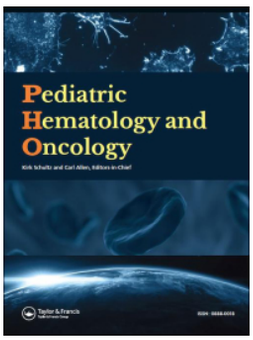

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Clinical and genetic characteristics of hemoglobin H disease in Iran

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ABSTRACT

Hemoglobin H (Hb H) disease is a subtype of α-thalassemia caused by deletional and/or non-deletional mutations in three alpha-globin genes in which the various genotypes determine the disease severity. This study was aimed to investigate the frequency of alpha gene mutations and genotypes and their correlation with hematological and clinical characteristics in Iran. Among 202 patients diagnosed with Hb H disease through a national study in Iran according to standard methods, we had access to the hematologic and clinical findings and genetic data of 101 patients in whom genetic study was performed. Genomic DNA from peripheral blood was extracted and analyzed for identification of α-globin gene mutations using Multiplex Gap Polymerase Chain Reaction, Reverse Hybridization Assay, and finally Direct DNA Sequencing method. Twenty-one different mutations and thirty genotypes were detected in 101 patients with Hb H disease. In total, 39 patients (38.6%) were deletional and 62 patients (61.4%) were non-deletional type of the disease. The $-^{MED}$ mutation was highly prevalent in almost half of the patients (56.4%). Among various genotypes, $-^{MED}/-a^{3.7}$ (29.7%) and $-\alpha^{20.5}/-\alpha^{5NT}$ (6.9%) were the most prevalent genotypes found in the studied group. Patients with non-deletional type presented with more severe hematological and clinical findings. Hb H percentage and serum ferritin levels were significantly higher in non-deletional patients in comparison to the deletional group ($p < 0.05$). 12 (11.9%) and 40 (39.6%) out of 101 patients were on regular and occasional transfusions, respectively. 83% of those with regular transfusion belonged to the non-deletional group. Among transfusion-dependent patients, $-^{MED}/\alpha^{CS}\alpha$ and $\alpha^{20.5}/-\alpha^{5NT}$ were the most common genotypes. In this study, two patients with $-\alpha^{20.5}/\alpha^{CS}\alpha$ and $-^{MED}/\alpha^{-5NT}$ genotypes experienced thrombotic events. This study indicated that although non-deletional genotypes of Hb H disease were responsible for more clinical severity of the disease, due to the presence of severe phenotypes even in deletional types, no definite correlation was found between genotype and phenotype.

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Introduction

The hemoglobin (Hb) molecule is a tetrameric protein including two alpha and two beta subunits that are synthesized independently by 2 multigene clusters located on chromosome 16 and 11 respectively.[1] Thalassemic mutations in globin genes lead to the reduced or absent synthesis of hemoglobin chains.[2] Alpha-thalassemia (α) as one of the most prevalent monogenic diseases in the world especially in Southeast Asia and the Middle East,[3,4] is characterized by changes in the level of α-globin gene expression due to deletions or point mutations in one or more alpha globin genes.[5–7] On the other hand, according to the frequency of missed or nonfunctional α-globin genes, α thalassemia can be classified to silent alpha thalassemia (single α-gene deletion (-α/αα)), alpha thalassemia trait (two alpha gene deletions in cis (–/αα) or in trans (- α/- α)), hemoglobin H disease (Hb H) (mutations in three α-globin genes (–/-α or –/α^{T}α) and Hb Bart's hydrops fetalis (deletion of four α-genes (- -/- -)).[8,9]

