# Sickle cell disease: Acute clinical manifestations in early childhood and molecular characteristics in a group of children in Rio de Janeiro

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***Objective:*** *To describe clinical events of sickle cell disease and the correlation with ß-globin haplotypes and α-thalassemia in under 6-year-old children.*

***Methods:*** *A retrospective study was conducted of under 6-year-old children from the neonatal screening program in Rio de Janeiro. Forty-eight male and 48 female children were enrolled in this study, 79 with sickle cell anemia and 17 with hemoglobin SC. The mean age was 29.9 (standard deviation = 20.9) months, 62 (16.2 ± 8.6) were aged between 0-3 years old and 34 (54.9 ± 11.3) were from 3-6 years old. Painful events, acute splenic sequestration, hemolytic crises, hand-foot and acute chest syndromes and infections were evaluated.*

***Results:*** *The events were more frequent in under 3-year-old children, 94% of children had at least one episode. Infection was the most common event affecting 88.5% of children. Acute splenic sequestration took place earlier, while painful crises and acute chest syndromes in under 6-year-old children. Thal-α 3.7 was observed in 20.9% of cases. Bantu was the most frequent haplotype found, followed by Benin. No correlation was observed between clinical events and ß-globin haplotypes. Children with sickle cell anemia and α-thalassemia have less infectious events. No correlation was found among these polymorphisms and clinical events, however, the majority of children with Bantu/Bantu and without α-thalassemia had more clinical events.*

***Keywords:*** *Anemia, sickle cell; Alpha-thalassemia; Haplotypes; Neonatal screening; Clinical evolution*

## Introduction

In Brazil, it is estimated that around 3500 children are born with sickle cell anemia (SCA) each year, with an overall incidence of one case per 1000 births[1]. In Rio de Janeiro, one infant per 21 births is a carrier of the sickle cell trait, whereas one infant per 1200 has sickle cell disease (SCD)[1]. The prevalence of SCA is variable in Brazil and depends on the geographical region as well as the ethnic group studied; prevalence is higher in regions that maintained trade routes with African countries, such as the northeastern states and Rio de Janeiro[2]. After gold was discovered in the state of Minas Gerais and the slave trade was prohibited north of the equator, the state of Rio de Janeiro became the most important center for the reception and distribution of slaves. Nowadays, Rio de Janeiro is one of the Brazilian states with the highest incidence of SCA[2].

In 2001, the inclusion of hemoglobinopathies in the National Neonatal Screening Program (NNSP) has enabled the early diagnosis and efficient follow-up of cases; this is of unquestionable benefit for children compared to diagnosis only after the first clinical manifestations of the disease.

Early diagnosis has allowed a better understanding of SCD in the Brazilian population as, despite the therapeutic advances achieved in recent years, SCD is still responsible for significant morbidity[3].

A retrospective study was performed with patients from Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti (HEMORIO) in Rio de Janeiro to study acute clinical manifestations of SCD in early childhood.

## Methods

A retrospective study was performed of SCD children who were diagnosed by the neonatal hemoglobinopathy screening program of the state of Rio de Janeiro, Brazil. The screening was performed by the Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti, Rio de Janeiro where children with SCD were provided clinical assistance. The Ethics Research Committee of the institution approved the study and informed consent was obtained from the children's parents. From October 2006 to December 2007, children diagnosed with SCD were randomly included in the study and clinical and demographic data were obtained from their medical records. All acute clinical events correctly recorded in the medical files from birth to the date of the final evaluation (July 2008) were included in the analyses. The following events were considered: painful and hemolytic crisis, splenic sequestration, hand-foot syndrome,

infection and acute chest syndrome. The criteria used to define the clinical events were as follows. (a) Painful crisis: a painful event that required hospital treatment. (b) Hemolytic crisis: decreases in the concentration of hemoglobin (Hb) and hematocrit. (c) Splenic sequestration crisis: the presence of splenomegaly associated with a significant decrease of Hb from baseline levels. (d) Hand-foot syndrome: swelling in the hands and feet with pain and/or local heat, which may also be associated with a decrease in Hb concentration. (e) Infection: fever accompanied by prostration and leukocytosis, with or without other laboratory tests and imaging. (f) Acute chest syndrome: chest radiography with recent pulmonary infiltrate associated with severe respiratory symptoms, low oxygen saturation and requirement of a blood transfusion for clinical and radiological improvement.

