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THALASSAEMIA SYNDROMES IN BOMBAY†

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Thalassaemia is an inherited erythrocyte abnormality due to a gene, which in the heterozygous condition, produces microcytosis with certain cytological abnormalities known as Thalassaemia minor, and in homozygous state, a profound anaemia known as Thalassaemia major which usually terminates fatally before of the age of ten. At times, family studies on clinically mild cases of thalassaemia indicate that one parent has the findings characteristic of thalassaemia and the other has, at best, doubtfut evidence for the diagnosis of thalassaemia. A number of such atypical parents have been shown to transmit a gene for abnormal haemoglobin. Largely through the use of paper electrophoresis many abnormal haemoglobins were found in association with thalassaemia gene. They include genes controlling the presence of haemoglobin S, haemoglobin C, haemoglobin D, haemoglobin E, haemoglobin G, haemoglobin H, haemoglobin Q, haemoglobin Bart's, haemoglobin F and Lepore trait. Some of these syndromes are clinically mild while others are almost symptomless. In India, interaction of thalassaemia with sickle cell gene with a presumptive diagnosis was reported from Bombay.[5] Cases of sickle cell-thalassaemia were also reported from Nagpur[10] and from Bengal.[3] Parekh *et al*[7]., reporting a case of haemolytic anaemia in a child, presented evidence of thalassaemia with sickle cell gene. Haemoglobin E associated with thalassaemia gene was reported from Bengal[2] and it may be said that upto now this is the only area in India from where haemoglobin E-thalassaemia cases have been reported. Haemoglobin D-thalassaemia has been reported from Bombay among the Gujarati-speaking and Sindhi-speaking Lohanas.[13] The only example in the literature of haemoglobin J associated with thalassaemia was also in a Gujarati Lohana.[9] Interaction of thalassaemia with high foetal trait was reported in a Christian family in Bombay.[12] The purpose of this paper is to present two groups of cases of double heterozygosity: one thalassaemia and haemoglobin S and the other thalassaemia and haemoglobin E. The former consists of three cases in two families, one of them of special interest being the only case in a Gujarati-speaking Caste Hindu (Dasasimali Bania) seen in Bombay. The second group consists of three cases of thalassaemia with haemoglobin E in a Muslim (Bohri) family from near Ahmedabad.

METHODS

Haematological investigations were carried out by the conventional methods. Foetal haemoglobin was estimated by the method of Singer *et al.*[11] Osmotic fragility was measured by using Simmel's tyrode in graded dilutions and percentage of lysis in various dilutions recorded.[9] 90-100% lysis occurred in large number of normal individuals tested in our laboratory at

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0.4 concentration and the amount of lysis at this critical dilution is presented in the table. Electrophoresis of haemoglobin was carried out by the method reported earlier.[14] A_2 fraction of haemoglobin was estimated by eluting the separated fraction after staining. Serum iron was estimated by the method of Davies *et al.*[4] Sickling test was done by using sodium metabisulphite.

Thalassaemia trait was identified by a combination of investigations. Peripheral blood in these cases is characterised by microcytosis due to reduction in the thickness of red cells which gives them the 'target' appearance. The hypochromia depends upon a low haemoglobin content of cells rather than upon low saturation. Red cell count may not be reduced and may indeed exceed normal. Packed cell volume may be reduced compared to the red cell count resulting in low MCV, but a normal MCHC. Flattened red cells give a reduced osmotic fragility. Serum iron value is normal in thalassaemia trait, but reduced in iron deficiency anaemia. Foetal haemoglobin may be normal or raised. A fraction of normal adult haemoglobin is found to be raised in contrast to a reduction reported in iron deficiency anaemia. The term 'thalassaemia trait' used throughout this paper would denote the above criteria for diagnosis and special reference will be made wherever they are incomplete.

I SICKLE CELL-THALASSAEMIA

T—Family: In September 1956, DJT, a Gujarati-speaking boy aged 4 years and 8 months was brought to the laboratory for investigation. The father, an intelligent person, noticed that the haemoglobin of this child, in spite of repeated and continuous administration of haematinics including iron, did not rise above 11.5 g.%. This refractory mild anaemia along with bone pains in the child were the manifestations responsible for further investigation. Clinically, slight pallor, a firm spleen of two fingers breadth below the costal margin and a just palpable liver, which according to the father, were persisting from the age of two years were observed. Past history suggested repeated attacks of fever diagnosed as tonsilitis, typhoid, malaria, etc.

Laboratory investigation on his blood showed red cell count to be 4.7 million per cmm. with haemoglobin 11.5 g.%. MCV was found to be 68 cu.μ. Osmotic fragility was decreased markedly. Reticulocyte count was 2.8%. Peripheral blood smear showed anisocytosis, poikilocytosis and fair number of target cells. Foetal haemoglobin was found to be raised to 15.6%. Haematinics did not produce any improvement on the hypochromic blood picture. Serum iron could not be estimated. Direct Coomb's test was negative. Sickling test was found to be positive. Paper electrophoresis revealed a single band simulating homozygous S pattern. To establish the haemoglobin genotype of this patient his parents and other members of the family were investigated.

The father I-4 (JMT) of the boy aged 43 years, Gujarati-speaking Ghanchi or Teli by caste, appeared to be healthy. His blood examination re-

