Case Studies

Three Different Co-existing α-Thalassemia Mutations and Sickle Cell Disease in a Pregnant Woman

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ABSTRACT
Thalassemia is the most frequent single-gene defect and overwhelmingly effects prenatal patients in Iran; the condition is caused by a diverse range of mutations. We discuss an extremely rare combination of α-globin gene disease and sickle cell trait. This combination may explain a mild form of thalassemia that presents with moderate anemia.

Keywords: α-thalassemia, mutation, determinants, sickle cell disorder

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Abbreviations
Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; HbA2, hemoglobin A2; Hbs, Sickle hemoglobin; HbAS, hemoglobin AS; HbSS, hemoglobin subserosal; Hbf, fetal hemoglobin; CBC, complete blood cell count; EDTA, ethylenediaminetetraacetic acid; PCR, polymerase chain reaction; ARMS, amplification refractory mutation system; RBD, RNA-binding domain; MLPA, multiplex ligation-dependent probe amplification; PND, prenatal diagnosis; HbH, hemoglobin H; RBCs, red blood cells; RDW, red blood cell distribution width; HbA1, hemoglobin A1; NA, not applicable

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α-thalassemia, caused by a defect on the short arm of chromosome 16, is the most common inherited hemolytic anemia worldwide.1 The disease is characterized by microcytic hypochromic anemia and is inherited as an autosomal recessive disorder. Healthy people have 4 α-genes (2 α1 and 2 α2 genes). Depending on deletions and mutations in 1 or more genes, patients have different clinical manifestations, from mild to severe anemia.2 All patients have variable degrees of anemia (ie, decreased hemoglobin [Hb]), decreased mean corpuscular hemoglobin (MCH), decreased mean corpuscular volume (MCV), and a normal or slightly decreased level of hemoglobin A2 (HbA2).1 α-thalassemia is highly prevalent throughout Southeast Asia, India, the Middle East, parts of Africa, and the Mediterranean countries. The most common mutations and deletions in Iran are -α3.7, -α4.2, --MED, -HBA2: c.*94A>G, -α2 PA4: A>G, -α2 CD19 [-G], and -α2 intervening sequence (IVS-II) -5nt.3 Sickle cell disease is one of the most prevalent hemoglobinopathies and is characterized by chronic hemolytic anemia. A point mutation in the β chain leads to the substitution of glutamic acid for valine, resulting in the production of an abnormal Sickle hemoglobin (Hbs; αβS2).4 Its clinical manifestations are different depending on the state of inheritance and heterozygocity or homozygocity for this mutation. Although the heterozygote form (ie, sickle cell trait or, hemoglobin AS [HbAS]) is generally asymptomatic, in homozygote form (sickle cell disease, or hemoglobin subserosal [HbSS] disease) the patient will have sickle cell crisis (ie, vaso-occlusion, sequestration, and aplasticity), acute chest syndrome, and increased susceptibility to infections. It has also been observed5 that pregnant women with sickle cell disease are at risk of pre-eclampsia, low birth weight, and fetal loss. Severity of disease in coinheritance of HbS with β-thalassemia and/or α-thalassemia determinants depends on the number and type of genes involved.6

In the electrophoresis of HbSS disease, HbA is not observed; however, HbA2 is observed at a level of less than 85%, along with a percentage of HbA2 in the normal
Range and an increased percentage of fetal hemoglobin (HbF). In patients with HbAS disease, the HbS is approximately 40%, HbA is approximately 60%, the HbA2 percentage is normal or slightly increased, and the HbF percentage is normal.

Because of demographic diversity, different ethnicities, and migration, we have observed a spectrum of the co-inheritance of a variety of mutations and deletions. This article reports a novel co-inheritance of 3 α-thalassemia determinants (deletion, gene triplication, and point mutation) in a pregnant woman with sickle cell disease.

Material and Methods

This patient was referred by hematologists to the Prenatal diagnosis (PND) center of Shafa Hospital of the Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, with suspicion of α-thalassemia and sickle cell disease. After preliminary testing, results for complete blood cell count (CBC), hemoglobin electrophoresis (ie, acetate cellulose), and iron levels ruled out iron-deficiency anemia, genetic tests were conducted to identify and confirm the presence of thalassemia and/or hemoglobinopathies.