Hb H disease also identified as alpha thalassemia intermedia which results from the imbalanced α-globin chains due to the molecular defects is a moderate clinical form of α-thalassemia. This disorder is mainly characterized by microcytic hypochromic hemolytic anemia, mild jaundice, splenomegaly, leg ulcers, gallstones, and, sometimes, thalassemic facies.[10] It can be classified into two main forms: deletional and non-deletional Hb H disease.[8,11–13] In deletional form; the most common type of the disease, two deleted α-globin genes are located on one chromosome 16 and the third deletion on another chromosome 16 (-α $^{3.7}$ or -α $^{4.2}$), while non-deletional Hb H disease occurs due to a combination of two α-globin genes deletion on the same chromosome and a point mutation or small insertion/deletion in alpha-globin gene (α2 or α1) on the other chromosome.[11,14] Furthermore, a less common form of Hb H disease in a small group of patients resulted from point mutations in α2-globin gene on both chromosomes. This type is also considered as non-deletional Hb H disease.[7] Studies have shown that symptoms and severity of Hb H disease are variables between deletional and non-deletional types, so that clinical manifestations such as anemia, hepatosplenomegaly and transfusion dependency in patients with non-deletional mutations are more severe than those with deletional types.[15–21] In a study from the southwest region of Iran, Khuzestan province, a highly diverse range of Hb H patients in terms of both genotype and phenotype has been reported.[22] Due to high incidence of alpha thalassemia genes in Iran and clinical significance of Hb H disease in public health, we aimed to carry out a national study collecting information on genotype and phenotype of patients with Hb H disease from the whole country in order to reach a comprehensive data of Hb H disease in Iran.

Materials and methods

Patient selection

Between January 2016 to February 2020, medical records of 202 cases of Hb H disease from various geographic regions of Iran who were referred to the thalassemia centers, with the age range of 4–70 years (55.4% female and 44.6% male) were reviewed. Patients with thalassemia major and Hb H disease who start blood transfusion since early

childhood are managed by pediatric hematologists in Iran and as a result pediatric hematologists have access to their data and records.

Based on the inclusion criteria considering complete genetic and diagnostic tests, only patients with a definite diagnosis of Hb H disease were analyzed in this study. In this regard, 101 patients met eligible criteria for the study. The current research was approved by the Ethical Committee of Shahid Beheshti Medical University. Each center had its own informed consent provided to the patients or their guardians.

The age, sex, age at diagnosis, spleen size, history of splenectomy and blood transfusion, hematologic, electrophoretic, and genetic data were extracted for each patient. All techniques including laboratory and molecular procedures, analytical methods such as analysis of red blood cell indices and hemoglobin analysis were performed according to the standard protocols.

Patients were divided into two main groups [transfusion dependent (TD) or non-transfusion dependent (NTD)] in terms of receiving blood transfusion. TD groups were subdivided into those who received regular or occasional transfusions. Furthermore, for comparison of the clinical outcomes, Hb H genotypes were classified into deletional and non-deletional types. It has to be noted that the non-deletional/non-deletional and deletional/non-deletional genotypes are considered as non-deletional type.[7,23]

Genetic diagnosis of Hb H disease by DNA analysis

Genomic DNA was extracted from peripheral blood samples using DNA isolation kit (QIAamp DNA Blood Mini Kit, Qiagen) according to the manufacturer's instructions. Multiplex Gap-polymerase chain reaction (Gap-PCR), assay was used for the detection of $-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $-^{MED}$ and $-^{SEA}$, as a primary screening test.[24] This was followed by reverse hybridization test strips (α-Globin Strip Assay; Vienna Lab Diagnostics, Vienna, Austria) to detect two point non-mutations on the α1 gene [codon 14 (TGG > TAG) (HBA1: c.44G > A); codon 59 or Hb Adana (GGC > GAC) (HBA1: c.179G > A)], and 11 point mutations on the α2 gene [initiation codon (ATG > ACG) (HBA2: c.2T > C); αcodon 19 α (GCG > GC–, α2) (HBA2: c.56delG), IVS-I, −5 nt (–TGAGG); codon 59 (HBA2: c.179G > A); codon 125 (CTG > CCG) (Hb Quong Sze, Hb QS, HBA2: c.377T > C; Hb Constant Spring (Hb CS, α142, Term → Gln, TAA > CAA, HBA2: c.427T > C, αCSα; Hb Icaria (α142, Term→Lys, TAA > AAA, HBA2: c.427T > A); Hb Paksé (a142, Term→Tyr, TAA > TAT, HBA2: c.429A > T); Hb Koya Dora (α142, Term→Ser, TAA > TCA, HBA2: c.428A > C; polyadenylation (polyA1) site αpolyA1α (AATAAA > AATAAG); polyA2 (αpolyA2α, AATAAA > AATGAA)].[25] Cases where no mutation could be identified, were further analyzed by direct genomic sequencing of the PCR-amplified α2- and α1-globin genes (ABI-3130; Applied Biosystems, Foster City, CA, USA).[26]

Statistical analysis

All statistical analyses were conducted using SPSS Statistical Software (version 23.0). Means and SD (standard deviation) were calculated in continuous data and the frequency proportion for categorical data. The distribution of the quantitative variables

Table 1. Frequency of mutated a-globin alleles in 101 Iranian patients with Hb H disease.

