Hemoglobin
international journal for hemoglobin research

ISSN: 0363-0269 (Print) 1532-432X (Online) Journal homepage: http://www.tandfonline.com/loi/ihem20

Hemoglobinopathies in the Çukurova Region and Neighboring Provinces

Sedefgul Yuzbasioglu Ariyurek, Sule Menziletoglu Yildiz, Ali Erdinc Yalin, Figen Guzelgul & Kiymet Aksoy

To cite this article: Sedefgul Yuzbasioglu Ariyurek, Sule Menziletoglu Yildiz, Ali Erdinc Yalin, Figen Guzelgul & Kiymet Aksoy (2016) Hemoglobinopathies in the Çukurova Region and Neighboring Provinces, Hemoglobin, 40:3, 168-172, DOI: 10.3109/03630269.2016.1155156

To link to this article: http://dx.doi.org/10.3109/03630269.2016.1155156

Published online: 17 Mar 2016.

www.tandfonline.com/ihem
ISSN: 0363-0269 (print), 1532-432X (electronic)

HEMOGLOBIN

Hemoglobin, 2016; 40(3): 168–172
 DOI: 10.3109/03630269.2016.1155156

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Hemoglobinopathies in the Çukurova Region and Neighboring Provinces

Sedefgul Yuzbasioglu Ariyurek[1], Sule Menziletoglu Yildiz[1], Ali Erdinc Yalin[2], Figen Guzelgul[3], and Kiymet Aksoy[3]

[1]*Department of Medical Services and Technics, Vocational School of Health Services, Çukurova University, Adana, Turkey*
[2]*Department of Biochemistry, Faculty of Pharmacy, Mersin University, Mersin, Turkey*
[3]*Department of Medical Biochemistry, Faculty of Medicine, Çukurova University, Adana, Turkey*

Abstract

To contribute to the creation of a mutation map of the region, we aimed to determine the mutation spectrum of thalassemias and abnormal hemoglobins (Hbs) in the Çukurova region and surrounding provinces. In this study, a total of 8135 samples from Adana, Hatay, Mersin, Konya and Kayseri provinces between 1993 and 2014 were analyzed. Complete blood cell (CBC) counts and Hb typing were carried out using automatic cell counters, cellulose acetate membrane electrophoresis and high performance liquid chromatography (HPLC), respectively. For the molecular analyses, genomic DNA was extracted using both manual and automated DNA extraction devices. Determination of Hb mutations were done by microarray, restriction fragment length polymorphism (RFLP), amplification refractory mutation system (ARMS) and gap-polymerase chain reaction (gap-PCR) methodologies. Samples were analyzed for abnormal Hb and thalassemia mutations. Out of 8135 samples, 1382 were observed to be carrying Hb mutations. It was identified that 826 mutation carriers included abnormal Hbs with a frequency of 59.7%, 416 carriers included β-thalassemia (β-thal) mutations with a frequency of 30.7% and 136 carriers included α-thalassemia (α-thal) mutations with a frequency of 9.9%. In this study, the most frequently observed abnormal Hb in the region was Hb S [β6(A3)Glu→Val (G*T*G > G*A*G), *HBB*: c.20T > A], whereas the most commonly observed mutations were the IVS-I-110 (G > A) (*HBB*: c.93–21G > A) point mutation in β-thal and the 3.7 kb deletion in α-thal.

Keywords

Abnormal hemoglobin (Hb), α-thalassemia (α-thal), β-thalassemia (β-thal), hemoglobinopathy

History

Received 3 July 2015
Revised 9 December 2015
Accepted 9 December 2015
Published online 8 March 2016

Introduction

Hemoglobinopathies, which are known to be the most prevalent hereditary blood diseases worldwide, occur as a result of the changes in globin chains or hemoglobin (Hb) molecules. Quantitative changes of globin chains create α- and β-thalassemias, while qualilative changes constitute structurally abnormal Hbs. Depending on the type and/or combination of mutation, a substantially variable clinical course and outcome of severe forms of hemoglobinopathies may cause death of patients at an early age (1,2).

