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Case Report

Hemoglobin I-Philadelphia [alpha 16 (A14) LYS→GLU] heterozygote among blood donors from Brazil

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ABSTRACT. We describe a heterozygous case of Hb I-Philadelphia [alpha 16 (A14) LYS→GLU] in a blood donor from the Acre State Blood Bank, in the Brazilian Amazon region. We confirmed the mutation by electrophoretic and chromatographic methods and by DNA sequencing. A literature search showed that this is the first description of this alpha globin mutant in a Brazilian Caucasian group. We also emphasize the importance of the hemoglobin study in blood donors for the purpose of the genetic counseling and quality assurance of the blood to be transfused. Screening tests for hemoglobin mutants are also important for gathering anthropological information about the Brazilian population.

Key words: Hb I-Philadelphia, Rare hemoglobin, HPLC, Blood donors

INTRODUCTION

Hemoglobin I-Philadelphia (Hb I) is a variant hemoglobin, with an isoelectric point less than that of normal hemoglobin (Hb A) as result of a mutation in alpha globin gene 2, in codon 16 (AAG→GAG). The difference between Hb A and Hb I is due to the substitution of a lysine for a glutamic acid at the 16th position of the alpha chain. The mutation involves the external surface of the molecule, and the mutant protein concentration is between 24 and 28% in heterozygous individuals, showing normal stability and causing no clinical symptoms in carriers (Honig and Adams III, 1986). Hb I-Philadelphia was described for the first time in 1955 in an Afro-American family from North Carolina (USA), and is characterized by faster electrophoretic mobility in alkaline pH similar to Hb H migration at the same pH (Rucknagel et al., 1955). After this report, it was identified in different countries and ethnic groups including Africans and descendants, Indians, Caucasians, and Asians (Boulard et al., 1961; Thompson et al., 1963; Saito et al., 1982; Piña-Flores and Ruiz-Reyes, 1991). However, its frequency is low in the majority of the countries where it was described. In the relevant literature, other names are given to the same mutant, such as: Hb I-Texas, Hb I-Burlington and Hb I-Skamania (Huisman et al., 1986).

REPORT

Blood of a Caucasian man, 32 years old and without hematological symptoms, was obtained from blood donations at blood bank in Rio Branco, Acre State, located in a northern region of Brazil that is an endemic area for malaria. This sampling was part of a large screening study of protein polymorphisms in the Brazilian Amazon population. Five milliliters of venous blood was collected in a tube with EDTA as anticoagulant, after informed consent. The samples were stored refrigerated until the time of analysis. Classical laboratory procedures for the identification of abnormal hemoglobin were used. These procedures for laboratory diagnosis of hemoglobinopathies follow a flowchart and protocols previously established and described by Bonini-Domingos (2003). One sample of lysed red blood cells was submitted to electrophoresis at alkaline pH, where it is possible to distinguish the migration of Hb A, and one diffuse band of abnormal hemoglobin, running faster than Hb A, resulting from the chemical characteristics acquired with the mutation. Because of initial suspicion of a mutant, complementary electrophoretic tests at acid and neutral pH were conducted to confirm the presence of the abnormal Hb fraction. The sequence of methodological tests for the confirmation of a possible mutant is based on the comparison of different electrophoretic profile comparison and the profile obtained for high-pressure liquid chromatography using the automated analysis system VARIANT (Bio-Rad Laboratories) with the β Thalassemia Short program (Bonini-Domingos, 2003). The chromatogram obtained showed the fraction of abnormal Hb to be 16.5% with a retention time of 1.29 min. According to the variant hemoglobin standard supplied by the manufacturer, the interpretation of the chromatographic profile suggested the presence of Hb I-Philadelphia as heterozygous, despite the low concentration of the mutant fraction (16.5%) when compared with the findings in the literature that point to 24-26% for the abnormal fraction. Differences in the concentration of alpha chain mutants have been frequently observed previously (Schneider et al., 1966; Esan et al., 1970; Molchanova et al., 1994). To assist in the identification of the modified globin chain, the electrophoresis of polypeptide chains at alkaline and acid pH was

carried out and confirmed the mutant. The comparative results of electrophoretic and chromatographic procedures suggested the presence of the alpha globin chain mutant. Krauss and Khankhanian (1989) demonstrated earlier the effectiveness of high-pressure liquid chromatography in the detection of Hb I-Philadelphia especially in the research and evaluation of Hb A_{1c} in monitoring the stages of the diabetic condition. The suspicion of this mutant using the combination of separation methods was confirmed by direct DNA sequencing in an IBI system, as previously described (Leoneli, 2001; Madeira and Gruber, 2005).

DISCUSSION

Hb I-Philadelphia is frequent and clinically mild variant hemoglobin. In Afro-American individuals the frequency is about 1 in 1000, while it is very rare in Europeans and Asians. In the present study, we report its occurrence in an individual of Caucasian origin, from a region originally of few African descendants. The ethnic composition of this population shows a strong Spanish influence, since the state belonged to Bolivia until its definitive incorporation as a Brazilian territory in the beginning of the 19th century. Several hemoglobin variants can produce varied interference in cation-exchange chromatography of Hb A1c, requiring careful interpretation of the results. In this case, due to the low percentage of the variant fraction, we suspected an interference due to a glycosylated hemoglobin. Clinical evaluation did not confirm this suspicion, but the results of electrophoretic and chromatographic methods combined with DNA sequencing confirmed the hemoglobin mutant.

A survey of the literature indicates that this is the first description of this mutant in a Brazilian population in a mesoendemic area for malaria. It should be noted that the occurrence of this variant was in a blood donor, which points out the importance of these hemoglobin studies in this group, following the guidelines of the World Health Organization, in a mixed population like in Brazil. The correct diagnosis of the hemoglobin polymorphisms led us to a genetic counseling and extends the knowledge of this protein marker in Brazil.

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