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Description of hemoglobin H disease mutations in alpha thalassemia patients in Sulaimani Region in Kurdistan Region, Iraq

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Abstract:

CONTEXT: Hemoglobin H (HbH) disease is induced by mutations in three out of the four α- globin genes. Most commonly, mutations are either deletional or nondeletional. While some deletions (3.7 and 4.2) induce α+ thalassemia, others induce (20.5, MED, THA1, FIL) α0 thalassemia. HbH disease is a combination of both.

AIMS: This study aimed to describe alpha-thalassemia (HbH disease) mutations in Suliamaniyah Province, Iraq.

MATERIALS AND METHODS: Fifty-one patients with hypochromic microcytic anemia were evaluated for HbH disease. For each patient, a 2-ml venous blood sample was taken for isolating DNA. The samples were inspected for HbH disease mutations by gel electrophoresis, applying the α-Globin Strip Assay from the Vienna Lab TM commercial kit.

STATISTICAL ANALYSIS: Microsoft Excel software was used to analyze data.

RESULTS: Clinical data from complete blood count, hemoglobin (Hb)-electrophoresis, and HbH test were measured. HbH patients had significantly low levels of mean corpuscular volume, mean corpuscular Hb, and Hb (HGB) compared to normal values, and all showed a positive result in the HbH test with a low level of HbA2. Both the Med double gene deletion (3.7/MED) and the 3.7 single-gene deletion were detected in 68.62% of patients. Single-gene deletion 4.2, double gene deletion 20.5 (4.2/20.5), double gene deletion Med, and point mutation α2 poly A2 (MED/α2 poly A2) were all found in 1.96% of patients.

CONCLUSION: There is no difference between the phenotypes of patients with different genotypes.

Keywords:

α-Thalassemia, genotype, hemoglobin H disease, mutations

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Introduction

As a group, thalassemia forms the main single-gene mutation documented, and these diseases are a compelling public health concern in many areas of the world.[1] These conditions are often more common in the Eastern Mediterranean, where about 4% of the population carries hemoglobin S, hemoglobin C, hemoglobin E, α0-thalassemia (α0-thal), and β-thalassemia (β-thal) which are of proven clinically importance. In addition, one in every thousand conceptions is affected with thalassemia including Hb H (β4) disease, homozygous α0-thal, and homozygous β-thal.[2]

Alpha-thalassemia is characterized by monogenic or multigenic deletions or mutations, leading to absent or reduced α-globin chain production as caused

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by the location of mutation(s).[3] Interestingly, the hematologic outcome varies according to type and degree of mutation. For instance, the α-globin monogenic deletion or inactivation generally results in minimal hematologic returns. However, when two alpha-globin genes are inactivated or removed, either one on each of the two chromosomes 16 (in trans) or both on the same chromosome 16 (in cis), the affected individual is still healthy but becomes having marginal anemia, and the red blood cells become microcytic and hypochromic.[3] Furthermore, when three alpha-globin genes get inactive as a result of deletions with or without collateral non-deletional mutations, the person affected will possess only one functional alpha-globin gene. These individuals typically demonstrate remarkable hypochromia, microcytosis, and moderate anemia. Affected adults are characterized by an additional β-globin chain inside their erythrocytes, forming β4 tetramer familiar as Hemoglobin H (HbH). This inherited illness is acknowledged as HbH disease.[4] Alpha-thalassemia type in fetuses has all alpha-globin genes missing,[5] thus considered the most serious type of α-thal.

HbH disease patients typically have moderate anemia with hepatosplenomegaly, eventually, a counted proportion require supplementation of folic acid[6] and periodic blood transfusion.[7]

HbH disorder is subdivided into two major types; deletional and nondeletional HbH disease.[8,9]

HbH disease is still the most serious, but nonfatal, type of disease. Furthermore, because most of the patients with HbH have buffered hemolytic anemia with typical Hb levels >9.0 g/dL, this clinical condition was previously determined as mild. Nevertheless, hemolytic crises occur frequently in the context of acute infections.[10]

Since HbH disease is considered a rare disease in all alpha-thalassemia patients in our region specifically and our country in general, we deemed it necessary to find the mutation types affected by HbH disease and describe their genotypes and phenotypes.

