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Original Article *Özgün Araştırma*

THE EVALUATION OF TERTIARY CARE CENTER HEMOGLOBIN VARIANT DATA FOR THREE YEARS PERIOD

Üçüncü Basamak Sağlık Merkezi Hemoglobin Varyant Verilerinin Üç Yıllık Değerlendirilmesi

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ABSTRACT

Objective: Hemoglobin disorders are one of the most common hereditary diseases in the world. In this study, we aimed to evaluate the hemoglobin variants detected by hemoglobin electrophoresis for a three years period in a tertiary care center.

Materials and Methods: Hemoglobin variant analysis results of 4804 di_erent variants for a three years period were evaluated retrospectively. Hemoglobin variant analysis was performed by capillary electrophoresis method on Minicap Flex Piercing analyzer (Sebia, Lisses, France).

Results: One thousand and six (20.94%) of hemoglobin variants were detected in the study. The number of the patients with thalassemia trait was 1028 (21.39%) and the number of the patients with Beta thalassemia major was 44 (0.91%). In the study, the most common hemoglobin variant was found to be HbF (45.72%). The other hemoglobin variants in decreasing order were HbD, HbS, HbE, HbC and HbH.

Conclusion: The place where the study was conducted is not the region where hemoglobinopathy is most commonly known in our country. However, the outcomes of the study indicated di_erent results than expected. It should be not-ed that the frequency of hemoglobinopathy and the regions where it is seen frequently may change due to the migration wave that occurs due to reasons such as sociocultural, economic and war conditions.

Keywords: *Hemoglobin disorders, sickle cell anemia, thalassemia, hemoglobinopathies*

ÖZ

Amaç: Hemoglobin bozuklukları dünyada en sık görülen kalıtsal hastalıklardan biridir. Bu çalışmada üçüncü basamak bir sağlık kuruluşunda üç yıllık süre boyunca hemoglobin elektroforezi ile saptanan hemoglobin varyant-larının değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Üç yıllık dönem için 4804 farklı Hemoglobin varyant analizi sonuçları geriye dönük olarak değerlendirilmiştir. Hemoglobin varyant analizi, Minicap Flex Piercing analiz cihazında (Sebia, Lisses, Fransa) kapil-er elektroforez yöntemiyle çalışılmıştır.

Bulgular: Çalışmada 1006 tane (%20.94) hemoglobin var-yantı tespit edilmiştir. Talasemi taşıyıcısı hasta sayısı 1028 (%21.39), Beta talasemi majör hasta sayısı 44 (%0.91) olarak bulunmuştur. En yaygın hemoglobin varyantı HbF (%45.72), diğer hemoglobin varyantları ise azalan sırayla HbD, HbS, HbE, HbC ve HbH olarak kaydedilmiştir.

Sonuç: Çalışmanın yapıldığı yer ülkemizde hemoglobinopatinin en sık görüldüğü bölgelerden değildir. Ancak, çalışma beklenenden farklı sonuçlar göstermiştir. Sosyokültürel, ekonomik ve savaş koşulları gibi nedenlerle oluşan göç dalgası nedeniyle hemoglobinopati sıklığı ve sık görüldüğü bölgelerin değişebileceği akılda tutulmalıdır.

Anahtar Kelimeler: *Hemoglobin bozuklukları, orak hücreli anemi, talasemi, hemoglobinopatiler*

