CASE REPORT

Coinheritance of Sicilian ($\delta\beta$)⁰-Thalassemia and Two Rare Hemoglobin Variants: A Complex Case of Hemoglobinopathy

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Abstract α -Thalassemia (α -thal) is considered as the most common inherited hemoglobin disorder worldwide. The present study describes the first observation of a combination of rare α -chain variants, and β -globin gene cluster deletion. A 21-year-old woman with thalassemia trait, marked microcytosis, mild anemia, and normal range of Hb F was referred to Amirkola genetic center in the North of Iran for routine molecular test of thalassemia in the context of carrier detection and prevention of thalassemia major birth. Nucleotide sequencing revealed a novel compound heterozygosity status for two non-deletional mutations on *HBA2*, Hb O Indonesia ($\alpha 116(\text{GH4})\text{Glu} \rightarrow \text{Lys}$), and Hb Matsue–Oki ($\alpha 75(\text{EF4})\text{Asp} \rightarrow \text{Asn}$), together with heterozygosity for the sicilian ($\delta\beta$)⁰-thal mutation. This finding highlights the necessity of deep molecular investigation of thalassemia in regions where thalassemia is abundant, and present highly heterogeneous population.

Keywords α -Thalassemia (α -thal) · *HBA2* · Mutation · HbF · Iran

Introduction

2 α -like and 2 β -like globin chains ($\alpha_2\beta_2$) would make human hemoglobin (Hb) at all the development stages. Mutations in the globin genes may cause blood disorder, during which the production, stability, and functional properties of Hb tetramers are affected. Unlike beta globin, 2 α -globin genes, i.e., $\alpha 1$ and $\alpha 2$ (*HBA1* and *HBA2*), regulate α -globin synthesis on each copy of chromosome 16 (16p13.3). α -Thalassemia (α -thal) is considered as the most common inherited hemoglobin disorder worldwide, which is caused by deletional or non-deletional mutations of one or both α -globin genes characterized by hypochromic microcytic anemia with marked phenotypic heterogeneity that ranges from asymptomatic to lethal in utero. Most α -thal mutations are associated with deletions of one or both α -globin genes unlike the domination of non-deletional mutations in β -thal [1]. However, compared to the deletional α -thal types, a more severely reduced α -chain synthesis may be resulted from the non-deletional α -thal. The $-\alpha^{3.7}$ (rightward) deletion in α -thal appears as the most frequent α -globin gene mutation in Iran, and is mostly asymptomatic. $-\alpha^{3.7}$ is an example of α^+ thalassemia, in which some α -globin chains are produced. When both α genes on a single chromosome are inactive, the α^0 thalassemia is designated, which can present a more severe phenotypic manifestation based on the deleted region size of α -globin gene cluster.

mRNA processing and translation, as well as α -globin stability of either $\alpha 2$ - or $\alpha 1$ -globin genes have been described to be influenced by many α -globin point mutations. Considering its higher level of expression, $\alpha 2$ -globin gene is mostly involved by the mentioned mutations. Generally, an abnormal α -chain Hb may be resulted from point mutations caused by the variant α -globin. Some of

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these hemoglobin variants are asymptomatic in the heterozygous state or may lead to only borderline hematological abnormalities. The phenotype is more evident when the patients are homozygous for such a variant or compound heterozygous with another α -thalassemic defect [2].

Here, we report the first observation of a combination of Hb O Indonesia ($\alpha 116(\text{GH4})\text{Glu} \rightarrow \text{Lys}$), and a rare α -chain variant, Hb Matsue–Oki ($\alpha 75(\text{EF4})\text{Asp} \rightarrow \text{Asn}$), in a 21-year-old female from Northern Iran, who was also heterozygous for the sicilian ($\delta\beta$)⁰-thal mutation with a normal range of Hb F. She presented thalassemia trait with a marked microcytosis and mild anemia that was referred to Amirkola genetic center, Babol, Iran, and the molecular basis of her anemia still remained unknown after applying a routine alpha globin gene mutation analysis.

Case Presentation

A non-consanguineous married couple from Mazandaran province in Northern Iran with a positive history of β -thalassemia major in the husbands' family was referred to Amirkola genetic center in Babol, Mazandaran for molecular analysis. The direct and indirect analyzes of α and β -globin gene mutations were performed for the husband and no mutations were detected. His wife, a 21-year-old female, was also screened for common β -globin gene mutations using an in-house reverse dot-blot assay and no mutations were found. Regarding the hematologic indices (Table 1), the presence of common deletional delta beta and α -globin gene mutations were also analyzed. The results revealed that the wife had sicilian ($\delta\beta$)⁰-thal mutation although the Hb F level was in the normal range. On the other hand, the hematological indices of the mentioned case were lower than an explainable mutation (Table 1), thus being indicative of carrying another α -globin gene defect. Therefore, the patient's α -globin gene was analyzed by a direct conventional sequencing of amplified DNA. The sequencing result revealed the presence of 2 variants

for the patient. Hb O-Indonesia [A2] [$\alpha 116(\text{GH4})\text{Glu} \rightarrow \text{Lys}$ (HBA2:c.394G > A)] and Hb Matsue–Oki [$\alpha 75(\text{EF4})\text{Asp} \rightarrow \text{Asn}$ (HBA2:c.226G > A)] (Fig. 1).

