Genotype–phenotype association in alpha-thalassemia cohort in a population with high prevalence of alpha and beta-thalassemia: importance of genetic screening

Ahmad Tamaddoni[a], Hassan Mahmoudi Nesheli[a], Alireza Paniri[b], Mandana Azizi[c], Ali Banihashemi[c], Reza Youssefi Kamangari[c], Haleh Akhavan-Niaki[b,c]

[a]Department of Hematology, Non-Communicable Pediatric Diseases Research Centre, Babol University of Medical Sciences, [b]Department of Genetics, Faculty of Medicine, Babol University of Medical Sciences, [c]Genetic Laboratory of Amirkola Children Hospital, Babol University of Medical Sciences, Babol, Iran

Correspondence to Haleh Akhavan-Niaki, Department of Genetics, Faculty of Medicine, Babol University of Medical Sciences, Babol, 4714957534, Iran.
Tel: + 98-1132345874; Fax: + 98-1132345874;
E-mail: halehakhavan@yahoo.com

Received 02 October 2020
Revised 25 January 2021
Accepted 27 April 2021
Published 09 October 2021

Middle East Journal of Medical Genetics
2021,10:24–31

Purpose
Alpha and beta-thalassemias are common inherited hemoglobinopathies caused by reduced synthesis of alpha and beta-globin chains, respectively. Based on the type of mutation, the spectrum of hematological indices could be variable. This study aimed to determine the influence of coinheritance of alpha and beta-globin mutations on anemia severity.

Patients and methods
A total of 1415 patients with thalassemia, including alpha, beta, and alpha-beta thalassemia that were referred to the genetic center of premarital and prepregnancy screening were enrolled in this retrospective study. Hematological indices including complete blood count and hemoglobin A2 levels as well as genotypes of alpha and beta globin genes were considered. Gap-PCR, reverse dot–blot, restriction fragment length polymorphism, and sequencing were recruited for molecular analysis.

Results
The frequency of participants with alpha-globin deletion ($n = 912$) was ~ 2.5 times more than those with a point mutation ($n = 392$). The most common alpha-globin gene deletion and point mutation were HBA2:c. 94_95delAG (or HBA1) (del 3.7 kb) and AATAAA > AATGAA; HBA2: c.*92 A>G (PA2), respectively. Patients with beta-globin mutation had a lower hematological index in comparison with those with alpha-globin mutation. Moreover, alpha-globin mutation could moderate the phenotype of beta-thalassemia carriers.

Conclusion
Considering both alpha-globin and beta-globin gene mutations in the diagnosis of beta-thalassemia, especially in high-prevalence thalassemia regions showing genetic heterogeneity of the disease, may lead to a more accurate genetic counseling in the context of premarital and prepregnancy screening for thalassemia prevention.

Keywords:
hematological indices, prepregnancy screening, α-thalassemia, genetic counseling, β-thalassemia

Middle East J Med Genet 10:24–31

2090-8571

Introduction

Thalassemia, an autosomal recessive inherited disorder, is a common hematological condition characterized by disrupted synthesis of globin chains (Martin and Thompson, 2013). Based on the mutated chain type, thalassemia is classified into alpha and beta-thalassemia form (Muncie and Campbell, 2009). Alpha-thalassemia affects 5% of the world's population and is common in regions with malarial outbreaks, including Mediterranean countries, South-East Asia, Africa, Middle East, and Indian subcontinent (Vichinsky, 2010). Hb Barts, which is accompanied by a hydrops fetalis syndrome, is a severe alpha-thalassemia disorder caused by deletion of all four alpha-globin genes (--/--) (Karakaş *et al.*, 2015). Hemoglobin H (HbH) disease occurs upon deletion of three α globin genes (-α/--) or two α globin gene deletions along with one point mutation ($\alpha^T\alpha$/--) (Gilad *et al.*, 2017). HbH consists of unstable β-globin chain tetramer leading to hemolytic anemia by precipitating in erythrocytes (Calderón-Brenes *et al.*, 2020). Individuals with two alpha-globin gene deletion or some point mutations (--/αα or $\alpha^T\alpha/\alpha^T\alpha$) show mild anemia only (Gilad *et al.*, 2017), whereas silent carriers show no significant hematological symptoms owing to inheritance of one defective alpha globin gene (Sirachainan *et al.*, 2016). A total of 25 000 infants are annually born with beta-thalassemia major worldwide (De Sanctis *et al.*, 2017). Beta-thalassemia occurs most often following point mutations in β-globin gene, whereas in rare cases, deletions also

lead to the disorder (Cao and Galanello, 2010). Precise detection of mutation type is very essential to choose the proper treatment for patients presenting with severe anemia, as some Hb variants (Hb S, Hb E, or Hb C) or coinheritance of alpha- and beta-thalassemia may modulate the anemia. Moreover, alpha globin gene triplication could worsen the severity of patients with beta-thalassemia and cause a broad range of red blood cell (RBC) abnormalities (hypochromasia, anisopoikilocytosis, and circulating normoblasts) and lower hemoglobin (Hb) levels.

