

Case Report: Silent β -thalassemia: Transition Mutation of the β -globin Gene (Promoter nt-101 C>T)



Zeynab Gharehdaghi¹ , Arghavan Hosseinpouri² , Narges Obeidi^{1,3*}

1. Department of Hematology, Faculty of Paramedicine, Bushehr University of Medical Sciences, Bushehr, Iran.
2. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Blood Transfusion Organization, Bushehr, Iran



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* Corresponding author:

Narges Obeidi, PhD.

Address: Department of Hematology, Faculty of Paramedicine, Bushehr University of Medical Sciences, Bushehr, Iran.

E-mail: nobeidi@yahoo.com

Abstract

Introduction: Heterozygote β -thalassemia is called carrier or β -thalassemia trait (BTT). Carriers have no clinical symptoms but sometimes have a mild anemia. They can often be identified with MCV<80 fl, MCH<27 pg and HbA₂> 3.5 %. However, these tests are not enough to diagnose some unexpected beta-globin mutations in premarital or prenatal screening.

Case Presentation: The mentioned case was one of the most common silent β -thalassemia mutations (promoter nt-101C>T).

Conclusion: It was the first report from Fars (Iran) and the second one from Iran. The case had normal hematologic indices and borderline hemoglobin A₂ values that may be mistakenly interpreted as normal. The presented case showed that electrophoresis and PCR sequencing methods should be applied for screening thalassemia.

Keywords: beta-Thalassemia, Genetic Carrier Screening, Heterozygote, Hematological Diseases, Anemia

1. Introduction

β thalassemia is defined as a hereditary blood disorder caused by defects in the synthesis of hemoglobin β -chains (HBB) [1]. More than 300 different mutations in the HBB gene have been reported so far, [2]. Some mutations cause complete loss of HBB (β^0), while some result in partial synthesis (β^+) [3]. Heterozygote β -thalassemia is called minor, carrier or β -thalassemia trait (β TT) [4]. Statistics suggest that 1.5% of the

world's population (80-90 million people) and approximately 2-3 million of the Iranian population are carriers [4, 5]. The risk of having a child with β -thalassemia major from the marriage of two carriers in each pregnancy is 25% [4]. Therefore, screening this silent disorder and people's awareness about their inherited trait is effective in controlling its progression. Carriers have no clinical symptoms or sometimes have a mild anemia. Laboratory diagnosis of BTT is carried out in three steps. In the first step, Complete Blood Counter (CBC) indices for BTT are mostly described as microcytosis (mean cor-

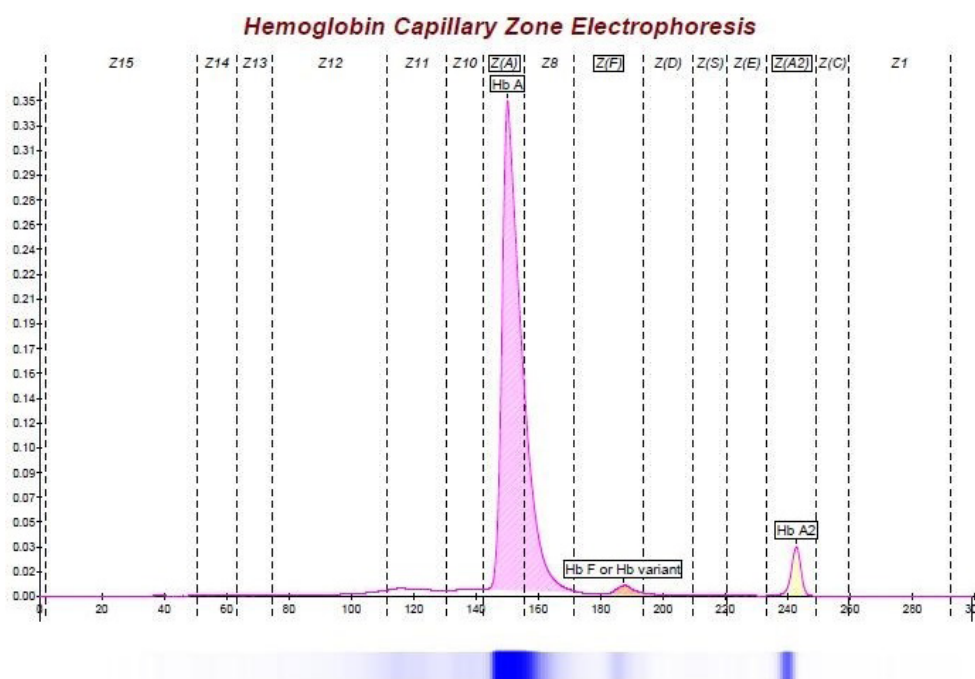


Figure 1. Hemoglobin capillary electrophoresis result of case with heterozygote -101 C to T mutation: HbA2: 3.6%, HbA: 95.4%, HbF: 1%

puscular volume [MCV]<80 fl) and Mean Corpuscular Hemoglobin (MCH)<27 pg [6]. If the mentioned case is observed, Hb electrophoresis is ordered in the second step. The Hb pattern of BTT is characterized by 92–95% HbA, HbA2>3.8%, and variable amount of HbF (0.5–4%). Some studies have confirmed normal CBC indices and HbA2<3% or borderline (3.1-3.9%) in BTT sample incorrectly diagnosed as normal [7, 8]. In the third step,

all mutations (on promoters/ exons/introns/ 3'UTR) such as point mutation, frameshift, deletion are detected by the Amplification Refractory Mutation System- Polymerase Chain Reaction (ARMS-PCR) methods and DNA sequencing [2, 9]. In this report, one of the silent β -thalassemia mutations (nt-101 C>T) with normal red cell indices and borderline HbA2 level while apparently healthy is presented.

