrier of beta thalassemia, Gap polymerase chain reaction (PCR) of mother showed her to be a carrier of Hb Lepore deletion and that of the boy showed compound heterozygous state forHb Lepore/β thalassemia. At the time of presentation, the boy did not show any complications or developmental delay. Thus regular blood transfusion was not recommended and the parents were advised for regular follow up.

Table 1: Hematological parameters of the patient and his parents.

CBC Parameters	Patient	Father	Mother
Hb (g/dl)	11.1	12.2	9.6
TLC (X 10^9/l)	7.55	8.42	10.36
Platelet count (X10^9/l)	236	268	268
RDW (fl)	42.1	37.1	38.4
MCV (fl)	72.8	66.4	67.5
MCH (pg)	24.7	21.6	21.5
MCHC (gm/dl)	33.9	32.5	31.9
Corrected reticulocyte count (%)	2.5	0.74	1.24

CBC: Complete blood count; Hb: Hemoglobin; TLC: Total leukocyte count; RDW: Red cell distribution width; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration.

Table 2: Comparative study of reported Indian cases and the present case.

Parameters	Sreedharanunni et al	Nadkarni et al	Italia et al	Shaji et al		Present case
Number of cases of Hb Lep/β-thal reported	1	1	1	2		1
Age at diagnosis	4 years	9 months	6 months	4 years	6 months	2 years
Sex	Male	Male	Male	Female	Female	Male
Region/State	Bharauch, Uttar Pradesh	Madhya Pradesh	West Bengal	-	-	Bihar
Clinical presentation	Pallor, frontal bossing, mild icterus, hepato-splenomegaly	Anemia (transfusion dependent)	Anemia (transfusion dependent)	Jaundice, hepato-splenomegaly	Anemia (transfusion dependent)	Fever, cough, coryza, easy fatiguability, pallor
Number of transfusions	0	Regularly required	Regularly required	0	>50	0
RBC (10^{12}/L)	3.53	3.3	4.29	-	-	3.5
Hb (g/dL)	11.1	9.4	11.5	8.8	-	11.1
MCV (fL)	82.3	85.5	79.3	69.7	-	72.8
MCH (pg)	24.3	28.5	26.8	-	-	24.7
MCHC (g/ dL)	29.5	-	33.8	-	-	33.9
Reticulocyte count (%)	4.3	-	-	-	-	2.5
RDW (fL)	32.7	-	15.1	-	-	42.1
Hb Lepore +HbA_2 of case (HPLC)	0.7 %	-	-	-	-	6.9 %
HbF of case (HPLC)	92.4 %	1.7 %	0.6 %	97.6 %	-	94.8 %
HbA_0 of case (HPLC)	4.1 %	-	-	-	-	1.9 %
Father's Hb (g/dL)	14.3	12.4	10.3	13.4	12.3	12.2
Father's HbA_2 (HPLC)	9.9 %	16.4 %	-	14.6 %	-	5.6 %
Father's HbF (HPLC)	2.7 %	1.7 %	17.2 %	3.0 %	0.5 %	0.7 %
Father's HbA_0 (HPLC)	77.8 %	-	-	-	-	84.2 %
Mother's Hb (g/dL)	10.4	9.3	10.4	10.5	10.4	9.6
Mother's HbA_2 (HPLC)	4.6 %	-	-	-	12.3 %	10.5 %
Mother's HbF (HPLC)	0.8 %	1.0 %	0.4 %	0.7 %	2.4 %	2.0 %
Mother's HbA_0 (HPLC)	84.9 %	-	-	-	-	78.2 %
Molecular analysis of case	Hb Lepore-Hollandia/ β-thalassemia(IVS-1-5)	Hb Lepore-Hollandia/ β-thalassemia (IVS-1-5)	Hb Lepore-Boston-Washington/ β-thalassemia(IVS-1-5)	Hb Lepore-Boston-Washington/ β-thalassemia(IVS-1-5)	Hb Lepore-Boston-Washington/codon 30 (G→C)	Hb Lepore / β-thalassemia
Molecular analysis of father	Hb Lepore-Hollandia	Hb Lepore-Hollandia	HbE +Hb Lepore-Boston-Washington	-	-	Heterozygous β^+-thalassemia
Molecular analysis of mother	Heterozygous mutation (IVS-1-5) β^+-thalassemia	Hetero-zygous mutation (IVS-1-5) β^+-thalassemia	Hetero-zygous mutation (IVS-1-5) β^+-thalassemia	-	-	Heterozygous Hb Lepore

