SICKLE CELL DISEASE SC IN NORTHEAST OF BRAZIL: A CLINICAL AND MOLECULAR CHARACTERIZATION

DOENÇA FALCIFORME SC NO NORDESTE DO BRASIL: CARACTERIZAÇÃO CLÍNICA E MOLECULAR

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The SC disease is really prevalent in Brazil, mainly in Bahia, being that the patients present a severe anemia but with less clinical complications than SS homozygous. The patients with SC disease have less painful crisis, infections, skeletal involvement, anemia, and priapism. The aim of the present study was to investigate the hemoglobin C and S globin gene haplotypes distribution among 63 individual with SC disease from Northeast Brazil, associating with their phenotype. Our results show that the studied patients have mild anemia (hemoglobin median=10.88 g/dL) and slightly high fetal hemoglobin levels (median=3.06%). The α-thalassemia$^{3.4Kb}$ deletion was found in 18 (28%) patients. The most frequent β^C and β^S globin gene haplotypes were CAR and Benin. We have not found any association among the globin haplotypes and clinical events of the patients, however further studies need to be developed to confirm the finding related to the SC disease patients.
Keywords: Sickle cell disease, SC disease, thalassemia, haplotypes.

A doença SC é muito prevalente no Brasil, principalmente na Bahia, sendo que os pacientes apresentam anemia grave, mas com menos complicações clínicas que os homozigotos SS. Os pacientes com doença de SC têm menos crises dolorosas, infecções, comprometimento ósseo e priapismo. O objetivo do presente estudo foi realizar a caracterização clínica e molecular dos haplótipos ligados ao gene das globinas β^S e β^C e da talassemia $\alpha^{3,7Kb}$ em 63 indivíduos com doença do SC da Bahia, Brasil. Os resultados obtidos demonstram que os pacientes estudados têm moderada anemia (média de hemoglobina=10,88g/dL) e níveis discretamente elevados de hemoglobina fetal (média=3,06%). A talassemia $\alpha^{3,4Kb}$ foi encontrada em 18 (28%) pacientes. Os haplótipos ligados aos genes da globina do gene da globina β^S e β^C mais frequentes foram CAR e Benin. Não foi encontrada associação entre os haplótipos da globina e eventos clínicos dos pacientes, porém estudos adicionais poderão confirmar os resultados obtidos com relação aos pacientes com doença SC.
Palavras-chave: doença falciforme, doença SC, talassemia, haplótipos.

The hemoglobinopathies result of molecular alteration in a globin gene and may be divided in two major groups, characterized by the presence of a structurally abnormal globin chain or by a reduction or absence of globin chains synthesis named thalassemias[7].

The hemoglobin S has a single GAT→GTA at the sixth codon of the β-globin gene, conducting to the glutamic acid to valin substitution ($\beta^{S\ 6\ Glu\rightarrow Val}$) and a variant β-globin chain. Sickle cell anemia disease, the homozygous state of HbS (*HBSS*) has heterogeneous clinical picture with vasooclusive crisis, hemolysis, painful episodes and other chronic complications such as leg ulcers and priapism and others in different degrees[25].

Sickle cell disease affects million of people worldwide, in Brazil, around 4 million of people has a sickle cell trait (HbAS)[1]. In Bahia, Northeast of Brazil, studies conducted in different population groups, described a frequency of 7.4 to 15.7% for heterozygous AS[2 9], the heterozygous state is found in a frequency of 6.5% among the African population[2]. The second most common variant hemoglobin described in Brazil is the C hemoglobin in which the sixth codon of the b-globin gene, the GAG is replaced by AAG, resulting in the glutamic acid to lysine ($\beta^{6Lys\rightarrow Glu}$) substitution at globin chain[18]. The β^C homozygous (CC) has a moderate hemolytic anemia. The presence of hemoglobin S and C in Brazilian population has contributed to a high prevalence of SC disease, mainly in Bahia where the heterozygous frequency is around 3.5%. The double heterozygous SC presents a severe anemia but with less clinical complications than SS homozygous[6].

All complications that are found in patients with sickle cell disease anemia have occurred in individuals with HbSC disease. Yet, most – but not all – of these complications are seem less often and appear at later time in HbSC disease compared with sickle cell anemia[17]. The patients with SC disease have less painful crisis, infections, skeletal involvement, anemia, and priapism. However, they have more thromboembolic events, renal papillary necrosis[26] and a particular incidence of retinopathy[8], aseptic necrosis of the head of long bones, and pathological events involving the spleen during adulthood[13 14].

