

Expression of Hb β -T and Hb β -E genes in Eastern India—Family studies

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MS received 24 May 1980; revised 14 November 1980

Abstract The distribution patterns of different haemoglobins were observed amongst the family members of β -thalassaemia homozygous and HbE- β -thalassaemia patients with the aid of gel electrophoretic and alkali denaturation techniques. Of the 18 families studied, four belonged to β -thalassaemia homozygous and 14 to HbE- β -thalassaemia patients. Interaction of HbE and β -thalassaemia genes resulted in major clinical abnormalities with increase in the percentages of haemoglobins F and E. The percentages of HbA₂ in homozygous β -thalassaemia were within the normal range. Although in Southeast Asia the β^o type of HbE-thalassaemia is more prevalent, only one individual with this type of thalassaemia was observed during this survey. In the rest of the patients examined the percentages of adult haemoglobin ranged from 5.2 to 42.5 indicating the presence of a β^+ type gene.

Keywords. β -thalassaemia; haemoglobin E; haemoglobin A

Introduction

Haemoglobin E is found in relatively high frequencies in the eastern part of India and has been suggested to act as a marker for the mongoloid element (Flatz *et al.*, 1972, Das and Flatz, 1975). It occurs both in heterozygous and homozygous forms and frequently in combination with β -thalassaemia. The effect of the genetic interaction between HbT and HbE has been known to result in more drastic clinical expression than β -thalassaemia heterozygote and HbE homo- and heterozygotes (Chatterjea *et al.*, 1956, Chernoff *et al.*, 1956, Lie-Injo, 1959 and Feldman and Reider 1973). In the present paper, an analysis has been made of 18 families carrying various combinations of these two genes and their effects on the percentages of the different haemoglobins.

Materials and methods

Haemoglobin analysis was carried out in the following way on four families. (IA to IVA) of β -thalassaemia homozygous and fourteen (IB to 14B) of HbE- β -thalassaemia patients (table 1)

Table 1. Total individuals studied of the β -thalassaemia homozygous and HbE- β -thalassaemia families.

Type	No. of families	No. of family members	No. of sibs
β -Thalassaemia homozygous	4	23	15
HbE- β -Thalassaemia	14	64	39
Total	18	87	54

Anticoagulated blood samples were collected. The haemoglobin percentage and blood grouping were determined and the sickling test carried out (Dacie and Lewis 1968). Haemolysates were prepared by standard techniques for electrophoresis and foetal haemoglobin estimation. Paper, starch and Polyacrylamide gels were used as media for comparison. Spectrophotometric quantitations were made after the elution of haemoglobins separated on Polyacrylamide gel electrophoresis (Ajmani *et al.*, 1976, 1977).

For the separation of different haemoglobins, a 5% Polyacrylamide gel solution was prepared by mixing one part of stock solution A containing acrylamide 20% and methylene bisacrylamide 0.53% and two parts of stock solution B (consisting of tris-HCl buffer—17.15 g tris and 24 ml of N.HCl/dl, pH 8.8 and 0.4 ml N, N, N', N' tetramethylene diamine and one part of aqueous ammonium persulphate solution 0.48%). Gel solution was immediately pipetted into glass gel tubes (12×0.5 cm). Bridge buffer pH 8.3 (Tris 0.6% and glycine 2.88%) was poured in both the electrode chambers and Polyacrylamide gel electrophoresis was carried out according to Davis (1964) and a current of 4 mA per tube was applied for a period of 120 min at 22°C. Two distinct bands appeared, a major band (HbA) and a minor one (HbA₂ or HbE) which moved slightly behind it; both were distinctly visible without further staining. The bands were cut out and placed in Drabkin's solution. The cyanmethaemoglobin contents of the respective haemoglobins were measured at 420 nm in a Beckmann spectrophotometer (model DU₂). Foetal haemoglobin was measured by the alkali denaturation technique of Betke *et al.* (1959). HbA₂ was considered negative (–) when not visible and hence not detectable by our method.

Results

Pattern of HbA₂ and HbF in β -thalassaemia homozygous families

The sickling test was negative in all the cases. Table 2 shows the distribution pattern of haemoglobins—HbA₂ and HbF of the members of four β -thalassaemia homozygous families.

