

# The prevalence and molecular basis of $\beta$ -thalassemia in Isparta province and region

Bahattin Tunç<sup>1</sup>, Hasan Çetin<sup>1</sup>, Fatma Gümrük<sup>2</sup>, Beysun İstanbullu<sup>1</sup>, Hasan Yavrucuoğlu<sup>1</sup>  
Umran Kurt<sup>1</sup>, Halide Genç<sup>1</sup>

<sup>1</sup>Department of Pediatrics, Süleyman Demirel University Faculty of Medicine, Isparta, and <sup>2</sup>Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

**SUMMARY:** Tunç B, Çetin H, Gümrük F, İstanbullu B, Yavrucuoğlu H, Kurt U, Genç H. The prevalence and molecular basis of  $\beta$ -thalassemia in Isparta province and region. *Turk J Pediatr* 2002; 44: 18-20.

The prevalence and molecular basis of  $\beta$ -thalassemia in the district of Isparta were determined in a total of 6,054 healthy high school students who were recruited from 21 randomly selected high schools in the Isparta province and region. In 182 subjects, naked eye single tube red cell osmotic fragility test (NESTROFT test) was positive HbA<sub>2</sub> was measured by high-performance liquid chromatography (HPLC) in these subjects and was found to be high in 149 subjects. The incidence of  $\beta$ -thalassemia was 149 in 6,054 (25%). The  $\beta$ -thalassemia frequency was lower in the city center than in neighboring towns, 1.7% vs. 2.2%, respectively. The most prevalent mutation of  $\beta$ -thalassemia in this region was IVS 1-110 (G-A), followed by Codon 39 (C-T) and IVSII-745 (C-G).

*Key words:* hemoglobinopathy, prevalence,  $\beta$ -thalassemia trait, screening, population.

The  $\beta$ -thalassemias are due to mutations of the beta globin gene that markedly decrease or completely prevent the production of beta globin chains<sup>1-3</sup>. Thalassemias are the most common inherited disorders in the world. A relatively high prevalence is also observed in Turkey. Although the overall estimated frequency of  $\beta$ -thalassemia in Turkey has been stated to be 2%<sup>4</sup>, the frequency varies from 0.6% to 10.7% between regions<sup>5-7</sup>. Numerous up-to-date surveys have been conducted in various regions of Turkey, but there have not been any systematic studies in Isparta. We planned this screening program in order to determine the prevalence and to establish the molecular basis of  $\beta$ -thalassemia trait, and to estimate the magnitude of disease and requirements for its control in this district.

## Material and Methods

An educational and screening program for the thalassemias was carried out in 21 randomly selected high schools with a total of 6,054 healthy students in different regions of Isparta. The ages of students ranged between 13 to 18 years. The number of male and female students were 3,360 (55.5%) and 2,694 (44.5%), respectively.

This screening program was structured in three steps: the first included information and educational aspects and the second involved blood sampling and detection of carriers. In the third phase, students were informed of the results, and genetic counselling was given.

During the second stage of the study, capillary blood was obtained from each participant's finger tip. The naked eye single tube red cell osmotic fragility test (NESTROFT test) (osmotic fragility of red blood cells in 0.4% saline solution) was applied as first-step screening test to detect  $\beta$ -thalassemia trait<sup>8</sup>. Further investigation was performed on subjects who were NESTROFT positive or doubtful. Their hemoglobin A<sub>2</sub> and hemoglobin F were then determined by high-performance liquid chromatography (HPLC) (Bio-Rad). For hemoglobin electrophoresis, 2 ml of venous blood sample anticoagulated with EDTA was obtained with parental consent.

We did not attempt to investigate other variant of hemoglobinopathy other than  $\beta$ -thalassemia. The students who were found to be carriers of thalassemia mutations were given an opportunity to receive additional information and counselling.

In addition, all carriers received a card printed with their thalassemia carrier status for their records and future reference.

DNA was extracted from the peripheral blood sample of the subjects collected in EDTA-vacutainer. Mutations in the beta globin gene were identified by polymerase chain reaction (PCR) based diagnostic strategies in Hacettepe University Faculty of Medicine and Faculty of Science<sup>9</sup>.

Although the study was designed primarily for the students, 76 teachers also asked to be tested.

## Results

A total of 6,054 blood samples were obtained and analyzed for  $\beta$ -thalassemia trait with NESTROFT test. Of 6,054 students 2,992 (49.4%) were from the center district of Isparta and 3,062 (50.6%) were from 11 different towns of Isparta. One hundred eighty-two out of 6,054 blood samples (3.12%) were positive for NESTROFT test. HbA<sub>2</sub> was found to be 2.5%. Distribution of carriers according to region is illustrated in Table I.

