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ORIGINAL ARTICLE

Alpha Thalassemia in Istanbul: Distribution of Deletions in Alpha-globin Gene Cluster

İstanbul'da Alfa Talasemi: Alfa-globin Gen Kümesi Delesyonlarının Dağılımı

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ABSTRACT

Background/Aims: Alpha thalassemia is an autosomal recessive congenital disease resulting from a globin protein disorder encoded by genes in the alpha thalassemia gene cluster. It presents a wide range of clinical conditions, from mild anemia to hydrops fetalis. Alpha thalassemia trait is common in Middle Eastern and Mediterranean countries. It is included in the premarital screening program in Türkiye. The aim of this study was to determine the spectrum of alpha thalassemia deletions observed in Istanbul.
Methods: This cohort included 169 patients who were clinically suspected to have alpha thalassemia disease or carrier, and whose mutation was detected by the Multiplex Ligation-dependent Probe Amplification (MLPA) method.
Results: The identified variants were listed according to their frequencies and compared to previous studies conducted in different regions of Türkiye. In a total of 338 alleles, 61.8 % (209/338) mutations were detected. The most common variant was −a3.7 and -aMED ranked second.
Conclusion: This study reports alpha thalassemia mutations in Istanbul and reveals a different spectrum for some variants compared to previous studies in the region. This situation has been evaluated as evidence that the demographic structure in Istanbul has changed as a result of migration. Additionally, presenting the detected variants and mean hematological findings will guide genetic counseling in region.

Keywords: alpha-Thalassemia, gene deletion, alpha globin, Hemoglobin A

ÖZ

Amaç: Alfa talasemi, alfa globin gen kümesindeki genler tarafından kodlanan bir globin protein bozukluğu nedeni ile ortaya çıkan otozomal resesif kalıtsal bir hastalıktır. Hafif anemiden hidrops fetalise kadar geniş bir klinik tabloda görülebilir. Alfa talasemi taşıyıcılığı, Orta Doğu ve Akdeniz ülkelerinde yaygındır ve Türkiye'de evlilik öncesi tarama programına dahil edilmiştir. Bu çalışmanın amacı, İstanbul'da gözlemlenen alfa talasemi delesyon spektrumunu belirlemektir.
Gereç ve yöntemler: Bu çalışmada klinik olarak alfa talasemi hastalığı veya taşıyıcısı olduğu şüphesi olan ve mutasyonları Multiple-Ligand Prob Amplifikasyonu (MLPA) yöntemiyle tespit edilen 169 hastanın sonuçları değerlendirilmiştir.
Bulgular: Çalışma sonucunda toplam 338 alel içinde %61.8 (209/338) mutasyon tespit edilmiştir. En yaygın varyant −a3.7 iken, -aMED ikinci sırada yer almıştır.
Sonuç: Bu çalışma, İstanbul'da alfa talasemi mutasyonlarını rapor etmektedir ve bölgedeki önceki çalışmalarla karşılaştırıldığında bazı varyantlar için farklı bir spektrumu ortaya koymaktadır. Bu durum İstanbul'da demografik yapının göç sonucu değişmesinin bir kanıtı olarak değerlendirilmiştir. Ayrıca saptanan varyantlar ve ortalama hematolojik bulguların sunulması genetik danışmada yol gösterici olacaktır.

Anahtar kelimeler: alpha-Talasemi, gen delesyonu, alfa globin, Hemoglobin A

Introduction

Alpha (a) thalassemia is an inherited, autosomal recessive (AR) disorder caused by mutations in the alpha globin gene cluster (HBZ, HBA1, HBA2 and HBQ1 genes) located on chromosome 16 (1). The alpha thalassemia genes (HBA1, HBA2) occur in duplicate and the alpha chains are located approximately 3.7 kilobases apart. Although point mutations in one or both a-globin genes are known to cause thalassemia, deletions in these genes often cause the disease (2).

Alpha-thalassemia (a-thalassemia) presents in four clinical variations: silent carrier (deletion/inactivation of one a-globin gene (-a/aa or a+ carrier), thalassemia trait(deletion/inactivation of two a-globin genes either in cis (--/aa, or -a0 carrier) or in trans (-a/-a), hemoglobin H (HbH) (most commonly arising due to the deletion/ inactivation of three a-globin genes; --/-a), and hemoglobin Bart's hydrops fetalis (Hb Bart) syndrome (resulting from the removal/inactivation of all four alpha

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globin [a-globin] genes; --/--). Hb Bart syndrome is the most severe form, which results from severe anaemia induced congestive heart failure and leads to death during the neonatal period. HbH disease is the second form. It can show symptoms in the first years of life, but can only be detected by routine haematological tests in asymptomatic adults (3).