One of the reasons of improper diagnosis of thalassemia mutation type is the genetic and clinical heterogeneity of the disease, especially in regions with higher thalassemia prevalence (Akhavan-Niaki *et al.*, 2011). As alpha- and beta-globin genes are located on different autosomes (Forget and Hardison, 2009). It is not surprising to encounter people who inherited both alpha- and beta-globin gene mutations in regions with high prevalence of alpha- and beta-thalassemia. This could raise the possibility that owing to overlapping hematological indices of beta- and alpha-beta thalassemia, only one phenotype may be suspected. In this condition, if the other partner has also a thalassemia trait, there may be possibility of birth of an affected child owing to inappropriate genetic counseling. Consequently, the exact recognition of patients' genotype could pave the way to determine their phenotype and thereby appropriate follow-up to prevent the birth of affected children with transfusion-dependent thalassemia.

Patients and methods

Patients

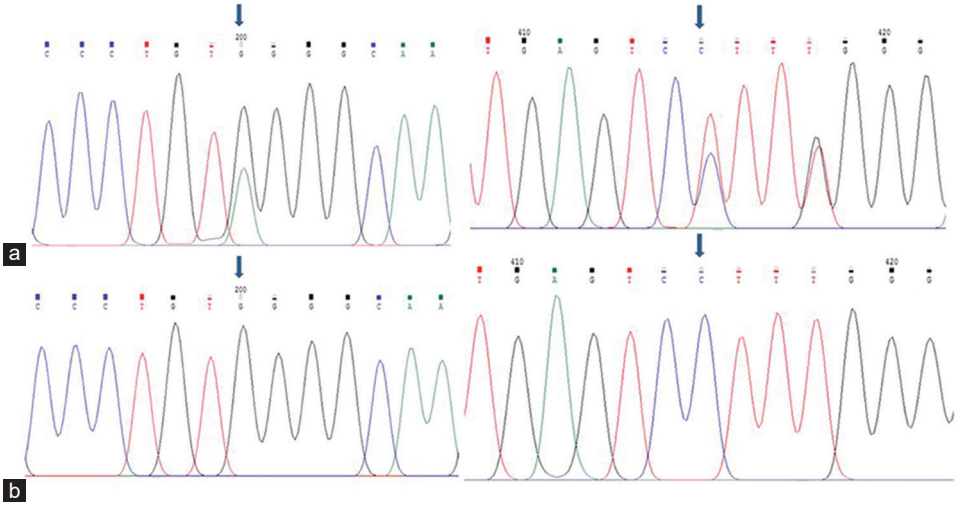
This retrospective study was conducted on a cohort of patients with anemia and involved 1415 individuals (728 men and 687 female) affected by alpha-, beta-, or alpha-beta-thalassemia who were referred to the genetics laboratory of Amirkola Children Hospital in the context of premarital counseling or prepregnancy screening from 2004 to 2019. All participants provided consent to participate in the study, and all procedures were performed according to the institutional ethics guidelines.

Hematological and molecular characteristics

Mutation detection was performed previously by gap-PCR, reverse dot–blot, restriction fragment length polymorphism (RFLP) (Akhavan-Niaki *et al.*, 2011), and sequencing (Fig. 1). Gap-PCR is commonly used for analysis of deletional alpha-thalassemia mutations, such as MED and 20.5. In this method, specific primers are designed for identified deletions, and primers are able to bind their target sequence, and then the corresponding DNA fragment amplification occurs only in presence of deletion (Zhou *et al.*, 2002). Reverse dot–blot technique is able to detect simultaneously several mutations with a single hybridization reaction. Relatively, patients' DNA is amplified using biotinylated primers, producing a pool of biotin-labeled amplicons for hybridization with allele-specific oligonucleotide probes prealably fixed on the membrane. Notably, visualization of hybridization has occurred following incubation with streptavidin horseradish peroxidase (Cai *et al.*, 1994). Another technique that has been used in the present study is restriction fragment length polymorphism, which could identify a mutation based on fragments with different lengths generated following treatment of the amplified DNA with a specific restriction enzyme (Sajadpour *et al.*, 2019) (Fig. 2).