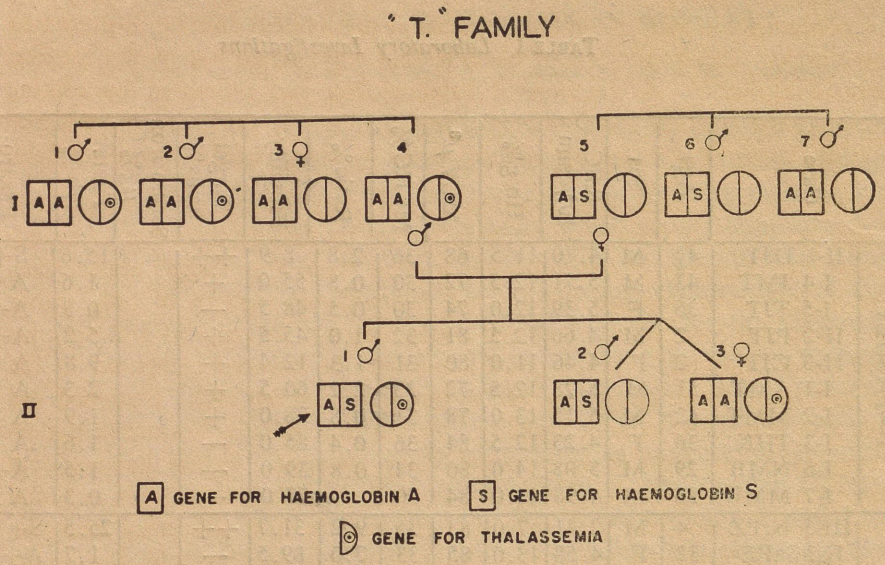


Fig. 1

vealed a thalassaemia trait picture on the criteria already mentioned in the beginning. Finding of a comparatively low haemoglobin, considering his red cell count, resulted in starting treatment for his mild anaemia. Repeated intake of iron and other haematinics is said to have showed no improvement in his haemoglobin. Serum iron was not done. Sickling was found to be negative on repeated examination. Electrophoresis of haemoglobin showed no abnormality. Foetal haemoglobin was 1.6%. The mother 1-5 (TJT) aged 36 years, a Gujarati convert Christian from Panchmahal district, was found to be apparently normal. Her blood examination showed sickling positive and electrophoresis revealed a trait pattern showing haemoglobin A and S. Osmotic fragility was found to be decreased with a microcytic picture of blood. Foetal haemoglobin was found to be normal. Sickle cell trait, confirmed by electrophoresis, was found in two more individuals in the family namely, the maternal uncle I-6 (NMB) aged 29 years and in another younger sib, a twin brother II-2 (PJT) aged 12 years. Except for a raised foetal haemoglobin of 5.18% in the twin brother the findings in both of them were essentially the same as that of the mother—a sickle cell trait. Thalassaemia trait was seen in two paternal uncles I-1 (AMT) and I-2 (MMT) aged 51 and 42 years respectively and also in the other younger sib, a twin sister II-3 (CJT) aged 2 years, in whom foetal haemoglobin was found to be 9.8%. Sickling was negative in all of them. Finding of raised foetal haemoglobin in both the twins is difficult to explain at this young age. The other two members in the family, paternal aunt I-3 (Mrs. THK) aged 36 years and the maternal uncle I-7 (MMB) aged 26 years were normal and did not reveal anything contributary to the diagnosis.

TABLE I *Laboratory Investigations*

	Case	Age	Sex	RBC 10^6/cmm	Hb g%	MCV μ^3	MCHC%	Retic%	Fragility	Target cells%	Serum iron μg%	Foetal Hb %	Electrophoretic pattern
T—FAMILY	II-1 DJT	4¾	M	4.70	11.5	68	36	2.8	8.9	++		15.6	S+F
	I-4 JMT	43	M	5.71	12.5	72	30	0.8	55.0	+		1.6	A
	I-5 TJT	36	F	5.39	12.0	74	30	0.5	48.5	—		0.8	A+S
	II-2 PJT	2	M	4.66	12.5	81	32	1.0	45.5	+		5.2	A+S
	II-3 CJT	2	F	4.46	11.0	80	31	1.3	12.4	+		9.8	A
	I-1 AMT	51	M	5.28	12.5	72	33	2.2	60.5	+		2.3	A
	I-2 MMT	42	M	5.15	13.0	78	31	0.8	28.0	+		1.9	A
	I-3 THK	36	F	4.23	12.5	84	36	0.4	85.0	—		1.6	A
	I-6 NMB	29	M	5.08	14.0	80	34	0.8	39.0	—		1.5	A+S
	I-7 MMB	26	M	4.98	13.0	84	30		77.0	—		0.3	A
Z—FAMILY	III-3 NrPZ	4	M	2.41	7.0	83	35	9.2	31.7	++		25.5	S+F
	II-3 SaPZ	32	F	4.58	13.0	85	33	2.0	89.5	—		1.7	A+S
	II-4 PHZ	43	M	5.71	13.0	74	31	3.2	35.3	+	90	0.7	A(A_2-6.4%)
	III-1 SoPZ	8	F	4.87	11.0	62	36	4.6	32.0	++		15.7	S+F
	III-2 BPZ	6	F	4.97	10.5	64	33	2.6	44.0	++		3.3	A(A_2-4.2%)
	III-4 NyPZ	1¼	F	4.34	12.0	83	33	0.4	90.0	—		1.3	A(A_2-1.3%)
	I-1 DHZ	62	F	4.46	11.0	76	32	1.2	55.0	a few	91	2.7	A
	II-2 KK	37	F	4.05	8.5	74	28	0.8	66.0	a few	45	1.7	A
	II-1 DM	44	M	4.62	13.0	90	31	0.5	100.0	—	135	1.7	A

Z-Family: In May 1957, a male child aged 4 years was brought to our laboratory with complaints of frequent attacks of cold and cough with progressive weakness and pallor for the last two years. Both the parents belong to Guparati-speaking Bania (Dasasimali) caste from Saurashtra. The child was said to be bright in colour till he was a year old. Since then, frequent attacks of cold and cough were followed by progressive pallor. In spite of repeated treatment with haematinics and iron he showed no improvement. General examination showed a poorly built, poorly nourished boy with pale conjuctiva and nails. Glands in the axilla were found to be enlarged and bossing of the skull was noticeable. Spleen was enlarged to 2½ fingers below the costal margin, was smooth and firm, and liver was just palpable, otherwise nothing abnormal was detected.

Laboratory examination revealed anaemia with haemoglobin 7 g. %, red cell count 2.41 million per cmm. and increased reticulocytes of 9.2%. Osmotic fragility was decreased. The peripheral smear showed marked anisocytosis, poikilocytosis, number of target cells and a few normoblasts. Direct Coomb's test was negative. Sickling test was found to be positive with a few cells of filamentous forms of sickling. Foetal haemoglobin was found to be 25.5%. Electrophoretic study of haemoglobin showed haemoglobin S pattern without any adult fraction.