All peripheral blood counts and fetal Hb levels registered in the medical records were recorded and the mean of these counts was used when analyzing the data.

Screening for SCD and fetal Hb were carried out by high-pressure liquid chromatography (HPLC, Variant II, Bio-Rad Laboratories, Hercules, CA, USA). Assessments of blood counts were performed using a Cell Dyn 3700 analyzer (Abbott).

Two to five milliliters of whole blood were collected in ethylenediaminetetraacetic acid (EDTA) during routine medical examinations of the children. DNA extraction was performed according to the instructions accompanying the commercial kit (Puregene® DNA Purification System From Whole Blood, Gentra Inc., Minneapolis, USA).

The ß-globin gene cluster haplotypes [Bantu or CAR (Central African Republic), Benin, Senegal, Arab-Indian, CI, CII, CIII, and Atypical] were investigated by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP).

A pattern of six polymorphic restriction sites was identified within the ß-globin gene complex (5´$\gamma^G$, $\gamma^G$, ´$\gamma^A$, ψß, 3´ψß, 5´ß). Each PCR reaction was performed with a final volume of 50 µL containing 100 ng of DNA, 1.5 µL of dNTPs (10 mM), 10 pmol of each primer, 0.5 µL (2.5 U) of Taq DNA polymerase (Qiagen), 5.0 µL of 10 x buffer containing 200 mM Tris-HCl (pH 8.4), 500 mM KCl, and 2.0 µL $MgCl_2$ at a final concentration of 50 mM. Amplification was performed using an Eppendorf Mastercycler Gradient Thermal Cycler®. The reaction conditions for the investigation of the 5´$\gamma^G$ region consisted of an initial denaturation step of 5 min at 94°C followed by 35 cycles at 94°C for 45 s, 60°C for 45 s, and 72°C for 1 min 30 s, and an additional extension at 72°C for 7 min. For the $\gamma^G$, $\gamma^A$, 3´ψß, and ψß regions the reaction conditions consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles at 94°C for 30 s, 55°C for 1 min and 72°C for 1 min and an additional extension at 72°C for 7 min. The reaction conditions for the 5´ß region consisted of an initial denaturation at 94°C for 10 min followed by 35 cycles at 94°C for 45 s, 57°C for 45 s and 72°C for 1 min 30 s, and a final extension step at 72°C for 10 min. Ten microliters of the products obtained were digested with restriction endonucleases to identify the polymorphisms (XmnI [5´$\gamma^G$], HindIII [$\gamma^G$, $\gamma^A$], HincII [ψß, 3´ψß], and HinfI [5´ß]) according to Sutton et al.[4].

α-Thalassemia (-$\alpha^{3.7kb}$, -$\alpha^{4.2kb}$) single-gene deletions were detected by PCR according to Chong at al.[5]. The technique used enabled identification of the normal allele (α2 gene) and the -$\alpha^{3.7}$ and -$\alpha^{4.2}$ deletions in the same reaction. In the presence of the normal allele, the $\alpha_2$/3.7F and $\alpha_2$R primers generated a 1800 bp DNA fragment, whereas in the presence of the mutated -$\alpha^{3.7}$ and -$\alpha^{4.2}$ alleles, the $\alpha_2$/3.7F, 3.7/20.5R and 4.2F, 4.2R primers generated 2022 bp (-$\alpha^{3.7}$) or 1628 pb (-$\alpha^{4.2}$) fragments. Each 25-µL reaction contained 100 ng DNA, 1.25 µL of each dNTP (10 mM), 1.5 µL of each primer (10 µM), 5 µL of 5x Q-solution (Qiagen), 0.5 µL (2.5 U) of Taq DNA polymerase (Qiagen), 2.5 µL of 10x buffer containing 200 mM Tris-HCl (pH 8.4), 500 mM KCl, and 1.0 µL of $MgCl_2$ (50 mM). Reactions were performed using a Eppendorf Mastercycler Gradient Thermal Cycler with an initial 3-min denaturation step at 95°C followed by 30 cycles of denaturation at 98°C for 45 s, annealing at 63°C for 1 min 30 s, and extension at 72°C for 2 min 15 s followed by a final extension at 72°C for 5 min.

