The prevalence and genotypes of alpha-thalassemia in Adıyaman: two rare alpha variants

Adıyaman’da alfa-talasemi genotipleri ve sıklığı: İki nadir alfa variant

Abstract

Introduction: Alpha-thalassemia (α-thal) is one of the most common diseases in the world, and is more common in the Mediterranean countries, the Middle East, Southeast Asia, and Africa. The aim of this study was to determine the prevalence and genotypes of α-thal in high school students in Adıyaman province, Southeast Turkey.

Methods: In this study, a total of 289 people were screened for α-thal mutations by multiplex gap-PCR and ARMS.

Results: α-thal mutation frequency was determined to be 13.15%. Five different mutations were identified and the most common mutation was found to be −3.7 deletions with a frequency of 11.07%. We identified two heterozygous α-globin variants. We described the second case of heterozygote Hb Fontainebleau of Turkish origin. Here, we also reported another heterozygote hemoglobin variant, Hb Q-Iran, previously reported in a few cases in another part of Turkey.

Discussion and conclusion: This study covers the frequency of α-thal disease, and the molecular analysis is the first study in the Southeastern Anatolia Region of Turkey. The prevalence of the α-thal trait is low in Adıyaman Province, compared to the other cities of Turkey.

Keywords: Hb Fontainebleau; Hb Q-Iran; Adıyaman; Alpha thalassemia frequency; Genotypes.

Introduction

Alpha-thalassemia (α-thal) is the most common recessively inherited globin disorder throughout the world and is characterized by deficient or absent synthesis of one or both functional α-globin genes located on the short arm of chromosome 16. It most frequently results from deletion

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Özet

GİRİŞ ve AMAÇ: Alfa talasemi başta Akdeniz, Ortadoğu, Güneydoğu Asya ve Afrika ülkelerinde olmak üzere, dünyada en yaygın görülen hastalıklardan biridir. Bu çalışmanın amacı ülkemizin güneydoğusunda yer alan Adiyaman ilinde okuyan lise öğrencilerinde alfa talasemi sıklığı ve genotiplerini belirlemektir.

YÖNTEM ve GEREÇLER: Bu çalışmada, toplamda 289 kişinin alfa-talasemi mutasyonların multipleks gap-PCR ve ARMS yöntemi ile taramanmıştır.

BULGULAR: Alfa-talasemi mutasyon sıklığı %13,15 olarak belirlenmiştir. Beş farklı mutasyon tanımlanmıştır ve en yaygın mutasyon −3,7 delesyonu %11,07 sıklıkta bulunmuştur. Ayrıca ikinci alfa hemoglobin varyantı heterozigot olarak tanınmıştır. Bu çalışmada tanınan Hb Fontainebleau ülkemizdeki ikinci vakasıdır. Diğer Hb varyantı heterozigot Hb Q-Iran olarak rapor edilmiş olup, bu varyant ülkemizin geçiş illerinde önceden rapor edilmişdir.

TARTIŞMA ve SONUÇ: Ülkemiz Güneydoğu Anadolu Bölgesi’nde alfa-talasemi hastalığının sıklığını ve genotiplerinin belirlendiği ilk çalışma olmuştur. Alfa talasemi sıklığı ülkemizdeki diğer ilerle kıyaslandığında Adiyaman ilinde düşük bulunmuştur.

Anahtar Kelimeler: Hb Fontainebleau; Hb Q-Iran; Adiyaman; Alfa talasemi sıklığı ve genotipleri.
of one (−α/αα) or both α-globin (−/αα) genes and, less frequently, from different non-deletional mutations [1]. It is more prevalent throughout the Mediterranean countries, the Middle East, Southeast Asia, and Africa [2]. The clinical severity of α-thalassemia varies depending on the number of affected α-globin genes [3]. The α-thal trait caused by the dysfunction or deletions of one or two α-globin genes commonly displays no symptoms or very mild anemia [4].

Distribution of mutations causing α-thal has been reported in various provinces in Turkey, and some studies have shown that the −α^2/αα genotypes are the most common mutations in Turkish patients [5–8]. No epidemiological study of α-thal and α-globin variants has been undertaken in Adıyaman province, in the Southeastern Anatolia Region of Turkey. Thus, the aim of the present investigation was to determine the α-globin variants and frequency of the α-thal mutations in the province of Adıyaman in high school students. Here, we describe the results of molecular analyses of 289 blood samples that had microcytosis and/or anemia. In this study, the frequency and genotypes of α-thal were determined. Also, we identified two rare α-globin variants.

