Diagnostic challenges in compound heterozygous Hb Lepore/ β thalassaemia presenting as thalassaemia major

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Introduction

Haemoglobin (Hb) Lepore [α2 (δβ) 2] is a structurally abnormal Hb, resulting from a fusion between a δ and a β globin gene during meiosis. 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. Three Hb Lepore variants have been identified, each with a different crossover breakpoint: Hb Lepore Washington Boston (δ87/β116), Hb Lepore Hollandia (δ22/β50) and Hb Lepore Baltimore (δ50/β86). Hb Lepore Washington Boston is the most common variant. In all three variants, synthesis of these δβ hybrid chains is substantially less than that of the normal β chain[1,2,3,4]. Hb Lepore occurs with a low frequency in a variety of ethnic groups, mainly in Mediterranean countries. It has rarely been detected in Southeast Asian countries[1]. Laboratory diagnosis of the carrier state of Hb Lepore is rarely a problem since HPLC is able to give a presumptive diagnosis. On HPLC, Hb Lepore co-elutes with HbA2 giving a combined level of less than 15%. 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[1,3,4]. We report an infant from a Sri Lankan family with Tamil ethnicity with compound heterozygote Hb Lepore and β-thalassaemia, and a clinical picture of transfusion dependent thalassaemia (TDT), highlighting the challenges in diagnosis.

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Case report

A five month old baby girl, the first baby of non-consanguineous parents, presented with severe pallor, hepatomegaly and splenomegaly. Her full blood count (FBC) revealed a Hb of 2.9g/dl, mean corpuscular volume (MCV) of 69.3fl, mean corpuscular haemoglobin (MCH) of 22.3pg and a mean corpuscular haemoglobin concentration (MCHC) of 32.2g/dl. Her reticulocyte count was elevated (18.8%). Serum ferritin was 171 ng/ml. Blood picture showed evidence of chronic haemolysis (Figure 1). Based on the clinical picture and preliminary haematology, the baby was suspected of having TDT. The baby was transfused with ABO and Rh compatible leuco-reduced blood to correct anaemia after sending samples for extended red cell phenotyping and high performance liquid chromatography (HPLC).

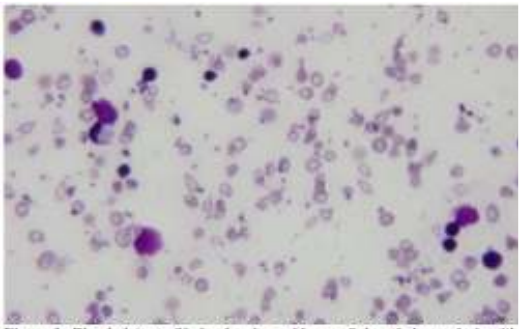

Figure 1: *Blood picture of baby showing evidence of chronic haemolysis with many polychromatic red cells, anisopoikilocytosis and nucleated red cells*

HPLC of the baby using a Bio-Rad Variant Hb Testing system with 'β-thalassaemia short programme' revealed raised Hb F (87.9%) and Hb A2 (9.9%). The retention time of Hb A2 was at 3.47 with a small shoulder at Hb A2 window. As there was a small shoulder at Hb A2 window, the presence of a variant Hb was suspected; capillary electrophoresis (Sebia) was performed, which showed raised Hb F (94.3%) and the presence of a variant Hb at zone 6 (4.5%) and Hb A2 of 1.2% (Figure 2).

Parental screening was arranged later which showed the father was a carrier for Hb Lepore and the mother was a carrier for β thalassaemia. Both were asymptomatic and not screened for thalassaemia before (Table 1 and Figure 3).