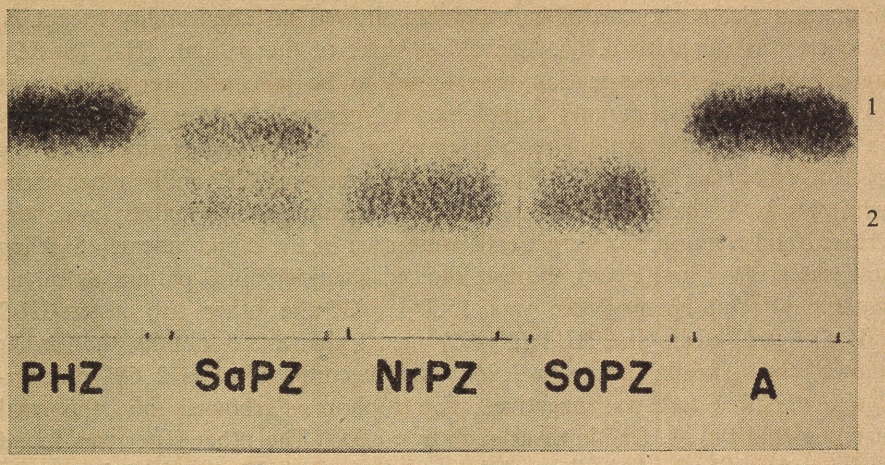


Fig. 2 1—Hb-A 2—Hb-S

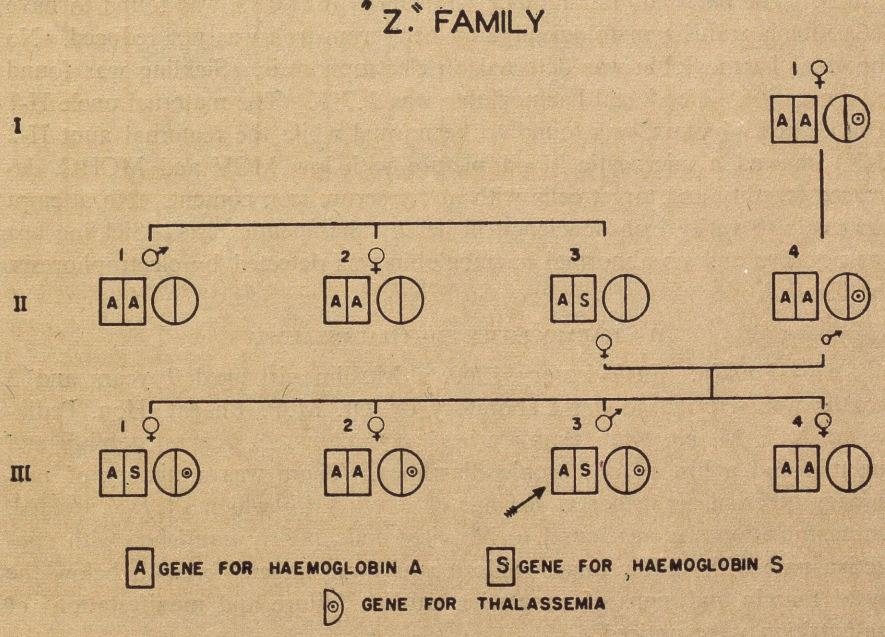


Fig. 3

Detailed study of the family showed the mother of the patient II-3 (SaPZ) aged 32 years to have a sickle cell trait showing haemoglobin A and S components on electrophoresis with positive sickling test. She is said to be healthy except for feeling of fatigue on exertion. Her blood examination showed nothing abnormal except the above findings. The father II-4 (PHZ), aged 43 years, showed thalassaemia trait with raised reticulocyte count of 3.2% and serum iron of 90 μ g.%. Foetal haemoglobin was within normal

limits and electrophoresis of haemoglobin showed no abnormality except A_2 fraction was raised to 6.4%. The eldest sister of the patient, III-1 (SoPZ), aged 8 years, on examination, was found to be pale and her spleen was just palpable. The parents complained of general weakness and lethargy in her. Blood examination revealed findings essentially the same as in the patient except for anaemia. Her red cell count was 4.87 million per cmm. with 11 g.% haemoglobin and 4.6% reticulocytes. Fragility was decreased and a number of target cells were seen in the blood smear. Sickling test was positive and electrophoresis showed haemoglobin S pattern as in the patient. Foetal haemoglobin was 15.7%. Of the other two sisters-III-2 (BPZ) aged 6 years and III-4 (NyPZ) aged 1 year and 3 months, the former showed thalassaemia trait with 3.3% foetal haemoglobin and 2.6% reticulocytes. Sickling test was negative. Electrophoresis showed no abnormal haemoglobin but A_2 fraction was slightly raised. The other sister was found to be normal, both clinically and haematologically. Electrophoretic pattern of her haemoglobin showed no abnormality and A_2 fraction was found to be normal. The paternal grandmother I-1 (DHZ), 62 years, was found to have thalassaemia trait haematologically and her serum iron was not reduced. No abnormal haemoglobin was detected on electrophoresis. Sickling was found to be negative. Her foetal haemoglobin was 2.7%. The maternal uncle II-1 (DM), aged 44 years, was found to be normal while the maternal aunt II-2 (KK) showed a microcytic blood picture with low MCV and MCHC, decreased fragility and target cells with a low serum iron content. No attempt was made to verify iron deficiency in her by therapeutic tests. Sickling test was negative and no abnormal haemoglobin was detected by electrophoresis. See Table I.

II HAEMOGLOBIN E—THALASSAEMIA

AR—Family: In February 1960, a Muslim girl aged 7 years and 3 months was referred to our Laboratory by Dr. M.P. Bhagat, Hon. Pediatrician, K.E.M. Hospital, Bombay, with complaints of general debility and anaemia and a history of chronic diarrhoea. There was a history of pulmonary tuberculosis in her at the age of 3 years for which she was treated. On examination she was found to be fairly built, poorly nourished with conjuctiva pale with yellow tinge. Spleen was three fingers' breadth below the costal margin and signs of congestive cardiac failure and manifestations of malnutrition were noticed.

Her blood examination revealed anaemia with haemoglobin 5.8 g.% and red cells 2.9 million per cmm. MCV was low with a low MCHC. Reticulocyte count was 11% with decreased osmotic fragility. Direct Coomb's test was negative. Sickling was negative on repeated examination. Her blood smear showed target cells, marked anisocytosis and poikilocytosis and plenty of normoblasts (35 for 100 WBC). Foetal haemoglobin was found to be 35%. (Fig. 4) Electrophoresis showed two fractions, one major component showing mobility similar to haemoglobin F and A while the other with slow mobility, slower than haemoglobin S. When compared with a

known haemoglobin sample having haemoglobin A and C, kindly made available by Dr. J. C. White of London, this component in the patient was found to move faster than the haemoglobin C fraction in the control. A comparison with the haemoglobin from a known case of thalassaemia-haemoglobin E disease showed the slow moving fraction to be haemoglobin E.

