Introduction of novel $\alpha_1$-hemoglobin gene mutation with transfusion-dependent phenotype

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\begin{abstract}
Objective and importance: Thalassemia is the most frequently monogenetic disorders around the world that is inherited as a recessive single-gene disease, resulting from mutations in $\alpha$- or $\beta$-globin gene clusters. The aim of this report was to present a new insertional mutation in the $\alpha_1$-globin gene which causes transfusion-dependent anemia in $\alpha$-thalassemic patients.

Clinical presentation: Two 5-year-old girls with blood transfusion-dependent $\alpha$-thalassemia anemia and another girl with moderate $\alpha$-thalassemia have been presented among patients who have been referred to Hematology and Thalassemia Research Center, Dastgheib Hospital, Shiraz, Iran. They were not relatives. All children were stunted and pale; they were put on regular blood transfusion every 14–21 days.

Intervention: Sequencing of the $\beta$-globin gene was normal in all cases and their parents; but, $\alpha$-globin gene sequencing results were remarkable. An insertion of 21 base pairs (+21nt)(+GACCGGTCAACTTCAAGGTG) in the $\alpha_1$-globin gene was detected in all three cases and one of their parents. In two cases, this insertion was accompanied by MED deletion and in one child by POLY A; mutation. MED deletion was detected by gap-PCR.

Conclusion: This new 21 base pair insertion cannot affect blood parameters on its own, but can present as continuous blood transfusion-dependent $\alpha$-thalassemia. Thus, it is important to take this point into account for detecting the carriers, like $\beta$-thalassemia carriers, which can present as transfusion-dependent children in parents with $\alpha$-thalassemia trait.
\end{abstract}

Introduction

Thalassemias are the most frequent monogenetic disorder around the world that is inherited as a recessive single-gene disease, resulting from mutations in $\alpha$- or $\beta$-globin gene clusters. About 7% of the world’s population are carriers of a globin gene mutation [1–3].

$\alpha$-Thalassemia is one of the hemoglobinopathies that is characterized by a quantitative reduction of the $\alpha$-globin chains [2–4]. $\alpha$-Thalassemia is most common in Southeast Asia but is also prevalent in the Mediterranean, Middle East, India and sub-Saharan Africa, with carrier frequencies ranging from 15 to 30% [5].

$\alpha$-Thalassemia can lead to reduction, deletion, or defect in $\alpha$-globin chain which causes an imbalanced chain. Thus, $\gamma$-globin and $\beta$-globin chains will deposit in red blood cells as tetramers and develop hemolytic anemia [1]. Mutations on this gene are point mutations or deletions with various sizes and that include one gene or both [2]. $\alpha$-Zero mutations are mostly common in Southeast Asia ($\alpha^\text{SE-A}$) and the Mediterranean ($\alpha^\text{MED}$) [6–8] The aim of this report was to present a new insertional mutation in the $\alpha_1$-globin gene that causes transfusion-dependent anemia in $\alpha$-thalassemic patients.

Case report

Among patients who have been referred to Hematology and Thalassemia Research Center, Dastgheib Hospital, Shiraz, Iran with an unknown anemia to different extents (mostly severe), three patients and their families were investigated. Patients consisted of two 5-year-old girls with severe anemia who need regular blood transfusion, and another girl and her mother with frequent transfusion-dependent anemia; suspicious to any type of anemia except thalassemia due to not matched paraclinical results (mild decrease in mean corpuscular value (MCV)). They were not relatives. All children were stunted and pale; they were put on regular blood transfusion every 14–21 days. After obtaining informed consent, the blood samples of the families were collected in the Hematology research laboratory of Dastgheib Hospital.

Primary laboratory tests such as complete blood count and hemoglobin electrophoresis were done before the first blood transfusion, the results of which are presented in Table 1. Anemia with mild decrease in MCV, slight anisocytosis, slight hypochromia, few target cells with the presence of Heinz body were revealed, and with the possibility of any known microcytic hypochromic anemia, different tests such as

\begin{table}
\caption{Hemoglobin electrophoresis}
\begin{tabular}{|c|c|c|}
\hline
Gene & Type & Result \\
\hline
$\alpha_1$ & Insertion & (21nt)(GACCGGTCAACTTCAAGGTG) \\
\hline
$\beta$ & Normal & \\
\hline
\end{tabular}
\end{table}
ferritin level, serum iron level, unsaturated iron-binding capacity, reticulocyte count, direct and indirect Coombs test and liver function test were performed, and except for mild increase in total bilirubin level (2.2 mg%) with normal direct bilirubin, all other tests were normal. Owing to the presence of an unknown hemoglobin variant and fast hemoglobin band in the hemoglobin electrophoresis, further studies were done in the area of thalassemia (Figure 1).

