Jornal Brasileiro de Patologia e Medicina Laboratorial
ISSN: 1676-2444
jbpml@sbpc.org.br
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Brasil

Nascimento, Rafael E.; Castelo, Natália M.; Bueno, Adriana C.; Mescouto, Larissa; Rodrigues, Artemis S. N.
Absence of atypical haplotype and presence of Senegal haplotype sickle cell disease in African-descent population in the northern Brazil
Jornal Brasileiro de Patologia e Medicina Laboratorial, vol. 51, núm. 2, marzo-abril, 2015, pp. 99-101
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Rio de Janeiro, Brasil

J Bras Patol Med Lab, v. 51, n. 2, p. 99-101, April 2015

10.5935/1676-2444.20150017

Absence of atypical haplotype and presence of Senegal haplotype sickle cell disease in African-descent population in the northern Brazil

Ausência do haplótipo atípico e presença do haplótipo Senegal da doença falciforme em uma população afrodescendente na região norte do Brasil

Rafael E. Nascimento; Natália M. Castelo; Adriana C. Bueno; Larissa Mescouto; Artemis S. N. Rodrigues

Universidade Federal do Amapá (UNIFAP).

ABSTRACT

Introduction: Sickle cell anemia (SCA) is the most severe form of sickle cell disease; it presents variants that are called haplotypes β^S. There are five major haplotypes β^S gene: Arab-Indian/Saudi, Senegal, Benin, Bantu, and Camaroon. **Objective:** Characterize the presence of haplotypes in patients with SCA in Amapá. **Method:** 46 sample were studied, all samples were amplified and analyzed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). **Results:** Bantu (61.2%), followed by Benin (26.5%) and Senegal (12.2%). **Conclusion:** We identified three haplotypes characteristic of African ethnicity, with the presence of Senegal. In our study we found the presence of atypical haplotype, suggesting concentration and semi-isolation of the founding groups with little mixing.

Key words: sickle cell disease; HBS gene; haplotypes; northern Brazil.

INTRODUCTION

The Brazilian population is characterized by having wide genetic heterogeneity, so the distribution of the hemoglobin variants is closely related to ethnic groups that compose it, i.e., it is due to high degree of miscegenation. Since the discovery of hemoglobin variants, great progress have been emerging, and, through them, we could confirm the importance of hemoglobinopathies as problem of medical relevance, among which the sickle cell disease stands out, it is considered the hematologic and hereditary condition with the highest prevalence rate in the world[1, 2].

The cause of sickle cell disease is a mutation in the gene that produces hemoglobin A, (HbA) originating a hemoglobin known as S (HbS), which is an autosomal recessive inheritance. Besides HbS there are other hemoglobins (C, D, E etc.), which combined with S are a group called sickle cell disease: sickle cell anemia (Hb SS), sickle cell S/beta thalassemia (Hb S/β Th), SC, SD, SE, and other rarer[3].

Among the sickle cell disease, the one of greater clinical significance is SCA or homozygous for hemoglobin S (Hb SS), the monogenic genetic disorder most common in Brazil, its cause is the point mutation (G<u>A</u>G $\rightarrow$ G<u>T</u>G) in the beta globin gene, resulting in HbS. This disease is notable for its clinical and hematological variability that occurs from the first year and extends throughout life. While some patients have clinical signs of high severity and are subject to numerous complications and frequent hospitalizations, other have milder evolution[3-5].

We can observe that the main clinical manifestations presented are chronic anemia accompanied by osteoarticular and abdominal pain, infections and pulmonary infarction, growth retardation, cerebrovascular accident (CVA), chronic involvement of multiple organs or systems, such as circulatory system, kidneys, eyes and skin, in addition to the development of ulcers in limbs and osteonecrosis[6].

Therefore, the SCA is characterized by chronic inflammatory manifestations; we observed that origin of most of clinical manifestations of this disease is linked to three mechanisms:

First submission on 29/01/14; last submission on 05/03/15; accepted for publication on 07/03/15; published on 20/04/15

a) adhesion of erythrocytes, granulocytes, monocytes and platelets to vascular endothelium; b) chronic inflammatory-reactive phenomena, exacerbated by acute episodes; c) production of inflammatory intermediaries, such as cytokines, and altered nitric oxide metabolism. The adhesion of erythrocytes leads to local obstruction and hypoxia, with consequent worsening of cell shape changes, while triggering inflammatory phenomena that are more intense when there is tissue necrosis, besides coagulation alterations and mobilization of acute (granulocytes) and chronic (monocytes) inflammatory cells (development of ulcers in limbs and osteonecrosis)[6, 7].