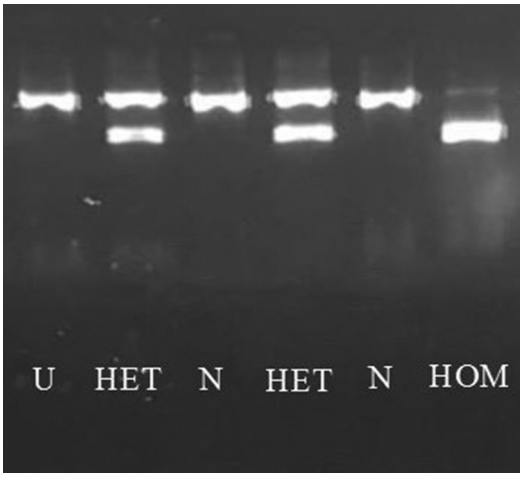
Patients were screened for 10 common alpha-globin mutation types, including del 3.7, del 4.2 (leftward deletion), NG_000006.1:g. 24664_41064del16401 (MED), NG_000006.1:g. 15164_37864del22701 (20.5), AATAAA> AATAAG; HBA2: c.*94 A>G (PA1), HBA2: c.*95 A>G (PA2), HBA2: c. 95+2_95 + 6delTGAGG (5NT), HBA2:c. 427 T>C (CS), HBA2:c. 56delG (C19), insertion of 21 base pair (IVS II + 3ins(+21nt) (+GACCCGGTCAACTTCAAGGTG) (ins 21) in the α1-globin gene, and 24 common types of beta globin gene mutation, including HBB: c.-138C>A (-88), HBB: c.-137C>A (-87), HBB: c. 135delC (Codon 44), HBB: c. 118 C>T (Codon 39), HBB: c. 114 G>A (Codon 37), HBB: c. 92 G>A (Codon 30), HBB: c. 67 G>T (Codon 22), HBB: c. 51delC (Codon 16), HBB: c. 47 G>A (Codon 15), HBB: c. 25_26delAA (Codon 8), HBB: c. 17_18delCT (Codon 5), HBB: c. 27_28insG [frameshift codons (FSC) 8/9 (+G)], HBB: c. 79 G>A (Hb E), HBB: c.-78A>G (-28), HBB: c.-29G>A (+22), HBB: c. 92+1 G>A (IVSI-1), HBB: c. 92+5 G > C (IVSI-5), HBB: c. 92+6 T>C (IVSI-6), HBB: c. 93-21 G>A (IVSI-110), c. 93-1 G>C (IVSI-130), HBB: c. 315+1 G>A (IVSII-1), HBB: c. 316-106 C>G (IVSII-745), HBB: c. 93-21_96del (IVSI-25bp del), HBB: c.-50A>C (Cap + 1), Hb lepore, as well as Asian and Indian δβ thalassemia. Moreover, hematologic indices including RBC, Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), HbA2, HbF, and hemoglobin H were examined to determine patients'

Figure 1



Electropherogram showing Sanger sequencing results of two patients with beta-thalassemia with rare mutations, including (a) HBB: c. 47 G>A (Codon 15) and (b) HBB: c. 135delC (Codon 44). Each sequencing result was compared with sequencing results of patients without the mentioned mutation.

Figure 2



Agarose gel visualization of fragments generated by the PCR-RFLP technique for screening of HBB: c.316-106C>G (IVSII-745) mutation. Normal alleles were not recognized by EcoRI and remained uncut while mutant alleles generated two fragments. U, undigested amplicon; N, no mutation; HET, heterozygous; HOM, homozygous.

phenotype. Data were extracted from patients' records to determine their genotype–phenotype correlation.

Statistical analysis

The results of hematologic indices and molecular examinations were analyzed, version 26, and *P* value less than 0.05 was considered significant.

Results

Of 1415 persons, 710 patients had one deletion in alpha-globin gene, 98 patients presented two alpha-globin gene deletion on one chromosome, 374 patients had one alpha-globin gene point mutation, 148 patients had two alpha-globin genes mutation, seven patients had three alpha globin genes mutation (Fig. 3 and Table 1), 40 patients had beta-globin mutation Table 2), and 38 patients had alpha-globin mutation along with beta-globin mutation, also known as αβ thalassemia (Table 3). The most prevalent deletion and point mutation in alpha-globin gene were 3.7 Kb deletion and PA2 point mutation, respectively. Furthermore, of 40 patients with beta-thalassemia, 26 patients were diagnosed with IVSII-1, which is the most common beta globin mutation in the study population. Furthermore, the most prevalent genotype among patients with alpha-beta thalassemia was -$\alpha^{3.7}$/IVSII-1.

The hematological indices of patients with one or two mutations were compared with each other. The minimum and maximum of hematological indices among the groups are presented in Table 4. Furthermore, individuals with one deletion in alpha-globin gene demonstrated higher levels of Hb, MCV, MCH, and MCHC in comparison with individuals who had two deletions, irrespective of their position on the same or different chromosomes ($P < 0.001$) (Tables 5 and 6). Comparison of hematological indices in patients with beta- and alpha-thalassemia has indicated that Hb ($P < 0.001$), MCV ($P < 0.001$), and MCH ($P < 0.001$) were remarkably lower in beta-thalassemia group, whereas their RBC ($P < 0.001$) and HbA2 ($P < 0.001$) were significantly higher in comparison with the alpha-thalassemia group (Table 7). Furthermore, the Hb, MCV, MCH, and HbA1 levels were higher in patients with two mutated alpha-globin

Table 1 An overview of the alpha-thalassemia mutation frequencies

Number and type of mutated globin gene	Genotype	Number of patients	Frequency (%)
One alpha mutation	-α3.7	631	45
	-α4.2	79	5.7
	--MED	92	6.6
	--20.5	6	0.4
	PA2	230	16.7
	PA1	19	1.3
	CS	72	5.2
	5NT	47	3.4
	ins 21	5	0.3
	CD19	1	0.07
Two alpha mutations	-α3.7/-α4.2	15	1
	-α3.7/-α3.7	79	5.7
	-α4.2/-α4.2	3	0.2
	αpα/αpα	18	1.3
	α-/αpα	33	2.4
Three alpha Mutations	--MED/-α3.7	5	0.3
	--MED/-α4.2	1	0.07
	--20.5/-α3.7	1	0.07
Total alpha-thalassemia		1337	

The statistics data are calculated among patients with alpha- and beta-thalassemia only.