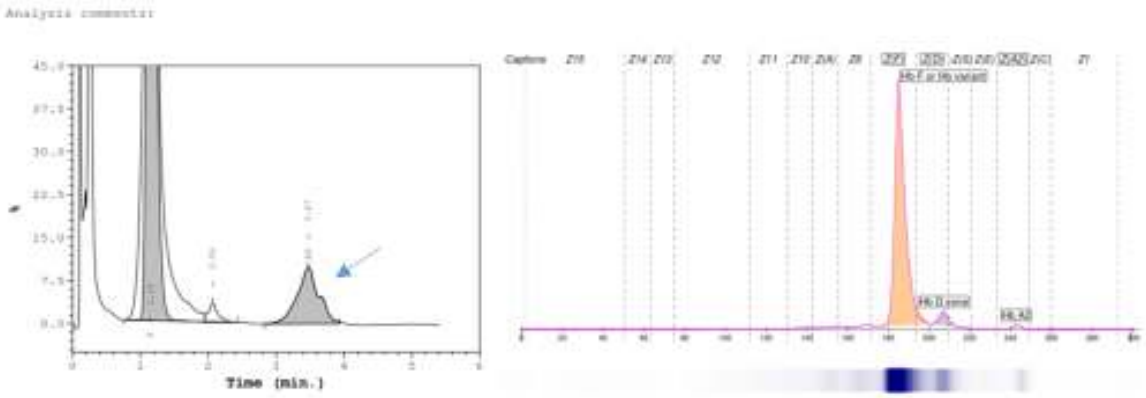

Figure 2: *HPLC and CE tracings of the baby*

HPLC: high performance liquid chromatography, CE: capillary electrophoresis

Table 1: *FBC / HPLC /CE parameters of the proband, father and mother*

FBC / HPLC / CE parameters	Proband	Father	Mother
Red blood cell count	1.30 x 10^6 /µL	6.01 x 10^6 /µL	5.27 x 10^6 /µL
Haemoglobin level	2.9g/dL	13.4g/dL	10.1g/dL
Mean corpuscular volume	69.3fl	83.0fl	58.0fl
Mean corpuscular haemoglobin	22.3pg	22.3pg	19.1pg
Mean corpuscular haemoglobin concentration	32.2g/dl	26.8g/dl	33.0g/dl
Red cell distribution width	39.1%	15.1%	15.4%
Haemoglobin A (HPLC)	1.4%	74.9%	84.0%
Haemoglobin A2 (HPLC)	9.9%	13.0%	5.5%
Haemoglobin F (HPLC)	87.9%	1.4%	1.1%
Haemoglobin A (CE)	94.3%	80.4%	CE not done
Haemoglobin A2 (CE)	1.2%	2.7%	
Haemoglobin F (CE)	94.3%	-	
Abnormal Haemoglobin (CE)	4.5%	13.1%	

FBC: full blood count, HPLC: high performance liquid chromatography, CE: capillary electrophoresis

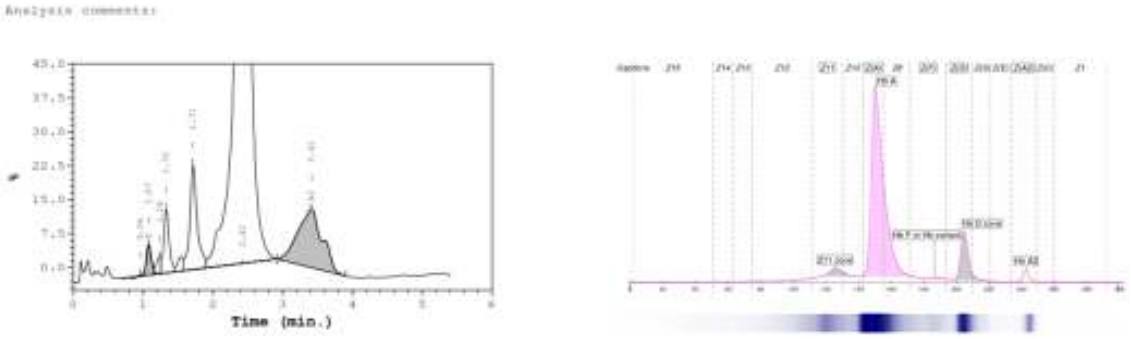

Figure 3: *Father's HPLC and CE tracings*

HPLC: high performance liquid chromatography, CE: capillary electrophoresis

The parents were counselled. The possibility of having a thalassaemic baby in a future pregnancy was explained. Long term management plan of the baby was explained highlighting the importance of adhering to transfusion protocol and the future need for iron chelation.

Discussion

In compound heterozygous states of Hb Lepore with other thalassaemias or haemoglobinopathies including β-thalassaemia, Hb S and Hb E, the patient's phenotypes can be a variation from Non-Transfusion Dependent Thalassaemia (NTDT) to Transfusion Dependent Thalassaemia (TDT). The haematological findings in these patients, including peripheral blood film features, are indistinguishable from severe forms of β-thalassaemia[1,4]. In the compound heterozygous state of Hb Lepore/β-thalassaemia, HPLC shows increased Hb F and Hb A2 with or without Hb A[1]. CE shows Hb F, Hb A2 and a variant Hb band at zone 6 (Hb Lepore) with or without Hb A.

In our patient, clinical presentation, blood counts and blood picture were compatible with β thalassaemia major. HPLC showed prominent Hb F, elevated Hb A2 and reduced Hb A, which was similar to β-thalassaemia major (homozygous β-thalassaemia). (Hb A2 percentage may be normal, elevated or occasionally reduced in β thalassaemia major[1]). In the proband's HPLC, the small shoulder in Hb A2 band was a lead to repeat analysis on CE which was able to detect the small Hb Lepore (4.5%).

For a presumptive identification of abnormal haemoglobin, at least two methods should be used[1]; these methods include Hb electrophoresis at alkaline and acid pH using cellulose acetate membrane, isoelectric focusing (IEF), HPLC, and CE. In Sri Lanka, most of the thalassaemia screening centres have only one presumptive Hb identification method; either HPLC or CE. When only one identification method is used, diagnosis of compound heterozygosity may be challenging, as in our case.

Parental screening is always helpful in confirming compound heterozygous haemoglobinopathies. In our case, parental screening confirmed the diagnosis of compound heterozygous Hb Lepore/ β-thalassaemia of the baby. Molecular genetic studies are also important in accurate characterization of complex haemoglobinopathies. In Sri Lanka, molecular genetic studies for haemoglobinopathy diagnosis are not freely available.

Management of compound heterozygous Hb Lepore/ β-thalassaemia depends on the clinical phenotype. Correct diagnosis of the haemoglobinopathy is essential for clinical management and genetic counselling. Our case was managed as TDT as the baby presented in early age with severe anaemia requiring blood transfusion

Our case highlights the importance of logical approach in haemoglobinopathy diagnosis and the importance of analysis by two methods if a variant Hb is suspected. Parental screening is always helpful in diagnosing difficult cases.

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