Short Communication

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Investigation of beta globin gene mutations in Syrian refugee patients with thalassemia major

Suriye'den göç eden talasemili hastalarda beta globin gen mutasyonlarının araştırılması

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Abstract

Objectives: This study, detection of beta globin gene mutations in thalassemia major patients who migrated from Syria to Kahramanmaraş region were planned.
Materials and methods: The study included 35 Syrian national beta thalassemia major patients. Beta globin gene mutations were detected by ARMS (Amplification Refractory Mutation System) method, RFLP (Restriction Fragment Length Polymorphism) method and DNA sequence analysis. Codon 15, codon 9/10, codon 5 and codon 8 mutations, which we could not detect with other methods in our study, were detected by sequence analysis.
Results: In beta thalassemia major patients, 16 types of mutations were detected, the most common being IVS-I-110 (n = 8). Other mutations are according to frequency order IVS-II-745 (n = 3), codon 44 (n = 3), codon 15 (n = 3), IVS-I-110/IVS-I-1 (n = 3), codon 5 (n = 2), IVS-I-1 (n = 2), codon 8/IVS-II-1 (n = 2), codon 44/codon 15 (n = 2), IVS-II-1 (n = 1), codon 39 (n = 1), IVS-I-6/codon 5 (n = 1), codon 9/10 (n = 1), IVS-I-110/codon 39 (n = 1), IVS-I-5/IVS-II-1 (n = 1), codon 39/IVS-II-745 (n = 1).
Conclusions: According to the results of our study beta-thalassemia mutations in Syrian immigrant groups show heterogeneity and mutation types of mutation map is similar to Turkey. The conclusion is to prevent families to have a second patient child by genetic counseling.

Keywords: Beta thalassemia; Beta globin gene mutation; Syrian immigrants; Children.

Öz

Amaç: Bu çalışmada Kahramanmaraş ve yöresinde Suriye'den göç eden beta talasemi majörlü hastaların beta globin gen mutasyonlarının tespit edilmesi planlanmıştır.
Gereç ve Yöntem: Çalışmaya 35 Suriye uyruklu beta talasemi majorlu hasta dahil edildi. Hastalardan tam kandan hemogram ve mutasyon çalışması için EDTA'lı tüplere kan alındı. Alınan kan örneklerinden ARMS (Amplification Refractory Mutation System) yöntemi, RFLP (Restriction Fragment Length Polimorfizm) yöntemi ve DNA dizi analizi ile beta globin gen mutasyonları saptandı. Çalışmamızda diğer yöntemlerle saptayamadığımız Kodon 15, Kodon 9/10, Kodon 5 ve Kodon 8 mutasyonları sekans yöntemi ile saptandı.
Bulgular: Çalışmaya dahil edilen 35 hastanın 19'u (%54,3) erkek, 16'sı (%45,7) kız idi. Tüm hastaların yaşları ortalaması 9,48 ± 4,51 yıl (2 yaş-17 yaş) idi. Beta talasemi majörlü hastalarda en yaygın IVS-I-110 (n = 8) olmak üzere 16 çeşit mutasyon tespit edilmiştir. Diğer mutasyonlar sıklık

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sırasına göre şöyledir; IVS-II-745 (n = 3), kodon 44 (n = 3), kodon 15 (n = 3), IVS-I-110/IVS-I-1 (n = 3), kodon 5 (n = 2), IVS-I-1 (n = 2), kodon 8/IVS-II-1 (n = 2), kodon 44/kodon 15 (n = 2), IVS-II-1 (n = 1), kodon 39 (n = 1), IVS-I-6/kodon 5 (n = 1), kodon 9/10 (n = 1), IVS-I-110/kodon 39 (n = 1), IVS-I-5/IVS-II-1 (n = 1), kodon 39/IVS-II-745 (n = 1).

Sonuç: Çalışmamızın sonuçlarına göre Suriye uyruklu göçmenlerde beta talasemi mutasyonları heterojenite göstermekte ve mutasyon çeşitleri Türkiye mutasyon haritasına benzemektedir. Sonuçlar ile amaçlanan, ailelere genetik danışmanlık verilerek ikinci bir hasta çocuk sahibi olmalarının önüne geçilmesidir.

Anahtar kelimeler: Beta talasemi; beta globin gen mutasyonu; Suriye'li göçmenler; çocuk.

Introduction

The thalassemias are a group of anemias that result from inherited defects in the production of hemoglobin [1]. Thalassemia are classified according to the affected globin chain or chains. Major subtypes are alpha (α) and beta (β) thalassemias [2, 3].

