α-chain hemoglobin variants with electrophoretic mobility similar to that of hemoglobin S in newborn screening programs

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Hemoglobin (Hb) S is a β-chain structural variant of human hemoglobin that undergoes polymerization in its deoxygenated form, making red blood cells stiff and undeformable and causing hemolysis and vaso-occlusion, with tissue damage and pain. Heterozygotes (Hb AS) are generally asymptomatic, while homozygotes (Hb SS) have sickle cell anemia (SCA). Combinations of Hb S and other hemoglobin variants, such as Hb C and D, or β-thalassemia can produce clinical pictures similar to that of SCA and are known as sickle cell diseases (SCD)[1].

Around 1 to 2% of the world's population are Hb S heterozygotes, but in some regions of Africa that are endemic for malaria (against which Hb AS individuals are protected) the frequency of the β^S gene can be greater than 40%[2]. In Brazil, the Southeast and Northeast regions have the greatest prevalence (around 8% of individuals of African descent are heterozygotes), although in some populations, such as that in Salvador, Bahia, the prevalence can be higher than 10%[3,4].

Correct diagnosis of this hemoglobinopathy is extremely important, particularly during the neonatal period, as early detection of SCD is fundamental if morbidity and mortality of these diseases are to be reduced. Laboratory diagnosis of SCD generally involves techniques based on the electric charge of the variants, such as cellulose acetate or gel electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC) and capillary electrophoresis[5]. However, as more than 1,000 variants have been described to date[6], the probability of finding another variant with electrophoretic behavior similar to that of Hb S (Hb S-like hemoglobins) is quite high, particularly in a population with a high degree of miscegenation, like that of Brazil. For this reason it is important to use confirmatory tests (which are also frequently used in screening programs), such as the sickling test and the hemoglobin solubility test (based on the insolubility of deoxy-HbS in high molarity phosphate buffer)[5]. In the case of Hb S in association with other variants, whether Hb S-like or not, correct identification of the second variant is fundamental to distinguish between those variants that will lead to SCD, those that evolve without any symptoms and those that produce specific symptoms[7]. Molecular techniques, such as restriction enzyme analysis and globin gene sequencing, are the most commonly used techniques for known mutations and new or rare mutations, respectively[8,9]. It should also be mentioned that some Hb S-like variants can cause red blood cell sickling, hemolysis and vaso-occlusion even in a heterozygous state. Examples include Hb Jamaica Plain[10] and Hb S São Paulo[11], the latter recently described in the Brazilian population. It should also be stressed that Hb S in association with an Hb S-like hemoglobin can lead to an incorrect diagnosis of SCA if the appropriate confirmatory tests are not carried out[12].

And what are the Hb S-like variants in the Brazilian population?

Some variants have a structural change in the β-chains, while in others the α-chains are affected. The former include Hbs D, Lepore, Korle-Bu, Osu-Christiansborg and Zürich[13,14], and the latter Hbs Hasharon, Stanleyville-II, G-Pest, Sunshine Seth, G-Philadelphia, West One and Daneshgah-Tehran[8,9,14]. In the case of the α-chain variants, some technical characteristics can help with the diagnosis; these include the presence of four bands in electrophoresis - two corresponding to Hb A_2 (A_2 and A_2') and two to Hb A (A and 'X') - and a lower concentration of 'Hb X' than normally observed in individuals with β-chain variants. Some α-chain variants are associated with α-thalassemia, with concomitant microcytosis and hypochromia, as is the case with the Hasharon (Figure 1) and Stanleyville-II variants, the most common in the Brazilian population[8,15].

Diagnosis during the neonatal period, however, involves further difficulties: the reduced concentration of α-chain variants makes it difficult to see the bands clearly, and the high concentration of fetal Hb (Hb F) interferes with confirmatory tests. The most commonly used screening methods in Brazil are isoelectric focusing and HPLC, followed by molecular biology techniques[16]. In the National Neonatal Screening Program operated by the Brazilian national health system (SUS), the heel prick, which is carried out in most Brazilian maternity wards, is used to screen for SCD and other genetic diseases[1]. With the aid of this screening program various α-chain variants have been detected, as the article published in this issue by Silva et al.[17],

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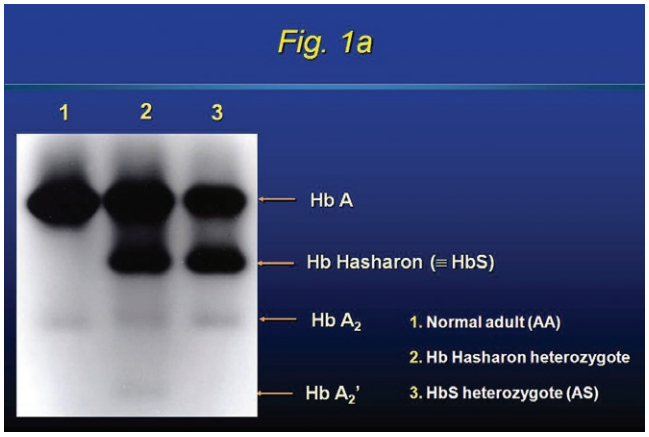

