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The frequency of β^S-globin haplotypes in the state of Paraná, Brazil, and clinical manifestations of sickle cell anemia

Frequência dos haplótipos da globina β^S no estado do Paraná, Brasil, e manifestações clínicas em pacientes com anemia falciforme

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ABSTRACT

Introduction: Haplotypes in the β^S-globin cluster are named according to their geographical origin as Central African Republic (CAR), Benin (BEN), Senegal (SEN), Cameroon (CAM) and Arab-Indian. They are considered to have influence on the diversity of clinical manifestations in sickle cell anemia (HbSS). **Objective:** To identify β^S haplotypes and genotypes, their frequencies and their probable association with clinical presentation in patients with sickle cell anemia in the state of Paraná. **Method:** Longitudinal and descriptive study for the definition of haplotypes, and associative study for analysis of their influence on clinical severity. By polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), polymorphic regions of 100 HbSS patients were identified. The association of haplotypes with clinical manifestations was analyzed in a subset of 52 pediatric patients. **Results:** In the state of Paraná, haplotype frequencies were: CAR: 76% BEN: 17.5% SEN: 0.5%, CAM: 0.5% and Atypical (Atp): 5.5%. Genotype frequencies were: CAR/CAR: 62%; CAR/BEN: 20%; CAR/Atp: 6%; CAR/SEN: 1%; CAR/CAM: 1%; BEN/BEN: 6%; BEN/Atp: 3%, Atp/Atp: 1%. The average percentage of fetal hemoglobin (HbF) in CAR/CAR and CAR/BEN patients was higher than in other studies. Clinical manifestations were not influenced by β^S haplotypes. Dactylitis and splenic sequestration occurred more frequently in children below 3 years of age. **Conclusion:** In this study, no association was found between haplotypes and clinical manifestations, probably given the almost absolute predominance of CAR and BEN haplotypes. However, this fact alerts to the possible influence of other polymorphisms and miscegenation in the Brazilian population.

Key words: sickle cell anemia; pediatrics; haplotypes.

INTRODUCTION

Sickle cell anemia is characterized by hemolysis with clinical manifestations resulting from tissue hypoxia and vaso-occlusive phenomena; the severity of clinical manifestations is variable[1]. Still in childhood, it is difficult foreseeing the frequency and intensity of these manifestations, what brings uncertainties and insecurity to relatives regarding prognosis. In Brazil, morbidity and mortality have decreased due to early diagnosis reached by neonatal screening programs, to the prophylactic use of antibiotics against invasive bacteria, and to continuous education[2].

The severity of clinical manifestations is attributed to the different concentrations of fetal hemoglobin (HbF) associated with the hemoglobin S (HbS) haplotypes that, historically, originated in African and Asian populations, whose nomenclature was based on geographical location: Benin (BEN), Central African Republic (CAR or Bantu), Cameroon (CAM) and Senegal (SEN), in Africa; and the Arab-Indian type or Asian, in Asia[3-5]. The haplotypes can be identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)[6].

Patients with the CAR/CAR genotype present low HbF concentrations and more severe clinical manifestations; those with the BEN/BEN genotype, moderate HbF and clinical manifestations; those homozygous for SEN and Arab-Indian haplotypes are associated with higher HbF concentrations[3, 4, 7] and mild clinical severity.

Considering the hypothesis that haplotypes can influence on the clinical expression of sickle cell disease[3, 4, 8-10], the objective of the present work was to determine the β^S-globin haplotypes,

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their frequencies and association with clinical manifestations in patients with sickle cell disease in the state of Paraná.

METHOD

The study was longitudinal, descriptive and analytical in the definition and frequency of β^S-globin haplotypes, and associative in the assessment of haplotype influence on clinical severity. Between March 2011 and January 2014, blood samples were consecutively collected from 100 patients with sickle cell anemia (HbSS) in Curitiba, treated at the outpatient clinics of pediatric hematology of Hospital de Clínicas (HC) of Universidade Federal do Paraná (UFPR) ($n = 52$), and of outpatients clinics of hemoglobinopathy of the coordinating blood center of the state of Paraná – Centro de Hematologia e Hemoterapia do Paraná (Hemepar) ($n = 48$). Genotyping of β^S-globin haplotypes was performed at Centro de Genética Molecular e Pesquisa do Câncer em Crianças (Cegempac). Clinical and laboratory data were retrospectively collected from records of 52 children treated at HC, born in the state of Paraná, who were not on hydroxyurea during the research. The information had been extracted according the consultation protocol for patients with sickle cell anemia in the latest 16 years. Adult patients of Hemepar, who were on hydroxyurea, were just genotyped.

