

CASE REPORT

Hb Lepore/ β^0 -thalassaemia with α^+ -thalassaemia interactions, a potential diagnostic pitfall

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Abstract

Haemoglobin (Hb) Lepore is a variant Hb consisting of two α -globin and two $\delta\beta$ -globin chains. In a heterozygote, it is associated with clinical findings of thalassaemia minor, but interactions with other haemoglobinopathies can lead to various clinical phenotypes and pose diagnostic challenges. We reported a pair of siblings from a Malay family, who presented with pallor and hepatosplenomegaly at the ages of 21 months and 14 months old. The red cell indices and peripheral blood smears of both patients showed features of thalassaemia intermedia. Other laboratory investigations of the patients showed conflicting results. However, laboratory investigation results of the parents had led to a presumptive diagnosis of compound heterozygote Hb Lepore/ β -thalassaemia and co-inheritance α^+ -thalassaemia ($-\alpha^{3.7}$). Hb Lepore has rarely been detected in Southeast Asian countries, particularly in Malaysia. These two cases highlight the importance of family studies for accurate diagnosis, hence appropriate clinical management and genetic counseling.

Keywords: Hb Lepore, β -thalassaemia, α^+ -thalassaemia

INTRODUCTION

Haemoglobin (Hb) Lepore [$\alpha_2(\delta\beta)_2$] is a structurally abnormal Hb, first described by Gerald and Diamond in 1958, that results from a fusion between a δ and a β globin gene during meiosis.¹ The hybrid gene is formed by the fusion of 5' end of the δ globin gene and the 3' end of the β globin gene. This fusion leads to a 7.4-kb deletion between the δ and β globin genes.² Three Hb Lepore variants have been identified, each with a different crossover breakpoint: Hb Lepore Washington Boston ($\delta 87/\beta 116$), Hb Lepore Hollandia ($\delta 22/\beta 50$) and Hb Lepore Baltimore ($\delta 50/\beta 86$).³ Hb Lepore Washington Boston is the most common variant and occurs with a low frequency in a variety of ethnic groups, mainly in Mediterranean countries. Hb Lepore

Baltimore was first described in one family with Black ancestry from Baltimore and afterwards in Yugoslavia, Spain and Northern Sardinia.² Hb Lepore Hollandia is a rare variant and has been found in New Guinea, Bangladesh and Thailand.⁴ In all three variants, the synthesis of these $\delta\beta$ hybrid chains is substantially less than that of the β chain, resulting in an overall reduction in the non- α globin chains and having clinically a β -thalassaemia-like condition.⁵

Hb Lepore has rarely been detected in Malaysia and other Southeast Asian countries, although sporadic cases of Hb Lepore heterozygotes and compound heterozygote for Hb Lepore/Hb E have been reported in Thailand and Malaysia.^{6,7} Laboratory diagnosis of the carrier state of Hb Lepore is rarely a problem since the combination of cellulose acetate at alkaline pH and HPLC are

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able to give a presumptive diagnosis. On cellulose acetate, the variant Hb will produce a faint band at the S region, while on HPLC it co-elutes with HbA₂ giving a combined level of less than 15%.⁴ However, in compound heterozygous states, the diagnosis may not be apparent as the variant Hb is not easily detectable due to a much lower level Hb Lepore being produced. We report two siblings from a Malay family with compound heterozygote β-thalassaemia and Hb Lepore with co-inheritance α⁺ thalassaemia (-α^{3,7}), who presented with thalassaemia intermedia, highlighting the challenges encountered during the diagnosis of such cases.

CASE REPORT

This report is of a pair of Malay siblings, a girl aged four years and five months and her younger brother aged one year and two months, who presented with pallor and hepatosplenomegaly.

Proband 1 is a 4-year 5-month-old girl who first presented with fever at the age of 21 months and was found to have pallor and hepatosplenomegaly (liver was 2 cm below the right subcostal margin and spleen 3 cm below the left subcostal margin). Her haematology profile showed a Hb level of 67 g/L, red cell count 3.12 x 10¹²/L, MCV 69.7 fL and MCH 23.4 pg (Table 1) using LH750 (Beckman Coulter; Miami, FL, USA).