Discussion

Both alpha and beta thalassemia are frequent in Northern provinces of Iran, which harbors a very heterogeneous population [3]. Hb O-Indonesia, known as Hb Oliviere or Hb Buginese-X, has been reported in the Iranian, Italian, South African, Chinese, Makassar and Bugis of Sulawesi populations [4]. This known variant was reported previously to occur in HBA1 in the HbVar database. However, through DNA sequencing of $\alpha 2$ -globin gene, we identified Hb O Indonesia resulting from HBA2 instead of HBA1 gene mutation. We found a G > A nucleotide change in codon 116 [HBA2: c. 116G > A ($\alpha 2$)] causing a change in glutamic acid $\rightarrow$ lysine. A similar observation was reported by Henderson et al. [5] in the UK screening program. The residue location is on the external surface of the corner between the G- and H-helices. This position is near the $\alpha\beta 1$ contact. The replacement of negatively charged glutamic acid by positively charged lysine at residue 116 is structurally significant and thus can potentially lead to a functional or assembly defect. The previous studies demonstrated that the Hb O mutation is associated with either the instability of α^{OIna} -globin chain or less efficient assembly of the hemoglobin in vivo [6], which is also observed as a consequence of Hb E mutation due to the changes in Glu26Lys amino acid in the β -globin chain. Hb O Indonesia was also found in combination with Sickie Hemoglobin (Hb S) in an Indian family, where the newborn infant was seen to have reduced Hb levels and need to be followed up. The baby's follow-up suggested no clinical problems [7]. The mentioned hemoglobin was also found in association with Hb D-Punjab, but the presence of abnormal hemoglobins was not associated with hemolytic disorders or obvious clinical symptoms. Carrying a

Table 1 $\alpha 2$ and β genotypes, hematological indices and phenotypes in the two subjects

Patients	$\alpha 2$ genotype	β Genotype	RBC ($10^{12}/\text{L}$)	Hb (g/ dL)	MCV (fL)	MCH (pg)	Hb (%)			Phenotype
							A	A2	F	
Husband	Normal	Normal	5.45	13.7	74.1	25.1	95.1	3	1.8	Mild hemolytic anemia
Wife ^a	HBA2:c.349G > A, HBA2:c.226G > A	Carrier sicilian ($\delta\beta$) ⁰	5.34	10.9	63.1	20.4	94.7	3.3	2	Mild hemolytic anemia

RBC red blood cell count; MCV mean corpuscular volume; MCH mean corpuscular Hb; Hb hemoglobin

^a The patient was heterozygous for sicilian ($\delta\beta$)⁰ mutation and two HBA2 variants (HBA2:c.349G > A, HBA2:c.226G > A)

Fig. 1 Sequence chromatograms of the $\alpha 2$ -globin gene. **a** codon75 (G > A) mutation in a heterozygous subject; **b** codon 116 (G > A) mutation in a heterozygous state in the same patient. The *arrow* indicates the site of the mutation

heterozygous Hb O mutation was also observed to be involved in a milder phenotype of a homozygous β -thalassemia individual [8].

We also found another transition at codon 75 (HBA2: c. 226G > A) changing the protein residue aspartic acid to asparagine at position α 75(EF4) Asp → Asn (GAC > AAC) on the $\alpha 2$ -globin gene that was previously reported as Hb Matsue–Oki. The aspartic acid residue is the 4th amino acid of the EF interhelical segment and is located in the external position of the Hb molecule. It is not involved in the heme or interchain contacts. The deletion of one of the aspartyl residues described in Hb Watts induces only a slight instability in vitro without any thalassemia-like phenotypes. Hb Matsue–Oki (M–O) had been reported from a few Japanese and African ethnic origins and also in the Dutch thalassemia screening program [9, 10]. However, this is the first time it is being reported in Iran. Hb G-Philadelphia [α 68(E17)Asn- > Lys] related to α -thal-2 (–3.7 kb) was found to occur in a black family in association with Hb M–O. Their clinical and hematological conditions could be compared with a mild heterozygosity of α -thalassemia-2 as a simple Hb

G-Philadelphia trait [9], but carriers whose chromosomes showed to bear this hemoglobin variant were asymptomatic. Nevertheless, the co-inheritance of 2 rare α -chain variants and a delta beta-thalassemia mutation can be at the origin of the low hematological indices of the present mentioned case. Therefore, considering a region with such a heterogeneous population and high thalassemia prevalence, it is necessary to evaluate more precisely both common and rare point mutations as well as gene deletions through the molecular characterization of the α - and β -globin gene clusters even if the Hb F value is within the normal range. A quick and precise recognition of globin gene mutations can lead to an improved genetic counseling quality. The co-inheritance of a sicilian delta beta deletion and 2 rare α -chain variants reflects the complexity of diagnosing and prevention of thalassemia in heterogeneous populations and the necessity for a wider range of clinical, hematological, and molecular approaches to addressing this issue. In the present case, although prenatal diagnosis of the fetus was not necessary, but regarding the 50% risk of inheritance of $\delta\beta$ thalassemia from the mother, molecular analysis of this mutation in all siblings of this family is

recommended. Also, those siblings carrier of $\delta\beta$ thalassemia have about 10% chance of marrying a beta thalassemia minor (regarding carrier prevalence in the population), and therefore being at risk of having an affected child. Moreover, molecular investigation of $\delta\beta$ thalassemia in couples with anemia in which the HbF level is not necessarily elevated is recommended regarding complex situations that may happen due to the interference of modifier genes such as alpha globin. Correspondingly, carrier couples with $\delta\beta$ thalassemia each or with $\delta\beta$ thalassemia and β thalassemia need to be offered prenatal diagnosis regarding the 25% risk of having a thalassemia major or intermedia child.

Compliance with Ethical Standards

Conflict of interest Authors declare no conflict of interest.

Ethical Standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution.

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