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Research Article

Beta-thalassemia mutation types and the relationship with the demographic factors in Sanliurfa, Turkey

Türkiye, Şanlıurfa'da Beta-talasemi mutasyon çeşitleri ve demografik faktörlerle ilişkisi

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Abstract

Introduction: Beta-thalassemia is an autosomal recessive disease that occurs as a result of a disorder in the (β-globin chains synthesis), and the gold standard method for diagnosis is genetic mutation analysis. It is important to know the distribution of mutations according to regions and races. The aim of this study is to document the mutations in the beta-globin gene of beta-thalassemia major and intermedia patients who were followed and treated in Sanliurfa province, and to examine the relationships between these mutations by defining them according to gender, nationality, consanguineous marriage, history of disease in siblings and blood type.

Methods: The files of 272 patients diagnosed with beta-thalassemia major and intermedia followed up in the Pediatric Hematology-Oncology outpatient clinic of Sanliurfa Training and Research Hospital between August 2016 and August 2017 were retrospectively reviewed and mutation analyzes were documented. Coding exons and exon-intron junction regions of beta globin-HBB gene were amplified by PCR method and then DNA sequencing was performed. Gender, nationality, consanguineous marriage, sibling history and blood type information were recorded.

Results: Out of 272 patients, 94.1% were thalassemia major and the others were thalassemia intermedia. Approximately one third of the patients (30.1%) were foreign nationals. A total of 27 different mutations in the beta-globin gene were detected. The most common mutation is IVS-I-110 c.93-21 (G> A) (23.1%), which is followed by IVS-I-1 c.92 + 1 (G> A) (15.8%) and Codon 39 c.118 (C> T) (11.5%). One hundred and forty-two individuals (52.2%) had no sibling history, while 103 (37.9%) had one sibling and 27 (9.9%) had two siblings with thalassemia disorder. First-degree, second degree and third degree consanguineous marriages were present in 42.6% (n = 116), 8.1% (n = 22), 11% (n = 30) of parents, respectively.

Conclusions: Beta-thalassemia disease is a common hematological condition in Sanliurfa. Approximately one-third of the patients who apply are foreign nationals. Correct identification of beta-globin gene mutations will guide genetic counseling and preventing prenatal disease. This data can contribute to the national thalassemia prevention program in Turkey.

Keywords: Beta-thalassemia major, beta-thalassemia intermedia, mutation, consanguineous marriage

Öz

Giriş: Beta-talasemi, β-globin zincir sentezindeki bir bozukluk sonucu ortaya çıkan otozomal resesif geçişli bir hastalık olup, tanı koyabilmek için altın standart yöntem genetik mutasyon analizidir. Bölgelere ve ırklara göre mutasyon dağılımlarının bilinmesi önem taşımaktadır. Bu araştırmanın amacı, Şanlıurfa ilinde takip ve tedavi edilen beta-talasemi majör ve intermedia hastalarının beta-globin geninde bulunan mutasyonları dökümante etmek ve bu mutasyonların cinsiyet, uyruk, akraba evliliği, kardeşte hastalık öyküsü ve kan grubuna göre tanımlamasını yaparak aradaki ilişkileri incelemektir.

Yöntem: Şanlıurfa Eğitim ve Araştırma Hastanesi Çocuk Hematoloji-Onkoloji polikliniğinde Ağustos 2016-Ağustos 2017 takip edilen beta-talasemi majör ve intermedia tanılı 272 hastanın dosyaları retrospektif olarak taranarak mutasyon analizleri dökümante edildi. Mutasyon analizleri, Beta globin-HBB geninin kodlama yapan ekzonları ve ekzon-intron bağlantı bölgeleri PCR yöntemi ile amplifiye edildikten sonra DNA dizi analizi ile yapıldı. Cinsiyet, uyruk, akraba evliliği, kardeş öyküsü ve kan grubu bilgileri kaydedildi.