Sequencing of the \( \beta \)-globin gene was normal in all cases and their parents; but, \( \alpha \)-globin gene sequencing results were remarkable. An insertion of 21 base pair (IVS II+3ins (+21nt) (+GACCCGGTCAAATTCAAGGTG) in the \( \alpha_1 \)-globin gene was detected in all three cases and one of their parents (results are represented in Table 1) (Figure 2). In two cases, this insertion was accompanied by MED deletion and in one child by POLY A1 mutation. MED deletion was detected by gap-PCR [9]. For the laboratories which do not have the possibility of DNA sequencing, we have designed an ARMS PCR method to detect this insertion which is described in Table 2 and Figure 3 and the results are presented in Figure 4. This study was approved by the local Ethical Committee of Shiraz University of Medical Sciences. Informed written consent was signed by a parent of the patient.

Discussion

In the present study, we report a new insertion of 21 base pair (IVS II+3ins (+21nt) (+GACCCGGTCAAATTCAAGGTG) in the \( \alpha_1 \)-globin gene that caused transfusion-dependent anemia in two 5-year-old children in combination with MED deletion and a frequent transfusion-dependent \( \alpha \)-thalassemia in another child and her mother in combination with POLY A1 mutation. MED deletion is a two-gene deletion which includes 1.26% of \( \alpha \) gene mutations in the south of Iran [10].

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**Table 1.** Primary laboratory test results of three cases and their families.

| Case 1 | RBC count (g/l) | MCV (fl) | MCH (pg) | HbA2 (%) | Unknown variant | Fast Hb (%) | \( \alpha \)-Globin genotype | Phenotype |
|--------|-----------------|----------|----------|----------|-----------------|-------------|---------------------------|-----------|
| Father | 6.88 | 130 | 65.5 | 19.0 | 2.2 | 0 | \( _{\text{Med}}/_{\alpha\alpha} \) | Minor \( \alpha \)-thalassemia |
| Mother | 5.19 | 125 | 77.5 | 24.1 | 2.5 | 0 | \( \alpha/\alpha_{1}\text{Int insertion} \) | Silent \( \alpha \)-thalassemia |
| Unaffected child | 5.02 | 119 | 71.7 | 23.7 | 2.3 | 0 | \( _{\text{Med}}/_{\alpha\alpha} \) | Silent \( \alpha \)-thalassemia |
| Affected child | 1.83 | 32 | 75.4 | 17.5 | 1.2 | 6.6 | \( _{\text{Med}}/_{\alpha\alpha} \) | Transfusion-dependent \( \text{Hb H} \) disease |

| Case 2 | RBC count (g/l) | MCV (fl) | MCH (pg) | HbA2 (%) | Unknown variant | Fast Hb (%) | \( \alpha \)-Globin genotype | Phenotype |
|--------|-----------------|----------|----------|----------|-----------------|-------------|---------------------------|-----------|
| Father | 5.21 | 136 | 77.0 | 26.1 | 2.5 | 0 | \( \alpha/\alpha_{1}\text{Int insertion} \) | Silent \( \alpha \)-thalassemia |
| Mother | 5.33 | 107 | 64.4 | 20.1 | 2.1 | 0 | \( _{\text{Med}}/_{\alpha\alpha} \) | Minor \( \alpha \)-thalassemia |
| Affected child | 4.04 | 85 | 70.8 | 21.0 | 1.3 | 3.8 | \( _{\text{Med}}/_{\alpha\alpha} \) | Transfusion-dependent \( \text{Hb H} \) disease |