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INTRODUCTION

Hemoglobin disorders are one of the most common hereditary diseases in the world and in our country (1,2). There are 300 million hemoglobin molecules in each red blood cell (3). A healthy person's blood contains 95-96% Hemoglobin A1, 2.5-3.5% Hemoglobin A2, and less than 1% Hemoglobin F. Normal human hemoglobins are HbA1, HbA2, HbF, Hb Gower 1, Hb Gower 2, and Hb Portland (4). Hb variants result from various mutations in genes encoding polypeptide chains. These mutations result in the formation of various hemoglobin variants (5). Genetic diseases of hemoglobin are divided into two major groups, abnormal hemoglobins, and thalassemias (3). The frequency of thalassemia and he-moglobinopathy in our country was found to be approximately 4%(6). Abnormal hemoglobins are caused by gene mutations. The known types of abnormal hemoglobin, result from a single amino acid change in a globin chain in more than 90%, and 60% of these occur in the beta globin chain of hemoglobin (7). For the analysis of the hemoglobin variant, methods such as isoelectric focusing, high pressure liquid chromatography (HPLC), and hemoglobin electrophoresis are used (8,9). The most common abnormal hemoglobins are S, C, E, and D. One of the most common hemoglobin variants is HbS. It occurs as a result of point mutations when the amino acid valine replaces glutamic acid in the 6th row of the beta chain of normal hemoglobin. Hemoglobin formed as a result of this change in amino acid sequence is called HbS. The incidence of HbS in Turkey, especially in the southern regions, is approximately 8% (3,5). There are several variants called HbD based on the di_erence in their electrophoretic migration. HbDs are together with HbS in alkaline cellulose acetate electrophoresis, and in acidic citrate agar electrophoresis migrates to the same place with HbA(10). This variant of hemoglobin has been reported in Austria, Spain, Greece, England, Germany, Portugal, Turkey, Iran, and India (3). Other known names for Hemoglobin N-Baltimore (HbN) are Hopkins-I, N-Memphis, Jekins and Kenwood. It occurs as a result of the replacement of AAG in the 95th cord of the beta globin gene with GAG. With this mutation, the amino acid Lys is replaced by the amino acid Glu. HbN can be separated from HbA by its rapid migration in electrophoresis at alkaline pH. HbN has been seen in the American black race and in Turkey in and around Antalya province (11).

In previous studies, it was reported that the total number of abnormal hemoglobin variants reached 750 (3). In the Çukurova region (Antakya, Adana, and İçel) located in the south of our country, the frequency of sickle cell anemia carrier (HbAS) was determined as 8.2%. HbE is another common abnormal hemoglobin in our country while HbD, one of the abnormal hemoglobins, is rarely seen in our country. Also, HbC has been detected in a few cases. Other abnormal hemoglobins detected in Turkey were determined as a result of the examination of cases with thalassemia or community screening. Of the abnormal hemoglobin variants, 33 have been observed in our country, and 7 of them were rst identied in the Turkish population (12-14). In this study, we aimed to evaluate the hemoglobin variants detected by hemoglobin electrophoresis for three years in Ankara City Hospital Medical Biochemistry Laboratory, the largest hospital in Turkey, where no screening was performed before and to compare our ndings with the literature.

MATERIALS AND METHODS

In this study, hemoglobin variant analysis results of 4804 different patients analyzed in Ankara City Hospital Medical Biochemistry Laboratory for three years period were evaluated retrospectively. The data of the cases were obtained from the laboratory information system (LIS). All of the cases of hemoglobin electrophoresis studied over three years period were determined. The patients with multiple analysis results were included in the study only once. The distribution and percentages of abnormal hemoglobin var-iants were found using Microsoft O_ce Excel (Microsoft, Washington, USA). Approval for our study was obtained from the ethics committee of Ankara City Hospital with the number of E2-22-1547.

Hemoglobin variant analysis was performed by capillary electrophoresis method on Minicap Flex Piercing analyz-er (Sebia, Lisses, France). Two to ve milliliters of fresh blood samples were collected into dipotassium ethylenedi-aminetetraacetic acid (EDTA) containing plasma tubes and centrifuged at 1500×g for ten minutes. Samples were analyzed with Minicap Flex Piercing capillary electrophoresis equipment to assess the hemoglobin fractions. According to the manufacturer's instructions, capillary electrophore-sis was done in an alkaline bu_er (pH 9.4±5). Samples are diluted in hemolyzing solution (pH 8.7 ± 0.5 bu_er solution). The diluted samples are then injected into the capillaries. Migration is carried out under constant voltage for approximately 8 minutes. The molecules are separated based on the electrolyte pH. Hemoglobin was detected at the wave length of 415 nm. The electrophoretic prole was displayed on the system screen.

Results were evaluated according to the manufacturers recommendations, Normal hemoglobin prole had between 96.8-97.8% of hemoglobin A, 2.2-3.5% of hemoglobin A2 and less than 2% of hemoglobin F.