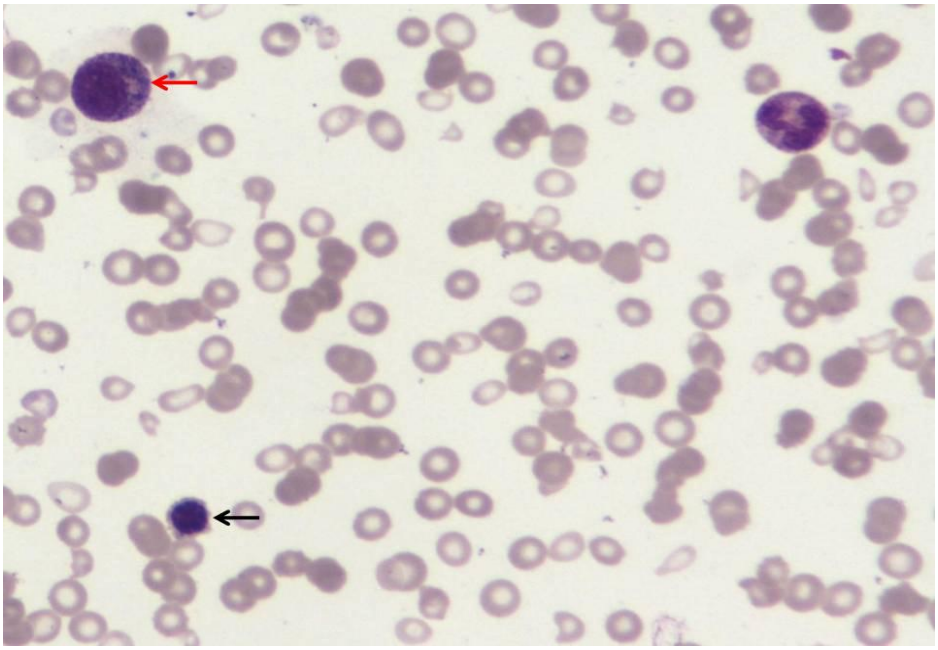

Figure 1: Wright stained peripheral smear showing leukoerythroblastic blood picture with shift to left (red arrow) and nucleated RBC (black arrow) (x1000).

