Heterozygosis for hemoglobin Porto Alegre identified by a combination of laboratory diagnostic methodologies

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Hemoglobin (Hb) Porto Alegre is a beta globin chain mutant [beta 9 (A6) Ser>Cys] that was initially described in a Caucasian Brazilian family in 1963[1]. It was subsequently identified in other families in Brazil and in other places such as Cuba and the Canary Islands. The origin of the mutation was reported as Portuguese by the co-inheritance of an intragenic polymorphism characteristic of the population[2].

The laboratory identification of the variant by routine methods is often difficult because the mutant fraction migrates to the same position as Hb A in alkaline electrophoresis, the most widely used method for identifying hemoglobins in Brazil. In this case report, we describe the identification of the globin variant by associating electrophoretic, chromatographic and molecular methods after suspicion of a hemoglobin variant by capillary electrophoresis.

The patient was a female, adult Caucasian. Blood tests did not identify anemia and the suspected variant was only investigated after her clinician requested hemoglobin electrophoresis.

With the presence of a fraction close to Hb A and with 41.0% identified as Hb Atlanta by capillary electrophoresis, an investigation was begun to confirm the suspicion. The fraction was not identified by cation-exchange high-performance liquid chromatography (HPLC) as elution had the same retention time of hemoglobin A, but with unknown peaks close to the elution time of Hb C. Only a diffuse fraction with migration close to Hb A was identified by electrophoresis in cellulose acetate at alkaline pH. The profile of Hb A was also confirmed by electrophoresis in agarose at an acid pH. Hb A2 was 4.2% by HPLC. Figure 1 shows the Hb profile for electrophoretic procedures at different pH, by capillary electrophoresis and HPLC.

The results of electrophoretic and chromatographic procedures were discordant with the initial suspicion of Atlanta Hb, because this Hb has an unstable component, and testing for thermal and isopropanol instability was negative. Given the inconsistency of findings of conventional laboratory methods, and how the mutant beta chain is suggested by the percentage of the fraction obtained by capillary electrophoresis, electrophoresis at alkaline pH was performed that showed the profile A alpha/A beta globin chain. The next step was to amplify the three exons of the gene for beta globin and perform nucleotide base sequencing. The result showed the presence of a mutation in the codon responsible for the amino acid number nine, with replacement of C by G (TCT > TGT).

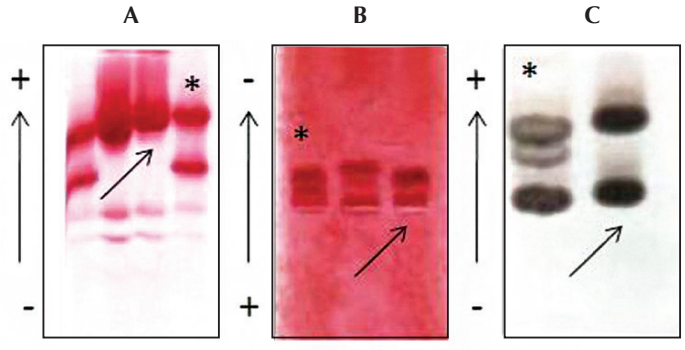

Figure 1 - Electrophoretic profile obtained by alkaline pH (A), acid pH (B) and globin chain in alkaline pH (C);

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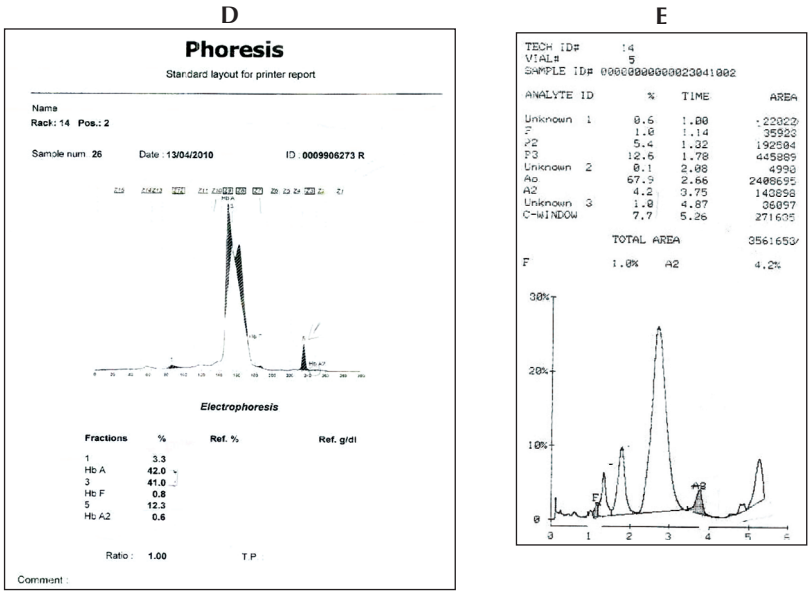

Figure 1 - The arrows identify the patient; (D) electrophoretic profile by capillary electrophoresis systems show the Hb fraction close to Hb A; (E) Chromatographic profile obtained by high-performance liquid chromatography; Standard (*) – hemoglobin AS.

The mutation in the exon leads to the beta globin amino acid substitution in the external portion of the molecule, which does not result in hematologic or clinical manifestations. The mutant fraction can be better seen by isoelectric focusing[3] due to the proximity of migration to other Hbs. Hb Porto Alegre presents normal Bohr effect, slightly decreased cooperativity and increased oxygen affinity[4]. Hb Porto Alegre is also found in association with beta thalassemia and other variants[5].

Confirmation of heterozygous Hb Porto Alegre and also the presence of an intragenic polymorphism at codon 27, reinforcing the contribution of Portuguese genetics for this mutant, were only possible with molecular analysis. Greater attention should be given to obtaining hemoglobin profiles using procedures with lower sensitivity, because the co-migration of fractions can hinder identification.

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