Original Articles

HEMOGLOBIN E-BETA THALASSEMIA IN UTTAR PRADESH

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Objective: To evaluate the molecular make up of hemoglobin E-Beta thalassemia to facilitate diagnosis, genetic counseling and prenatal diagnosis in Uttar Pradesh. Design: DNA analysis. ***Setting:*** *Referred hemolytic anemia cases to Genetics OPD of a tertiary care center. Subjects:* 21 *families of HbE-thalassemia of which 19 were of UP origin. Methods: The patient and obligate carriers in their families were evaluated at hematological, biochemical and molecular level. A total of 62 cases were evaluated which included the index cases and their family members. Red blood cell indices, osmotic fragility, hemoglobin electrophoresis, quantitation of fetal hemoglobin, HbA2/E, serum iron and total iron binding capacity estimation were carried out in all the blood samples. DNA analysis was done for HbE and beta thalassemia mutations. Results: The commonest, 1VSI-5 (G—>C) mutation (57%) was found along with HbE mutation. Only* 23/26 *cases belonged to the group of common P-thal mutations as described in literature. Conclusion: Establishment of antenatal diagnostic services is necessary* ***in*** *those parts of India where both these mutations are commonly seen.*

Key words: *Amplification refractory mutation system, Antenatal diagnosis, beta thalassemia, Hemoglobin E, Mutations.*

HEMOGLOBIN E-Beta thalassemia is a disorder with a high frequency in South-East Asia and has been reported from different parts of India(l-14).Various factors decide the outcome of the interplay between this combination of a qualitative and a quantitative defect of hemoglobin. The clinical picture produced by the interaction is heterogeneous. We report here 26 patients with HbE thalassemia disease from 21 families where parents are obligate heterozygotes for HbE and **p-thal** mutations. An effort has been made to characterize the molecular make up of these patients with a view to develop antenatal services for such families.

Subjects and Methods

Twenty six individuals from 21 families were diagnosed to be cases of [3-thalassemia HbE interaction. These patients had been referred to the Genetics OPD from 1987-1995 for investigation of refractory anemia. The index cases and available family members were clinically evaluated and investigated for the cause of anemia.

Patients Groups

Red blood cell indices were measured on automated Sysmax 800. Osmotic fragility was calculated according to the test described by Dacie and Lewis(15). Fetal hemoglobin (HbF) was estimated by alkali denaturation technique(16) and also by the methodology of Singers *et al.(17).* Quantitative estimation of hemoglobin A_2 (HbA2) was carried out by column chromatography(18). Prepared hemolysates were subjected to Hb electrophoresis on cellogel at pH 8.5 in tris-glycine buffer for 90 minutes. DNA was extracted from peripheral blood leucocytes(19). Amplification refractory mutation system (ARMS) technique(20) was applied to confirm the presence of HbE mutation and to characterize the (3-thalassemia mutations.

Follwing primers were used to detect the presence of HbE:

5' ACC TCA CCC TGT GGA GCC AC - *

5' AA CC TG CCC AGG GGC TT <-

Primers used for detection of the 10 commonest (3-thalassemia mutations found in Indians were the same as described earlier(21,22).

ARMS

The basis of the system is the observation that oligonucleotides that are complementary to a given DNA sequence, except for a mismatch at their 3'-OH residue, will not function as primers in the PCR under appropriate conditions. A typical ARMS test consists of two complementary reactions, one primer for specific normal sequence and another for specific mutant sequence. The genotype of an individual can be determined by analysis of the amplification products. A normal individual generates PCR products only in the normal reaction, a heterozygote gives products in both reactions, and a homozygoe mutant individuals does so only in the mutant reaction.

Results

Of the 21 families with 26 affected individuals, 19 belonged to Uttar Pradesh and one each to Bihar and Haryana. These included one Christian, 2 Muslim and 18 Hindu families. The patients were placed in 2 groups on the basis of age at onset and requirement for regular blood transfusfons. Patients with milder phenotype, later age of onset, without regular need for blood transfusions were placed in Group A. Group B consisted of patients with severe anemia and early age of onset who required regular blood transfusions to maintain Hb levels beyond 6g/dl, except for one 18 year old girl who became symptomatic at *8-9* year of age but now requires regular blood support. The age range in this group was 2-18 years with 7 out of 11 patients aged below 5 years. In contrast, the age range in Group A was 4-38 years with 12 of the 15 patients being above 5 years and 9 out of 15 subjects older than 10 years of age.

The hematological parameters of the two groups are summarized in *Table I.* The mean Hb levels in Groups A and B were *7.9* and 5.0 g/dl, respectively. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were similar in the two groups. Osmotic fragility (OF) was reduced in all patients to < 80% at 0.36% saline, except for two patients, one in each group.