Table 1. Erythrocyte indices and hemoglobin electrophoresis

Clinical Parameters	Mean±SD
Age (years)	21
Hb (g/dl)	12.5±0.5
Hematocrit (%)	38.4±1.3
MCV (fl)	85.9±1.8
MCH (pg)	27.9±0.5
MCHC (g/dl)	32.5±0.9
RBC (x10 ⁶ /L)	4.48±0.2
RDW (%)	12.7±0.3
HbA (%)	95.4 ±0.07
HbA2 (%)	3.6±0.03
Hb F (%)	0.9±0.14

2. Case Presentation

A 21-year-old girl referred to central laboratory (Fars, Iran) for premarital hematological tests in July 2019. CBC showed normal red cell indices (MCV: 87.4 fl, MCH: 28.4 pg). Apparently, there was no doubt about the existence of trait thalassemia. Since her fiancé was a typical case of β -thalassemia minor (RBC: $7.05 \times 10^6/\mu\text{l}$, MCV: 63.3 fl, MCH: 18.6 pg), both were referred for Hb electrophoresis and re-CBC testing. β -thalassemia minor was diagnosed in the boy with an HbA2 value of about 5%. HbA2 in the girl was borderline (3.6%) as shown in Table 1. As the case was serious, the test was repeated for which similar results were found. Deficiency of vitamin B12 and folic acid, which could induce increased HbA2, was ruled out (folic acid: 7.24 ng/ml, vitamin B12: 288.9 pg/ml). Clinical examination excluded other etiology such as liver disease, medications, and iron deficiency. However, hypothyroidism was revealed by the thyroid function test (TSH: 4.77 $\mu\text{IU/ml}$, normal range 0.3- 3.94 $\mu\text{IU/ml}$). Molecular studies on DNA were prioritized to indicate the unusual β -thalassemia genotype. DNA was extracted from peripheral blood sample to detect the 20 most common Iranian β -thalassemia mutations by ARMS-PCR. No mutation was identified in this case. Therefore, we performed beta-globin gene DNA sequencing. Mutational analysis proved to be heterozygous for -101 C>T β -thalassemia trait mutation.

3. Discussion

β -thalassemia trait was identified by reduced β -globin chain synthesis, having high incidence in the Middle East and Mediterranean origin. No significant hematological or clinical consequence has been found in the majority of carriers. Therefore, laboratory indicators were required for accurate diagnosis [10]. One of the most effective solutions to reduce thalassemia is to prevent the birth of children with thalassemia by identifying the carrier parents before marriage or prenatal diagnosis [11]. The thalassemia prevention program has formally been implemented in Iran since 1997 with the purpose of identifying couples who carry beta thalassemia for premarital and prenatal counseling. According to β -thalassemia screening guidelines, more accurate tests such as Hb electrophoresis are ordered for individuals with MCV<80fl and MCH <27 pg [12]. Despite the fact that in recent years BTT has been found in individuals with normal CBC indices, it could indicate non-silent heterozygote β -thalassemia [8]. The first identified case with the silent β -globin mutation, -101 C>T in 1989 had normal hematological indices and normal or borderline HbA2 and

HbF value [13]. This mutation within the distal CACCC box decreased by approximately 20% in β -globin production due to decreased binding and transactivation of EKLF to the mutant promoter [14]. Although this mutation is the most common in the Mediterranean origin (specially Italy and Greece), its report has been limited so far [8]. In the case mentioned here, we presented normal CBC parameters and borderline HbA2. After exclusion of other etiologies, DNA sequencing demonstrated promoter nt-101 C>T mutation. Earlier, Najmabadi et al. reported a homozygous silent -101 C>T mutation individual with Turkish ethnic origin in Iran [15]. Heydari et al. (2017) detected this mutation in five individuals with non-silent heterozygote β -thalassemia (MCV<80fl and MCH <27 pg) [16]. In conclusion, this silent (promoter nt-101 C>T) β -thalassemia mutation case was the first report in Fars and the second one in Iran. The presented case showed that -101 C>T leads to normal hematological indices and clinical awareness. Meanwhile, with normal MCV and MCH, thalassemia trait cannot be rejected. Familial history was not checked in this report; however, if a history of thalassemia is available, DNA molecular analysis seems necessary even if the HbA2 is not to be elevated.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles are considered in this article. The participants were informed of the purpose of the research and its implementation stages. They were also assured about the confidentiality of their information and were free to leave the study whenever they wished, and if desired, the research results would be available to them.

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Author's contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declare no conflict of interest.

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