In Turkey, because of its geographical location, the Çukurova region and neighboring cities have been populated by various ethnicities. Consequently, the region has a heterogeneous structure in respect to population genetics (3). Accordingly, the region has a socioculturally conservative structure and consanguineous marriages are commonly seen in the area. The rate of consanguineous marriages, which are the major reason of frequent occurrence of autosomal recessively inherited diseases, is around 20.0–25.0% in Turkey (4). There are hundreds of thousands of hemoglobinopathy carriers with severe clinical forms being diagnosed every year but sufficient treatment has not yet been developed. The most effective way to prevent the birth of hemoglobinopathy carriers is prenatal diagnosis (PND).

In Turkey, Prevention of Hereditary Blood Diseases Law No. 3960 was introduced in the Official Gazette No. 21804 (dated December 30 1993). The Hemoglobinopathy Control Program was launched to prevent the birth of children with hemoglobinopathies by mutation screening tests before marriage. Hemoglobinopathy screening centers were established to identify carriers with the intention of reducing the frequency of disease, especially in 33 cities at risk such as Adana, Gaziantep, Hatay and Mersin that have a high frequency of carriers (5).

In this present study, a total of 8135 cases were screened for mutation types in Adana, Hatay, Mersin, Konya and Kayseri (Figure 1). The State Health Institutions have recently started hemoglobinopathy screening programs in Konya and Kayseri. We wanted to determine the mutation distribution of populations settled in Konya and Kayseri as

Address correspondence to Sedefgul Yuzbasioglu Ariyurek, Ph.D., Department of Medical Serives and Technics, Vocational School of Health Services, Çukurova University, 01330 Adana, Turkey. Tel: +90-322-338-65-38. Fax: +90-322-338-65-39. E-mail: syuzbasioglu@cu.edu.tr

Figure 1. Geographical locations of the samples collected in this study.

well as provinces of the Çukurova region. Therefore, determination of the mutation types and carrier frequencies observed in these provinces were targeted. In addition, it was also our aim to constitute a map of Hb mutations in Turkey.

Materials and methods

This cross sectional study was carried out on blood samples collected from a total of 8135 cases in the Çukurova region and neighboring provinces between 1993 and 2014. We aimed to determine the mutation pattern of hemoglobinopathies in individuals who volunteered themselves for screening. For this purpose, daily mobile camps were organized at these cities of the Çukurova region and neighboring provinces by the Department of Biochemistry, Çukurova University, Adana, Turkey. The research protocol was approved by the Ethics Committee of the Medical Faculty of Çukurova University.

Blood samples were collected in vacutainers containing EDTA as anticoagulant, and the hematological findings were obtained by a blood cell counter (model T890; Coulter Electronics, Miami, FL, USA). Hemoglobin typing was performed by cellulose acetate membrane electrophoresis and high performance liquid chromatography (HPLC) (6,7). Hb A_2 and Hb F levels were measured by microcolumn chromatography and by a modification of the Betke method, respectively (8,9). The DNA to be used in molecular analyses were isolated by the procedure described by Poncz *et al*. (10) and a MagnaPure LC automated DNA isolation device (Roche Diagnostics GmbH, Penzberg, Germany), and then were stored at 4 °C (11). Hemoglobinopathies commonly seen in our region were characterized by microarray, amplification refractory mutation system (ARMS), restriction fragment length polymorphism (RFLP) and gap-polymerase reaction (gap-PCR) methodologies (12–15).

Results

In this study, the analyses of a total of 8135 samples from the cities of Adana, Hatay, Mersin, Kayseri and Konya were done at the Department of Medical Biochemistry, Faculty of Medicine, Çukurova University, Adana, Turkey. Seven thousand, seven hundred and eighty-nine samples came from provinces of the Çukurova region (Adana, Mersin and Hatay), 277 samples from Konya and 69 from Kayseri. We observed that 1382 chromosomes of the cases sampled from these provinces carried Hb mutations. We identified 826 carriers of abnormal Hbs with a frequency of 59.7%, 416 carriers of β-thal with a frequency of 30.1% and 136 carriers of α-thal with a frequency of 9.9% (Table 1).

In our study, we observed that some cases carried homozygous Hb mutations, and some cases carried compound or double heterozygous mutations. The chromosome number and types of mutations carried in the homozygous state are shown in Table 2, and the cases carried as compound or double heterozygous mutations are shown in Table 3. The most frequent mutations observed in the cities where samples were collected is presented in Table 4.

