Hb H disease resulting from the association of an $\alpha^0$-thalassemia allele [-(\(\alpha^{20.5}\))] with an unstable $\alpha$-globin variant [Hb Icaria]: First report on the occurrence in Brazil

Elza M. Kimura1, Denise M. Oliveira1, Kleber Fertrin2,3, Valéria R. Pinheiro3, Susan E.D.C. Jorge1, Fernando F. Costa2 and Maria de Fátima Sonati1

1Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brazil.
2Centro de Hematologia e Hemoterapia, Universidade Estadual de Campinas, Campinas, SP, Brazil.
3Centro Infantil Dr. Domingos A. Boldrini, Campinas, SP, Brazil.

Abstract

Hb H Disease is caused by the loss or inactivation of three of the four functional $\alpha$-globin genes. Patients present chronic hemolytic anemia and splenomegaly. In some cases, occasional blood transfusions are required. Deletions are the main cause of this type of thalassemia ($\alpha$-thalassemia). We describe here an unusual case of Hb H disease caused by the combination of a common $\alpha^0$ deletion [-(\(\alpha^{20.5}\))] with a rare point mutation (c.427T > A), thus resulting in an elongated and unstable $\alpha$-globin variant, Hb Icaria, (X142K), with 31 additional amino-acid residues. Very high levels of Hb H and Hb Bart’s were detected in the patient’s red blood cells (14.7 and 19.0%, respectively). This is the first description of this infrequent association in the Brazilian population.

Key words: hereditary hemoglobinopathies, alpha-thalassemia, Hb H disease, Hb Icaria.

Received: December 10, 2008; Accepted: May 21, 2009.

The alpha ($\alpha$)-globin genes are duplicated ($\alpha_2$ and $\alpha_1$) and located on the short arm of chromosome 16 (16p13.3). Alpha-thalassemia ($\alpha$-thal) is a hereditary condition resulting from deficient synthesis of $\alpha$-globin chains. It has a worldwide distribution and reaches frequencies as high as 80% or more in some populations, reflecting positive selection after exposure to malaria (Higgs and Weatherall, 2009). Deletions resulting from unequal crossing-over between homologous sequences in the $\alpha$-gene cluster are the main cause of this hemoglobinopathy and may affect one or both $\alpha$ genes in the haploid genome ($\alpha^+$ or $\alpha^0$ alleles, respectively). Nondeletional mutations are less frequent and usually correspond to more pronounced hematological alterations (Steinberg et al., 2001; Weatherall and Clegg, 2001).

The combination of an $\alpha^0$ allele with an $\alpha^+$ allele, affecting three of the four functional $\alpha$ genes, leads to Hb H disease (–/–$\alpha$), a moderate to severe chronic hemolytic anemia with the presence of 5%-25% of Hb H ($\beta_4$) in peripheral blood erythrocytes (Steinberg et al., 2001; Weatherall and Clegg, 2001). Hb H is unstable and precipitates in all circulating cells when submitted to any oxidant stress. Herein, we describe a rare case of Hb H disease resulting from the association of the [-(\(\alpha^{20.5}\))] deletion, an $\alpha^0$ deletion commonly found in Mediterranean and Central Asian populations, with a point mutation (c.427T > A), thus leading to the synthesis of an elongated and unstable $\alpha$-globin variant, Hb Icaria, (X142K), with 31 additional amino-acid residues. Very high levels of Hb H and Hb Bart’s were detected in the patient’s red blood cells (14.7 and 19.0%, respectively). This is the first description of this infrequent association in the Brazilian population.

The patient here described is a 2-year-old boy of mixed Italian and African origin, followed up at Centro Infantil Dr. Domingos A. Boldrini, in Campinas, state of São Paulo, southeastern Brazil. He presented chronic hemolytic anemia, pallor, jaundice and spleen enlarge-
Peripheral blood analysis revealed a remarkable degree of anisocytosis with microcytosis, hypochromia and 6.8% of reticulocytes. Serum ferritin was normal (45 ng/mL). The hematological data of the proband and his mother are summarized in Table 1. His father was not available for study.

Hb H, as well as its fetal version Hb Bart’s - γ4, were both detected by alkaline electrophoresis and quantified by cation-exchange HPLC (High Performance Liquid Chromatography) (Variant II - β-Thalassemia Short Program; Bio-Rad Laboratories, Hercules, CA, USA). The percentages for Hb H and Hb Bart’s were 14.7% and 19.0%, respectively (Figure 1). The presence of these abnormal variants was further confirmed by electrophoresis at neutral pH (Dacie et al., 2006). Hemoglobin instability was demonstrated by n-butanol, isopropanol and heat tests. Heinz and Hb H inclusion bodies were observed in the patient’s red blood cells (Dacie et al., 2006) but no further anomalous hemoglobin was identified in his peripheral blood sample. No abnormal variant whatsoever was detected in the mother’s blood sample.