Mutation	Type of mutation	Number	*N* (%)
$--^{MED}$	Deletion	57	32.5
$-\alpha^{3.7}$	Deletion	41	23.4
$-\alpha^{20.5}$	Deletion	20	11.4
$-\alpha^{4.2}$	Deletion	1	0.6
$-\alpha^{5NT}$	Point mutation	12	6.8
$\alpha^{poly\text{-}A1}$ α	Point mutation	12	6.8
α^{CS} α	Point mutation	10	5.7
$\alpha^{poly\text{-}A6}$ α	Point mutation	6	3.4
α^{polyA4} α	Point mutation	3	1.7
α^{cd59} α	Point mutation	2	1.1
α^{cd19} α	Point mutation	1	0.6
$\alpha^{IVSII+4}$ α	Point mutation	1	0.6
α^{cd99} α	Point mutation	1	0.6
α^{cd142} α	Point mutation	1	0.6
α^{21nt}	Point mutation	1	0.6
α^{cd108} α	Point mutation	1	0.6
α^{cd90} α	Point mutation	1	0.6
α^{CD130} α	Point mutation	1	0.6
$\alpha^{IVSII\text{-}I}$ α	Point mutation	1	0.6
$\alpha^{cd36/37}$ α	Point mutation	1	0.6
$\alpha^{Hb\ Setif}$ α	Point mutation	1	0.6

was analyzed using the Kolmogorov–Smirnov test. Depending on whether the data were normally distributed or not, parametric, or nonparametric tests were employed. The Mann–Whitney and independent *t*-test were used to determine whether there is a relationship between Hb H genotypes (deletional and non-deletional) and hematological parameters. Furthermore, categorical variables were analyzed by Chi-square or Fisher's exact test. The *p*-value was considered statistically significant at a level of <0.05.

Results

In this retrospective study, demographic data, hematologic parameters, clinical characteristics and genetic data of 101 cases who were diagnosed with Hb H disease with the age range of 4–70 years were documented.

The characteristics of the 101 Iranian patients with Hb H disease (42 males and 59 females), with the mean age of 30.77 ± 15.95 whom their genetic analysis was performed are provided. Molecular findings including 21 allelic mutations and 30 genotypes are presented in Tables 1 and 2.

Based on the results of the mutation analysis, $--^{MED}$ double gene deletion was the most common mutation with a frequency of 32.5%, followed by $-\alpha^{3.7}$(23.4%), $-\alpha^{20.5}$ (11.4%), $-\alpha^{5NT}$ (6.8%), $\alpha^{poly\text{-}A1}\alpha$ (6.8%), $\alpha^{CS}\alpha$ (5.7%), $\alpha^{poly\text{-}A6}\alpha$ (3.4%) among all reported α-globin mutant alleles in this study. The $--^{MED}/-a^{3.7}$ (29.7%) was found as the most frequently encountered deletional genotype, followed by $-\alpha^{20.5}/-\alpha^{3.7}$ (5.9%), while $-\alpha^{20.5}/-\alpha^{5NT}$ and $\alpha^{poly\text{-}A6}\alpha/\alpha^{poly\text{-}A6}\alpha$ were the most common non-deletional genotypes (6.9 and 5.9%, respectively). Deletional genotypes were observed in 38.6% of the patients, while 61.4% of them had non-deletional genotypes.

Comparison of hematological parameters between Hb H patients showed that patients with deletional genotypes had lower Hb H than non-deletional genotypes (Table 3).

Table 2. Alpha globin genotypes in 101 Iranian patients with Hb H disease.