Ninety-six children (48 boys and 48 girls) were included in the study, 79 of whom had SCA and 17 had Hb SC disease.

Analyses were performed using the Statistical Package for Social Sciences (SPSS). Comparisons of categorical data were carried out with the chi-square test, including odds ratios (OR) and 95% confidence intervals (CI); Student's t-test was used to compare quantitative variables between groups and p-values ≤ 0.05 were considered significant.

## Results

The children's ages ranged from 1-80 months with a mean age of 29.9 months (SD ± 20.9) and a median of 24 months. The children were divided into two groups based on their age: the younger children included 62 (64.6%) children aged 0-3 years and the older children 34 (35.4%) children aged 3-6 years old.

Ninety (94%) of the 96 children experienced at least one acute event. Clinical manifestations occurred from age 2-79 months and were more frequent among younger children. Table 1 shows the distribution of clinical events according to age group and Hb (SS or SC). During the study period, two deaths were recorded; one was caused by splenic sequestration in a 13-month-old child with SCA and the other was of unknown cause in a 67-month-old child with Hb SC disease. One child with SCA suffered a stroke and another had an episode of priapism at 34 and 43 months of age, respectively.

Infection, occurring in 88.5% of children, was the most frequent event in both groups. Considering only children with SCA, painful episodes and acute chest syndrome were significantly more frequent in the older children, whereas splenic sequestration was more prevalent in younger children (Table 1).

In the current study, splenic sequestration crises were recorded 89 times, affecting almost half (47.9%) of all children. This event was the one most clearly associated with the age of the children as it was observed in 58.1% of the younger children (p-value = 0.0001). Furthermore, splenic sequestration had a high frequency of relapse: 63% of patients experienced more than one episode.

The most prevalent clinical events per child were painful episodes (2.9 events/child), infection (2.3 events/child) and hemolytic crises (2.1 events/child). Splenic sequestration crises first occurred at a younger mean age (12.8 months), than acute chest syndrome (34.1 months) and painful episodes (29.4 months).

Table 1 - Distribution of acute events according to children age and hemoglobin type