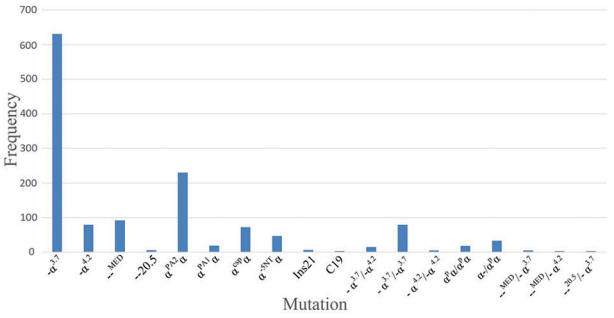
Table 2 Frequency of β-thalassemia genotypes

Number and type of mutated globin gene	Genotype	Number of patients	Frequency (%)
Beta mutation	IVSII-1 (heterozygous)	27	67.5
	IVSI-5 (heterozygous)	7	17.5
	FR8/9 (heterozygous)	1	2.5
	CD44 (heterozygous)	1	2.5
	CD30 (heterozygous)	1	2.5
	CD15 (heterozygous)	1	2.5
	CD8 (heterozygous)	1	2.5
	–28 (heterozygous)	1	2.5
Total beta-thalassemia		40	

The statistical data are calculated among patients with alpha- and beta-thalassemia, only.

genes (deletion or point mutation) in comparison with patients with alpha-beta thalassemia regardless of their sex ($P < 0.001$) (Tables 6 and 7). The variations of MCV and MCH values for the studied partients are also shown in Fig. 4a–c. Comparison of individuals who had αβ-thalassemia with individuals who had one point mutation in the alpha-globin gene revealed that Hb ($P < 0.01$), MCV, MCH, and HbA1 ($P < 0.001$) in patients with alpha-thalassemia were higher. Similar results were observed in patients with alpha-beta thalassemia in comparison with those presenting one alpha globin mutation (deletion or point mutation). On the contrary, the Hb ($P < 0.01$), MCV ($P < 0.001$), and MCHC ($P < 0.01$) in patients with alpha-beta thalassemia were significantly higher in comparison with beta-thalassemia carriers. Some individuals presenting borderline HbA2 levels (near 3.5%) along with

Figure 3



Bar plot of frequency of genotypes of patients with α-thalassemia.

microcytosis were revealed to have a beta-thalassemia mutation along with alpha-thalassemia.

Discussion

Thalassemia challenges the decision of genetic counselors and health care professionals about the condition of a couple in premarital screening owing to genetic heterogeneity. Hematological indices could pave the way for a rapid and accurate diagnosis through reflecting the patients' genotype. We have demonstrated that -α3.7, IVSII-1, and -$\alpha^{3.7}$/IVSII-1 were the most prevalent genotypes, accounting for 631, 27, and eight cases in patients with alpha-, beta-, and alpha-beta-thalassemia, respectively. Similar to our results, a recent study including 1706 patients with thalassemia has revealed that of 539 patients with alpha-thalassemia, 130 (%24.12) showed -α3.7/αα genotype, which was one of the three most common alpha-globin mutation in the studied population (Zhu *et al.*, 2020). It is important to mention that – 3.7α and IVSII-I in the North of Iran, and – 3.7α and IVSI-5 in the South of Iran are the most common alpha- and beta-globin mutations, respectively (Akhavan-Niaki *et al.*, 2011; Eftekhari *et al.*, 2017; Ebrahimi *et al.*, 2020). Our data showed that Hb levels were higher in patients with two alpha-globin gene mutations in comparison with those with alpha-beta thalassemia. This is consistent with a study conducted from 2000 to 2013 that involved 4010 Iranian patients (Dehbozorgian *et al.*, 2015). These findings have proposed that the severity of beta-globin mutations is more than alpha-globin mutations. Moreover, data revealed that alpha-beta thalassemia cases have Hb, MCV, and MCH levels lower than alpha-thalassemia cases. On the contrary, the comparison of beta-thalassemia group with alpha-beta thalassemia showed higher levels of MCV and MCH in the latter group. Intriguingly, these findings are in line with the results of an investigation including a comparison of hematological indices and genetic analysis of 17 581 couples with