The project was approved by the research ethics committee of HC/UFPR. Participation in the study occurred after signature of the free consent term by the patient and/or legal representative.

The diagnosis of sickle cell anemia was defined through cellulose acetate alkaline electrophoresis, measurement of hemoglobin A2 (HbA2) with elution, and determination of HbF percentage by the alkali-resistance Singer-Chernoff technique. The values considered for analysis were those obtained from patients over 1 year of age, to avoid the influence of physiological variations that happen with this parameter in the first year of life. The assessed clinical manifestations were: cerebrovascular accident (CVA), painful crises, dactylitis, lung diseases, urinary tract infections, and splenic sequestration. The analyzed hematological parameters were: Hb (g/dl), mean corpuscular volume (MCV) (fl), leukocyte count (cells/mm^3), platelet count (cells/mm^3), reticulocytes (%), and HbF (%), collected from patients at baseline. Blood samples were collected in filter paper and dried at room temperature[11]. From the leukocyte-extracted deoxyribonucleic acid (DNA), polymorphisms in β^S-globin were identified by the PCR-RFLP technique. Regions of β^S-globin cluster were amplified: 5'γ^G, γ^G, γ^A, φ, 3'$\varphi\beta$, 5'β. The polymorphic fragments obtained by the activity of restriction enzymes XmnI, HindIII, HincII and HinfI (Fermentas, Promega)[6] were analyzed according to a pre-established protocol. The undefined haplotypes were analyzed with enzymes HincII, AvaII and BamHI for polymorphisms in regions ε, β and 3'β, respectively[5, 12, 13], and considered atypical (Atp).

In order to assess the association of clinical manifestations with age, the patient cohort was divided into two: group 1, made of children aged 0-3 years ($n = 52$); and group 2, of children older than 3 up to 15,9 years of age ($n = 46$). Group 2 children are also represented in group 1, but in distinct chronological moments. Among the 52 children, five of group 1 and one of group 2 presented no clinical manifestation until the moment of analysis. The cut-off point at 3 years of age was defined by a quote from the literature about higher frequency of dactylitis in children up to that age[14].

Statistical analysis

Clinical and laboratory data underwent statistical treatment with the program Statistical Package for Social Sciences (SPSS) version 17.0. Descriptive and inferential statistical analyses were carried out, considering the significance level of 5% ($p < 0.05$). Parametric and non-parametric tests were employed according to data normality.

RESULTS

The frequencies of β^S-globin haplotypes in the state of Paraná were: 76% CAR; 17.5% BEN; 0.5 % SEN; 0.5% CAM and 5.5% Atp. The frequencies of genotypes were: 62% CAR/CAR; 20% CAR/BEN; 6% BEN/BEN and CAR/Atp; 3% BEN/Atp; and 1% CAR/SEN, CAR/CAM and Atp/Atp.

Of the 52 children, 24 (46%) were males, and 28 (54%), females. The median age was 92.5 months (17-191 months). The **Figure** presents the distribution of children according to age, during the analysis of clinical manifestations.

The verified laboratory parameters (mean + standard deviation) were: Hb = 7.33 ± 0.76 g/dl; MCV = 87.01 ± 6.96 fl; leukocyte count = 15,298 ± 4,088/mm^3; platelet count:

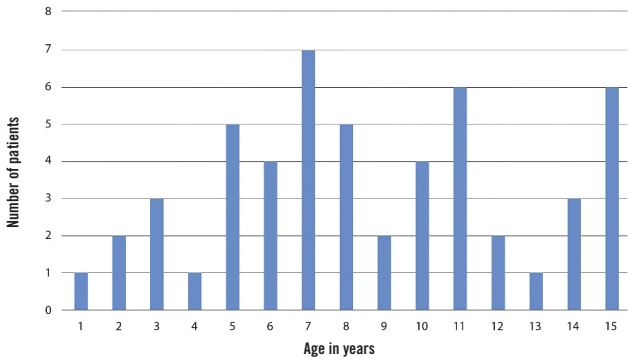

FIGURE – *Distribution of patients with sickle cell anemia according to age*

Ordinate: number of patients in each age, during the analysis; abscissa: age of patients in years.

484,182 ± 147,551/mm³; reticulocytes = 17.3% ± 5.57%; HbF = 12% ± 7.25%. **Table 1** presents the values of these parameters for the different haplotypes.

