

## Identification of a Neonate with Thalassemia Intermedia Despite Premarital Screening Program in Mazandaran Province (Co-inheritance of Hb<sup>Knossos</sup> and IVS II-1 G> A Mutations)

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### Abstract

**Background:** Beta thalassemia is a common health problem in Iran especially in Northern provinces. Premarital screening for thalassemia is compulsory in Iran and identification of the carriers is based on primary CBC (Cell Blood Count) and hemoglobin electrophoresis. Silent mutations on  $\beta$ -globin gene have borderline or normal hematological indices that cannot be detected in premarital screening.

**Materials and methods:** A four years old boy affected with  $\beta$ -thalassemia was referred to the lab for molecular analysis. During the screening program for  $\beta$ -thalassemia, his father and mother were diagnosed as  $\alpha$ - and  $\beta$ -thalassemia carrier respectively.

**Results:** The results of molecular analysis showed that in addition to the  $\alpha$ -globin single gene deletion ( $\alpha^{3.7}$ ), the father had also carried a silent mutation on his  $\beta$ -globin gene named HBB c.82G>T or Hb<sup>Knossos</sup> that was missed in screening program.

**Conclusion:** The presented case shows that using CBC and hemoglobin electrophoresis in premarital screening program for detecting  $\beta$ -thalassemia carriers is not a valid approach and individuals who are carriers of silent  $\beta$ -globin gene mutations are missed in this procedure. Hence, in premarital screening program precise molecular investigation especially when the partner is a typical  $\beta$ -thalassemia carrier is recommended.

**Keywords:**  $\beta$ -thalassemia; Hb<sup>Knossos</sup>; Premarital screening

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### Introduction

Beta thalassemia is an autosomal recessive monogenic disease with high incidence rate among Mediterranean, Middle Eastern and Indian population. The gene responsible for this anemia is located on chromosome 16 and up to now, more than 200 mutations have been identified related to the disease (1, 2). Beta thalassemia is also a common health problem in Iran especially in Northern provinces (3, 4).

Premarital screening program for thalassemia has been started since 1997 in Iran (5). This program encourages  $\beta$ -thalassemia carriers to participate in counseling and prenatal diagnosis (PND). At screening process, first the hematological indices of the couples are

measured in health center's labs and if they have atypical hematological indices, the subsequent hemoglobin electrophoresis will be used to identify whether the cases are carriers of  $\alpha$ - or  $\beta$ - thalassemia. In this protocol, if one of the couple is being a carrier of beta thalassemia and the partner has normal hematological indices, further molecular investigations usually will not be followed (6).

There are some rare silent mutations that do not change the hematological indices of the carriers and as a result they cannot be identified in primary analysis (7). Compound heterozygotes for silent mutations and other severe beta-thalassemia alleles are

phenotypically severe enough to necessitate appropriate therapy and counseling (8). In the present study, we report a couple with a child affected with  $\beta$ -thalassemia in which the father was misdiagnosed as  $\alpha$ -thalassemia carrier while in addition to  $\alpha$ -thalassemia he carried a silent mutation on  $\beta$ -globin gene too.

#### Case presentation

A four years old boy from Mazandaran Province was referred to Fajr Medical Laboratory for CBC (Cell

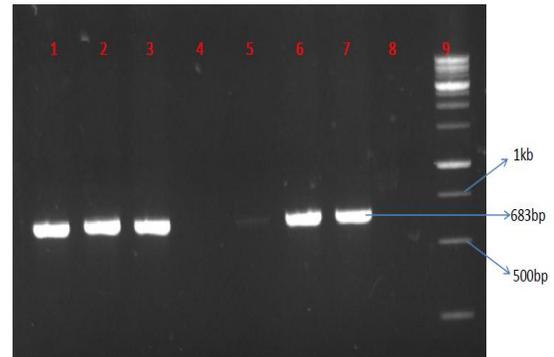
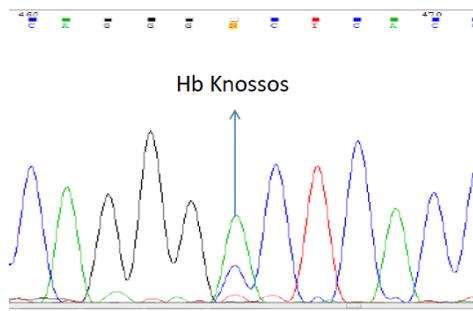
Blood Count) test as a routine checkup. The primary hematological parameters were compatible with Thalassemia, so his parents were also tested. The mother was a carrier for  $\beta$ -thalassemia, while father's hematological indices were indicative of  $\alpha$ -thalassemia silent carrier (Table1). The parents had attended at national premarital screening program for thalassemia. Considering the hematological indices, the father and the mother were also diagnosed as  $\alpha$ - and  $\beta$ - thalassemia carriers respectively with no risk for having a child with  $\beta$ - thalassemia. So, further molecular analysis was not followed.