Materials and Methods

Fifty-one hypochromic microcytic anemia patients with HbH disease were admitted to Thalassemia Center from the period between 2016 and 2019 for the screening of alpha thalassemia mutations and for periodic follow-up in Suliamaniyah Province, KRG. Some of the patients had received blood transfusion throughout their life. For each patient, a 2-ml venous blood sample was drawn into the EDTA tubes for complete blood count (CBC) using Swe laboratory, Sweden. Hb-electrophoresis was measured by Hb electrophoresis capillary's Sebia, France. DNA isolation was done using available kits according to the manufacturer's protocol. The polymerase chain reaction (PCR) multiplex method was employed for *in vitro* DNA amplification. Amplified products were then examined for alpha globulin genes mutations, including 21 alpha thalassemia mutations, using gel electrophoresis, and α-Globin Strip from Vienna Lab Assay TM commercial kit. Single-tube multiplex PCR assay for prevalent deletion of alpha-thalassemia was used by making use of the primers illustrated in Table 1.

Results

The core analysis of this study is descriptive statistics; fifty-one of the patients admitted to Thalassemia Center in Sulaymaniyah Province were identified with alpha thalassemia (HbH) disease. These patients included infants (4%), toddlers (4%), preschoolers (13.4%), school-aged children (23%), and adolescents (11.5%). Females counted for 28.8% of the patients, whereas males counted for 15.3%.

Clinical data from CBC, Hb-electrophoresis, and HbH tests are summarized in Table 2.

As shown in Table 2, HbH patients had significantly low levels of Mean Corpuscular Volume (MCV), Mean Corpuscular Hb (MCH), and HGB as compared to normal values, and all patients showed a positive result in the HbH test with a low level of HbA2.

Deletion mutations were recognized in 80.39% of the studied samples, whereas non-deletion mutations were detected in 19.60%. The patients' genetic results demonstrated a deletional and non-deletional type of HbH disease, as shown in Table 3.

The predominant mutation was the Med double-gene deletion and the 3.7 single-gene deletion (3.7/MED) found in 35 patients (68.62%), as shown in Figures 1 and 2.

Table 1: Primers used for polymerase chain reaction analysis in gel electrophoresis method

Name	5'-3' sequence	Product size (bp)
4.2-forward	GGACCCATGTGGTGCCTC	1628
4.2-reverse	CCCGTTGGATCTTCTCATTTCCC	
α 2/3.7-forward	CCCCTCGCCAAGTCCACCC	2022/2029
3.7-reverse	AAAGCACTCTAGGGTCCAGCG	
α 2-reverse	AGACCAGGAAGGGCCGGTG	1800
MED-forward	TACCCTTTGCAAGCACACGTAC	807
MED-reverse	TCAATCTCCGACAGCTCCGAC	
20.5-forward	GCCCAACATCCGGAGTACATG	1007
20.5 -reverse	AAAGCACTCTAGGGTCCAGCG	

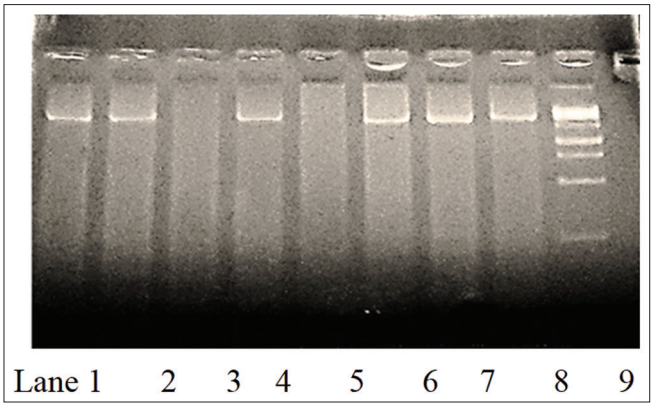

Figure 1: Analysis of the α-globin gene cluster on agarose gel electrophoresis. Indication of 3.7 kb mutations, Lanes (1, 2, 4, 6, 7): positive samples Lanes (3, 5): negative samples, Lane (8): positive control and Lane (9): Ladder 1000 kb

Table 2: Clinical tests and hemoglobin - electrophoresis values for patients

Parameter	Minimum	Maximum	Mean±SD	HbH test
A2 (%)	0.0	2.9	1.519±0.704	+
A (%)	77.5	99.0	93.429±5.237	+
F (%)	0.0	16.4	1.239±2.430	+
MCV (fl)	49.2	78.8	60.043±7.788	+
MCH (pg)	14.9	29.0	18.364±3.372	+
HGB (g/dl)	6.3	28.7	9.376±3.045	+

(+) = Positive test: Represent inclusion bodies test for HbH disease patients, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, HGB: Hemoglobin, SD: Standard deviation, HbH: Hemoglobin H