Genotype	Type of mutation	Frequency	*n* (%)
$--^{MED}/-\alpha^{3.7}$	Deletional	30	29.7
$-\alpha^{20.5}/-\alpha^{3.7}$	Deletional	6	5.9
$--^{MED}/-\ \alpha^{4.2}$	Deletional	1	1
$-\ \alpha^{3.7}\ /-\ \alpha^{3.7}$	Deletional	1	1
- - /- $\alpha^{3.7}$	Deletional	1	1
$-\alpha^{20.5}/-\alpha^{5NT}$	Non- Deletional	7	6.9
$\alpha^{poly\text{-}A6}\alpha/\alpha^{poly\text{-}A6}\alpha$	Non- Deletional	6	5.9
$--^{MED}/\alpha^{CS}\alpha$	Non- Deletional	5	4.9
$\alpha^{poly\text{-}A1}\alpha/\alpha^{poly\text{-}A1}\alpha$	Non- Deletional	5	4.9
$--^{MED}\ /\alpha^{polyA2}\alpha$	Non- Deletional	5	4.9
$--^{MED}\ /\ \alpha^{cd19}\ \alpha$	Non- Deletional	4	3.9
$--^{MED}/\alpha^{-5NT}$	Non- Deletional	4	3.9
$-\alpha^{20.5}/\ \alpha^{CS}\alpha$	Non- Deletional	3	2.9
$--^{MED}\ /\ \alpha^{polyA4}\alpha$	Non- Deletional	3	2.9
$-\alpha^{20.5}/\ \alpha^{poly\text{-}A1}\alpha$	Non- Deletional	3	2.9
$\alpha^{CS}\alpha\ /\ \alpha^{CS}\alpha$	Non- Deletional	2	1.9
$\alpha^{poly\text{-}A1}\alpha\ /\ \alpha^{cd59}\ \alpha$	Non- Deletional	2	1.9
$--^{MED}/\ \alpha^{IVSII+4}\ \alpha$	Non- Deletional	1	1
$-\alpha^{20.5}/\ \alpha^{cd99}\ \alpha$	Non- Deletional	1	1
$--^{MED}\ /\ \alpha^{cd142}\ \alpha$	Non- Deletional	1	1
$--^{MED}\ /\alpha^{21nt}$	Non- Deletional	1	1
$--^{MED}\ /\ \alpha^{cd108}\ \alpha$	Non- Deletional	1	1
$--^{MED}\ /\ \alpha^{cd90}\ \alpha$	Non- Deletional	1	1
$-\alpha^{3.7}/\ \alpha^{poly\text{-}A1}\alpha$	Non- Deletional	1	1
$-\alpha^{3.7}/\ \alpha^{-5NT}$	Non- Deletional	1	1
$\alpha^{CD130}\ \alpha\ /\alpha^{CD130}\ \alpha$	Non- Deletional	1	1
$\alpha^{IVSII\text{-}I}\ \alpha\ /\ \alpha^{IVSII\text{-}I}\ \alpha$	Non- Deletional	1	1
$\alpha^{cd19}\ \alpha\ /\ \alpha^{cd19}\ \alpha$	Non- Deletional	1	1
$\alpha^{cd36/37}\ \alpha\ /\ \alpha^{cd36/37}\ \alpha$	Non- Deletional	1	1
$\alpha^{Hb\ Setif}\ \alpha\ /\ \alpha^{Hb\ Setif}\ \alpha$	Non- Deletional	1	1

The RBC count was considerably lower in patients with non-deletional type of the disease compared to the deletional type ($p = 0.03$). Also, patients with non-deletional type had significantly higher ferritin compared to the deletional group ($p = 0.02$).

The clinical features of patients with deletional and non-deletional types are shown in Table 4. There was no significant difference in gender, age at diagnosis, age at first transfusion, splenomegaly/splenectomy between the two mentioned groups ($p > 0.05$). As shown in Table 4, 38.4% and 59.6% of patients in deletional and non-deletional groups received blood transfusions (regularly or occasionally), respectively. Among Hb H disease patients, 48.5% had no history of blood transfusion, 39.6% had occasional transfusions and the rest were on regular transfusions. The $-^{MED}/\alpha^{CS}\alpha$ and $-\alpha^{20.5}/-\alpha^{5NT}$ were the most prevalent genotypes in TD patients. The $-^{MED}/-a^{3.7}$ was the dominant genotype in NTD patients (38.7%). Significantly, TD patients had lower hemoglobin and higher ferritin levels compared to NTD patients ($p = 0.009$ and $p = 0.001$, respectively). Three patients with non-deletional genotype underwent laparoscopic cholecystectomy who two of them were TD. The obtained results revealed that 21.1% of transfused patients underwent splenectomy in contrast to 4% in NTD patients. Furthermore, mean ferritin levels were significantly higher in splenectomized patients compared to the non-splenectomized group ($p = 0.004$). The following genotypes were detected in splenectomized patients: $-^{MED}/-a^{3.7}$, $-^{MED}/\alpha^{cd19}\ \alpha$, $-^{MED}/\alpha^{CS}\alpha$, $-\alpha^{20.5}/\alpha^{CS}\alpha$, $-\alpha^{20.5}/-\alpha^{3.7}$, $-^{MED}/\alpha^{-5NT}$, $-^{MED}/\alpha^{polyA2}\alpha$, $-^{MED}/\alpha^{21nt}$, $\alpha^{CD130}\ \alpha/\alpha^{CD130}\ \alpha$, $\alpha^{IVSII\text{-}I}\ \alpha/\alpha^{IVSII\text{-}I}\ \alpha$ and $-^{MED}/\alpha^{IVSII+4}\ \alpha$.