| | 0 to 3-year-old children n (%) | 3 to 6-year-old children n (%) | Total n (%) | $\chi 2^{\#}$ | p-value |
|---|---|---|---|---|---|
| **Painful events** | 18/62 (29.0) | 19/34 (55.9) | 37/96 (38.5) | | |
| Hb SS | 16/49 (32.7) | 17/30 (56.7) | 33/79 (41.8) | 4.412 | 0.03 |
| Hb SC | 2/13 (15.4) | 2/4 (50.0) | 4/17 (23.5) | | |
| **Splenic sequestration** | 36/62 (58.1) | 10/34 (29.4) | 46/96 (47.9) | | |
| Hb SS | 35/49 (71.4) | 9/30 (30.0) | 44/79 (55.7) | 12.943 | 0.0001 |
| Hb SC | 1/13 (7.7) | 1/4 (25.0) | 2/17 (11.8) | | |
| **Hemolytic crisis** | 37/62 (59.8) | 23/34 (67.6) | 60/96 (62.5) | | |
| Hb SS | 35/49 (71.4) | 22/30 (73.3) | 57/79 (72.2) | 0.034 | 0.85 |
| Hb SC | 2/13 (15.4) | 1/4 (25.0) | 3/17 (17.6) | | |
| **Foot-hand syndrome** | 21/62 (33.9) | 14/34 (41.2) | 35/96 (36.4) | | |
| Hb SS | 19/49 (38.8) | 14/30 (46.7) | 33/79 (41.8) | 0.476 | 0.49 |
| Hb SC | 2/13 (15.4) | 0/4 (0) | 2/17 (11.8) | | |
| **Infection** | 53/62 (85.5) | 32/34 (94.1) | 85/96 (88.5) | | |
| Hb SS | 42/49 (85.7) | 28/30 (93.3) | 70/79 (88.6) | 1.070 | 0.47 |
| Hb SC | 11/13 (84.6) | 4/4 (100) | 15/17 (88.2) | | |
| **Acute chest syndrome** | 2/62 (3.2) | 8/34 (23.5) | 10/96 (10.4) | | |
| Hb SS | 2/49 (4.1) | 8/30 (26.7) | 10/79 (12.6) | 8.585 | 0.005 |
| Hb SC | 0/13 (0) | 0/4 (0) | 0/17 (0) | | |

Hb SS = Hemoglobin SS (sickle cell anemia); Hb SC = hemoglobin SC; # chi-square calculated only for events in children with Hb SS

Table 2 - Laboratory data according to gender, age, presence of thalassemia and beta globin gene haplotypes

| | n | Hb* (g/dL) Mean ±SD [95% CI] | p-value | MCV* (fl) Mean ±SD [95% CI] | p-value | Fetal Hb* (%) Mean ±SD [95% CI] | p-value |
|---|---|---|---|---|---|---|---|
| **Gender** | | | | | | | |
| Male | 48 | 8.2 ± 1.2 [7.9-8.6] | | 80.8 ± 12.3 [77.3-84.4] | | 12.0 ± 8.3 [9.5-14.5] | |
| Female | 48 | 8.9 ± 1.3 [8.5-9.2] | < 0.05 | 81.2 ± 9.4 [78.4-83.9] | > 0.05 | 12.3 ± 5.9 [10.4-14.2] | > 0.05 |
| **Age group** | | | | | | | |
| 0-3 years old | 62 | 8.7 ± 1.4 [8.3-9.0] | | 78.4 ± 10.3 [75.8-81.0] | | 13.1 ± 7.6 [10.9-15.2] | |
| 3-6 years old | 34 | 8.3 ± 1.2 [7.8-8.7] | > 0.05 | 85.7 ± 10.6 [82.0-89.4] | < 0.05 | 10.7 ± 6.3 [8.5-12.9] | > 0.05 |
| **α-Thalassemia** | | | | | | | |
| αα/αα | 72 | 8.5 ± 1.2 [8.2-8.8] | | 83.7 ± 9.0 [81.6-88.5] | | 12.5 ± 7.1 [10.7-14.2] | |
| -α/αα or -α/-α | 19 | 8.9 ± 1.7 [8.1-9.7] | > 0.05 | 71.7 ± 11.9 [66.0-77.4] | < 0.05 | 12.5 ± 8.0 [8.1-16.9] | > 0.05 |
| **ß- globin haplotype** | | | | | | | |
| Bantu/Bantu | 39 | 8.0 ± 1.0 [7.6-8.3]# | | 84.8 ± 9.7 [81.7-87.9]# | | 11.9 ± 5.3 [10.2-13.8]# | |
| Bantu/Benin | 32 | 8.7 ± 1.2 [8.2-9.1] | < 0.05 | 83.1 ± 7.3 [80.4-85.7] | > 0.05 | 16.1 ± 8.1 [12.9-19.2] | < 0.05 |
| Bantu/CI | 7 | 10.5 ± 0.5 [10.0-11.0] | | 71.8 ± 8.6 [63.8-79.9] | | 4.1 ± 3.4 [0.5-7.8] | |
| Bantu/Atypical | 9 | 8.5 ± 1.5 [7.4-9.7] | | 74.0 ± 10.7 [65.8-82.3] | | 7.9 ± 3.9 [4.7-11.2] | |
| Benin/Benin | 2 | 7.8 ± 1.5 | | 86.6 ± 14.6 | | 15.9 ± 14.4 | |
| Benin/CI | 2 | 10.4 ± 0.6 | | 66.2 ± 21.5 | | - | |
| Benin/Atypical | 2 | 9.5 ± 1.2 | | 67.7 ± 15.9 | | 5.3 ± 0.7 | |
| Atypical/CI | 2 | 8.3 ± 0.6 | | 80.9 ± 4.2 | | 4.2 ± 0.5 | |