Bulgular: İki yüz yetmiş iki hastanın %94,1'ini talasemi majör, diğerlerini talasemi intermedia olguları oluşturmaktaydı. Hastaların yaklaşık üçte biri (%30,1) yabancı uyrukluydu. Beta-globin geninde toplam 27 farklı mutasyon saptandı. En sık görülen mutasyon IVS-I-110 c.93-21 (G>A) (%23,1) olup, bunu IVS-I-1 c.92+1 (G>A) (%15,8) ve Codon 39 c.118 (C>T) (%11,5) takip etmekteydi. Yüz kırk iki kişide (%52,2) kardeş öyküsü bulunmazken 103 kişide (%37,9) bir kardeşte, 27 kişide ise (%9,9) iki kardeşte hastalık bulunmaktaydı. Hastaların anne ve babalarının %42,6'sında (n=116) birinci derece, %8,1'inde (n=22) ikinci derece, %11'inde (n=30) ise üçüncü derece akraba evliliği söz konusu idi.

Sonuç: Şanlıurfa'da beta-talasemi hastalığı, sık görülen bir hematolojik durumdur. Başvuran hastaların yaklaşık üçte biri yabancı uyrukludur. Beta-globin gen mutasyonlarının doğru tanımlanması genetik danışma ve doğum öncesi hastalığın engellenebilmesi açısından yol gösterici olacaktır. Bu veriler, Türkiye'de ulusal talasemi önleme programına katkı sağlayabilir.

Anahtar kelimeler: Beta-talasemi majör, beta-talasemi intermedia, mutasyon, akraba evliliği

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Introduction

Beta-thalassemia major is a public health problem that is observed frequently in Mediterranean countries including Turkey. It is manifested by findings of microcytosis and hemolytic anemia and renders patients' transfusion-dependent. Although the disease can be diagnosed prenatally, approximately more than 300000 babies are born with thalassemia each year throughout the world. In Turkey, there are about 5500 thalassemic patients [1-3]. So that the treatment of this disease is difficult and highly expensive, it is very important to prevent occurrence of pregnancies that might give birth to potentially ill babies. Providing genetic counseling for prenatal diagnosis to the couples who have had affected child previously may help to prevent recurrent pregnancies with thalassemia.

Beta-thalassemia major is a group of genetic disease that is transmitted by autosomal recessive inheritance and characterized by defective synthesis of the beta globin chain of hemoglobin. Individuals with the defect in a single chain are specified as thalassemia carriers. They generally have an asymptomatic course and do not need any treatment. However, homozygous or compound heterozygous thalassemia alleles result in beta-thalassemia major or intermedia disease. Patients with thalassemia major generally become transfusion-dependent after 6 months, whereas cases with thalassemia intermedia can be diagnosed at any time after the age of 2 years. Although hemoglobin electrophoresis is still a very helpful method for the diagnosis of beta-thalassemia disease, mutation analysis is currently considered as the gold standard diagnostic tool [4-8]. Because of autosomal recessive inheritance, consanguineous marriage increases the risk of occurrence of thalassemia. In concordance with this, beta-thalassemia major is a significant problem in the Southeastern Anatolia Region of Turkey where consanguineous marriages are common.

The aim of this study was to document the mutations related to beta-thalassemia and to identify the relationship between these mutations and demographic properties like sex, nationality, consanguineous marriage, history of affected sibling and blood groups.

Methods

Beta-thalassemia major and intermedia patients, who were followed up in Pediatric Hematology-Oncology Outpatient Clinic of Sanliurfa Education and Research Hospital, were included in this study. The patients' mutation types, and the homozygous or heterozygous status of the mutations were examined. After the encoder exons of the Beta globin-HBB gene [Reference sequence: NM_000518.5 (GRCh37)] and exon-intron binding sites were amplified by way of the PCR method, DNA sequence analysis was performed. The independent variables consisted of sex, nationality (Turkish Republic/other), consanguineous marriage (none/1st degree/2nd degree/3rd degree) and history of having a sibling with the disease (none/1 sibling/2 siblings).

Ethical Approval

The patient files were analyzed retrospectively. Ethical approval was obtained from Harran University Faculty of Medicine Ethics Committee (Date: 03.08.2017, Number: 08). Additionally, written informed consent forms were obtained from the patients' families.

Statistical analysis

The data were analyzed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA) statistical software. The findings were presented as numbers and percentages. In comparison of the patients' categorical sociodemographic and clinical characteristics with mutation types, chi-square and Fisher's exact tests in crosstabs were used. A p value of <0.05 was considered statistically significant.