Venous blood was drawn from the patient and her husband and collected in an ethylenediaminetetraacetic acid (EDTA) tube for hematologic parameters determination (Sysmex K1000 Hematology Analyzer, Sysmex Corporation, Kobe, Japan) and Hb electrophoresis (cellulose acetate in alkaline pH of 8.6); also, an amniotic-fluid sample was withdrawn from the fetus. Samples for molecular DNA analysis were extracted using AccuPrep Genomic DNA Extraction Kit (Bioneer Corporation, Daejeon, South Korea).

α- and β-globin genes were analyzed using a reverse dot-blot assay as instructed (ViennaLab Diagnostics Gmbh, Vienna, Austria). Briefly, in this method the normal and mutant sequences were amplified using biotinylated primer sets by polymerase chain reaction (PCR), then hybridized to fixed probes on a nitrocellulose strip and finally, visualized by an enzymatic reaction benefiting from biotin-avidin affinity. Using the blood-analysis kit, 21 deletions, various point mutations, and an α-gene triplication could be identified simultaneously. Separate gap PCRs for the 3 most frequent deletions (3.7, 4.2, and med) were performed to verify the strip assay result. In cases in which the point mutation seen in the strip assay was present, α-globin genes were sequenced for further confirmation. The β-globin gene was primarily studied by direct sequencing; the amplification refractory mutation system (ARMS) and linkage analysis were used as secondary methods. After detection of α-gene triplication by RNA-binding domain (RBD), the heterozygous status was checked by multiplex ligation-dependent probe amplification (MLPA) genetic testing. Further, for β-globin gene sequencing, the forward and reverse strands were both sequenced to confirm cd6 homozygosity. Written informed consent was obtained from the patient involved in this study and her husband. This study was approved by Ahvaz Jundishapur University of Medical Sciences ethics committee.

Case History

A 24-year-old pregnant woman, at 7 weeks’ gestation, from Ahvaz, a city in the southwestern region of Iran, was sent to our hospital for fever, anemia, and arthralgia. The patient underwent PND assessment because of her thalassemia and her HbS background. Via genetic testing, she was found to have a –α3.7 deletion, anti-3.7 gene triplication, and α-gene point mutation HBA2: c.*94A>G (Figure 1). In β-globin gene studies, the patient was shown to have the HBB: c.20A>T, Glu6Val mutation in homozygous form (Figure 2). Finally, the fetus (proband) was found to have a single (−α3.7) and a double (−MED) deletion on the α gene, which was concomitant with heterozygous HBB: c.20A>T, Glu6Val mutation.

Family Study

The hematological data, clinical manifestation, and result of hemoglobin analysis of the parents are summarized in Table 1. The patient’s husband was 26 years old and a carrier of α-thalassemia (Table 1). The mother tested homozygous for HbS (ie, she does not have sickle cell trait) and appeared to have no symptoms of sickle cell anemia. Her α-globin mutation may modify her risk for symptoms of sickle cell disease but these factors shouldn’t put her at risk for significant thalassemia symptoms. However, the fetus, at 7 weeks’
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**Figure 1**

α-globin strip assay by reverse dot-blot results in the study patient, showing anti-3.7 α-gene triplication, α2 poly A-1, and -α3.7 gene deletion.

**Figure 2**

β-globin sequence analysis. The arrow indicates the homozygous Sickle hemoglobin (HbS) [A>T] mutation in the study patient.

**Table 1. Hematological Data and Genotypes of the Parents and the Proband With HbS and α-Thalassemia**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>RBCs (µL)</th>
<th>RDW (%)</th>
<th>HbA2 (%)</th>
<th>HbF (%)</th>
<th>HbA1 (%)</th>
<th>HbS</th>
<th>α-Genotype</th>
<th>HbS Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>24 year</td>
<td>69.1</td>
<td>19.7</td>
<td>28.5</td>
<td>4.11 × 10⁶</td>
<td>21.8</td>
<td>3.4</td>
<td>1.9</td>
<td>NA</td>
<td>94.7</td>
<td>-α⁻³⁷ / -α⁻³⁷</td>
<td>cd6 (GAG&gt;GTT)/cd6 (GAG&gt;GTT)</td>
</tr>
<tr>
<td>Father</td>
<td>26 y</td>
<td>70.0</td>
<td>22.4</td>
<td>27.6</td>
<td>5.22 × 10⁶</td>
<td>23.1</td>
<td>2.5</td>
<td>1.8</td>
<td>96.7</td>
<td>NA</td>
<td>α⁻³⁷ / -α⁻³⁷</td>
<td>NA</td>
</tr>
<tr>
<td>Proband</td>
<td>7 wk</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-α⁻³⁷ / -α⁻³⁷</td>
<td>cd6 (GAG&gt;GTT)/N</td>
</tr>
</tbody>
</table>