Table 3. Laboratory and hematological parameters in 101 Hb H disease patients.

Parameter	Deletional Hb H ($n=39$)	Non-deletional Hb H ($n=62$)	p value
RBC	5.06 ± 0.67	4.53 ± 0.89	0.03
Hb (g/dl)	9.04 ± 1.89	8.99 ± 1.15	0.89
MCV (27)	60.37 ± 5.75	66.12 ± 8.16	0.00
MCH (pg)	18.03 ± 2.47	19.30 ± 3.31	0.03
Ferritin	347.02 ± 373.94	603.40 ± 715.52	0.02
Bilirubin (mg/dL)	2.44 ± 2.01	2.54 ± 1.61	0.86
HbA (%)	91.34 ± 7.85	87.32 ± 8.19	0.03
HbA2 (%)	1.62 ± 0.57	1.40 ± 0.68	0.13
HbF (%)	0.80 ± 0.58	0.98 ± 0.76	0.33
Hb H (%)	7.17 ± 6.96	11.16 ± 7.02	0.03

Note. Data are shown as mean ± SD.

Table 4. The clinical presentation of 101 patients with deletional and non-deletional Hb H disease.

Clinical presentations	Deletional Hb H ($n=39$)	Non-deletional Hb H ($n=62$)	p-value
Sex			
Male	14 (36%)	28 (45%)	
Female	25 (64%)	34 (55%)	0.35
Age at diagnosis	14.85 ± 12.62	12.84 ± 13.77	0.46
Spleen size			
Not palpable	17(43.5%)	24(38.7%)	
Just palpable	4 (10%)	2 (3%)	0.11
2 cm below LCM	9 (23%)	9 (14.5%)	
≥3 cm below LCM	9 (23%)	27 (43.5%)	
Splenectomy	2 (5.1%)	11 (17.7%)	0.06
Age of splenectomy	37 ± 15.55	22.27 ± 11.25	0.13
Age of first blood transfusion	21.22 ± 15.34	16.27 ± 18.37	0.46
Transfusion			
Non-transfused	24 (61.5%)	25 (39.6%)	
Regular transfusion	2 (5.1%)	10 (16.1%)	0.07
Occasional transfusion	13 (33.3)	27 (43.5%)	
Jaundice	8 (20%)	11(18%)	0.72
Growth failure	0	2 (3.2%)	0.25
Cardiovascular disease	0	4 (6.4%)	0.10
Facial changes	2 (5.1%)	8 (12.9%)	0.20
Thrombotic event	0	2 (3.2%)	0.24
Diabetes Mellitus	2 (5.4%)	1)1.6%)	0.55
Leg ulcer	0	0	–

Note. The data are presented as mean ± SD or number (percent).

Our results showed that the non-deletional genotypes were more common in splenectomized patients. All TD patients except one, had ferritin concentrations of more than 500 ng/ml. In addition, 10 out of 12 TD patients received iron chelation therapy that all except one belonged to the non-deletional group. A limited number of the patients with Hb H disease developed clinical manifestations such as growth failure, cardiovascular disease, facial changes, hypothyroidism, and diabetes mellitus (Table 4). The $-^{MED}/-\alpha^{3.7}$, $\alpha^{poly\text{-}A1}\alpha/\alpha^{poly\text{-}A1}\alpha$, $\alpha^{poly\text{-}A6}\alpha/\alpha^{poly\text{-}A6}\alpha$, $-^{MED}/\alpha^{CS}\alpha$, $\alpha\ ^{CD130}\ \alpha/\alpha\ ^{CD130}\ \alpha$, $-\alpha^{3.7}/-\alpha^{-5NT}$and $-\alpha^{20.5}/\alpha^{CS}\alpha$ genotypes were reported in patients with dysmorphic facial features. Two patients with $-^{MED}/\alpha^{-5NT}$ and $-\alpha^{20.5}/\alpha^{CS}\alpha$ genotypes experienced thrombotic events. Two siblings who were TD with $-\alpha^{20.5}/-\alpha^{5NT}$ genotype developed renal failure for which no conclusion could be drawn. None of the patients in our study showed any evidence of leg ulcers.

