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Prevalence and molecular characterization of alpha-thalassemia among newborns in Ardabil Province

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Abstract

Background and objective: Alpha-thalassemia is one of the most recessively congenital hemoglobin disorders in the world, and is characterized by decreased or absence of alpha globin chains production. Although it has been suggested that the frequency of alpha-thalassemia in Iran is greater than worldwide, its exact rate is unknown. Due to lack of more studies on this topic in this area, the aim of the present study was to determine prevalence and molecular characterization of alpha-thalassemia among newborns in Ardabil Province.

Methods: In this cross-sectional study, one thousand newborns were referred for screening of alpha thalassemia at a pediatric unit in Ardabil province between April 2016 and March 2018. Cases with Mean Corpuscular Volume (MCV) <100 fL and Mean Corpuscular Hemoglobin (MCH) < 33 pg were referred for serum Ferritin measurement, Hb electrophoresis and then genetic analysis. Collected data were analyzed by statistical methods such as number, percent and Mean±SD in SPSS version 21.

Results: The prevalence of α-thalassemia in studied newborns was 3.3% in Ardabil province. The most common mutation was the 3.7 single gene deletions that were found in 42.4% (14 cases) of newborns with α-Thalassemia.

Conclusions: Results showed that, the prevalence of α-thalassemia in Ardabil province was lower than the average rate for the country and the most common mutation was $-\alpha^{3.7}/\alpha\alpha$, which was similar to other places in Iran.

Keywords: Alpha-thalassemia; Mutation; Gene Deletion; Prevalence

Abbreviations / Acronyms:
EDTA: Ethylenediaminetetraacetic acid; **HbA:** Hemoglobin A; **HbA2:** Hemoglobin A2; **HbF:** Fetal hemoglobin; **MCH:** Mean Corpuscular Hemoglobin; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **MCV:** Mean Corpuscular Volume; **MLPA:** Multiplex Ligation-dependent Probe Amplification

1. Introduction

Alpha-thalassemia is one of the most recessively congenital hemoglobin disorders in the world, which is characterized by decreased or absence of alpha globin chains production (1, 2). Worldwide, up to around 5% of the general population are affected, but it is commonly prevalent in populations from Southeast Asia, the Mediterranean and the Middle East, with prevalence rates ranging from 1 to 40% (2-4). Although the frequency of α-thalassemia in

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Iran is greater than it is worldwide, it is not exactly determined (5). Alpha-thalassemia syndrome results from deletion or mutation of one or both α-globin genes that are located on 16p13.3 (6). The phenotype of the disease is related to the number of α-globin genes deficiency as well as the type of mutation, ranging from asymptomatic phenotype to a fatal in utero disease (7-8). The loss of one (-α) and two (-) genes of α-globin as the most common cause of a-thalassemia are not associated with clinical symptoms. While, the loss of three genes of α-globin (--/-α) leads to hemoglobin H disease, which sometimes results in moderate anemia and the need for transfusion, the loss of four genes of α-globin (--/--) leads to Hb Bart's Hydrops Fetalis Syndrome, which can be fatal (9-10). Synthesis Reduction of α-globin less than 25%, may result in a moderately severe hemolytic anemia (11). Worldwide, more than 770 mutations have been recognized in α-globin gene clusters, and of those, more than 19 mutations documented in Iran (5). Gene sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) techniques can be useful for better detection of novel suspected α-thalassemia mutations (12). Due to the lack of more studies on α-thalassemia in this area, and effective mutation in its prevalence, the aim of the present study was to determine the prevalence and molecular characteristic of α-thalassemia among newborns in Ardabil Province.

2. Material and Methods

2.1. Study design

In this cross-sectional study, one thousand newborns were screened for α- thalassemia in the pediatric unit (the only research center for thalassemia cases) of Bu-Ali hospital in Ardabil city between April 2016 and March 2018. Informed consent was obtained from the parents.