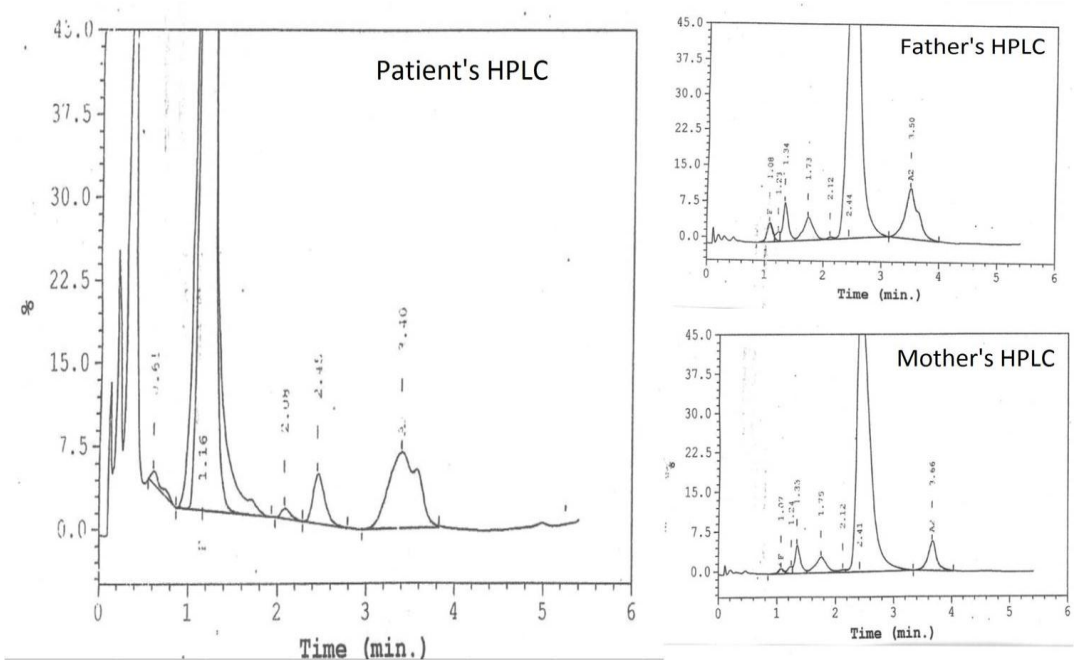

Figure 2: High performance liquid chromatography of patient and his parents.

DISCUSSION

Haemoglobin Lepore is a rare structurally abnormal haemoglobin (Hb) resulting from unequal crossing over during meiosis causing deletion of approximately 7.4 kb.[1] Based on the deletion breakpoints ten different δβ rearrangements are known of which Hb Lepore–Washington–Boston, Hb Lepore-Baltimore, and Hb Lepore-Hollandia are more common.[3] The compound heterozygous state of Hb Lepore/β-thalassemia usually produces Hb Lepore levels ranging 5-15%.[4] Cases with heterozygosis of Hb Lepore are usually clinically healthy, with the hematological picture of mild microcytic hypochromic anemia almost similar to that of β thalassemia. In Hb Lepore, δβ hybrid chains are synthesized in lesser quantities as compared to β chains with an overall reduction in non-α globin chains and a clinical phenotype of mild β-thalassemia.[5] However, clinical presentation of Hb Lepore/β-thalassemia patients varies widely depending on the type of β chain mutation.[6] Patients with severe β-thalassemia mutations present as thalassemia major requiring regular blood transfusions while those with mild β-thalassemia mutations have the clinical phenotype of thalassemia intermedia presenting as non-transfusion dependent thalassemias (NTDT) as seen in the present case.

NTDT are cases in which regular blood transfusions are not required except for instances like infection, pregnancy, surgery or developmental delay. β-thalassemia intermedia, haemoglobin E (HbE) β-thalassemia, HbH disease, HbS β-Thalassemia and HbC Thalassemia are the possible causes.[7] Compound heterozygosity for Hb Lepore and β-thalassemia is a rarer and an unusual cause of NTDT. It is essential to recognize NTDT in order to avoid life-long transfusion therapy to the affected children. Intercurrent infections in children may aggravate the underlying anemia transiently and may give a false picture of a requirement of regular blood transfusion.[7] Hb Lepore has also been found to be coinherited with HbS, HbC and HbE, with variable clinical phenotypes.[5,8,9]

A presumptive diagnosis of Hb Lepore can be made on citrate agar electrophoresis (CAE), capillary electrophoresis (CE) and HPLC.[4] In HPLC with the VARIANT™ Haemoglobin Testing System (Bio-Rad Laboratories), Hb Lepore elutes in the region of HbA_2 as a broad-based peak of 10.0–15.0% in heterozygotes.[3] A useful indicator helping identify Hb Lepore (without any compound heterozygosity) is the shorter retention time of this Hb (3.34-3.42 min) in comparison to that of HbA_2 (3.59–3.65 min) giving rise to lesser or no gap between HbA and HbA_2 on HPLC.[2] Separation from HbA_2 is facilitated by reversed phase HPLC.[10] However, in Hb Lepore/β-thalassemia, HbA_2 and Hb Lepore get separated as low level of Hb Lepore does not merge with HbA_2.[11] In all heterozygotes for Hb Lepore, HbF levels are raised, the increase being higher in Hb Lepore Baltimore than in Hb Lepore Washington-Boston. Possible association of Hb Lepore Baltimore with $XmnIG_\gamma$ (C→T) polymorphism is the cause for this relative increase in HbF.[3] Hb Lepore is confirmed by gap-Polymerase Chain Reaction (PCR) and multiplex ligation-dependent probe amplification (MLPA) technique.[10] The variants of Hb Lepore are identified by multiplex-PCR and gene sequencing.[12] Parents can opt for prenatal diagnosis in cases where their first child might have moderate to severe anemia. In such cases, HPLC analysis of parents may not always turn out to be sacrosanct. Before undertaking chorionic villous sampling (CVS), further confirmation of parents' status by DNA analysis is an important step. Instances, where there is a mismatch between findings of HPLC and DNA analysis, further extended family studies of couples referred for the interventional procedure, is important.[13] Thus, the hematological, biochemical and molecular correlation becomes important in such cases to avoid misdiagnosis possible out of mere HPLC.