The white blood cells and platelet counts were normal. The patient’s peripheral blood smear showed hypochromia, microcytosis, anisopoikilocytosis, polychromasia with some target cells and a few nucleated red blood

cells. Hb electrophoresis on cellulose acetate at alkaline pH (pH8.6) showed a dense band at F region. Quantification of Hb F by high-performance liquid chromatography (HPLC) using a Bio-Rad Variant Haemoglobin Testing system with β-thalassaemia Short Programme (Bio-Rad Laboratories, Hercules, CA, USA) revealed a level of 88.6% and a borderline Hb A₂ level (3.5%).

Proband 2, a younger brother of proband 1, was brought to medical attention at the age of 14 months for thalassaemia screening as part of the family study. He was also noted to be pale. Physical examination revealed mild hepatosplenomegaly. His full blood counts showed a Hb of level 63 g/L, red cell count 3.32 x 10¹²/L, MCV 62.7 fL and MCH 18.8 pg. The peripheral blood film showed hypochromia, microcytosis, severe anisopoikilocytosis, polychromasia with some target cells and nucleated red blood cells (Fig. 1). Hb electrophoresis on cellulose acetate at an alkaline pH (pH 8.6) showed a prominent band at F region (Fig. 2). Hb quantification by HPLC revealed Hb F level of 92.9% and Hb A₂ level of 5.0%. There was 3.8% unknown Hb at retention time (RT) of 3.62 minutes (Fig. not shown). Further testing by the capillary zone electrophoresis (CE) system (Sebia, Inc., Norcross, Ga) showed presence of Hb variant peak in zone 6 (3.9%) (Fig. 3). DNA analysis for the α-globin genotype revealed a single deletional α-gene mutation for both probands (αα/-α^{3,7}).

Their father’s full blood count showed a Hb of 133 g/L, red cell count 5.94 x 10¹²/L, MCV

TABLE 1: The haematology parameters and laboratory results of the probands and their parents

	Father	Mother	Proband 1	Proband 2
β-genotype	β/β ^{IVS1-5}	β/β ^L	β ^{IVS1-5} /β ^L	β ^{IVS1-5} /β ^L
α-genotype	αα/-α ^{3,7}	αα/αα	αα/-α ^{3,7}	αα/-α ^{3,7}
Rbc (x10 ¹² /L)	5.94	5.40	3.12	3.32
Hb (g/L)	133	114	67	63
MCV (fl)	71.2	67.9	69.7	62.7
MCH (pg)	22.4	21.0	23.4	18.8
HbA ₂ /variant (%) (HPLC)	Not available	11.3	3.5	5.0
HbF (%) (HPLC)	Not available	3.7	88.6	92.9
Unknown (%) (HPLC)	Not available	–	–	3.8
HbA ₂ (%) (CE)	5.1	2.4	Not available	3.0
HbF (%) (CE)	<1.0	4.3	Not available	90.9
Hb Lepore (%) (CE)	–	9.4	Not available	3.9

*note: β^L =beta lepore gene, β^{IVS1-5}=beta gene with IVS1-5 mutation, HPLC=high performance liquid chromatography, CE=capillary electrophoresis

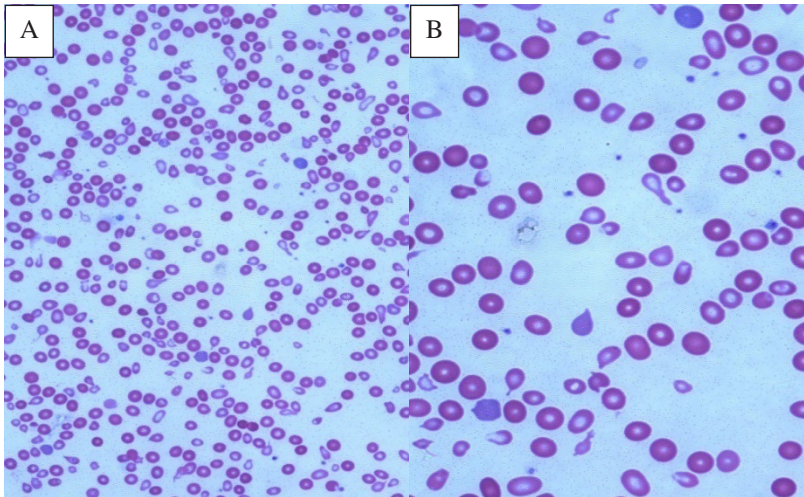


FIG 1: Peripheral blood film of proband 2 showing hypochromia, microcytosis, anisopoikilocytosis and polychromasia. Wright stain (A) x200, (B) x400.