Five major *β^S-globin* gene haplotypes defined by the presence of restriction endonuclease polymorphics sites located throughout the *β-globin* gene cluster have been described. The haplotypes are associated with the African geographic origin of the mutation[20] with description of the Benin (BEN) type in the Midwestern Africa, the Bantu in Central Africa Republic (CAR) in South Central and Eastern

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Africa, the Senegal (SEN) in Atlantic West Africa, the Cameron along west coast and the Saudi Arabia-India on Indian subcontinent and Arabian peninsula[10 18].

The *β^C-globin* gene haplotypes have been devided among the groups: I, II and III[11]. A single origin of the mutation followed by the spread to other haplotypes by meiotic recombination of 5' to the *β-globin* gene has been proposed by Nagel and Rannel[18]**.** The *β^C-globin* gene haplotype when combined with the Benin *β^S-globin* gene haplotype, commonly presents very low HbF levels[17].

Other common hemoglobin disorder with a high worldwide distribution is caused by reduction or absence of the globin chain synthesis, known as thalassemia syndromes. The α-thalassemia has different molecular bases and the α_2-thalassemia followed by a 3.7 Kb deletion has been described in 20-25% of the black Brazilian population[23].

Based on the high prevalence of sickle cell disease and SC disease among the Northeast of Brazil population, we investigated the hemoglobin C and S globin gene haplotypes distribution among SC disease patients associating with the patients' phenotype.

Material and Methods

We studied 126 chromosomes of SC disease patients, aged 23 ± 15.3 years, after obtaining the approval of the Oswaldo Cruz Foundation Institutional Ethical Committee (protocol number 142). They attended the out patient's clinic of the Bahia Blood Center Foundation (HEMOBA) and the peripheral blood samples were obtained during a regular clinic visit. Hematological data were analyzed by an automated cell counter *(Coulter-Counter* T890), hemoglobin profile was investigated by high-performance liquid chromatography (HPLC) (*Bio-Rad VARIANT™* II, CA, USA) and DNA was isolated from the peripheral blood leukocytes by *GFX™ Genomic Blood DNA Purification KIT* (*Amersham Pharmacia Biotech.* NJ, USA). *β^S* and *β^C* globin gene haplotypes were determined by polymerase chain reaction (PCR) and RFLP techniques as previous described[5 24]. The statistical analyses were developed at the software EPI Info version 6.04.

Results

In a group of 63 (39 female and 24 male) the patients median hemoglobin concentration was 10.88 (± 1.67)g/dL; median hematocrit (Hct) 34.48 (± 7.9)%; median cell volume (MCV) 79.93 (± 11.3); median cell hemoglobin (MCH) 27,5 (± 12.80); median cell hemoglobin concentration (MCHC) of 34.66 (± 17.56) and median fetal hemoglobin concentration 3.06%. Table 1 shows the β -globins haplotypes distribution among the 63 SC disease patients.

The α-thalassemia was studied in 60 patients and we observed five (8.3%) homozygous and six (10.0%) heterozygous. The result of α-thalassemia in the different haplotypes is described on Table 2.

Table 1. The β^C and β^S globin gene haplotypes among 45 SC disease patients from Salvador, Bahia (Northeast-Brazil).

β^CHaplotypes	β Haplotypes – n(%)			Total
	Benin	CAR	Athypical	n(%)
β^C I	29 (46.1)	20 (31.7)	2 (3)	48 (80.9)
β^C II	7 (11.1)	5 (7.9)	0	12 (19.1)
Total	33 (57.2)	25 (39.7)	2 (3.1)	63 (100)

Table 2. Association between a-thalassemia and β^S globin gene haplotypes among 45 SC disease patients from Salvador, Bahia (Northeast-Brazil).

Thalassemia	Globin gene haplotypesn(%)			
	Ben I	Ben II	CAR I	CAR II
Normal	21(35)	4(6.7)	1(1.7)	18 (30)
Homozygous	3(5)	1(1.7)	0	1(1.7)
Heterozygous	3(5)	1(1.7)	1(1.7)	1(1.7)

The patients' phenotypes and hematological data are shown in Table 3. There was no statistic significance between gender and hematological data.