Sickle cell trait is one of the most common genetic conditions in Brazilian populations, affecting 6%-10% of African, and about 1% of the general population. This sickle cell trait resulting from heterozygous state for HbS gene, shows one normal gene and one affected in the beta chain, and produces about 60% of HbA and 40% of HbS. The fact that HbA is present in a higher percentage justifies the absence of symptoms in people heterozygous for hemoglobin S[6].

Therefore, the identification of individuals with sickle cell trait or heterozygous for hemoglobin S is very relevant. Carriers must have the knowledge of their genetic condition and know that they have a high risk of having children with SCA and heterozygous children, who would be parents of other future patients with sickle cell[8].

Early diagnosis of SCA through newborn screening allows the monitoring of patients before occurring manifestations and symptoms and, thus, preventing complications and squeals. Prophylaxis includes drug treatment, administration of vaccines, early identification, and proper management of febrile episodes. These measures significantly reduce the mortality associated with SCA, on average from 30% to 1%, especially due to infection[8].

This study aimed to characterize the haplotype of β^S gene in a group of individuals with SCA in the state of Amapá, and to provide important information regarding the genetic characteristics of these carriers, and contribute to the implementation of early diagnosis, contributing to suitable treatment.

METHOD

This is a cross-sectional cohort study performed in 46 volunteer patients of both sexes, with clinical and laboratory diagnosis of sickle cell disease (homozygous form SS), assisted at the ambulatory of Hematology of Hematology and Hemotherapy Center of Amapá (HEMOAP). The project was submitted to analysis the reporter of the Committee on Ethics in Research Involving Human Beings of the Federal University of Amapá and had favorable opinion for execution.

Blood samples (n = 46) collected from SS patients assisted at HEMOAP underwent laboratory screening for hemoglobin (electrophoresis at alkaline and acid pH; high-performance liquid chromatography [HPLC]), then the extraction of deoxyribonucleic acid (DNA) for confirmation of HbSS and determination of haplotype by polymerase chain reaction-restriction fragment length polymorfism (PCR-RFLP), with restriction endonuclease: *Xmn I, Hind III, Hinc II, Hinf I* for analysis of six polymorphic sites.

RESULTS

Among the 46 samples analyzed three kinds of haplotypes were identified, among which, more often, Bantu (61.2%) haplotype stands out, followed by Benin (26.6%) and Senegal (12. 2%), we observed the absence of Cameroon, Saudi, atypical or indeterminate haplotypes.

DISCUSSÃO

Among the 46 DNA samples analyzed for haplotypes identification of β^S gene, the more common was Bantu haplotype. Our results, regarding the frequency of this haplotype, are consistent with those described in the literature that emphasizes the frequency of Bantu haplotype in regions of Brazil[2, 8, 9].

In our study, there were no Cameroon and Saudi haplotypes, similar to the study of Adorno *et al*. (2003)[10] held in Bahia, they also determined atypical haplotypes, differing from data of Amapá studies, in which atypical haploid were not found.

Atypical haplotypes observed in association with β^S gene are generated by several genetic mechanisms: a) nucleotide change in one restriction-site polymorphism; b) single and double crossing-over between two typical β^S haplotypes, or more often, between a typical βS haplotype and a different β^A haplotype; and c) nonreciprocal transfer of sequences conversion. In a study by Romana *et al*. (2000), the authors observed that 15 from 20 different atypical haplotypes were originated by recombination[9, 11].

A study involving 244 patients with SCA (156 Brazilian and 88 Benineses) analyzed 488 chromosomes, 15 (3.2%) atypical haplotypes were found. The frequency of atypical haplotypes

observed in the Brazilian sample was 3.9% and in the Benin sample, 2.2%. The percentage of atypical haplotypes found in both populations ranged between 5.3% and 7.0%[9].

The nature of the slave trading practiced in the American Continent disintegrated the slave family structure, allowing people mix among from different African ethnic. Mixing with non-African or non-African descent populations can be estimated at a rate of 1.3%, 2.5%, and 3% per generation in the United States, Brazil and Cuba, respectively. This high degree of miscegenation may have favored the appearance of atypical haplotypes by recombination[3-5, 10, 12].

