۳۰ درصد تخفیف نوروزی ویژه کارگاه‌ها و فیلم‌های آموزشی

اصول تنظیم قراردادها

پروپوزال نویسی

آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Abstract

We report the case of a 23-year-old man and 3 members of his family with Hb J-Iran confirmed by electrophoresis, chain separation by high-performance liquid chromatography, and sequencing. Alpha thalassemia was also confirmed in two family members. The substitution at β77 leads to a higher negative charge of the βJ-Iran subunit, which enhances its electrostatic attraction for the normally positively charged α subunit. Therefore, more Hb J-Iran than Hb A forms in the red blood cells of heterozygotes. In α-thalassemia, the more attractive βJ-Iran subunit out-competes βA subunits in forming assemblies with deficient α subunits, so even more Hb J-Iran is formed.

Keywords: Hb J-Iran; Alpha-thalassemia; Hemoglobin variants; Electrophoresis; Chromatography; High performance liquid

Introduction

Hb J-Iran (β77 His→Asp) is one of the first hemoglobin variants that have been discovered in Iran. The substitution at β77 leads to a higher negative charge on the surface of the βJ-Iran subunit, which enhances its electrostatic attraction for the normally positively charged α subunit. As a result, red cells in heterozygous individuals contain more Hb J-Iran than Hb A.

Case report

A 23-year-old man with mild jaundice and anemia was referred to the Hematology Research Center and the Genetic and Prenatal Diagnosis of Thalassemia and Hemophilia Service of Dastgheib and Nemazee Hospital (the main reference hospitals affiliated with Shiraz University of Medical Sciences in Shiraz, Southern Iran) for premartial screening for thalassemia. On physical examination he was asymptomatic and had no clinical manifestations. However, a hemoglobin electrophoresis at pH 8.6 (Helena, Process-24, Beaumont, TX, USA) detected a band located between Hb A1 and Hb H (Figure 1). The proportion of this unknown band was determined by capillary electrophoresis (Sebia, Capillaries 2, Norcross, GA, USA). To determine the nature of the unknown Hb, chain separation by high-performance liquid chromatography (HPLC) was used. The lysate was injected in a Waters system (Waters, Breeze, Milford, MA, USA), and comparison of the retention times identified the unknown band as a β variant (Figure 2). The β gene was sequenced with an ABI 310 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA) to detect the mutation or deletion that caused the formation of this hemoglobin. By comparing sequencing graphs for normal and sample sequences, we detected a mutation in codon 77 (CAC > GAC), which caused a β77 His→Asp substitution in the β chain (Hb J-Iran; Figure 3). Molecular studies of the alpha globin gene found the homozygous form of the 3.7-kbp deletion (-α3.7/-α3.7) as the probable cause of mild hypochromic microcytic anemia in the proband (diagnosed by a blood smear prepared from fresh blood and stained with Wright stain). To detect long deletions in the alpha gene, gap-PCR was conducted, and to detect mutations or deletions, the reverse dot blot method was used according to the manufacturer’s protocol for the strip assay α globin gene kit (Viennalab, Vienna, Austria). Further studies were performed on five of the proband’s siblings and his mother (his father had died before he
was referred to our center). The results are summarized in Table 1.

**Discussion**

Because there are two genes for β-globin, an individual heterozygous for a β-globin variant would be expected to have equal proportions of normal and abnormal hemoglobins. However, some β variants are synthesized significantly less than normal β globin, so the level of these variants (such as E, Lepore, Knossos, K-Woolwich, and Vicksburg) will be below that of normal Hb A, a situation associated with the thalassemic phenotype. In heterozygous individuals with forms of unstable hemoglobin such as Koln and Zurich, the proportion of Hb variants is also below that of normal hemoglobin. Differences between the levels of normal and other variant hemoglobins reflect the different tendencies of variants that assemble with normal alpha subunits (1). In heterozygous forms of hemoglobinopathies with the β-variant chain, the normal β chain compared with the abnormal β chain, has a greater affinity to combine with the α chain and form normal Hb A at so the amount of Hb A is usually greater than abnormal hemoglobin. This situation

is more obvious in individuals with both α-thalassemia and a heterozygous form of hemoglobinopathy because the normal β chain has a stronger tendency to assemble with the deficient α chain present in α-thalassemia and is thus more successful in forming Hb A than in individuals with only heterozygous forms of hemoglobinopathies. The J-Iran variant (β77 His → Asp), first reported in Iran (2), was subsequently found in Turkey (3) and in a Russian-Armenian family (4). The substitution at β77 leads to a higher negative charge on the surface of the βJ-Iran subunit, which enhances its electrostatic attraction for the normally positively charged α subunit. As a result, red cells in heterozygous individuals contain more Hb J-Iran than Hb A. In the presence of α-thalassemia, the more attractive βJ-Iran subunit will be even more successfully combined with the deficient α subunits so that more Hb J-Iran is formed (unlike the situation with other β variants such as Hb S and Hb C). The same mechanism is responsible for the greater proportion of Hb J-Baltimore than Hb A. Competition experiments with the [A and βJ-Baltimore subunits in vitro showed that when α subunits are deficient, Hb J formation will be greater than in individuals without α-thalassemia (1, 5).

