Hemoglobin Interlaken in combination with beta thalassemia trait

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Abstract

We report a rare α1 globin gene variant (Hb Interlaken) found in a 63-year-old woman of Italian ancestry living in Buenos Aires Province, Argentina. The variant, a missense mutation at cd15 (GGT → GAT) causing a Gly → Asp amino acid substitution and also known as Hb J Oxford, was found in combination with the common thalassemia trait cd 39 (C → T). The clinical picture of the patient was that of a β-thalassemia trait.

Introduction

In 1964 Liddell first described hemoglobin Interlaken in an English family.1 Only isolated reports exist in which the same mutation was named Hb J Oxford or Hb N-Cosenza.2 The variant has thus far been described in combination with β-thalassemia homozygosity and HbS but not in combination with β-thalassemia trait and never before in Argentina, a multi ethnic country with many ethnic components.

Hb Interlaken is a stable α1 chain variant caused by a GAC → CAC transversion at codon 15 of the α1 gene causing a Gly → Asp amino acid substitution (HGVS nomenclature HBA1c.47G>A).3

Case Report

We report the identification of Hb Interlaken in a 63-year-old Argentinian woman of Italian ancestry, referred to our laboratory because of a microcytic hypochromic anemia. Hb Interlaken was found in this patient in combination with the common Mediterranean β-thalassemia trait cd 39 (C → T) (HGVS nomenclature HBB.e118C>T), the most frequent β-thalassemia mutation in Argentina.4 Complete blood count (CBC) was obtained with a Coulter Counter model ACT10 (Beckman Coulter Inc, Brea, CA, USA): Hb (g/L) 11.6, RBC (1012/L) 5.88, MCV (fL) 62.4, HCM (pg) 19.7, reticulocytes (%) 1.0.

The separation of the Hb fractions was done on alkaline cellulose acetate electrophoresis (Figure 1A). The presence of a J like minor fraction was suggestive for an α chain variant. Hb A2 measured by elution from electrophoresis followed by spectrophotometric measurement of the absorbance at 415 nm was estimated at 3% while Hb X was 28%. In spite of the normal HbA1 level, the CBC, the slightly elevated HbF (1.3%) estimated according to Betke et al.5,6 indicated a possible β-thalassemia trait while the very low mean corpuscular hemoglobin could be an indication for a β/α-thalassemia combination. Iron parameters were measured as previously described:7 serum iron 56 µg/dL, total iron-binding capacity 328 µg/dL, transferrin saturation 17% and serum ferritin 4 ng/mL.8 Isopropanol9 heat stability and sickle tests were performed and were all negative.

DNA was extracted from peripheral blood sample.10 Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR)11 was used to confirm the presence of the β^39 mutation (data not shown).

Alpha thalassemia deletion analysis was done using Gap-PCR12 taking into account the ethnic origin and hematological data of the patient while for point mutation analysis amplification of the α2- and c1-globin genes was performed as previously described by using oligonucleotide primers (CyberSyn, Lenni, PA, USA); the common forward primers Fa2: 5’-CCGCCTCGCGCCGCGGCAC-3’, and reverse specific primers for the α2 gene: 5'-GGGGAGCCCATCGGGCAGGAGGAAC-3' and α1 gene: 5'-GGGGAGGGCCCAAGGGGCGAAGA-3’. Sequencing was done using a Big Dye Terminators Ready Reaction Kit (Perkin-Elmer Cetus, Norwalk, CT, USA) in an ABI PRISM 310 sequencer (Perkin-Elmer Cetus). Primers used for sequencing the two genes were the following: exon 1, common forward primers Fa2; exon 2, primer S2 (5’-CCGCCGCCGCCACACA-3’); exon 3, primer S3 (5’-GCCGTTTGCGGGAGGCT-3’). The reverse specific primers for the α1 gene were used to confirm the mutation.

GAP-PCR were used to detect α thalAS - (α)2.5, —MED and c2 α deletions -α^2,12 and were all negative. Sequencing of α1 gene revealed a GAC → CAC (Asp → His) substitution at codon 15, corresponding to Hb Interlaken (Figure 1B).

Even though Hb Interlaken is detectable by electrophoresis a correct characterization of the genotype requires better methods, especially in combination with α thalassemia. Then more sophisticated systems able to measure Hb fractions more precisely by high-performance liquid chromatography or capillary electrophoresis13 are needed before molecular analysis.

In our case the estimation of the HbA1 that should have been elevated was typically normal risking misdiagnosis. The underestimation was due to the fact that part of the delta chain is bound by the mutated α chain and this abnormal HbA1 fraction migrates on a different spot and is lost for measurement. Therefore the measured HbA2 value (3%) should be augmented by 28% resulting into a 3.84% a still ambiguous but elevated HbA2. Even though our value (28%) for hemoglobin variant J-Oxford is high, the consulted references showed values between 21 and 25%.14,15

It is the first time that this abnormal hemoglobin is described in our country. The low red blood cell indexes observed in this case are due to co-inheritance of β^3 thalassemia and the underestimation of HbA1 could be caused
by iron deficiency in the patient at the time of diagnosis and lost of the abnormal HbA2 fraction.

References


Figure 1. A) Hemoglobin electrophoresis at alkaline pH. B) Sequencing of the \( \alpha_{1} \)-globin gene showed one mutation corresponding to Hb Interlaken (Hb J-Oxford, Hb N-Cosenza), \([\alpha_{1}Gly \rightarrow Asp, GGT \rightarrow GAT]\).