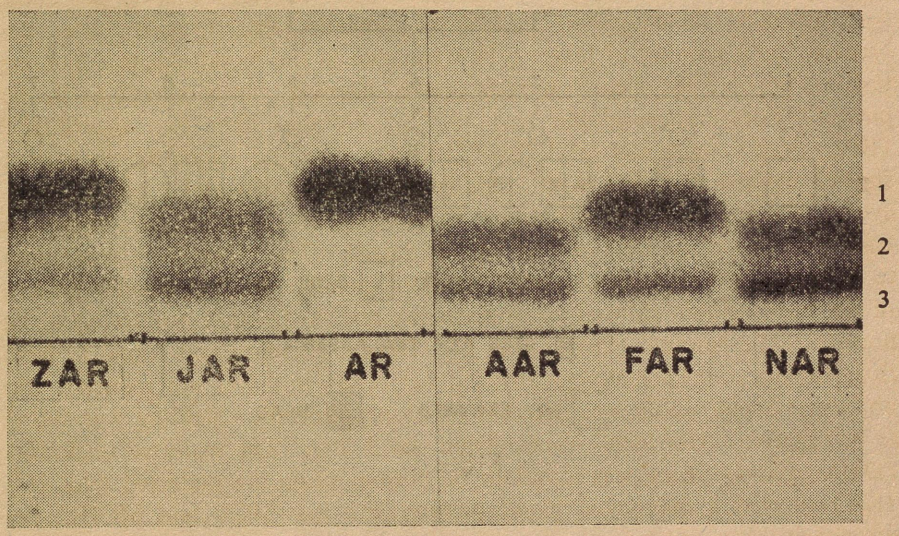


Fig. 4 1 Hb-A 2 Hb-F+A 3 Hb-E

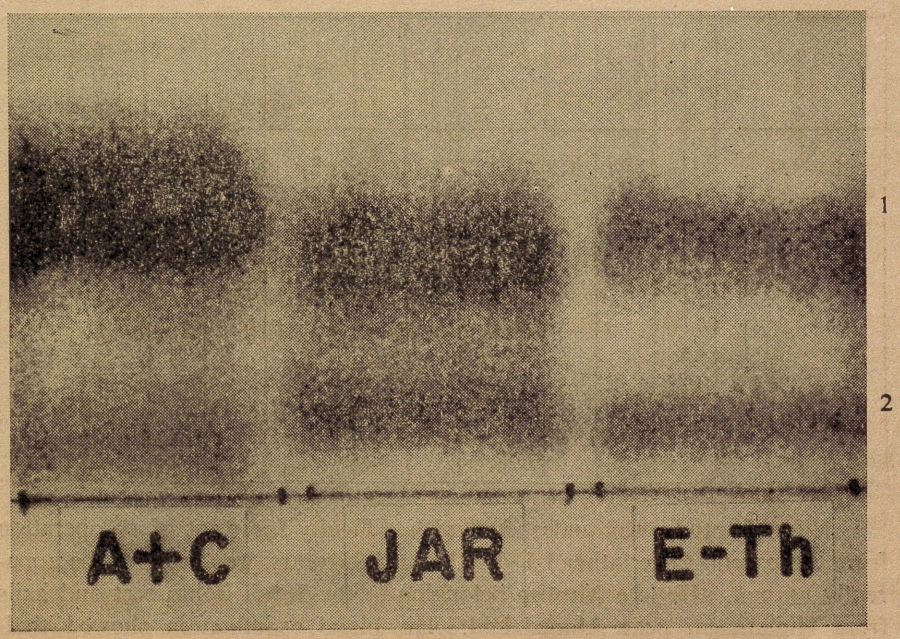
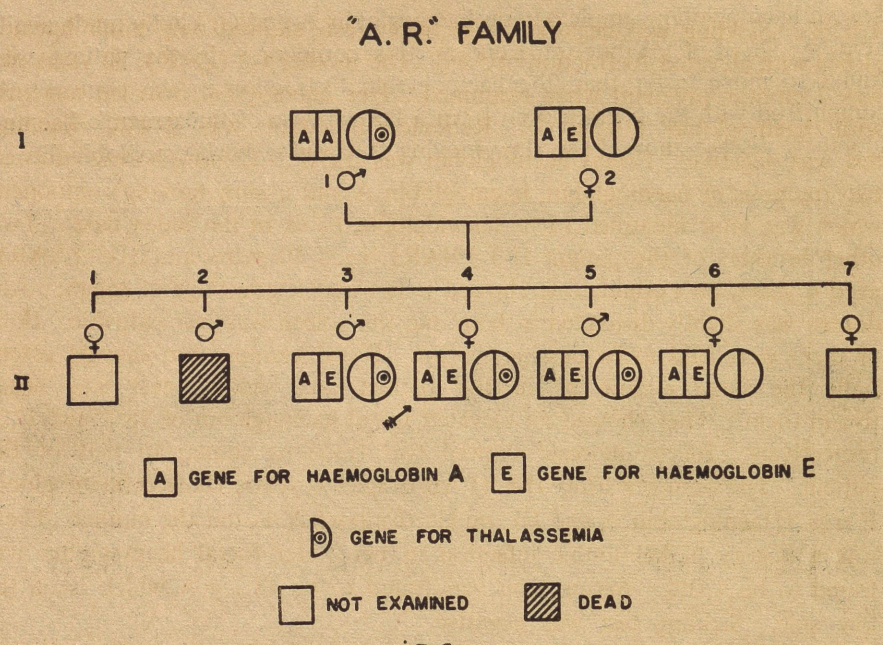


Fig. 5 1 Hb-F+A 2 Hb-E



Fig·6

TABLE II *Laboratory Investigations*
Ar-Family

Case	Age	Sex	10^6/cmm RBC	Hb g%	MCV μ^3	MCHC%	Retic%	Fragility	Target cells %	Serum iron μg%	Foetal Hb %	Electrophoretic pattern
II-4 JAR	7¼	F	2.90	5.8	76	27	11.0	21.6	+		35.0	E+F
I-1 AR	35	M	6.50	14.2	71	31	2.0	40.5	++	86	4.0	A(A_2-5.8 %)
I-2 ZAR	30	F	4.42	6.4	55	26	3.2	65.0	a few		2.3	A+E
II-5 AAR	6	M	3.88	7.1	64	29	3.4	14.0	+++		38.0	E+F
II-3 NAR	10	M	4.41	7.4	61	27	2.6	11.0	+++		32.0	E+F
II-6 FAR	4	F	5.41	11.6	70	30	2.0	21.5	a few	48	4.0	A+E

A family study was undertaken with difficulty for want of co-operation from the parents. The father I-1 (AR), aged 35 years, was found to be apparently normal. He is a Muslim belonging to the Sunni Bohra sect and hails from Himatnagar near Ahmedabad. His blood examination showed thalassaemia trait with serum iron level of 86 μ mg.%. Foetal haemoglobin was

4.03%. Sickling was negative. Electrophoresis showed only adult haemoglobin with elevated A_2 fraction of 5.8%. The mother I-2 (ZAR), 30 years old was 7 months pregnant when examined. Her haemogram revealed anaemia with haemoglobin 6.4 g. %. Reticulocyte count was 3.4%. Sickling test was negative. Foetal haemoglobin was 2.3%. Electrophoresis showed two fractions of haemoglobin, haemoglobin A and a slow moving component which was later identified to be haemoglobin E, as in the index case. Two other brothers of the patient II-3 (NAR), aged 10 years and II-5 (AAR), aged 6 years, on examination, showed pallor and icteric tinge of sclera. Abdomen was slightly distended in both and the spleen was just palpable. Both of them were said to be passing worms. Blood examination showed essentially the same picture as in the patient and sickling test was negative in them. They showed an elevated foetal haemoglobin of 38% and 32% respectively. Electrophoresis showed two fractions similar to that of the patient. The younger sister II-6 (FAR), aged 4 years, showed haemoglobin E trait (Haemoglobin A and E), on electrophoresis, as did the mother. There was a history of helminthic infection in her. Her foetal haemoglobin was found to be 4.0%. Serum iron was found to be 48 μg.% which is on the low side. Sickling test was negative.

TABLE III *Thalassaemia Syndromes–Comparative Data on Laboratory Findings*

Syndrome	Case	Age	Sex	RBC 10^6/cmm	Hb g %	MCV μ^3	MCHC %	Retic%	Fragility	Target cells%	Serum iron μg%	Foetal Hb%	Electrophoretic pattern
	DJT	4¾	M	4.70	11.5	68	36	2.8	8.9	++		15.6	S+F
Thal+Hb S	Nr PZ	4	M	2.41	7.0	83	35	9.2	31.7	++		24.5	S+F
	SoPZ	8	F	4.87	11.0	62	36	4.6	32.0	++		15.7	S+F
	JAR	7½	F	2.90	5.7	76	27	11.0	21.6	+		35.0	E+F
Thal+Hb E	AAR	6	M	3.88	7.2	64	29	3.4	14.0	+++		38.0	E+F
	NAR	10	M	4.41	7.4	61	27	2.6	11.0	+++		32.0	E+F
	SDJ	34	M	6.48	13.0	65	31	3.4	5.0	+++	173	1.7	D
	JJT	25	F	5.68	7.2	50	26	2.2	13.0	+++	41	0.8	D
Thal+Hb D	NMT	16	M	6.33	13.3	70	31	2.0	9.0	+++	138	6.5	D
	MMT	11	F	5.21	10.2	65	30	2.8	9.0	++	129	3.4	D
	BIR	31	F	5.20	10.0	65	30	3.0	41.0	++	116	60.5	F
Thal+High F	CR	23	M	4.69	9.0	61	30	3.2	31.4	++	88	47.2	F
Thal+Hb J	MK	28	F	5.61	12.0	68	31	2.8	38.0	+	125	2.8	A+J

DISCUSSION

The data presented show sufficient evidence of double heterozygosity for genes for thalassaemia and haemoglobin S and haemoglobin E. This is observed in some sibs besides the propositus in each of the families. These genes found in heterozygous state in the parents add to the evidence. Finding of sickle cell gene in a family of Gujarati-speaking Dasasimali Bania

—is of special significance as this gene so far is reported almost exclusively in Adivasis i.e. tribal population from Gujarat.[14] Haemoglobin E is found confined to Bengal and Assam in India and findings of this gene in other parts of the country have to be accepted with caution. This is exemplified from the study presented on 'A.R.' family. It is said that I-1 (AR) the head of the family hails from Himatnagar near Ahmedabad and conventionally the finding of haemoglobin E in the family would be construed as found in that area. Careful interrogation revealed that the late maternal grand-mother of the propositus of this family originated from Bengal, which is a fair indication of the origin of haemoglobin E gene in this family through the mother.