In 1987 Rahbar et al. presented a 14-year-old Persian girl who had 65 % Hb J-Iran but who also had Hb H disease with three α-gene deletions, which probably explain the difference in the amount of Hb J-Iran between this girl and the family we are reporting here. Her two siblings had 50 % Hb J-Iran and probably silent or minor α-thalassemia (1). In Turkey, the first person with Hb J-Iran (β77 His → Asp) was reported in 1986 by Arcasoy et al. (3). Since then, five cases have been reported in the Turkish population, from Ankara, Antalya, and Mugla (6). In the current study, the proband, his mother, and his siblings did not need blood transfusions and were advised to take 5 mg of folic acid daily. They had no apparent health problems related to the hemoglobin variant, and we assume that Hb J-Iran functions correctly in their bodies. To investigate Hb J-Iran functioning, O2 saturation and erythropoietin (EPO) levels are useful because when O2 saturation is normal, EPO secretion and thus serum EPO

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**Table 1. Hematological results in the proband and family members**

| Genotype of e-globin chain | Amount of J-Iran Hb | Presence of J-Iran Hb | MCHC, g/dL | RBC count, 10^12/μL | MCH, pg | Hct, % | MCV, fl. | Hb, g/dL | Presence of Hb A1 | Genotype of α-globin chain | MCV, fl. | Hct, % | MCH, pg |
|---------------------------|--------------------|----------------------|-----------|---------------------|--------|-------|--------|--------|---------------|--------------------------|--------|-------|--------|
| ∆a37/∆a37               | 49.5               | No                   | 13.4      | 44.4                | 86.2   | 26.0  | 30.2   | 5.15   | Yes           | ∆a37/aa                  | 13.2   | 43.0  | 72.0   |
| ∆a37/aa                 | 15.8               | No                   | 13.4      | 46.3                | 72.0   | 20.4  | 29.2   | 4.80   | Yes           | ∆a37/aa                  | 12.2   | 41.8  | 87.1   |
| ∆a37/aa                 | 0                  | No                   | 13.4      | 48.0                | 80.0   | 26.3  | 32.0   | 6.00   | No            | ∆a37/aa                  | 15.8   | 48.0  | 80.0   |
| ∆a37/aa                 | 13.1               | Yes                  | 13.4      | 44.9                | 90.7   | 27.9  | 30.7   | 4.95   | Yes           | ∆a37/aa                  | 13.2   | 43.0  | 72.0   |
| ∆a37/aa                 | 13.2               | No                   | 13.4      | 44.4                | 86.2   | 26.0  | 30.2   | 5.15   | Yes           | ∆a37/aa                  | 13.2   | 43.0  | 72.0   |
| ∆a37/aa                 | 13.4               | No                   | 13.4      | 43.3                | 86.9   | 26.9  | 30.9   | 4.98   | No            | ∆a37/aa                  | 13.2   | 43.0  | 72.0   |
| ∆a37/aa                 | 13.4               | No                   | 13.4      | 44.9                | 90.7   | 27.9  | 30.7   | 4.95   | Yes           | ∆a37/aa                  | 13.2   | 43.0  | 72.0   |
| ∆a37/aa                 | 13.4               | No                   | 13.4      | 43.3                | 86.9   | 26.9  | 30.9   | 4.98   | No            | ∆a37/aa                  | 13.2   | 43.0  | 72.0   |
| ∆a37/aa                 | 13.4               | No                   | 13.4      | 44.9                | 90.7   | 27.9  | 30.7   | 4.95   | Yes           | ∆a37/aa                  | 13.2   | 43.0  | 72.0   |
| ∆a37/aa                 | 13.4               | No                   | 13.4      | 43.3                | 86.9   | 26.9  | 30.9   | 4.98   | No            | ∆a37/aa                  | 13.2   | 43.0  | 72.0   |

**Figure 1.** Hb electrophoresis of the proband (Patient no. 3) at pH: 8.6 detected a band located between Hb A1 and Hb H
The β-chain retention time was shorter than the α-chain time, and because the total proportion of the βA and variants was almost equal to the proportion of the total α chain, we concluded that the variant was a β variant.

Figure 2. Chain separation in the proband by HPLC

The oxygen saturation levels are normal. However, when O2 saturation is below normal (as in some hemoglobin disorders), the increased EPO secretion stimulates red blood cell production to compensate for this defect. When the compensatory mechanism operates, no anemia or obvious health problems are apparent aside from an increase in serum EPO levels.

Financial support

None declared.

Conflict of Interest

None declared.

Acknowledgements

We thank Shirin Parand of the Hematology Research Archive of SID www.SID.ir
A mutation was detected in codon 77 (CAC > GAC), which caused the β77 His→Asp substitution in the β chain (Hb J-Iran). The arrows show codon 77.

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