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Thalassaemia Intermedia: A Genetic Study in 11 Patients*

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Beta-thalassaemia is a hereditary defect in the synthesis of beta-polypeptide chains of haemoglobin. Most patients with beta-thalassaemia can be classified, both genetically and haematologically, into those with the homozygous form of the disease, and those with the heterozygous disease or thalassaemia minor. However, in clinical practice one occasionally encounters a patient with thalassaemia of intermediate severity, namely thalassaemia intermedia.

It is the purpose of this paper to confirm the previously reported genetic heterogeneity of 'thalassaemia intermedia'.

Methods

The haematological methods were standard. Haemoglobin analyses were performed by starch gel electrophoresis using borate buffer at *p*H 8·6 according to the method of Smithies (1955). Quantitative estimations of Hb A_2 were determined by the method of DEAE-cellulose chromatography according to Huisman and Dozy (1961), by starch block electrophoresis, and also by starch gel electrophoresis according to Kunkel and Wallenius (1955) and Aksoy and Erdem (1965a). The maximum values for Hb A_2 in these methods were in the first two, 3%, and in the last one, 4%. Hb F was determined by the method of Singer, Chernoff, and Singer (1951). The relative concentrations of haemoglobin solutions were finally estimated in a Unicam spectrophotometer at 415 mμ. G6PD activities were determined qualitatively or quantitatively by the methods of Tönz and Betke (1962) and Gadsden and Cannon (1964). Osmotic fragility was determined either quantitatively by the method of Dacie (1960) or qualitatively by the technique described by Wintrobe (1956).

Results

Diagnostic features of patients studied. Table I gives some clinical data and haematological findings of each patient with thalassaemia intermedia. Our series consisted of 5 males and 6 females from 6 to 43 years of age.† Five of them were more than 20 years of age. The study contained two pairs of sibs (Cases 1 and 2, and Cases 7 and 8). All patients were of Turkish origin. Infantilism was found in 2 (Cases 2 and 9), and leg ulcers in 2 (Cases 1 and 9). In 10 of the 11 patients, a mild splenomegaly was found in one (Case 8), moderate splenomegaly in 5 (Cases 4, 5, 6, 7, and 10), and massive splenomegaly in 4 (Cases 1, 2, 3, and 9). In 4 of them (Cases 3, 4, 6, and 9) splenectomy was performed; and they had a pronounced erythroblastaemia. In 3 patients (Cases 3, 6, and 9) gallstones were seen, and in one (Case 9) cholecystectomy was performed. In 3 patients (Cases 3, 6, and 9) mild haemosiderosis was found. Six patients (Cases 1, 2, 5, 7, 8, and 10) had never been transfused; 2 of these patients (Cases 1 and 2) had their first transfusion in our department because of their anaemia. The remaining 5 patients (Cases 3, 4, 6, 9, and 11) were rarely transfused—not more than two or three times a year; usually once or twice between 1 and 2 years. The anaemia in our patients with thalassaemia intermedia was moderate in degree. Because Cases 1 and 2 were never transfused before admission, their anaemia was comparatively severe. The clinical manifestations and haematological findings in four patients (Cases 5, 7, 8, and 10) were comparatively mild. The results of haemoglobin analysis varied greatly in 11 patients with thalassaemia intermedia. Hb F varied between 1·3 and 67%; in 3 patients (Cases 4, 6, and 11) it was below 10%, and in 4 (Cases 5, 7, 8, and 9) it was above 50%.

Results of genetic studies. Table II gives the haematological data of the family members of each patient with thalassaemia intermedia in nine families. As can be seen from this Table, in only 6

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† Many of these patients with thalassaemia intermedia (Cases 1, 2, 4, 6, 7, 8, 9, and 10) are mentioned elsewhere (Aksoy, 1959; Aksoy, Eğribozlu, and Alpüstün, 1961a; Aksoy *et al.*, 1961b; Aksoy, 1964; Aksoy, 1965; Aksoy and Erdem, 1965b).

TABLE I

CLINICAL AND HAEMATOLOGICAL DATA IN 11 PATIENTS WITH THALASSAEMIA INTERMEDIA (HOMOZYGOUS, APPARENT HETEROZYGOUS, AND HETEROZYGOUS TYPES)