Dactylitis ($p < 0.001$) and splenic sequestration ($p = 0.001$) were more frequent in the group of children up to 3 years of age, while cardiac alterations, in children over 3 years ($p = 0.05$). Occurrences of CVA ($p = 0.16$), painful crises ($p = 0.104$) and lung diseases ($p = 0.547$) were not influenced by age (**Table 2**).

Except for CVA, clinical manifestations were not associated with genotypes (**Table 3**).

The HbF mean of CAR/CAR genotype was significantly lower than the HbF mean of CAR/BEN genotype (**Table 4**).

TABLE 1 – Laboratory parameters × β^S-globin haplotypes according to genotypes ($n = 52$)

β^S haplotypes	CAR/CAR ($n = 32$)		CAR/BEN ($n = 10$)		CAR/SEN ($n = 1$)		CAR/Atp ($n = 4$)		BEN/BEN ($n = 3$)		BEN/Atp ($n = 2$)	
	Mean ± SD	*p*	Mean ± SD	*p*		*p*	Mean ± SD	*p*	Mean ± SD	*p*	Mean	*p*
Hb (g/dl)	7.23 ± 0.86	0.579	7.39 ± 0.67	0.817	7.57	0.756	7.71 ± 0.7	0.337	7.60 ± 0.4	0.547	7.23	0.854
MCV (fl)	86.89 ± 7.29	0.94	87.66 ± 6.94	0.788	90.5	0.621	85.03 ± 9.25	0.593	87.1 ± 2.52	0.982	94.3	0.148
Leukocyte count (cells/mm³)	15,147 ± 4,516	0.875	16,015 ± 3,326	0.604	12,066	0.437	13,708 ± 5,080	0.463	16,702 ± 1,182	0.558	15.52	0.94
Platelet count (cells/mm³)	490,989 ± 128,114	0.83	499,466 ± 186,935	0.775	389,666	0.529	386,542 ± 253,338	0.231	555,333 ± 101,177	0.416	395,666	0.404
Reticulocytes (%)	18.19 ± 5.35	0.472	16.9 ± 4.2	0.83	22.1	0.397	8.99 ± 5.97	0.006	17.07 ± 6.55	0.945	18.13	0.835
HbF (%)	9.6 ± 6.54	< 0.001	16.85 ± 6.28	< 0.001	12.3	0.88	16.18 ± 9.89	< 0.001	16.33 ± 5.63	< 0.001	=	=

Analysis using Student's t *test;* $p < 0.05$.

CAR: Central African Republic; BEN: Benin; SEN: Senegal; Atp: Atypical; MCV: mean corpuscular volume; HbF: fetal hemoglobin.

TABLE 2 – Clinical manifestations according to age group

Clinical manifestations HbSS	Children aged 0-3 years ($n = 47$) (group 1)		Children older than 3 years and teenagers ($n = 46$) (group 2)		*p*
	n	%	*n*	%	
CVA	1	2.1	4	8.7	0.16
Cardiac alterations	3	6.4	13	28.2	0.005
Painful crises	41	87.2	34	73.9	0.104
Dactylitis	24	51	1	2.1	< 0.001
Urinary tract infection	7	14.9	2	4.3	0.085
Lung disease	16	34	13	28.2	0.547
Splenic sequestration	13	27.66	1	2.17	0.001

Analysis using the binomial test of comparison of proportions.

HbSS: sickle cell anemia; CVA: cerebrovascular accident.

TABLE 3 – Sickle cell genotypes and clinical manifestations

Genotype	Clinical manifestations						
	Cardiac alteration	CVA	Pain	Lung disease	Dactylitis	Urinary tract infection	Splenic sequestration
CAR/CAR	11/32	4/32	29/31	20/32	19/32	4/32	7/32
CAR/BEN	2/10	0/10	10/10	4/10	3/10	3/10	4/10
CAR/SEN	0/1	0/1	1/1	0/1	0/1	0/1	0/1
CAR/Atp	1/4	1/4	3/3	2/3	1/4	1/3	1/4
BEN/BEN	1/3	0/3	3/3	0/3	2/3	0/3	1/3
BEN/Atp	1/2	0/2	1/2	0/2	0/2	0/2	1/2
p	0.901	0.047	0.275	0.113	0.223	0.606	0.812

Analysis using the X^2 *test,* $p < 0.05$.

Numerator: number of clinical manifestations; denominator: number of analyzed patients; CVA: cerebrovascular accident; CAR: Central African Republic; BEN: Benin; SEN: Senegal; Atp: Atypical.

