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Hereditary Persistence of Foetal Haemoglobin with Haemoglobin-E in a Maratha family from Bombay.

BY

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Hereditary persistence of foetal haemoglobin (HPFHb) is an inherited condition in which high levels of foetal haemoglobin is present throughout life and is not usually associated with anaemia. Absence of anaemia and associated abnormal red cell morphology, perhaps prompted the description of this condition as "non-microcythaemic thalassaemia" by Edington and Lehmann (1955) who first described this entity. Presence of foetal haemoglobin evenly distributed in all red cells rather than in separate clones and absence of increased Hb-A levels with microcytic hypochromia distinguished this condition from β-thalassaemia.

Simple heterozygote of HPFHb as well as instances of double heterozygosity with β-thalassaemia ; α thalassaemia ; Hb-S and Hb-C have been described (for references see Wasi et al., 1968). Only three cases of homozygous HPFHb have been hitherto reported. Clinical severity of this condition both in homozygous state and in mixed heterozygotes with thalassaemia or haemoglobin variants, was reported to be absent or at best mild. In contrast, cases of HPFHb with thalassaemia reported from this country presented severe and other clinical manifestations (Sukumaran et al., 1961; Parekh et al., 1963 and Bird et al., 1964). Schroeder and his group (1968) have shown that two or possibly four of the γ- chain loci which control the synthsis of two types of this chain are involved in the production of foetal haemoglobin. Based on this they have classified HPFHb into various groups (Huisman et al., 1969). First case of HPFHb with haemoglobin E was described from Thailand (Wasi et al., 1968).

Haemoglobin E is not uncommonly found in India. Besides regions in and around Bengal, presence of this gene was reported from U.P., Orissa, Punjab, Kerala and Madras in very few cases. In Maharashtra, cases of Hb-E with thalassaemia are not infrequent and were reported from Marathawada and Bombay region (Sukumaran et al., 1961; Udani et al., 1961, Lele et al., 1962). We have found cases of Hb-E trait, Hb-E thalassaemia and one case of homozygous E disease in Maratha families near Bombay. (Sukumaran, Unpublished observation). Recently we investigated a Maratha family with HPFHb and haemoglobin E and the carrier of this mixed heterozygous condition presented clinical manifestation characteristically distinct from one

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case reported earlier. This paper presents a report of this case with unusual features.

Materials and Methods

Haematological studies were carried out using standard techniques. Quantitative osmotic fragility was determined using Simmels Tyrode and percentage haemolysis in 0.4 dilution was found critical for evaluation (Sanghvi et al., 1958). In this dilution 80 – 100% haemolysis was noticed in normal subjects. Acid elution staining of Betke and Kleihauer (1958) was used to demonstrate individual cells containing foetal haemoglobin. Alkali-resistant haemoglobin was measured by the one-minute method (Singer et al., 1951). Electrophoreses were carried out using veronal buffer pH 8.6 and also discontinuous veronal-Tris buffer for paper, and Tris-Borate EDTA pH 8.6 for cellulose acetate. With the latter Hb-A_2 could be separated satisfactorily. Haemoglobin A_2 was estimated by elution after electrophoretic run on cellulose acetate and also paper elution method was used in parallel, for earlier observations showed that the results were comparable (Sukumaran & Master, 1971).

Results

Propositus, II-1 (fig. 1) was an 18-year old Maratha boy born of non-consanguineous parents. He was referred by a local doctor to one of us (A.S.V.) on 15-5-70 for evaluation of a heart murmur. The patient gave history of having had an attack of jaundice 5-6 months back and also having had low grade fever for some time. He complained of easy fatigu-ability. On examination he was found to be average built and nourished, conjunctive yellow and pale. Examination of cardio-vascular system

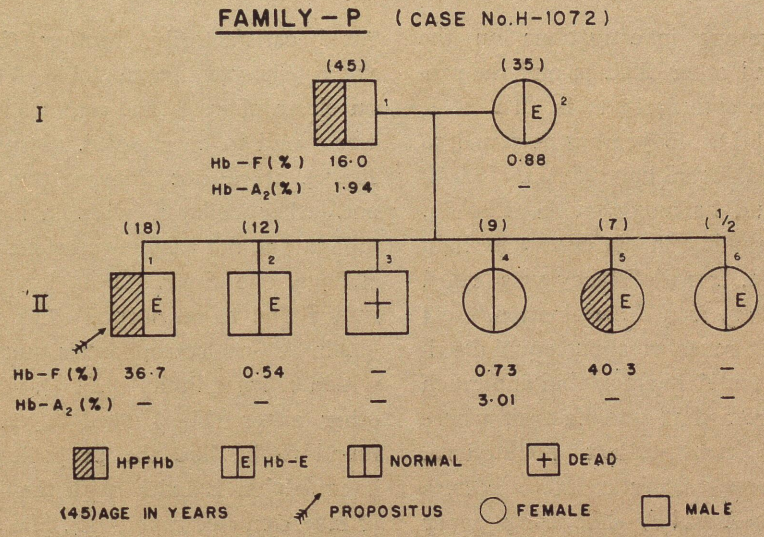

Fig. 1.— Showing pedigree of family-P.

indicated normal heart sounds, and a Gr. II/VI systolic murmur at the base of the heart. The ECG and X-ray were basically normal. The murmur was therefore considerd functional. His liver was found palpable 2 fingerbreadths and spleen 4 fingerbreadths below costal margin. It was decided to invstigate him for the cause of anaemia with special reference to haemoglobinopathy.

