Thalassaemia in Azerbaijan

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Abstract

β thalassaemia is present throughout the southern regions of the former USSR. We have defined the clinical picture of the disorder, the spectrum of β thalassaemia mutations, and the role of customary consanguineous marriage in Azerbaijan, where thalassaemia presents a public health problem of the same order as that in Greece. Contrary to earlier suggestions, we found that the common form of the disorder is typically severe. Typical Turkish, Mediterranean, Azeri, Kurdish, and Asian Indian mutations were found, consistent with the history of the region. The common Mediterranean β^0 thalassaemia mutation (codon 39) was not found. Three mutations (codon 8-AA, IVS2-1 and IVS1-110) account for over 80% of β thalassaemia genes. Consanguineous marriage appears to contribute relatively little to the frequency of affected births. These observations provide the basis for a thalassaemia prevention programme in Azerbaijan.

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8%.⁴ Hb S is also present,⁵ and Hb E has been reported. The birth rate of children with thalassaemia major is thought to be about 1/1000, and over 200 affected births are expected annually. In short, the problem seems to be similar to that in Greece.⁶ In Baku at least 60 affected births are expected annually. The Children's Hospital provides a thalassaemia service, and a day transfusion unit has recently been established at the Institute of Haematology.

Published evidence suggests that a relatively mild form of thalassaemia may be common in Azerbaijan. Only half of 82 patients described by Akhundova³ were transfusion dependent, and 18 had laboratory and clinical findings similar to those in patients homozygous for the mild "Portuguese" (IVS1-6) β thalassaemia mutation.⁷ "Silent" carriers with a normal Hb A₂ level have been reported,⁴ and globin biosynthesis studies have shown unusually high (30%) Hb A production in some patients.⁸ In addition, it has been said that many patients are "cured" by splenectomy (G Abdullaev, personal communication). If the common type of thalassaemia is mild, recommendations for treatment and indications for prenatal diagnosis might differ from those in other areas.

We have studied the clinical picture of thalassaemia and the frequency and types of the associated β thalassaemia mutations in Azerbaijan. The usual form of thalassaemia is severe; there is a wide variety of thalassaemia mutations but only four are really common. Only a minority of affected births are associated with parental consanguinity. The findings indicate the need for a thalassaemia control programme⁶ and provide a foundation for patient management, population screening, genetic counselling, and prenatal diagnosis.

Population and methods

Between 1989 and 1992, 120 thalassaemic patients in 99 families were examined at the Children's Hospital in Baku or at the Genetics Unit of the Institute of Obstetrics or both. The sample of patients seen is thought to be representative, as no other centres provide services for thalassaemia. A history was taken verbally and from the notes, patients were assessed clinically, and recommendations were made for future management.⁹ Parental consanguinity was noted.

Haematological indices were measured using a Coulter CBC5 particle size analyser. Hb A₂ estimations were made by the column method. Blood samples from 13 children (eight with β thalassaemia major, five with thalassaemia intermedia) were labelled for globin biosynthesis studies, which were per-

Haemoglobin disorders and G6PD deficiency are common in the transcaucasian and central Asian regions of the former USSR.¹ On average, β thalassaemia trait is carried by 1 to 2% of Armenians, up to 3% of Georgians and Uzbeks, 3.5% of the population of Dagestan, up to 5% of Tadjiks, and over 6% of Azerbaijanis, though there are marked local variations in prevalence. Hb S, Hb D, Hb E, and Hb H disease also occur. In addition, in Azerbaijan and central Asia the homozygote birth rate for a given carrier frequency may be increased by customary consanguineous marriage.

The population of Azerbaijan in 1984 was 6.3 million, and with a birth rate of 26/1000, about 165 000 children are born annually. Infant mortality is less than 30/1000.² Historically, Azerbaijan is a crossroads of the Middle East and Asia: it is close to the silk road, and for many hundreds of years was a centre of pilgrimage for Indian (Zoroastrian) fire worshippers. Malaria, previously endemic because of the proximity of the Caspian sea and extensive marshes at the estuary of the river Kura, was eradicated in the 1950s. In such an area a high frequency and considerable heterogeneity of haemoglobin disorders are to be expected. Thalassaemia major has long been a cause of concern in Azerbaijan.³ The prevalence of β thalassaemia trait ranges from 0 to 20% in relation to altitude: in the capital Baku, which contains 30% of the total population, it is 6 to