TABLE 4 – Comparison among HbF values in the different SS genotypes in the state of Paraná

Genotype (*n*)	HbF (%) (mean ± standard deviation)	*p*
CAR/CAR ($n = 32$) × CAR/BEN ($n = 10$)	9.6 ± 6.54 × 16.85 ± 6.28	0.004
CAR/CAR ($n = 32$) × BEN/BEN ($n = 3$)	9.6 ± 6.54 × 16.33 ± 5.63	0.095
CAR/CAR ($n = 32$) × CAR/Atp ($n = 4$)	9.6 ± 6.54 × 16.18 ± 9.89	0.081
CAR/BEN ($n = 10$) × BEN/BEN ($n = 3$)	16.85 ± 6.28 × 16.33 ± 5.63	0.9

Analysis using Student's t *test.*

HbF: fetal hemoglobin; CAR: Central African Republic; BEN: Benin; Atp: Atypical.

DISCUSSION

The predominance of CAR (76%) haplotype, followed by BEN (17.5%), in the state of Paraná, is similar to the results found in South and Southeast Regions of Brazil. In Triângulo Mineiro, a region of Minas Gerais, the frequency of CAR haplotype was 64.8% and 22.1% of BEN[15]; in Ribeirão Preto, 66.2% of CAR and 23% of BEN[8]; in the city of São Paulo, 55% of CAR and 34% of BEN[16]; in Rio de Janeiro, 72.9% of CAR and 20.3% of BEN[17]; and in Rio Grande do Sul, 66% of CAR and 27% of BEN[18].

On the other hand, greater representation of the BEN haplotype is observed in Salvador (55.2%)[19, 20], in Recôncavo Baiano (52.9%)[20], and in Ceará (43.2%)[21]. The frequency of HbS haplotypes in the different regions of the country reflects the flow of enslaved Africans in the Brazil of the 18th and 19th centuries. Salvador and Rio de Janeiro served as their ports of entry. Those born in the region of Benin, northwest of the African country, arrived in Brazil at the port of Salvador, causing a higher frequency of the BEN haplotype in the North Region of the country. Those born in the present-day Central African Republic arrived in Brazil also through Salvador, but their most frequent entry port was Rio de Janeiro. Later on, they were taken to São Paulo, Paraná, Santa Catarina and Rio Grande do Sul, besides Espírito Santo and north of Rio de Janeiro[22], justifying the higher frequency of the CAR haplotype in the South and Southeast Regions of the Brazil.

Among the clinical manifestations, it was possible to identify that dactylitis (51%) and splenic sequestration (27.6%) happened most frequently up to 3 years of age ($p < 0.001$) (Table 2), as expected[14, 23]. There is a downward bias in identifying manifestations specific of the age group older than 3 years, because some children who at 5 years, for example, still did not present cardiac alterations, can present them in the subsequent years.

No difference was observed on clinical manifestation among haplotypes. One explanation can be the small number of patients with homozygous genotype different from CAR/CAR, with BEN/BEN being just 6%. The CAR haplotype, present in 90% of genotypes, can have promoted the occurrence of more severe clinical manifestations, compatible with CAR. Silva Filho *et al*. (2012)[24] also found no clinical association with type of haplotype in the studied population.

The presence of α-thalassemia, which could interfere with prognosis, was not analyzed in this study. However, in the population with sickle cell disease in Paraná, its frequency is just 9.67% [Tormen (2015), data not published], with low probability of altering the observed results.

Higher concentrations of HbF protect patients because they inhibit HbS polymerization, attenuating clinical manifestations[3, 25-27]. In this study, CAR/CAR children ($n = 32$) presented lower mean HbF (9.6 ± 6.54) (Table 1) than the patients of group SS (12 ± 7.25) ($p < 0.001$), but there was no association of HbF levels with the type of assessed clinical manifestations.

The only identified tendency in this series of patients was the greater occurrence of CVA in CAR/Atp patients ($p = 0.047$), data similar to those described by Sarnaik and Ballas[10]. In 41 children, whose first CVA happened in the age group of 5.6 ± 3.2 years, the greatest frequency of the event occurred in those who had at least one Atp allele [10]. Deletions of the α globin chain reduced the occurrence of CVA for they inhibited formation of intracellular polymers; excess of these α chains could be a risk factor for this event[10]. In the state of Rio de Janeiro, children with the CAR/Atp genotype, with mean age of 6.6 years (3.2-15 years), had 15 times more chance [odds ratio (OR) = 15.4] to present CVA than those with other genotypes, but the α globin chains did not influence the event occurrence[28]. The association of CVA with the CAR/Atp haplotype in the state of Paraná must be assessed carefully, given the small size of the sample ($n = 4$).