The electrophoretic pattern found in double heterozygosity for thalassaemia and haemoglobin S is almost indistinguishable from that seen from homozygous sickle cell diseases. Suppression of the expressivity of haemoglobin A by thalassaemia gene is the explanation given for such findings where this gene is associated in double heterozygous state with abnormal haemoglobin. The various thalassaemias encountered by us in Bombay, where haemoglobin abnormality is involved, included haemoglobin D-thalassaemia, haemoglobin J-thalassaemia, and thalassaemia with gene for high foetal haemoglobin. Haemotological data on the 13 cases in this group is presented in the table for comparison. It will be seen that the degree of anaemia is most in haemoglobin E-thalassaemia, progressively less in sickle cell thalassaemia, thalassaemia with high foetal haemoglobin, thalassaemia-haemoglobin D and lastly thalassaemia-haemoglobin J. Anaemia with variable severity was noticed amongst the cases within each of the group. Foetal haemoglobin content varied in these groups. It is well known that in thalassaemia major, foetal haemoglobin varies from 35 to 90% of the total haemoglobin. In thalassaemia-haemoglobin E diseases foetal haemoglobin ranges from 10 to 40% and the three cases reported here showed 30 to 38%. High foetal haemoglobin upto 60% is seen in the newly established syndrome, a double heterozygous state, with thalassaemia and high foetal gene. Our cases show 40 to 60% in two individuals reported. Similar high foetal contents were seen in cases investigated at Liberia.[6] High foetal trait is evidenced by the persistence of high foetal haemoglobin in the absence of any haematological abnormality, target cells, microcytosis and decreased fragility. Foetal haemoglobin in thalassaemia-haemoglobin S is found to vary from 5 to 25% while in our cases it varied from 15 to 25%. Our cases of thalassaemia-haemoglobin D showed 0.6 to 6% of foetal haemoglobin. In all these cases the gene action of thalassaemia resulting in the suppression of adult haemoglobin and resultant enhancement of abnormal haemoglobin production is noticeable. Thalassaemia-haemoglobin J stands as an exception to this. It may be mentioned that haemoglobin J falls into the category of fast moving haemoglobin while the others mentioned above are with mobility slower than adult haemoglobin in alkaline medium. In the case of haemoglobin J in association with thalassaemia, a solitary example reported, shows no suppression of haemo-

globin A or increase in foetal haemoglobin. Study on more cases would be necessary before any conclusion is drawn on this exceptional finding. In this connection, it may be said that in haemoglobin H-thalassaemia where haemoglobin H is a fast moving component, no suppression of haemoglobin A is noticed. Perhaps haemoglobin H falls in a different category as this haemoglobin is not found singly in heterozygous state. Variability of foetal haemoglobin found in the different syndromes poses a question whether this is a direct result of the action of thalassaemia gene or incidental, being the result of suppression of adult haemoglobin by this gene. It has been suggested by Rich[8] that foetal haemoglobin is produced to compensate for the marked decrease in the amount of normal adult haemoglobin synthesised by people with thalassaemia major. In normal infants a switch-over mechanism exists which causes foetal haemoglobin production to be replaced completely by adult haemoglobin production within about 6 months of birth. On the other hand, certain factors are believed to enhance the expressivity of haemoglobin F and among these thalassaemia is most active. Great variability of thalassaemia in clinical manifestations and haematological abnormality suggests that there is not one kind of thalassaemia minor or major but more, one of which may be responsible for the expression of foetal haemoglobin. Findings of persistently high foetal haemoglobin with no haematological abnormality and inherited in three generations in the family studied by us, supports the existence of a separate gene for high foetal haemoglobin. Evidence of this gene being independent of thalassaemia gene is found in the case reported wherein this gene was associated with sickle cell trait. So far, gene for high foetal haemoglobin has been reported only in Negroes, and finding of this gene in Bombay among a Christian family is interesting. Considering the clinical severity of various thalassaemia syndromes, cases of thalassaemia-haemoglobin D seem to be least affected in spite of the suppression of adult haemoglobin, the difference being the lower concentration of foetal haemoglobin in them. Of the individuals investigated, two were adults who had children. Our studies indicate that there is a close bearing on the clinical severity and foetal haemoglobin content present in them. Further data may be necessary to draw a definite conclusion. Haemoglobin D is reported to exist in three forms ($D\alpha$, $D\beta$ & $D\gamma$) having same properties when studied by the physical method available, but differing in the finer composition of the poly-peptide chains. Haemoglobin D found in the Punjab is said to be $D\gamma$ form and it would be interesting to know the form to which haemoglobin D found in Bombay belongs and if found to be γ type, its relationship with thalassaemia. As long as genetics of thalassaemia are not fully unravelled, thalassaemia syndromes would remain, interestingly enough, a problem to clinicians and geneticists.

SUMMARY

Two groups of thalassaemia syndromes associated with abnormal haemoglobins are presented in detail. The first group consists of three cases in two Gujarati-speaking families where haemoglobin S is involved. Of this,

one is of special interest being found in a caste Hindu (Dasasimali Bania). The second group presented gives evidence of interaction of haemoglobin E with thalassaemia in three cases from a Muslim family. Other thalassaemia syndromes encountered in Bombay include association of thalassaemia with (a) haemoglobin D, (b) haemoglobin J and (c) high foetal trait. Some salient features of these various syndromes with special reference to haematological and genetical aspects of these conditions are discussed.

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THALASSAEMIAS

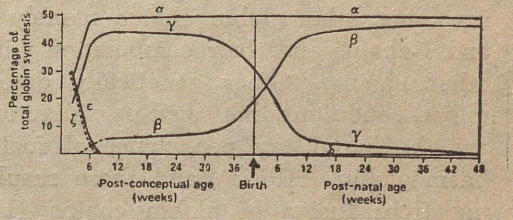
P. K. Sukumaran

Cooley and Lee[1] in 1925 described a type of blood disorder of infancy and childhood showing chronic progressive anaemia, characteristic facies, splenomegaly and familial incidence. Findings of this disease in the Mediterranean region led to the introduction of the term 'Thalassaemia' (thalassa, the sea + haima, blood). The clinical severity of the conditions classified under thalassaemia is however, highly variable. At one extreme thalassaemia causes death in utero and ranges from death in children through chronic anaemia to the other extreme when it is asymptomatic and virtually undiagnosable. Nevertheless, they are all characterized by reduced rate of synthesis of varying degree of one or the other of the globin chains of the haemoglobin.