Discussion

Hb H disease, a subtype of alpha thalassemia, is one of those diseases that may not be considered as a benign disorder.[15,19] The Middle East and the Mediterranean area are among the areas where Hb H disease seems relatively common.[1,27,28] The clinical severity of the disease is determined based on the type of a-globin gene mutations and is a reflection of different combinations of these mutations.[10,21,29]

It is of great importance to recognize the frequency and distribution of different mutations as well as genotype-phenotype correlations in various populations for better diagnostic approach and clinical management. For this purpose, a comprehensive study on clinical findings and genetic analysis of patients with Hb H disease was carried out in Iranian patients. Our findings revealed that the $--^{MED}$ mutation was the most common deletional mutation causing Hb H disease. This result is in agreement with a previous study from Southwest of Iran.[22] Numerous studies have reported –SEA, –THAI, and –FIL as common deletional mutations, while none of them were found in our study,[28,31–33] likely because of the differing ethnicity and origin of the Iranian population. Based on the obtained data from the current study, $-\alpha^{5NT}$ and $\alpha^{poly\text{-}A1}\alpha$ were the most mutant alleles among non-deletional mutations. Similar results for $-\alpha^{5NT}$ were reported by Valaei et al.[30] In contrast, some studies have reported $\alpha\ ^{CS}\ \alpha$ as the most frequent mutation.[18,31]

In the current study, we found that non-deletional genotypes were more common than deletional types (61.4% vs. 38.6%). It is supposed that patients with the deletional type of the disease have less degrees of anemia and might be referred to a hematologist or specialist thalassemia center less than patients with non-deletional types. However, this is in contrast to reports from other studies which show deletional types of the disease have been reported more frequently.[21,22,32,33]

We found that in deletional Hb H patients, $--^{MED}/-\alpha^{3.7}$ was the most prevalent genotype. Our results showed that this genotype was found in 11 TD patients (who were on either regular or occasional transfusion) similar to another study by Ebrahimkhani et al.[34] Valaei et al reported that patients with this genotype did not require blood transfusions.[30] As we mentioned, those 11 TD patients with –MED/-α3.7 genotype did not have exactly similar clinical features. Some were transfusion-dependent regularly and some of them received blood on occasions. A possible explanation for these differing findings is that there are many other modifier factors that could contribute to the severity of the disease, similar to what is seen in beta-thalassemia major patients.

Patients with non-deletional type showed higher levels of Hb H, ferritin, and MCV in the current study. Similarly, in one study on 90 Taiwanese patients with Hb H disease; Hb, MCV, Hb H, and ferritin levels were significantly higher in the non-deletional group.[20] Also, patients with non-deletional type were more likely to require blood transfusion. As we showed in the results, 38.4% of our patients in the deletional group and 59.6% in the non-deletional group received blood transfusions (regularly or occasionally). Clinical severity in non-deletional Hb H is caused by the production of unstable α-globin chain due to the specific type of the mutations that contribute to damage in membrane of RBCs, leading to hemolytic anemia, splenomegaly and increased blood transfusion demand.[35] In comparison to our study, several other studies have

also reported that patients with non-deletional Hb H disease may need regular blood transfusions or even splenectomy.[36–38]

Data analysis showed that all patients with Hb levels less than 7 g/dL fitted to the non-deletional group with the following genotypes: $\alpha^{poly\text{-}A1}\alpha/\alpha^{poly\text{-}A1}\alpha$, $-^{MED}/\alpha^{21nt}\ \alpha$, $\alpha^{poly\text{-}A1}\alpha/\alpha\ ^{cd59}\ \alpha$, $-^{MED}/\alpha^{CS}\alpha$, $-^{MED}/\alpha\ ^{cd90}\ \alpha$ and $-^{MED}/\alpha\ ^{cd19}\ \alpha$. Interestingly, the lowest level of Hb belonged to patients with $-\alpha^{3.7}/\alpha^{CS}\alpha$ genotype according to a study from Iran.[39]