In our study we did not observed individuals with SCA and the atypical haplotype or associated with other β^S haplotype, it means that there was enough mixing between African and non-African in Amapá to emerge atypical haplotypes, thus determining that results from Amapá exhibit unique characteristics when related to haplotypes from other regions, with the presence of Senegal haplotype and absence of atypical, Cameroon and Saudi, confirming that Brazil has a diversity of ethnic origin, as well as different haplotype frequencies.

ACKNOWLEDGEMENTS

To Dr. Claudia Bonini for their support and technical assistance, to all people with sickle cell disease who agreed to participate and to directors and employees of HEMOAP.

RESUMO

Introdução: *A anemia falciforme (AF) é a forma mais grave da doença falciforme; apresenta formas variantes que são chamadas de haplótipos* β^S*. Existem cinco principais haplótipos desse gene: árabe-indiano/saudi, Senegal, Benin, Bantu e Camarões.* ***Objetivo:*** *Caracterizar a presença dos haplótipos em pacientes com AF no Amapá.* ***Método:*** *Foram estudadas 46 amostras, todas amplificadas e analisadas pela técnica de reação em cadeia da polimerase-polimorfismo de tamanho de fragmentos de restrição (PCR-RFLP).* ***Resultado:*** *Bantu (61,2%) seguido de Benin (26,5%) e Senegal (12,2%).* ***Conclusão:*** *Foram identificados três haplótipos característicos da etnia africana, com a presença do Senegal. Em nosso estudo não encontramos a presença de haplótipos atípicos, sugerindo a concentração e semi-isolamento de grupos fundadores com pouca miscigenação.*

Unitermos: *doença falciforme; gene HBS; haplótipos; região norte.*

REFERENCES

1. Naoum PC. Interferentes eritrocitários e ambientais na anemia falciforme. Rev Bras Hematol Hemoter. 2000; 22: 5-22.

2. Neto GCG, Pitombeira MS. Aspectos moleculares da anemia falciforme. JBPML. 2003; 39: 51-6.

3. Agência Nacional de Vigilância Sanitária. Manual de diagnóstico e tratamento de doença falciforme. Brasília; 2002. 142 p.

4. Castilhos JK. Análise de haplótipos βS em pacientes com anemia falciforme de Porto Alegre-RS. [trabalho de conclusão de curso]. Nova Hamburgo: Centro Universitário Feevale, Instituto de Ciências da Saúde; 2008.

5. Cipolotti R, et al. Diversidade clínica e laboratorial no haplótipo bantu da anemia falciforme. Rev Bras Hematol Hemoter. 2006; 28: 40-4.

6. Mendonça AC, Garcia JL, Almeida CM, et al. Muito além do teste do pezinho. Rev Bras Hematol Hemoter. 2009; 31(2): 88-93.

8. Pinto ACS, Zago MA. Fisiopatologia das doenças falciformes: da mutação genética à insuficiência de múltiplos órgãos. Rev Bras Hematol Hemoter. 2007; 29: 207-14.

9. Silva MAL. Estudo da identificação de haplótipos e a relação com as manifestações clínicas em pacientes com doença falciforme. 2008. [dissertação]. Programa de pós-graduação em Ciências Médicas, Universidade Federal do Rio Grande do Sul; 2008.

10. Adorno EV, Gonçalves MS, Bonfim GC, et al. ßS-Haplotypes in sickle cell anemia patients from Salvador, Bahia, Northeastern Brazil. Braz J Med Biol Res. 2003; 36: 1283-8.

11. Zago MA, Silva WA Jr, Dalle B, et al. Atypical βS haplotypes are generated by diverse genetic mechanisms. Am J Hematol. 2000; 63: 79-84.

12. Cardoso GL, Guerreiro JF. African gene flow to North Brazil as revealed by HBB*S gene haplotype analysis. Am J Hum Biol. 2006; 18: 93-8.

MAILING ADDRESS

Artemis Socorro do Nascimento

Universidade Federal do Amapá; Rod. Juscelino Kubitschek, KM 02; Jardim Marco Zero; CEP: 68903-419; Macapá-AP, Brazil; e-mail: artemis@unifap.br.