Frequency of hereditary hemochromatosis gene mutations (C282Y and H63D) in hemoglobin S carrier from Brazil

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Abstract:

Brazil is a multiethnic country and the prevalence of hereditary hemochromatosis is unknown, in special associated with other autosomal recessive disorder. The hemoglobinopathies screening was performed by electrophoretical and HPLC procedures, in blood samples from three Brazilian regions and was selected the Hb AS profile. The HH mutations was verified by PCR-ASO and Hb S genotype by PCR-RFLP and PCR-ASO. Were analyzed by molecular biology, 48 blood samples and The C282Y HFE mutations were found in 8,33% and H63D in 14,58% of the samples. The total value of HFE alterations in Hb S carriers was 22,9%. Mutations screening in populations of different ethnic background are recommended to define its contribution to hereditary hemochromatosis in non-caucasian people. The history and structure of the Brazilian population, to which contributed a large number of ethnic components in increasing miscegenation, are frequency and distribution of hereditary disease. These results can be explain the diverse symptom of Hb S carries.

Key Words: hereditary hemochromatosis, Hb S, Molecular diagnosis

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Introduction

Structural hemoglobin (Hb) variants typically are based on a point mutation in a globin gene that produces a single amino acid substitution in a globin chain. Although most are of limited clinical significance, a few important subtypes have been identified with some frequency. One of the most important hereditary hematological diseases in Brazilian population is sickle cell anemia (Hb SS) with prevalence estimated of 1 in 1000 birth. The sickle cell traits (Hb AS) don't have significant clinical manifestation (CLARKE & HIGGINS, 2000; BONINI-DOMINGOS, et al., 2000). The globin genes frequency varies considerably in Brazil, from 1.9% in the general population of the Southeast to 7.5% in the Northeast, while among Afro-Brazilian selected samples it varies from 5.2 to 10.2 (ZAGO et al., 1999). The clinical manifestation are the direct result of vaso-occlusive phenomena, by the Hb S polymerization and membrane damage.

Hereditary Hemochromatosis (HH) is one of the most common genetic diseases in individuals from Northern European descent. It is an autossomal recessive disorder of iron metabolism and expressed more severely in males than in females, characterized by exaggerated absorption of intestinal iron. The HFE gene is found in region 21.3 on the short (p) arm of human chromosome 6 [6p21.3] In HH, iron accumulation in a variety of organs leads to organ failure. Signs and symptoms are a result of progressive tissue iron accumulation. Early symptoms may be vague and easily overlooked. A single mutation C282Y in the HFE gene explains 80–90% of all diagnosed HH cases in population of Northwestern European ancestry. The importance of another frequent mutation in this gene, H63D is still a matter of debate (GIRONARD, 2002). The frequency of

carriers for this mutation in Northern European is 1 in 8 -10 for carrier. Screening of persons with any of the heterogeneous and nonspecific problems potentially representing iron overload, including weakness, fatigue, arthropathies, liver disease, heart disease, or diabetes, is necessary.

In this study we estimated the prevalence of these two HH mutations in carriers of Hb S, from Brazil.

Materials and Methods

Specimens:

Blood samples (EDTA anti coagulated) sent to the laboratory for Hb analysis. The study was in accordance with CONEP, and the sample was obtained after consent.

Analytical Methods

After hemolysis with a provided reagent [50 ul of whole blood + 50 ul saponin 1%] the samples were submitted to electrophoretical procedures at alkaline and acid pH (BONINI-DOMINGOS, 1993)

Automated cation exchange HPLC (VARIANT^{TM} hemoglobin system beta thalassemia short program; BIO-RAD Laboratories) was used for the identification of abnormal hemoglobins and Hb A_2 and F quantification (VARIANT MANUAL 2002).

Genomic DNA was extracted from whole blood samples using the BIO-RAD mDx Instagene^{TM} Genomic DNA kit (BONINI-DOMINGOS, et al., 2001).