Literature review in Indian context shows only few case reports with compound heterozygosity for Hb Lepore and β-thalassemia. Sreedharanunni et al reported a case of Hb Lepore/β-thalassemia presenting as β-thalassemia intermedia (βTI).[10] Nadkarni et al reported one such case out of eight cases of Hb Lepore-Hollandia presenting as β-thalassemia major (βTM).[3] Shaji et al reported two cases of compound heterozygous Hb Lepore/β-thalassemia out of 320 patients of thalassemia, one of whom presented with βTI while the other presented with βTM.[11] Italia et al reported one case of Hb Lepore/β-thalassemia out of three compound heterozygous Hb Lepore cases (the other being Hb Lepore/HbE) who were clinically managed as βTM.[13] Table 2 presents clinical, hematological and molecular details of these cases vis-a-vis the present case for a better analysis. As can be seen, with the few reported cases it might be inferred that compound heterozygosity for Hb Lepore/β-thalassemia is rarer in the Indian population. However, with the possibility of misdiagnosis by taking into consideration of findings of only HPLC and rare availability of molecular studies, the probability of missing such cases can also not be ruled out.

Compound heterozygous Hb Lepore/β-thalassemia patients, as discussed above may present with βTM or as NTDT. The present case was clinically a patient of NTDT. Although patients with NTDT are not dependent on transfusion therapy, many complications have been documented and hence early recognition and timely management of these by regular follow up is important. Ineffective erythropoiesis and expansion of erythron in the bone marrow lead to osteoporosis and bone deformities while the proliferation of erythron in the liver and spleen causes hepatosplenomegaly. Extramedullary hematopoiesis other than liver and spleen causes pseudotumors which are more commonly associated with NTDT than transfusion dependent anemias.[14] Hypercoagulability and thrombotic vascular disease are more relevant to NTDT patients, with the prevalence reaching upto 20% as compared to less than 1% in cases of βTM.[14] Apart from overt and silent cerebral ischemic diseases, pulmonary hypertension is specifically documented to be more prevalent in NTDT patients than that seen in patients with βTM.[15,16] Although slower than that observed in transfusion related iron overload, increased intestinal absorption of iron in NTDT may still cause clinically significant siderosis. Involvement of liver is more pertinent in NTDT patients with documented reports of hepatic fibrosis and even hepatocellular carcinoma.[14]

Recent studies have highlighted management of NTDT. Blood transfusions may be required in acute states as mentioned earlier. Thus hasty decision on transfusion requirements without adequate follow up may lead to unnecessary iron overload which would add on to already existing siderosis.[7] Hepatic iron overload can be prevented by iron chelation therapy. However, due to slower iron absorption and hence slower rate of iron overload, iron-related morbidities are unusual under ten years of age. Therefore, iron chelators are indicated from ten years and above.[14] The present case being 2 years of age has not yet been recommended for the iron chelation therapy. Deferasirox has been clinically tried in NTDT patients and has also received approval from the US

Food and Drug Administration (FDA).[17] Hydroxyurea not only induces fetal haemoglobin production but may also improve the associated hypercoagulable state.[18] Newer modalities being developed are JAK2 inhibitors, hepcidin modulators, and transferrin.[14]

CONCLUSION

Compound heterozygous state of Hb Lepore/β-thalassemia in the Indian population seems to be rare due to few available case reports. However, its existence can easily be missed and misdiagnosed if complete workup including those involving the family, is not performed. If presenting as β-thalassemia major, the management guidelines can easily be followed. However, patients may present with non-transfusion dependent thalassemia as the present case, the management of which can be challenging, especially in the Indian set up. Regular follow up, early recognition of complications and avoidance of overtreatment (transfusion therapy) are pertinent in such patients.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

