Prevention of hemoglobinopathies in Turkey

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Abstract

Hemoglobinopathies are the most common genetic disorders in Turkey. The incidence of beta thalassemia and sickle cell trait (HbAS) is 2.0% and 0.3% respectively. In addition to HbS, 51 abnormal hemoglobins and 42 different beta thalassemia mutations have been detected by DNA analysis. In Turkey, beta thalassemia and sickle cell anemia cause major health problems. For thirty years, screening programs for carriers, genetic counseling and prenatal diagnosis have sought to prevent hemoglobinopathies. In 1983, the first prenatal diagnosis center was established for sickle cell anemia and beta thalassemia at Hacettepe University, Ankara. After many population-screening studies, a law was passed in 1993 by the Turkish Parliament for the eradication of hemoglobinopathies. Forty-one premarital screening centers were set up by the Ministry of Health in the 33 provinces where most of the transfusion-dependent thalassemic patients live. The mothers at risk for hemoglobinopathies were given genetic counseling and directed to prenatal diagnosis centers. Since 1990, four prenatal diagnosis centers have been established at university hospitals in Adana, Antalya, stanbul and zmir. A total of 5255 prenatal diagnoses have been made for sickle cell anemia and beta thalassemia in 5 centers; 1338 fetuses have been diagnosed as homozygous or compound heterozygotes for hemoglobinopathies. Prenatal diagnosis was performed on families who had decided to terminate the pregnancy if it were to be found that the fetus was affected.

Introduction

Turkey is a large country located on the European and Asian continents. The country has a population of 73,722,988 and covers a total area of 783,562 km^2. Due to the presence of various ancient civilizations, there is great genetic diversity. Twenty percent of the beta thalassemia mutations (42 of 200) and 5% of abnormal hemoglobins (52 of 1000) reported worldwide have been detected in Turkey.[1-11] Beta thalassemia is seen all over the country but sickle cell anemia is peculiar to the Çukurova region in southern Turkey. The incidence of beta thalassemia and sickle cell trait for the country as a whole is 2% and 0.3%, respectively.[4,7,9] Different regions report different percentages of beta thalassemia trait. The west and south of the country are at risk for beta thalassemia. These areas include Thrace, and the Aegean, the Mediterranean and the southeastern parts of Turkey.[12-14] Although the overall frequency of beta thalassemia is 2%, it reaches as high as 10% in the Thrace region. Sickle cell hemoglobin (HbS) is common in southern Turkey. Average HbAS is approximately 8.2% in the Çukurova region (Adana, Hatay and Mersin). The prevalence of carriers ranges from 3% to 44% in some villages and towns in Hatay.[15-17]

After the first sickle cell patient was diagnosed in 1946, several population-screening studies were carried out by different study groups.[12-18] In 1983, the first prenatal diagnosis center was established at Hacettepe University in Ankara. Over eight years, a total of 101 prenatal diagnoses were made by *in vitro* globin chain analysis to prevent sickle cell anemia and beta thalassemia.[19] This method was used at Hacettepe University for some couples whose beta globin gene mutation had been unknown until 2000.[20] This was the main diagnostic procedure adopted and was performed by using fetal blood samples taken during the 18th-20th weeks of gestation. In the Gurgey 1991 study, the majority of the pregnant women had given birth to one or more affected offspring. Since the beginning of the 1990s, amplification of genomic DNA by polymerase chain reaction (PCR) techniques has been adopted in prenatal diagnosis.[21,22] These PCR techniques are still being adopted by using DNA isolated from chorionic villus sampling (CVS) at the first trimester of pregnancy. Later, 2 prenatal diagnosis centers were established in Turkey, in 1990 in stanbul and in 1992 in Adana. While many prenatal diagnoses were performed by PCR, molecular diversity was well documented in hemoglobinopathies.[23,24]