The ?-globin gene and HFE gene were amplified using master mix by the PCR process in the presence of biotinyled primers, for the Hb S, H63D and C282Y

mutation (MDx instruction Manual, 2002). The PCR products were submitted to hybridization with ASOs for the specific mutations (mDx kit BIO-RAD).

In some cases, the Hb S mutation was detected by PCR-RFLP with specific primers and Dde I digestion (BONINI-DOMINGOS, et al., 2001).

Results and Discussion

Were analyzed 48 whole blood samples by electrophoretic and HPLC procedures, and the profile of Hb AS were found in 36 samples. Association between Hb AS and alpha thalassemia (Hb ASH) was found in 12 samples.

The alpha thalassemia presence was confirmed by electrophoretic and cytological procedures. The Hb A_2 and Hb F value was in accordance to the heterozygous estate for that sample with Hb variant in special for Hb AS. The presence of Hb S was shown to influence Hb A_2 quantification with the HPLC method used, probably because of Hb S adducts collating with Hb A_2.

All the samples were from individual with discreet anemia, which is not common in sickle cell trait. 35% are from female and 65% are from male. Most of then are Afro-descendents, but in some cases, is very difficult determinate the racial origin in Brazilian population. For these 48 blood samples, the individual related that 20% had a Caucasian origin and 78% were Afro-descendent.

The Hb S was confirmed by the presence of the allele that encodes $?^S$-globin chain, using the principle of allele specific oligonucleotide (ASO) hybridization. After this procedure confirmation, five samples weren't present a secure result and PCR-RFLP were used to confirm the Hb S mutation.

With the Hb S presence confirmed, the HFE gene was amplified by the PCR process with multiplex amplification master mix included in the kit, which

contains primers necessary for the amplification of both the C282Y and the H63D mutation. The PCR process generates two fragments of 395 bp and 207 bp that correspond to C282Y and H63D mutations fragment. After the amplification the hybridization steps was processed in accordance with kit methodology.The distributions of HFE mutations observed are shown in table1.

Table 1. Genotypic frequency for HFE gene mutations in Hb S carriers.

Hemoglobin	Hereditary Hemochromatosis		Total
	C282Y	H63D	
AS (36)	3	4	7
ASH (12)	1	3	4
Total 48	4 (8,33%)	7 (14,58%)	11 (22,91%)

We found 22,91% of HFE mutation in blood samples with Hb S. For the Hb AS profile 19,44% present HH mutation, with 3 (8,33%) C282Y and 4 (11,11%) H63D. In that samples with Hb ASH we found 1 C282Y and 3 (25%), in a total defects of 33,33%.

The vast majority of Caucasian patients with HH demonstrate a single homozygous missense mutation in the HFE gene (C282Y). The underlying genetic defects in hemochromatosis patients of non-Caucasian origin are largely unknown. Mutation screening in populations of different ethnic background is recommended to precisely define its contribution to HH in non-Caucasian patient's (STEINER et al., 2002).

It has been proposed that iron overload may adversely affect liver disease outcome. HFE gene mutation might be an additional factor to be considered

among those implicated in the determination of a worse prognosis of the lives disease (MARTINELLI et al., 2002).

The excess absorbed iron can be lead to accumulation in tissue and organs (lives, heart, pancreas) and subsequent organ dysfunction and failure. The disease can also cause other serious illnesses as cirrhosis, hepatomas, diabetes, cardiomyopathy, arthritis and hipogonadotrophic hypogonadism. Most of this dysfunction was observed in sickle cell anemia patients too.

Conclusion

The history and structure of the Brazilian population, to which contributed a large number of ethnic components in increasing miscegenation, are frequency and distribution of hereditary disease. Sickle cell anemia is the most common hereditary hematological disease in Brazil; the HH distribution is unknown. We found in a group of sickle cell anemia carriers a high frequency of – HFE mutation – H63D (14,58%) and C282Y (8,33%) as a result of multiethnic process population. It can be explain the diverse symptom of Hb S carries.

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