Case No.	Age (yr.)	Sex	RBC (10^6/ cu. mm.)	Hb (g./ 100 ml.)	Hct (%)	MCV (μ^3)	MCH ($\gamma\gamma$)	Hb A_2 (%)	Hb F (%)	NCR/ 100 WBC	Trans-fusion	Sple-nectomy	Haemo-side-rosis
1	23	M	2·70	5·5	21	77	20	3·9*	43·4	19	Never	No	—
2	12	M	2·00	4·3	15·5	77	21	5·5*	47·2	110	Never	No	—
3	43	F	3·00	6·5	24	80	21	6·3†	15	175	Rarely	Yes	Mild
4	14	F	3·70	8·2	24	65	22	5‡	7·6	180	Rarely	Yes	—
5	13	M	3·80	8·7	31	81	22	1·7†	67	5	Never	No	—
6	36	F	3·00	5·1	21	70	17	5·2‡	4·8	333	Rarely	Yes	Mild
7	9	F	3·82	7·9	29	76	26	2*	55·3	3·5	Never	No	—
8	6	M	4·00	7·9	31	77	20	1·9*	63	14	Never	No	—
9	35	F	3·10	6·5	26	80	21	1·9‡	59	382	Rarely	Yes	Mild
10	15	M	4·10	7·7	29	70	19	8·5*	20	4	Never	No	—
11	33	F	3·90	8	29	74	20	6·7*	1·3	0	Rarely	No	—

Cases 1 and 2, and 7 and 8 are sibs.
* Hb A_2 was estimated by starch gel electrophoresis according to the method of Aksoy and Erdem (1965a).
† Hb A_2 was estimated by DEAE-cellulose chromatography according to the method of Huisman and Dozy (1961).
‡ Hb A_2 was determined by starch block electrophoresis according to the method of Kunkel and Wallenius (1955).

of these 9 families (those of Cases 1, 2, 4, 5, 6, 7, 8, and 10) were genetic studies performed in both parents. On the other hand, in two families (those of Cases 3 and 9) only one of the parents was investigated. In the family of Case 11, only the children and the patient's husband were studied.

Table III gives the possible types of thalassaemia intermedia in these 11 patients according to the results of the family studies. According to these results, Cases 1, 2, 4, and 5 were homozygous thalassaemia intermedia, and Cases 6, 7, and 8 apparently heterozygous for thalassaemia intermedia. The family study of Case 10 showed that one parent (mother) was a beta-thalassaemic heterozygote, and the other (father) was an asymptomatic individual with a decreased level of Hb A_2. Case 3 was possibly homozygous thalassaemia intermedia because her father and one offspring had asymptomatic

TABLE II

HAEMATOLOGICAL DATA IN FAMILY MEMBERS OF 11 PATIENTS WITH THALASSAEMIA INTERMEDIA

Subject	RBC (10^6/ cu. mm.)	Hb (g./ 100 ml.)	Hct (%)	MCV (μ^3)	MCH ($\gamma\gamma$)	Osmotic Fragility (% NaCl)	Target Cells	Hypo-chromia	Micro-cytosis	Hb A_2 (%)	Hb F (%)	Haemo-globin Pattern
Cases 1 and 2							±					
Father	4·60	12·2	44	95	26	0·4–0·3		−	−	5*	0	A
Mother	4·50	10·4	40	80	23	ND	++	+	+	4*	0	A
Case 3												
Father	5·30	12·5	44	82	23	ND	+	+	+	4.2†	0	A
Husband	5·00	14	45	88	28	ND	−	−	−	2·6†	0	A
Offspring	5·20	12	43	82	23	ND	++	+	+	4·3†	0	A
Case 4												
Father	5·20	12	48	92	23	0·46–0·23	+	+	+	5·5*	0	A
Mother	5·40	10·8	41	74	20	ND	+	++	++	4·6‡	3	A
Sister	5·00	11	40	80	22	ND	+	++	++	6·1*	0	A
Case 5												
Father	5·20	11·2	45	86	21	ND	++	++	+	5·7†	0	A
Mother	4·70	9·4	39	82	20	ND	+	+	+	4·7†	4·5	A
Case 6												
Father	5·10	10	40	79	20	0·42–0·26	+	+	+	6·1*	0	A
Mother	5·00	14	45	90	28	0·42–0·30	−	−	−	2·5*	0	A
Cases 7 and 8												
Father	4·60	10	38	82	22	0·4–0·15	+	++	+	6*	0	A
Mother	4·20	12·1	40	95	29	0·4–0·3	−	−	−	3·1*	0·2	A
Case 9												
Father	5·00	14	47	90	28	ND	−	−	−	2·9*	0	A
Case 10												
Father	4·85	14·2	46	95	30	0·45–0·3	−	−	−	1·3*	0	A
Mother	4·50	11·5	40	88	25	0·4–0·28	+	+	+	6*	0	A
Case 11												
Husband	4·60	13·6	43	93	29	0·4–0·3	−	−	−	2·5*	0	AS
Offspring 1	3·30	7·6	25	71	21	0·42–0·16	+++	++	+++	7·3*	22·75	AFS
Offspring 2	3·40	7·5	26	75	22	0·4–0·18	+++	++	++	4·72*	25	AFS
Offspring 3	4·50	11·6	36	80	25	0·44–0·22	+	+	+	11·7*	0	A

* Hb A_2 was determined by starch gel electrophoresis according to the method of Aksoy and Erdem (1965a).
† Hb A_2 was determined by DEAE-cellulose chromatography according to the method of Huisman and Dozy (1961).
‡ Hb A_2 was determined by starch block electrophoresis according to the method of Kunkel and Wallenius (1955).

