Hemoglobin LeporeBoston in Two Iranian Families

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Hemoglobin LeporeBoston was found in two Iranian families presenting the clinical manifestations of \( \beta \)-thalassemia trait. Electrophoretic examination of the patients' hemoglobin revealed the presence of an abnormal hemoglobin with Lepore characteristics. Fingerprinting and amino acid analysis of selected peptides of this variant suggest that it is similar to LeporeBoston.

LEPORE HEMOGLOBINS are the result of unequal crossing over between structural genes coding for \( \delta \) and \( \beta \) polypeptide chains of human hemoglobin during meiosis. The abnormal polypeptide chain \( \delta \beta \) produced after this crossing over has part of the \( \delta \) chain at its N terminus, and the remaining part is similar to the \( \beta \) chain. The point of fusion of the two polypeptide chains and the proportion of the \( \delta \) and \( \beta \) in the \( \delta \beta \) hybrid chain varies in different types of Lepore hemoglobins. For example, in LeporeHolland crossing over is between residue 22 of the \( \delta \) chain and residue 50 of the \( \beta \) chain; in LeporeBaltimore crossing over has occurred between residue 50 of the \( \delta \) chain and residue 86 of the \( \beta \) chain. In LeporeBoston crossing over is somewhere between residue 87 of the \( \delta \) chain and 116 of the \( \beta \) chain.

The inverse of this crossing over results in the synthesis of another polypeptide chain with a \( \beta \) chain at its beginning and a \( \delta \) chain at the end (\( \beta \delta \)) called anti-Lepore. Hemoglobin Miyada is a good example of an anti-Lepore hemoglobin type in which the \( \beta \delta \) crossing over occurred between threonine 12 of the \( \beta \) chain and alanine 22 of the \( \delta \) chain. Hemoglobin P Congo identified by Lehmann and Charlesworth and hemoglobin P-Nilotic described recently by Badr, Lorkin, and Lehmann, are other examples of anti-Lepore hemoglobin types with \( \beta \delta \) structure. Figure 1 illustrates the sites of crossing over in various types of Lepore and anti-Lepore hemoglobins.

Hemoglobin LeporeBoston was first described by Gerald and Diamond in 1958. Since then it has been reported in Italy, Romania, Yugoslavia, Greece, and Cyprus under different names: Hb Pylos, Hb Cyprus, Hb Augusta and LeporeWashington. All these turned out to be LeporeBoston after identification. Structural studies of this hemoglobin have been carried out by Baglioni, and completed by Labie et al.

In this article we report the occurrence of LeporeBoston in two Iranian families.

CASE HISTORIES

The probands were two children of age 10 and 13 from two unrelated families admitted to the Children's Hospital presenting clinical manifestations of \( \beta \)-thalassemia trait. Routine hema-
Lepore Hollandia

Lepore Baltimore

Lepore Boston

Hemoglobin Miyada

Hemoglobin Nilotic

Fig. 1. Cross-over sites in various types of Lepore and anti-Lepore hemoglobins.

tological examinations were as follows: RBC 5.1 x 10^6 cu mm, hemoglobin 12.9-13.1 g/100 ml, hematocrit 37%, 40%, and reticulocytes 4%. Red cells were microcytic, and no inclusion bodies were detected in them after incubation in brilliant cresyl blue or methyl violet. Red cell’s enzyme assays for G6PD and PK were within the normal ranges.

Electrophoresis of the patients’ hemolysates revealed the presence of a slow-moving hemoglobin in addition to the normal hemoglobin (Hb A). The abnormality was found in the hemolysate of the mother of one of the propositi, presenting the same clinical manifestations of β-thalassemia trait. The parents of the other patient were not available for study.

Investigation of the Abnormal Hemoglobin

Hemolysates were prepared by washing the red cells three times with 0.9% saline and lysing by the addition of 1 volume of distilled water and 0.5 volume of carbontetrachloride followed by centrifugation.

Fetal hemoglobin was less than 1%; the solubility test was within the normal range. Heat denaturation, carried out according to the method of Grimes and Dacie and Carrell was negative.

Electrophoresis on cellulose acetate at pH 8.9 and in starch gel at pH 8.6 revealed a slow-moving hemoglobin in the same position as Hb S (Fig. 2). The proportion of this fraction was estimated to be 12.5%, of the total hemoglobin. After elution of hemoglobin bands from cellulose acetate following electrophoresis, Hb A2 was 2.3%, and Hb A was 85.2%.

On agar gel electrophoresis at pH 6.2, the abnormal hemoglobin moved with Hb A.

Separation of hemoglobin α and β chain by electrophoresis according to the method of Ueda and Schneider using buffers containing 6 M urea and 0.05 M 2-mercaptoethanol, as well as treatment of hemolysates with p-hydroxymercuribenzoate (PMB) followed by starch gel electrophoresis, revealed that the abnormality exists in the β chain (non-α chain). With such a low proportion of the abnormal fraction, Lepore hemoglobin was suspected.

The abnormal hemoglobin was purified by column chromatography of the hemolysates on DEAE cellulose (Whatman DE-52) using Tris-HCl buffer pH 8.10 and the protein was concentrated in vacuo. Globin was prepared by treatment of the purified hemoglobin with 2 M HCl in acetone at -20°C, and the precipitate was washed with cold acetone and freeze dried.
Fig. 2. Starch gel electrophoresis. Tris-EDTA-Borate buffer pH 8.6. Amido-Black staining, cathode to the left. Above, normal hemolysates. Below, Lepore trait; note the low quantity of Hb A₂.

Fig. 3. Fingerprints of the S-aminoethylated non-α chain of Hb Lepore (above), and normal β' chain (below). (1) indicates the usual place of the peptide β'Tp3. (2) indicates the peptide δTp3.
Peptide chains of the globin were prepared by CMC column chromatography of the globin in phosphate buffers at pH 6.8, containing 8 M urea and 0.05 M 2-mercaptoethanol, according to the method of Clegg et al. The chains were aminoethylated, and tryptic digestion and fingerprinting of the S-aminoethylated chains were carried out. Figure 3 shows the fingerprints of the non-α chain of hemoglobin Lepore as well as the normal β chain. It can be seen that the peptide β Tp3 is missing from its usual place, 1, and has shifted towards the cathode, 2.

Peptide T3 and T5 were eluted from six preparative fingerprints of the non-α chain of Lepore hemoglobin with 6N HCl and hydrolysed for 20 hours at 110°C in micropipettes. The amino acid composition of each peptide was determined in an automatic amino acid analyser (Hitachi, KLA-5).

The results of the amino acid analysis of the tryptic peptides T3 and T5 indicate the same amino acid composition found in peptides β Tp3 and β Tp5, respectively. An alanine was found in β Tp3 instead of glutamic acid in the β Tp3, and a serine was found in β Tp5 instead of threonine in the peptide β Tp5. This serine at residue 50 is a good index of the presence of hemoglobin Lepore Boston.

DISCUSSION

Clinical manifestations in our patients were similar to the β-thalassemia minor which is frequently found among Iranians. From the results of the surveys carried out on 13,000 individuals during the last 8 yr, an estimate of 100,000 cases of β-thalassemia trait could be predicted in Iran with a total population of 30 million.

The diagnosis of Lepore trait was made on the basis of the presence of a slow-moving hemoglobin with the same mobility of Hb S in electrophoresis, a proportion of 12.5%, and the low percentage of Hb A2, and fingerprints of the abnormal hemoglobin. This is the first report of the occurrence of Lepore hemoglobin among Iranians.

REFERENCES

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