**Table1.** The hematological indices of the case and his parents.

|               | Age (Year) | RBC (x 10 <sup>6</sup> / $\mu$ l) | Hb (g/dL) | Hct (%) | MCV (fl) | MCH (pg) | MCHC (g/dl) | Hb-A1 (%) | Hb-A2 (%) | Hb-F (%) |
|---------------|------------|-----------------------------------|-----------|---------|----------|----------|-------------|-----------|-----------|----------|
| <b>Case</b>   | 4          | 3.86                              | 7         | 21.2    | 54.9     | 18.1     | 33          | 52.9      | 1.2       | 45.9     |
| <b>Father</b> | 37         | 5.05                              | 12.1      | 38.1    | 75.5     | 24       | 31.8        | 98.1      | 1.9       | <0.5     |
| <b>Mother</b> | 36         | 4.62                              | 9.1       | 28.5    | 61.7     | 19.7     | 31.9        | 92.8      | 5.3       | 1.9      |

For molecular analysis, at first, genomic DNA was extracted using QIA amp DNA mini kit (Qiagen-Germany). Multiplex- GAP-PCR and PCR sequencing techniques were applied for the detection of common deletions on  $\alpha$ -globin genes and probable point mutations respectively. For the identification of the IVSII-1 G>A mutation (the most common mutation in the region), ARMS-PCR method was used and PCR-Sequencing method was applied to detect other mutations.

The results of DNA analysis on  $\beta$ -globin gene showed that the patient was compound heterozygote for HBB: c.82G>T (Hb<sup>Knossos</sup>) (Figure1) and HBB: c.315+1G>A (IVS II-1 G> A) mutations and no mutation was detected on his  $\alpha$ -globin genes. The mother was heterozygote for IVSII-1 G>A mutation on  $\beta$ -globin gene and she had no mutation on her  $\alpha$ -globin genes. The father carried Hb<sup>Knossos</sup> on  $\beta$ -globin and  $\alpha$ 3.7 single gene deletion on  $\alpha$ -globin genes.



**Figure 1.** Left, PCR-Sequencing results showing Hb Knossos in heterozygote state. Right: The results of ARMS-PCR method for the detection of c.315+1G>A mutation. Lanes 1 and 5 (normal subject), 2 and 6 (heterozygote control), 3 and 7 (the case), 4 and 8 (Non template control), 9 (Ladder 1kb).

#### Discussion

Silent  $\beta$ -globin mutations are very mild pathogenic variants associated with normal RBC indices and normal or borderline HbA2 level. However, homozygosity for these variants or compound heterozygosity for a silent  $\beta$ -globin mutation and a typical  $\beta$ -globin pathogenic variant leads to mild non-transfusion-dependent forms of  $\beta$ -thalassemia. Since

carriers of silent  $\beta$ -globin mutations have hematological parameters close to normal ranges, it is hard to identify them using primary CBC test (7). Hb<sup>Knossos</sup> or  $\beta$ 27 (B9) Ala $\rightarrow$ Ser [HBB: c.82G>T] is a rare Hb variant that its RNA transcripts are abnormally processed (9). This variant for the first time was reported in a Greek family in which thalassemia intermedia was diagnosed in three members who were

compound heterozygous for this variant and other mutations responsible for  $\beta$ -thalassemia (10). Hb<sup>Knossos</sup> has been identified in Africa and Mediterranean region at incidence rate lower than 1 % and with a relatively higher frequency in Jordan (3.3 %) (11). Alpha and  $\beta$ -thalassemia are common health problems in Mazandaran province (12, 13). Besides, several cases with Hb<sup>D</sup> (14), Hb<sup>S</sup> (15), Hb<sup>J-Toronto</sup> (16), and Hb<sup>Setif</sup> (17) were reported from the region. At 2012, we reported another case with co inheritance of Codon 8 and a mild  $\beta$ -globin mutation +22 5'UTR (G>A) that was missed in screening program (13). In Iran, Hb<sup>Knossos</sup> for the first time was reported from Mazandaran and in a case that like the presented case was compound heterozygote for c.82G>T and c.315+1G>A mutations (18). In that study, the Hb<sup>Knossos</sup> carrier had normal hematological indices. Hence in the presented case the slight reduced CBC parameters in Hb<sup>Knossos</sup> carrier subject was related to  $\alpha^{3.7}$  deletion. These cases show that premarital screening for thalassemia based on primary CBC test cannot detect all  $\beta$ -thalassemia carriers especially silent carrier ones. Since  $\beta$ -thalassemia is a common health problem in Mazandaran province, precise DNA analysis of subjects with normal hematological indices is recommended in premarital screening program especially when the partner is a typical  $\beta$ -thalassemia carrier in order to control child births with anemia.

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#### Author Contributions

HK introduced the case to the lab and wrote the article, HJ and MM carried out the tests. MRM supervised the article.

#### Conflict of Interest

The authors have no conflict of interest.

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