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Detection of Hb Bart's and Hb H Diseases Caused by -α3.7 Prevalent Deletion Using Capillary Electrophoresis in Ardabil Province

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Abstract

***Background and Objective*:** Alpha-thalassemia (α-thal) appears to be the most common monogenic disorder worldwide. The diagnosis of α-thalassemia depends on the detection of Hemoglobin Bart (Hb Bart's) in newborns, which indicates one or more defective or absent α-globin genes. In addition, in patients with Hemoglobin H (Hb H), the Hb H range usually varies between 7-10 g / dL. Therefore, tracking Hb Bart's and Hb H can be useful in diagnosing thalassemia α. This study was performed to evaluate Hb Bart's and Hb H in infants with α thalassemia in Ardabil province, northwestern Iran.

Material and Methods: In this cross-sectional descriptive study, 33 infants with alpha thalassemia mutation, including infants born in Ardabil province, Iran in the years 2019 to 2020. Hemoglobin analysis was performed by capillary electrophoresis system.

Results: Hb H and Hb Bart's were detected in only two cases (6%) and three cases (9%). In this study, only 5 patients (15.15) were observable by detection of Hb Bart's and Hb H levels by electrophoresis. In cases of Hb Bart disease, -α3.7 was the most common genotype. Therefore, most infants with alpha thalassemia were lost when electrophoresis alone was used.

Conclusion: This study showed that molecular analysis of Hb Bart's newborns is necessary to confirm α-thalassemia. Capillary electrophoresis is a way to prevent the diagnosis of rare Hb H and Bart's disease.

Keywords: Alpha-Thalassemia [MeSH], Hemoglobin Bart's [MeSH], Hemoglobin H [MeSH], Electrophoresis Capillary [MeSH]

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Highlights

- The differentiation of α-thalassemia is essential for appropriate management of patients.
- The molecular analysis is useful for diagnostic confirmation and genotype-phenotype correlation.
- Capillary electrophoresis is a good method for Hb H and Hb Bart's diseases detection

Introduction

Thalassemia is a group of inherited blood diseases that lead to abnormal production of hemoglobin, an oxygen-carrying molecule in the blood (1). Alpha thalassemia is one of the most congenital disorders of congenital hemoglobin, which is characterized by reduced or no production of alpha globin chain (2). More than 750 different variants have been identified in the α-globin genes that lead to alpha thalassemia worldwide (3, 4). α-Thalassemia is commonly found in Africa, the Mediterranean region, the Middle East, the Indian subcontinent, East and Southeast Asia, and immigrants to these regions.

Iran is located in the Middle East between Iraq and Pakistan and the incidence of alpha thalassemia in Iran is high. Although the frequency of alpha thalassemia carriers in Iran has not been well identified, a report from northern Iran has estimated its frequency to be around 15.0% (3, 5). The diagnosis of α-thalassemia is based on the diagnosis of Hb Bart's in infants, which indicates one or more defective or missing α-globin genes (1). Hb Bart's hemoglobin level was found to be correlated with the number of defective globin α genes and is used to screen for alpha thalassemia blood conditions in infants (2, 6). Hb Bart's has been reported to have a greater affinity for oxygen and therefore is unable to deliver effective oxygen to tissues (7). Hb Bart's levels in carriers of α genes range from zero to a small amount (up to 1%) and may even be found in people with normal α genes (8, 9). The three defective alpha globin genes cause mild to moderate anemia, as well as microcytosis and hypochromia as Hb H disease (10). Because Hb Hb is fast moving, Hb electrophoresis analysis can reveal its presence in the range of 5-30 (11, 12). However, Hb H is unstable and may not be detected by Hb electrophoresis.