The high average HbF levels in CAR/CAR and CAR/BEN patients are noteworthy, as demonstrated in **Table 5**, in contrast to initial publications, which characterized these genotypes as more severe for the low HbF levels[7, 9].

TABLE 5 – HbF in HbSS patients from different Brazilian states

Genotype	Paraná[a]	Paraná[29]	Rio de Janeiro[24]	Minas Gerais[30]	Ceará[31]	Bahia[32]
	$n = 52$	$n = 17$	$n = 79$	$n = 200$	$n = 47$	$n = 77$
CAR/CAR	9.6 ± 6.54	9.5 (3.8-15.9)	11.9 ± 5.3	17.3 ± 8.4	6.14 ± 3.46	7.544 ± 4.342
CAR/BEN	16.85 ± 6.28	14.3 (1.2-24.2)	16.1 ± 8.1	19.1 ± 7.7	6.47 ± 3.59	8.146 ± 4.631
BEN/BEN	16.33 ± 5.63	NR	NR	19.2 ± 9.1	8.56 ± 1.93	9.882 ± 3.558
CAR/Atp	16.18 ± 9.89	NR	15.9 ± 14.4	NR	10.88 ± 2.78	4.18 ± 2.25
BEN/Atp	NR	NR	5.3 ± 0.7	NR	1.43 ± 1.17	NR

HbF: fetal hemoglobin; HbSS: sickle cell anemia; a: present study; NR: not reported; CVA: cerebrovascular accident; CAR: Central African Republic; BEN: Benin; Atp: Atypical.

In order to explain such discrepancies, the following hypotheses are proposed:

• higher HbF values can be associated with polymorphisms found in the $\beta^{S\text{-}CAR}$-globin cluster, called atypical CAR[33]. Srinivas *et al*. (1988) identified seven subtypes of CAR haplotypes in patients from the Central African Republic: Bantu A1, Bantu A2, Bantu A3, Bantu A4, Bantu A5, Bantu A6 and Bantu A7[34]. These subtypes showed strong correlation between the percentage of γ^G and HbF ($r = 0.093, p < 0.000001$). In that study, the Bantu A4 haplotype with γ^G levels higher than 50% presented HbF of up to 30%[34]. Eventually, in Iran, five types of CAR haplotypes were identified: Bantu A1, A2, A2a, A4, A6; associated with the Arab-Indian haplotype, presented high concentrations of HbF (27.83 ± 12.32)[35];

• polymorphisms in the locus control region of HS2 of different haplotypes can modify HbF levels for a certain genotype[2], as verified in CAR/CAR, CAR/BEN and BEN/BEN patients with high HbF[2, 19, 32]. The analysis of these polymorphisms and the determination of γ^G concentrations in HbSS patients in the state of Paraná, in the genotypes that presented higher HbF, could be a tool for understanding biomolecular mechanisms;

• polymorphisms in other regions of the β^S-globin gene also influenced the increase in HbF[28, 36], what was deduced from the observation of carriers of hereditary persistence of fetal hemoglobin[36]. Thein *et al*. (2009) suggested that the quantitative trait loci (QTL) XmnI-HB-G2, linked to the β^S-globin gene, could be associated with severity of the disease, as well as genes not related to its cluster, such as *HBS1L-MYB* (6q23) and *BCL11A* (2p)[36, 37]. Recently, association was demonstrated between genes *HBS1L-MYB* and *BCL11A* and high levels of HbF in Cameroon patients with CAM haplotypes[38], confirming the hypothesis that alterations in different genes can influence clinical manifestations in sickle cell anemia[4].

In the initial researches carried out among native Africans, patients homozygous for the SEN haplotype presented mild clinical picture, particularly in relation to hemolysis and high HbF[3]. In the present work, the only child with SEN haplotype is heterozygous, presenting the genotype CAR/SEN and the following laboratory results: Hb = 7.57 g/dl, HbF = 12.3%, HbA_2 = 2.1%; reticulocytes = 22.1% and MCV = 90.5 fl. Hospitalized at an intensive care unit (ICU), the patient needed exchange transfusions. These results show a severe clinical picture.