Investigations by Yu-Hua Chao et al, showed that serum ferritin levels in all patients with the deletional type of Hb H disease were less than 800 ng/mL.[20] In our study, the same results were obtained for all deletional types except four patients who had received blood transfusions: one regularly and three in occasions. According to our findings, obviously, transfused patients had higher ferritin levels; however, two studies found no association between increased serum ferritin levels and history of blood transfusions.[16,40] Fucharoen and colleagues reported that 25% of Hb H patients with Constant Spring were more likely to have severe phenotypes of the disease.[41] The $-^{MED}/\alpha^{CS}\alpha$ and $-^{MED}/\alpha^{polyA2}\alpha$ were reported as the most common genotypes in TD patients by Valaei et al.[30] Genetic analyses of our patients indicated that $-^{MED}$, $\alpha^{CS}\alpha$, $-\alpha^{20.5}$ and $-\alpha^{5NT}$ were the most prevalent mutations in TD patients. The combination of $-^{MED}$ with CS mutation has also been reported as one of the most common genotypes in TD patients in another study from Iran.[34] Similarly, our study showed that $-^{MED}/\alpha^{CS}\alpha$ and also $-\alpha^{20.5}/-\alpha^{5NT}$ were the most common genotypes in our TD patients.

In our study, two patients developed clinical evidence of thrombosis. They showed $-\alpha^{20.5}/\alpha^{CS}\alpha$ and $-^{MED}/\alpha\text{-}^{5NT}$ genotypes, and splenectomy was performed in the former case. It should be mentioned that splenectomy may increase the risk of thrombotic events in some types of thalassemia.[42–44] Based on our findings, patients with $\alpha^{poly\text{-}A6}\alpha/\alpha^{poly\text{-}A6}\alpha$, $\alpha\ ^{Hb\ Setif}\ \alpha/\alpha\ ^{Hb\ Setif}\ \alpha$, $-^{MED}/\alpha\ ^{cd142}\ \alpha$, $-^{MED}/\alpha\ ^{cd108}\ \alpha$, $\alpha\ ^{cd19}\ \alpha/\alpha\ ^{cd19}$ and $-\ \alpha\ ^{3.7}/-\ \alpha\ ^{3.7}$ genotypes were not TD.

Our findings revealed that a high percentage of TD patients (83.3%) required iron chelation therapy. In one study in Taiwan, 73.9% of Hb H disease patients without frequent transfusions had evidence of iron overload.[45] In the Mediterranean region, iron overload rarely occurs in Hb H disease patients that might be related to genetic and environmental factors.[46–48]

Yu-Hua Chao and colleagues reported that hepatosplenomegaly and jaundice were more frequent in non-deletional Hb H disease patients.[20] Lal et al showed that patients with deletional types did not experience splenectomy, while 5% of deletional Hb H patients in our study underwent splenectomy.[19] The data about splenomegaly considering even the different sizes of the spleen has been shown in Table 4. Regarding the percentage of splenectomy among deletional patients of our cohort, we need to mention that these patients were managed by different hematologists from different parts of the country and the decision on splenectomy goes back to their clinical judgment and we gathered the data according to the medical records available. However, since we found that 23% of patients had splenomegaly more than 3 cm below costal margins, this may explain the decision on splenectomy in those 5%, particularly in cases causing hypersplenism.

Conclusion

Although Hb H is a growing disease in the world, it has not yet been fully understood. Due to insufficient reporting of Hb H patients, the exact prevalence and correlation between genotype-phenotype, as well as the natural history of this disease is not quite clear.

Our findings showed that α-globin genotypes were associated with diverse clinical manifestations of Hb H disease, but there was no definite evidence behind this association. Some patients with the same genotypes showed different clinical features of the disease. Therefore, understanding the relationship between genotype and phenotype in Hb H patients remains a matter of debate. This relation may also be a consequence of the interaction between different genetic and environmental factors.

However, our conclusions are limited due to genetic data not being available in 50% of our patients, so it is possible that the frequency and clinical findings associated with certain genotypes could have been quite different if genotype data had been available for the whole cohort.

We propose performing prospective studies even since early infancy in those with hypochromic-microcytic anemia and following them to be able to diagnose cases with Hb H disease since early childhood and in this regard, register all their clinical findings, correlate with genotype, and draw a more documented algorithm for management of this disease.

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Disclosure statement

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