To date, some research has been conducted to determine the efficiency of electrophoresis in the diagnosis of α-thalassemia. For example, Wu et al. (2015) used automated capillary electrophoresis to determine the level of Bart hemoglobin in umbilical cord blood and then used molecular analysis to detect different α-thalassemia genotypes. A total of 70 infants were registered out of 1170 infants with amplified Hb Bart's in whom the diagnosis was confirmed by PCR. Among the remaining neonates, 45 alpha thalassemia carriers were identified by PCR. All of these have only a 3.7 KB deletion mutation (6). Therefore, the authors suggested that Hemoglobin Bart could not be a reliable α gene mutation for screening infants with α-gene mutation. Hafiza Aladdin et al., (2017) used capillary electrophoresis with HPLC to detect and determine the amount of Hb Bart in cord blood samples and they confirmed the results by multiplex ARMS PCR. Among 600 infants, 5.3 infants showed the presence of Hb Bart peak using electrophoresis while 5.5 with positive by HPLC and electrophoresis. PCR confirmed that all positive samples had α-thalassemia genetic mutations. However, three of the fifty Hb Bart-negative samples were detected for α-globin-positive mutant genes (2).

The authors showed 92% sensitivity and 100% specificity for capillary electrophoresis. In general, previous research has shown that Hb Bart's in cord blood can be used as a suitable marker of alpha thalassemia, so that Bart hemoglobin levels increase in proportion to defective α genes (13, 14). However, the effectiveness of this method has never been tested in our population. In this study, we used an electrophoresis system to detect alpha thalassemia from cord blood and then measured the output using PCR. Finally, our group determined the probability of α-thalassemia in Bart's hemoglobin screening program.

Materials and Methods

In this cross-sectional study, 33 infants with low levels of MCV (<100 fl) and MCH (<33 pg) were referred for molecular analysis. Written consent was provided for each patient. Venous blood samples were taken from each case of α-thalassemia in the first 3 days and their Hb analysis was performed using capillary electrophoresis system. Hemoglobin analysis was performed by capillary electrophoresis, which can isolate charged molecules at alkaline pH with electroosmotic current, electrophoretic mobility, and electrolyte pH. Each peak appears in a specific area. Barthes' hemoglobin was automatically located in the twelfth region according to the Hb A fraction. Until PCR evaluation, the remains of each blood sample were frozen and then stored at -20 ° C.

Genomic DNA extraction from EDTA anticoagulant peripheral blood samples was performed through a DNA extraction kit (Qiagen GmbH, Germany) according to the manufacturer's instructions. DNA purity and concentration were calculated at 280 and 260 nm, and finally the samples were stored at -30 ° C before use. PCR technique was used to detect deletion of α-globin gene by primers created by Chong et al. (15). PCR conditions were as follows: 1) Initial denaturation: 96 ° C for 15 minutes. 2) Color change: 30 cycles at 98 ° C for 45 seconds; 3) Baking: 67 ° C for 1 minute; 4) Spread at 72 ° C for 2 minutes. Finally, PCR products were isolated using agarose gel electrophoresis.

This study is based on the approval of the Medical Ethics Committee of Ardabil University (Ref: IR.ARUMS.REC.1396.236). Screening and identification of thalassemia was performed according to the latest national protocol for thalassemia prevention in Iran presented in 2012.

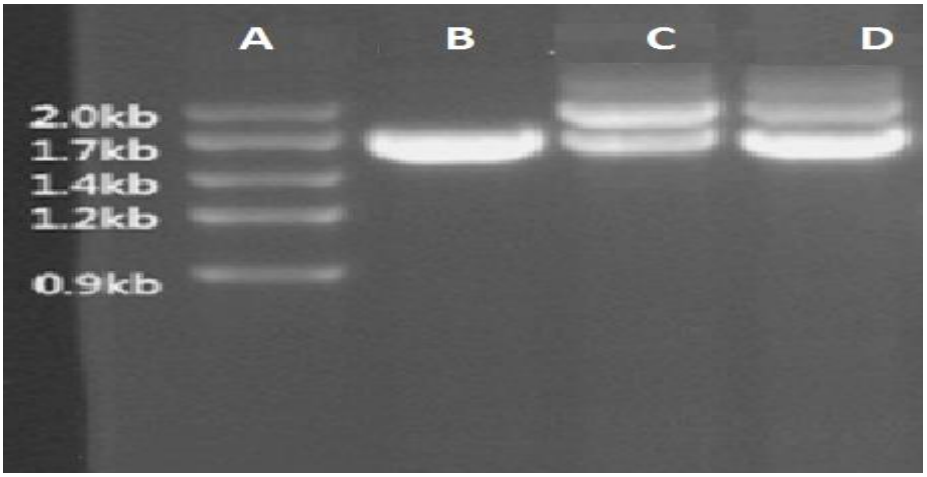

Figure 1. Multiplex PCR for screening of the most common α-thall alleles. A- Ladder marker; B- Natural genotype control (αα / αα); C- patient (α -3.7 / αα); D-positive control to remove α -3.7.