RESULTS

In the present study, 4804 hemoglobin electrophoresis results for three years period were evaluated retrospectively. The results of the patients admitted to the tertiary care hospital medical biochemistry laboratory were includ-ed in the study. No screening test for hemoglobinopathy is performed in the present laboratory. One thousand and six (20.94%) of hemoglobin variants were detected in the study. The numbers and the percentages of hemoglobin variants detected in the study are presented in Figure 1.

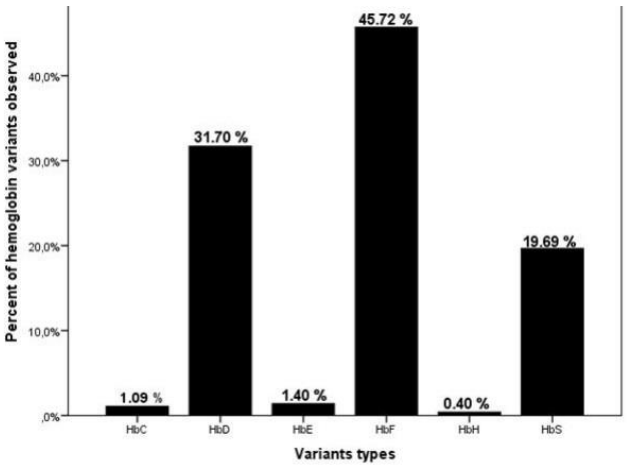

Figure 1. The percentages of hemoglobin variants in the present study

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The number of the patients with thalassemia trait was 1028 (21.39%) and the number of the patients with Beta thalassemia major was 44 (0.91%) (Figure 2B and 2C). The most common hemoglobin variant was HbF (45.72%). The other hemoglobin variants in decreasing order were HbD, HbS, HbE, HbC and HbH (Figure 3).

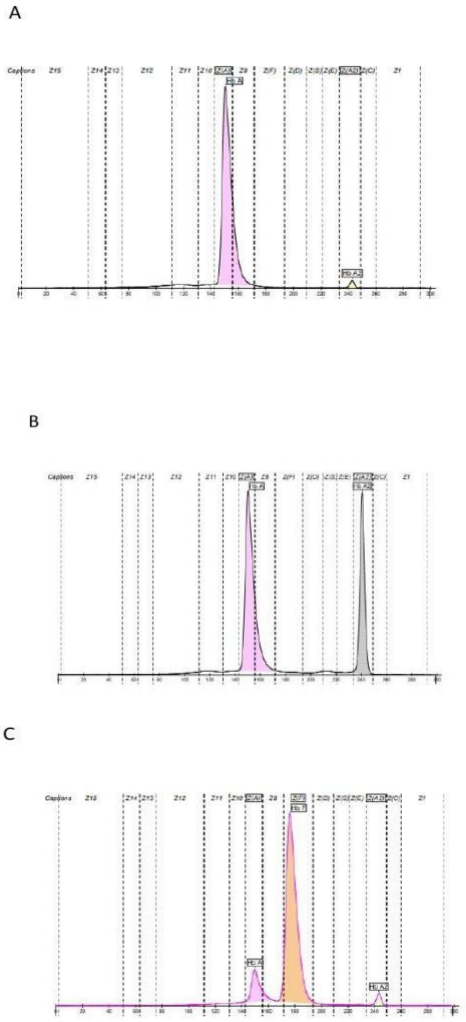

Figure 2: Capillary electrophoresis pattern of normal hemoglobin (A), Thalassemia trait (B) and Thalassemia majör (C).