Thalassaemias are spread widely throughout the world population. The geographical distribution of the gene offers evidence that heterozygotes are protected against malaria, though the nature and timing of the protective effect are still not clear. Because of the frequency and severity of the anaemia in the homozygous state, such conditions produce major public health problems in many countries. In spite of the better knowledge of the basic defect of the disease the treatment still remains largely symptomatic and empirical.[2]

Heterogeneity of Normal Human Haemoglobin

Human haemoglobin is heterogeneous. All the normal human haemoglobins have one pair of α-chains and another pair of non-α-chains. In adult haemoglobin, Hb A ($\alpha_2\beta_2$) forms about 97%; Hb A_2 ($\alpha_2\delta_2$) about 2.5% and traces of Hb F ($\alpha_2\gamma_2$) are found. In intrauterine life Hb F forms the major component and there are also Hb $Gower_2$ ($\alpha_2\epsilon_2$): Hb Portland ($\zeta_2\gamma_2$) and Hb $Gower_1$ ($\zeta_2\epsilon_2$). Globin chain synthesis during human development is shown in Fig. 1. It is now known



α, β, γ, δ, ε and ζ refer to types of globin chain. Interrupted lines show the probable pattern of globin synthesis in early fetuses, the ζ-chain pattern being based on the assumption that Hb Gower I has the composition $\zeta_2\varepsilon_2$ (Huehns & Farooqui, 1975)

Fig. 1. Globin chain synthesis during human development (Ref. No. 28).

that there are single loci controlling the structure of the ß- and δ-chains. In the case of γ-chains there are two forms which are products of separate structural genes. Evidences indicate the presence of more than one locus on the same chromosome controlling α-chains atleast in some population. Recent DNA/DNA hybridization data are consistent with these views.[3]

Classification of Thalassaemias

Disorders of haemoglobin in thalassaemia are due to the reduced rate of synthesis of one or more of the globin chains of the haemoglobin leading to un-

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balanced globin chain synthesis. Unbalanced synthesis of globin chains has been demonstrated in peripheral blood reticulocytes by measurement of the incorporation of a radio-active amino acid into the globin chains.

There are no electrophoretically distinguishable abnormal haemoglobins present. Most of the clinical features of thalassaemias can be attributed to the deleterious effects on erythropoiesis caused by the precipitation of the globin chains that are produced in excess. Commonly found thalassaemias are those affecting ß-chain synthesis (ß-thalassaemia) and those affecting α-chains (α-thalassaemia). There are also those involving other globin chains namely γ and δ-chains and their combination with other chains. Other genetically determined conditions that need consideration are the hereditary persistence of foetal haemoglobin (HPFH) and those resulting from fusion of globin chains (Hb Lepore δß-crossover; Hb Kenya γß-crossover). In areas where there are structural haemoglobin variants (Hbs S, C, D, E, Q) and thalassaemia, the latter existing in combination with haemoglobin variants are liable to present difficulties in diagnosis.

ß-thalassaemia. There is considerable variability in the genetic and clinical manifestations of ß-thalassaemia. In true ß-thalassaemia ß-chain synthesis alone is affected. Those in which there appears to be a total absence of ß-chains are called ß°-thalassaemia and those wherein some ß-chains are produced are ß+-thalassaemia. Available evidences suggest that there is a defect in transcription or mRNA processing which results in complete absence of ß-chain mRNA production in ß°-thalassaemia while in ß+-thalassaemia there is reduced activity of viable mRNA.[4, 5] The levels of haemoglobin A is variable in ß+-thalassaemia. Their severity is also variable. Beta°-thalassaemia is severe requiring blood transfusions. Even in this group heterogeneity is reported using a variety of techniques.[6]

Thalassaemia major or Cooley's anaemia, a homozygous condition, is characterized by the onset of anaemia at about three months of life. Progressive anaemia, splenomegaly and hepatomegaly, characteristic bone changes and stunted growth are the main manifestations. As age advances they may suffer from endocrine deficiencies, liver damage and finally die of cardiac failure due to haemochiomatosis of the cardiac muscle. Children with thalassaemia intermedia, a clinically mild disease, may grow and develop normally although they have splenomegaly and associated skeletal changes.

Blood picture is characteristic with marked hypochromia, nucleated red cells, reticulocytosis and marked erythroid hyperplasia of the bone marrow presenting typical X-ray changes. Inclusion bodies made up of precipitated α-chains in red cell precursors in the bone marrow can be seen when stained with methyl violet.[7] Peripheral blood contains variable amounts of foetal haemoglobin ranging from 20-100% of the total haemoglobin depending on the type of thalassaemia. Pathophysiology of ß-thalassaemia is schematically represented in Fig. 2.

Individuals with thalassaemia minor are fit, but they may occasionally present with severe anaemia when additional strain is thrown on the haemopoietic system. They may show severe anaemia when associated with malnutrition, parasitic infestations or chronic infection. Thalassaemia carriers may deve-

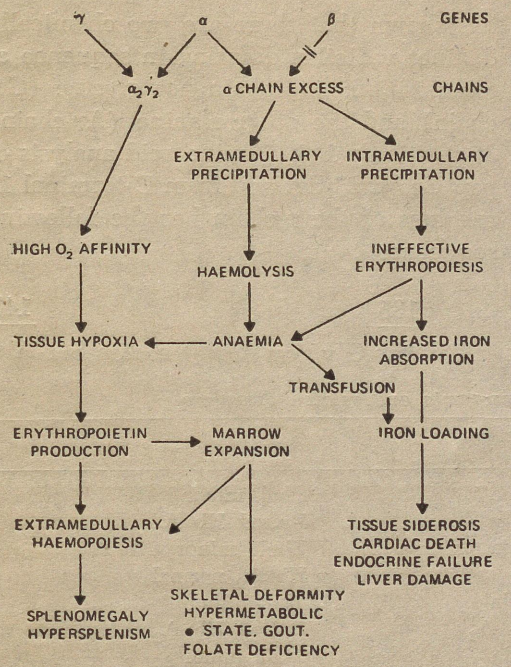

Fig. 2. A schematic representation of the pathophysiology of ß-thalassaemias.

lop quite significant anaemia during pregnancy and this may lead to abortions at times.

Blood picture in thalassaemia traits show microcytosis, hypochromia and can be mistaken for iron deficiency. Serum iron studies show normal values. Osmotic fragility is decreased and there is a definite increase of Hb A_2 levels. Iron deficiency causes a decrease in Hb A_2 levels and in ß-thalassaemia trait with iron deficiency, Hb A_2 levels may go down to normal levels.[8, 9] Hb F may or may not be raised, depending on the gene variability of the condition.

Present day management of thalassaemia is based on correcting recurrent anaemia and its effects by repeated blood transfusions for life. The effect of iron overload is eleminated with chelating agents. A new iron-chelating agent that can be taken by mouth (2, 3 dihydroxybenzoic acid) now undergoing clinical trials seems to be promising.[10] In view of the limited scope of treatment, recent advances with intrauterine diagnosis of thalassaemia and subsequent therapeutic abortions may become more practical in future.

A gene for common abnormal haemoglobin variants (Hbs S, C, D and E) can exist in the same individual with ß-thalassaemia gene as double heterozygote. In such cases little or no Hb A will be found and sometimes may be mistakenly diagnosed as homozygotes for abnormal haemoglobins.

Though $ß^+$-thalassaemia is the common type found in the West, $ß^0$-thalassaemia is said to be most common among the Orientals, considering the severity of the disease.