*Mean value of mean of each case; # between Bantu/Bantu and Bantu/Benin groups. Analysis by student t-test. Hb: hemoglobin; MCV: mean corpuscular volume.

Table 2 presents the mean Hb concentration, mean corpuscular volume (MCV) and mean fetal Hb concentration of patients according to their gender, age, presence of α-thalassemia and ß-globin gene haplotype.

The α-thalassemia 3.7 kb deletion was observed in 20.9% (19/91) of patients (16 heterozygous, 3 homozygous) but the 4.2 kb deletion was not detected. The most common ß-globin gene haplotypes were Bantu/Bantu (41.0%) and Bantu/Benin (33.7%). ß-Globin gene haplotypes and α gene deletions could not be identified in one and five cases, respectively, due to partial DNA degradation.

The mean Hb concentration was significantly higher among girls than boys and MCV was significantly lower in the younger group of children as well as in those with α-thalassemia. The mean Hb and fetal Hb concentrations were significantly lower in children with the Bantu/Bantu compared with the Bantu/Benin ß-globin haplotypes (Table 2).

There was no association between the ß-globin gene haplotypes and the occurrence of clinical events. Children with SCA and α-thalassemia experienced fewer infection episodes (Table 3). However, it must be emphasized that among the six asymptomatic children, namely those who were not affected by any clinical events, four had co-inheritance with α-thalassemia and one had persistent microcytosis without the $-\alpha^{3.7}$ or $-\alpha^{4.2}$ deletions. Of the two children who died, neither had thalassemia.

Table 3 - Distribution of clinical events according to beta-globin haplotypes and alpha-thalassemia

| Events | ß-globin haplotype | | α- thalassemia | |
|---|---|---|---|---|
| | Bantu/Bantu n = 39 n (%) | Bantu/Benin n = 32 n (%) | αα/αα n = 72 n (%) | -α/αα and -α/-α n = 19 n (%) |
| Painful events | 16 (41.0) | 12 (37.5) | 32 (35.9) | 8 (42.1) |
| Splenic sequestration | 21 (53.8) | 17 (53.1) | 34 (47.2) | 9 (47.3) |
| Hemolytic crises | 25 (64.1) | 25 (78.1) | 46 (63.4) | 10 (52.6) |
| Hand-foot syndrome | 16 (41.0) | 12 (37.5) | 29 (40.2) | 4 (21.1) |
| Infection | 35 (89.7) | 27 (84.3) | 66 (91.7) | 14 (73.6)* |
| Acute chest syndrome | 2 (5.1) | 6 (18.7) | 6 (8.3) | 3 (15.8) |

*p-value < 0.05

## Discussion

The literature shows that morbidity and mortality due to SCA is very high, mainly during childhood. In the USA, the median survival is 42 years for men and 48 years for women[6]. Platt et al. reported that in the USA the peak age at death among children with SCA was between 1–3 years and that infection was the primary cause of death among under 20-year-old patients[6]. In Jamaica, the survival rate for men and women is 53 and 58.3 years, respectively[7], and the highest rate of infant mortality occurs between the ages of 6 months and 1 year[8]. In Brazil, it is estimated that 25% of children will not reach 5 years old given the high perinatal mortality rate, which ranges from 20–50%[9].