Discussion

Hemoglobinopathies are common in regions with tropical climates as well as all over the world. According to the data of the World Health Organization, the prevalence of hemoglobinopathies is 7.0% and approximately 300,000–500,000 carriers with severe clinical symptoms are born every year (1). In the Mediterranean countries such as Greece, North and South Cyprus, Italy and in Canada where β-thal was a major health problem, the control program that was implemented in the 1980s has resulted in almost nil homozygous births by the 1990s (16). While the number of newborn with thalassemias and hemoglobinopathies was 272 in 2002, it had dropped to 25 in 2010. There has been a 90.0% reduction on the affected births in the last 10 years (17). In the studies conducted in

Turkey, 60 abnormal Hb variants, more than 42 β-thal and 12 α-thal mutations has been identified (18–22). We found that seven abnormal Hb variants, five α-thal and 12 β-thal mutations were determined. Unrelated individuals constitute the sample population.

In the studies by Ozturk *et al.* (23) and Bahadır *et al*. (24) aimed to investigate the β-globin gene cluster haplotypes of the abnormal Hbs and β-thalassemias observed in Turkey and found the highest ratio of Hb S [β6(A3)Glu→Val (G*T*G>G*A*G), *HBB*: c.20T>A] and (G>A) (*HBB*: [IVS-I-110 c.93–21G>A) mutations as we did in our study. In a study by Altay *et al.* (25), it was reported that the most frequent abnormal Hb variants observed in Turkey are Hb S, Hb D-Los Angeles) [β121(GH4)Glu→Gln (*G*AA>*C*AA), *HBB*: c.364G>C], Hb E [β26(B8)Glu→Lys (*G*AG>*A*AG), *HBB*: c.79G>A] and Hb O-Arab [β121(GH4)Glu→Lys (*G*AA>*A*AA), *HBB*: c.364G>A]. According to our findings, the most frequent mutation is Hb S with a 57.5% frequency. Following that, Hb D-Los Angeles (0.8%), Hb E (0.6%) and Hb O-Arab (0.3%) are commonly observed abnormal Hbs, especially in the Çukurova region (Tables 1 and 4). In the study by Guvenc *et al*. (26), it was pointed out that the frequently observed abnormal Hb variants are Hb S, Hb D-Los Angeles and Hb E in the Adana region. The result of our study determined 807 Hb S carriers with a 10.4% frequency of that mutation (Table 4). In a study carried out by Guler *et al*. (27), they reported 2.0% β-thal and 0.05% Hb S carriers in Konya. In our study, we found only one heterozygous Hb S carrier and one compound heterozygote for the Hb D-Los Angeles/IVS-I-110 mutations with a frequency of 0.4% for each one (Table 4). Moreover, Hb S was the most frequently observed abnormal Hb that was inherited together with the compound heterozygous forms of thalassemia mutations (Table 3).

Table 1. Hemoglobin variants and their frequencies observed in the Çukurova region and neighboring provinces.

Mutation	Chromosome (*n*)	%
Hb S (*HBB*: c.20T>A)	795	57.5
IVS-I-110 (G>A) (*HBB*: c.93–21G>A)	285	20.6
$-\alpha^{3.7}$	59	4.3
Codon 8 (−AA) (*HBB*: c.25_26delAA)	30	2.2
Codon 39 (C>T) (*HBB*: c.118C>T)	26	1.9
$-\alpha^{4.2}$	25	1.8
IVS-I-1 (G>A) (*HBB*: c.92+1G>A)	23	1.7
$--^{\text{MED I}}$	19	1.4
$--^{\text{MED II}}$	17	1.2
$-(\alpha)^{20.5}$	16	1.2
IVS-I-6 (T>C) (*HBB*: c.92+6T>C)	16	1.2
Hb D-Los Angeles (*HBB*: c.364G>C)	13	0.9
Codon 5 (-CT) (*HBB*: c.17_18delCT)	10	0.7
Hb E (*HBB*: c.79G>A)	9	0.7
−30 (T>A) (*HBB*: c.-80T>A)	9	0.7
IVS-II-745 (C>G) (*HBB*: c.316-106C>G)	9	0.7
IVS-II-1 (G>A) (*HBB*: c.315+1G>A)	9	0.7
Hb O-Arab (*HBB*: c.364G>A)	4	0.3
Hb G-Coushatta (*HBB*: c.68A>C)	2	0.1
Hb E-Saskatoon (*HBB*: c.67G>A)	2	0.1
Hb D-Iran (*HBB*: c.67G>C)	1	0.1
Codon 44 (-C) (*HBB*: c.135delC)	1	0.1
−87 (C>G) (*HBB*: c.−137C>G)	1	0.1
Total	1382	100.0

Table 2. Homozygous hemoglobin variants and their frequencies.