Results

In this study, the data belonging to a total of 272 individuals were analyzed retrospectively. The mean age was 7.33 (1-18) years. Female to male ratio was 0.73. Majority of the patients (n=256, 94.1%) had a diagnosis of thalassemia major and the others (n=16, 5.9%) had been followed for thalassemia intermedia. Most of the subjects (69.1%, n=188) were citizens of the Republic of Turkey (TR) and approximately one third (30.9%, n=84) were foreign national people. Twenty seven different mutations were found in the beta-globin gene. The most common mutation was IVS-I-110 c.93-21 (G>A) (23.1%). It was followed by IVS-I-1 c.92+1 (G>A) (15.8%) and Codon 39 c.118 (C>T) (11.5%). These three mutations constituted approximately half of all mutations (50.4%). When the mutations were analyzed, it was noted that especially common mutations were mostly homozygous (n=186). However, compound heterozygous mutations were found in 86 individuals. The patients' mutation analysis distributions are summarized in Table 1 by the status of homozygosity and compound heterozygosity.

One hundred and tree patients (37.9%) had one and 27 individuals (9.9%) had two affected siblings. Among the patients' parents, 42.6% (n=116) had first-degree, 8.1% (n=22) had second-degree and 11% (n=30) had third-degree consanguinity marriages. When the blood group distribution was analyzed, it was observed that 127 patients (46.6%) had blood group O, 71 patients (26.1%) had blood group A, 58 patients (21.3%) blood group B and 16 patients (5.8%) had blood group AB. When the variables were compared in terms of the disease's genetic type (homozygous/compound heterozygous), there was no difference in terms of sex, nationality, history of an affected sibling, and blood groups; but consanguineous marriage was found more frequent among the parents of homozygous subjects (Table 2).

Table 1. Beta-thalassemia mutation distribution in 544 alleles

Mutation Type	Homozygous	Compound Heterozygous	Total*
IVS-I-110 c.93-21 (G>A)	98	28	126(23.1%)
IVS-I-1 c.92+1 (G>A)	60	26	86(15.8%)
Codon 39 c.118 (C>T)	44	19	63(11.5%)
IVS-I-5 c.92+5 (G>C)	26	13	39(7.1%)
IVS-II-1 C.315+1 (G>A)	18	11	29(5.3%)
Codon 8 c.25-26 del (-AA)	16	13	29(5.3%)
Codon 9/10 c.30-31 ins (+T)	14	12	26(4.7%)
IVS-I-130 c.93-1 (G>C)	18	8	26(4.7%)
IVS-I-6 c.92 +6 (T>C)	4	15	19(3.4%)
Codon 5 c.17-18 (C>T)	16	3	19(3.4%)
Codon 44 c.135.del	14	1	15(2.7%)
Codon 9 c.27-28 ins (+G)	10	1	11(2.9%)
Codon 8/9 c.27-28 ins G	6	3	9(1.6%)
Turkish 7.6.kb β0- Thal	8	0	8(1.4%)
Codon 25-26 c.78-79 ins T	8	0	8(1.4%)
Codon 17 c.52 (A>T)	6	1	7(1.2%)
Codon 15 c.47(G>A)	2	3	5(0.9%)
IVS-II-745 c.316-106 (C>G)	2	3	5(0.9%)
Codon 6 c.20 (A>T)	2	1	3(0.5%)
Codon 27 c.82 (G>T)	0	3	3(0.5%)
-28 c.118-78 (A>C)	0	2	2(0.3%)
IVS-I-128 c.93-3 (T>G)	0	1	1(0.1%)
IVS-II-675 (C316-185) (A>G)	0	1	1(0.1%)
IVS-II-537(c.316-314) (T>C)	0	1	1(0.1%)
IVS II-848 (G>A)	0	1	1(0.1%)
Codon 82/83 c.251 del (-G)	0	1	1(0.1%)
5' UTR +20 (C>T)	0	1	1(0.1%)
TOTAL	372	172	544

***** Column percentage

Table 2. Relationship between being homozygous or combined heterozygous and demographic features