Table 3 Frequency of different αβ-thalassemia genotypes

Number and type of mutated globin gene	Genotype	Number of patients	Frequency (%)
Alpha and beta mutation	$\alpha^{3.7}$/IVSII-1-	8	0.5
	α^{PA2}/IVSII-1	5	0.3
	MED/IVSII-1	5	0.3
	$\alpha^{3.7}$/+22-	2	0.1
	$^{3.7}$/CD8-α	2	0.1
	$\alpha^{3.7}$/IVSI-5-	2	0.1
	-$\alpha^{4.2}$/CD8	2	0.1
	20.5/IVSII-745	2	0.1
	-$\alpha^{3.7}$/CD30	1	0.07
	-$\alpha^{3.7}$/F8.9	1	0.07
	-$\alpha^{3.7}$/Hb S	1	0.07
	-$\alpha^{3.7}$/Cap	1	0.07
	-$\alpha^{3.7}$/IVSI-110	1	0.07
	-$\alpha^{3.7}$/IVSI-1	1	0.07
	-$\alpha^{3.7}$/IVSII-745	1	0.07
	α^{PA2}/CD22-	1	0.07
	MED/CD30	1	0.07
	MED/CD22	1	0.07
Total alpha-beta-thalassemia		38	

The statistics are calculated among patients with αβ-thalassemia, only.

alpha-, beta-, and alpha-beta-thalassemia (Nezhad *et al.*, 2018). This could be explained by the fact that beta-globin mutations are more severe relative to alpha-globin mutations, and alpha-globin mutation is able to moderate the effects of beta-globin mutation. Moreover, our results demonstrated that those patients who had borderline HbA2 levels (near 3.5%) along with microcytosis (MCV < 80 fl) had alpha- and beta-thalassemia simultaneously. In this context, a prospective study conducted by Nezhad *et al.* (2018) revealed similar findings. Given that the individuals with HbA2 more than or equal to 3.5% and MCV less than 80 fl are considered as a beta-thalassemia trait, the cutoff of 3.5 to detect patients with beta-thalassemia seems to not be exactly reliable. Given the possibility of coinheritance of both alpha- and beta-globin gene mutations in high-prevalence zones, couples with beta-thalassemia may be at risk of having HbH disease in their offspring (Fig. 5). Therefore, it raises the importance of screening alpha-globin gene mutations alongside beta-globin gene mutations in couples with beta-thalassemia in high-prevalence regions during premarital or prepregnancy screening. Moreover, it is noteworthy to mention that the prevalence of αβ-thalassemia might be higher than we reported in the present study, as we did not study systematically the alpha-globin gene mutation in all beta-thalassemia cases referred to our center (>10 000 cases during 2000–2020). The high variability of alpha- and beta-thalassemia in regions with high prevalence complicates genetic counseling in premarital or prepregnancy screening. However, genotype–

Table 4 Minimum and maximum of hematological indices of patients with α-thalassemia with one, two, and three defective alpha globin genes

Hematological indices / Mutation	-$\alpha^{3.7}$	-$\alpha^{4.2}$	α α^{PA2}	α α^{PA1}	α^{esp}	α α^{SNT}	Ins^{21}	-$\alpha^{3.7}$/-$\alpha^{3.7}$	-$\alpha^{4.2}$/-$\alpha^{4.2}$	-$\alpha^{3.7}$/-$\alpha^{4.2}$	- - MED	- -/20.5	α α^{P}/α α^{P}	α -/α α^{P}	Three alpha mutations
RBC	3.91-7.43	4.4-7.28	4.05-7.46	4.94-6.63	4.34-7.56	4.28-6.55	5.27-6.84	4.47-6.9	5.38-6.54	4.65-6.8	4.6-7.44	5.7-7.1	4.4-6.83	4.16-6.97	5.22-7.24
Hb	8.4-17.2	9.9-16.8	8.4-16.2	12.1-15.2	8.7-17.4	8.1-16.6	12.4-15.8	10.7-16.6	11.3-14.1	10.9-14.6	8.5-15.6	11.2-14.5	8.1-14.6	9.6-15.9	8.1-14.1
MCV	52-91.9	63-89.2	57-86.9	69.8-81.3	56.7-82.5	58.3-80.7	68.1-76.1	57-82.1	66.8-72.9	63.4-77.6	55.9-78.9	61-70.8	64.7-82.5	62.9-82.5	54.51-70.2
MCH	16.2-31.4	15.4-29	17.2-35.1	21.3-25.8	16.1-26.7	17-26.4	19.7-25.7	18.2-26.97	21-22.9	20.9-25.3	17.3-26.4	19.6-21	17.9-25	18.9-28.3	15.3-21.2
MCHC	24.9-36.5	25.1-34.2	24.3-35.2	30.3-32.9	29.3-35.3	27.9-34.1	31.6-34.6	25.7-34.9	30.1-32.3	30.2-33.2	26.5-39.7	28.5-33	27.7-32.2	29.3-33.7	26.5-32.37
HbA1	91.8-98.3	88.7-97.8	93.4-98.3	92.6-97.6	93.7-97.91	92.6-97.9	95.2-97.2	91.4-99	96.4-96.7	96.5-97.9	93.4-98.7		95.5-97	95.5-99	78.2-96.7
HbA2	0.8-6.6	1.6-4.8	0.8-6.4	1.5-6.4	1.60-5.5	1.8-6.5	2.2-3	1.4-5.8	2.5-4.2	1.7-4.7	1-5.4	2.4-2.8	2.1-3.70	0.5-4.2	0.5-2.5
HbF	0.1-4.1	0.1-1.8	0.2-4	0.6-1.4	0.1-1.8	0.2-1.9	0.5-1.8	0.2-1.8		0.3-0.9	0.4-4.9		0.2-1.5	0.3-1.8	0.6-1.4
HbH															2.3-20

HbH, hemoglobin H; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell.