Hb Mutations	*n*	%
Hb S/Hb S (β^S/β^S)	48	61.0
IVS-I-110(G>A)/IVS-I-110(G>A)	14	18.0
$-\alpha^{3.7}/-\alpha^{3.7}$	8	10.0
Hb O-Arab(G>A)/Hb O-Arab(G>A)	2	2.5
Hb E-Saskatoon(G>A)/Hb E-Saskatoon(G>A)	1	1.3
Hb E/Hb E (β^E/β^E)	1	1.3
Hb D-Los Angeles(G>C)/Hb D-Los Angeles(G>C)	1	1.3
Hb D-Iran(G>C)/Hb D-Iran(G>C)	1	1.3
Codon 5(−CT)/codon 5(−CT)	1	1.3
Codon 39(C>T)/codon 39(C>T)	1	1.3
IVS-I-6(T>C)/IVS-I-6(T>C)	1	1.3
Total	79	100.0

Table 3. Compound heterozygous hemoglobin variants and their numbers.

Compound Heterozygous Variants	*n*
Hb SS(β^S/β^S)/$-\alpha^{3.7}/-\alpha^{3.7}$	7
IVS-I-110(G>A)/IVS-I-6(T>C)	7
IVS-I-110(G>A)/codon 8(−AA)	6
$-(\alpha)^{20.5}/-\alpha^{4.2}$	5
$--^{\text{MED I}}/-\alpha^{3.7}$	5
$-(\alpha)^{20.5}/-\alpha^{3.7}$	3
IVS-I-110(G>A)/IVS-II-745(G>A)	3
$-\alpha^{4.2}/-\alpha^{3.7}$	2
Hb SS (β^S/β^S)/IVS-I-110(G>A)	2
Hb AS ($\beta^A\beta^S$)/IVS-I-110(G>A)	2
IVS-I-110(G>A)/−30(T>A)	2
IVS-I-110(G>A)/codon 39(C>T)	1
Hb EE (β^E/β^E)/IVS-I-110(G>A)	1
Hb AE ($\beta^A\beta^E$)/IVS-I-110(G>A)	1
Hb AD-Los Angeles($\beta^A/\beta^{\text{D-Los Angeles}}$)/IVS-I-110(G>A)	1
IVS-I-110(G>A)/$-\alpha^{3.7}/\alpha\alpha$	1
IVS-I-6(T>C)/IVS-II-1(G>A)	1
IVS-I-1(G>A)/IVS-I-1(G>A)	1
Hb AS(β^A/β^S)/IVS-I-1(G>A)	1
IVS-I-1(G>A)/codon 8(−AA)	1
IVS-I-6(T>C)/−30(T>A)	1
Hb SS($\beta^S\beta^S$)/$-(\alpha)^{20.5}/\alpha\alpha$	1
$--^{\text{MED I}}/-\alpha^{4.2}$	1
Total	57

Table 4. The most frequently observed mutation types in the provinces where the samples were collected.

		Most Frequently Observed Mutation Types		
Province	*n*	Abnormal Hb (%)	α-Thal (%)	β-Thal (%)
Çukurova	7789	Hb S (5.1)	$-\alpha^{3.7}$ (0.4)	IVS-I-110 (1.0)
Kayseri	69	–	–	IVS-I-110 (42.0)
Konya	277	Hb S, Hb D-Los Angeles (0.2)	$-\alpha^{3.7}$ (0.2)	IVS-I-110 (12.5)