The β-globin gene is located on the short arm of chromosome 11. There are two but one for each homologous chromosome. Recently more than 200 mutations in the β globin gene have been reported. The most common mutations are point mutations. Clinical variability in beta thalassemia is due to variability of mutations, not the number of functional genes unlike alpha thalassemia [4]. Beta thalassemia major is usually manifested by severe microcytic anemia, mild jaundice and hepatosplenomegaly in infants less than 2 years of age. Carriers are sometimes asymptomatic although they may be mildly anemic [3].

Beta thalassemia patients can be detected by clinical and laboratory data. Complete blood count and Hb electrophoresis are routine test to detect the disease. DNA sequence analysis now used as the gold standard for mutation analysis [3]. IVS-I-110 is the most common of beta thalassemia mutation [5]. The most common mutations found in a study conducted in Syria were as follows: IVS-I-110 (G > A) (17%), IVS-I-1 (G > A) (14.7%), codon 39 (C > T) (14.4%), IVS-II-1 (G > A) (9.8%), codon 8 (−AA) (6.2%), IVS-I-6 (T > C) (5.2%), IVS-I-5 (G > C) (4.9%), codon 5 (−C) (3.2%), IVS-I-5 (G > A) (3.2%) and codon 37 (G > A) (2.2%) [6].

Beta thalassemia patients who migrated from Syria did not have any report of beta thalassemia gene mutation which is mandatory for diagnosis. The aim of this study was to provide genetic counseling for families who want to have healthy children by determining the type and distribution of mutations in refugee patients living in Kahramanmaraş and neighboring cities.

Materials and methods

A total of 35 refugee children with diagnosed beta thalassemia major who were living Kahramanmaraş or neighboring cities were enrolled in the study. This study started in March 2015 and was completed in February 2017. This study was approved by the Local Ethics Committee, parents of all patients were informed with the assistance of an interpreter. Written consent was taken from all parents of children that participated in the study. Blood was collected from the patients with EDTA tubes for hemogram and mutation studies. Patients were evaluated by age, gender, splenectomy status, chelation therapy, ferritin values, viral serologies, antiviral treatment, cardiac and endocrine complications. Beta globin gene mutations were detected by Amplification Refractory Mutation System (ARMS) method, Restriction Fragment Length Polymorphism (RFLP) method and Deoxyribose Nucleic Acid (DNA) sequence analysis from the blood samples. Mutations of codon 15, codon 9/10, codon 5 and codon 8, which we could not detect by other methods, were detected by DNA sequence analysis.

Results

A total of 35 refugee children with diagnosed beta thalassemia major who were living Kahramanmaraş or neighboring cities were enrolled in the study. Nineteen (54.3%) were males and 16 (45.7%) were females. The mean age of patients was 9.48 ± 4.51 years (between 2 years and 17 years). Eighty percent of the patients came from halep, 20% from idlip. The mean hemogram value profile before erythrocyte suspension transfusion was hemoglobin 8.64 ± 1.66 g/dL, RBC 3.24 ± 0.58 × 10^6/mm^3 MCV 80.95 ± 5.10 fL. Mean ferritin was 5030 ± 2919 ng/mL (between 1414 and 12,595 ng/mL). HCV infection was positive in 12 patients (34.3%).

Sixteen different mutations were detected in our patients while the most common being IVS-I-110 (n = 8). The frequency of other mutations are: IVS-II-745 (n = 3), codon 44 (n = 3), codon 15 (n = 3), IVS-I-110/IVS-I-1 (n = 3), codon 5 (n = 2), IVS-I-1 (n = 2), codon 8/IVS-II-1 (n = 2), codon 44/codon 15 (n = 2), IVS-II-1 (n = 1), codon 39 (n = 1),

IVS-I-6/codon 5 (n = 1), codon 9/10 (n = 1), IVS-I-110/codon 39 (n = 1), IVS-I-5/IVS-II-1 (n = 1), codon 39/IVS-II-745 (n = 1).

Discussion

Thalassemic children are more than expected in Turkey because of high incidence of consanguineous marriages and high birth rate [7]. Since the onset of the civil war in Syria in March, 2011, over 3.5 million Syrians have migrated to Turkey. During this time, many refugees with thalassemia are being treated in our country. There are a lot of studies about the frequency and diversity of beta thalassemia mutation in Turkey and worldwide. This is the first study carried out for the determination of beta thalassemia mutations in migrating Syrian refugees to Turkey.

A total of 35 refugee children with diagnosed beta thalassemia major who were living Kahramanmaraş or neighboring cities were enrolled in the study. Mean ferritin values and HCV positivity found very high (34.3%) due to uncontrolled blood transfusion and irregular iron chelation therapy. Hb values before blood transfusion were below the Hb values that should be because the patients did not come to regular control on time.

