

Molecular and Hematological Characterization of α -Thalassemia in Denizli Province

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ABSTRACT

Aim: Alpha thalassemia, a common monogenic disorder, occurs with defective synthesis of the α -globin chain and has a very wide clinical spectrum depending on the disorders in the globin genes. This study aims to determine the frequency of α -globin gene mutations in patients suspected of having alpha thalassemia in Denizli province and to evaluate the phenotypic effects of detected mutations.

Methods: A total of 93 patients (55 female, 38 male) with suspected alpha thalassemia based on anemia, family history, premarital screening and high-performance liquid chromatography results were analyzed for α -thalassemia gene deletions. DNA was isolated from peripheral blood samples, and the results were evaluated using the "Seqline Alpha Thalassemia Diagnostic Kit" to detect common α -globin gene deletions: large deletions of 3.7 kb ($-\alpha 3.7$), 4.2 kb ($-\alpha 4.2$), and 20.5 kb ($-(\alpha)20.5$) in *HBA1-2* genes, as well as MED1 ($--MED$) and SEA ($--SEA$).

Results: Among the 93 patients, mutations were detected in 38 patients, yielding a deletion detection rate of 40.9%. The most common mutation was $-\alpha 3.7$ (40.7%), followed by $-(\alpha)20.5$ (17.1%), $--MED$ (5.2%), and $-\alpha 4.2$ (2.6%). No $--SEA$ deletions were identified.

Conclusion: This study represents the first molecular characterization of α -thalassemia in Denizli province, identifying four different α -thalassemia deletions and eight distinct genotypes. The findings provide valuable insights into the regional distribution and clinical implications of these mutations.

Keywords: Alpha thalassemia, alpha-globin gene, gene deletion, genotype-phenotype correlation

Introduction

Hemoglobinopathies are the most common autosomal recessive disorders in the world [1]. Alpha thalassemia (α -thalassemia), which is seen with high frequency in the population living in the Mediterranean, Southeast Asia, and the Middle East, is a common type of hemoglobinopathy characterized by deficiency or absence of alpha globin chain synthesis [2]. The α -globin gene cluster, located on chromosome 16p13.3, consists of two α -globin genes ($\alpha 1$ and $\alpha 2$) on each homologous chromosome, totaling four functional α -globin genes [3,4].

Alpha thalassemia syndromes result from mutations in one or more α -globin genes, and the molecular defects are usually gene deletions; however, point mutations might also be found [5]. α -Thalassemia is quite heterogeneous at the clinical and molecular levels. The form resulting from deletion/inactivation of one of the four α -globin genes is called a silent carrier state. It usually leads to insignificant hematological findings; in this form, patients' hemoglobin levels, erythrocyte parameters, and hemoglobin electrophoresis are normal. Deletion/inactivation of two α -globin genes in cis or trans leads to α -thalassemia trait, and patients show mild microcytic, hypochromic anemia. When three of the α -globin genes are deleted or inactivated,

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hemoglobin (Hb) H disease, characterized by severe anemia, is observed. Deletion/inactivation of four α -globin genes results in the most severe form, Hb Bart syndrome, which causes hydrops fetalis [6,7]. Although the phenotype of alpha thalassemia mutations is related to the number of affected alpha globin genes, its expression and genotype can be more complex and variable. Large deletions, non-deletion mutations, mutations in regulatory regions, mutations in epigenetic genes, and unstable mutations can all be effective in increasing the severity of the phenotype [8-10].

According to the World Health Organization (WHO) data, it is estimated that hemoglobin disorders have a frequency of 5% worldwide. More than 330,000 newborns are affected each year, 83% by sickle cell diseases and 17% by thalassemias [11]. Türkiye has a high prevalence of hemoglobinopathies, exacerbated by consanguineous marriages, making these disorders a significant public health concern. However, research on α -thalassemia in Türkiye remains limited, and no molecular studies on α -globin gene mutations have been conducted in Denizli province. Understanding the α -globin mutation spectrum in different regions is crucial for improving genetic counseling and establishing national health policies for α -thalassemia screening.

This study aims to investigate gene deletions in patients with suspected α -thalassemia referred to our center, analyze the molecular spectrum of deletions, and evaluate genotype-phenotype correlations.

Methods

This retrospective study included 93 patients (55 females, 38 males) who were referred to the Pamukkale University Genetic Diagnosis Center with a preliminary diagnosis of α -thalassemia between August 2020 and December 2024. Ethical approval was granted by the Pamukkale University Non-interventional Clinical Research Ethics Committee (decision no: E-60116787-020-657799, date: 19.02.2025).