HbE percentage was significantly higher in Group A with a mean of 53.9% and range of 24.6-81.0%. Group B showed a mean value of 41.2% with a range of 17.8-57.9%. However, the Group B patients with a severe phenotype had slightly higher mean HbF level of 25.1% as compared to 22.0% in Group A. The implication is difficult to interpret due to the small sample size.

TABLE *Y-Hemalological, Biochemical and Molecular Comparison ofHbE-Beta Thalassemia Families.*

	S. No.	Age/ Sex	Hb (g'/dl)	MCV (fe)	MCH (Pg)	MCHC (g/dl)	OF (%)	HbE/A2 (%)	HbF (%)	E/p-thal
Group A:	1.	29/M	7.6	65.1	17.3	26.5	45.7	81.0	10.6	.E/?
Mild	2.	6/M	7.3	55.0	–	–	39.0	55.7	26.6	E/VIS1-5 (G->C)
	3.	4/F	10.0	76.9	20.8	27.0	33.0	24.6	. 3.3	E/Co30 (G-^C)
	4.	12/M	10.8	63.9	24.3	38.2	-	41.8	30.1	E/IVS 1-5 (G->C)
	5.	34/F	7.9	61.0	16.2	26.0	44.0	59.0	18.4	E/IVS1-5 (G->C)
	6.	22/F	8.1	54.0	20.2	36.8	61.0	47.3	31.4	E/IVS 1-5 (G-^C)
	7.	34/M	7.2	63.0	19.5	31.0	42.4	69.9	19.0	E/CO 15 (G->A)
	8.	30/F	6.9	73.0	20.2	29.2	83.0	54.2	20.3	E/IVSI-5 (G->C)
	9.	7/M	7.1	70.6	-	–	15.4	52.0	23.7	E/Co30 (G->C)
	10.	5/M	7.3	57.9	-	–	24.8	60.0	12.5	E/Co30 (G->C)
	11.	19/M	9.2	78.0	22.0	30.0	48.0	51.8	22.6	E/Co 15 (G->A)
	12.	11/M	7.3.	71.0.	19.4	27.3	59.1	60.9	19.4	E/FSH 41-42
	13.'	9/M	7.9	74.9	21.9	29.2	72.2	35.6	45.6	E/FSH 41-42
	14.	20/M	6.9	60.0	17.1	28.9	38.7	52.9	23.6	E/?
	15.	38/M	7.9	62.0	16.2	25.6	41.0	63.8	23.1	E/?
Mean		--	7.9	65.7	19.6	29.6	46.2	53.9	22.0	
Group B:	1.	3/M	5.4	68.0	21.3	31.4	37.4	39.9	29.9	E/Co 16 (-C)
Severe	2.	9/M	5.2	61.5	-	–	55.5	52.0	23.8	E/IVSI-5 (G->C)
	3.	**7/F**	5.6	69.5	21.6	35.0	55.6	46.8	22.0	E/IVSI-5 (G->C)
	4.	2.5/F	5.6	62.3	14.2	23.0	62.0	17.8	24.0	E/IVSI-5 (G->C)
	5.	9/M	5.6	60.0	14.0	. 23.3	65.6	34.2	31.3	E/IVSI-5 (G->C)
	6.	3/F	3.0	77.5	-	–	51.0	57.9	15.6	' E/Co 15 (G->A)
	7.	2/M	5.6	77.0	24.0	31.1	–	19.2	7.0	E/Co 15 (G->A)
	8.	4/M	4.8	63.0	18.3	30.0	73.6	48.3	37.4	E/IVS I-5(G->C)
	9.	3/M	5.0	63.0	20.8	33.3	74.2	42.6	43.3	E/IVS 1-5 (G->C)
	10.	5/M	4.6	44.5	20.7	32.1	58.0	46.1	5.1	E/IVS 1-5 (G->C)
	11.	18/F	4.6	57.0	20.8	26.7	86.7	48.2	36.8	E/IVSI-5 (G->C)
Mean		-	5.0	63.9	19.5	29.5	62.0	41.2	25.1	

Presence of HbE mutation was confirmed in all patients, on analysis by ARMS. The (i-thalassemia mutation could only be characterized in 12 of the 15 patients in Group A and all in Group B with a panel of probes for the 10 most commonly found mutations in India (Fig. 1).