71.2 fL, MCH 22.4 pg, and peripheral blood smear revealed hypochromic microcytic picture, mild anisopoikilocytosis with occasional pencil cells and target cells. His Hb electrophoresis on cellulose acetate (pH 8.6) and CE were consistent with the diagnosis of β -thalassaemia trait. DNA analysis for the α -globin genotype revealed a single deletional α -gene mutation ($\alpha\alpha/\alpha^{3.7}$).

The mother's Hb was 114 g/L, red cell count $5.40 \times 10^{12}/L$, MCV 67.9 fL, MCH 21.0 pg and the peripheral blood smear showed anisopoikilocytosis with hypochromic microcytic red cells and presence of ovalocytes, target cells as well as occasional fragmented red cells. Her Hb electrophoresis on cellulose acetate at alkaline pH showed a band at the S region (Fig. 2), while electrophoresis on citrate agar

at acid pH excluded HbS. HPLC of the mother revealed 11.3% of an abnormal Hb that co-eluted with Hb A2 at the retention time of 3.48 minutes (Fig. 4a) while on the CE, there was a 9.4% variant Hb peak at zone 6 with a slightly raised Hb F level of 4.3% (Fig. 4b). These findings were consistent with Hb Lepore trait. The mother had a normal α genotype.

In view of the Hb analysis results of both parents, a presumptive diagnosis of compound heterozygous Hb Lepore with β -thalassaemia and α^+ -thalassaemia were made for both siblings. However, contradictory to the presumptive diagnosis, the results of the DNA sequencing of the siblings showed homozygous IVS1-5 β -globin gene mutation. The mother, a Hb Lepore carrier was shown to only carry the



FIG. 2: Cellulose acetate haemoglobin electrophoresis at alkaline pH (8.6). The mother exhibited a discrete band at the S region, while proband 2 had only Hb F band visible. The father is the classical β -thalassaemia carrier with a mildly raised HbA₂ level.

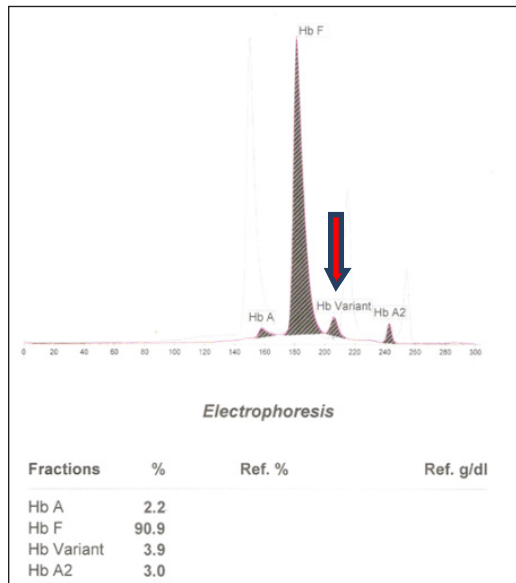


FIG. 3: Capillary zone electrophoresis (CE) of proband 2 showed presence of Hb variant peak in zone 6 (3.9%) (↓) and Hb F level of 90.9%.

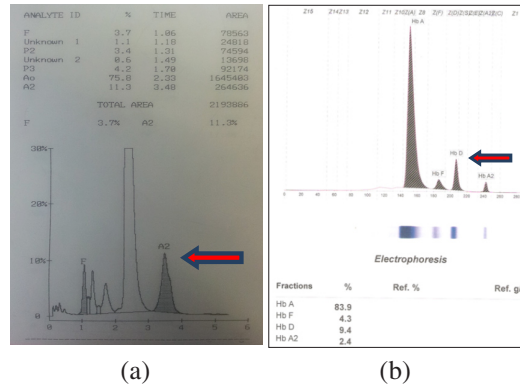
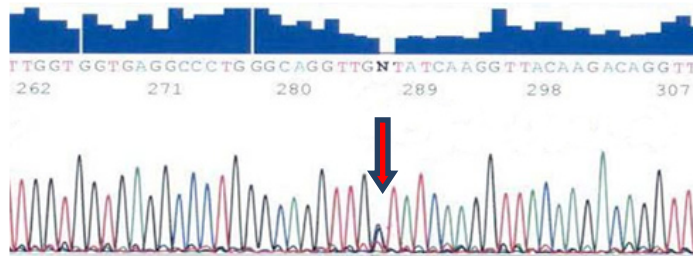
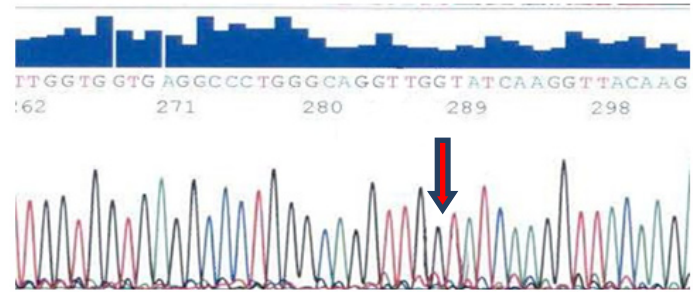