It was found that affected births had not been eradicated completely, despite the studies carried out between 1983 and 1992. Due to the high burden on the state of the medical costs of thalassemic patients, in 1993 the Turkish Parliament passed a law for the prevention and eradication of human genetic disorders. Consequently, premarital screening centers were established as pilot studies by thalassemia associations and health authorities in Adana, Hatay and Mersin in the Çukurova region.[25-27] Given the success of the pilot studies, a hemoglobinopathy scientific board was set up, and in 2002 the Ministry of Health published regulations to govern the centers' activities. Since 2003, premarital screening tests have been carried out in the 33 provinces with the highest incidence of carriers in Turkey. For the past ten years, prenatal diagnosis procedures have been performed in coordination with the Ministry of Health and university hospitals.[20,28-30]

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Materials and Methods

Prenatal diagnosis is still the only option that can prevent hemoglobinopathies. After screening couples who manifest the sickle cell or beta thalassemia trait, prenatal diagnosis is performed at the first trimester of pregnancy, and affected fetuses are aborted as early as possible.

Blood samples and hematologic procedures for premarital screening

Many population-screening studies were carried out in the regions in which beta thalassemia and sickle cell anemia were reported between 1993 and 2002. The main screening tests carried out were complete blood count and analysis of hemoglobin variants. Blood samples (5 mL) with EDTA as anticoagulant were taken for hematologic and hemoglobin analysis. A complete blood count (CBC) was taken using a cell counter. Hemoglobin variants were characterized by cellulose acetate electrophoresis.[31] HbA_2 was quantified by micro column chromatography for detection of beta thalassemia trait.[32]

Over the last ten years, the fully automated high performance liquid chromatography

(HPLC) systems have been used for both determination of hemoglobin variants and quantification of the Hb levels.[32,33] These HPLC systems are used for hemoglobin analysis and have made it easier to set up premarital screening centers. Forty-one centers have been set up in the 33 provinces with the highest incidence of beta thalassemia trait over the last decade (Figure 1).[34]

Fetal samples and molecular procedures for prenatal diagnosis

The reason for setting up the first prenatal diagnosis center for sickle cell anemia and beta thalassemia at Hacettepe University, Ankara, in 1983, was mainly to help multiparous pregnant women at risk of having a baby affected with hemoglobinopathies. Fetal blood samples were taken between the 18th-20th weeks of gestation. By using fetal blood samples, *in vitro* hemoglobin chain analysis was performed using ^{3}H-Leucine by conventional methods.[19] This proved to be a safe and reliable procedure for prenatal diagnosis for sickle cell anemia and beta thalassemia. The method was also used for some couples whose mutation had been unknown until 2000. After detection of the beta globin gene mutations of the parents, PCR-based methods are used for prenatal diagnosis.

Genomic DNA is isolated from leukocytes by conventional methods.[35] Fetal DNA is isolated from CVS obtained in the 10th-11th weeks of pregnancy. Beta globin gene mutations are detected by PCR-based techniques, such as amplification refractory mutation systems (ARMS) and restriction fragment length polymorphism (RFLP).[22,36] Some rare or unknown beta thalassemia mutations were characterized by DNA sequencing with the dideoxy chain termination reaction procedure.[37]

Results

In Turkey, there are eighty-one provinces, including cities, towns and villages. Population size and the incidence of beta thalassemia tra in 33 of these provinces are shown in Table 1.[38-45] Some provinces have more than one center: 2 in Ankara, 3 in Antalya, 2 in Aydın, 2 in stanbul, and 4 in Mugla. Istanbul is the most heavily populated (total population 13,255,685), while Ankara is the capital and the second largest city (total population 4,771,716) in Turkey.

Forty-one premarital screening centers have been set up in 33 provinces by the Ministry of Health over the last ten years. The couples at risk for hemoglobinopathies have been given genetic counseling and directed to prenatal diagnosis centers. The percentage of the cou-

Table 1. Population size and frequency of -thalassemia trait in the 33 provinces.