δß-thalassaemia and Hb Lepores: This form of thalassaemia is the result of the defective synthesis of δ-and ß-chains. As a result of this, heterozygotes show Hb A, Hb A_2 and Hb F. Levels of Hb F may range from 5 to 20%. In homozygotes only Hb F is seen with no Hb A and Hb A_2, and may have clinical findings similar to thalassaemia intermedia. Distribution of Hb F in red cells is heterogeneous. Beta-thalassaemia associated with δß-thalassaemia show extremely variable clinical features with haemoglobin ranging from 5 to 13 gm% with Hb F in the range of 40 to 80%. Hb A_2 is normally low and Hb A may or may not be demonstrable.

Hb Lepore is a haemoglobin containing two normal α-chains paired with two abnormal chains. Each of the abnormal chains consists of an initial segment of normal δ-chain terminated by part of the normal ß-chain and characterized as $\alpha_2(\delta ß)_2$. $(\delta ß)_2$. Different Hb Lepores are known, each having a crossover at differ-

ent positions. Homozygotes show Hb F and Hb Lepore with absence of Hb A and Hb A_2. Clinical manifestations of this condition resemble ß-thalassaemia major. Examples of $\alpha_2(ß\delta)_2$ haemoglobins with N-st terminal ß-chain sequences and C-terminal δ-chain sequences are known, and designated as Hbs Anti-Lepore.[2] Data based on haemoglobin synthesis studies on some ß- and δß-thalassaemia and related disorders are shown in Table 1.

TABLE I—*The ß- and δß-thalassaemia and related disorders*

Type of thalassaemia	Homozygote	Heterozygote
ß-thal°	Cooley's anaemia Hbs F + A_2	Thal. minor Raised Hb A_2
(δß)-thal°	Cooley's anaemia Hb F only	Thal. minor; Hb F 5-15% Hb A_2 normal
ß-thal$^+$	Cooley's anaemia Hbs A + F + A_2	Thal minor Raised Hb A_2
ß-thal$^+$ (Negro)	Thal. intermedia Hbs A + F + A_2	Thal. minor Raised Hb A_2
ß-thal$^+$ (High F)	Thal. intermedia Hbs A + F + A_2	Thal. minor; Hb F 5-12% Raised Hb A_2
δß-Lepore	Cooley's anaemia Hbs F + Lepore	Thal. minor Hbs. A + Lepore + A_2
δß-thal$^+$ (Silent)	Not described	Minimal blood changes Normal Hbs F and A_2
Excess α-chain production	Not described	Thal. intermedia and inclusion bodies Raised Hb A_2

Reference No. 29.

Hereditary persistence of foetal haemoglobin (HPFH): This is a condition in which process of switching-off of the γ-chain and turning-on of the ß-and δ-chain production fail to occur. This results in the continued production of Hb F in adult life. Heterozygotes for this condition are found distributed across Africa (where it was first described), the Mediterranean, India to South East Asia. Homozygotes are rather rare, while double heterozygotes along with abnormal Hbs S, C or E as well as ß-thalassaemia are found.

Structural analysis of γ-chain of Hb F has shown that there are two chemically distinct varieties differing in sequence at one position; one chain has glycine (Gγ-chain) and the other alanine (Aγ-chain) at position γ 136. Amino acid analysis of peptide γCB3 derived from C-terminal 13 residues of the γ-chain has the following sequence:

135 140

Val-Thr-Gly (or Ala) Val-Ala-Ser-Ala-

145

Leu-Ser-Ser-Arg-Tyre-His.

Based on the structural differences, Hb F can be atleast of three types (Gγ; Aγ and Gγ Aγ) and depending on the amounts of Hb F they can be further classified.[11] One important finding in HPFH condition is the pattern of distribution of Hb F in the red cells, which is homogeneous in contrast to other conditions showing raised Hb F. In the latter the distribution is in separate population indicating the clonal nature.

HPFH with ß-thalassaemia in double heterozygous state can be mistaken for ß-thalassaemia major. A more benign clinical course combined with a family study will place the diagnosis beyond doubt. A form of HPFH in which Hb F is homogeneously distributed is Hb Kenya consisting of N-terminal residues of the γ-chain fused to C-terminal residue of the ß-chain.[12]

Recently a new type of foetal haemoglobin carrying a replacement of isoleucine with threonine at position 75 (E19) of the γ-chain has been described. Possibility that the γ-chain with threonine at position 75 (Tγ-chain) represents the product of an additional γ locus has been suggested.[13] Further classification of Hb F based on the above findings can be rewarding.

α-thalassaemia: There are distinct genes called α-thalassaemia 1 (α-thal 1); α-thalassaemia 2 (α-thal 2) and Hb Constant Spring (Hb CS) responsible for different types of α-thalassaemia wherein α-chain synthesis is found to be impaired.[6, 14] Gene for α-thal 1 is completely inffective in directing α-chain synthesis while α-thal 2 and Hb CS are responsible for only reduced rate of production of α-chains and thus less severe. Confirmation on the molecular defect in these forms of α-thalassaemia comes from studies of DNA/RNA hybridization and also hybridization to complementary DNA probes using pure globin mRNA with viral reverse transcriptase techniques.[8]

Homozygote for α-thal 1 is incompatible with life and results in hydrops foetalis —a common cause of intrauterine deaths. Affected infants carry only Hb Bart's (γ_4) Hb H ($ß_4$) and Hb Portland ($S_2 Y_2$). Double heterozygous state for α-thal 1 and α-thal 2 or α-thal 1 and Hb CS result in Hb H disease.

Infants with hydrops foetalis due to α-thal 1 homozygozity show oedema, anaemia, massive enlargement of liver and spleen. Blood picture is that of severe thalassaemic disorder. Haemoglobin analysis reveals about 80% Hb Bart's and small amounts of Hb H and Hb Portland. Hb H disease (α-thal 1 and α-thal 2 or α-thal 1 and Hb CS) presents clinical features of thalassaemia intermedia with typical thalassaemic blood picture. Precipitated Hb H ($ß_4$) inside red cells are seen as inclusion bodies. Low levels of Hb Bart's and Hb A_2 are also found. Where Hb CS is involved traces of this halmoglobin is also found. Carrier state of α-thal 1 shows only mild haematological changes with low MCH while α-thal 2 carrier shows almost normal red cells. Hb CS trait shows this haemoglobin which is an elongated α-chain of 172 residues as a result of termination mutation. Normally α-chain termination codon at position 142 is UAA and in the case of Hb CS it undergoes mutation by a single base change to CAA, a codon for glutamine, which is the first amino acid for the additional 31 residues in this haemoglobin.[15] A brief classification of α-thalassaemias is shown in Table 2.

Thalassaemias in India

In India thalassaemia was first reported in a $2\frac{1}{2}$ year old Bengali boy.[16] Since then reports of thalassaemia were recorded from different parts of this country.[17, 18] They included mostly collected series of cases seen at hospitals and belonged to different communities. Very little information is available on the incidence of thalassaemia based on studies in different endogamous groups. Even the limited data provide credence to the

TABLE 2.—*The Alpha-thalassaemias*

Genotype	Clinical Severity	Hb Bart's in Cord Blood (%)	Hb H in Adults (%)	Other
$\alpha\,\alpha$	Normal	0.5	0	—
α-thal 1	Thal. minor	5-6	0	—
α-thal 2	"Silent"	1-2	0	—
α-thal 2 α-thal 2	Thal. minor	5-6	0*	—
α-thal 1 α-thal 1	Hydrops fetalis	80-90	±	Hb A ± Hb Portland
α-thal 1 α-thal 2	Hb H disease	25	4-30	—
$\alpha\,\alpha$-CS	Nil	0	0	1% Hb CS
α-thal 1 α-CS	Hb H disease	-	13-19	Hb CS 2.5%
α-CS α-CS	Thal. minor	-	-	Hb $CS_3$5—6%

Hb Constant Spring (Hb CS) is an α-chain termination mutant. Others are Hb Icaria; Hb Koya Dora and Hb Seal Rock.

