Comparing electrophoresis at alkaline pH and high performance liquid chromatography to diagnose Hb S-like hemoglobin

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Conflict-of-interest disclosure:
The authors declare no competing financial interest

Submitted: 12/29/2011
Accepted: 7/2/2012

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DOI: 10.5581/1516-8484.20120039

Hemoglobin (Hb) variants are caused by point mutations, usually characterized by a change in the nucleotide sequence, resulting in an amino acid substitution in the globin chain. Due to the high genetic variability of the Brazilian population, several cases of rare Hb variants have been reported; the most common are known as 'Hb S-like', defined because the electrophoretic profile is similar to that of Hb S.[1] Some examples of these specific variants in the population are Hb D-Los Angeles, Hb Korle-Bu, Hb Hasharon and Hb Lepore.[2,3] Given this diversity, there is a need for accurate Hb identification to assist therapeutic and counseling procedures.

This paper is intended to alert professionals who work with the diagnosis of hemoglobinopathies of the importance of precise characterization of rare Hb variants, in particular S-like Hb that, by classical diagnostic methods, may be mistakenly classified as Hb S.

We analyzed 838 peripheral blood samples collected in EDTA in the period from January to June 2011 of patients suspected of having anemia. After informed consent, the samples were submitted to classical diagnostic procedures including electrophoresis at alkaline pH in an automated hemoglobin analyzer (Bio-Rad Variant®) using the Short β-Thalassemia Program.

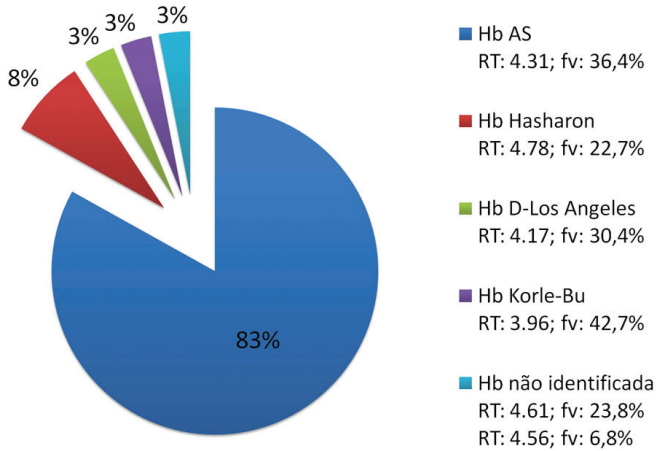

Figure 1 – Percentage of hemoglobin variants found, which migrate to the same position of Hb S (Hb S-like) by electrophoresis at alkaline pH

By electrophoresis at alkaline pH, 45.1% (378) of the samples showed a normal Hb migration pattern (Hb AA) and 7.76% (65) were characterized as Hb AS. After this preliminary analysis, the samples initially considered Hb AS were submitted to additional testing by high performance liquid chromatography (HPLC) and 16.92% (11) were characterized as variants that co-eluted with Hb S (Hb S-like). The data obtained in the chromatographic analysis, such as retention time (RT), percentage of Hb variant and the characteristic peaks were analyzed using the Library Variants software version 1.0 (Bio-Rad Laboratories) that characterizes some variants (Figure 1). All variants found were confirmed by molecular tests (PCR-RFLP).[4-6]

In addition, we found two unidentified rare Hb variants; chromatographic analysis identified the first with a fraction of 23.8% and RT 4.61 minutes and the second with a variant fraction of 6.8% and RT of 4.56 minutes, both with alpha globin chain mutations. These samples require family studies and subsequent DNA sequencing for better characterization.

The diagnosis of hemoglobinopathies based only on methods such as electrophoresis at alkaline pH in groups such as the Brazilian population formed after intense migration, incurs 17% of mistaken identity. We emphasize that this is the method of choice for most clinical laboratories because of its low cost and ease of handling. In recent years HPLC has proved to be reliable in the characterization of these variants. Furthermore, it is an automated and easy-to-use and thus has been shown to support the main alternative in the diagnosis of hemoglobinopathies, allowing more accurate results and appropriate guidance.[6]

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