Table 2. Distribution pattern of HbA₂ and HbE in β -thalassaemia homozygous families

	β -Thalassaemia homozygous	β -Thalassaemia heterozygous	Normal
No. of individuals	5	18	0
HbA ₂ %	3.45 \pm 1.38	7.31 \pm 2.16	0
HbF%	41.77 \pm 18.70	1.75 \pm 0.16	0

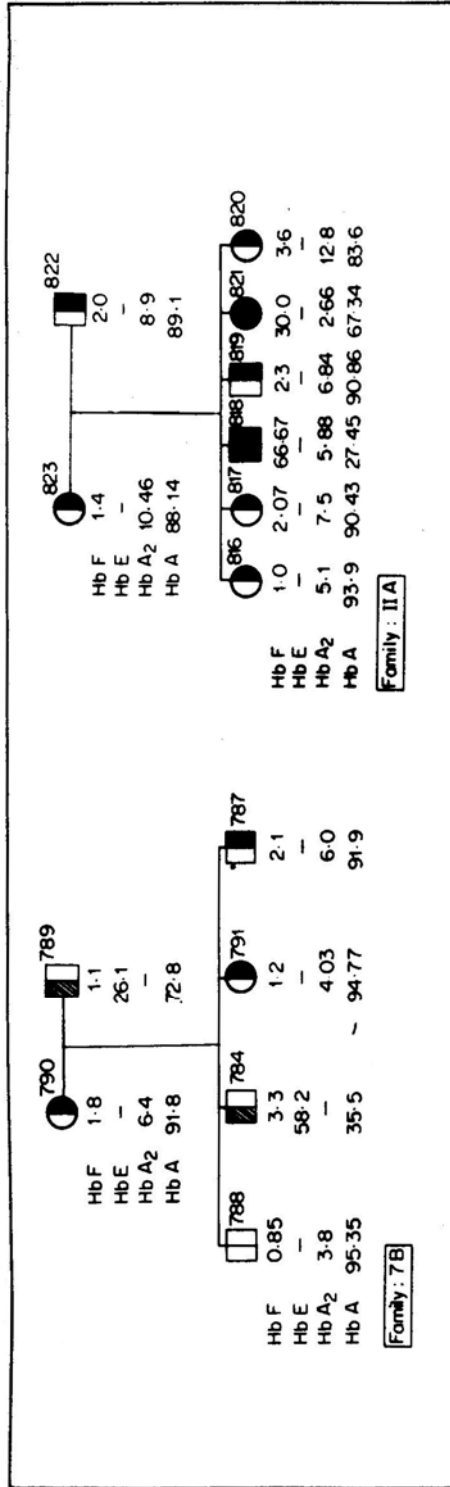
Table 3 shows the distribution pattern of different haemoglobins HbA₂, HbE and HbF among the family members of the 14 HbE- β -thalassaemia families.

Table 3. Distribution pattern of HbA₂, HbE and HbF in HbE- β -thalassaemia families.

	HbE- β Thalassaemia	HbE- β Heterozygous	β -Thalassaemia heterozygous	Normal
No. of individuals	16	17	22	9
HbA ₂ %	—	—	5.53 \pm 1.24	3.17 \pm 0.90
HbE %	48.48 \pm 10.72	29.51 \pm 4.58	—	—
HbF %	31.14 \pm 14.26	0.99 \pm 0.64	1.66 \pm 1.49	1.24 \pm 0.90

The age of the patients ranged from 10 months to 17 years and their HbA₂ percentage was always within the normal range. The only exception was family IIA (figure 1) where one of the brothers (818) of the patient was later diagnosed as a β -thalassaemia homozygote with high percentages of both haemoglobins F and A₂. The patients available did not show total absence of adult haemoglobin (HbA). None of the cases received any blood transfusion before the study.