REFERENCES

1. Weatherall DJ, Clegg JB**.** The Thalassaemia Syndromes: Wiley, 2008.
2. Chaibunruang A, Srivorakun H, Fucharoen S, Fucharoen G, Sae-ung N, Sanchaisuriya K**.** Interactions of hemoglobin Lepore (deltabeta hybrid hemoglobin) with various hemoglobinopathies: A molecular and hematological characteristics and differential diagnosis. Blood Cells Mol Dis, 2010; 44: 140-5.
3. Nadkarni A, Italia K, Sawant P, Ghosh K, Colah R**.** Hemoglobin Lepore Hollandia in India. Int J Lab Hematol, 2012; 34: 148-53.
4. Efremov GD**.** Hemoglobins Lepore and anti-Lepore. Hemoglobin, 1978; 2: 197-233.
5. Viprakasit V, Pung-Amritt P, Suwanthon L, Clark K, Tanphaichitr VS**.** Complex interactions of δβ hybrid haemoglobin (Hb Lepore-Hollandia) Hb E (β26 G→A) and α+ thalassaemia in a Thai family. Eur J Haematol, 2002; 68: 107-11.
6. Efremov DG, Efremov GD, Zisovski N, Stojanovski N, Kutlar F, Diaz-Chico JC, et al. Variation in clinical severity among patients with Hb Lepore-Boston-beta-thalassaemia is related to the type of beta-thalassaemia. Br J Haematol, 1988; 68: 351-5.
7. Weatherall DJ**.** The definition and epidemiology of non-transfusion-dependent thalassemia. Blood Rev, 2012; 26(Suppl 1): S3-6.
8. Huisman TH. Compound heterozygosity for Hb S and the hybrid HbS Lepore, P-Nilotic, and Kenya; comparison of hematological and hemoglobin composition data. Hemoglobin, 1997; 21: 249-57.
9. Schoentag R, Pedersen J, Ballard H**.** Double heterozygosity for hemoglobins C and Lepore in an American black man. Arch Pathol Lab Med, 1985; 109: 777-9.
10. Sreedharanunni S, Chhabra S, Hira JK, Bansal D, Sharma P, Das R**.** beta-Thalassemia Intermedia caused by compound heterozygosity for Hb Lepore-Hollandia and beta-Thalassemia is rare in the Indian population. Hemoglobin, 2015; 39: 362-5.
11. Shaji RV, Edison ES, Krishnamoorthy R, Chandy M, Srivastava A**.** Hb Lepore in the Indian population. Hemoglobin, 2003; 27: 7-14.
12. Nussenzveig RH, Vanhille DL, Hussey D, Reading NS, Agarwal AM. Development of a rapid multiplex PCR assay for identification of the three common Hemoglobin-Lepore variants (Boston-Washington, Baltimore, and Hollandia) and identification of a new Lepore variant. Am J Hematol, 2012; 87: E74-5.
13. Italia K, Sheth J, Sawant P, Nadkarni A, Ghosh K, Colah R**.** Prenatal diagnosis of HbE-Lepore and Hb Lepore-beta-thalassemia: the importance of accurate genotyping of the couple at risk. Prenat Diagn, 2012; 32: 703-7.
14. Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA**.** Non-transfusion-dependent thalassemias. Haematologica, 2013; 98: 833-44.
15. Musallam KM, Taher AT, Karimi M, Rachmilewitz EA**.** Cerebral infarction in beta-thalassemia intermedia: breaking the silence. Thromb Res, 2012; 130: 695-702.
16. Farmakis D, Aessopos A**.** Pulmonary hypertension associated with hemoglobinopathies: prevalent but overlooked. Circulation, 2011; 123: 1227-32.
17. Taher AT, Porter J, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, et al. Deferasirox reduces iron overload significantly in nontransfusion-dependent thalassemia: 1-year results from a prospective, randomized, double-blind, placebo-controlled study. Blood, 2012; 120: 970-7.
18. Singer ST, Vichinsky EP, Larkin S, Olivieri N, Sweeters N, Kuypers FA**.** Hydroxycarbamide-induced changes in E/beta thalassemia red blood cells. Am J Hematol, 2008; 83: 842-5.