Genomic DNA was obtained from peripheral blood leukocytes. Multiplex PCR for the most common α-thal alleles (Tan et al., 2001) revealed the presence of the - (α)20.5 deletion in the patient’s DNA sample (Figure 2), which was confirmed by specific gap-PCR (Kattamis et al., 1996). The deletion removes a 20.5 kb fragment of DNA containing the entire α2 gene and part of the α1 gene, the latter, however, is not expressed (Steinberg et al., 2001; Weatherall and Clegg, 2001).

Table 1 - Hematological data of the patient and his mother, both of African ancestry.

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Patient</th>
<th>Mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (million/L)</td>
<td>4.57</td>
<td>4.74</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>7.6</td>
<td>12.9</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>29.3</td>
<td>39.7</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>64.1</td>
<td>83.8</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.6</td>
<td>27.2</td>
</tr>
<tr>
<td>RDW-CV (%)</td>
<td>27.6</td>
<td>13.0</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>6.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Serum ferritin (ng/mL)</td>
<td>45.85</td>
<td>36.75</td>
</tr>
<tr>
<td>Hb Profile</td>
<td>A2 + A + Bart’s + H</td>
<td>A2 + A</td>
</tr>
<tr>
<td>Hb Barts (%)</td>
<td>19.0</td>
<td>-</td>
</tr>
<tr>
<td>Hb H (%)</td>
<td>14.7</td>
<td>-</td>
</tr>
<tr>
<td>α-genotype</td>
<td>-(α)</td>
<td>-(α)20.5</td>
</tr>
<tr>
<td></td>
<td>/α</td>
<td>/Hb Icaria α</td>
</tr>
<tr>
<td></td>
<td>/αα/α</td>
<td>/Hb Icaria α</td>
</tr>
</tbody>
</table>

RBC = Red Blood Cells; Hb = Hemoglobin; Hct = Hematocrit; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; RDW-CV = Coefficient of Variation of the Red Cell Distribution Width.

Figure 1 - Cation-exchange HPLC chromatogram of the patient’s blood sample showing hemoglobins Bart’s and H beside the normal hemoglobins (Hb A2 and Hb A).

Figure 2 - Multiplex PCR for screening of the most common α-thal alleles (Tan et al., 2001). A - 250 bp ladder marker; B - Normal Genotype Control (αα/αα); C - Patient -(α)20.5 /α /Hb Icaria α; D - Positive control for the -(α)20.5 deletion; E - 100 bp ladder marker.
ing the opposite strand of the DNA, this resulting in an elongated (and unstable) α-chain constituted by 31 extra residues: (142)Lys-Ala-Gly-Ala-Ser-Val-Ala-Pro-Pro-Ala-Arg-Trp-Ala-Ser-Gln-Arg-Ala-Leu-Leu-Pro-Ser-Leu-His-Arg-Pro-Phe-Leu-Val-Phe-(172)Glu-COOH. A stop codon was found at the new codon 173 (Hardison et al., 2002).

Hb Icaria is a rare Hb structural and thalassemic variant described in Greek, Yugoslavian and Macedonian families (Clegg et al., 1974; Efremov et al., 1990; Kanavakis et al., 1996). It is difficult to detect in peripheral blood samples by the more commonly used techniques, due to its very low concentration and electrophoretic mobility, which is slower than that of Hb A2 at alkaline pH (Clegg et al., 1974). The pathophysiology of these elongated chains has been attributed to mRNA instability (Waggoner and Liebhaber, 2003), but more recent studies have shown that it could be due to defective interaction with AHSP (alpha-hemoglobin stabilizing protein) (Turbpaiboon et al., 2006). In the patient investigated here, the low availability of α-chains was probably responsible for the high levels of Hb H and Hb Bart’s observed (33.7% of the total hemoglobin). Despite this, the alteration does not give rise to important clinical manifestations in heterozygous individuals, the case of our patient’s mother, who has the αα/αα αα Hb Icaria αα genotype and is clinically silent.

This is the first description of Hb H disease caused by a combination of -(α)20.5 deletion with Hb Icaria -(α)20.5/αα αα Hb Icaria αα in the Brazilian population. It is also the first description of this variant in an individual of Italian and African origin. Our findings illustrate the importance of investigating these atypical cases and identifying their molecular basis and pathophysiological mechanisms. They also give us an idea of how frequent these mutations and associations are in our population.

Acknowledgments

We would like to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant n. 02/13801-7) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

References