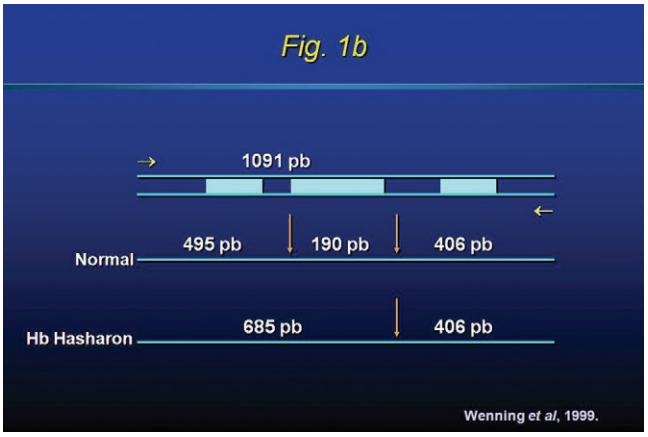

Figure 1 – (a) Cellulose acetate electrophoresis at alkaline pH; (b) restriction analysis with *Taq I* to confirm Hb Hasharon

belonging to the research group of Prof. Marcos Borato Viana, of the of the Pediatrics Department of the Universidade Federal de Minas Gerais (UFMG), shows us. The neonatal screening program run by the Nucleus of Actions and Research in Diagnosis at this university has helped to provide a greater understanding of the hemoglobinopathies found in different regions of Brazil - each one with its own ethnical characteristics and diversity - and has highlighted the importance of screening in itself and of correctly identifying other variants that can be confused with Hb S.

References

1. Sonati MdeF, Costa FF. The genetics of blood disorders: hereditary hemoglobinopathies. J Pediatr (Rio J). 2008;84(4 Suppl):S40-51.
2. Berry PA, Cross TJ, Thein SL, Portmann BC, Wendon JA, Karani JB, et al. Hepatic dysfunction in sickle cell disease: a new system of classification based on global assessment. Clin Gastroenterol Hepatol. 2007;5(12):1469-76.
3. Di Nuzzo DV, Fonseca SF. [Sickle cell disease and infection]. J Pediatr (Rio J) [Internet]. 2004[citado 2009 Dez 21];80(5):347-54. Comment in: J Pediatr (Rio J). 2005;81(1):88. Disponível em: http://www.jped.com.br/conteudo/04-80-05-347/ing.asp
4. Adorno EV, Couto FD, Moura Neto JP, Menezes JF, Rêgo M, Reis MG, et al. Hemoglobinopathies in newborns from Salvador, Bahia, Northeast Brazil. Cad Saude Publica. 2005;21(1):292-8.
5. Dacie JV, Lewis SM. Hematologia prática de Dacie e Lewis. 9th ed. Porto Alegre: Artmed; 2006.
6. Globin Gene Server [Internet]. Pennsylvania: Laboratories of Computer Science & Engineering and Biochemistry & Molecular Biology; 2013. [cited 2013 February 14] Available from: http://globin.cse.psu.edu/
7. Calder D, Etienne-Julan M, Romana M, Watkins N, Knight-Madden JM. Sickle retinopathy in a person with hemoglobin s/New York disease. Case Rep Genet. 2012;2012:136582.
8. Wenning MR, Kimura EM, Costa FF, Saad ST, Gervásio S, de Jorge SB, et al. Alpha-globin genes: thalassemic and structural alterations in a Brazilian population. Braz J Med Biol Res. 2000;33(9):1041-5.
9. Wenning MR, Kimura EM, Jorge SB, Costa FF, Sonati MF. Molecular characterization of hemoglobins Kurosaki [alpha7 Lys-->Glu], G-Pest [alpha74 Asp-->Asn], Stanleyville-II [alpha78 Asn-->Lys] and J-Rovigo [alpha53 Ala-->Asp]. Acta Haematol. 1999;102(4):203-5.
10. Geva A, Clark JJ, Zhang Y, Popowicz A, Manning JM, Neufeld EJ. Hemoglobin Jamaica plain--a sickling hemoglobin with reduced oxygen affinity. N Engl J Med. 2004;351(15):1532-8. Comment in: N Engl J Med. 2004;35(15):1490-2.
11. Jorge SE, Petruk AA, Kimura EM, Oliveira DM, Caire L, Suemasu CN, et al. Hb S-São Paulo: a new sickling hemoglobin with stable polymers and decreased oxygen affinity. Arch Biochem Biophys. 2012;519(1):23-31.
12. Costa FF, Zago MA, Sonati MF, Bottura C. The association of Hb Stanleyville II with alpha thalassemia and Hb S. Nouv Rev Fr Hematol. 1987;29(6):387-90.
13. de Oliveira RG, Zamaro PJ, Bonini-Domingos CR. Comparing electrophoresis at alkaline pH and high performance liquid chromatography to diagnose Hb S-like hemoglobin. Rev Bras Hematol Hemoter. 2012;34(2):170-1.
14. Kimura EM, Oliveira DM, Jorge SE, Abreu CF, Albuquerque DM, Costa FF, et al. Identificação e caracterização de variantes novas e raras da hemoglobina humana. Rev Bras Hematol Hemoter. 2008;30(4):316-9.
15. Silva MR, Sendin SM, Pimentel FS, Velloso-Rodrigues C, Romanha ÁJ, Viana MB. Hb Stanleyville-II [α78(EF7)Asn→Lys (α2); HbA2: c.237C>A]: incidence of 1:11,500 in a newborn screening program in Brazil. Hemoglobin. 2012;36(4):388-94.
16. Silva MR, Sendin SM, Araujo IC, Pimentel FS, Viana MB. Clinical and molecular characterization of hemoglobin Maputo [beta 47 (CD6) Asp > Tyr HBB: c.142G > T] and G-Ferrara [beta 57 (E1) Asn > Lys HBB: c.174C > A] in a newborn screening in Brazil. Int J Lab Hematol. 2012 Dec 27. Forthcoming.
17. Silva MR, Sendin SM, Araujo IC, Pimentel FS, Viana MB. Alpha chain hemoglobins with electrophoretic mobility similar to that of hemoglobin S in a newborn screening program. Rev Bras Hematol Hemoter. 35(2):109-14.