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# HEMOGLOBIN E B-THALASSEMIA IN A PAKISTANI FAMILY

Pages with reference to book, From 301 To 303

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## Abstract

Hemoglobin E is a slow moving B chain variant of hemoglobin, first discovered by Itano<sup>1</sup>. Characterized by Hunt et al<sup>2</sup> showed glutamic acid at B 26 to be replaced by lysine. It is a common variant of hemoglobin in the world and reported in high frequency from South-East Asia<sup>3-6</sup>. Cases of Hb E, in combination with thalassemia have been reported on the basis of electrophoretic pattern only. In this communication a case of Hb E with B thalassemia is reported on the basis of amino acid sequencing of the abnormal peptide.

## METHODOLOGY

Blood sample of propositus was collected in EDTA. Hematological parameters were determined by normal methods. Radiological examination of the skeletal system was also carried out. Hemolysate was prepared by the classical method<sup>7</sup>. Electrophoretic separation of hemoglobin was carried out on cellulose acetate membrane<sup>8</sup> and on 10% polyacrylamide gel at pH 8.3<sup>9</sup>. Hemoglobin components were separated by chromatography on DEAE-Sephacel column (15x2.5cm) with 0.05M Tris/HCl buffer pH 8.5. Sample was eluted with a linear gradient of 0-0.1 M NaCl<sup>10</sup>

Reversed phase HPLC was used for the separation of globin chains. A column of Nucleosil-C4 was equilibrated with 0.1% aqueous trifluoroacetic acid (TFA). Sample was eluted with a linear gradient of acetonitrile from 35—60% in 60min, at a flow rate of 1ml/min.

The abnormal (3 chain was oxidized and digested with trypsin (TosPheCH<sub>2</sub>C1-treated, Worthington) at pH 10.5 for 1h, followed by pH 9.5 for 2h with enzyme to substrate ratio of 5:100<sup>11</sup>

Separation of tryptic peptides was carried out by reversed phase HPLC<sup>12</sup> on a LiChrosorb RP2 column equilibrated with 0.05M ammonium acetate. Peptides were eluted with a linear gradient of 0—40% acetonitrile in 60 min at a flow rate of 1ml/min.

Amino acid composition of the abnormal peptides was determined by an automatic amino acid analyzer Model LC 5000, (Biotronik GmbH, West Germany).

The amino acid sequence was determined in a liquid-phase sequencer Model 890B, (Beckmann Instrument) according to the method of Edman and Begg<sup>13</sup>

## RESULTS AND DISCUSSION

Hemoglobin E is the third most common variant of hemoglobin. Association of Hb E with (3-thalassemia produces severe clinical problems. In the present study hematological and biochemical examinations revealed the following: Hemoglobin 63g/dl, reticulocytes 11%, PCV 0.241/1, MCHC 26g/dl, bilirubin total 4.2mg/dl and direct 2.6mg/dl, serum iron 220ig/dl, TIBC 320j.Lg/dl.

The morphology of the erythrocytes showed severe hypochromia, anisochisto-and poikilocytosis, film suggestive of thalassemia.

Radiological examination of the skeletal system showed markedly generalized osteoporosis and blood dyscrasia.

The electrophoretic pattern of hemolysate on cellulose acetate membrane and polyacrylamide gel showed an elevated Hb F and a slow moving hemoglobin at the position of Hb A 2 (Figure 1).

Separation of hemoglobin components was achieved by chromatography on DEAE-Sephacel confirmed the results of electrophoresis (Figure 2).

Reversed phase HPLC of hemolysate resulted in the separation of abnormal globin chain approx. 55% (Figure 3)

and showed the absence of BA chain.

Two abnormal peaks were observed in the fingerprint of peptides by RP-HPLC (Figure 4).

Amino acid analysis is presented in Table.

**TABLE : Amino Acid Composition of the Peptides .**  
 $\beta^E T3a$  and  $\beta^E T3b$ .

Amino acid	$\beta^E T3a$	$\beta^E T3b$	$\beta^E T3b$	$\beta^E T3b$	$\beta^E T3b$
Asp	1.97 (2)	—	—	—	(2)
Glu	1.02 (1)	—	—	—	(2)
Gly	2.03 (2)	0.99 (1)	(1)	(1)	(3)
Ala	—	1.05 (1)	(1)	(1)	(1)
Val	2.98 (3)	—	—	—	(3)
Leu	—	1.01 (1)	(1)	(1)	(1)
Lys	0.98 (1)	—	—	—	—
Arg	—	0.93 (1)	(1)	(1)	(1)
Sum	9	4			13

Amino acid sequence study of abnormal peptides confirmed it to be a case of Hb E.

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