* Hb H disease found in some population (Ref. No. 30).

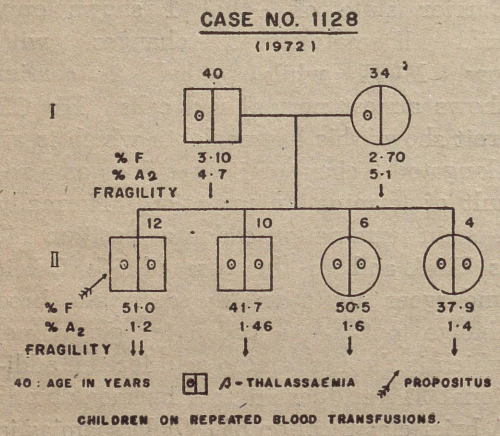

Fig. 3. Pedigree of a Gujarati (Halai Lohana) family showing all four children suffering from ß-thalassaemia major.

fact that thalassaemia is widely distributed in this country.

In the absence of any information on the globin chain synthesis and other related studies classification of thalassaemia on molecular basis has not been possible. However, based on the clinical severity, very early onset of the disease requiring repeated blood transfusions, marked haematological abnormality together with Hb F and Hb A_2 status, most of the thalassaemia cases appear to be ß°-thalassaemia. Less severe cases were also reported answering the description of ß +-thalassaemia and related variants. An example of a severe type of ß-thalassaemia in all the four children requiring blood transfusions at frequent intervals in a Gujarati (Halai Lohana) familiy depicted in a pedigree is shown in Fig. 3. Pedigree of another, a Sindhi family, showing only two out of ten children suffering from ß-thalassaemia major is shown in Fig. 4. These two children re-

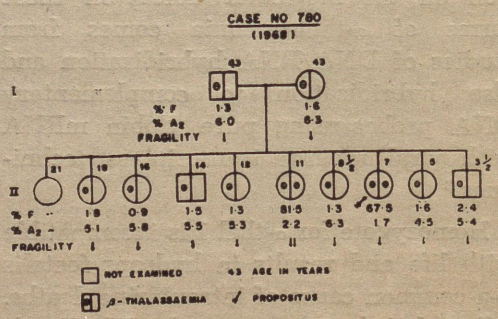

Fig. 4. Pedigree of a family with two of the ten children suffering from ß-thalassaemia major (mild type).

ceived no blood transfusions since the diagnosis six years ago when one among them (the proband) was hospitalised and given blood transfusion.

Information on some ethnic groups for ß-thalassaemia trait has shown that it is comparatively higher in some of them. Data on ß-thalassaemia incidence is shown in Table 3. More planned retrospective studies are indicated considering the heterogenicity of our population.

tion on the exact point of fusion was not elucidated.[20]

Hereditary persistence of foetal haemoglobin (HPFH) in heterozygous state and double heterozygosity for HPFH and ß-thalassaemia, as well as with Hb E were reported.[17, 21] Chemical examination of the γ-chain in some such cases indicated the presence of Gγ as well as $A_\gamma G_\gamma$ types. One case of homozygous Gγ-type, first known so far, was dectect-

TABLE 3.—*Beta-thalassaemia trait in some Indian population*

Group	Number tested	ß-thalassaemia trait per cent	References
Sikh (Vancouver)	80	6.3	31
Bengali (Calcutta)	100	3.7	32
Cochin Jews (Israel)	66	15.2	33
Bnei Jews Israel)	172	13.9	33
Cutchi Bhanusali (Bombay)	296	14.9	34
Mixed group (Trivandrum)	1000	0.6	35
G. S. Brahmin (Mangalore)	113	0.9	36
„ „ (Bombay)	154	2.6	36
Chitrapur Saraswat (Bombay)	202	5.9	36
G. C. Brahmin* (Bombay)	118	4.3	36
Cutchi Lohana (Bombay)	121	10.7	37
Halai Lohana (Bombay)	117	17.2	37
Sindhi Lohana (Bombay)	134	6.8	37
Punjabi Khatri (Bombay)	194	5.2	37
Sindhi (Ulhasnagar, Thana)	82	12.4	18
Varli**	134	2.3	38
Dhodia**	80	3.0	38
Kokana**	84	3.6	38

* Goan Catholic Brahmin. ** From Dadra & Nagar Haweli.

Among the variants of ß-thalassaemia seen in this country include δ ß-thalassaemia in heterozygous as well as with ß-thalassaemia seen in Bombay. Some atypical cases with presumptive diagnosis of various combinations (ßδ/α; ß/α; /ßδß and δß -homozygous) were reported from Calcutta.[17, 19]

Haemoglobin Lepore resulting from a cross-over of ß-chain was detected in a family from Coondapur, though informa-

ed in a Muslim (Davoodi Bohra) family in Bombay.[22, 23]

Other variants of ß-thalassaemia reported include those existing in combination with ß-chain haemoglobin variants (Hbs S, D, E and J). Beta-thalassaemia cases with Hb S reported were mostly severe although few cases in adults with minimum clinical manifestations were also found. An α-chain haemoglobin variant designated as Hb Q India (α64—

Asp→His) was found along with ß-thalassaemia in a few Sindhi-speaking families in Maharashtra.[17, 19]

Very little information is available of the existence of α-thalassaemia in Indian population. First case of Hb H disease ($\alpha_1\alpha_2$) was reported from Calcutta.[24] Two more such cases were found in Western India.[25, 26] Haemoglobin H inclusion bodies in one such case is shown in Fig. 5. Limited studies on cord blood samples indicate that α-thalassaemia is not uncommon. A recent study using globin chain synthesis technique carried out showed the presence of α-thal 1 in some Gujarati Indians in U.K.[27] Information on α-thalassaemia in Indian population based on cord blood studies is shown in Table 4.

TABLE 4.—*Alpha-thalassaemia in Indian population*

Group	No. of Cord Bloods tested	Hb. Bart's (Number)	References
Bengali	100	4	39
Bombay (Mixed group)	438	9	26
Bombay (Mixed group)	219	2	18
Bombay (Mixed group)	240	1	40

Alpha-thalassaemia 1 reported in Gujarati Indians (Ref. No. 27).

CONCLUSIONS

Thalassaemias are spread widely throughout the world and is a major public health problem in many countries. Commonly found thalassaemias are ß-thalassaemia and α-thalassaemia. They are further classified into subgroups using globin chain synthesis and nucleic acid hybridization techniques. Still much is to be understood of the molecular basis of different thalassaemias. Information so far available is briefly described.

Various types of thalassaemia seen in India are presented in brief. Importance of detailed family studies and wherever necessary globin chain synthesis studies are stressed, for better evaluation of some of the atypical cases seen in this country. Most of the thalassaemias seen are of ß-type and its variations although evidence for the presence of α-thalassaemia exists as seen from cord blood studies.

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See Fig. Art Paper I