DISCUSSION

Hemoglobinopathies are one of the most serious health problems in our country as well as in the world. According to the screening studies conducted in the Turkish population, the incidence of abnormal hemoglobin has been reported as 0.6-13% (15-17). The region where hemoglo-binopathies are the most common in our country is the Mediterranean region, the South of Turkey. According to a hemoglobinopathy control program, there are approximately 1.5 million thalassemia and hemoglobinopathy patients in Turkey (18) . In the present study, the frequency of the hemoglobinopathy was found as 20.94% among the total hemoglobin electrophoresis results for three years period. We found the hemoglobinopathy frequency higher than expected. Ankara is the capital city of Turkey in the middle of the country, far from the Mediterranean region. The hospital where the study was conducted is the biggest tertiary care hospital in Turkey, even in Europe. As screening tests for hemoglobinopathy are not performed in the hospital and also the patients with the diagnosis or clinical suspicion of hemoglobinopathies all over the country are admitted to the hospital, it should be expected to be more frequent. Also, the migration wave that occurs due to reasons such as sociocultural, economic and war conditions might have contributed to the change in the frequency of hemoglobinopathies seen in our hospital.

Various studies have been carried out for hemoglobinop-athies outside the regions where hemoglobinopathies are frequently seen. According to the study of Güngör et al., in Erzurum, a city in east region of Turkey; it was found that abnormal hemoglobin types HbD, HbS, HbWood, Hb-Malmo and HbN-Baltimora existed in Erzurum region and their incidence was calculated as 0.20% and it was emphasized that this rate is quite low in our country compared to the Mediterranean region, where hemoglobinopathies are common. Abnormal hemoglobins were found in 0.057% HbD type, 0.028% HbAS type and 0.028% HbS type in this study (3). In the study of abnormal hemoglobin variant frequency conducted by Dikker et al., 1894 hemoglobin variant analyzes were examined and abnormal hemoglobin variants were detected in 18 cases. The result of this study indicated that the most common Hb variant was HbS and it was followed by the Hb D-Los Angeles variant (6). In the hemoglobinopathy frequency study conducted by Topal et al.in young adults in Muğla region. Approximately 155000 students over the age of 16 were screened, and abnormal hemoglobin variants were found in approximately 3.8% of them. In this study, the most common variant found was Hb F (19). In the study of Bircan et al. in which they included 1616 patients aged 15-60 years randomly selected in Antalya; they found the most common variant as Hb F (%0.8) and the second most common variant was Hb D Los Angeles (20). In another study, Sarper et al., performed premarital hemoglobinopathy screening in Kocaeli. Approximately 90.000 patients who applied to primary health care institutions before marriage in Kocaeli and its districts for three years were evaluated in the study. As a result of the study, they found the most common to be Hb D variant and the second most common variant was Hb E (21).

Yuregir et al., conducted a hemoglobinopathy screening study in Kahramanmaraş. They included 1491 patients in their study. According to the results of their studies, they found Hb D (% 0.28) variant as the most frequent variant and Hb O Arab (%0.013) variant was in the second place in Kahramanmaraş region (22). Similarly, in our study the most common hemoglobin variant was HbF, followed by HbD. We also found HbS, HbE, HbC and HbH variants. However the frequencies of the hemoglobin variants in our study were higher than the mentioned studies.

One of the limitations of our study was that genetic analysis results and mutation types were not included in the study. Our study was conducted in the biggest hospital of Turkey. We evaluated all the hemoglobin electrophore-sis results requested from the patients who applied to the hospital for three years period. Although the frequency of hemoglobin variants is the highest in the Mediterranean region in our country, according to the results of the present study, a high number of hemoglobin variants were detected in Ankara, the Central Anatolia region, which is not considered as a risky region for hemoglobinopathies. It should be noted that the frequency of hemoglobinopathy and the regions where it is seen frequently may change due to the migration wave that occurs due to reasons such as sociocultural, economic and war conditions. These conditions should be considered when planning studies. Thus, new comprehensive studies covering all regions will contribute to the literature and also it will raise awareness about an important public health problem and shape the measures to be taken.