In a group of 60 SS Afro-American adult patients, with at least one of the SEN haplotypes, the laboratory tests (mean ± standard deviation) were: Hb (9.6 ± 1.1 g/dl), MCV (91.2 ± 9.2 fl) and HbF (9.9% ± 5.4%)[3]. In Rio de Janeiro, a CAR/SEN patient aged 3 years presented HbF = 21.6%; another, a BEN/SEN aged 17 years, HbF = 6.8%[25]. In Ceará[21], an adult patient with BEN/SEN genotype presented Hb = 8.9 g/dl, HbF = 13.4% and MCV = 92 fl, values similar to those of the child in the present study. One can observe, therefore, that heterozygosity modifies the expression of the SEN haplotype, traditionally considered to induce mild clinical manifestations.

In the countries where heterozygosity predominates for the different haplotypes, and miscegenation is the rule, the association between haplotype and clinical severity becomes less clear[4, 30]. On the other hand, the prognosis of clinical morbidity cannot be given just based on haplotypes[9, 14, 30] and HbF[38]. Other genetic factors must be involved, interfering in clinical expression[4, 18, 24, 30] of sickle cell anemia.

Thus, in the patients studied in the state of Paraná, predominance of CAR and BEN haplotypes, the lack of association with the type of clinical manifestations, and the possible influence of miscegenation suggest the need to extend molecular studies, aiming at the comprehension of clinical variability in sickle cell diseases.

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RESUMO

Introdução: *A variabilidade nas manifestações clínicas da anemia falciforme (HbSS) pode ser influenciada pelos haplótipos no grupamento da globina β^S, nomeados de acordo com a origem geográfica: República Centro-Africana (CAR), Benin (BEN), Senegal (SEN) Camarões (CAM) e Árabe-indiano.* ***Objetivo:*** *Identificar haplótipos e genótipos da globina β^S, suas frequências e as possíveis associações com manifestações clínicas em pacientes com anemia falciforme no estado do Paraná.* ***Método:*** *Estudo longitudinal e descritivo na distribuição dos haplótipos e associativo na análise da influência destes sobre as manifestações clínicas. Identificaram-se as regiões polimórficas da globina β^S de 100 pacientes HbSS pela técnica da* polymerase chain reaction-restriction fragment length polymorphism *(PCR-RFLP). A associação dos haplótipos com as manifestações clínicas foi analisada em um subgrupo de 52 pacientes pediátricos.* ***Resultados:*** *As frequências dos haplótipos foram CAR: 76%; BEN: 17,5%; SEN: 0,5%; CAM: 0,5% e Atípico (Atp): 5,5%. Os genótipos foram CAR/CAR: 62%; CAR/BEN: 20%; CAR/Atp: 6%; CAR/SEN: 1%; CAR/CAM: 1%; BEN/BEN: 6%; BEN/Atp: 3% e Atp/Atp: 1%. A porcentagem média de hemoglobina fetal (HbF) dos pacientes CAR/CAR e CAR/BEN foi maior que em outros estudos. Os haplótipos da globina β^S não tiveram influência nas manifestações clínicas. A dactilite e o sequestro esplênico ocorreram com mais frequência nas crianças abaixo de 3 anos de idade.* ***Conclusão:*** *Na população estudada, não foi possível identificar associação dos haplótipos com as manifestações clínicas. Esse fato pode ser decorrente do predomínio quase absoluto dos haplótipos CAR e BEN, de diferentes polimorfismos e da miscigenação da população brasileira.*

Unitermos: *anemia falciforme; pediatria; haplótipos.*

REFERENCES

1. Van der Tweel XW, Van der Lee JH, Heijboer H, Peters M, Fijnvandraat K. Development and validation of a pediatric severity index for sickle cell patients. Am J Hematol. 2010; 85: 746-51. Pubmed PMID: 20806231.

2. Belisário AR. Genótipos da talassemia alfa e haplótipos do grupamento de genes da globina beta como moduladores da gravidade na doença falciforme em crianças do Programa Estadual de Triagem Neonatal de Minas Gerais, matriculadas no Hemocentro de Belo Horizonte da Fundação Hemominas [dissertation]. Belo Horizonte: Faculdade de Medicina, Universidade Federal de Minas Gerais; 2010.

3. Nagel RL, Erlingsson S, Fabry ME, et al. The Senegal DNA haplotype is associated with the amelioration of anemia in African-American sickle cell anemia patients. Blood. 1991; 77(6): 1371-75. Pubmed PMID: 2001460.

4. Steinberg MH. Predicting clinical severity in sickle cell anaemia. Br J Haematol. 2005; 129(4): 465-81. Pubmed PMID: 15877729.

5. Lapouméroulie C, Dunda O, Ducrocq R, et al. A novel sickle cell mutation of yet another origin in Africa: the Cameron type. Hum Genet. 1992; 89(3): 333-7. PubMed PMID: 1376298.

