Hemoglobin Interlaken in combination with beta thalassemia trait

Mara J. Ojeda,[1] Susana M. Perez,[1] Karina L. Calvo,[1] Arianna F. Pratti,[1] María E. Voss,[1] Angela C. Milani,[1] Gustavo Chiappe,[2] Beatriz Erramouspe,[2] Irma M. Bragós[1]

[1]Cátedra Hematología, Facultad Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina; [2]Unidad Asistencial Dr. César Milstein, Ciudad Autónoma de Buenos Aires, Argentina

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 HBA1:c.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 hypocromic anemia. Hb Interlaken was found in this patient in combination with the common Mediterranean β^0 thalassemia trait cd 39 (C→T) (HGVS nomenclature HBB:c.118C>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 (10^{12}/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 HbA_2 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 corpuscolar hemoglobin could be an indication for a β-/α thalassemia combination. Iron parameters were measured as previously described:[7] serum iron 56 ug/dL, total iron-binding capacity 328 ug/dL, transferrin saturation 17% and serum ferritin 4 ng/mL.[8] Isopropanol,[9] 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 $\beta^0$39 mutation (*data not shown*).

Alpha thalassemia deletion analysis was done using Gap-PCR[12] taking into account the ethnic origin and hematological data of the patient while for point mutation analysis amplification of the α2- and α1-globin genes was performed as previously described by using oligonucleotide primers (CyberSyn, Lenni, PA, USA); the common forward primers FAa2: 5'-CGCGCTCGCGGCCCGGCAC-3', and reverse specific primers for the α2 gene: 5'-GGGAGGCCCATCGGGCAGGAGGAAC-3' and α1 gene: 5'-GGGGGGAGGCCCAAGGGGCAAGAA-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 FAa2; exon 2, primer S2 (5'-CCCGCCCGGACC-CACA-3'); exon 3, primer S3 (5'-GCGGGTTGCGGGAGGT-3'). The reverse specific primers for the α1 gene were used to confirm the mutation.

GAP-PCR were used to detect α^0 deletions -(α)20.5, —MED and α^+ deletions -$\alpha^{3.7}$ and -$\alpha^{4.2}$ 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 and α thalassemia. Then more sophisticated systems able to measure Hb fractions more precisely like high-performance liquid chromatography or capillary electrophoresis[13] are needed before molecular analysis.

In our case the estimation of the HbA_2 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 HbA_2 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 HbA_2.[6] Even though our value (28%) for hemoglobin variant J-Oxford is high, the consulted references showed values between 21 and 25%.[14-16]

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^0 thalassemia and the underestimation of HbA_2 could be caused

Correspondence: Irma M. Bragós, Cátedra Hematología, Facultad Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina.
Tel. +54.341.4804592/3 - Fax: +54.341.4804598.
E-mail: ibragos@fbioyf.unr.edu.ar

Key words: thalassemia, hemoglobinopathies, microcytic hypocromic anemia, Hb Interlaken.

Acknowledgments: we are very grateful to Dr M.C. Rosatelli from the Istituto di Clinica e Biologia dell'Età Evolutiva, Università degli Studi Cagliari, Italy who provided DNA samples with known mutations.

Contributions: IMB, MJO, conception and design; IMB, SMP, MJO, BE, GC, AFP, MEV, development of methodology; IMB, GC, BE, acquisition of data; IMB, SMP, MJO, KLC, GC, BE, AFPratti, MEV, analysis and interpretation of data; IMB, SMP, MJO, KLC, ACM, writing, review and/or revision of the manuscript; IMB, KLC, AFP, MEV, ACM, administrative, technical, or material support; IMB, SMP, MJO, ACM, study supervision.

Conflict of interests: the authors report no conflict of interests.

Received for publication: 10 October 2012.
Revision received: 14 December 2012.
Accepted for publication: 7 January 2012.