Abbreviation: MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBCs, red blood cells; RDW, red blood cell distribution width; HbA1, hemoglobin A1; HbS, Sickle hemoglobin; NA, not applicable.
gestation, was at risk for thalassemia symptoms (ie, hemoglobin H [HbH] disease).

**Discussion**

Herein, we report the case of a patient with an infrequent hereditary arrangement of 4 various genetic anomalies of hemoglobin genes: the patient and her husband had 3 mutations on the α-gene and a sickle-cell anomaly on the β-gene, which is a rare combination.9,10 The co-occurrence of sickle-cell anemia with α-thalassemia (due to the 3.7 kb deletion) is extremely common in ethnic African populations. What makes this case potentially unique is that the unusual α-globin point mutation and triplication in the mother is typically seen in patients of Asian ethnic origin. The mother’s c.*94A>G (a2PA6) mutation is presumably located on the same chromosome as the triplication, which had previously been reported11 in association with an α-globin triplication. The mutation in the present case, which was comprised of the combination of c.*94A>G/triplication and the 3.7 kb deletion, might mean that the patient has a more significant level of α-thalassemia than most individuals with reported sickle cell disease and α-thalassemia.

Sickle cell disease is itself quite variable; factors such as genetic and environmental influences contribute to this variability. One of the most important genetic factors is thalassemia.

Individuals with sickle cell trait are heterozygous for HbS; therefore, they have a 50% chance of transmitting the mutation to their son or daughter. For children to develop sickle cell disease, both of their parents almost always must carry a mutation. The risk of transmitting α-thalassemia depends on the nature of the mutation.

As can be observed from the data in this report, this case is a classic example of moderately severe sickle α-thalassemia disease. Thalassemia is very common among people of Mediterranean ethnic origin. The sickle cell gene also exists more commonly in people of the same ethnic origin. In the United States, 10% of the population is at risk of sickle cell anemia; in northwestern Europe, between 2% and 9% are at risk for hemoglobin disorders. In some Southeast Asian countries, as much as 40% of the population may carry significant hemoglobin mutations, resulting in increased rates of infants born with thalassemia.12 Hemoglobin analyses of the parents of such infants have shown that the fraction of HbS was extremely high, namely, 94.7% in the mother, whereas the MCV was decreased in both parents to less than 71 fl.

The complete or partial deletion of both α-genes in cis deletion results in no α-chain synthesis; hence, rare deletions that cause α*-thalassaemia remove the regulatory region, which lies 40 to 50 kb upstream of the α-globin gene cluster, leaving the α-genes intact. Moreover, the coexistence of the anti-3.7 gene triplication at a point mutation in cis deletion is a rare entity that is observed in our case individual.

Patients with HbH disease have only a single active α-globin gene and manifest moderate to severe anemia. Deletion of all 4 α-globin genes (–/–/–), has the most severe manifestation, known as Hb Bart’s hydrops fetalis syndrome, which is generally associated with death in utero.2

The consequences of α-thalassemia on sickle-cell anemia are possibly connected to an essential effect on mean corpuscular hemoglobin concentration (MCHC) and polymerization; further, simultaneous effects on other properties of sickle cells and the possibility that the MCHC differences may be secondary to sickling effects cannot be overlooked. Studies of the phenotypic results of the interaction of α-thalassemia and sickle-cell anemia will provide further insight into the causes of clinical diversity in sickle cell disease.13 Co-existing α-thalassemia and sickle cell disease significantly improved hematological parameters and resulted in fewer blood transfusions,14 may cause musculoskeletal pain,15 and decreases the risk of cerebrovascular disease in children with sickle cell anemia.16,17 In conclusion, we report an extremely rare combination of α-globin gene disease and sickle cell trait. This combination may explain the mild form of thalassemia disease that manifests with moderate anemia. LM

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Conflict of interest
The authors declare no conflict of interest.

References