Table 5 Variation in hematologic indices of patients with α-thalassemia with one defective alpha-globin gene

Mutation type	Genotype	Phenotype	RBC	Hb	MCV	MCH	MCHC	HbA1	HbA2	HbF
Deletion	$-\alpha^{3.7}$	silent carriers	5.47±0.59	13.29±1.47	76.35±5.01	24.41±2	31.97±1.3	96.69±0.82	2.7±0.73	0.78±0.49
Deletion	$-\alpha^{4.2}$	silent carriers	5.44±0.54	13.26±1.42	76.52±4.69	24.31±2.11	31.86±1.48	96.56±1.36	2.55±0.58	0.74±0.35
Point mutation	$\alpha\ \alpha^{PA2}$	silent carriers	5.44±0.6	13.24±1.46	76.43±4.28	24.38±1.82	31.83±1.32	96.74±0.92	2.61±0.62	0.81±0.61
Point mutation	$\alpha\ \alpha^{PA1}$	silent carriers	5.65±0.51	13.74±0.79	75.26±3.39	23.81±1.2	31.67±0.72	96.36±1.51	2.53±1.23	0.85±0.31
Point mutation	α^{CSP}	silent carriers	5.39±0.59	13.24±1.91	75.82±4.63	24.18±1.98	31.92±1.55	96.82±0.83	2.84±0.77	0.66±0.34
Point mutation	$\alpha\ \alpha^{5NT}$	silent carriers	5.52±0.55	13.24±1.63	75.12±5.16	23.85±2	31.85±1.2	96.53±1.21	2.95±0.99	0.76±0.39
Point mutation	Ins 21	silent carriers	5.8±0.67	13.62±1.33	73±1.03	23.58±2.35	32.82±1.36	96.17±0.93	2.64±0.32	1.12±0.67

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell.

Table 6 Variation in hematologic indices of patients with α-thalassemia with two and three defective alpha-globin genes

Mutation type	Genotype	Phenotype	RBC	Hb	MCV	MCH	MCHC	HbA1	HbA2	HbF	HbH
Deletion	$-\alpha^{3.7}/-\alpha^{3.7}$	Mild anemia	5.76±0.61	12.71±1.45	71.04±4.83	21.88±1.88	30.74±1.87	96.72±1.32	2.57±0.8	0.78±0.52	
Deletion	$-\alpha^{4.2}/-\alpha^{4.2}$	Mild anemia	6.02±0.59	13.16±1.61	69.86±3.05	21.83±0.97	31.26±1.1	96.55±0.21	3.16±0.9		
Deletion	$-\alpha^{3.7}/-\alpha^{4.2}$	Mild anemia	5.76±0.57	12.96±1.41	70.73±4.08	22.46±1.66	31.26±1.21	96.9±1.14			
Deletion	$--/^{MED}$	Mild anemia	5.87±0.63	12.17±1.44	68.15±5.26	20.73±1.88	30.74±2.13	96.64±1.4	2.58±0.73	0.9±0.78	
Deletion	$--/^{20.5}$	Mild anemia	6.22±0.55	12.65±1.28	68.41±3.72	20.23±0.54	29.68±1.69		2.55±0.16		
Point mutation	$\alpha^{p}\alpha/\alpha^{p}\alpha$	Mild anemia	5.24±0.73	11.73±1.66	73.81±5.78	22.43±2.25	30.37±1.25	96.65±0.56	2.71±0.52	0.74±0.43	
Deletion/Point	$\alpha-/\alpha^{p}\alpha$	Mild anemia	5.7±0.73	12.6±1.4	71.61±4.42	22.2±1.71	31.06±1.28	96.66±1.06	2.76±0.71	0.82±0.4	
Deletion/Point	*Three alpha mutation	Microcytic hypochromic anemia	5.99±0.81	10.98±2.07	60.51±4.97	17.93±2.26	29.69±2.15	90.72±7.34	1.65±0.78	1.02±0.31	8.5±7.84