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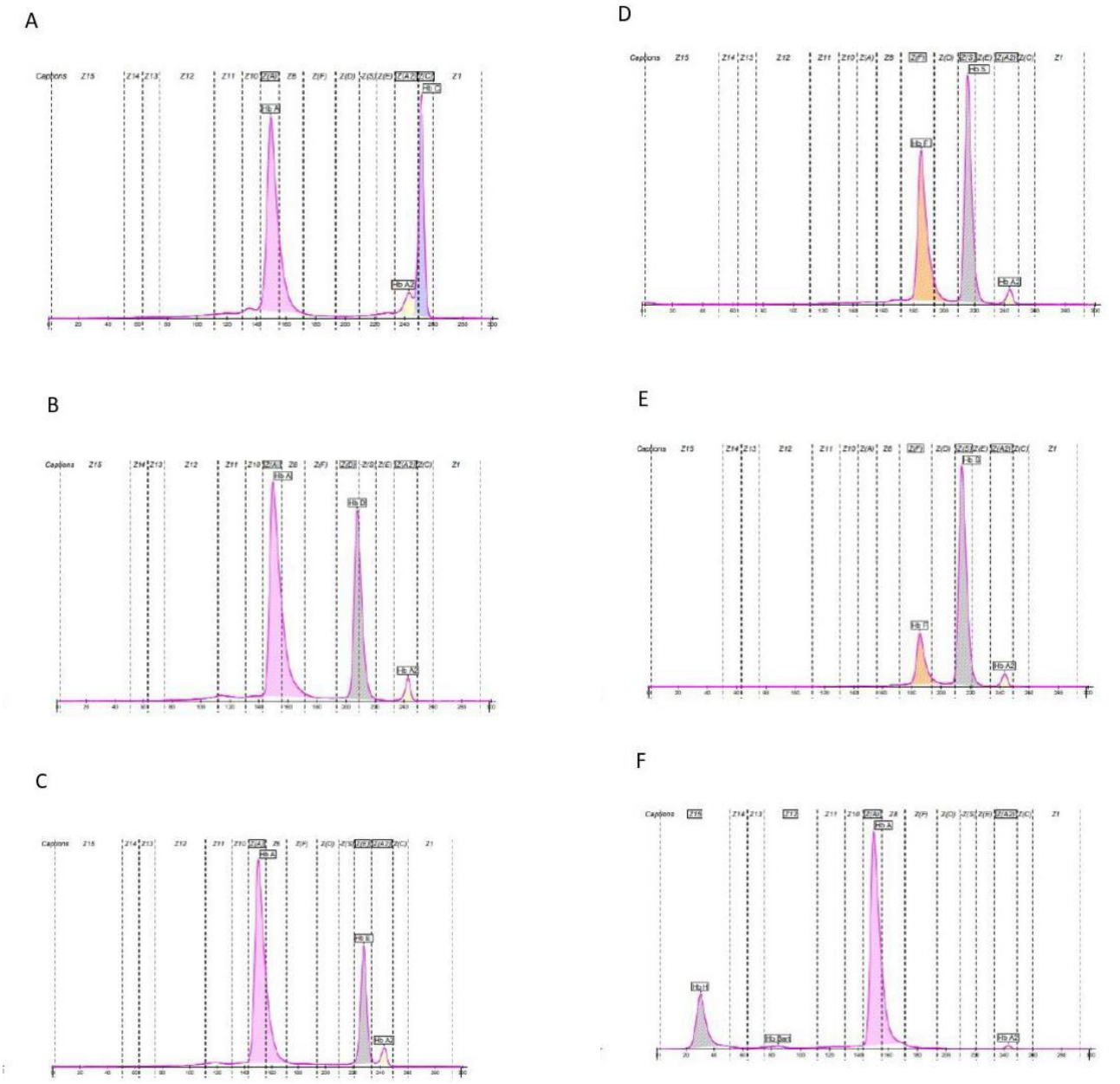

Figure 3. Capillary electrophoresis pattern of hemoglobin variants. Hemoglobin C (A), Hemoglobin D (B), Hemoglobin E (C), Hemoglobin F (D), Hemoglobin S (E), Hemoglobin H (F)

Ethics Committee Approval: Approval for our study was obtained from the ethics committee of Ankara City Hospital with the number of E2-22-1547.

Informed Consent: Written informed consent was obtained from all participants who participated in this study

Conflict of Interest Statement: The author has no con_icts of interest to declare.

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REFERENCES

1. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704-12.