Infection, which occurred in 88.9% of children, was the most frequently observed event in the current study; this is in agreement with the main studies reported in the literature. In Jamaica, infection occurred more frequently within the first 3 years of life and was one of three primary causes of death[10,11]. In the Cooperative Study of Sickle Cell Disease (CSSCD), infections caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* were the most common causes of death in children[6,12]; in France, infections, the second main cause of death, were more common in under 5-year-old children[13]. In Rio de Janeiro, infectious events were observed in 100% of patients[14]. After the introduction of penicillin prophylaxis, there was a considerable decrease in the frequency of infectious episodes[15,16]; however, infection remains one of the main causes of infant mortality among children with SCA. Despite the high frequency of infectious events in our study, no deaths were caused by an infectious episode.

A splenic sequestration crisis is an acute complication that is responsible for high morbidity; it is potentially fatal if not promptly treated[17]. In the current study, splenic sequestration crises occurred in almost half of the patients and were significantly more prevalent in the younger group of children. Data from Jamaica indicate that splenic sequestration is the leading cause of death among young children with a high frequency of relapse. In a study of 216 children, 24% experienced a sequestration crisis with the events occurring most frequently between 6 and 12 months of age and decreasing progressively thereafter[18,10]. On the other hand, the CSSCD reported a lower frequency of splenic sequestration, but their data agree with the age of the highest incidence, between 6 months and 3 years[12]. In France, the leading cause of acute anemia is aplastic crisis (23%) and splenic sequestration is represented by only 13% of clinical events[13]. In Britain, in two studies conducted in the 1980s reported that acute anemia represented 2.5% and 3.5% of clinical events[19,20]. According to numerous reports in the literature, the prevalence of splenic sequestration varies; however, the studies agree that these episodes are more frequent between 6 months and 3 years of age.

Painful episodes are the main cause of hospitalization among SCD patients; they are associated with all genotypes but occur most frequently in carriers of the homozygous disease. Hematologic risk factors for pain crises include a low basal level of fetal Hb and a high hematocrit[6,12]. In Britain, 250 children aged less than 16 years experienced painful crises more frequently than any other event in all age groups, with the highest frequency being seen at age 3-6 years[16]. In Jamaica, painful crises were the most frequent event after age 2 years[21]. In our study, painful crises affected more children in the older age group, was observed more frequently after 2 years of age and peaked at the age of 4 years.

Acute chest syndrome, although an infrequent event in our study, was significantly more prevalent among the older group of children. Acute chest syndrome is one of the most common clinical manifestations of SCA and is responsible for 25% of premature deaths; it is the second leading cause of hospitalization[22]. The frequency of acute chest syndrome observed in this study was lower than that found in other studies. In the USA, studies have reported incidences of 25% and 50%[22,12]. In Jamaica, acute chest syndrome reportedly occurs in 83% of patients[23], whereas in France, Neonato et al.[13] described at least one episode among 44.8% of the children studied. These results show the enormous variation in the prevalence of this event; however, all studies report that acute chest syndrome is more frequent among over 3-year-old children, as was seen in our study.

The most frequent ß-globin haplotype detected in the current study was Bantu, followed by Benin. These data corroborate published findings for the population of Rio de Janeiro, historical records on $ß^S$ gene flow to Brazil and the results of DNA mitochondrial analyses among Black populations in Brazil[2,24,25].