2.2. Measurements

Using EDTA-containing tubes, a total of 2 ml venous blood was taken from each infant for analysis during routine hematological tests (RBC, Htc, Hb, MCH and MCHC as well as ferritin) and the remainder was kept at 4˚C and sent for laboratory analysis in the first three days of life. There is a general agreement that the α-thalassemia trait is considered typical and could be present when MCV (Mean Corpuscular Volume) is below 94FL and MCH (mean corpuscular hemoglobin) is below 30 pg, respectively, except in neonates with iron deficiency. But in the current study, cases with MCV and MCH below 100 fL and 33 pg respectively, were referred to laboratory for measurement of serum ferritin level, acetate cellulose electrophoresis and molecular analysis. Molecular techniques have been applied to identify genetic mutations and genomic DNA was extracted from whole blood.

2.3. Statistical analysis

Collected data were analyzed by statistical methods in IBM© SPSS© Statistics version 21 (IBM© Corp., Armonk, NY, USA).

2.4. Ethical approval

This study was financially supported and approved by the Ethical committee of Ardabil University of Medical Sciences (Ref: IR.ARUMS.REC.1396.236).

3. Results

In total, 97 newborns had MCV < 100 fL and MCH <33 pg. Hb electrophoresis was carried out on all cases. Based on these results, each suspected newborn was referred for genetic analysis. In summary, we found 33 newborns with α-thalassemia. The mean of Hemoglobin A2 (HbA2) and Fetal Hemoglobin (HbF) were 0.97±0.41 and 73.53±12.3, respectively. The prevalence of α-thalassemia in studied newborns was 3.3 % in Ardabil province. The most common mutation was the 3.7 single gene deletions that were found in 42.4% (14 cases) of newborn with α-thalassemia. HbH was only detected in two cases (14.7% and 15.6%) and Hb Bart's was seen only in three neonates (2.5%, 4.5% and 5.5%) (Table 1). The most mutations in studied samples in our study was - $\alpha^{3.7}$/ α α, with 14 cases (42.4%) (Table 2).

Table 1. The mean of hematological parameters in studied samples

Number	RBC	Hb	Htc	MCV	MCH	MCHC	HbA	HbA2	HBF	Ferritin
33	4.50± 0.52	13.68±1.82	41.04± 5.47	92.6182± 3.70	31.20±1.63	33.78±0.82	22.36± 4.84	0.97± 0.41	73.53± 12.2	187.5± 75.5

HbA: Hemoglobin A; HbA2: Hemoglobin A2; HbF: Fetal hemoglobin; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean Corpuscular Volume

Table 2. The frequency of found mutations in studied samples

Genotype	N	%	HbA2 (%)
- $\alpha^{3.7}$/ α α	14	42.4	0.8
- $\alpha^{3.7}$/ - $\alpha^{3.7}$	6	18.2	0.72
aaa anti $^{3.7}$/ aa	2	6.1	1
- α^{PA2}/ α α	1	3.0	0.9
- $\alpha^{4.2}$/ α α	4	12.2	1.75
α2 IVS1(−5nt)	1	3.0	0.8
α 2 cd19 [-G] het	1	3.0	1.5
het deletion med1	1	3.0	0.8
HET.C.427T>C at hbA2	1	3.0	2.9
del G at codon 126/Wt	1	3.0	1.8
het -a 20.5	1	3.0	1.5

4. Discussion

According to WHO reports, approximately, 20% of the global population are α-thalassemia carriers (13). Although no compressive study has been performed about the prevalence of α- thalassemia in Iran (14), the rate of α-thalassemia carriers was estimated to be about 7.8% in the general population (15). The thalassemia prevention program in Iran has been started since 1997 which deal to significant success in decreasing the rate of talassemic infants (16). Since the Iranian population is a mixture of different ethnic group, the rate of α-thalassemia can be different in different regions. Alpha-thalassemia is more prevalent around the Caspian Sea and Persian Gulf (North, and South of Iran). The rate of α-thalassemia differs from 2.7% (in East Azerbaijan; north-west of Iran) to 15.3% (in Mazandaran; northern Iran (Caspian Sea coast). Alpha-thalassemia is prevalent in populations in South East Asia, the Mediterranean and the Middle East. It has been reported that the prevalence of α-thalassemia in Thailand, Morocco and turkey is 30-40%, 0.96% and 0.25%-4.1%, respectively.