DNA Isolation and Quantification

Genomic DNA was extracted from peripheral blood samples collected in K2EDTA tubes using the Qiacube automated DNA isolation system (Qiagen, Germany) based on the spin-column method. The concentration and purity of the extracted DNA were determined via spectrophotometric measurement using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). DNA samples with concentrations between 80 and 100 ng/ μ L were used for subsequent analyses.

α -thalassemia Deletion Analysis

Alpha-globin gene deletion analysis was performed using the “ α -thalassemia Diagnostic Kit” (Seqline, Türkiye) which targets the detection of gross deletions in the *HBA1* and *HBA2* genes. The kit uses the Gap-polymerase chain reaction (PCR) method to detect deletions. This method detects deletions of 3.7 kb ($-\alpha 3.7$), which results in a deletion of part of the *HBA2* gene; 4.2 kb ($-\alpha 4.2$), which results in the deletion of the entire the

HBA2 gene; and deletions like ($-(\alpha)20.5$), MED ($--MED$), and SEA ($--SEA$) that result in deletions both the *HBA2* and *HBA1* genes.

All procedures were performed according to the manufacturer’s protocol. PCR amplification products were subjected to gel electrophoresis using a 1.4% agarose gel with a 50 bp-5000 bp DNA ladder. Electrophoresis was carried out at 110 V for 80 minutes, and bands were visualized under UV imaging.

The deletion regions examined and the expected band sizes in gel electrophoresis are given in Table 1.

This methodological approach ensured the accurate detection of common α -thalassemia deletions in the studied population.

Statistical Analysis

The overall group and subgroups (e.g., patients with and without mutations, male and female patients) were analyzed for mean and median age. Mutation distributions, allele frequencies, and hematological findings (with median values calculated) are presented as percentages.

Results

In this study, gross deletions in α -thalassemia genes were investigated in 93 patients, using the Gap-PCR method, and the distribution of the results was evaluated. While deletions were detected in 38 of the patients included in the study, no deletions were detected in 55 patients. This means that the deletion detection rate in alpha globulin genes in our study was 40.9% (Table 2).

The allelic frequencies of the determined α -thal gene deletions were as follows: $-\alpha 3.7$, 40.7% (n=31) $-(\alpha)20.5$, 17.1% (n=13) $--MED$, 5.2% (n=4) $-\alpha 4.2$, 2.6% (n=2). $--SEA$ deletion was not detected in the patients. Table 3 presents the allelic frequencies of the deletions found in the patients.

Table 1. Examined deletion regions and expected band sizes in gel electrophoresis	
Deletion type	Band size (bp)
$-\alpha 3.7$	2020
$\alpha 2$ (wild-type)	1800
$-\alpha 4.2$	1630
$--SEA$	1350
$-(\alpha)20.5$	1010
$--MED$	800

Table 2. Overview of our cohort (n=93)			
	Patients (n)	Age at diagnosis Age (mean)	Frequency (%)
Male	38	30.8	40.9
Female	55	33	59.1
Deletion detected	38	28.6	40.9
No deletion detected	55	34.6	59.1
Total patient	93	32.2	100

Hematological parameters were analyzed by averaging according to the genotypes of the patients. It was observed that mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and MCH concentration (MCHC) values were related to the number of functional α -globin genes, and that a decrease in the number of genes caused these values to decrease. The mean values of Hb, MCV, MCH, and MCHC were higher in individuals with the $-\alpha 3.7/\alpha\alpha$ genotype carrying a single gene deletion, while they were lowest in individuals with the $-\alpha 3.7/-(\alpha)20.5$ genotype carrying three gene deletions. The results of all hematological parameters are given in Table 4.

Discussion

α -thalassemia is one of the most prevalent genetic disorders worldwide, with significant differences in prevalence and mutation spectrum influenced by ethnic and geographical factors. The WHO reported the global prevalence of α -thalassemia as 44.6% in 2008 [12]. However, regional prevalence varies considerably, with the highest carrier frequencies observed in the Middle East and the Mediterranean, reaching up to 40% in some populations [13,14]. In Türkiye, the carrier frequency of hemoglobinopathies also shows regional variation, with the highest rates found in the southern part of the country [15,16]. Studies conducted in Adana, a southern city of Türkiye, reported beta-thalassemia and alpha-thalassemia carrier frequencies as high as 13.5% and 7.5%, respectively [17,18].

Despite the high prevalence of α -thalassemia in Türkiye, very few studies have specifically focused on this disorder, and

no prior study has investigated alpha-globin mutations in the Denizli region. Given the ethnic diversity of the Turkish population, it is crucial to determine the spectrum of alpha-globin mutations across different regions. Our study represents the first molecular characterization of alpha-thalassemia deletions in the Denizli region, providing valuable insights into the distribution and clinical implications of these mutations.