Distribution of β-thal trait in our country shows a variable prevalence depending on the regions. Thrace, Aegean, Mediterranean and southeast Turkey are especially at risk for β-thal trait. Although the overall frequency of β-thal is 2.0%, it reaches about 10.0% in the Thrace region (19). The most frequent mutations are IVS-I-110, IVS-I-6 (T > C, *HBB*: c.92 + 6T > C), codon 8 (−AA, *HBB*: c.25_26delAA), IVS-I-1 (G > A, *HBB*: c.92 + 1G > A), IVS-II-1 (G > A, *HBB*: c.315G > A) and IVS-II-745 (C > G, *HBB*: c.316–106C > G) in Turkey and these comprise 70.0% of all reported mutations (27). In our study, it was determined that the most frequently observed β-thal mutation in all provinces was IVS-I-110 with a rate of 20.7%, and subsequent mutations were codon 8 (2.2%), codon 39 (C > T, *HBB*: c.118C > T) (1.9%), IVS-I-1 (1.7%), and IVS-I-6 (1.3%) (Table 1). The study by Guvenc *et al*. (25) reported 18 different β-thal mutations with a 13.46% frequency for all in Adana. Aldemir *et al*. (28) pointed out that with respect to other regions of Turkey, Hatay had a considerable difference in frequencies of mutations due to migrants from Syria and Iraq, and a 75.8% frequency was reported for the IVS-I-110, IVS-I-6, codon 123 (−A, *HBB*: c.370delA), IVS-I-1 and codon 8 β-thal mutations. Yapıcı *et al*. (29) determined the frequency of β-thal trait in a population screening program in Mersin. In our study, out of 7789 subjects, we observed 243 heterozygous β-thal carriers with a 3.1% frequency. The most frequent mutation, IVS-I-110, has a 1.9% heterozygous state. In the Çukurova region, frequencies of heterozygous states of the other mutations were determined to be as follows: IVS-I-1 (0.3%), codon 39 (0.3%), codon 8 (0.2%), IVS-I-6 (0.1%), −30 (T > A, *HBB*: c.−80T > A) (0.1%), IVS-II-1 (0.1%), and IVS-II-745 (0.1%). The most common mutation in Konya was IVS I-110 with an unexpectedly high 26.0% frequency, which may be considered to be due to low sample size. Karakukcu *et al.* (30) observed a 1.7% prevalence of β-thal trait but we found higher than that in Kayseri due to a similar reason as for Konya. Cases with compound heterozygosity for Hb S/β-thal have a more severe phenotype than other mutational combinations and Tadmouri *et al*. (31) reported a 1:1000 ratio of compound heterozygotes in a newborn study. We determined two compound heterozygotes with Hb S/IVS-I-110 and only one compound heterozygote for Hb E/IVS-I-110 in our study.

In studies carried out in Turkey, the α-thal carrier frequency was reported to be 2.0%, and five types of deletional mutations were detected, namely, $-\alpha^{37}$ (rightward), $-\alpha^{4.2}$ (leftward), $-(\alpha)^{20.5}$, $--^{\text{MED I}}$ and $--^{\text{MED II}}$ (32). Guvenc *et al.* (33) reported that α-thal carrier frequency was 7.5% and the most common mutation in the Adana region was the $-\alpha^{3.7}$ deletion. Another investigation carried out by Bozdogan *et al.* (21), showed 12 different α-thal mutations and these were: $-\alpha^{3.7}$ (63.3%), $--^{\text{MED}}$ (11.7%), $-(\alpha)^{20.5}$ (10.7%), IVS-I (−5 nt) (−TGAGG), (3.9%) and the polyadenylation (polyA2) site (AATAAA > AATGAA (3.5%). According to the results of our study, the most frequently observed deletional α-thal mutations in Çukurova region are $-\alpha^{3.7}$ (4.3%), $-\alpha^{4.2}$ (1.8%), $--^{\text{MED I}}$ (1.4%), $--^{\text{MED II}}$ (1.2%) and $-(\alpha)^{20.5}$ (1.2%) deletions, respectively (Table 1).

Hemoglobinopathies constitute an important public health problem in a large majority of provinces in Turkey. Although their distribution varies from one region to another, the southeastern coast of the Mediterranean Sea (Çukurova region) is the most affected area. National prevention programs are of prime importance in the prevention and eradication of hemoglobinopathies. They should be improved by means of public education about hemoglobinopathies, extended investigations and screening programs, enhanced genetic counseling and establishing national patient and carrier registration for hemoglobinopathies.