Blood exmination showed haemoglobin 10.4 gms. %. Blood picture was microcytic as seen from the indices and marked morphological abnormality of red cells including the presence of a few mucleated red cells. Osmotic fragility of red cells markedly decresed. Foetal haemoglobin was found to be increased (36.7 %). Acid elution staining revealed foetal haemoglobin in all the cells, though not homogeneously distributed. Electrophoresis showed haemoglobin F. No. haemoglobin A was detected (Fig. 2).

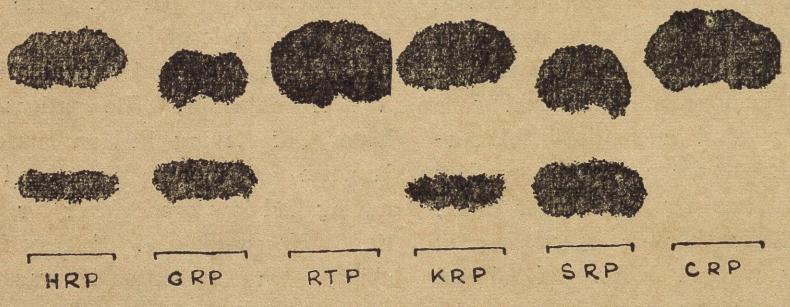

Fig. 2.— Paper electrophoretic pattern, Mother (HRP); Propositus (GRP); Father (RTP); Brother (KRP); and Sisters (SRP & CRP).

Laboratory investigations on the parents and other siblings and the propositus are summarised in Table 1. Father (I-I), 45 years was found to have raised foetal hemoglobin (16.0 %) with normal haemoglobin A_2 (1.94 %), Osmotic fragility in his case was slightly reduced. Iron deficiency was ruled out by serum iron determination. Acid elution test revealed about equal distribution of foetal haemoglobin and much different from thalassaemia trait where one finds two distinct populations. Electrophoresis using cellulose acetate showed Hb-A and F, while using paper electrophoresis this could not be separated satisfactorily. Mother (1-2) 35 years, showed haemoglobin A and E on electrophoresis and osmotic fragility was found to be reduced. Serum iron studies were not done as the mother and other children were in a far off village and not easily accessible. Of the siblings, one sister, (11-4), 9 years old was found to be normal with haemoglobin A on electrophoresis and Hb-A_2 within normal limits (3.01 %), while the other sister (11-5) aged 7 years was found to be like the propositus with haemoglobin E and F with the absence of Hb-A on electrophoresis. She showed increased Hb-F (40.3 %) and haemato-

TABLE 1.

HEMATOLOGICAL DATA OF FAMILY - P

Case	Sex & age in years.	Hb.%	RBC $10^6/mm^3$	MCV um^3	MCH pg	MCHC %	Retics %	Abnormal red cell Morphology.	Osmotic Fragility (%Haemolysis)	Hb-F %	Hb-A^2 %	Electrophoresis (Cellulose Acetate)
I-1 (RTP)	M 45	14.8	5.55	77.5	26.7	34.5	0.6	+	D (69.5)	16.0	1.94	A+F
I-2 (HRP)	F 35	11.0	5.04	77.2	21.8	28.3	0.2	+	D (46.6)	0.88		A+E
II-1 (GRP)	M 18	10.4	4.44	74.2	23.5	31.5	1.8	+++*	D (24.9)	36.7		E+F
II-2 (KRP)	M 12	14.8	5.54	86.0	27.2	31.5	1.2	—	N (82.5)	0.54		A+E
II-4 (CRP)	F 9	12.4	5.09	81.5	24.4	30.0	0.4	—	N (99.0)	0.73	3.01	A
II-5 (SRP)	F 7	8.4	4.05	71.5	20.8	28.9	3.0	++	D (54.0)	40.3		E+F

* 3 Nucleated Red Cells/100 WBC.

logical stigmata similar to her brother (propositus). Unfortunately no clinical examination could be done on her but was reported to be apparently healthy but somewhat pale according to her father. One brother, (II–2), 12 years old was found to be Hb–E trait with no haematological abnormality. A similar electrophoretic pattern showing Hb–A and E was found in the youngest sister (II–6) 6 months old. No detailed haematogical studies could be carried out on her for want of co–operation from the parents.