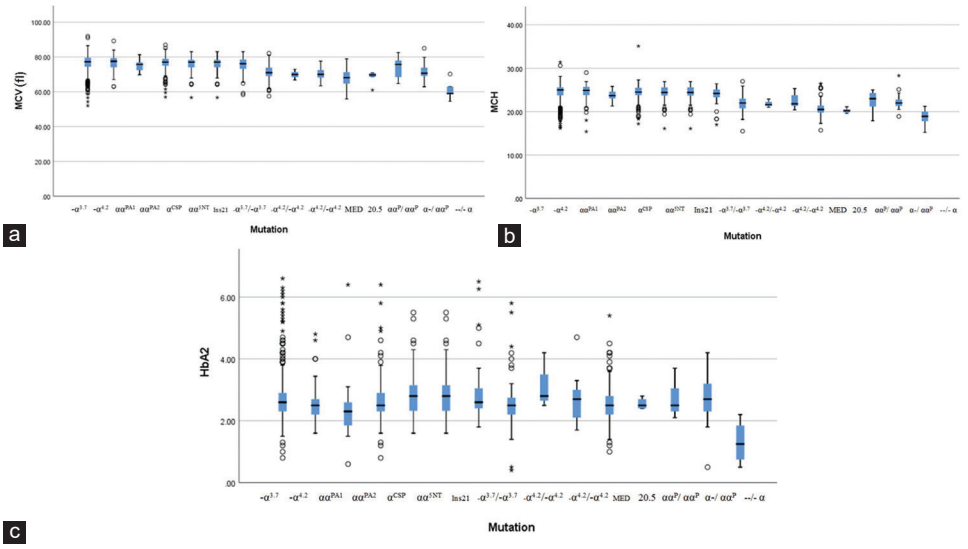
HbH, hemoglobin H; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell.

Table 7 Variation in hematologic indices of patients with β and αβ-thalassemia with defective alpha- and beta-globin gene

Mutation type	RBC	Hb	MCV	MCH	MCHC	HbA1	HbA2
Beta	6.09±0.68	12.09±1.12	64.20±5.09	20.07+1.92	31.29±1.18	93.46±2.45	4.95±1.08
Alpha-beta	5.43±0.87	12.18±1.78	71.09±4.96	22.14±2.07	31.42±1.16	91.53±12.76	1.84±1.34

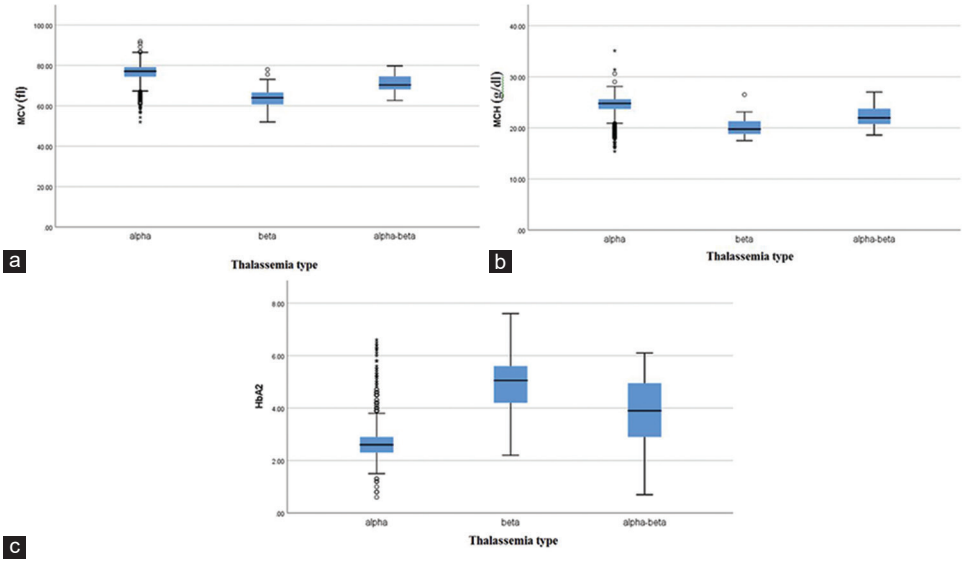
Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell.

Figure 4



Hematological features of patients with various α-thalassemia mutations. (a) MCV; (b) MCH; (c) HbA2. Data are presented as mean ± SD. MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

Figure 5



Hematological features of patients with α, β, and αβ-thalassemia mutations. (a) MCV; (b) MCH; (c) HbA2. Data are presented as mean ± SD. MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

phenotype correlations could pave the way of diagnosis of couples who are at high risk for having an affected child. The best correlation between thalassemia genotypes and phenotypes is characterized in line with hematological parameters, such as MCV and MCH (Akhavan-Niaki *et al.*, 2012). Correspondingly, our results have revealed that hematological indices and consequently the clinical manifestations are remarkably related to the type of the mutation. Collectively, it is worth emphasizing on the importance of both alpha- and beta-thalassemia screening in patients presenting with anemia for a more accurate genetic counseling in highly heterogeneous populations. It is noteworthy that molecular analysis in the context of

prenatal diagnosis plan could prevent from birth of affected neonates especially in populations with high prevalence of thalassemia mutations. A limitation of the present study is the absence of molecular studies for triplication/quadrupling of alpha-globin genes, although according to another investigation, the prevalence of triplication is relatively low (1.7%) in Iran (Abedini *et al.*, 2018).