	Homozygous (n/%)	Compound Heterozygous n (%)	X^2;P*
Gender			
Male	108 (68.8)	49 (31.2)	0.079; 0.778
Female	78 (67.8)	37 (32.2)	
Nationality			
Turkish	128 (68.1)	60 (31.9)	0.005; 0.944
Foreign	58 (69)	26 (31)	
Consanguineous marriage			
No	52 (50.5)	51 (49.5)	33.041; **0.001**
First degree	99 (85.3)	17 (14.7)	
Second degree	17 (77.3)	5 (22.7)	
Third degree	18 (60)	12 (40)	
Presence of an affected sibling			
No	102 (71.8)	40 (28.2)	3.044; 0.218
One	64 (62.1)	39 (37.9)	
Two	20 (74.1)	7 (25.9)	
Blood Groups			
O	90 (70.9)	37 (29.1)	5.078; 0.166
A	44 (62)	27 (38)	
B	44 (75.9)	14 (24.1)	
AB	8 (50)	8 (50)	
Total	186	86	

*Statistical method: Fischer exact test and chi-square test

The distributions of the two most common mutations including homozygous IVS-I-110 c.93-21 (G>A) and homozygous IVS-I-1 c.92+1 (G>A) in different groups were compared. Accordingly, both were more common in the subjects whose parents had first-degree consanguinity. Otherwise, a significant difference was not found between the groups (Table 3).

Table 3. Comparison of the two most common homozygous mutations in terms of gender, nationality, consanguineous marriage, history of disease in siblings and blood types

	Homozygous IVS-I-110 c.93-21 (G>A) (n /%)		X^2; P*	Homozygous IVS-I-1 c.92+1 (G>A) (n /%)		X^2; P*
	No	Yes		No	Yes	
Gender						
Male	124 (79)	33 (21)	2.269; 0.132	102 (88.7)	13 (11.3)	0.015; 0.901
Female	99 (86.1)	16 (13.9)		140 (89.2)	17 (10.8)	
Nationality						
Turkish	156 (83)	32 (17)	0.047; 0.524	167 (88.8)	21 (11.2)	0.012; 0.912
Foreign	67 (79.8)	17 (20.2)		75 (89.3)	9 (10.7)	
Consanguineous marriage						
No	94 (91.3)	9 (8.7)	**15.238; 0.002**	96 (93.2)	7 (6.8)	**12.240; 0.007**
First Degree	83 (71.6)	33 (28.4)		102 (87.9)	14 (12.1)	
Second Degree	19 (86.4)	3 (13.6)		15 (68.2)	7 (31.8)	
Third Degree	26 (86.7)	4 (13.3)		28 (93.3)	2 (6.7)	
Presence of an affected sibling						
No	113 (79.6)	29 (20.4)	1.174; 0.556	131 (92.3)	11 (7.7)	3.265; 0.195
One	87 (84.5)	16 (15.5)		88 (85.4)	15 (14.6)	
Two	23 (85.2)	4 (14.8)		23 (85.2)	4 (14.8)	
Blood Groups						
O	101 (79.5)	26 (20.5)	1.076; 0.783	112 (88.2)	15 (11.8)	1.320; 0.724
A	60 (84.5)	11 (15.5)		62 (87.3)	9 (12.7)	
B	49 (84.5)	9 (15.5)		54 (93.1)	4 (6.9)	
AB	13 (81.3)	3 (18.8)		14 (87.5)	2 (12.5)	

* Statistical method: Fischer exact test and chi-square test

Discussion

The beta-globin gene encoding the beta chain of human adult hemoglobin, is located on the short arm of the 11th chromosome (11p15.4) in the beta-globin gene cluster. Beta-thalassemia mutations are quite variable and expanded in different regions of the gene. More than 300 mutations causing beta–thalassemia have been reported until this time and more than 43 mutation types have been identified in our country [9-11]. The distribution of the mutations may show variance according to region. Among these mutations, IVS-I-110 was the most common one with a rate of 40% [12-14]. In a study conducted at a University Hospital in Ankara, 18 different mutations were found, and the most common mutation was reported as IVS I.110 (G>A) with a percentage of 35.3 [15]. In this study, we detected a wide molecular heterogeneity of thalassemic subjects. IVS-I-110 c.93-21 (G>A) was the most prevalent mutation in all nationalities, and it is consistent with previous results. IVS-I-1 c.92+1 (G>A) and Codon 39 c.118 (C>T) were other frequent mutations. IVS I.6 (T>C) mutation, which was reported to be the second most common mutation in previous studies conducted in Turkey [13,16], was found with a rate of only 4.4% in the present study. These different results may be associated with presence of various geographic regions and nationalities in the same country.