Wherever both parents of the HbE- β -thalassaemia patient were available for studies, one parent was invariably found to be a carrier of HbE-disease and the other of β -thalassaemia. Amongst the brothers and sisters of the patients all types of combinations were observed including HbE carrier (HbAE), β -thalassaemia carrier (HbAT), HbE- β -thalassaemia diseased and also normal individuals. The ages of the patients ranged from 1 year and 10 months to 28 years with total haemoglobin percentages between 1.1 and 6.3. A few normal cases, having HbA₂ and HbF in the normal range, were reported as well, except sample 597 of family IB who showed foetal haemoglobin as 3.3% of the total haemoglobin.



Female: Normal \odot , β -Thalassaemia heterozygous \ominus , Hb E: heterozygous $\omin�$, Hb E- β -Thalassaemia $\omin�$, β -Thalassaemia homozygous \bullet , Not studied \circ Deceased \oplus , Propositus \blacktriangle
 Male: Normal \square , β -Thalassaemia heterozygous \boxminus , Hb E: heterozygous \boxplus , Hb E- β -Thalassaemia \boxplus , β -Thalassaemia homozygous \blacksquare , Not studied \square Deceased \boxplus

Figure 1. Pedigree analysis and haemoglobin composition of two typical families.

In family 4B, a patient (771) showed total absence of adult haemoglobin. This was the only case available with complete lack of adult haemoglobin. In family 6B, a patient (763) had a very low percentage of haemoglobin A (5.2). Family 7B had the usual pattern in the parents, one of them being a carrier of HbE and the other of β -thalassaemia (figure 1). The patient had a very high percentage of HbE (higher than HbE carrier) with little increase in the percentage of foetal haemoglobin (3.3). A brother (787) and a sister (791) carried β -thalassaemia but another brother (818) had relatively normal haemoglobin components.

Discussion

In the expression of β -thalassaemia two types of genes are involved. The gene β° suppresses β^A globin formation and results in the complete absence of HbA in homozygotes while the β^+ gene causes a decrease in the production of β^A globin but not its complete suppression (Conconi *et al.*, 1970; Weatherall and Clegg 1972). Earlier Chernoff *et al.* (1956), Wasi *et al.*, (1969) and Feldman and Reider (1973) suggested that β° type is more common in South east Asia since patients with HbE- β -thalassaemia usually show a complete absence of haemoglobin A.

From the results, as shown in table 1, which compares the percentages of different haemoglobins of the thalassaemia patients with those of HbE- β -thalassaemia, the earlier contention, that Hb β E and Hb β T interact to result in more drastic symptoms than HbE or HbT heterozygotes, is fully borne out. In all cases the HbE and HbF percentages are increased in the double heterozygotes (figure 1). The only exception is family 7B in which the patient, though diagnosed as a HbE-thalassaemic, shows a lower level of haemoglobin F than other similar cases. Apparently, in this patient the interaction between Hb β T and HbBE is either not complete or is influenced by some other factors.

In all the cases of homozygous β -thalassaemia studied here haemoglobin A₂ has been observed to be well within the normal range and below the percentage shown by the thalassaemia carrier of the same family. These observations support those of Weatherall and Clegg (1972), though Chatterjea *et al.* (1959) had earlier recorded an increased level of haemoglobin A₂ (8.0%) in thalassaemia homozygotes as well. The present investigation does not support the effort of Chatterjea *et al.* (1959) except in one case (family IIA, sample 818), where relatively high percentages of both haemoglobins F and A₂ were present in a β -thalassaemia homozygote. In the initial patient of the same family (821), however, haemoglobin A₂ was low though the individual had been diagnosed as a β -thalassaemia homozygote.

In Eastern India, on an average, β -thalassaemia is of quite frequent occurrence. The haemoglobin E gene occurs frequently with Hb β T and has been suggested to indicate the mongoloid element in the populations of this region (Flatz *et al.* 1972; Das and Flatz, 1975). As far as the present data show, haemoglobin A is present in rather high quantities even in HbE- β -thalassaemia patients (5.2 to 42.5%). The only exception is family 4B, patient (771), where it is totally absent. This patient had an enlarged spleen which had to be later removed. Taking the criterion that

the presence of β° type is denoted by the total absence of haemoglobin A, only in this case it appears that the gene involved is of β° type. In all other families studied here, apparently the gene is of β^{+} type since haemoglobin A has been recorded in appreciable quantities. This observation may indicate that these families do not have any Southeast Asian affiliations.

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