The prevalence of α-thalassemia in the current study was 3.3%. Our results are similar to the study of East Azerbaijan province, in the north-west of Iran. (17). Data showed that -$\alpha^{3.7}$(6.1%) and -α4.2 (4.68%) are the most common mutation, causing α-thalassemia among 10,849 cases of α-thalassemia carriers in a comprehensive study of Iran (14). Like other regions, -$\alpha^{3.7}$ (60.6%) (-$\alpha^{3.7}$/ αα and -$\alpha^{3.7}$/ -$\alpha^{3.7}$) was the most common mutation in this study, followed by - α4.2/ α α (12.2%). The second most common mutation in other studies was aa/–a4.2 (4.6% in East Azerbaijan), poly A2 (18.2%, Gilan), deletion $^{--MED}$ (9.7%, Khuzestan), α-5 nt (16.8%, Sistan and Baluchistan). The most common mutation in the world is -$\alpha^{3.7}$ which was similar to our study results (18).

The mean of MCV and MCH was 92.62±3.7 and 31.20±1.63, respectively. This is an agreement that α- thalassemia trait could be present when MCV is below 94fL (19-20). In the current study, 15.15% (5 case) and 84.8% (28 cases) had MCV above 94fL and MCH above 30, respectively. So, by using these cut-off points (MCV<94 and MCH<30), a large number of cases are not detected. So it cannot be confidently said that there is clear demarcation of the level of MCV and MCH to find α-thalassemia (21). But since using these parameters is more readily available in most laboratories, they can be used for primary screening of thalassemia in newborns. On the other hand, these parameters are highly predictive when MCV < 90fL and MCH<30pg (21-23). The values of MCV and MCH are strongly affected by the number of α- genes, cases with one α gene deletion have slightly decreased of these parameters and can be overlapped with normal values (24).

The mean of HbA2 and HbF were 0.97±0.4 and 73.53±12.3, respectively. HbH was only detected in two cases (14.7% and 15.6%) and Hb Bart's was seen only in 3 neonates (2.5%, 4.5% and 5.5%). The level of HbA2 in newborns with α-thalassemia carrier is normal or lower. Especially in HbH disease, it can drop to less than 2% (94% of cases in our study had HbA2 below 2%). Alpha-thalassemia carriers can be identified by detection of Hb Bart's in newborns. The concentration of Hb Bart's in newborns with α-thalassemia carriers is about 1-8% of total Hb (7, 24). The level of Hb Bart's is found in a large number of newborns with α-thalassemia and it is correlated with the number of defective α-globin genes (26). In this study, Hb Bart's was detected only in three cases (9.09%) and the majority of cases were negative. It is important to note that the absence of Hb Bart's does not exclude the presence of α-thalassemia in newborns (especially in mild α-3.7/αα interactions) and diagnosis can only be confirmed by the molecular analysis (27).

5. Conclusions

Results of this study showed that, the prevalence of α-thalassemia in the current study was 3.3% in Ardabil province. The most common mutation causing α-thalassemia in this study was $-\alpha^{3.7}/\alpha\alpha$ (n=14, 0.8%). Hematological parameters can be normal in some cases with α-thalassemia in first screening and so the disease cannot be detected. In general, the hematological routine tests and Hb electrophoresis can be useful together for detection of α-thalassemia carriers but the absence of any abnormality of analysis does not exclude the α-thalassemia. Alpha-thalassemia can only be confirmed by molecular analysis. Conducting more multi center studies in Iran is necessary in future.

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Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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