Results

The capillary electrophoresis system led to detect the different quantities of Hemoglobin Bart's in 3 (9.09%) of the total 33 infants (Table.1). In the current study, Hb Bart's was traced only in three (9.09%), which all of them having double gene mutations. The level of Hemoglobin Bart's in three cases was 2.5%, 4.5% and 5.5%. All the three cases were verified through the PCR technique to carry α-thalassemia. Moreover, Hb H was observed only in two cases (6.06%) by using electrophoresis. The level of Hb H in two cases was 14.7% and 15.6%. All the two cases were verified through the PCR technique to carry α-thalassemia. The 3.7 kb deletion carrier was found

the most common genotype (Fig. 1). The findings revealed that the Hemoglobin Bart's level was enhanced proportional to the number of defect α-genes.

Table 1. Correlation of Hb Bart level and hematological data with genotype at a-globin locus in 33 cord blood samples

Patients No.	Genotype	MCH (pg)	MCV (fL)	Hb (gldL)	Hb Bart's
1	het -a 3.7 single	32.5	94	10.1	4.3
2	het -a 3.7	30	96.4	7.33	2.1
3	-a 3.7 - a 3.7	28	83	9.25	0.3
4	het -3.7a	31.7	92	8.21	0.7
5	anti 3.7 het	32	93.5	7.79	0.4
6	het a2polyA-z (AATAAA>AATGAA)	30	88.8	10.8	3.6
7	hem -3.7a	29.8	87	6.28	3.3
8	het -3.7a	30.9	93	9.44	0.6
9	hom -3.7a	26	89	6.28	1.7
10	het -3.7a	30	88.8	10.2	2.8
11	het -3.7a	31	91	6.43	4.65
12	het -a 3.7	33	99	8.30	19.5
13	het -3.7a	30.9	89.7	6.88	2.5
14	het -4.2a	29	87	7.36	0.6
15	a2 IVS1 [-5nt] WT	30.9	91	10.5	0.4
16	het - 3.7 single	30.9	88.1	11.3	3.6
17	het - 3.7 single	30.6	91.5	8.49	2.2
18	het - 3.7 single	33.3	96.9	12.2	1.50
19	het -3.7 a single	31.7	95.5	7.85	2.1
20	a2 cd19 [-G] het	31.7	96.6	9.58	6.5
21	het deletion med1	30	91	10.44	10.4
22	het -3.7a	24.9	72.6	9.56	8.3
23	het -a 20.5	21.9	66.9	8.8	2.3
24	hom -a 3.7	16.9	55.4	11.35	0.2
25	aaa anti 3.7 / aa	25.9	76.6	8.50	5.7
26	IVS 1.1/wt	20.6	77	10.40	2.8
27	hom -a 3.7	24	77.8	6.28	0.6
28	a-3.7 a/aa	22	75	7.79	0.3
29	-a/aa	25.1	74.7	8.78	0.8
30	-a/aa	25.8	77.1	10.8	5.9
31	HET.C.427T>C at hbA2	26	75.9	9.44	8.8
32	del G at codon 126/Wt	22.8	67.7	10.7	2.50
33	-4.2 single gene DEL	24.4	75.6	9.70	3.9

Discussion

Despite significant clinical success in the diagnosis of premarital thalassemia, 3 to 100 patients per 100,000 thalassemia are carriers of thalassemia in Iran (16). Hb Bart's disease is not accurately diagnosed in infancy because it disappears quickly after birth. Therefore, determining the amount of Hb Bart's after infancy is not reliable (17-19). On the other hand, Hb Bart's levels have been shown to vary between different ethnic groups (20). Hb H and Hb Bart's Hb are also fast moving, appearing on electrophoresis or HPLC. As a result, some reports indicate that these two parameters are unstable and may not be detectable by conventional methods (21). Therefore, it seems

that molecular analysis of Hb Bart's infants is necessary to confirm α-thalassemia.