	Province	Population size in 2010 Total	Districts and city centers	Towns and villages	Frequency of β-thalassemia trait % (ref.)
1	Adana	2,085,225	1,836,432	248,793	3.7 (39)
2	Ankara	4,771,716	4,641,256	130,460	2.0 (40)
3	Antalya	1,978,333	1,392,974	585,359	13.1 (39)
4	Aydın	989,862	588,552	401,310	5.1 (39)
5	Batman	510,200	373,388	136,812	-
6	Bilecik	225,381	173,389	51,992	-
7	Burdur	258,868	159,508	99,360	-
8	Bursa	2,606,495	2,308,574	296,921	1.7 (39)
9	Çanakkale	490,397	269,035	221,362	-
10	Denizli	931,823	641,093	290,730	2.6 (39)
11	Diyarbakır	1,528,958	1,090,172	438,786	3.6 (39)
12	Düzce	338,188	194,128	144,060	-
13	Edirne	390,428	261,920	128,508	6.4 (39)
14	Erzurum	769,085	489,486	279,599	0.6 (41)
15	Eski ehir	764,584	681,854	82,730	-
16	Gaziantep	1,700,763	1,501,566	199,197	1.84 (42)
17	Hatay	1,480,571	743,439	737,132	4.6 (39)
18	Isparta	448,298	311,064	137,234	2.4 (39)
19	Istanbul	13,255,685	13,120,596	135,089	4.5 (39)
20	İzmir	3,948,848	3,606,326	342,522	4.8 (39)
21	Kahramanmara	1,044,816	636,828	407,988	2.8 (43)
22	Karaman	232,633	159,834	72,799	-
23	Kayseri	1,234,651	1,064,164	170,487	-
24	Kırklareli	332,791	219,333	113,458	3.4 (39)
25	Kocaeli	1,560,138	1,459,772	100,366	0.8 (44)
26	Konya	2,013,845	1,486,653	527,192	2.0 (45)
27	Kütahya	590,496	383,572	206,924	-
28	Manisa	1,379,484	924,267	455,217	
29	Mersin	1,647,899	1,281,048	366,851	2.3 (39)
30	Muğla	817,503	350,050	467,453	4.5 (39)
31	Sakarya	872,872	646,899	225,973	-
32	Şanlıurfa	1,663,371	922,539	740,832	6.4 (39)
33	Tekirda	798,109	545,481	252,628	-
Total	(33 provinces)	53,662,316	44,465,192	8,905,394	-
Turkey (81 provinces)		73,722,988	56,222,356	17,500,632	-

Table 2. Results of prenatal diagnosis centers in Turkey.

Province	No. fetuses	No. affected fetuses	Center
Adana	3616	903	Çukurova University
Ankara	947	261	Hacettepe University
Antalya	407	105	Akdeniz University
İstanbul	70	14	Bogaziçi University
İzmir	215	55	Ege University
Total	5255	1338	5

ples screened at the centers has reached 81 per cent at six years.[46]

A total of 5255 prenatal diagnoses have been made for sickle cell anemia and beta thalassemia in five centers at university hospitals since 1983. A summary of the results is presented in Table 2.[20,23,29,30]

A total of 1338 affected fetuses were homozygous or compound heterozgotes for hemoglobinopathies.[20,23,29,30] Two hundred and seventeen fetuses were diagnosed by *in vitro* globin chain analysis at Hacettepe University Hospital in Ankara.[20] The rest of the results (3.739) were detected by DNA analysis in 5 prenatal diagnosis centers.

Frequency and type of beta thalassemia mutations in couples at risk are shown in Table 3. [20,23,29,30] The IVS1-110 G>A mutation was the most common. The ten most common mutations account for 77.1-95.2% of all the beta thalassemia mutations.

In Turkey, genetic heterogeneity of hemoglobinopathies was well documented during the prenatal diagnosis and population screening. In addition to HbS, 51 hemoglobin variants and 42 different beta thalassemia mutations were found in the country as a whole. The lists of beta thalassemia mutations and hemoglobin variants are shown in Tables 4 and 5, respectively.[6-11,20,30,40,47-50]

Discussion

Hemoglobinopathies are the most common genetic diseases causing health problems in the world. Sickle cell anemia and beta thalassemia constitute the majority of the hemoglobin disorders in Turkey. Unfortunately, there is no ultimate cure for these diseases, and prevention is the best option for families at risk. Since 1983, prenatal diagnosis has been a well-accepted solution in Turkey for prevention of the hemoglobinopathies.