FIG. 4: Mother's results (a) HPLC revealed 11.3% of an abnormal Hb (Hb Lepore) that co-eluted with Hb A2 (RT=3.48 min) (←). (b) Capillary electrophoresis of the mother showed presence of abnormal peak (Hb Lepore) at zone 6 (9.4%) (←) with a mildly raised Hb F level of 4.3%.

Father:
Peaks G & C
(IVS1-5 Heterozygous)



Mother:
Only Peak G
(Wild type)



Patient:
Only Peak C
(Appear as
IVS1-5 Homozygous)

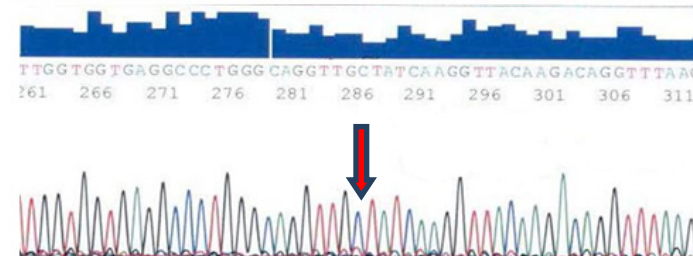


FIG. 5: DNA sequencing results of β -globin gene flanking the IVS 1-5 region of the father, mother and proband 2, highlighted by the arrows (↓).

wild-type alleles for β -globin gene. The father, as expected, showed IVS1-5 β -globin mutation in a heterozygous state (Fig. 5). All the laboratory results are summarized in Table 1.

Both patients behaved phenotypically major, requiring monthly transfusion.

DISCUSSION

Heterozygotes of Hb Lepore are healthy individuals who usually present with mild anaemia, hypochromia and microcytosis, similar to that of β -thalassaemia trait. The mean cell Hb (MCH) is thought to be the most reliable parameter in the Hb Lepore heterozygotes (range 20-25 pg).⁶ The mother of the probands in our cases, a Hb Lepore carrier, was asymptomatic with the Hb level of 11.4 g/dl and MCH of 21.0 pg.

Hb Lepore shows electrophoretic mobility similar to Hb S on cellulose acetate electrophoresis at alkaline pH.⁴ The other Hbs that run in this position are Hb D and Hb G, which are differentiated from Hb S by sickle solubility test and Hb electrophoresis at acid pH. On electrophoresis at acid pH, Hb Lepore migrates at a similar position to Hb A. On HPLC analysis with the Bio-Rad Variant Hb testing system, Hb Lepore has a similar retention time (RT) to Hb A₂ and Hb E. In a study by Chaibunruang *et al*, Hb Lepore was identified as having a shorter retention time on HPLC, which elutes at 3.34-3.42 minute whilst that of Hb A₂ is 3.59-3.65 minutes, therefore, appearing as a single peak with a small shoulder in the HbA₂ window.⁷

Diagnosis of the carrier state is rarely complicated as was demonstrated by the mother's result (Fig. 3a and 4a). The use of capillary zone electrophoresis system further helped to establish the presumptive diagnosis, since this Hb variant is clearly separated as a peak in zone 6.⁷ However, DNA analysis is still required for confirmation.