A wide range of hemoglobin levels can be observed in people with a-thalassemia, ranging from normal to severe anemia (Hb 15.5-7.5 g/dl). Additionally, these individuals have a decrease in mean corpuscular volume (MCV < 79 fl) and mean corpuscular hemoglobin (MCH < 27 pg) (4).

The World Health Organization (WHO) estimates that about 5% of the global population carries a mutate gene for a hemoglobinopathy, and over 330,000 newborns are impacted, primarily by sickle cell diseases (83%) and thalassemias (17%) every year (5). This condition is especially common in Southeast Asia, but also in the Mediterranean countries, the Middle East, Central Asia, India, Southern China, North Africa and South America (6).

Southern blotting, cloning of breakpoints, PCR amplification and direct sequencing have been used in the molecular diagnosis of alpha thalassemia. Next Generation sequencing (NGS) detects single point mutations, small deletions, and duplications, while Multiplex Ligation-dependent Probe Amplification (MLPA) detects copy number variants. Since mutations in alpha thalassemia may be population-specific, the most common mutation type in the region should be considered in the test selection and sequencing (4).

The high number of consanguineous marriages in our country increases the incidence of thalassemia, which is an AR disease. The prevalence rate reported for carriers of a thalassemia 0.25% in Türkiye and within the scope of National Health Policies, there is a comprehensive National Hemoglobinopathy Control Program for the prevention of hemoglobinopathies through prenatal diagnosis, genetic counseling and public education (7). In this study, we aimed to present the spectrum of alpha-globin gene deletions using MLPA method for patients suspected of having alpha thalassemia in Istanbul, the most populous city in Türkiye (province/country population: 18,65%) (8).

Material and Methods

This study included 169 individuals (85 females, 84 males) who were examined for alpha thalassemia at the Haseki Genetic Diagnosis Center between February 2021 and February 2023. The individuals underwent molecular testing because of their family history, premartial screening program, and clinical findings and hemoglobin characteristics. We performed mutation analysis in the alpha globin gene using the MLPA method. Written informed consent was obtained from the patients or their legal guardians. The study was approved by the Haseki Research and Training Hospital ethical committee (Protocol no:194-2023, dated November 01, 2023).

Blood samples (4 ml) were collected from the participants, and genomic DNA was extracted from EDTA-treated peripheral blood samples using a spin column method with the DNA isolation kit (PureLink® Genomic DNA Kits) according the manufacturer's instructions. DNA samples were stored at -20°C until further use. MLPA was performed using SALSA MLPA Probemix P140 HBA (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol. Amplification products were analyzed by capillary electrophoresis on an ABI 3130 Genetic Analyzer (Thermo Fisher Scientific, MA, USA). Data were analyzed using GeneMapper 4.0 and Coffalyser.net software.

Results

In this study, the distribution of the results from 169 patients was evaluated using the MLPA method in the alpha thalassemia genes. In a total of 338 alleles 61.8 % (209/338) mutations were detected. The most common variant was −a3.7 and second most common was MED-- . All identified alpha globin variants are listed in Table 1. The subtypes of the −a3.7 mutation were also determined according to the product information sheet. Accordingly, the most common type was −a3.7 (D). Although this distinction is not official, it does indicate the breakpoints.

The average age of the patients was 24,6 years and 85 were female and 85 were male. The most common genotype found in patients was −a3.7 (34%), and the second most common variant was –MED1(9.1). The most common genotype in patients carrying mutations in both alleles was −a3.7/MED-- . The distribution of genotypes and mean value of haematological parameters (hemoglobin Hb, mean corpuscular volume (MCV), red cell distribution width (RDW), mean corpuscular hemoglobin (MCH)) of the patients is shown in Table 2. Screen shots of the Coffalyser.net software are shown in Figure 1.

Table 1: Mean values of haematologic findings of patients.

Genotype	n	%	Hb (13.4-17.2) (g/dl)	MCV (79-92.2)(fl)	RDW (11.4-14.6)(%)	MCH(27-31 pg) (%)
−a3.7/aa	59	34.9	12.2	73.4	16.9	21,3
−aMED/aa	38	22.4	9.9	69.1	20.5	19,5
−a20.5/aa	25	14.7	11.3	67.6	16.6	20,6
−a3.7/−aMED	21	12.4	8.6	69.4	27	21,1
−a3.7/-a3.7	15	8.8	11.5	68.3	14.8	20,9
−a4.2/aa	3	1.7	12.1	69.3	17.2	21,4
−a20.5/−a3.7	3	1.7	9.2	61.8	21.8	20,7
−aSEA /aa	2	1.1	11.9	69.4	18.3	21,8
−a4.2/−aMED	1	0.6	8.9	57.4	32.8	19,7
aaaanti-3.7/aa	1	0.6	11.8	67.5	16.9	28,1
aaaanti-4.2/aa	1	0.6	12.3	74.6	-	-

