

## Co-inheritance of $--^{MED}$ Double Gene Deletion and $\alpha\alpha^{Anti3.7}$ Triplication 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  $--^{MED}$  double gene deletion are at risk of having a child with haemoglobin H (HbH) disease, if they marry a silent carrier. Co-inheritance of  $\alpha\alpha^{Anti3.7}$  triplication 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  $--^{MED}$  double gene deletion and  $\alpha\alpha^{Anti3.7}$  triplication 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;  $\alpha\alpha^{anti3.7}$  triplication;  $--^{MED}$  double gene deletion

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### Introduction

Alpha thalassemia is one of the most frequent inherited hemoglobin disorders worldwide (1). Healthy individuals have four copies of  $\alpha$ -globin genes that are located on 16p13.3. Based on the copies of mutated  $\alpha$ -globin genes, a wide spectrum of clinical manifestations ranging from asymptomatic carriers to severe forms of anemia incompatible with life is anticipated. The disease is categorized into four types according to the copies of  $\alpha$ -globin gene deletions: silent carrier ( $-\alpha/\alpha$ ), alpha-thalassemia trait ( $-\alpha/-\alpha$  or  $--/\alpha\alpha$ ), HbH disease ( $--/-\alpha$ ), and hemoglobin Bart's hydrops fetalis syndrome ( $--/--$ ) (2-5).  $--^{MED}$  and  $--^{20.5}$  are double gene and  $-\alpha^{3.7}$  and  $-\alpha^{4.2}$  are single gene deletions reported from Iran.

$\alpha$ -globin gene deletions have high incidence rate in the north of Iran and our previous work showed that 15.29% (CI 95%: 11.81-18.77) of neonates in Mazandaran province carry one of the five common mutations on  $\alpha$ -globin gene (6). The  $-\alpha^{3.7}$  single gene deletion was the most common mutation among neonates, none of them had  $--^{MED}$  double gene deletion and 2.2 % carried  $\alpha\alpha^{Anti3.7}$  triplication.

Carriers of  $--^{MED}$  double gene deletion are at risk of having a child with HbH disease if they marry a silent carrier individual and there is 25% likelihood of childbirth with thalassemia intermedia if carriers of  $\alpha\alpha^{Anti3.7}$  triplication mate with a  $\beta$ -thalassemia minor subject (7). In individuals with  $\alpha\alpha^{Anti3.7}$  triplication, hematological parameters are similar to normal subjects (8). Using precise molecular

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 QIA amp DNA mini kit (Qiagen- Germany). In order to detect mutations responsible for  $\alpha$ -

thalassemia, two multiplex-Gap- PCR reactions were applied. The first one could simultaneously detect the presence of six deletions including  $^{-MED}$ ,  $^{-20.5}$ ,  $^{-FIL}$ ,  $^{-SEA}$ ,  $^{-\alpha^{3.7}}$ , and  $^{-\alpha^{4.2}}$  (9) while the second one could just identify  $\alpha\alpha^{Anti3.7}$  triplication (10). Reverse hybridization assay with a commercial kit (Vienna Lab, Austria) was also used for the detection of 22 common  $\beta$ -globin gene mutations in the region.

### Ethics Statement

The patient signed an informed consent.

### Results

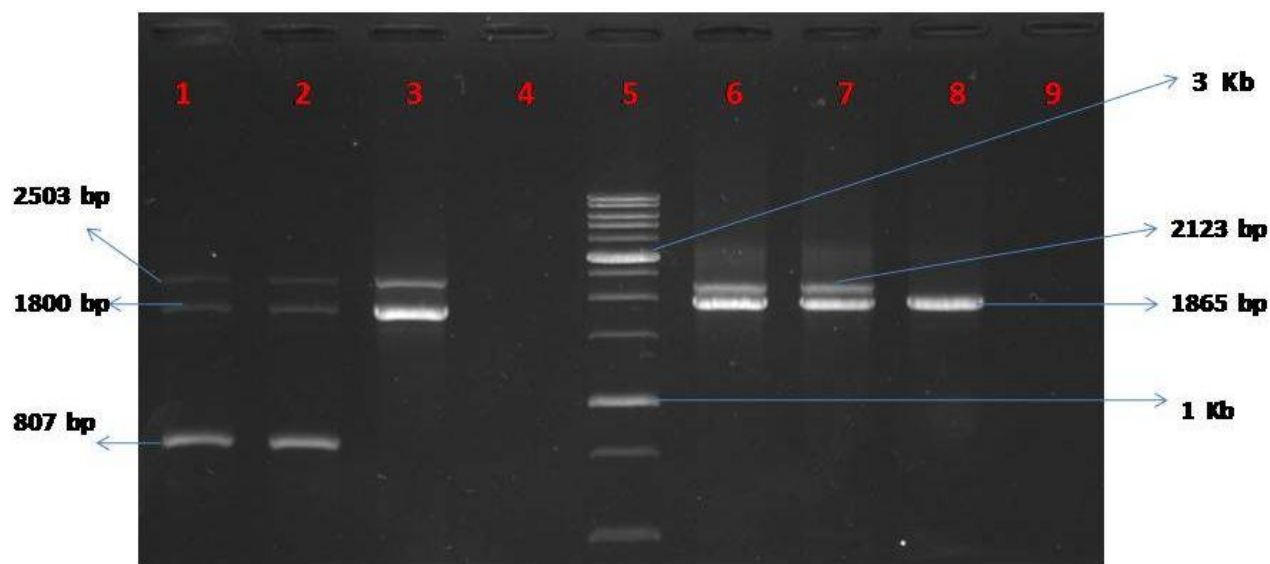
CBC and hemoglobin electrophoresis results were suggestive of  $\alpha$ - thalassemia pattern (Table 1).