Table I. Distribution of  $\beta$ -thalassemia Traits

Region	Subjects	Traits	%
Center of Isparta	2992	52	1.74
Aksu	147	4	2.72
Atabey	151	5	3.31
Barla	87	2	2.29
Eğirdir	600	19	3.16
Gelendost	205	11	5.36
Gönen	179	12	6.70
Keçiborlu	342	9	2.63
Senirkent	208	9	4.32
Sütcüler	142	6	4.22
Şarkikaraağaç	542	12	2.21
Yalvaç	459	8	1.74
Total	6054	149	2.46

In order to establish the molecular basis of  $\beta$ -thalassemia, DNA samples of 14 carriers were analyzed. Three different point mutations were identified. These mutations, their frequency in this study and in Turkey overall are given in Table II.

Table II. Results of the Mutation Analysis

Molecular pathology	Number of % subjects	Overall frequency of mutation for Turkey (%)
IVS I-110 (G-A)	10	38.06
Codon 39 (C-T)	3	2.6
IVSII-745 (C-G9)	1	4.33

## Discussion

$\beta$ -thalassemia constitutes one of the most serious health problems in Turkey. It is preventable by carrier screening and prenatal diagnosis<sup>10</sup>.  $\beta$ -thalassemia major remains a serious physical, emotional, and financial burden for patients, their families and countries<sup>11</sup>. The related health care cost are very high. The success observed in some countries in reducing the prevalence of thalassemia major by genetic counselling shows that screening can have a major impact in communities in which the thalassemias are common. That is, screening of the population of thalassemia is the first step in eradication.

We determined the prevalence of  $\beta$ -thalassemia trait as 2.5% in the Isparta region. The prevalence of  $\beta$ -thalassemia in our study is slightly higher than that seen in Turkey as a whole, but is lower than that of neighboring provinces. Previous studies have reported the prevalence of thalassemia in Antalya<sup>12</sup>, Konya<sup>13</sup>, and Denizli<sup>3</sup> as 10.2%, 3.8%, and 3%, respectively.

It was noted that the prevalence in the city center is lower than that in neighboring towns and villages (Table I). This difference may be due to immigration to the city center from other cities or villages with low  $\beta$ -thalassemia frequency. It appears that heterozygotes for  $\beta$ -thalassemias are protected from the severe effects of falciparum malaria<sup>14</sup>, and natural selection has increased and maintained their gene frequencies in these malarious regions as seen around Eğirdir Lake and some other towns (Table I).

We selected high school students as the target population for this survey. The ideal age for population screening for autosomal recessive disease is early adulthood, before marriage<sup>15</sup>. Therefore, screening of high school students for common genetically inherited disorders is generally recommended<sup>16</sup>. The present study indicates that most high school students as well as their parents and teachers, after being given proper information, are willing to participate in a screening program for thalassemia.

The tests in developing countries for screening of hemoglobinopathies must be reliable, easy to perform, adaptable and cost-effective. It is recommended that 0.4% buffered saline solution be used as a first screening test for the detection of  $\beta$ -thalassemia trait in a population, as was used in this study<sup>17,19</sup>. However, this test is not

enough to differentiate hypochrome anemias of various etiology. Therefore, electronic whole blood count, electrophoretic studies and HbA<sub>2</sub> determination are recommended as a first step in many screening studies.

Our results show that in Isparta the IVS-I-110 (G-A) mutation is the most common  $\beta$ -thalassemia defect, followed by Codon 39 (C-T) and IVSII-745 (C-G). The frequencies of these mutations are higher than those reported for Turkey overall. However the number of carriers was too small to represent the thalassemic population<sup>20-24</sup>.

In conclusion, the prevalence of  $\beta$ -thalassemia is relatively high in Isparta. Therefore, continuation of population screening programs and establishment of premarital screening programs, along with education and counselling, are necessary in this region.

#### Acknowledgements

We thank Çiğdem Altay MD for her support and encouragement, and we are indebted to all the students and their teachers for their participation in this project.