Table 3: Genotypes and phenotypes of the patients with hemoglobin H disease

Genotype	Mutation type	Phenotype
3.7/MED	Deletional	HbH
4.2/MED	Deletional	HbH
MED/α1 Cd 59	Deletional/nondeletional	HbH
α2/(+107 A>G)	Deletional/nondeletional	HbH
α2/α2 poly A1	Deletional/nondeletional	HbH
3.7/20.5	Deletional	HbH
MED/α2 IVS1-5nt	Deletional/nondeletional	HbH
4.2/20.5	Deletional	HbH
MED/α2 poly A2	Deletional/nondeletional	HbH

α: Alpha, HbH: Hemoglobin H

Other common mutations were the 4.2 single-gene deletion and Med double gene deletion (4.2/MED) that were found in 3 patients (5.88%). A nondeletional type of Med double gene deletion and Cd 59 mutation (MED/α1 Cd 59) was also found in 3 patients (5.88%). There were other mutations found involved (+107 A > G), (7.84%), (α2 poly A1) with (5.88%), (3.7/20.5) and (MED/α2 IVS1-5nt) (3.92%), and (4.2/20.5) and (MED/α2 poly A2) (1.96%).

In this research, there was no determination of a number of deletions (-SEA and -FIL) and mutations (α2 cd19, α1 cd14, and gene triplication) as shown in Table 4.

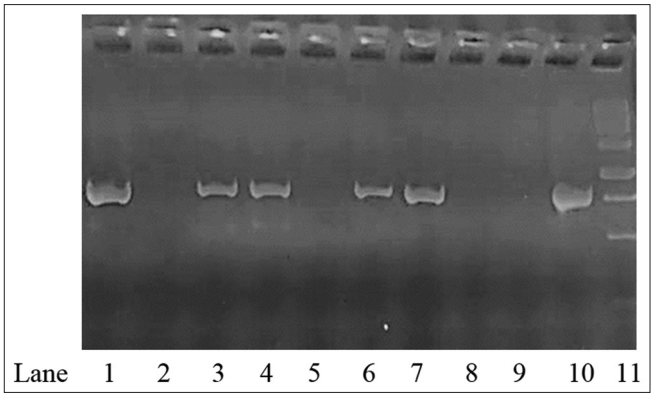

Figure 2: Analysis of the α-globin gene cluster on agarose gel electrophoresis. Indication of med mutation, Lanes (1, 3, 4, 6, and 7) positive samples, Lanes (2, 5, 8, and 9) negative sample, Lanes (10) positive control, and Lane (11) ladder 1000 kb

Discussion

Thalassemia is globally recognized as the most prevailing monogenetic disease. About 20% of the world population are α + thalassemia carriers, and 5.2% of the population are carrying a substantial variant of hemoglobin disease including α0 thalassemia and β thalassemia.[11] In Iraq, the rate of the β -thal gene carrier varies around 3.7 and 4.6 percent. Even then, no data on the carrier percentage of α-thal in Iraq have been published.[12] Measurements of MCV, MCH and quantity of Hb A2 and HbF, along with an understanding of the hematological features of the various forms of thalassemia genes and their interactions, are the basic hematological measures commonly used. The red cell MCV is markedly reduced in HbH deletion patients.[13] All affected individuals showed variable degree of anemia, decreased MCH/pg, decreased MCV/fl, and a normal to slightly lowered level of HbA_2.[14] All patients also showed lower MCV values. Where the mean reference intervals for the male healthy adults was MCH=29.7±1.6 and healthy females was 29.9±1.6 who based in Sulaymaniyah, Iraq. Furthermore, the reported mean MCV levels were lower as compared to the mean reference values 86.6 ± 3.9 and 87.3 ± 4.1 for adult males and females, respectively.[15] Furthermore, patients with two functional α-globin genes had lower MCV and MCH compared with patients with one mutated α-globin gene. Therefore, the values of MCV and MCH may help select the convenient molecular assessment to resolve the genotype of α-thalassemia carriers.[16] The level of HbA2 for all patients in this study was below 3.5.[17]

Analysis has shown that the coinheritance of HbH disease could affect not only the hematological parameters (Hb, MCV, and MCH) but also the HbA2 levels. This is shown in the low level of HbA2 in all patients.[18]

The HbH disease arises from imbalanced production of globin chain, while the reduced α-globin chain synthesis