Discussion

Diagnosis of hereditary persistence of foetal haemoglobin in this family is made by the presence of high level of foetal haemoglobin (16 %) in the father. This is supported by the distribution pattern of Hb–F in red cells more or less similar in all cells, as opposed to distinct heterogeneous distribution found in thalassaemia or artifical mixture of adult and cord blood cells. Propositus and another sibling (II–5) seem to have inherited a gene for Hb–E from the mother and another gene for HPFHb from father resulting in a mixed heterozygote state in both of them. Hb–E trait is segregated in two children and one child is found to be normal. Haemoglobin status of the deceased child is not known and is reported to have died of dog–bite.

Marginal difference in the morphological abnormalities of red cells and decreased osmotic fragility generally found in– βthalassaemia trait, exhibited in the father with normal levels of haemoglobin A_2, in the absence of iron deficiency, are some of the features that make this case different from earlier reported traits of this condition. Distribution pattern of foetal haemo-globin in red cells rules out the possibility of this case being β-thalassaemia or even for that matter βζ-thalassaemia trait. Propositus produced no haemoglobin A but produced Hb-E in TRANS to the condition for HPFHb. Abnormal red cell morphology, decreased osmotic fragility and clinical severity as evidenced by mild anaemia and hepato-splenomegaly are features presented by the patient. The only family of similar condition reported so far is from Thailand wherein haemoglobin pheno type E + F showed target cells, decreased osmotic fragility, red cell indices similar to thalassaemia trait but no severe anaemia was found in them. Thus it appears that there can be cases of the same condition exhibiting different clinical and haematological manifestations. This is also true in the cases of HPFHb and β-thalassaemia found in India where most of them show moderate severity clinically and haematologically distinct from those reported from other parts of the world. In those cases anaemia and other clinical manifestations are said to be similar to those found in β-thalassaemia minor (Weatherall, 1965).

Like racial differences in thalassaemia, there are similar differences reported in HPFHb as found in Greeks and Negroes. Thus mean level of Hb-F in the Negro (25 %) is considerably higher

than that found in the Greeks (14.5 %) and similarly when found along with β-thalassaemia the Negro-type show higher levels (about 70%) while that of Greek or Caucasian-type was much less (about 35 %). The Caucasian type was found to be clinically more severe as well.

It is known that HPFHb can be divided into different groups depending on the structural differences of the gama-chain. Schroeder et al (1968) presented evidence that Ɣ-chain of the foetal haemoglobin is chemically heterogeneous. This conclusion was based on the structural analyses of a peptide termed CB3 at the carboxyl-terminal end of the Ɣ-chain. Cyanogen bromide (CB) used in treating globin F, cleaved wherever there was methionin residue and thus produced three peptides. The third peptide (CB3) consists of 13 amino acid residues starting with valine at 134th position. Sequence analysis showed there can be a glycyl or analyl residue at the 136th position of this peptide. The heterogeneity, therefore, consists in the presence of two kinds-of Ɣ-chains, one of which having a glycyl at position 136 termed G-gamma and the other at the same position with analyl termed A-gamma chains. These workers have shown that heterogeneity of human Ɣ-chain results from the activity of non-allelic structural genes. Table II shows the comparison of data on the subjects with different types of HPFHb conditions. With this in view work is in progress on the studies of various HPFHb including the present case on the structural analyses of their gamma chains. Preliminary data indicate that this case could be GƔ-type (Sukumaran et al, in press).

Table 2.

Comparison of Data on Subjects with Different Types of the HPFH Condition.

CRITERION/SUBGROUPS	1 CAUCASIAN	2 CAUCASIAN	3 NEGRO	4 NEGRO	5 NEGRO
Red cell morphology	normal	normal	normal	normal	normal
% Hb-A_2 in heterozygote	normal	decreased	decreased	decreased	decreased
% Hb-F in heterozygote	4-7	15-20	10-15	15-32	22-30
Distribution of Hb-F in red cells	about equal	equal	equal	equal	equal
No. of Gly residues in CB-3 (mean for simple heterozygotes)	0.09	0.09	1.06	0.15-0.51	0.53-0.69
Type of Gamma chain	AƔ	AƔ	GƔ	GƔ + AƔ	CƔ + AƔ

Sequence of Amino acid residues in CB-3 of Gamma chain originating from the C-terminal,

135					140				145	
Val - Thr	- GLY (ALA)	- Val -	Ala -	Ser -	Ala -	Leu -	Ser -	Arg	Try	- His

Similarly, finding of fair number of unrelated families in Marathas and other communities in Maharashtra showing Hb-E in whom, so far as can be ascertained, there existed ~~an~~ no indication of migration and resultant hybridization. It is known that the type of Hb-E found in South East Asian region is the type having an amino acid substitution Glutamic acid with Lysine at 26th position of the β-chain. It is assumed that the similar type is found in Burma and adjoining Bengal and its neighbouring states. In the light of the finding of a structurally different Hb-E (E Saskatoon) with substitution of similar amino acid residues as Glutamic acid with Lysine but at position 22 of the β-chain (Vella et al, 1967). Structural study of the Hb-E variant found in this area will perhaps give useful information on origin and spread of this gene in India.

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