Revista Brasileira de Hematologia e Hemoterapia
Brazilian Journal of Hematology and Hemotherapy
www.rbhh.org

Original article

Molecular analysis and association with clinical and laboratory manifestations in children with sickle cell anemia

Roberta Faria Camilo-Araújo, Olga Maria Silverio Amancio, Maria Stella Figueiredo, Ana Carolina Cabanãs-Pedro, Josefina Aparecida Pellegrini Braga*

Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 1 August 2013
Accepted 31 March 2014
Available online 9 July 2014

Keywords:
Anemia, Sickle Cell
Alpha-thalassemia
Beta-globins
Haplotypes
Child

ABSTRACT

Objectives: To analyze the frequency of β^S-globin haplotypes and alpha-thalassemia, and their influence on clinical manifestations and the hematological profile of children with sickle cell anemia.

Method: The frequency of β^S-globin haplotypes and alpha-thalassemia and any association with clinical and laboratorial manifestations were determined in 117 sickle cell anemia children aged 3–71 months. The confirmation of hemoglobin SS and determination of the haplotypes were achieved by polymerase chain reaction-restriction fragment length polymorphism, and alpha-thalassemia genotyping was by multiplex polymerase chain reaction (single-tube multiplex-polymerase chain reaction).

Results: The genotype distribution of haplotypes was 43 (36.7%) Central African Republic/Benin, 41 (35.0%) Central African Republic/Central African Republic, 20 (17.0%) Rare/atypical, and 13 (11.1%) Benin/Benin. The frequency of the α3.7 deletion was 1.71% as homozygous ($-\alpha3.7/-\alpha3.7$) and 11.9% as heterozygous ($-\alpha3.7/\alpha\alpha$). The only significant association in respect to haplotypes was related to the mean corpuscular volume. The presence of alpha-thalassemia was significantly associated to decreases in mean corpuscular volume, mean corpuscular hemoglobin and reticulocyte count and to an increase in the red blood cell count. There were no significant associations of β^S-globin haplotypes and alpha-thalassemia with clinical manifestations.

Conclusions: In the study population, the frequency of alpha-thalassemia was similar to published data in Brazil with the Central African Republic haplotype being the most common, followed by the Benin haplotype. β^S-globin haplotypes and interaction between alpha-thalassemia and sickle cell anemia did not influence fetal hemoglobin concentrations or the number of clinical manifestations.

* *Corresponding author at:* Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), Rua Botucatu, 703, Vila Clementino, 04023-062 São Paulo, SP, Brazil.
E-mail address: robertafc.dped@epm.br (R.F. Camilo-Araújo).
http://dx.doi.org/10.1016/j.bjhh.2014.06.002

Introduction

Sickle cell anemia (Hb SS) is characterized by homozygosity for hemoglobin S (Hb S), consequent to a point mutation in the 6th codon of the β-globin gene on chromosome 11.[1,2] It is the most prevalent hereditary hematologic disease in Brazil with elevated morbidity and mortality. It is estimated that 3500 children are born with Hb SS annually, one in every 1000 live births, whereas the frequency of heterozygotes in the general population is 2–8%.[3,4] The clinical state of these patients is exceptionally variable, which may be influenced by different factors, such as the haplotypes of the β^S-globin gene, the presence of alpha-thalassemia, and the fetal hemoglobin level (Hb F).[1,2]

Haplotypes of the β^S-globin gene are determined by specific polymorphism patterns in the β gene complex. They originate from different regions of Africa, from which they receive their denominations: CAR or Bantu (Central African Republic), Benin (West Africa), Senegal (West Africa), Cameroon (West Africa) and Saudi-Indian (Asia, India and the Arabian Peninsula). Polymorphism sets not recognized by these classical patterns are referred to as atypical (ATP) and occur at a rate of 5–10%.[5,6] In Brazil, due to the forced migration of African descendants, CAR and Benin (BEN) haplotypes are the most common.[5,7–11]