Acknowledgments

In this article, findings from the studies titled 'Establishing the infrastructure of the new methods to be used in the diagnosis of hemoglobinopathies: microarray and bioinformatics,' project no. 2005K120320-E by DPT, 'Screening of β thalassemic gene frequency and determination of mutation focuses in Çukurova,' project no. TAG 0758 by TÜBİTAK, 'Determination of miRNA expression levels by RT-PCR in sickle cell anemia cases,' project no. TF2012D7 from the Çukurova University Research Fund, Adana, Turkey, 'Typing of the hemoglobinopathies and informing study in high schools in the region of Hatay-Samandağ,' project no. TF2008LTP14, 'Determination of hemoglobinopathies with microarray method,' project no. TF2006YL9, 'Determination of abnormal hemoglobin and thalassemia mutation types of Konya region,' project no. SBE2004D4, 'Typing of β-thalassemia mutations of Anamur region,' project no. SBE2002YL1, 'Screening of thalassemic mutations in Kayseri region,' project no. TF2001U32, 'The analysis of the mutations that cause the deficiency of α thalassemia and G6PD enzyme at the molecular level,' project no. SBE99D2, 'The typing of α thalassemia deletions by PCR method in the Çukurova region,' project no. TF 9522 and 'Determination of α thalassemia mutations at the molecular level in the Çukurova region,' project no. TFE 94-4, have been utilized. In this study, Dr. S. Yuzbasioglu Ariyurek, Dr. S. Menziletoglu Yildiz, Associate Professor A. Erdinc Yalin, Dr. F. Guzelgul and Professor Dr. K. Aksoy (Department of Medical Services and Technics, Vocational School of Health Services, Çukurova University, Adana; Department of Biochemistry, Faculty of Pharmacy, Mersin University, Mersin and Department of Medical Biochemistry, Faculty of Medicine, Çukurova University, Adana, Turkey) were involved in carrying out the experiments and in writing this article.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

1. Weatherall DJ, Clegg JB. Inherited hemoglobin disorders: An increasing global health problem. Bull World Health Organ. 2001; 79(8):704–712.
2. Steinberg MH, Forget BG, Higgs DR, Weatherall DJ, Eds. Disorders of Hemoglobin Genetics, Pathophysiology, and Clinical Management, 2nd ed. New York, NY: Cambridge University Press, 2009.
3. Alakoc YD, Akar N. The importance of studying inherited hematological disorders in ancient Anatolian populations. Turk J Hematol. 2011;28(4):257–263.