All the 8/11 (73%) characterized mutations in Group B were IVS-I, 5 (G—>C), whereas this was present in only 5/12 (42%) characterized mutations in Group A. Two siblings from Group A revealed a frameshift 41-42 (-CTTT) mutation. One was with Col6 in Group B and two each with Col5 from both groups were identified. However, three cases were found to have an uncommon mutation Co30 (G—>C)

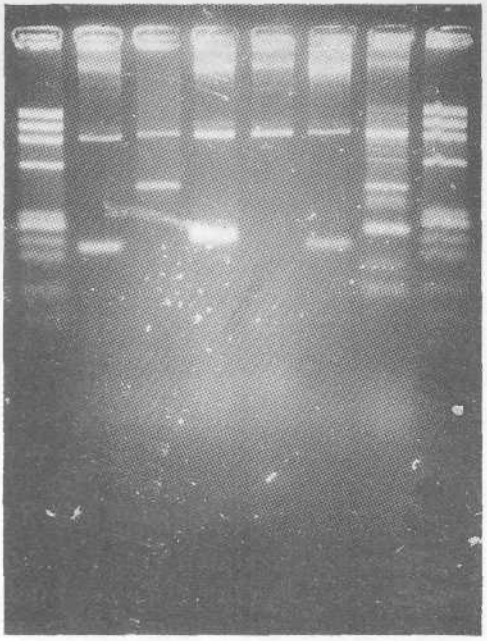

Fig.] ***Ethidiwn*** *bromide stained PCR products on 2% agarose gel. Lane 1 - Hae. IB digested x 174 DNA Moluculnr weight marker. It yields fragments of 1353, 1078, 872. 603, 310, 281, 234, 194 and 118 bp. Lane 2 & 6-861 and 239 bp fragments corresponding to Codon 16 (-c) mutation. Lane 3-861 and 443 bp fragments corresponding to Frame shift codon 41-42 (-CTTT) mutation. Lane 4-861 and 285 bp fragments corresponding to IVS 1-5 (G-C) mutation. Lane 5-861 bp fragment in normal sample used as positive internal control for PCR amplification.*

which was detected by DNA sequencing in Dr. Hattori's Laboratory, (i-thalassemia mutations in other three patients of Group A could not be characterized by the panel of probes for these 10 mutations.

Discussion

The heterogenecity in clinical and hematological picture of HbE/(3-thalassemia interaction is apparent from the results. Those with severe anemia and early age of onset have a disease course similar to homozygous p-thalassemia while those with milder anemia and later age of onset get away with occasional need for blood transfusion or completely asymptomatic course of disease, hemolytic facies being the only indicator to presence of disease.

High HbF values are known to reduce the severity of symptoms. In our patients, however the HbF levels were not very different in the two groups. Hence the factor responsible for milder phenotype may be different from the protective values of HbF.

The percentage and range of HbE is significantly higher in Group A and is probably one of the factors responsible for the mild symptoms of these patients. HbE is a mild structural variant of (3 globin chain, being asymptomatic in homozygous state. The severity of phenotype is, therefore, dependent on the type of (3 thai mutation, HbE and HbF levels and number of alpha-globin genes which tend to reduce the severity of disease by altering the ratio of imbalance of alpha and (3-globin chains. The two alpha-gene deletions in association of (3° type could be of less severe type as compared to either none or one alpha gene deletion in association with p^{+}(12,23,24).

The interaction of genes for alpha and (J-thal, HbE, Hb-constant spring and other mutations results in a complex variety of phenotypes(25-27). A remarkable high incidence of deletion and non-deletion type of alpha thalassemia mutants and its interaction with P-thal have been found in Indians(27,28). However, in our patients interplay of alpha-thalassemia genes possibily affecting the EB^{T} phenotype, has not been ruled out.

The commonest thalassemia mutation in our patients was IVS-1,5 (G->C). Surprisingly, only 23/26 mutations belonged to the group of 10 most commonly found

(3-thal mutations responsible for 97-98% of all cases of (J- thai in India. The 8 mutations characterized in Group B and 5 in Group A were IVS-1,5 (G->C). This is a B^+ mutation and is probably contributory to the severity of the phenotype.

One family with two affected children (Christian) showed a frameshift mutation [FSC 41-42-(CTTT)]. Three patients were identified to be carrying an uncommon mutation, Co 30 G—>C. However, 4 cases were carrying mutation at Col5 and only in one case Col6 mutation was identified. Thus only 4 of the 10 commonest mutations contributed to our 26 cases of E/p thai interaction.

Our plan is to try and further characterize the remaining 3 undiagnosed (J thai mutations in these patients. Molecular diagnosis of HbE is required for first trimester antenatal diagnosis besides characterization of P-thal mutation. An establishment of this kind of antenatal diagnostic services is the ultimate aim of the study.

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