Co-inheritance of $^{\text{MED}}$ Double Gene Deletion and $\alpha\alpha\alpha^{\text{Anti3.7}}$ Triplcation on $\alpha$-globin Gene in Mazandaran at 2016

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Abstract

Background: Alpha Thalassemia is one of the most prevalent disorders worldwide with a high carrier rate in Mazandaran province (north of Iran). Carriers of $^{\text{MED}}$ double gene deletion are at risk of having a child with haemoglobin H (HbH) disease, if they marry a silent carrier. Co-inheritance of $\alpha\alpha\alpha^{\text{Anti3.7}}$ triplcation that cannot be detected using hematological indices and $\beta$-globin gene mutations, in heterozygote states, leads to intermediate form of thalassemia. Using precise molecular analysis, the mutations that do not change the hematological parameters can be identified. The diagnosis of these mutations is important in screening programs.

Materials and Methods: Multiplex Gap-PCR and reverse hybridization assay analysis were applied for the detection of mutations on $\alpha$ and $\beta$-globin genes in a patient with abnormal hematological indices from Sari at 2016.

Results: A rare co-inheritance of $^{\text{MED}}$ double gene deletion and $\alpha\alpha\alpha^{\text{Anti3.7}}$ triplcation was identified.

Conclusion: The presented case can be at risk of having a child with HbH disease and thalassemia intermedia. So, the presented case shows the importance of precise molecular analysis in premarital screening in order to prevent having a child with thalassemia.

Keywords: Alpha Thalassemia; $\alpha\alpha\alpha^{\text{Anti3.7}}$ triplcation; $^{\text{MED}}$ double gene deletion

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analysis, the mutations that do not change the hematological parameters can be identified. The identification of these mutations is important in screening programs and decreases the chance of having a child with thalassemia.

Materials and methods
A 22-year-old male from Sari (Mazandaran Province, north of Iran) was referred to a medical laboratory for routine screening of hemoglobin disorders in 2016. After CBC analysis (Cell Blood Count), hemoglobin electrophoresis was performed using capillary method (Sebia, France). Genomic DNA was extracted from peripheral blood using QIAamp DNA mini kit (Qiagen - Germany). In order to detect mutations responsible for α-thalassemia, two multiplex-Gap-PCR reactions were applied. The first one could simultaneously detect the presence of six deletions including $–^{\text{MED}}$, $–^{20.5}$, $–^{\text{FR}}$, $–^{\text{SEA}}$, $–^{\text{α3.7}}$, and $–^{\text{α4.2}}$ (9) while the second one could just identify $\alpha^{\text{Anti3.7}}$ triplication (10). Reverse hybridization assay with a commercial kit (Vienna Lab, Austria) was also used for the detection of 22 common β-globin gene mutations in the region.

Ethics Statement
The patient signed an informed consent.

Results
CBC and hemoglobin electrophoresis results were suggestive of α-thalassemia pattern (Table 1).

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (year)</th>
<th>RBC (x106/μl)</th>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>HbA (%)</th>
<th>HbA2 (%)</th>
<th>HbF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>22</td>
<td>5.76</td>
<td>12.6</td>
<td>41.7</td>
<td>72</td>
<td>21.8</td>
<td>30.2</td>
<td>97.3</td>
<td>2.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The results of the multiplex-Gap-PCR tests showed that the subject is compound heterozygote for $–^{\text{MED}}$ double gene deletion and $\alpha^{\text{Anti3.7}}$ triplication ($–^{\text{MED}}/\alpha^{\text{Anti3.7}}$) (Figure 1).

Besides, the patient did not carry the 22 common β-globin gene mutations (Figure 2).

Discussion
Since thalassemia is one of the most common health problems in Iran, premarital genetic counseling, screening for identification of β-thalassemia carriers and reducing affected childbirths have been started since 1997 in Iran (11). The precise and deep molecular analysis of the suspected cases helps decreasing the number of childbirth with thalassemia. Tamadoni et al. reported that the frequency of $–^{\text{MED}}$ double gene deletion was 4.3% among 255 patients with hypochromic and microcytic anemia (12), but our previous study in Mazandaran province showed that from the 416 randomly selected neonates no one was carrier of $–^{\text{MED}}$ double gene deletion and 2.2% (CI 95%: 0.78-3.62) of the newborns had $\alpha^{\text{Anti3.7}}$. 

Figure 1. Multiplex Gap-PCR for the detection of –Med double gene deletion (lanes 1 to 4) and $\alpha^{\text{Anti3.7}}$ triplication (lanes 6 to 9). Lane 1: positive control, 2: the case, 3: the normal subject, 4: NTC, 5: ladder, 6: positive control, 7: the case, 8: normal subject, and 9: NTC.
triplication (6). It should be mentioned that the carrier frequency of $\text{aaa}_{\text{anti}3.7}$ triplication in Tehran was 2.14% (8). Hence, co-inheritance of these mutations is a rare event in the region. The presence of $\text{aaa}_{\text{anti}3.7}$ triplication can lead to extra production of $\alpha$-globin chains and co-inheritance of this triplication with $\beta$-thalassemia mutations could end up with severe anemia (8 and 11).

Coexistence of $\text{aaa}_{\text{anti}3.7}$ triplication and $-\alpha^3.7$ single mutation was reported in an Omani person who had children with $\beta$-thalassemia intermedia phenotype (13). Giordano et al. have also reported two cases with $-\alpha^3.7$/aaa$_{\text{anti}3.7}$ genotype with hematological indices similar to those with $-\alpha$/aa genotype (14). Interestingly, in the presented case the hematological indices are similar to patients with $-\alpha$/aa genotype. The presented case co-inherited --MED double gene deletion and $\text{aaa}_{\text{anti}3.7}$ triplication. Around 5.5% of Mazandaran populations are carriers of $\beta$-thalassemia and 15% of newborns are silent carriers for $\alpha$-thalassemia (6 and 15) and if the presented case mates with a subject from the mentioned population there is the probability of the birth of children affected with HbH or thalassemia intermedia. Since hematological indices of this case is affected with HbH or thalassemia intermedia due to co-inheritance of triplicated alpha-globin genes (alpha alpha/alpha alpha (anti 3.7)) and beta-thalassemia trait (IVS-II-1 G-->A), ZhonghuaXue Ye XueZaZhi. 2000; 21(4):195-7. PMID: 11876981


