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. Kimura$^1$, Denise M. Oliveira$^1$, Kleber Fertrin$^{2,3}$, Valéria R. Pinheiro$^3$, Susan E.D.C. Jorge$^1$, Fernando F. Costa$^2$ and Maria de Fátima Sonati$^1$

$^1$Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brazil.
$^2$Centro de Hematologia e Hemoterapia, Universidade Estadual de Campinas, Campinas, SP, Brazil.
$^3$Centro 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.

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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).

Two α-globin genes still remained. Direct α-globin gene sequencing (ABI PRISM 377 DNA Automated Sequencer, Applied Biosystems, Foster City, CA, USA) with primers described elsewhere (Dodé et al., 1990) identified base substitution (TAAaAAA) at the 142nd (termination) codon of the α2-globin gene (Figure 3). This mutation, also found in the patient’s mother, was confirmed by sequenc-

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

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

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/αHb 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-Val-Pro-Pro-Ala-Arg-Trp-Ala-Ser-Gln-Arg-Ala-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) (Turpbaiboon 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/αHb Icariaα 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/αHb Icariaα] 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.

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References

Clegg JB, Weatherall DJ, Contopolou-Griva I, Caroutsos K, Poungouras P and Tsevrenis H (1974) Haemoglobin Icaria, a new chain-termination mutant which causes alpha thalassemia. Nature 251:245-247.

Dacie JV, Lewis SM, Bain BJ and Bates I (2006) Practical Haematology. 9th edition. Artmed, Porto Alegre, 572 pp.

Dodé C, Rochette J and Krishnamoorthy R (1990) Locus assignment of human alpha globin mutations by selective amplification and direct sequencing. Br J Haematol 76:275-281.

Efremov GD, Josifovska O, Nikolov N, Codrington JF, Oner C, Gonzalez-Redondo JM and Huisman TH (1990) Hb Icaria-Hb H disease: Identification of the Hb Icaria mutation through analysis of amplified DNA. Br J Haematol 75:250-253.

Hardison RC, Chui DHK, Giardine B, Riemer C, Patrinos GP, Anagnou N, Miller W and Wajcman H (2002) Hb Var: A relational database of human hemoglobin variants and thalassemia mutations at the globin gene server. Hum Mut 19:225-233.

Higgs DR and Weatherall DJ (2009) The alpha thalassemias. Cell Mol Life Sci 66:1154-1162.

Kanavakis E, Traeger-Synodinos J, Papasotiropoulos I, Vrettou C, Metaxotou-Mavromati A, Stamoulakatou A, Lagona E and Kattamis C (1996) The interaction of alpha zero thalassemia with Hb Icaria: Three unusual cases of haemoglobinopathy H. Br J Haematol 92:332-335.

Kattamis AC, Camaschella C, Sivera P, Surrey S and Fortina P (1996) Human alpha-thalassemia syndromes: Detection of molecular defects. Am J Hematol 53:81-91.

Schrier SL, Bunyaratvej A, Khuhapinant A, Fucharoen S, Aljurf M, Snyder LM, Keifer CR, Ma L and Mohandas N (1997) The unusual pathobiology of hemoglobin constant spring red blood cells. Blood 89:1762-1769.

Steinberg MH, Forget BG, Higgs DR and Nagel RL (2001) Disorders of Hemoglobin - Genetics, Pathophysiology and Clinical Management. Cambridge University Press, New York, 1268 pp.

Tan AS, Quah TC, Low PS and Chong SS (2001) A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for alpha-thalassemia. Blood 98:250-251.
Turbpaiboon C, Limjindaporn T, Wongwiwat W, U-Pratya Y, Siritanaratkul N, Yenchitsomanus PT, Jitrapakdee S and Wilairat P (2006) Impaired interaction of alpha-haemoglobin-stabilising protein with alpha-globin termination mutant in a yeast two-hybrid system. Br J Haematol 132:370-373.

Waggoner SA and Liebhaber SA (2003) Regulation of alpha-globin mRNA stability. Exp Biol Med 228:387-395.

Wajcman H, Traeger-Synodinos J, Papassotiriou I, Giordano PC, Harteveld CL, Baudin-Creuza V and Old J (2008) Unstable and thalassemic alpha chain hemoglobin variants: A cause of Hb H disease and thalassemia intermedia. Hemoglobin 32:327-349.

Weatherall DJ and Clegg JB (2001) The Thalassaemia Syndromes. 4th edition. Blackwell Science, Oxford, 864 pp.