Table 1 Patients examined, diagnosis, age group, and estimated survival

Age group (y)	<i>β thalassaemia</i>			<i>Hb S/β thal</i>	<i>Hb H disease</i>	<i>Total patients</i>	<i>Estimated survival (%)</i>
	<i>Major</i>	<i>Intermedia</i>					
		<i>Possible</i>	<i>Definite</i>				
0–5	56	3	0	1	0	60	100
5–10	16	4	5	1	1	27	45
10–15	1	2	11	1	1	16	27
15–20	1	0	5	0	0	6	10
20–25	0	1	5	0	0	6	10
25–30	0	0	3	0	0	3	5
30+	0	0	2	0	0	2	<5
Total	74	10	31	3	2	120	

formed using standard methods.^{10,11} Blood samples were obtained for DNA analysis from 38 of the 99 families with full clinical details, from nine patients encountered in the course of surveys in villages (clinical details incomplete), and from 46 unrelated single heterozygotes found in these surveys and among pregnant women. As the amount of DNA available from most persons was very limited, this preliminary study was limited to the β globin genes.

Common Mediterranean and Turkish β thalassaemia mutations (β⁰ codon 8 (-AA), β⁰ IVS1 nt 1 (G→A), β⁺ IVS1 nt 110, β⁰ codon 39 (C→T), β⁰ IVS2 nt 1 (G→A), β⁺ IVS2 nt 745 (C→G)) were defined by dot blot analysis using amplified DNA with either ³²P¹² or horse radish-peroxidase labelled oligonucleotide probes.¹³ Mutations not defined by this approach were characterised by denaturing gel electrophoresis^{14,15} or direct sequencing of amplified single strand DNA, by the dideoxy chain termination method of Sanger with the enzyme T7 DNA polymerase (Sequenase USB).¹⁶ Sequencing gel and autoradiography were by standard techniques.¹⁷

Results

THE FAMILIES

The 120 patients (in 99 families) studied are listed in table 1. Three had Hb S/β thalassaemia and two had Hb H disease, leaving 115 patients with homozygous β thalassaemia, in 94 families. For these, a clinical diagnosis of thalassaemia major or intermedia was made on the basis of age at presentation, spleen size, Hb level, and severity of symptoms. Seventy four (64%) had definite thalassaemia major and 31 (27%) definite thalassaemia intermedia. Ten could not be confidently said to have one or the other. Table 1 also gives the patients' age distribution, from which mortality can be calculated (last column). In Azerbaijan until very recently the cumulative mortality from homozygous β thalassaemia has been at least 75% before 10 years of age. Survivors are likely to have milder disease, and in fact 79% (26/33) of the patients over 10 years old had definite thalassaemia intermedia, while 83% (72/87) of the thalassaemic children under 10 had definite thalassaemia major.

Eighteen couples had two, and one had three, thalassaemic children. Most sets of sibs had the same clinical picture, but three families showed differences in severity that were not the result of hypersplenism in the more seriously affected children. In two families one

patient had clear thalassaemia major and one clear thalassaemia intermedia. In the family with three affected children, one had very mild and two had severe thalassaemia intermedia. In two of these three families, one parent and the less severely affected child were both reported to have Hb H on electrophoresis, as well as typical findings of β thalassaemia trait or β thalassaemia major. The parents were closely related (mostly first cousins) in 28 of the 99 families studied.

CLINICAL PICTURES

The two children with Hb H disease had severe problems. One was a 12 year old girl, previously quite well, who now had a spleen palpable 10 cm and Hb 6.4 g/dl: she has since undergone splenectomy. The second was a child of 6 years old with a spleen enlarged 8 cm, who has been transfusion dependent since 3 months of age.

Patients with thalassaemia major were managed on a low transfusion scheme (mean pretransfusion Hb 6 to 6.5 g/dl: mean Hb maintained by transfusion varied from 7 to 9.5 g/dl). Most had a typical thalassaemic appearance and most over 3 to 4 years old had a large spleen and some growth retardation. Splenectomy, hitherto rarely done for fear of overwhelming infection, was recommended for 27 patients with a spleen palpable more than 6 cm and a blood consumption over twice that expected.⁹ Regular iron chelation therapy is not available, but some families obtain desferrioxamine at the time of transfusion, or for intramuscular injection twice a week.

