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CASE REPORT

Haemoglobin Lepore in a Malay family: a case report

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Abstract

A 2-year-old Malay boy was brought to the University Malaya Medical Centre for thalassaemia screening. Physical examination revealed thalassaemia facies, pallor, mild jaundice, hepatomegaly and splenomegaly. Laboratory investigations on the patient including studies on the parents lead to a presumptive diagnosis of homozygous Haemoglobin Lepore (Hb Lepore). The aim of this paper is to increase awareness of this rare disorder, this being the first case documented in Malaysia in a Malay. The case also demonstrates the need for this disorder to be included in the differential diagnosis of patients presenting clinically like thalassemia intermedia or thalassemia major. Accurate diagnosis would provide information necessary for prenatal diagnosis, proper clinical management and genetic counseling. The clinical, haematological and laboratory features of this disorder are discussed in this paper.

Key words: Haemoglobin Lepore, Thalassaemia-like disorder

INTRODUCTION

Haemoglobin Lepore is a structurally abnormal haemoglobin in which the abnormal globin chain is a hybrid or fused globin chain that has the N-terminal amino acid sequence of a delta chain and the C- terminal amino acid sequence of a beta chain.[1] This abnormal globin chain is the product of a hybrid gene that results from an unequal crossing over between the delta and beta globin genes because of a misalignment of homologous chromosome during meiosis. This leads to a 7.4 kb deletion between the delta and beta globin genes.[1, 2]

Three different Lepore haemoglobins have been identified so far and they are characterized by different delta to beta sequence transitions at the fusion junction.[1,2,3] Hb Lepore Washington Boston (δ87/β116), Hb Lepore Hollandia (δ22/β50), Hb Lepore Baltimore (δ50/β86). Hb Lepore Washington Boston is the most common and occurs worldwide.[1,2] Hb Lepore Baltimore was first described in one family with black ancestry from Baltimore, and afterwards in Yugoslavia, Spain and Northern Sardinia. Hb Lepore Hollandia is a rare variant and has been found in New Guinea, Bangladesh and in Thailand.[2,3] In all three variants, the synthesis of these hybrid chains is substantially less than that of the beta chains, resulting in an overall reduction in the non alpha globin chains and having clinically a beta thalassaemia-like condition. However, the Hb Lepore condition can be differentiated from a beta thalassaemia by the presence of the distinct Hb Lepore band on cellulose acetate electrophoresis.[4,5]

There are numerous large series and single case reports describing the heterozygous state for Hb Lepore and compound heterozygous state for Hb Lepore with thalassemia and other haemoglobinopathies. However homozygous Hb Lepore has been rarely reported, with only 22 patients recognized worldwide.[5] This report is the first case documented in Malaysia in a Malay.

The aim of this paper is to increase awareness of this rare disorder which should be included in the differential diagnosis of patients presenting clinically like a mild or severe thalassaemia intermedia/major, to provide accurate prenatal diagnosis, proper clinical management and genetic counseling. We describe a 2-year-old Malay boy whose clinical presentation was like a beta thalassaemia major. Laboratory

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investigations revealed the diagnosis to be homozygous Haemoglobin Lepore. The clinical, haematological and laboratory features of this disorder are discussed in this paper.

CASE REPORT

A 2-year-old Malay boy was brought to University Malaya Medical Centre for thalassemia/ haemoglobinopathy screening. Physical examination revealed thalassemia facies, pallor, mild jaundice, hepatomegaly (3 cm below the right subcostal margin) and splenomegaly (3 cm below the left subcostal margin). His height was at 3rd percentile and his weight was at 3-10th percentile. His haematological profile showed haemoglobin 74 g/L, red cell count 3.3 x 10^{12} L, MCV 77 fl, MCH 22 pg and a reticulocyte count of 5.8%. The white blood cell count and platelet count were normal. The patient's blood smear revealed hypochromia, microcytosis, anisopoikilocytosis, polychromasia, basophilic stippling and a few target cells (Fig.1).

Haemoglobin electrophoresis on cellulose acetate at alkaline pH (pH 8.6) showed a prominent F band and an additional band in the S region (Fig. 2). There was no haemoglobin A or haemoglobin A2 bands seen. Quantification of haemoglobin F revealed a level of 90% and the unknown haemoglobin constituted 9.3%. Electrophoresis at acid pH (pH 6.0) on citrate agar was carried out to identify the band in the S region. There was no Hb S detected. However, a prominent band was noted in the F region and a faint band in the A region. As the patient did not have any A band on the cellulose acetate strip, the faint band in the A region on citrate agar was suggestive of Hb D/G or Hb Lepore.