| Case 3 | RBC count (g/l) | MCV (fl) | MCH (pg) | HbA2 (%) | Unknown variant | Fast Hb (%) | \( \alpha \)-Globin genotype | Phenotype |
|--------|-----------------|----------|----------|----------|-----------------|-------------|---------------------------|-----------|
| Father | 5.76 | 145 | 76.4 | 25.2 | 2.5 | 0 | \( \alpha/\alpha \) | Normal |
| Mother | 3.96 | 93 | 87.1 | 23.5 | 1.2 | 3.9 | \( \alpha/\alpha_{1}\text{Poly A1} \) | Frequent transfusion-dependent \( \text{Hb H} \) disease |
| Affected child | 4.02 | 89 | 70.8 | 22.1 | 1.1 | 4.1 | \( \alpha/\alpha_{1}\text{Poly A1} \) | Frequent transfusion-dependent \( \text{Hb H} \) disease |

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Because of frequent blood transfusion which was not predictable, RBC indices are not reliable.

Hemoglobin H disease.

**Figure 1.** The electrophoresis diagram of the second case of Table 1: the presence of a fast Hb and an unknown variant is remarkable. Hb electrophoresis was done by Hydrasys2 system, Sebia, France.
As shown in Figure 2, the new insertion location is in intron part, which normally cannot translate, or may make new stop codon; but the presence of variant chain in hemoglobin electrophoresis may only be explained by changing RNA splicing location with the insertion, as it is so close to exon part of the gene. On the other hand, the $\alpha_1$-globin gene is functionally weaker than the $\alpha_2$-globin gene, and we expect the new $\alpha_1$ insertion mutation has only a mild phenotypic effect with mean corpuscular volume in the range of 79–80 $\mu l$; similar to codon 59 or codon 14 mutations, which can completely turn-off $\alpha_1$-globin gene.

This mild phenotypic presentation was also observed in parents of our patients who have only

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**Table 2.** ARMS PCR method to detect 21 base pair (IVS II+3ins (+21nt) (+GACCGGTCAACTCAAGGTG)) in $\alpha_1$-globin gene.

| Primer Type   | Primer Sequence                                      |
|--------------|-------------------------------------------------------|
| Common R primer | 5′CTCAAAGCACCTAGGGTCCA3′                            |
| Normal F primer | 5′GTCAACTCAAGGTGAGCGGC3′                            |
| Mutant F primer  | 5′GTCAACTCAAGGTGACCCGGT3′                           |
| Common F primer  | 5′CCAAGCATAAACCCTGGCGCGCT3′                         |

PCR cycles:
- 94°C ......... 5 min for 1 cycle
- 94°C ......... 1 min for 30 cycle
- 65°C ......... 1 min for 30 cycle
- 72°C ......... 1 min for 30 cycle
- 72°C ......... 5 min for 1 cycle

For each sample two PCR reaction was done, M (Mutant) and N (Normal):

Tube M contents: Each 50 µl reaction contained 20 mmol/l Tris–HCl pH 8.4, 50 mmol/l KCl, 1.5 mmol/l MgCl$_2$, 1 mol/l betaine, 0.2 µl of Common F primer, 0.2 µl of Common R primer, 0.2 µl of Mutant F primer, 0.2 mmol/l of each dNTP, 2.5 units of polymerase, and 100 ng of genomic DNA. In Tube N, Mutant F primer is replaced by Normal F primer and all the other contents are the same as tube M.
heterozygote form of new insertion. The presentation of severe anemia by coinheritance of the new insertion with other \( \alpha \)-gene mutations (MED, POLY A1) can amplify the role of new insertion in production of very unstable hemoglobin, that presents as an unknown variant hemoglobin in patients’ hemoglobin electrophoresis. Our theory is supported by normal hemoglobin electrophoresis of parents without variant hemoglobin. This could confirm that heterozygote form of new insertion produces a small amount of unstable hemoglobin with no specific effect on hemoglobin level and RBC indices, but double heterozygote form of 21 base pair insertion in \( \alpha \)-globin gene with MED deletion in another allele which causes the complete deletion of \( \alpha \)-globin gene which leads to not presenting a band in N tube.

**Conclusion**

This new 21 base pair insertion cannot affect blood parameters on its own, but can present as continuous blood transfusion-dependent \( \alpha \)-thalassemia. Thus, it is important to take this point into account for detecting the carriers, like \( \beta \)-thalassemia carriers, which can present as transfusion-dependent children in parents with \( \alpha \)-thalassemia trait.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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