Materials and methods

The study was approved by the Non-Invasive Research Ethics Committee of Firat University (date: 11.03.2014, number: 05/01). It involved 289 blood samples in Adıyaman Province. Screening tests and the collection of blood have been described in a previous study [9]. Students with low 2.5% HbA2 below 80 fL mean corpuscular volume (MCV); and considered possible carriers of α-thal were included in the study. As our financial support did not allow for determining plasma iron and ferritin levels, we only took microcytosis as a sign of iron deficiency.

In cases with possible α-thal, mutation screening was performed by the gap-PCR and ARMS [10, 11]. DNA sequencing was used to verify IVSI −5nt and polyA2 (AATAAA > AATGAA) mutations and also to characterize the abnormal α-variants [12].

Results

Of the 289 Adıyaman high school students, 38 (13.15%) were identified as carriers of α-thal. Molecular analysis of 38 samples revealed five different mutations [−α^3, IVSI −5nt, −20.5, −α^3; and polyA2 (AATAAA > AATGAA)] and the most common mutation was 3.7 deletions with a frequency of 11.07%. The frequency of genotypes of α-thal mutations and hematological parameters is shown in Table 1.

We observed two abnormal HPLC-elution patterns while screening high school students for thalassemia. Chromatographic analysis of red cell lysates revealed that one of these variants comprised 18.0% of the total Hb. An abnormal Hb with mobility between that of HbA and HbA2 and adjacent to HbA was detected by HPLC. Retention time (RT) was determined as 2.82 min (Figure 1A). Sequencing data of the α2 globin gene for the second case showed the presence of the GCT → CCT mutation in heterozygous form at codon 21, corresponding to the heterozygous Hb Fontainebleau [α2(II)Ala→Pro; GCT→CCT] (Figure 2A and B). The other abnormal Hb was 18.4% of total Hbs with 1.1% HbA2, and RT time was 4.82 min (Figure 1B). This case was identified as Hb Q-Iran in the α2 gene [α75 (EF4) Asp→His; GAC→CAC] by sequencing (Figure 2C and D). Hematological parameters are also given in Table 2.

Discussion

Although our country is in the thalassemia belt, there are only a few provinces that determine the frequency and genotypes of α-thal. In particular, there are no studies to determine the prevalence and genotypes of α-thal in the

| α-thalassemia mutations | n | Present study Adıyaman, % | İstanbul % (n=95) (Karakaya et al. [13]) | Isparta % (n=8) (Gulen et al. [7]) | Hatay % (n=97) (Celik et al. [8]) | Adana % (n=225) (Guvenc et al. [6]) |
|-------------------------|---|---------------------------|------------------------------------------|-----------------------------------|---------------------------------|---------------------------------|
| −α^3/αα                 | 32| 11.07                     | 23.1 (19)                                | 10.9 (5)                          | 57.73 (56)                     | 53.33 (120)                    |
| α^3−5nt/αα              | 3 | 1.04                      | 6.3 (3)                                  | –                                 | 2.06 (2)                       | –                              |
| −α^2.5/αα               | 1 | 0.35                      | 9.4 (13)                                 | 4.3 (2)                           | –                              | –                              |
| −α^2/αα                 | 1 | 0.35                      | –                                        | –                                 | 1.03 (1)                       | 0.44 (1)                       |
| α^2.5−α/αα              | 1 | 0.35                      | 0.5                                      | 2.2 (1)                           | 1.03 (1)                       | 4.0 (9)                        |

n, patient number.
Eastern and Southeastern Anatolia Regions in Turkey. In our country, the incidences of α-thal in Adana and Isparta province, respectively, are 7.5% (n = 225) and 17.4% (n = 8) [6, 7]. Besides, α-thal mutation screening is performed in a few centers, and many mutations have been identified with different frequency.