Legends: n: number of patients, **Hb**: Hemoglobin, **MCV**: Mean Corpuscular Volume, **RDW**: Red cell distribution width, **MCH**: Mean Corpuscular Hemoglobin

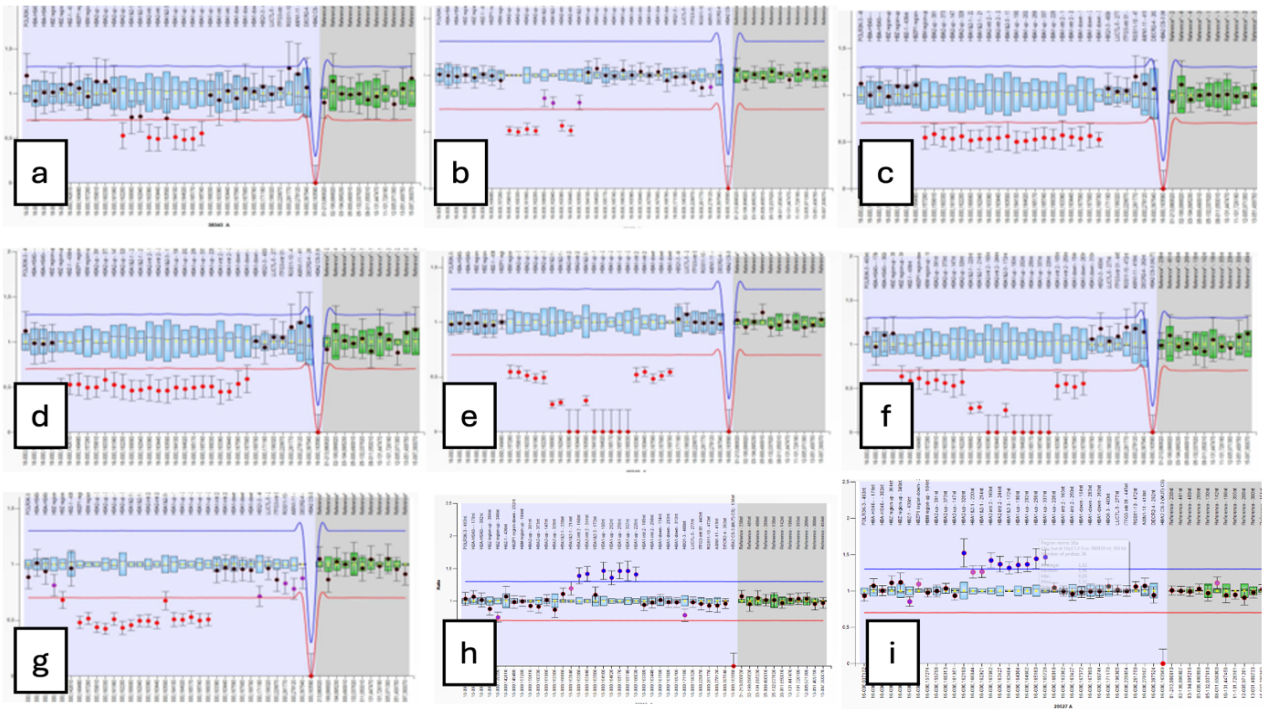

Figure 1: The images of analysis of MLPA

Legends: a: −a3.7, b: −a4.2, c: -aMED1, d: -aMED2, e: −a20.5/−a3.7 f: −a3.7/−aMED2, g: -a20.5, h: anti aaa 3.7, i: anti aaa 4.2

Table 2 Distribution of alpha globin gene deletions and allele frequencies found in previous studies in Türkiye

Variant	This study % (AC:338)	Güvenç et.al. 2010 Adana % (AC:426)	Demir et.al. 2021 Trakya % (AC:156)	Keser et.al. 2021 Antal-ya % (AC:214)	Barış et.al. 2023 West Aegean % (AC:186)
aa	37.6(127)	41 (175)	42 (65)	45(96)	41(76)
−a3.7	34(115)	43(183)	35.2(55)	42(90)	44.6(83)
−aMED	17.8(60)	10.1(43)	2.5(4)	3.7(8)	7.5(14)
−a20.5	8.2 (28)	4(17)	5.7(9)	7.5(16)	3.7(7)
−a4.2	1.2 (4)	0.7(3)	0.6(1)	-	1.1(2)
−aSEA	0.6(2)	-	1.9(3)	-	1.6(3)
aaaanti-3.7	0.3(1)	1.2(5)	9.6(15)	0.5(1)	0.5(1)
aaaanti-4.2	0.3(1)	0	-	-	-
−aHS40	-	-	0.6(1)	-	
Whole gene deletion	-	-	1.9(3)	-	
FIL/aa	-	-	-	1.4(3)	-