Thalassemia Reports 2013; 3:e3
doi:10.4081/thal.2013.e3

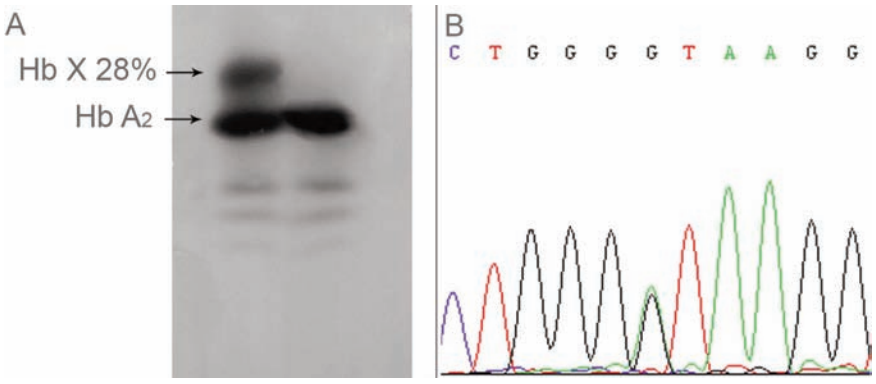

Figure 1. A) Hemoglobin electrophoresis at alkaline pH. B) Sequencing of the α1-globin gene showed one mutation corresponding to Hb Interlaken (Hb J-Oxford, Hb N-Cosenza), [α15Gly → Asp, GGT>GAT].

by iron deficiency in the patient at the time of diagnosis and lost of the abnormal HbA_2 fraction.

References

1. Liddell J, Brown D, Beale D, et al. A new Haemoglobin-Ja Oxford found during a survey of an English population. Nature 1964;204:269-70.
2. Schiliro G, Musumeci S, Pizzarelli G, et al. A new alkali-resistant hemoglobin α2J Oxford gammaF2 in a Sicilian baby girl with homozygous β^0 thalassemia. Blood 1976;48:639-51.
3. Molchanova TP, Pobedimskaya DD, Huisman, TH. The differences in quantities of α2- and α1-globin gene variants in heterozygotes. Br J Haematol 1994;88:300-6.
4. Bragós I, Noguera N, Morisoli L, Milani A. Most frequent mutations in β-thalassemia in a population in Rosario, Argentina. Haematologica 2000;85:101-2.
5. Betke K, Marti HR, Schlicht I. Estimation of small percentages of foetal haemoglobin. Nature 1959;184 Suppl 24:1877-8.
6. Stephens AD, Angastiniotis M, Baysal E, et al. International Council for the Standardisation of Haematology (ICSH). ICSH recommendations for the measurement of haemoglobin A_2. Int J Lab Hematol 2012;34:1-13.
7. International Committee for Standardization in Haematology (ICSH). The measurement of total and saturated Iron-binding capacity in serum. Br J Haematol 1978b;38:281-7.
8. Cazzola M. Metodologia e strategia per lo studio di laboratorio del metabolismo del ferro. Clinica e Laboratorio 1989;13:2.
9. Carrell RW, Kay R. A simple method for the detection of unstable haemoglobins. Br J Haematol 1972;23:615-9.
10. Noguera N, Tallano C, Bragós I, Milani A. Modified salting-out method for DNA isolation from newborn cord blood nucleated cells. J Clin Lab Analysis 2000;14;280-3.
11. Old JM, Varawalla NY, Weatherall DJ. Rapid detection and prenatal diagnosis of β-thalassaemia: studies in Indian and Cypriot populations in the UK. Lancet 1990 336:834-7.
12. Liu YT, Old JM, Miles K, et al. Rapid detection of -thalassaemia deletions and -globin gene triplication by multiplex polymerase chain reactions. Br J Haematol 2000;108:295-9.
13. Van Delft P, Lenters E, Bakker-Verweij M, et al. Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations. Int J Lab Hematol 2009;31:484-95.
14. Caruso D, Crestani M, Da Riva L, et al. Mass spectrometry and DNA sequencing are complementary techniques for characterizing hemoglobin variants: the example of hemoglobin J-Oxford. Haematologica 2004;89:608-9.
15. Schiliró G, Musumeci S, Pizzarelli G, et al. A new alkali-resistant hemoglobin α2J Oxford gammaF2 in a Sicilian Baby Girl With Homozygous β^0 Thalassemia. Blood 1976;48:639-44.
16. Gilcher RO, Bromberg PA, Finn FM, Jensen WN. Hemoglobin JOxford: effects on hemoglobin and erythrocyte function. Blood 1968;32:260-70.