Hemoglobinopathies are quite heterogeneous in the Turkish population. Up to now, more than 42 different point mutations in the beta globin gene and 52 hemoglobin variants have been characterized in the country. The seven most common beta thalassemia mutations alone account for approximately 72% of the total number of mutations found in the Çukurova region. In addition to HbS, there are some rare Hb variants, such as HbC, HbD and HbE, and also, more rarely, Hb Sarrebourg.[50] Due to molecular diversity, in some couples at risk for hemoglobinopathies prenatal diagnosis requires time. For this reason, it is strongly recommended that mutation analysis should be detected before pregnancy.

All premarital screening centers were certified over nine years by the Turkish Ministry of Health. Up to now, eleven of the centers (Adana, Mersin, Hatay, Izmir, Denizli, Gaziantep, Konya, Mugla, Kocaeli, Kahramanmara , Erzurum) have published their experiences and results.[25-27,41-45,51-54] Although Erzurum is included in the 33 provinces, here the incidence of beta thalassemia trait was only 0.68 per cent.[41]

Couples at risk for hemoglobinopathies have been directed by the centers to university hospitals for prenatal diagnosis. A large number of fetuses are diagnosed each year. It became apparent that some of the couples did not fully understand the importance of screening and genetic counseling, and so did not go to university hospitals for prenatal diagnosis. Approximately 100 affected babies are born annually because the couples had married before 2000. Consanguineous marriage also contributes significantly to affected births all over Turkey. Overall, consanguinity is approximately 21%, but rates may be as high as 46-63% in some regions.[30,40]

In conclusion, in Turkey, a national hemoglobinopathy screening program has played a major role in lowering the rate of affected births. Last year, the Turkish Ministry of Health adopted a policy of Family Medicine

Table 3. Frequency of β-thalassemia mutations detected at the University Hospitals.

	Akdeniz University no. 411	Bogazici University no. 140	Cukurova University no. 714	Hacettepe University no. 1114
IVS1-110 G>A	42.3	37.1	50.6	49.0
IVS1-1 G>A	5.1	7.1	8.1	7.9
Cod 39 C>T	4.9	6.4	7.1	3.6
Fsc 5 -CT	3.4	2.8	6.0	2.5
Fsc 8 -AA	3.2	5.7	5.5	7.6
IVS2-1 G>A	8.8	5.7	4.2	5.9
IVS1-6 T>C	7.0	7.1	4.2	4.6
-30 T>A	3.4	0.7	4.2	1.4
IVS2-745 C>G	6.8	3.5	3.5	7.0
Fsc 44 -C	3.2	1.4	1.8	3.2
Total	88.1	77.1	95.2	92.7

IVS, intervening sequence; Cod, codon; Fsc, frameshift codon.

Table 4. Human β-globin gene mutations in Turkey.

	Position	Mutation
1	-101	C→T
2	-88	C→T
3	-87	C→G
4	-30	T→A
5	-28	A→C
6	5'-UTR	+22 G→A
7	FSC-5	-CT
8	FSC-6	-A
9	FSC-8	-AA
10	FSC-8/9	+G
11	Cd 15	G→A
12	FSC 22-24	-AAGTTGG
13	Cd 30	G→C
14	IVS-I-1	G→A
15	IVS-I-1	G→C
16	IVS-I-1	G→T
17	IVS-I-2	T→A
18	IVS-I-5	G→A
19	IVS-I-5	G→C
20	IVS-I-5	G→T
21	IVS-I-6	T→C
22	IVS-I-110	G→A
23	IVS-I-116	T→G
24	IVS-I-130	G→A
25	IVS-I-130	G→C
26	FSC-36/37	-T
27	Cd 37	G→A
28	FSC 37-39	-7 bp
29	Cd 39	C→T
30	FSC-44	-C
31	FSC-74/75	-C
32	FSC-82/83	-G
33	IVS-II-1	G→A
34	IVS-II-654	C→T
35	IVS-II-745	C→G
36	IVS-II-848	C→A
37	IVS-II-849	A→G
38	3'-UTR	-13 bp
39	Poly A	AATAAA→AATGAA
40	Poly A	AATAAA→AATAAG
41	Poly A	AATAAA→AACAAA
42	290 bp	Deletion

IVS, intervening sequence; Cod, codon; Fsc, frameshift codon.