References :13,14,16,17, **AC**: Allele count

Discussion

Previous studies conducted in different regions of Türkiye have reported 14 different mutations, including the 3.7 kb deletion, which is the most common in alpha thalassemia (9). In studies conducted in Adana and its surroundings, where alpha thalassemia is common, the carrier rate was found as 4.1%-2.9% (10,11). In a study conducted on cord blood, the frequency of deletional alpha thalassemia carrier status was 3.6% (12). In Türkiye, the reverse dot blot method (alpha globin strip assay) has been mainly used in research studies, but recently the use of the MLPA method has increased. The comparison of the mutation frequencies we found with the frequencies from several studies conducted in Türkiye is shown in Table 2.

Due to its location, Türkiye acts as a bridge between Asia, Europe and Africa, and the province of Istanbul is the transition point of all these locations. The population of Istanbul consists of many ethnic and demographic groups due to its geographical location. The frequency of thalassemia carriage is observed high, both with migration from Anatolia and with migration from northern Iraq, Syria and other Middle Eastern countries.

In this study eight different CNV mutations were found, and one of them was triplication the others were deletions. −a3.7 deletional mutation was the most common alpha globin gene deletion (34%). In a study conducted in Istanbul in 2015, the frequency of the −a3.7 mutation was reported as 39% (13). Another study conducted in the southern region found similar diversity of mutations and reported the −a3.7 deletion frequency as 52,28% (2). The frequency of −a3.7 deletions was reported as 35,2% in a study in the Trakya region of Türkiye (14). In a study performed in the southern region of Türkiye, −a3.7 deletion frequency was reported as 44,6% and in a study performed in the Aegean Region of Türkiye was reported 43.2% (15,16). Although its frequencies differ, this mutation was the most common in all studies.

The second most common MED1/2 mutation frequency was 17.8%. In a previous study conducted in Istanbul, the rate of MED double deletion was reported as 17.9% (13). MED1/2 mutations were found with a higher frequency compared to other previous

studies (cases with point mutations are excluded) in our country (14,15,17,18). Both domestic and foreign immigrant population is concentrated in Istanbul. Although Istanbul is located in the Trakya region, detailed information on ethnic backgrounds was not available, which may be the reason for the different rates in the study conducted by Demir et al. (2021) (14).

In this study, unlike other reported studies in Turkey, an individual with the anti aaa4.2 variant (triplication) was identified, and the patient's hemoglobin level was normal. While the frequencies of other reported variants are largely similar to previous studies, regional differences are observed in their rankings (Table 1). These differences are thought to be related to regional demographic variations.

Deletions are detected in approximately 90% of alpha thalassemias (3). Therefore, presenting only deletions in this study does not cause a serious lack of data in terms of the scope of this study.

The mean hematological values provided in this study are important for genotype-phenotype correlation and will serve as a guide for genetic counseling in the region.

In regions where the carrier frequency is high, it is important to screen the population for potentially lethal diseases, especially alpha thalassemia before marriage. Nowadays, carriers of thalassemia can be determined by screening with complete blood count and hemoglobin electrophoresis before marriage in Türkiye. Molecular genetic tests are also performed depending on the results. If both spouses are thalassemia carriers, information about prenatal diagnosis and preimplantation genetic testing (PGT) should be provided, and genetic counseling should be given considering all possibilities.

Conclusion

Istanbul is the most populous city and receives a significant number of immigrants from both within and outside the country. The data we have collected is valuable as it illustrates the evolving demographic structure over the past decade. Migration due to war in Middle East countries and economic reasons from east to west have reshaped the population genetics. Consequently, while the types of mutations observed remain relatively constant due to the location of Türkiye, there has been a shift in their frequency. To track these changes more comprehensively, further studies are required.

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Approvals were obtained from Haseki Training and Research Hospital Ethics Committee (Protocol no:194-2023,November 01, 2023) Study design: FNO; Data Collection: FNO; Analysis: FNO; Manuscript Preparation: FNO.

Conflict of Interest Statement

There are no conflicts of interest in this article.

Data Availability Statement

The data from the findings of the study are available from the corresponding author upon reasonable request.

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