6. Sutton M, Bouhassira EE, Nagel RL. Polymerase chain reaction amplification applied to the determination of β-like globin gene cluster haplotype. Am J Hematol. 1989; 32(1): 66-9. PubMed PMID: 2757004.

7. Nagel RL, Fabry ME, Pagnier J, et al. Hematologically and genetically distinct forms of sickle cell anemia in Africa. The Senegal type and the Benin type. N Engl J Med. 1985; 312(14): 880-4. PubMed PMID: 2579336.

8. Zago MA, Figueiredo MS, Ogo SH. Bantu β^S cluster haplotype predominates among Brazilian Blacks. Am J Phys Anthropol. 1992; 88(3): 295-8. PubMed PMID: 1642317.

9. Powars D, Meiseman HJ, Fisher TC, Hiti A, Johnson C. β^S gene cluster haplotypes modulate hematologic and hemorheologic expression in sickle cell anemia. Use in predicting clinical severity. J Pediatr Hematol Oncol. 1994; 16(1): 55-61. PubMed PMID: 7508688.

10. Sarnaik SA, Ballas SK. Molecular characteristics of pediatric patients with sickle cell anemia and stroke. Am J Hematol. 2001; 67(3): 179-82. Pubmed PMID: 11391715.

11. Manual de normas técnicas e rotinas operacionais do Programa Nacional de Triagem Neonatal. Brasília (DF): Ministério da Saúde; 2005. p. 18-22. Available at: http://bvsms.saude.gov.br/bvs/publicacoes/triagem_neonatal.pdf.

12. Orkin SH, Kazazian JR HH, Antonarakis SE, et al. Linkage of β-thalassaemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. Nature. 1982; 296: 627-31. PubMed PMID: 6280057.

13. Guerreiro JF, Figueiredo MS, Santos SEB, Zago MA. β gene cluster haplotypes in Yanomama Indians from the Amazon region of Brazil. Hum Genet. 1992; 89(6): 629-31. PubMed PMID: 1511980.

14. Miller ST, Sleeper LA, Pegelow CH, et al. Prediction of adverse outcomes in children with sickle cell disease. N Engl J Med. 2000; 342(2): 83-9. Pubmed PMID: 10631276.

15. Leal AS, Martins PR, Balarin MAS, Pereira GA, Resende GAD. Haplotypes βs globin and its clinical-haematological correlation in patients with sickle cell anemia in Triângulo Mineiro, Minas Gerais, Brazil. J Bras Patol Med Lab. 2016; 52(1): 6-10. On-line version ISSN 1678-4774.

16. Lyra IM, Gonçalves MS, Braga JAP, et al. Caracterização clínica, hematológica e molecular de crianças portadoras de anemia falciforme em duas diferentes cidades do Brasil. Cad Saúde Pública. 2005; 21(4): 1287-90. Pubmed PMID: 15726590.

17. Okumura JV, Lobo CLC, Bonini-Domingos CR. Beta-S globin haplotypes in patients with sickle cell anemia: one approach to understand the diversity in Brazil. Rev Bras Hematol Hemoter. 2013; 35(1): 71-2. PubMed PMID: 23580889.

18. Silva MAL, Friedrisch JR, Bittar CM, et al. ß-Globin gene cluster haplotypes and clinical severity in sickle cell anemia patients in Southern Brazil. OJBD. 2014; 4: 16-23. DOI: 104236 OJBD 201442003.

19. Adorno EV, Zanette A, Lyra L, Seixas MO, Reis MG, Gonçalves MS. Clinical and molecular characteristics of sickle cell anemia in the northeast of Brazil. Genet Mol Biol. 2008; 31(3): 621-5. On-line version ISSN: 1678-4685.

20. Silva WS, Klautau-Guimarães MN, Grisolia CK. β-globin haplotypes in normal and hemoglobinopathic individuals from Recôncavo Baiano, State of Bahia, Brazil. Genet Mol Biol. 2010; 33(3): 411-7. Pubmed PMC: 3036130.

21. Galiza-Neto GC, Pitombeira MS, Vieira HF, Vieira MLC, Farias DAB. Análise dos haplótipos do gene da β^S-globina no Ceará. J Bras Patol Med Lab. 2005; 41(5): 315-21. On-line version ISSN: 1678-4774.

22. Florentino M, Ribeiro AV, Silva DD. Aspectos comparativos do tráfico de africanos para o Brasil (séculos XVIII e XIX). Afro-Asia. 2004; 31: 83-126. ISSN: 1981-1411.