Table 5. Human hemoglobin variants in Turkey.

	Hemoglobin	Mutation
1	O-Podova	α30 Glu→Lys
2	Hasharon	α47Aspu→His
3	Monthgomery	α48Leu→Arg
4	Adana	α59 Gly→Asp
5	J-Anatolia	61 Lys→Thr
6	Ube-2	α68 Asn→Asp
7	Q-Iran	α75 Asp→His
8	Moabit	α86 Leu→Arg
9	M-Iwate	α87 His→Tyr
10	Çapa	α94 Asp→Gly
11	Setif	α94 Asp→Tyr
12	G-Georgia	95 Pro Leu
13	Bronovo	103 His→Leu
14	Strumica	α112 His→Arg
15	J-Meerut	α120 Ala Glu
16	Tyne	β5 Pro Ser
17	S	β6 Glu→Val
18	C	β6 Glu Lys
19	Ankara	β10 Ala→Asp
20	D-Ouled Rabah	β19 Asn→Lys
21	E-Saskatoon	β22 Glu→Lys
22	G-Cousatta	β22 Glu Ala
23	D-Iran	β22 Glu→Gln
24	E	β26 Glu→Lys
25	Knosos	β27 Ala→Ser
26	Volga	β27 Ala→Asp
27	Siirt	β27 Ala→Gly
28	Hakkari	β31 Leu→Arg
29	G-Copenhagen	β47 Asp→Asn
30	Summer Hill	β52 Asp→His
31	Hamadan	β56 Gly→Arg
32	J-Antakya	β65 Lys→Met
33	City of Hope	β69 Gly→Ser
34	J-Iran	β77 His→Asp
35	Yaizu	β79 Asp→Asn
36	G-Szuhu	β80 Asn→Lys
37	Pyrgos	β83 Gly→Asp
38	Istanbul	β92 His→Gln
39	N-Baltimore	β95 Lys→Glu
40	Köln	β98 Val→Met
41	D-Punjab	β121 Glu→Gln
42	O-Arab	β121 Glu→Lys
43	Beograd	β121 Glu→Val
44	Tunis	β124Pro→Ser
45	Sarrebourg	β131 Gln→Arg
46	Brocton	β138 Ala→Pro
47	A2Yialousa	D82 Ala→Ser
48	Baskent	128 Ala→Thr
49	Lepore-Boston	Hybrid
50	P-Nilotic	Hybrid
51	Costant Spring	Elonged chain
52	Antalya	Deletion and insertion

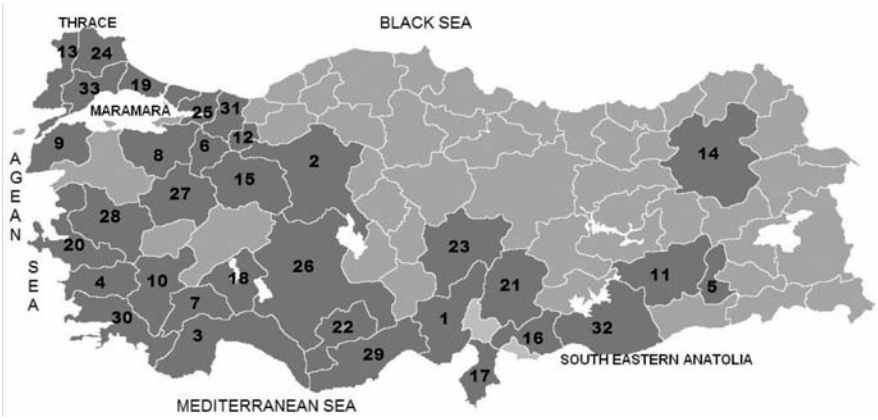

Figure 1. Provinces of premarital screening centers in Turkey.

throughout the country. Family Medicine is the medical speciality that provides continuing, comprehensive health care for individuals and their families. The scope of Family Medicine encompasses all ages, both sexes and every disease entity. If physicians examine and obtain blood samples for CBC and HPLC analysis from all pregnant women, it will be possible to eradicate hemoglobin disorders in Turkey.

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