2. Akar E, Akar N. A review of abnormal hemoglobins in Turkey. Turk J Haematol. 2007;24(4):143-5.

3. Güngör AA, Demir Y, Demir N. Anormal Hemoglobinler'in Farklı Hemoglobin Elektroforezleri ile Belirlenmesi. Süleyman Demirel Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi. 2011;6(1):40-54.

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Doi: 10.24938/kutfd.1128242

4. Huisman TH. 1 The structure and function of normal and abnormal haemoglobins. Baillieres Clin Haematol. 1993;6(1):1-30.

5. Flint J, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. Baillieres Clin Haematol. 1998;11(1):1-51.

6. Dikker O, Vardar M, Sandıkçı R, Basat B, Sucu V, Vurgun E, et al. Okmeydanı Eğitim ve Araştırma Hastanesi Tıbbi Biyokimya Laboratuvarında HPLC Yöntemi ile Saptanan Anormal Hemoglobin Varyantları. Okmeydanı Tıp Dergisi. 2016;32(4):185-9.

7. Fucharoen S, Winichagoon P. Thalassemia and abnormal hemoglobin. Int J Hematol. 2002;76(Suppl 2):83-9.

8. The laboratory diagnosis of haemoglobinopathies. Br J Haematol. 1998;101:783-92.

9. Wajcman H, Préhu C, Bardakdjian-Michau J, Promé D, Riou J, Godart C, et al. Abnormal hemoglobins: laboratory methods. Hemoglobin. 2001;25(2):169-81.

10. Bunn HF, Forget BG. Hemoglobin--molecular, genetic, and clinical aspects: WB Saunders Co.; 1986.

11. Cürük MA, Yüregir GT, Asadov CD, Dadasova T, Gu LH, Baysal E, et al. Molecular characterization of beta-thalassemia in Azerbaijan. Hum Genet. 1992;90(4):417-9.

12. . Altay Ç. Abnormal hemoglobins in Turkey. Turk J Hae- matol. 2002;19(1):63-74.

13. Atalay EÖ, Atalay A, Koyuncu H, Öztürk O, Köseler A, Özkan A, et al. Rare hemoglobin variant Hb Yaizu observed in Turkey. Med Princ Pract. 2008;17(4):321-4.

14. Akar N. An updated review of abnormal hemoglobins in the Turkish population. Turk J Haematol. 2014;31(1):97-8.

15. Kilinç Y, Kumi M, Gurgey A, Altay C, Webber B, Wilson J, et al. Hemoglobin o-padova or? 230 (b11) GLU› LYSß2 observed in members of a Turkish family. Hemoglobin. 1985;9(6):621-5.

16. Akar E, Özdemir S, Hakki Timur I, Akar N. First observation of homozygous hemoglobin Hamadan (B 56 (D7) GLY-ARG) and beta thalassemia (-29 G> A)-hemoglobin Hamadan combination in a Turkish family. Am J Hematol. 2003;74(4):280-2.

17. . Canatan D. Thalassemias and hemoglobinopathies in Turkey. Hemoglobin. 2014;38(5):305-7.

18. Canatan D, Kose MR, Ustundag M, Haznedaroglu D, Ozbas S. Hemoglobinopathy control program in Turkey. Community Genet. 2006;9(2):124-6.

19. Topal Y, Topal H, Ceyhan MN, Azik F, Çapanoğlu M, Ko-cabaş CN. The prevalence of hemoglobinopathies in young adolescents in the province of Muğla in Turkey: results of a screening program. Hemoglobin. 2015;39(4):247-50.

20. Bircan I, Şişli S, Güven A, Çali Ş, Yeğin O, Ertuğ H, et al. Hemoglobinopathies in the district of Antalya, Turkey. Pediatr Hematol Oncol. 1993;10(3):289-91.

21. Sarper N, Şenkal V, Güray F, Bayram J. Premarital hemoglobinopathy screening in Kocaeli, Turkey: a crowded industrial center on the north coast of Marmara Sea. Turk J Haematol. 2009;26(2):62-6.

22. Yuregir GT, Kilinc M, Ekerbicer H, Bilaloglu N, Tekin N. Screening of Hemoglobinopathies in Kahramanmaras, TURKEY. Turk J Haematol. 2001;18(2):79-83.