In our study, we found, IVS-I-110 as the most common β-globin gene mutation similar to other studies done previously in Syria [6, 8–10]. In a study conducted by el-Hazmi et al. [10], in eight different countries, IVS-I-1 and IVS-II-745 mutations were found only in Egypt, Jordan and Syria. The IVS-I-1 mutation was the most common B-globin gene mutation in the study performed by Murad et al. [11] whereas this mutation was found the second most common mutation in the study performed by Jarjour et al. [6]. In our study we found IVS-II-745 mutation in the second frequency and IVS-I-1 mutation in the third frequency.

In another study done by Murad et al. [12] 38 β-different globin gene mutations responsible for β-thalassemia in Syria has been shown. Most commonly IVS-I-110 [G > A] (22.2%) mutations were detected. Ten new mutations, –86 [C > G], –31 [A > G], –29 [A > G], 5′UTR; +22 [G > A], CAP + 1 [A > C], codon 5/6 [–TG], IVS-I (–3) or codon 29 [C > T], IVS-I-2 [T > A], IVS-I-128 [T > G] and IVS-II-705 [T > G] have been first time detected in Syria during this study.

IVS-I-110 have also been detected the most common mutations in several studies conducted in Turkey with Turkish people [13–16]. In a study done by Fettah et al. [15] from a tertiary center that receive patients from different region of Turkey, the most common β-globin mutation was found IVS-I-110 (G > A) (35.3%). Other common mutations detected in this study were codon 8 (–AA) (10.4%), IVS-II-1 (G > A) (8%), IVS-I-1 (G > A) (7.5%), codon 39 (C > T) (7.1%) and codon 5 (–CT) (6.6%). According to the results of our study beta thalassemia mutations of Syrian refugees show heterogeneity and mutations frequency is resembling to the Turkey's. The most common 6–7 mutations account for about 70% of all mutations. In our study, no new mutation was detected. The aim of this study is to prevent sick childbirth by giving genetic counseling to Syrian Refugees with thalassemic children.

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References

1. Thein SL. Genetic modifiers of beta-thalassemia. Haematologica 2005;90:649–60.
2. Cao A, Galanello R. Beta-thalassemia. Genet Med 2010;12:61–76.
3. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis 2010;5:11.
4. Kazazian HH Jr. The thalassemia syndromes: molecular basis and prenatal diagnosis in 1990. Semin Hematol 1990;27:209–28.
5. Basak N. The molecular pathology of β-thalassemia in Turkey: the Bogaziçi University experience. Hemoglobin 2007;31:233–41.
6. Jarjour RA, Murad H, Moasses F, Al-Achkar W. Molecular update of β-thalassemia mutations in the Syrian population: identification of rare β-thalassemia mutations. Hemoglobin 2014;38:272–6.
7. Cai SP, Wall J, Kan YW, Chehab FF. Reverse dot blot probes for the screening of beta-thalassemia mutations in Asians and American blacks. Hum Mutat 1994;3:59–63.
8. Hamamy HA, Al-Allawi NA. Epidemiological profile of common haemoglobinopathies in Arab countries. J Community Genet 2013;4:147–67.
9. Kyriacou K, Al Quobaili F, Pavlou E, Christopoulos G, Ioannou P, Kleanthous M. Molecular characterization of beta-thalassemia in Syria. Hemoglobin 2000;24:1–13.
10. el-Hazmi MA, Warsy AS, al-Swailem AR. The frequency of 14 beta-thalassemia mutations in the Arab populations. Hemoglobin 1995;19:353–60.
11. Murad H, Moassas F, Jarjour R, Mukhalalaty Y, Al-Achkar W. Prenatal molecular diagnosis of β-thalassemia and sickle cell anemia in the Syrian population. Hemoglobin 2014;38:390–3.
12. Murad H, Moasses F, Dabboul A, Mukhalalaty Y, Bakoor AO, Al-Achkar W, et al. Geographical distribution of β-globin gene mutations in Syria. Hematology 2018;23:697–704.
13. Mendilcioglu I, Yakut S, Keser I, Simsek M, Yesilipek A, Bagci G, et al. Prenatal diagnosis of β-thalassemia and other hemoglobinopathies in southwestern Turkey. Hemoglobin 2011;35:47–55.

14. Ulasli M, Oztuzcu S, Kirkbes S, Bay A, Igci YZ, Bayraktar R, et al. Novel beta (β)-thalassemia mutation in Turkish Children. Indian J Hematol Blood Transfus 2015;31:218–22.
15. Fettah A, Bayram C, Yarali N, Isik P, Kara A, Culha V, et al. Beta-globin gene mutations in Turkish children with beta-thalassemia: results from a Single Center Study. Mediterr J Hematol Infect Dis 2013;5:e2013055.
16. Ayçiçek A, Koç A, Özdemir ZC, Bilinç H, Koçyiğit A, Dilmeç F. Beta-globin gene mutations in children with beta-thalassemia major from Şanlıurfa province, Turkey. Turk J Haematol 2011;28:264–8.