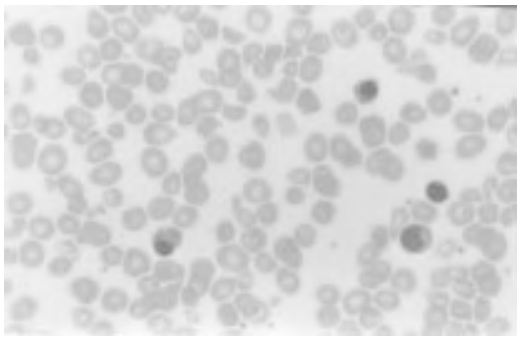

FIG. 1: Peripheral blood film of patient showing hypochromasia, microcytosis, anisopoikilocytosis, target cells and nucleated red blood cell. Wright stain X 400

Further testing to identify and quantify the abnormal haemoglobin variant was carried out by high performance liquid chromatography (HPLC) Bio-Rad Variant Haemoglobin testing system (Fig. 3). The unknown haemoglobin had a retention time (RT=3.59) similar to Hb A2 on the "Beta Thal Short Program" and was quantified as 10.7 %. There was no haemoglobin A synthesized and the rest of the haemoglobin present was hemoglobin F. Hb Lepore is known to have the same retention time as Hb A2 on the "Beta Thal Short Program" and therefore a presumptive diagnosis of homozygous Hb Lepore was made for the patient.

Screening was carried out for both parents. The father's blood studies showed haemoglobin 140 g/L, MCV 72 fl, and MCH 24 pg while the mother's haemoglobin was 111g/L, MCV 65.0fl, MCH 19 pg. The blood smear of both parents revealed hypochromia, microcytosis, few target cells and occasional basophilic stippling (Fig. 4). Haemoglobin electrophoresis on cellulose acetate (pH 8.6) of both parents (Fig. 5) showed bands at A, A2, and F regions and an additional band in the S region while electrophoresis on citrate agar at acid pH showed a prominent A band and a faint band in the F region. There was

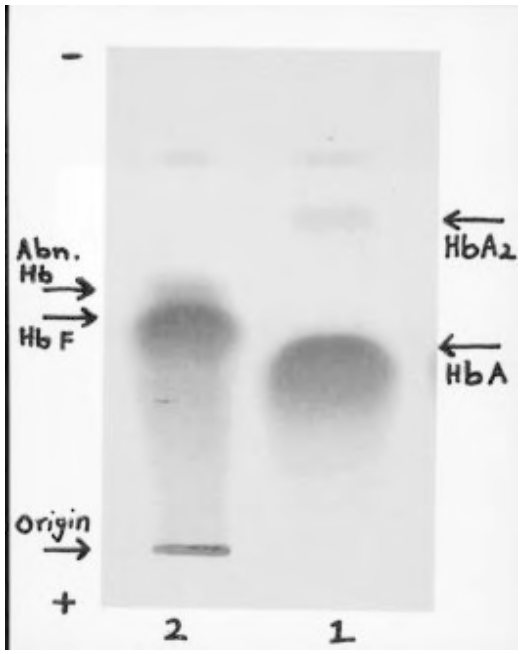

FIG. 2: Haemoglobin electrophoresis on cellulose acetate at pH 8.6 Lane 1: normal control showing Hb A and Hb A2. Lane 2: patient: absence of Hb A and Hb A2, increased Hb F and an abnormal Hb (unknown band).

no Hb S detected. The father's Hb F level was 1.6%, Hb A2 level 2.7% while the mother's Hb F level was 2.1%, Hb A2 level 3.0 %. High performance liquid chromatography (Fig. 6) revealed the unknown haemoglobin to be Hb Lepore (RT= 3.59) and the level in the father and mother was 13.1% and 13.8% respectively.

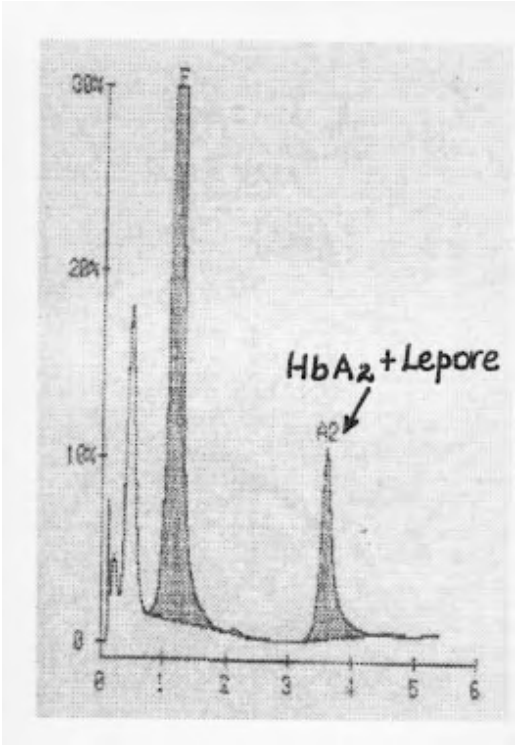

FIG. 3: High performance liquid chromatography analysis using the VARIANT Hemoglobin Testing System (Bio-Rad Laboratories). Chromatogram of patient (homozygous). No Hb A, increased Hb F, Hb A2 + Lepore retention time 3.59