#### REFERENCES

- Modell B, Mauzouras M, Ward RH, Fairweather DV. Population screening for carriers of recessively inherited disorders. *Lancet* 1980; 11: 806-809.
- Lipkin MJ, Fischer L, Rwley PT, Iker HP. Genetic counselling of asymptomatic carriers in a primary care setting. The effectiveness of screening and counselling for  $\beta$ -thalassemia trait. *Ann Intern Med* 1986; 105: 115-120.
- Davies SC, Wonke B. The management of hemoglobinopathies. *Clin Haematol* 1991; 4: 361-389.
- Çavdar AO, Arcasoy A. The prevalence of  $\beta$ -thalassemia and abnormal hemoglobins in Turkey. *Acta Haematol* 1971;45: 312-317.
- Kürkçüoğlu M, Dağcı A, Gencelli Y, Arcasoy A, Ağbaş A. Doğu Anadolu Bölgesinde  $\beta$ -thalassemia ve anormal hemoglobin taraması. *Doğa Bilim Dergisi* 1986; 8: 319-325.
- Aksoy M, Kutlar F, Dinçol G, Erdem S, Baştanbilici S. Batı Trakya Türklerinde hemoglobin varyantları,  $\beta$ -thalassemia, G6PD eksikliği ve haptoglobin tipleri. *Doğa Bilim Dergisi* 1985; 9: 45-49.
- Altay Ç, Gürgey A. Distribution of hemoglobinopathies in Turkey. *Türk J Pediatr* 1986; 28: 219-229.
- Kattamis C, Efromov G, Pootrakul S. Effectiveness of one tube osmotic fragility screening in detecting  $\beta$ -thalassemia trait. *J Med Gen* 1981; 18: 266-268.
- Pnocz M, Solowiejczyk D, Harpel B, Mory Y, Schwartz E, Surrey S. Construction of human gene libraries from small amounts of peripheral blood: analysis of beta-like globin genes. *Hemoglobin* 1982; 6: 27-36.
- Cao A, Saba L, Galanello R, Rosatelli MC. Molecular diagnosis and carrier screening for beta thalassemia. *JAMA* 1997; 278: 1273-1277.
- Modell B, Kuilev AM. A scientific basis for cost-benefit analysis of genetics services. *Trends Genet* 1993; 9: 46-52.
- Bircan I, Sisli S, Güven A, et al. Hemoglobinopathies in the district of Antalya, Turkey. *Ped Hematol Oncol* 1993; 10: 289-291.
- Turan C, Topal B, Gürgey A, Altay Ç. Konya ve Denizli yöresinde beta talasemi sıklığı. *Çocuk Sağ ve Hast Dergisi* 1991; 34: 9-11.
- Lilleyman JS. Hematologic effects of systemic disease and toxins. In: Lilleyman JS, Hann MI, Blanchette VS (eds). *Pediatric Hematology* (2<sup>nd</sup> ed) London: Churchill Livingstone; 1999: 771-789.
- Altay C, Yılgör E, Beksaç S, Gürgey A. Premarital screening of hemoglobinopathies: a pilot study in Turkey. *Hum Hered* 1996; 46: 112-114.
- Clayton EW, Steinberg KK, Khoury MJ, et al. Informed consent for genetic research on stored tissue samples. *JAMA* 1995; 274: 1786-1792.
- Raghavan K, Lokeshwar MR, Birewar N, Nigam V, Manglani MV, Raju NB. Evaluation of naked eye single tube red cell osmotic fragility test in detecting beta thalassemia trait. *Indian Pediatr* 1991; 28: 469-472.
- Manglani M, Lokeshwar MR, Vani VG, Bhatia N, Mhaskar V. "NESTROFT" an effective screening test for beta thalassemia trait. *Indian Pediatr* 1997; 34: 702-707.
- Canatan D, Arcasoy A, Çavdar AO. Tek tüp osmotik fragilitite test ile  $\beta$ -thalassemia trait taraması. *Doğa Bilim Dergisi* 1985; 9: 130-135.
- Tadmouri GO, Tüzmen S, Özçelik H, et al. Molecular and population genetic analyses of beta-thalassemia in Turkey. *Am J Hematol* 1998; 57: 215-220.
- Nişli G, Kavaklı K, Aydınok Y, Öztöp S. Beta-thalassemia alleles in Aegean region of Turkey. *Pediatr Hematol Oncol* 1997; 14: 59-65.
- Altay Ç, Öner C, Öner R, Mesci L. Genotype-phenotype analysis in Hb S-beta thalassemia. *Hum Hered* 1997; 47: 161-164.
- Akar N, Cavdar AO, Dessi E, Loi A, Pirastu M, Cao A.  $\beta$ -thalassemia mutations in the Turkish population. *J Med Gen* 1987; 24: 378-379.
- Gürgey A, Altay C, Diaz-Chico JC, Kutlar A, Huisman TH. Molecular heterogeneity of beta-thalassemia intermedia in Turkey. *Acta Haematol* 1989; 81: 22-27.
- Altay Ç, Başak AN. Molecular basis and prenatal diagnosis of hemoglobinopathies in Turkey. *Int J Pediatr Hematol Oncol* 1995; 2: 283-290.