TABLE III

GENETIC DATA AND POSSIBLE TYPES OF THALASSAEMIA INTERMEDIA IN 11 PATIENTS WITH THALASSAEMIA INTERMEDIA

Case No.	Genetic Data	Diagnosis
1 and 2	Both parents thalassaemia minima with increased Hb A_2	Homozygous thalassaemia intermedia
3	Mother dead; father has thalassaemia minima with increased Hb A_2; one of children is beta-thalassaemic heterozygote with increased Hb A_2	Homozygous thalassaemia intermedia
4	Both parents are beta thalassaemic heterozygotes (increased Hb A_2); one sister is beta-thalassaemic heterozygote (increased Hb A_2)	Homozygous thalassaemia intermedia
5	Father has thalassaemia minima with increased Hb A_2; mother is beta-thalasaemic heterozygote (increased Hb A_2 and F)	Homozygous thalassaemia intermedia
6	Mother is apparently normal; father has thalassaemia minima with increased Hb A_2	Apparent heterozygous thalassaemia intermedia
7 and 8	Mother is apparently normal; father is beta-thalassaemic heterozygote (increased Hb F and A_2)	Apparent heterozygous thalassaemia intermedia
9	Mother dead; father apparently normal	Apparent heterozygous thalassaemia intermedia
10	Mother has thalassaemia minima with increased Hb A_2; father apparently normal	Apparent heterozygous thalassaemia intermedia
11	Parents dead; husband has sickle cell trait; two of children have sickle cell-beta thalassaemia disease; one is beta-thalassaemic heterozygote	Possible heterozygous thalassaemia intermedia

forms of heterozygous beta-thalassaemia (the mother was dead and her husband was normal). Case 9 was apparently heterozygous for thalassaemia intermedia, because her father was normal and her mother was dead. The parents of Case 11 were not investigated. She had married someone with sickle cell trait, and two of her offspring, described elsewhere, had sickle cell beta-thalassaemia disease (Aksoy, 1963a; Aksoy and Erdem, 1967). One of her children was a beta-thalassaemic heterozygote.

Discussion

The criteria for the diagnosis of thalassaemia intermedia in these 11 thalassaemic patients were as follows:

(1) The absence of scarcity of transfusion requirements: either the patients had never been transfused before the first observation, as was the case in 6 patients (Cases 1, 2, 5, 7, 8, and 10), or they were rarely transfused, at the most two or three times in a year, as in the remaining patients. In addition to that, 3 of these patients (Cases 3, 6, and 11) were never transfused before the age of 18.

(2) Five of the patients with thalassaemia (Cases 1, 3, 6, 9, and 11) were more than 20 years, though they never had a regular or a large number of blood transfusions.

(3) The clinical manifestations and haematological findings of these patients with thalassaemic syndromes were moderate in degree. In other words, less severe than those seen in thalassaemia major and more serious than encountered in usual forms of heterozygous beta-thalassaemia.

Therefore, the clinical manifestations and haematological findings of these 11 patients were consistent with the diagnosis of thalassaemia intermedia.

The genetic pattern of these 11 patients with thalassaemia intermedia was heterogeneous. We may classify and discuss them in three categories as summarized in Table III.

Homozygous thalassaemia intermedia. This was observed in Cases 1, 2, 4, and 5 as both parents were beta-thalassaemic heterozygotes. Though in Case 3 the family study was incomplete, the differences concerning severity of the clinical and haematological pictures of the patient and her beta-thalassaemic father and the one offspring rule out the possibility of the presence of only one thalassaemic gene in the patient. We are, therefore, strongly inclined to accept that this patient is also a case of homozygous thalassaemia intermedia.