In this study, only five cases (15.15%) were detected by Hb Bart's diagnosis and also Hb H by electrophoresis. Therefore, most infants with α-thalassemia are missed when electrophoresis alone is used. In some normal infants, Hb Bart's can be detected in about 0.5-1.5% of cases (22). Various factors may play a role in the variability of Hb Bart levels, and among them, the amount of γ-β globin change may play an important role (17). Hb Bart's is diagnosed in many infants with alpha thalassemia, but not in patients with a 3.7 KB deletion. It was reported that most α-thalassemia not detected by electrophoresis had 3.7 KB of single α α globin genes (6). However, in this study, Hb Bart's was not detected in any of the mutations except in cases with deletion of the 3.7-kb gene.

As recorded in current research, all cases of Bart's detectable hemoglobin had the disease. In addition, the Hb Bart level can isolate a carrier of two or more α gene defects as well as an alpha gene defect. Although the accuracy of Hb Bart umbilical cord blood was recorded as a disease parameter in previous reports (23-25), the absence of Hb Bart could not make the diagnosis of α-thalassemia impossible. Our findings show that all infants with two alpha gene defects have elevated Hb Bart levels, while a large proportion of single-defect alpha gene carriers do not show traceable Hb Bart. Remarkably, it was found that all carriers fleeing the disease have a 3.7 KB deletion mutation. Silent α-thalassemia, the loss of one of the two alpha genes on a chromosome, has two major types: -α3.7 and -α4.2. The recombination process between homologous boxes non-X homologous boxes results in the removal of 4.2 KB, while the process in highly homologous boxes results in the removal of 3.7 KB (26-27). The data of this study show that the -α3.7 mutation is the most common deletion that causes α -tal in Ardabil province, as in the Derakhshan study in northwestern Iran (28).

Previous studies have shown that the -α4.2 allele causes a considerable synthesis imbalance of α-/non-α-globin chains and the considerable production of the γ chain in patients than the -α3.7 allele. According to such results, the level of Hb Bart in newborns with -α3.7 was 0.2 ± 0.5 was, while in newborns with -α4.2, the level of Hb Bart was represented in 0.3 ± 0.7. Therefore, our group guessed that a small amount of Hb Bart related to the -α3.7 allele is not technically reliable, while the α4.2 allele is reliably detectable due to the high level of hemoglobin Bart's.

Remarkably, the mean HbA2 level in this study was 0.97±0.41. HbA2 levels in infants with thalassemia carriers are normal or slightly lower, which can distinguish α-thalassemia from thalassemia in particular. In Hb H disease, HbA2 levels can be reduced to less than 1 (19, 20). Normal or low HbA2 levels combined with decreased mean body hemoglobin (MCH) (<33pg) and mean cell volume (MCV) (<100fl) may be a good chance to detect α-thalassemia carriers (18). In the current study, the recorded data belong only to people who met the inclusion criteria and lack of access to the initial number of people is another limitation of the study.

Conclusion

It is worth noting that although the evaluation of Hb Bart's and Hb H are direct methods for the diagnosis of α thalassemia α, only a few cases of this study were detected and most of them were missed. Therefore, molecular analysis of Hb Bart's infants is necessary to confirm α-thalassemia and determine the number of defective α genes. As a matter of fact, normal or low HbA2 levels combined with decreased MCV and MCH appear to be a good chance to detect α-thalassemia carriers. The CE result may be used as evidence of Hb Hb and Hart Bart's disease, whether derived from known genotypes or new mutation genotypes. Due to the specificity of anemia in blood analysis, capillary electrophoresis is a good way to diagnose Hb H and Hb Bart's disease.

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Conflicts of interest

The authors did not report any relationship that could be interpreted as a conflict of interest.

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