Table 4: Positions of the 21 alpha-gene mutations in the StripAssay kit

Number	Position	Gene mutation/deletion
1**	–3.7	Single gene deletion
2**	–4.2	Single gene deletion
3**	–20.5	Double gene deletion
4**	--MED	Double gene deletion
5*	--SEA	Double gene deletion
6*	--THAI	Double gene deletion
7*	--FIL	Double gene deletion
8*	α1 cd 14	G>A
9**	α1 cd 59	G>A (Hb Adana)
10*	Anti -3.7	Gene triplication
11*	α2 init.cd	ATG>ACG
12*	α2 cd 19	-G
13**	α2 IVS1-5nt	5-bp deletion
14*	α2 cd 59	G>A
15*	α2 cd 125	T>C (Hb Quong Sze)
16*	α2 cd 142	T>C (Hb Constant Spring)
17*	α2 cd 142	T>A (Hb Icaria)
18*	α2 cd 142	A>T (Hb Pakse)
19*	α2 cd 142	A>C (Hb Koya Dora)
20**	α2 polyA-1	AATAAA>AATAAG (Saudi type)
21**	α2 polyA-2	AATAAA>AATGAA (Turkish type)

*Not detected in this study, **Detected in this study

causes excess β- or γ-globin chains to precipitate in the RBC membrane and eventual hemolysis.[19]

In regional areas, a study of the genotype of HbH disease in Iraq showed that by all of 51 persons with anonymous microcytosis and/or hypochromia from Dohuk region- northern Iraq, specifically nine of the investigated HbH cases encountered the genotype (–α3.7/––MED-I).[20]

In Erbil, 6224 couples underwent premarital screening at Erbil Marriage Screening Center. Only three cases were discovered to have HbH disease, these three cases were females only.[21] In Baghdad, no cases with HbH disease were identified in the studied cases in a study involved 502 randomly selected pregnant women admitted to a major maternity care clinic in the city.[22]

In neighbor countries, the HbH disease genotype in Bahrain for instance shows a single consistent cause of HbH disease; αpoly A1α/αpoly A1α.[23] In Egypt, one thousand Egyptian newborns were screened to detect α-thalassemia (α-thal) deletions using PCR-based DNA analysis of cord blood samples. In the studied group, HbH disease with 3 α-globin genes deleted accounted for as low as 1.8% of the cases.[24] In Jordan, among 430 α-thalassemic patients investigated, only 33 patients were found with HbH disease (60.6%), while the (α poly A1α/αpoly A1α), (αpoly A1α/––MED-I), (–α3.7/––MED-I), and (α–5ntα/––MED-I) was encountered by 15.1%, 12.2%, and 12.2% of the patients, respectively.[25] However, this result is different from our common ratio of 68.6% for (Med/3.7) mutation type. Among 17 HbH patients in Kuwait, 70.8% held the (αpoly A1α/αpoly A1α), 25% held the (αpoly A1α/–α3.7), while 4.2% were undetermined.[26] In Tunisia, five out of seven families having HbH disease held the (αpoly A1α/αpoly A1α) genotype, and two held the (–α3.7/––MED-I).[27] In Oman, a total of 52 patients were molecularly verified as HbH disease carriers, involving 27 females and 25 males. Eight genotype consolidations were distinguished with α2 polyadenylation signal mutation (polyA1) (AATAA*A* > AATAA*G* ($\alpha^{PA1}\alpha/\alpha^{PA1}\alpha$), usually known as $\alpha^{T\text{-}Saudi}\alpha/\alpha^{T\text{-}Saudi}\alpha$, being the most frequent (53.8%), and $-\alpha^{3.7}$/--MED-I coming next (28.8%). Nondeletional HbH disease due to the α^{PA1} mutation is the most common in Omanis.[28] In the nearest neighbor country Iran, a study of HbH disease patients was analyzed showing that HbH disease is the most prevalent form of thalassemia intermediate in Iran. This study, performed at the Hemoglobinopathy and Thalassemia Center of the Ahvaz University of Medical Science, included a total of 80 patients who were suspected to have thalassemia based on their mild-to-moderate anemia, microcytosis, and average levels of iron. Twelve mutations were revealed in the explored population, where the genotype -α3.7/--MED (45%) being the most prevailing, followed by αPoly A2 Homozygote (17.5%).[29]

On the other hand, Sorour *et al*. found that routine molecular screening for all forms of alpha thalassemia trait was unjustified in antenatal screening. Their study included 5092 women presented at an antenatal care, out of which, 425 were found to have an MCH < 27 pg in the absence of b-thalassaemia trait. In addition, after partner testing for patients, homozygousa0-thalassaemia was not detected in any couples.[30]

Conclusion

The present study suggests that HbH disease is considered a rare disease in Sulaymaniyah. In addition, the geographical distribution and ethnic groups might be a factor affecting types and ratios of mutations in HbH disease. However, the rate of mutations found previously in the neighborhood countries shows a different ratio of these mutations compared to the results of this work.

Further genetic studies to determine the precise α-thal predictors causing HbH disease are necessary in Sulaymaniyah and other parts of Iraq, as they are essential in predicting phenotype intensity.

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Conflicts of interest

There are no conflicts of interest.

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