In our study, we identified four alpha-thalassemia deletions and eight genotypes among the study population. Notably, 59.1% of the patients referred to our clinic due to either anemia, family history, premarital screening, or abnormal high-performance liquid chromatography results did not have any alpha-globin gene deletions, while 40.9% carried a deletion. The most frequent deletion identified was $-\alpha 3.7$, with a prevalence rate of 40.7%. The other detected deletions were $-(\alpha)20.5$ (17.1%), $--MED$ (5.2%), and $-\alpha 4.2$ (2.6%). The $--SEA$ deletion, which is commonly observed in Asian populations, was not present in our cohort. Our findings align with previous alpha-thalassemia studies conducted in Türkiye, in which the $-\alpha 3.7$ deletion was the most frequently observed mutation. Similar results were reported in a study conducted in Antalya by Keser et al. [19]. According to studies from different Turkish regions, the frequency of the $-\alpha 3.7$ deletion ranges between 35.2% and 52.28% [20-24].

Previous studies have reported regional variations in the prevalence of other deletions. Onay et al. [21] identified $-(\alpha)20.5$ as the second most common mutation after $-\alpha 3.7$ in the Aegean region. Similarly, Keser et al. [19] found that the $-(\alpha)20.5$ deletion was the second most common in their study in Antalya, which is consistent with our results. However, unlike our findings, Onay et al. [21] detected the $--SEA$ deletion in one patient, while we did not observe this mutation in our study population. Additionally, we identified the $-\alpha 4.2$ deletion in two patients, whereas it was not reported in Onay et al.'s [21] study. Interestingly, studies conducted in other southern regions of Türkiye [9,16,19] reported a higher frequency of the $--MED$ double gene deletion than the $-(\alpha)20.5$ deletion, likely due to ethnic diversity in these regions. A comparative analysis of our mutation frequency data with those from other Turkish studies is presented in Table 5.

Table 3. Allele frequency of α -thalassemia deletions

Deletion	Allele	Frequency %
$-\alpha 3.7$	31	40.7
$-(\alpha)20.5$	13	17.1
$--MED$	4	5.2
$-\alpha 4.2$	2	2.6
$--SEA$	-	-

Table 4. Distribution of α -thalassemia deletions and mean values of hematological findings of patients according to this distribution

	Patients (n)	Frequency (%)	Hb (g/dL) (12-18) ^a	Hct % (37-52) ^a	MCV fL (80-100) ^a	MCH (pg) (27-31) ^a	MCHC (g/dL) (31-37) ^a	RDW (11.5-14.5) ^a	RBC M/uL (4.2-6.1) ^a
$-\alpha 3.7/\alpha\alpha$	13	14	13.18	40.53	73.79	23.95	32.44	15.47	5.50
$-(\alpha)20.5/\alpha\alpha$	12	12.9	12.09	38.19	66.21	20.78	31.52	16.00	5.77
$-\alpha 3.7/-\alpha 3.7$	6	6.5	11.08	34.60	68.60	21.82	32.10	15.30	5.09
$-\alpha 3.7/--MED$	3	3.3	10.90	34	57.33	18.36	32	24.56	5.86
$-\alpha 3.7/-\alpha 4.2$	2	2.2	11.35	35.55	68.25	21.75	31.90	14.65	5.21
$-\alpha 3.7/-(\alpha)20.5$	1	1	8.20	26.50	51.00	15.60	30.70	21.30	5.21
$--MED/\alpha\alpha$	1	1	12.60	39.60	65.10	20.60	31.70	16.40	6.09

^aNormal values.

Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red cell distribution width, RBC: Red blood cell