In large cohorts, the level of fetal Hb is higher in females than males, and this has been explained by the linkage of the FCP locus on the X chromosome[13]. Other studies have found that this phenomenon is related to factors such as age, and number of α genes and ß-globin haplotypes[26]. In this study, no difference in fetal Hb levels was observed between genders, although the Hb concentration was significantly higher in

the girls (p-value < 0.05). Previous reports have confirmed an association between ß-globin haplotype and variations in fetal Hb levels, with the greatest expression being found in individuals with the Senegal and Arab-Indian haplotypes. Thus, carriers of the Senegal and Arab-Indian haplotypes normally present with higher levels of fetal Hb; Bantu individuals have lower levels, and those with the Benin haplotype present with levels between those with the Bantu and Senegal/Arab-Indian haplotypes[27]. In the current study, children with the homozygous Bantu haplotype had lower levels of Hb and fetal Hb than those of Bantu/Benin individuals, suggesting that carriers of 2 Bantu chromosomes have lower levels of fetal Hb than those with only one chromosome. These results are in agreement with the results of published studies conducted in Brazilian populations[14,28].

In this study, no correlation was found between ß-globin haplotypes and clinical manifestations. The sample size, the large number of heterozygous individuals, the strong miscegenation of the Brazilian population, and the multiplicity of clinical expression in SCA may explain this lack of association.

The frequency of the $-\alpha^{3.7}$ deletion was high; 20.9% of children were found to have α-thalassemia, which is in agreement with previous reports of a frequency ranging from 20% to 25% in Afro-Brazilian populations[29]. α-Thalassemia is probably the most common genetic mutation in humans[30]. Therefore, the $-\alpha^{3.7}$ deletion is the most frequent worldwide and is very prevalent in tropical and subtropical regions; in Africa and America its prevalence can reach 40%[1]. Numerous studies have shown a clear impact of α-thalassemia on several hematological parameters, including decreases in MCV, mean corpuscular Hb, fetal Hb and reticulocytes and increases in hematocrit, Hb, $A_2$ Hb and the number of red blood cells[31-34]. However, the effect of co-inheritance on the clinical manifestations of SCD is variable. A previous review reported that the occurrence of occlusive diseases, such as stroke, leg ulcers and splenic manifestations, decreases in the presence of α-thalassemia. However, the prevalence of clinical manifestations is dependent on blood viscosity; painful episodes, acute chest syndrome, and osteonecrosis are slightly affected by the presence of α-thalassemia[27].

In a previous study on patients from Rio de Janeiro, it was not possible to demonstrate any effect of α-thalassemia; however, it was found that children with SCA without thalassemia had more bone pain events than did children with thalassemia[35]. No correlation between the presence of α-thalassemia and clinical manifestations was found in the current study, with the exception of infectious episodes in the entire group (Hb SS and Hb SC), which were more frequent in children without thalassemia. However, when only children with SCA were considered, there was no difference in the occurrence of infectious episodes. The majority of asymptomatic children had α-thalassemia, and the occurrence of painful episodes, splenic sequestration crises, hand-foot syndrome, and acute chest syndrome was less frequent among children with α-thalassemia; however, this difference was not statistically significant.

The ß-globin haplotypes studied did not show any correlation with clinical manifestations. However, the frequency of clinical events was higher among Bantu/Bantu children without α-thalassemia.

SCA presents with high morbidity, even in the earliest years of life since most acute clinical events occur in younger children. Infectious episodes were the main clinical event observed here. Splenic sequestration was more frequent in younger children, whereas acute chest syndrome and painful episodes occurred more frequently among older children.

The most commonly observed ß-globin haplotypes were Bantu followed by Benin; this is in agreement with studies of historical records of $ß^S$ gene flow to Rio de Janeiro as well as the results of analyses of mitochondrial DNA in black Brazilian populations. The $-\alpha_2^{3.7kb}$ deletion confirmed the high frequency of co-inheritance with Hb S in Rio de Janeiro.

The clinical expression of SCD is very variable in Brazil; few studies have attempted to characterize its clinical manifestations in childhood, and systematic early diagnosis was only possible because of the introduction of neonatal screening of hemoglobinopathies. Genetic factors probably contribute to the great variation in clinical presentation; further molecular characterization of patients in cohort studies is necessary to provide a clinical and genetic profile of these patients.

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