4. Tuncbilek E, Ozguc M. Application of medical genetics in Turkey. Turk J Pediatr. 2007;49(4):353–359.
5. Canatan D, Kose MR, Ustundag M, *et al*. Hemoglobinopathy control program in Turkey. Community Genet. 2006;9(2):124–126.
6. Kohn J. Separation of hemoglobin on cellulose acetate. J Clin Pathol. 1969;22(1):109–110.
7. Huisman THJ. High performance liquid chromatography as a method to identify haemoglobin abnormalities. Acta Haematol. 1987;78(2–3):123–126.
8. Huisman THJ, Schroeder WA, Brodie AN, *et al*. Microchromatography of hemoglobin. A simplified procedure for determination of Hemoglobin A_2. J Lab Clin Med. 1975; 86(4):700–702.
9. Singer K, Chernoff AA, Singer L. Studies on abnormal hemoglobins. Alkali denaturation. Blood. 1951;6(5):413–423.
10. Poncz M, Solowiejczyk D, Harpel B, *et al*. Construction of human gene libraries from small amounts of peripheral blood: Analysis of β-like globin genes. Hemoglobin. 1982;6(1):27–36.
11. Kessler HH, Muhlbauer G, Stelzl E, *et al*. Fully automated nucleic acid extraction: MagnaPure LC. Clin Chem. 2001; 47(6):1124–1126.
12. Foglieni B, Cremonesi L, Travi M, *et al*. β-Thalassemia microelectronic chip: A fast and accurate method for mutation detection. Clin Chem. 2004;50(1):73–79.
13. Saiki RK, Scharf S, Faloona F, *et al*. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science. 1985; 230(4735):1350–1354.
14. Newton CR, Graham A, Heptinstall LE, *et al*. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Res. 1989;17(7):2503–2516.
15. Chong SS, Boehm CD, Higgs DR, Cutting GR. Single-tube multiplex-PCR screen for common deletional determinants of α-thalassemia. Blood. 2000;95(1):360–362.
16. Arpacı A, Aytac N, Yüregir GT, *et al*. An education programme on sickle cell anemia and β-thalassemia for the 8th grade students. Turk Haematol. 2003;20(1):19–24.
17. Canatan D. Thalassemias and hemoglobinopathies in Turkey. Hemoglobin. 2014;38(5):305–307.
18. Akar N. An updated review of abnormal hemoglobins in the Turkish populations. Turk J Hematol. 2014;31(1):97–98.
19. Çürük MA, Yalin E, Aksoy K. Prevention of hemoglobinopathies in Turkey. Thalassemia Reports. 2013;3:e1. doi: 10.4081/thal.2013.e1.
20. Guzelgul F, Yalin AE, Aksoy K. The first observation of two α globin mutations in Turkey: Hb Stanleyville II and a homozygous 5nt deletion. Turk J Biochem. 2014;39(4):523–528.
21. Bozdogan ST, Yuregir OO, Buyukkurt N, *et al*. α-Thalassemia mutations in Adana Province, Southern Turkey: Genotype-phenotype correlation. Indian J Hematol Blood Transfus. 2015; 31(2):223–228.
22. Ariyurek SY, Yildiz SM, Aksoy K. Identification of Hb Hamadan Mutation (β56 GGC→CGC, (D7) Gly→Arg) which was detected in Çukurova Region for the First Time with Microarray Method. Turk J Hematol. 2009;34(4):256–259.
23. Ozturk O, Atalay A, Koseler A, *et al*. β Globin gene cluster haplotypes of abnormal hemoglobins observed in Turkey. Turk J Hematol. 2007;24(4):146–154.
24. Bahadır A, Ozturk O, Atalay A, Atalay EO. β Globin gene cluster haplotypes of the β thalassemia observed in Denizli Province of Turkey. Turk J Hematol. 2009;26(3):129–137.
25. Altay Ç. Abnormal hemoglobins in Turkey. Turk J Haematol. 2002; 19(1):63–74.
26. Guvenc B, Canataroglu A, Unsal C, *et al*. β-Thalassemia mutations and hemoglobinopathies in Adana, Turkey: Results from a single center study. Arch Med Sci. 2012;8(3):411–414.
27. Guler E, Karacan M. Prevalance of β-thalassemia and sickle cell anemia trait in premarital screening in Konya urban area, Turkey. J Pediatr Hematol Oncol. 2007;29(11):783–785.
28. Fettah A, Bayram C, Yarali N, *et al*. β-Globin gene mutations in Turkish children with β-thalassemia: Results from a single center study. Mediter J Hematol Infect Dis. 2013;5(1):e2013055. doi: 10.4084/MJHID.2013.055.
29. Aldemir O, Izmirli M, Kaya H. The spectrum of β-thalassemia mutations in Hatay, Turkey: Reporting three new mutations. Hemoglobin. 2014;38(5):325–328.
30. Yapici G, Aksoz FT, Kizilok AK. Frequency of carriers of β thalassemia and sickle cell trait in Mersin. Anatol J Clin Invest. 2007;1(4):255–259.
31. Karakukcu C, Kocer D, Torun YA, *et al*. Premarital hemoglobinopathy screening in Kayseri: A city in middle Anatolia region of Turkey. J Pediatr Hematol Oncol. 2012; 34(2):e49–e52.
32. Tadmouri GO, Başak AN. β-Thalassemia in Turkey: A review of the clinical, epidemiological, molecular, and evolutionary aspects. Hemoglobin. 2001;25(2):227–239.
33. Cürük MA, Genc A, Huseynova P, *et al*. Genotypes of α thalassemia and Hb H disease in Çukurova. Turk Klin J Pediatr Sci. 2007;3(10):17–23.
34. Guvenc B, Yildiz SM, Tekinturhan F, *et al*. Molecular characterization of α-thalassemia in Adana, Turkey: A single center study. Acta Haematol. 2010;124(4):197–200.