Our study demonstrated that consanguineous marriage was important in terms of mutation type and the status of being homozygous or compound heterozygous. The most common beta-globin gene mutation was IVS-I-110 c.93-21 (G>A) in the subjects whose parents had first-degree consanguinity and IVS-I-1 c.92+1 (G>A) in the subjects whose parents had second-degree consanguinity. In concordance with this, most common mutations are observed in children born because of consanguineous marriages. The contribution of consanguineous marriage to the occurrence of this disease has been known for a long time [17] and consanguineous marriage is very common in the region where this study was conducted. In genetic disorders, presence of the same disease in the siblings is one of the probable conditions. The considerable number of families with more than one affected offspring in the present study indicated that the local community does not have adequate information about genetic counseling.

Blood group analysis is important for beta-thalassemia patients. Knowing blood group distribution in transfusion-dependent patients with hemoglobinopathies may be significant in the strategic plan to supply blood products for these patients. In our study, the most common blood group was group O, and this appeared to be compatible with other studies published before [18]. In the current study, a relationship could not be found between the status of being homozygous or compound heterozygous and blood groups.

As beta-thalassemia is observed commonly at the countries in the Mediterranean region like Cyprus, Turkey, Syria, Egypt, these regions are known as “Thalassemia Zone” [19]. Sanliurfa is also inside this area. On the other hand, nearly four million Syrian immigrants registered in Turkey live in the cities like Sanliurfa bordering Syria. When compared to previous epidemiological studies, the increase in the number of patients is remarkable [12, 20-23]. It may be stated that the number of immigrant patients in this region might have contributed to this finding.

Limitations

In this study, the patients who were followed up in a certain period (August 2016-August 2017) in the province of Sanliurfa were included in a cross-sectional design. They reflect the genetic characteristics belonging only to this region and can not be generalized to the entire population in Turkey.

Conclusion

Current data indicate that a significant number of beta-thalassemia patients were followed up in an outpatient clinic in a public hospital in Sanliurfa and about one third of these patients were foreign national people. In the province of Sanliurfa, where beta-thalassemia is encountered commonly, it is important to provide and maintain the necessary conditions for the diagnosis, treatment, and follow-up of this disease. These data may contribute to the national thalassemia prevention program in Turkey.

Especially consanguineous couples, who have a family history of beta-thalassemia carrier status, should definitely receive genetic counseling before planning pregnancy. As the probability of having a child with thalassemia major is 25% in couples who are beta-thalassemia carriers, families should be informed about invasive prenatal diagnosis [chorionic villus sampling (CVS) or amniocentesis (ASI)] and preimplantation genetic diagnosis (PGD) during genetic counseling. Termination of pregnancy due to detection of the disease as a result of invasive prenatal diagnosis may also lead to physical and psychological problems in the families. Therefore, the method of PGD may be a choice.

In conclusion, accurate identification of beta-globin gene mutations will be a guide in terms of premarital screening, genetic counseling, and prenatal prevention of the disease.

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Author Contributions		Author Initials
SCD	Study Conception and Design	BA, FDY, ET, OO
AD	Acquisition of Data	BA, FDY, ET, OO
AID	Analysis and Interpretation of Data	BA, FDY, ET, OO
DM	Drafting of Manuscript	BA
CR	Critical Revision	BA