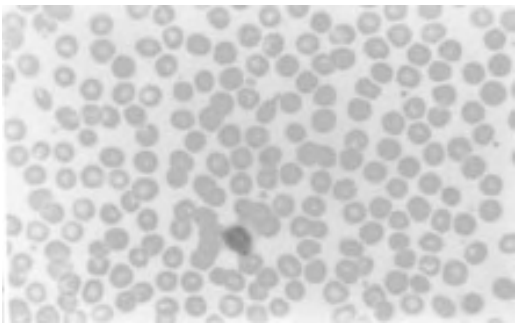

FIG. 4: Peripheral blood film (mother) showing hypochromasia, microcytosis and target cells. Wright stain x 400

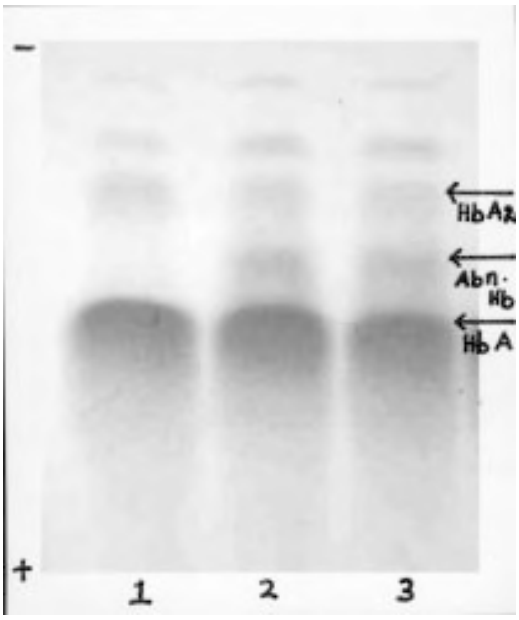

FIG. 5: Haemoglobin electrophoresis on cellulose acetate at pH 8.6. Lane 1: normal control showing Hb A and Hb A2. Lane2: mother, heterozygous showing HbA, Hb A2 and an abnormal Hb (unknown). Lane 3: father, heterozygous showing similar findings.

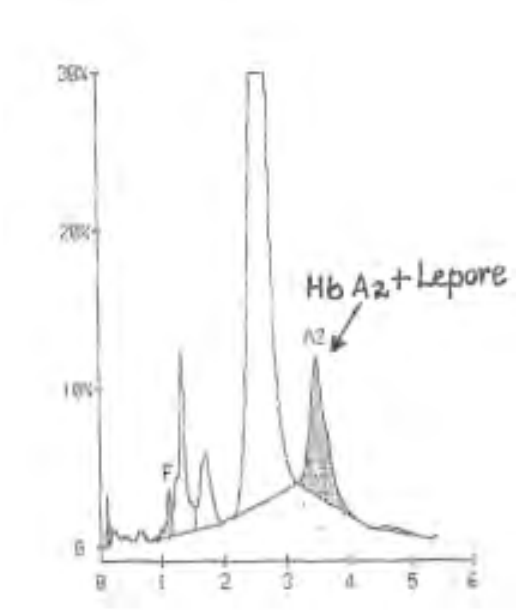

FIG. 6: High performance liquid chromatography analysis using the VARIANT Hemoglobin Testing System (Bio-Rad Laboratories) . Chromatogram of mother (heterozygous). Hb A2 + Lepore retention time 3.59

A diagnosis of heterozygous haemoglobin Lepore was made for both parents. The parents are first degree relatives. The patient's and parents' samples were sent for DNA sequencing which confirmed this unknown haemoglobin to be Hb Lepore Washington Boston. The patient is on regular follow-up and has received 3 blood transfusions at 4 -5 monthly intervals since the time of diagnosis.

DISCUSSION

Hb Lepore is a common abnormal haemoglobin seen in the Mediterranean region. It is the most common abnormal haemoglobin in Caucasians in Central Portugal and in the Spanish Alta Extremadura.[1] It is a variant form of human haemoglobin that contains delta beta hybrid chains or a fused globin chain, from the product of a hybrid gene: the unequal crossing over between the delta and beta globin genes resulting in a deletion of 7.4kb in the beta globin gene cluster.[1,2]

Three different types of Hb Lepore have been described so far, each with a different crossover breakpoint: Hb Lepore Washington Boston, Hb Lepore Baltimore and Hb Hollandia.[1,2,4] The basic defect in all the Hb Lepore disorders is the inefficient synthesis of the delta beta fusion chains of Hb Lepore, which leads to a variable degree of globin imbalance, excess alpha chain production and the clinical phenotype of beta thalassaemia. The ineffective erythropoiesis and the shortened red cell survival results from the deleterious effects of excess alpha chains. The selection of cells which are synthesizing relatively more gamma chains follow the same mechanism as occurs in beta thalassemia.[5] It has been postulated that the deletion which resulted in the delta beta fusion is somehow responsible for increasing the absolute output of gamma chains.[6] This is compatible with the observation that Hb Lepore heterozygotes produce significantly more Hb F than beta thalassaemia heterozygotes.