In Istanbul, 14 different mutations were found in the largest city, and the most common mutation was reported as $-\alpha^{3.7}$ with a 23.1% frequency rate (Table 1) [13]. Onay et al. reported 12 different mutations in the α-globin gene cluster and the highest incidence of $-\alpha^{3.7}$ deletion (52.2%) [14]. Other studies were performed with selected samples.
Table 2: Hematological data of Hb Fontainebleau and Hb Q-Iran subjects.

| Parameters          | Hb Fontainebleau | Hb Q-Iran   | Reference range |
|---------------------|-------------------|-------------|-----------------|
| Age/sex             | 16/F              | 15/F        | –               |
| RBC (10^{12}/L)     | 4.51              | 4.8         | 4.0–5.20        |
| Hb (g/dL)           | 12.3              | 12.8        | 12.0–16.0       |
| HCT (%)             | 34.1              | 34.9        | 36.0–48.0       |
| MCV (fl)            | 75.7              | 72.7        | 80.0–96.0       |
| MCH (Pg)            | 27.3              | 26.7        | 28.0–34.0       |
| MCHC (g/dL)         | 36.1              | 36.7        | 32.0–36.0       |
| RDW (%)             | 16.2              | 16.5        | 12.0–15.0       |
| HbA2 (%)            | 2.8               | 1.1         | 2.20–3.50       |
| HbF (%)             | 0.3               | 0.1         | <1.0           |
| Unknown Hb (%)      | 18.0              | 18.4        | –              |
| Genotype            | α2Cd21 Ala→Pro; GCT→CCT | α2Cd75 Asp→His; GAC→CAC | –             |
who attended clinic and/or hematology had anemia [6–8, 13, 14]. Although there was no population screening for α-thal to determine prevalence and mutations, this study was conducted with 3571 high school children's blood samples, and we reported that α-thal prevalence was 1.06% (total 38 students/3571) in Adıyaman Province [9]. The most common mutation in the α-globin genes was the 3.7 single gene deletion, detected in 32 patients with 11.07% frequency (Table 1). While high school students were screened for thalassemia, two α-globin variant was detected in two students.

Hb Fontainebleau results from a G→C substitution (GCT→CCT) at codon 21 of the α-globin gene, changing alanine to proline. The variant was first described in an Italian family, which was identified as a family with hereditary spherocytosis [15], and subsequently described independently as one case in an Iraqi man living in New Zealand [16]. Another case was reported in Cyprus while screening the Greek Cypriot population for thalassemia [17]. The heterozygote individual had normal hematological parameters and the abnormal Hb amounted to 25–29% of the total Hb in six adult cases [15–17]. The fourth occurrence was a 3-day-old female baby and her mother, who reported with sickle hemoglobin during newborn screening in India. The other two cases in India were reported in the heterozygous in a 35-year-old pregnant woman and a case of heterozygous with iron deficiency anemia [18, 19]. Furthermore, the amount of this variant and HbS combination was reported to be 8.5% of the total Hb in the proband’s mother [20].

The other case, coinheritance of the β-globin chain variant (Hb D-Punjab) with heterozygous Hb Fontainebleau, was reported in an infant by Rodriguez-Capote et al. [21]. Turner et al. reported the first homozygous a case for Hb Fontainebleau; six cases of heterozygote of this variant; four cases with –α13/αα; and one case that combined heterozygous with αT Saud α [22]. The variant was first described in a 37-year-old female by Canatan et al. [23]. However, the latest information provides details about the second case of heterozygous Hb Fontainebleau with mild microcytosis in population screening in Adıyaman, Turkey. Furthermore, we report another rare alpha variant, heterozygous Hb Q-Iran.

The case of Hb Q-Iran [α75 (EF4) Asp→His;GAC→CAG] was reported for the first time with two cases in North Central Turkey and Ankara with normal hematology [24]. This variant was also reported as homozygous in one case that originated from Sivas, Central Anatolia in Turkey [25]. The other two cases were a case of acute lymphoblastic leukemia with Hb S and a case of spinal ischemia with α-thal combination [26, 27]. This variant is found sporadically in our country [28]. Individuals carrying Hb Q-Iran are hematologically normal, but in this case had microcytosis and low HbA2 values (Table 2).

The cases of Hb Fontainebleau and Hb Q-Iran occurred in Turkish girls living in the Gölbaşı district of Adıyaman Province and the city center, southeastern Turkey, respectively. Therefore, this case different α-thal deletion or iron deficiency anemia had probably coexisted. As a result, we can conclude that the identification of alpha variants is important for the clinical management of Hb disorder.

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