**Table1.** Hematological indices of a case with  $^{-MED}$  double gene deletion and  $\alpha\alpha^{Anti3.7}$  triplication.

Gender	Age (year)	RBC (x106/ $\mu$ l)	Hb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	HbA (%)	HbA2 (%)	HbF (%)
Male	22	5.76	12.6	41.7	72	21.8	30.2	97.3	2.5	0.3

The results of the multiplex-Gap- PCR tests showed that the subject is compound heterozygote for  $^{-MED}$

double gene deletion and  $\alpha\alpha^{Anti3.7}$  triplication ( $^{-MED}/\alpha\alpha^{Anti3.7}$ ) (Figure 1).



**Figure 1.** Multiplex Gap- PCR for the detection of  $^{-Med}$  double gene deletion (lanes 1 to 4) and  $\alpha\alpha^{Anti3.7}$  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.

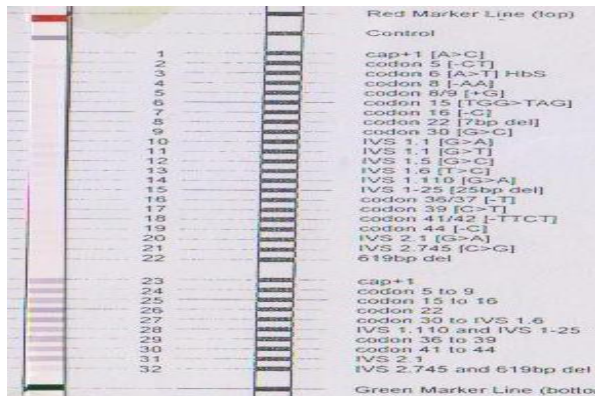
Besides, the patient did not carry the 22 common  $\beta$ -globin gene mutations (Figure 2).

### Discussion

Since thalassemia is one of the most common health problems in Iran, premarital genetic counseling, screening for identification of  $\beta$ -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  $^{-MED}$  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  $^{-MED}$  double gene deletion and 2.2% (CI 95%: 0.78-3.62) of the newborns had  $\alpha\alpha^{Anti3.7}$

triplication (6). It should be mentioned that the carrier frequency of  $\alpha\alpha^{\text{Anti3.7}}$  triplication in Tehran was 2.14% (8). Hence, co-inheritance of these mutations is a rare event in the region. The presence of  $\alpha\alpha^{\text{Anti3.7}}$  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).



**Figure 2.** The result of reverse strip assay for  $\beta$ -globin gene mutations. The patient did not carry 22 investigated mutations.

Coexistence of  $\alpha\alpha^{\text{Anti3.7}}$  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  $-\alpha^{3.7} / \alpha\alpha^{\text{Anti3.7}}$  genotype with hematological indices similar to those with  $-\alpha / \alpha\alpha$  genotype (14). Interestingly, in the presented case the hematological indices are similar to patients with  $-- / \alpha\alpha$  genotype. The presented case co-inherited  $--^{\text{MED}}$  double gene deletion and  $\alpha\alpha^{\text{Anti3.7}}$  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 disorders. Since hematological indices of this case is similar to the subjects with double gene mutation (low MCV and MCH), precise molecular screening of  $\alpha$ -globin gene is recommended for couples with atypical hematology. Besides, molecular analysis for the detection of  $\alpha\alpha^{\text{Anti3.7}}$  triplication is also suggested to be considered in screening programs even in individuals with normal hematological parameters.

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### Author contributions

MK introduced the case to the lab. MM carried out

the test. HJ wrote the article. M.R.M supervised the article.

### Conflicts of Interest

The authors declare no conflict of interest.

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