The Hb Lepore heterozygotes are usually asymptomatic. In a large Italian series no abnormal physical signs were reported, but a few of the Greek and Yugoslavian heterozygotes had mild splenomegaly.[7,8] Heterozyogotes are therefore healthy individuals with only a slight decrease in haemoglobin levels, but with a distinct microcytosis and hypochromia of their red blood cells. The mean cell haemoglobin (MCH) is thought to be the most reliable parameter that is altered in the Hb Lepore heterozygotes (range 20-25 pg).[9] In this series, the red cell indicies and morphological appearances of the blood film of beta thalassaemic carriers were compared with that of heterozygous Hb Lepore. It was found to be similar in both conditions. Studies suggest that there are no major differences in the haematological findings between carriers of the different molecular varieties of Hb Lepore.[1] Red cell survival and ferrokinetic data available reveal red cell survival is slightly shortened and Fe ^{59} utilization is increased.[10]

The Hb Lepore homozygotes vary in the severity of their clinical manifestations.[6,7,8] At one end of the spectrum are patients who present within the first 5 years of life with severe anaemia with haemoglobin values ranging from 4 to 7 g/dl. Significant splenomegaly, hepatomegaly, and skeletal abnormalities indistinguishable from those of homozygous beta thalassaemia are seen. These patients require regular blood transfusion and exhibit shortened life span. At the other end of the spectrum are patients who have a milder disorder indistinguishable from beta thalassemia intermedia, who are anaemic throughout childhood and require only occasional blood transfusions. The homozygous state for Hb Lepore therefore varies from a transfusion dependent disease like beta thalassaemia major through the milder spectrum of thalasaemia intermedia that appears to be compatible with longevity of life.[5] The haematological findings in these patients are peripheral blood film features indistinguishable from the severe forms of beta thalassaemia. Bone marrow smears show marked erythroid hyperplasia due to high level of erythropoietin production in response to the anaemia.[6]

The multifaceted approach for the presumptive identification of haemoglobin variants includes a scrutiny of blood counts/red cell indices and haemoglobin analysis. This approach easily identifies Hb Lepore. On cellulose acetate electrophoresis at alkaline pH, Hb Lepore shows electrophoretic mobility similar to Hb S.[5] The other haemoglobins that run in this position are Hb D and Hb G, which are differentiated from Hb S by sickle solubility test and electrophoresis at acid pH. Hb Lepore has a similar retention time (RT) value as Hb A2 on high performance liquid chromatography (HPLC) analysis with the Bio-Rad Variant Haemoglobin testing system.[2] Values greater than 10% suggest the presence of variant

haemoglobin. In Malaysia the most common haemoglobin variant seen is Hb E that is differentiated from Hb Lepore by cellulose acetate electrophoresis at alkaline pH. Hb E runs in the same position as HbA2, whereas Hb Lepore runs at the position of Hb S. Therefore a multifaceted approach which includes haemoglobin electrophoresis and high performance liquid chromatography provides sufficient information for the presumptive identification of Hb Lepore. The characterisation of the type of Hb Lepore requires analysis of the globin chains by reversed phase HPLC and DNA studies would confirm the diagnosis of this variant haemoglobin.[4]

In the homozygous state, Hb A and Hb A2 are absent and the haemoglobin is made up of Hbs F and Lepore only. The level of Hb Lepore ranges from 8 to 30% with a mean value of approximately 15%, the remainder of the haemoglobin being Hb F. In the heterozygous state the haemoglobin contains Hbs A, Lepore, A2 and a variable amount of Hb F. The reported level of Hb Lepore is between 5 to 15%, with a mean level around 10%. The mean level of Hb A2 is about 2% and the reported values for Hb F range from between 1-14%.[5]

In conclusion, Hb Lepore is a structural haemoglobin variant coded for by a hybrid gene formed by the fusion of delta and beta genes. The homozygous state is clinically similar to beta thalassaemia intermedia or major. Presumptive identification can be easily made in the laboratory by a multifaceted approach that includes the methods, scrutiny of blood counts/ red cell indices, haemoglobin electrophoresis at alkaline and acid pH and haemoglobin analysis by high performance liquid chromatography. However, confirmation requires DNA characterization. A correct diagnosis is essential for proper clinical management and genetic counseling.

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