Apparent heterozygous thalassaemia intermedia. In Cases 6, 7, and 8 one of the parents was normal and the other one was a beta-thalassaemic heterozygote. These patients with thalassaemia intermedia were thus considered as being cases apparently heterozygous thalassaemia intermedia.* These patients appeared to be heterozygous because there were considerable differences in the severity of the clinical and haematological pictures, and in the levels of fetal haemoglobin found in the patients and their affected beta-thalassaemic parents. To explain these significant differences, the present author (Aksoy *et al.*, 1961a; Aksoy, 1965) suggested that the parent who was apparently lacking the thalassaemic

* Non-paternity should also be considered in the above-mentioned patients with apparent heterozygous thalassaemia intermedia. Despite the absence of blood group data, this possibility is not to be considered in these patients (Cases 6, 7, and 8) because their normal parents were the mothers.

gene might have a 'silent' thalassaemia-like or thalassaemic gene responsible for beta-chain abnormality. Furthermore, today it is generally accepted that nearly 5 to 10% of cases of beta-thalassaemia fail to show increased levels of Hb A_2 (Marks and Gerald, 1964; Weatherall, 1965). Therefore, these patients with apparent heterozygous thalassaemia intermedia are, in reality, homozygous for thalassaemia intermedia. On the other hand, there was a difference between Case 6, and Cases 7 and 8. This was due to a high level of fetal haemoglobin, the latter being 55·3 and 63%, and the former 4·8%. We suggested, therefore, (Aksoy, 1963b; Aksoy, 1964) that the apparently normal parent of Case 6 had a 'silent' thalassaemia-like or thalassaemic gene responsible for alpha-chain abnormality. Recently, Pearson (1965) showed clearly that double heterozygous alpha-beta thalassaemia had clinical and haematological findings no more severe than in either trait alone. Thus, the presence of a thalassaemia-like or thalassaemic gene responsible for alpha-chain abnormality in Case 6, which showed the clinical and haematological findings of thalassaemic intermedia, is not acceptable. It would be better to accept the presence of a 'silent' thalassaemic gene responsible for beta-chain abnormality in Case 6 and her apparently normal parent, as discussed in Cases 7 and 8.

Genetic study of Case 10 showed that one of the parents (mother) was a beta-thalassaemic heterozygote and the other one (father) was an asymptomatic individual with a decreased level of Hb A_2, namely 1·3%. With the exception of some acquired states such as iron-deficiency anaemia and leukaemia (Chernoff, 1964; Aksoy and Erdem, 1967; Weatherall, Edwards, and Donohoe, 1968; Weatherall and Clegg, 1969), the decrease of Hb A_2 without other alteration in haemoglobin pattern is evidence for the presence of a gene responsible for delta-thalassaemia (Fessas and Stamatoyannopolous, 1962; Thompson *et al.*, 1965; Weatherall, 1967). Unfortunately, because of the uncooperative attitude of the parents, we were not able to confirm the decrease of Hb A_2 in the father by methods other than starch gel electrophoresis. So, for Case 10, both of the following possibilities can be considered: (1) heterozygous thalassaemia intermedia as discussed in Cases 6, 7, and 8; (2) double heterozygous form of delta- and beta-thalassaemia, though the increased level of Hb A_2 in Case 10 makes the second possibility unlikely (Thompson *et al.*, 1966).

Though only one of the parents of Case 9 was investigated, the presence of the same 'silent' thalassaemia-like or thalassaemic gene responsible for the beta-chain abnormality can be postulated for the patient and her normal appearing father.

Heterozygous thalassaemia intermedia. Case 11 had the clinical and haematological findings of thalassaemia intermedia. She had her first transfusions only a few years ago. Some acquired factors, such as iron and folic acid deficiencies and haemachromatosis were not seen, and the results of the trials with folic acid and pyridoxine were negative. Because her parents were dead, her genetic study was incomplete. From the genetic standpoint, there are two possibilities: (a) heterozygous thalassaemia intermedia and (b) homozygous thalassaemia intermedia. We are in favour of the former because of the similarity of the results of haemoglobin analysis and haematological findings in the patient and her beta-thalassaemic offspring, as they both had increased Hb A_2 levels only and no increase in Hb F and no splenomegaly. The only difference was the presence of a moderate microcytic anaemia in the mother, which increased with age.

Considering the results obtained in our patients with thalassaemia intermedia, we believe that the type of thalassaemia intermedia is determined by the type of thalassaemic genes inherited from the parents. Some beta-thalassaemia genes in the homozygous state result in thalassaemia intermedia, and others result in the heterozygous state, producing a similar condition.

Summary

Eleven patients with thalassaemia intermedia are described. The results of genetic studies in these patients were heterogeneous; and the patients could be classified into three categories:

(1) Homozygous thalassaemia intermedia.

(2) Apparent heterozygous thalassaemia intermedia: these patients were apparently heterozygous because there were considerable differences in the severity of the clinical and haematological picture and the levels of fetal haemoglobin found in the patients and their affected beta-thalassaemic parents or offspring. It is thought that the parent who is apparently free from the thalassaemic gene may have a silent thalassaemia-like or thalassaemic gene responsible for the beta-chain abnormality. These patients with apparent heterozygous thalassaemia intermedia are, in reality, cases of homozygous thalassaemia intermedia.

(3) Heterozygous thalassaemia intermedia.

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