LABORATORY RESULTS

Thirty of the 32 blood samples from parents of children with homozygous β thalassaemia examined showed typical microcytosis and an Hb A₂ above 3.5%. However, in two cases the Hb A₂ was 3.4% (borderline). DNA studies showed a typical β⁰ thalassaemia gene (codon 8-AA) in both cases. One was a very anaemic woman (Hb 8.2 g/dl), and the other had an MCH of 26 pg, suggesting coincidental α thalassaemia.¹⁸

Globin biosynthesis studies showed β⁺ thalassaemia in two of five children with severe thalassaemia major and in three of eight patients with definite or possible thalassaemia intermedia. The remainder had β⁰ thalassaemia.

Table 2 gives the results of DNA studies of 38 families with known clinical details, nine families without known clinical details, and 41 unrelated heterozygotes, 135 chromosomes in all. The mutation was identified for 126 chromosomes. Nine remain unknown, but in five cases this was because the DNA available was exhausted before investigations were complete. Fifteen different mutations (including known Turkish, Mediterranean, Kurdish, Azeri, and Indian mutations, and Hb S) were found. The common Mediterranean mutation codon 39 (C→T) was not found at all. Results

Table 2 Results of DNA analysis of 135 thalassaemic chromosomes in Azerbaijan (2 chromosomes/family)

				Homozygous β thalassaemia							
Mutation				Total	Heterozygotes	Total	No clinical data	Thal major	Thal intermedia	Uncertain	S/thal
Codon 8 (–AA)	β^0	severe	(Turkish)	44	18	26	2	18	4	1	2
IVS2-1 (G→A)	β^0	?	(Med)	29	5	24	2	11	10	1	
IVS1-110 (G→A)	β^+	severe	(Med)	17	2	15	4	8	2	1	
IVS1-6 (T→C)	β^+	mild	(Med)	8	3	5	2		3		
Codon 8/9 (+G)	β^0	severe	(Indian)	6	5	1	—			1	
IVS1-5 (G→C)	β^+	severe	(Indian)	2	—	2	—	2			
–30 (T→A)	β^+	mild	(Turkish)	2	—	2	2				
Codon 5 (–CT)	β^0	severe	(Med)	1	—	1	—		1		
Codon 36-37 (–T)	β^0	severe	(Kurdish)	1	1	—	—				
Codon 15 (TGG→TGA)	β^0	severe	(Indian)	3	3	—	—				
Codon 82-83 (–G)	β^0	?	(Azer)	5	2	3	1			1	
–28 (A→C)	β^+	mild	(Kurdish)	1	—	1	—		1		
–88 (C→A)	β^+	mild	(American black, Indian)	2	—	2	1		1		
+22 (G→A)	β^+	?mild	(Turkish, Bulgarian, Italian)	2	1	1	—			1	
Unknown				9	—	9	3	3	3		
S				3	1						2
Total				135	41	92	17	42	26	6	4

for thalassaemia intermedia and major are also shown separately in table 2.

The “Turkish” β^0 mutation (codon 8-AA), the Mediterranean β^0 mutation IVS2-1 (G→A), and the Mediterranean β^+ mutation IVS1-110 (G→A) accounted for two thirds of all mutations. In patients under 10 years old, they accounted for 82% of mutations. Codon 8-AA was the commonest mutation found in patients with thalassaemia major. IVS2-1 was equally associated with thalassaemia major and thalassaemia intermedia. It was the commonest mutation found in patients with thalassaemia intermedia though, as in other studies, many different mutations including mild β^+ mutations like IVS1-6 (T→C) and –88 (C→A) were also found in these patients. The severe codon 8-AA mutation did occur in three patients with thalassaemia intermedia (one was even homozygous), but in two of these families there was evidence of an independent moderating factor (probably coincidental α thalassaemia), as a sib had more severe disease.

In 24 of the 38 families with DNA studies the parents carried different mutations. Both parents carried the same mutation in 6/10 families where the parents were consanguineous, and in 8/28 where they were not.