Table 5. Allel frequency of α -thalassemia mutation in Turkish population

Variant	This study % (AC: 76)	Öztürk [26] 2024 İstanbul % (AC: 338)	Barış et al. [24] 2023 West Aegean % (AC: 206)	Keser et al. [19] 2021 Antalya % (AC: 224)	Demir et al. [23] 2021 Trakya % (AC: 156)	Onay et al. [21] 2015 Aegean % (AC: 285)	Karakaş et al. [22] 2015 İstanbul % (AC: 190)	Celik et al. [20] 2013 Hatay % (AC: 194)	Guvenc et al. [17] 2010 Adana % (AC: 450)
Method	Gap-PCR	MLPA	MLPA	StripAssay + MLPA	MLPA	StripAssay	StripAssay	StripAssay	StripAssay
- α 3.7	40.7 (31)	34 (115)	40.3 (83)	40.1 (90)	35.3 (55)	52.28 (149)	23.15 (44)	43.81 (85)	40.6 (183)
- α 20.5	17.1 (13)	8.2 (28)	3.4 (7)	7.1 (16)	5.8 (9)	14.74 (42)	10.5 (20)	0.51 (1)	3.7 (17)
- α MED	5.2 (4)	17.8 (60)	6.8 (14)	3.5 (8)	2.6 (4)	10.53 (30)	8.9 (17)	5.67 (11)	9.5 (43)
- α 4.2	2.6 (2)	1.2 (4)	1 (2)	-	0.6 (1)	-	1 (2)	0.51 (1)	0.6 (3)
- α SEA	-	0.6 (2)	1.4 (3)	-	1.9 (3)	0.35 (1)	-	-	-
$\alpha\alpha\alpha$ anti-3.7	ND	0.3 (1)	0.48 (1)	0.44 (1)	9.6 (15)	3.6 (9)	3.6 (7)	1.54 (3)	1.1 (5)
$\alpha\alpha\alpha$ anti-4.2	ND	0.3 (1)	-	-	-	-	-	-	-
- α HS40	ND	-	-	-	0.6 (1)	-	-	-	-
Whole gene deletion	ND	-	-	-	1.9 (3)	-	-	-	-
FIL/ $\alpha\alpha$	ND	-	-	1.33 (3)	-	2.81 (8)	1 (2)	0.51 (1)	-
Point mutation			4.85 (10)						

AC: Allele count, ND: No data, PCR: Polymerase chain reaction, MLPA: Multiplex ligation-dependent probe amplification

The deletions examined in our study represent the most common mutations in α -thalassemia. For example, in the study by Keser et al. [19] (2021), 92.8% of the total mutations consisted of the three deletions included in our analysis: - α 3.7 (73.3%), -(α)20.5 (13.0%), and --MED (6.5%). This may also indicate the importance of selecting molecular techniques based on the common mutations present in the target population to ensure cost-effectiveness and diagnostic accuracy.

The clinical impact of alpha-thalassemia is closely associated with hematological parameters. El-Kalla and Baysal [25] demonstrated that a reduction in the number of functional α -globin genes significantly decreases MCV values. Similarly, Guvenc et al. [17] emphasized that MCV, MCH, and MCHC values consistently correlate with the number of functional α -globin genes. In our study, we analyzed hematological parameters according to genotype and observed that the mean MCV was highest in individuals with the - α 3.7/ $\alpha\alpha$ genotype (single gene deletion) and lowest in those with the - α 3.7/-(α)20.5 genotype (three gene deletions). MCHC and MCH values also followed a similar pattern. This correlation between hematological indices and functional gene count further supports the clinical significance of molecular analysis in alpha-thalassemia diagnosis. However, hematological parameters alone are insufficient to determine the genotype of α -thalassemia, and definitive diagnosis requires molecular analysis. The correlation between genotype and hematological parameters in our study population is illustrated in Table 4.

Study Limitations

We used Gap-PCR analysis to detect common deletions in alpha-globin genes. While this technique effectively identifies the most prevalent mutations, it cannot detect rarer deletions or point mutations. Advanced molecular techniques such as next-generation sequencing (NGS) and multiplex ligation-dependent probe amplification (MLPA) could provide a more comprehensive understanding of the molecular spectrum of α -thalassemia by detecting less common mutations.

Conclusion

Our study provides critical insights into the molecular spectrum and distribution of alpha-thalassemia mutations in the Denizli region. The identification of four different deletions and eight genotypes highlights the genetic diversity of alpha-thalassemia in this region. Our findings are consistent with previous studies conducted in other parts of Türkiye, with the - α 3.7 deletion being the most prevalent mutation. The correlation between genotype and hematological indices emphasizes the importance of molecular analysis in the accurate diagnosis and clinical management of α -thalassemia.

This study serves as a foundation for future research on α -thalassemia in Türkiye, particularly in regions where data is scarce. Expanding molecular analysis with more advanced techniques, such as NGS and MLPA, and increasing the study population size will provide a more comprehensive understanding of the alpha-thalassemia mutation spectrum.

Additionally, the integration of genetic screening programs and improved genetic counseling strategies based on regional mutation profiles can contribute to better disease management and prevention efforts. Future studies should focus on identifying rarer mutations and exploring their clinical implications to enhance our understanding of alpha-thalassemia in Türkiye and beyond.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki. Ethics committee approval for this study was received from Pamukkale University Non-invasive Clinical Research Ethics Committee (decision no: E-60116787-020-657799, date: 19.02.2025).

Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: N.A.A., T.D., Concept: D.K., T.U.E., N.A.A., T.D., Design: D.K., Data Collection or Processing: D.K., T.U.E., Analysis or Interpretation: D.K., T.U.E., T.D., Literature Search: D.K., T.D., Writing: D.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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