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References

1. Canatan D. Thalassemias and hemoglobinopathies in Turkey. Hemoglobin. 2014;38(5): 305-7. https://doi.org/10.3109/03630269.2014.938163
2. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood. 2010;115(22):4331-6. https://doi.org/10.1182/blood-2010-01-251348
3. Canatan D, Aydinok Y. [Thalassemia and hemoglobinopathies diagnosis and treatment book] (in Turkish), 1st ed. Antalya. Thalassaemia Federation, 2007.
4. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet 2018; 391(10116):155–67. https://doi.org/10.1016/S0140-6736(17)31822-6
5. Higgs DR, Engel JD, Stamatoyannopoulos G. Thalassaemia. Lancet. 2012; 379(9813):373–83. https://doi.org/10.1016/S0140-6736(11)60283-3
6. Rachmilewitz EA, Giardina PJ. How I treat thalassemia. Blood. 2011;118(13):3479–88. https://doi.org/10.1182/blood-2010-08-300335
7. Cao A, Galanello R. Beta-thalassemia. Genet Med. 2010;12(2):61-76. https://doi.org/10.1097/GIM.0b013e3181cd68ed
8. Taher A, Isma'eel H, Cappellini MD. Thalassemia intermedia: Revisited. Blood Cells Mol. Dis. 2006;37(1):12-20. https://doi.org/10.1016/j.bcmd.2006.04.005
9. Canatan D, Kose MR, Ustundag M, Haznedaroglu D, Ozbas S. Hemoglobinopathy control program in Turkey. Community Genet. 2006;9(2):124-6. https://doi.org/10.1159/000091493
10. Giardine B, Borg J, Viennas E, Pavlidis C, Moradkhani K, Jolly P, et al. Updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Res. 2014;42(D1):1063-9. https://doi.org/10.1093/nar/gkt911
11. Cappellini MD, Cohen A, Porter J, Viprakasit V. Guidelines for the management of transfusion dependent thalassaemia (TDT), 3rd ed. Nicosia, Thalassaemia International Federation, 2014.
12. Aydinok Y, Oymak Y, Atabay B, Aydogan G, Yesilipek A, Unal S, et al. A National registry of thalassemia in Turkey: demographic and disease characteristics of patients, achievements, and challenges in prevention. Turk J Hematol 2018;35(1):12-8. https://doi.org/10.4274/tjh.2017.0039
13. Basak AN. The molecular pathology of beta-thalassemia in Turkey: The Bogazici University experience. Hemoglobin. 2007;31(2):233-41. https://doi.org/10.1080/03630260701296735
14. Akar N, Cavdar AO, Dessi E, Loi A, Pirastu M, Cao A. Beta thalassaemia mutations in the Turkish population. J Med Genet. 1987;24(6):378–81. https://doi.org/10.1136/jmg.24.6.378
15. Fettah A, Bayram C, Yarali N, Isik P, Kara A, Culha V, et al. Beta-globin gene mutation in Turkish children with beta-thalassemia: results from a single center study. Mediterr J Hematol Infect Dis. 2013;5(1):e2013055. https://doi.org/10.4084/MJHID.2013.055
16. Altay C. The frequency and distribution pattern of beta-thalassemia mutations in Turkey. Turkish J Haematol. 2002;19(2):309-15. https://ncbi.nlm.nih.gov/pubmed/27264774
17. Kayahan M, Simsek Z, Ersin F, Gozukara F, Kurcer MA. [Prevalence of consanguineous marriage and its effect on deaths under 5 years of age in Sanliurfa Tilfindir Health Center] (in Turkish). C.U. Hemsire Yuksek Derg. 2003;7(1):1-4. https://dergipark.org.tr/tr/pub/dtfd/issue/48273/611151
18. Mondal B, Maiti S, Biswas BK, Ghosh D, Paul S. Prevalence of hemoglobinopathy, ABO and rhesus blood groups in rural areas of West Bengal, India. J Res Med Sci. 2012; 17(8):772-6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3687885
19. De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, et al. β-thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. Mediterr J Hematol Infect Dis. 2017;9(1):e2017018. https://doi.org/10.4084/mjhid.2017.018

20. Syrian Regional Response—Inter-agency Information sharing Portal [Internet]. Available from: https://data2.unhcr.org/en/situations/syria/location/113 (Date of access:22.09.2020)
21. Aras B, Duman Y. I/NGOs' Assistance to Syrian Refugees in Turkey: Opportunities and Challenges. J Balk Near East Stud. 2019;21(4):478-91. https://doi.org/10.1080/19448953.2018.1530382
22. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of β-thalassemia. Eur J Haematol. 2020;00:1-12. https://doi.org/10.1111/ejh.13512
23. Aycicek A, Koc A, Ozdemir ZC, Bilinc H, Kocyigit A, Dilmec F. Beta-globin gene mutations in children with beta-thalassemia major from Sanliurfa province, Turkey. Turk J Hematol 2011;28(4):264-8. https://doi.org/10.5152/tjh.2011.86