Discussion

Until recently in Azerbaijan most thalassaemic children died very young. However, in recent years infant mortality has dropped and diagnosis and treatment are becoming available. Consequently patient numbers are rising, and thalassaemia is emerging as a public health problem comparable, for example, to that in Greece. There (in the absence of prevention) 150 to 200 affected births would occur annually in a population of 10 million⁶: in Azerbaijan over 200 affected births are expected annually in a population of 6.5 million.

Earlier observations suggested that a mild form of β thalassaemia is common in Azerbaijan, but we have found that the disease is as severe as elsewhere. High mortality among young children with thalassaemia major leading to selective survival of patients with thalassaemia intermedia accounts for the earlier findings. It also explains the observation that many patients are “cured” by splenectomy.

Hypersplenism is common in thalassaemia intermedia and can cause transfusion dependency: splenectomy can then restore such patients to tolerable health. Our DNA results show clear selection with age of mutations associated with thalassaemia intermedia. We conclude that in most developing countries, only studies of the families of young affected children, or of heterozygotes detected by population screening (rather than through family studies), can give a true picture of the pattern of mutations present.

The β thalassaemia mutations detected resembled those reported in Turkish studies.¹⁹⁻²¹ However, the common Mediterranean β^0 (codon 39) mutation was not found, and the common Mediterranean β^+ mutation (IVS1-110) is far less common in Azerbaijan than in Turkey. Mutations not found in Turkish studies include the Azeri mutation codon 82-83 –G,²² the Indian mutation codon 15 TGG→TGA,²¹ and the Kurdish mutation codon 36-37 –T.²¹ The fact that only one third of the patients studied were homozygous for the same mutation, and a considerable proportion of consanguineous couples carried different thalassaemia mutations, confirms the high frequency and diversity of β thalassaemia genes in the population.

A thalassaemia control programme including improved patient care, a patients’ and parents’ support group, and information, screening, genetic counselling, and prenatal diagnosis is planned on the basis of established models in the Mediterranean area.²³ Prevention is particularly important in Azerbaijan because adequate transfusion can be provided (in principle), but the iron chelation therapy needed for long term survival cannot. A programme of carrier screening and counselling, and the offer of DNA based prenatal diagnosis to carrier couples is planned. Carrier couples will be tested for the three mutations (codon 8-AA, IVS2-1, and IVS1-110) which account for over 80% of β thalassaemia mutations in young patients. If these are not found the rarer mutations will be sought, or RFLP linkage studies will be used.

To provide a sound basis for genetic counselling, it is also desirable to assess the severity of the different β thalassaemia mutations present, and to define the frequency and nature of

other genetic modifying factors, such as α thalassaemia.²⁴ In principle, β^0 thalassaemia mutations are severe (table 2). In Azerbaijan we found that the common β^0 codon 8-AA mutation is predominantly associated with thalassaemia major, but the common β^0 IVS2-1 mutation is equally associated with thalassaemia major and intermedia: an association with thalassaemia intermedia has also been noted in Turkey.²⁵ It appears that some but not all patients with this mutation make unusually large amounts of fetal haemoglobin. This requires further investigation, for example, of a possible association with an *Xmn* polymorphism upstream of the γ genes that can permit γ gene derepression.²⁶

When α thalassaemia is coinherited it often moderates the severity of homozygous β thalassaemia.²⁵ There are no published reports of α thalassaemia in Azerbaijan, but the presence of patients with haemoglobin H disease, and the observation of Hb H in some β thalassaemia heterozygotes and patients with thalassaemia intermedia, suggest that it is common. The severe clinical picture in the two Hb H disease patients described here suggests a non-deletional mutation of the Saudi Arabian type,²⁷ but we were unable to perform molecular studies of the α genes on this occasion because of the limited amount of DNA available. A separate study of the types and frequency of α thalassaemia mutations in Azerbaijan is planned.

A high frequency (about 20%) of consanguineous marriage in Azerbaijan has been suggested, but not documented. In principle this would lead to (1) more affected births than expected from the heterozygote frequency alone, (2) a disproportionate number of affected children having consanguineous parents, and (3) most such children being homozygous for a specific mutation.²⁸ However, only 28% of the parents of the affected children seen were related, and only six of the 10 consanguineous couples studied carried the same mutation. Thus, in this study, consanguineous marriage was associated with a less than 15% increase in affected births above expectation from the gene frequency alone. Possibly the frequency of consanguineous marriage has been overestimated, or there may have been recent change. Such changes can be very rapid.²⁹

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