23. Quinn CT. Sickle cell disease in childhood: from newborn screening through transition to adult medical care. Pediatr Clin North Am. 2013; 60(6): 1363-81. Pubmed PMID: 24237976.

24. Silva Filho IL, Ribeiro GS, Moura PG, Vechi ML, Cavalcante AC, Andrada-Serpa MJ. Sickle cell disease: acute clinical manifestations in early childhood and molecular characteristics in a group of children in Rio de Janeiro. Rev Bras Hematol Hemoter. 2012; 34(3): 196-201. Pubmed PMID: 23049419.

25. Fleury MK. Haplótipos do cluster da globina beta em pacientes com anemia falciforme no Rio de Janeiro: aspectos clínicos e laboratoriais. Rev Bras Anal Clin. 2007; 39(2): 89-93. ISSN 0370-369 X.

26. Costa PJMS, Vilela RQB, Cipolotti R, Figueiredo MS. Diversidade clínica e laboratorial no haplótipo Bantu da anemia falciforme. Rev Bras Hematol Hemoter. 2006; 28(1): 40-4. ISSN: 1806-0870.

27. Zago MA, Silva Pinto AC. Fisiopatologia das doenças falciformes: da mutação genética à insuficiência de múltiplos órgãos. Rev Bras Hematol Hemoter. 2007; 29(3): 207-14. On-line version ISSN: 1806-0870.

28. Silva Filho IL, Leite ACCB, Moura PG, et al. Genetic polymorphisms and cerebrovascular disease in children with sickle cell anemia from Rio de Janeiro, Brazil. Arq Neuropsiquiatr. 2011; 69(3): 431-5. Pubmed PMID: 21755116.

29. Shimauti ELT, Silva DGH, Souza EM, Almeida EA, Leal FP, Bonini-Domingos CR. Prevalence of β^S-globin gene haplotypes, α-thalassemia (3.7 kb) deletion and redox states in patients with sickle cell anemia in the state of Paraná, Brazil. Genet Mol Biol. 2015; 38(3): 316-23. PubMed PMID: 26500435.

30. Belisário AR, Martins ML, Brito AMS, Rodrigues CV, Silva CM, Viana MB. β-globin gene cluster haplotypes in a cohort of 221 children with sickle cell anemia or $S\beta^0$-thalassemia and their association with clinical and hematological features. Acta Hematol. 2010; 124(3): 162-70. Pubmed PMID: 20938172.

31. Silva LB, Gonçalves RP. Características fenotípicas dos pacientes com anemia falciforme de acordo com os haplótipos do gene da β^S-globina em Fortaleza, Ceará. Rev Bras Hematol Hemoter. 2010; 32(1): 40-4. Print version: ISSN 1516-8484.

32. Gonçalves MS, Bonfim GC, Maciel E, et al. β^S-haplotypes in sickle cell anemia patients from Salvador, Bahia, Northeastern, Brazil. Braz J Med Biol Res. 2003; 36(10): 1283-8. PubMed PMID: 14502357.

33. Naoum PC, Naoum FA. Doenças das células falciformes. 1 ed. São Paulo: Sarvier; 2004.

34. Srinivas R, Dunda O, Krishnamoorthy R, et al. Atypical haplotypes linked to the β^S gene in Africa are likely to be the product of recombination. Am J Hematol. 1988; 29: 60-2. PubMed PMID: 3177370.

35. Rahimi Z, Karimi M, Haghshenass M, Merat A. β-globin gene cluster haplotypes in sickle cell patients from southwest Iran. Am J Hematol. 2003; 74(3): 156-60. PubMed PMID: 14587041.

36. Thein SL, Menzel S, Lathrop M, Garner C. Control of fetal hemoglobin: new insights emerging from genomics and clinical implications. Hum Mol Genet. 2009; 18(2): 216-23. Pubmed PMID: 19808799.

37. Belini Jr. E, Cançado RD, Domingos CRB. The XmnI polymorphic site 5' to the gene γ^G in a Brazilian patient with sickle cell anaemia-fetal hemoglobin concentration, haematology and clinical features. Arch Med Sci. 2010; 6(5): 822-5. PubMed PMID: 22419945.

38. Bitoungui VJ, Pule GD, Hanchard N, Ngogang J, Wonkam A. Beta-globin gene haplotypes among Cameroonians and review of the global distribution: is there a case for a single sickle mutation origin in Africa? OMICS: a Journal of Integrative Biology. 2015; 19(3): 171-9. Pubmed PMID: 25748438.

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