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

Abedini SS, Forouzesh Pour F, Karimi K, Ghaderi Z, Farashi S, Tavakoli Koudehi A, *et al.* (2018). Frequency of α-globin gene triplications and coinheritance with β-globin gene mutations in the Iranian population. *Hemoglobin* **42**:252–256.

Akhavan-Niaki H, Derakhshandeh-Peykar P, Banihashemi A, Mostafazadeh A, Asghari B, Ahmadifard MR, *et al.* (2011). A comprehensive molecular characterization of beta thalassemia in a highly heterogeneous population. *Blood Cells Mol Dis* **47**:29–32.

Akhavan-Niaki H, Youssefi Kamangari R, Banihashemi A, Kholghi Oskooei V, Azizi M, Tamaddoni A, *et al.* (2012). Hematologic features of alpha thalassemia carriers. *Int J Mol Cell Med* **1**:162–167.

Cai SP, Wall J, Kan YW, Chehab FF (1994). Reverse dot blot probes for the screening of beta-thalassemia mutations in Asians and American blacks. *Hum Mutat* **3**:59–63.

Calderón-Brenes M, Porras-Moreno A, Granados-Alfaro P, Cartín-Sánchez W (2020). Hemoglobin H disease: Frst case of double heterozygous hemoglobin Constant Spring/Southeast Asian in Costa Rica. *Acta Méd Costarr* **62**:38-42.

Cao A, Galanello R (2010). Beta-thalassemia. *Genet Med* **12**:61–76.

Dehbozorgian J, Moghadam M, Daryanoush S, Haghpanah S, Imani Fard J, Aramesh A, *et al.* (2015). Distribution of alpha-thalassemia mutations in Iranian population. *Hematology* **20**:359–362.

De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, *et al.* (2017). β-thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. *Mediterr J Hematol Infect Dis* **9**:e2017018.

Ebrahimi M, Mohammadi-Asl J, Rahim F (2020). Molecular spectrum and distribution of hemoglobinopathies in southwest of Iran: a seven-year retrospective study. *J Hematop* **13**:97–103.

Eftekhari H, Tamaddoni A, Mahmoudi Nesheli H, Vakili M, Sedaghat S, Banihashemi A, *et al.* (2017). A comprehensive molecular investigation of α-thalassemia in an iranian cohort from different provinces of North Iran. *Hemoglobin* **41**:32–37.

Forget BG, Hardison RC (2009). The normal structure and regulation of human globin gene clusters. In: Steinberg M, Forget B, Higgs D, Weatherall D. *Disorders of hemoglobin: genetics, pathophysiology, and clinical management*. 2nd edition. Cambridge: Cambridge University Press; 46–61.

Gilad O, Shemer OS, Dgany O, Krasnov T, Nevo M, Noy-Lotan S, *et al.* (2017). Molecular diagnosis of α-thalassemia in a multiethnic population. *Eur J Haematol* **98**:553–562.

Karakaş Z, Koc B, Temurhan S, Elgun T, Karaman S, Asker G, *et al.* (2015). Evaluation of alpha-thalassemia mutations in cases with hypochromic microcytic anemia: the İstanbul perspective. *Turk J Haematol* **32**:344–350.

Khodaei GH, Farbod N, Zarif B, Nateghi S, Saeidi M (2013). Frequency of thalassemia in Iran and Khorasan Razavi. *Int J Pediatr* **1**:45–50.

Martin A, Thompson AA (2013). Thalassemias. *Pediatr Clin North Am* **60**:1383–1391.

Muncie HLJr, Campbell J (2009). Alpha and beta thalassemia. *Am Fam Physician* **80**:339–344.

Nezhad FH, Nezhad KH, Choghakabodi PM, Keikhaei B (2018). Prevalence and genetic analysis of α- and β-thalassemia and sickle cell anemia in Southwest Iran. *J Epidemiol Glob Health* **8**:189–195.

Sajadpour Z, Amini-Farsani Z, Motovali-Bashi M, Yadollahi M, Yadollahi F (2019). Investigation of RFLP haplotypes β-globin gene cluster in beta-thalassemia patients in central Iran. *Int J Hematol Oncol Stem Cell Res* **13**:61–67.

Sirachainan N, Chuansumrit A, Kadegasem P, Sasanakul W, Wongwerawattanakoon P, Mahaklan L (2016). Normal hemostatic parameters in children and young adults with α-thalassemia diseases. *Thromb Res* **146**:35–42.

Vichinsky E (2010). Complexity of alpha thalassemia: growing health problem with new approaches to screening, diagnosis, and therapy. *Ann N Y Acad Sci* **1202**:180–187.

Zhou YQ, Xiao GF, Li LY, Li WD, Liu ZY, Zhu LF, *et al.* (2002). Evaluation of clinical application of gap-PCR as a routine method for alpha-thalassemia carrier detection. *Di Yi Jun Yi Da Xue Xue Bao* **22**:434–436.

Zhu Y, Shen, N, Wang X, Xiao J, Lu Y (2020). Alpha and beta-thalassemia mutations in Hubei area of China. *BMC Med Genet* **21**:6.