In Hb Lepore heterozygotes, Hb electrophoresis shows 5 to 15% of Hb Lepore, with HbA₂ being reduced to about half of the normal level.⁴ The percentage of Hb Lepore Baltimore in heterozygotes is slightly but significantly higher than the percentage of Hb Lepore Washington Boston. Hb F is sometimes mildly increased. This may be because of linkage to a polymorphism that determines Hb F percentage.⁸ Viprakasit *et al* also reported that Hb F can be increased (1 to 3%) in Hb Lepore heterozygotes. It has been postulated that the deletion which resulted

in the $\delta\beta$ fusion is somehow responsible for increasing the absolute output of γ - chains. This is compatible with the observation that Hb Lepore heterozygotes produce significantly more Hb F than β -thalassaemia heterozygotes.⁶

In homozygous or compound heterozygous states with other thalassaemias or haemoglobinopathies including β -thalassaemia, Hb S and Hb E, the patients' phenotypes can be a variation from mild thalassaemia intermedia to thalassaemia major.⁷ The haematological findings in these patients are peripheral blood film features, indistinguishable from the severe forms of β -thalassaemia. Bone marrow smears may show marked erythroid hyperplasia due to high level of erythropoietin production in response to the anaemia. Individuals with thalassaemia intermedia present later than thalassaemia major, have milder anaemia and by definition do not or only occasionally require transfusion. At the severe end of the clinical spectrum, patients present between the ages of two and six-years-old old, and although they are capable of surviving without a regular blood transfusion, growth and development are retarded. On the other hand, there are patients who are completely asymptomatic until adult life with only mild anaemia.⁹ As for the probands in our cases, both patients behaved phenotypically major. They presented in early childhood at the age of one to two years old. They required two to three monthly transfusions at the initial period. Proband 1 had pre transfusion haemoglobin of less than 7g/dL at monthly follow up and proband 2 had failure to thrive. Thus, both patients received monthly transfusion subsequently.

Diagnosis of such interactions may sometimes be challenging, especially if only one identification method is used. The cellulose acetate electrophoresis of the probands showed only prominent Hb F band, similar to the finding in homozygous β -thalassaemia cases. Hb Lepore band was totally undetected. Fortunately, the CE was able to detect the presence of a low level of Hb Lepore in our case.

In homozygous states, Hb A and Hb A₂ are absent and the Hb is made up of Hb F and Hb Lepore only. The level of Hb Lepore ranges from 8 to 30% with a mean value of approximately 15%, the remainder of the Hb being Hb F.⁵

The compound heterozygous state of Hb Lepore/ β -thalassaemia usually produces Hb Lepore levels varying from 5 to 15%.¹⁰ The author described two cases of compound heterozygotes Hb Lepore/ β -thalassaemia with

different phenotypes of thalassaemia. One had thalassaemia intermedia, while the other had thalassaemia major. The difference in phenotypes in these two patients might be due to the types of β -thalassaemia mutations, the fusion genes, and their associated haplotypes. The one with thalassaemia intermedia was a compound heterozygote for Hb Lepore Hollandia and IVS-1-5 (G \rightarrow C) mutation, with 1.4% Hb Lepore, 1.0% Hb A2 and 97.6% Hb F levels. The other, who had thalassaemia major, was a compound heterozygote for Hb Lepore Washington Boston and codon 30 (G \rightarrow C) mutation.¹⁰

Viprakasit *et al* reported a case of Hb Lepore/Hb E interaction which also posed a diagnostic challenge similar to our cases. The initial diagnosis of Hb E/ β -thalassaemia was made based on the initial chromatography results. However, contradictory to the earlier results, the DNA analysis showed homozygosity for codon 26 G>A mutation. They hypothesized that the probands had inherited a large deletion which removed a region within the β -globin gene, resulting in amplification from only a single allele carrying the codon 26 G>A mutation. They further confirmed the presence of $\delta\beta$ -thalassaemia gene deletion using GAP-PCR.⁶ Similarly, we also postulated that the large deletion involving $\delta\beta$ -globin gene had resulted in amplification from one allele carrying the IVS 1-5 point mutation, leading to the inaccurate conclusion of homozygosity for IVS 1-5 β -globin point mutation, confusing the earlier diagnosis. Even the proband's mother was unable to show her carrier state of Hb Lepore gene by the molecular analysis performed.

The association of Hb Lepore and α^+ -thalassaemia (3.7 kb deletion) has so far been rarely reported. Patients with this genotype had a very similar haematological phenotype to that of the Hb Lepore heterozygote.⁷ The co-existence of α^+ -thalassaemia and Hb Lepore did not further contribute to the severity of the patients' symptoms. The reason for the low level of Hb Lepore (3.9%) observed in our case might have been due to the co-inheritance of α -thalassaemia.¹⁰

In conclusion, a possible misdiagnosis of Hb Lepore compound heterozygote states with other haemoglobinopathies could occur in a routine setting. However, an accurate diagnosis could be reached with the help of family studies and a careful scrutiny of the patients' history and various laboratory results.

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