Alpha-thalassemia is the result of deficient synthesis of α globin chains in hemoglobin (Hb). The α chain genes are located on chromosome 16, and normal individuals have two α genes on each chromosome with this genotype being represented as αα/αα.[12] The α3.7 deletion, which causes the most common form of alpha-thalassemia in Brazil,[13] is the result of an unequal cross-over exhibiting a loss of a 3.7 kb DNA fragment.[14] Recent Brazilian studies show different prevalences for the α3.7 genotype according to the geographical ancestry of the individuals studied: 4.5% in European and 21.5% in African descendants,[15] data similar to those obtained in populations of African descents by Sonati et al.[16] Brazilian studies analyzing alpha-thalassemia in individuals with Hb SS present prevalences of α3.7 heterozygous individuals of from 17.6 to 28.2%, according to the geographic region.[9–11,13,17]

The interaction between Hb SS and alpha-thalassemia is associated with an inhibition of Hb S polymerization[2,18,19] which decreases hemolytic episodes[2,18] entailing reductions in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) compared to Hb SS individuals without alpha-thalassemia.[20] An improvement in the patients' clinical state is observed with reductions in sickle cell crises.[18,21] The presence of alpha-thalassemia is also significantly associated with a reduction in white blood cell (WBC)[15] and reticulocyte[2,17] counts, and increased levels of Hb A_2.[17,22–25] Although there is no defined relationship, increased levels of Hb F are also reported.[2,24]

The objective of this study was to assess the prevalence of β^S-globin haplotypes and alpha-thalassemia in a group of children with Hb SS and their influence on hematological profile and clinical manifestations.

Methods

This was a cross-sectional study with convenience sampling involving 117 of 122 under 6-year-old children with Hb SS, no matter the severity of the disease, attended at the Pediatric Hematology Outpatient Clinic of the Escola Paulista de Medicina, Universidade Federal de São Paulo. The samples were collected from September 2007 to December 2009; the age varied from 3 to 71 months (median age of 35.5 months) and 62 (52.99%) of the children were male. The study was approval by the Research Ethics Committee of the Escola Paulista de Medicina, Universidade Federal de São Paulo.

Exclusion criteria

Patients who had received red blood cell transfusions in the 3 months prior to sample collection and those receiving hydroxyurea were excluded from the study.

Clinical and laboratorial variables

Clinical and laboratorial variables were obtained from the patient's medical records. The following laboratorial criteria were analyzed: Hb level, hematocrit (Ht), red blood cell (RBC), WBC, MCV, MCH, reticulocyte, and platelet counts, and Hb F levels.

The clinical variables were analyzed considering whether the patients had <3 or ≥3 types of manifestations per year including vaso-occlusive crisis (VOC), acute chest syndrome/pneumonia (ACS/PNM), infections (acute osteomyelitis and urinary tract infection) and episodes of acute splenic sequestration.

Molecular analysis

DNA was extracted from WBCs in peripheral blood using phenol–chloroform extraction and ethanol precipitation.[26] The Hb S mutation was confirmed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).[27] Single-tube multiplex-PCR was used for alpha-thalassemia genotyping using primers standardized by Chong et al.[28] Determination of the haplotypes of the β^s gene complex was performed by PCR-RFLP analysis using primers as proposed by Sutton et al.[29] All tests were conducted using a thermal cycler from Veriti Applied Biosystems® (Foster City, CA, USA).

Statistical analysis

Non-parametric tests were used for statistical analysis taking into account the variability and distribution of the study variables. Kruskal–Wallis with the Dunn test was used to compare more than two samples, and the Mann–Whitney test for two independent samples. Significance was set for alpha errors of 5% (p-value <0.05); statistically analysis was performed using the InStat-2 (GraphPad®, San Diego, CA, USA) and SigmaStat 3.11 software (Systat® Software, Chicago, IL, USA).