Table 4 shows association among the β^C/β^S haplotypes and the phenotype of 63 SC disease patients. There was not found any association between these data.

Table 5 compares the present study with other reports worldwide about SC disease clinical events.

Table 3. Hematological Data and β^C and β^S globin gene haplotypes among SC disease patients from Salvador, Bahia (Northeast-Brazil).

Haplotypes (n)	Gender (M/F)	MEANS (± SD) Age	%HbS	%HbF	%HbC
CAR I (20)	12/8	25 (15.2)	48 (2.5)	2.4 (3.1)	44.7 (1.9)
CAR II (5)	5/0	29 (18.8)	46.5 (2.7)	4.7 (4.2)	43.9 (2.6)
Ben I (29)	17/12	18.1 (13.8)	45.2 (96)	3.4 (3.9)	44.6 (2.3)
Ben II (7)	4/3	34.4 (17.8)	49.2 (3.4)	3.0 (3.5)	44.0 (3)
Athipical I (2)	1/1	23.0 (4.2)	49.7 (0.6)	0.7 (0.1)	44.0 (0.6)
p value	-	0.87*	0.22**	0.47*	0.45*

*Anova; **Kruskal-Wallis test.

Discussion

Bahia, a Northeast Brazilian state received immigrants from Portugal, Holland and France, but the major important racial group in the Bahia population is the Black African. About of 1,200,000 slaves were estimated to have been imported to Bahia from 1678 to 1851. Historical data suggest that about 90% of slaves imported into northern Brazil were from Angola, Congo and Mozambique, where the CAR or Bantu haplotype predominates[12 19]. Northeast region of Brazil (Bahia,

Table 4. The β globin gene genotypes and phenotypes among a group of 63 SC disease patients from Salvador, Bahia (Northeast-Brazil).

β^C/β^S haplotypes	N cases	Clinical features – n(%)				
		Retinopaty	Hepatomelgaly	Splenomegaly	Pain	Leg ulcer
CAR I	20	2	2	5	11	2
Ben I	29	4	5	7	18	0
P value	-	0.52*	0.68*	0.60*	<0.06*	0.17*
CAR II	5	0	1	2	3	0
Ben II	7	1	1	1	3	0
P value	-	1*	1*	0,52*	1*	-
Ben I	29	4	5	7	18	0
Ben II	7	1	1	1	3	0
P value		1*	1*	1*	<0.04*	-
CAR I	20	2	2	5	11	2
CAR II	7	0	1	2	3	0
P value		1*	0.50*	0,59*	1*	1*

*Fisher Exact Test.

Table 5. SC disease phenotype and description in several studies worldwide.

Clinical features	**Country**, Author (year)(reference) [n cases]					
	Jamaica, Serjeant et al.[22] (1973) [n=?]	**Ghana**, Konothey-Ahulu et al. (*apud* 3 15 22 27) (1974) [n=?]	**USA**, Ballas et al. [3] (1982) [n=27]	**Brazil**, Zago et al.[27] (1983) [n=26]	**Brazil**, Marmitt et al.[15] (1986) [n=32]	**Brazil**, present study [n=63]
	Percentage cases (%)					
Hepatomegaly	38	22	16	65	59	14.3
Splenomegaly	60	0	52	58	50	25.4
Bone or joint pain	82	92	0	35	72	58.7
Leg ulcers	20	2	0	0	6	3.2
Retinopaty	?	?	75	?	?	11.1
Cardiopaty	19	0	4	0	13	3.2

(?) No related.

Pernambuco and Maranhão) was heavily supplied by slaves from Central West Africa until the middle of 19th century. The Sudans composed the mayor part of Bahia's population and Pernambuco in minor part and the Bantus occupied Maranhão and center-south of Brazil[12], because of the local where arrived the slave African route in spite of economical development in Brazil.

The β^C allele is found almost exclusively among African-Americans and West Africans from Northern Ghana and the Volta territory and to a much lesser degree, Western Nigeria. The β^C allele presence in West African make up less than 10% of the haplotypes[16]. Although it has been found rarely in individuals from Italy, particularly from Sicily, its geographic distribution when compared to the other common b-globin variants such as βS, βE, βD is quite localized[19].

Haplotypes analysis is a useful tool important to describe the molecular background and is association with normal and variant β-globin alleles, providing clues about the origins of several β-globin variants[5].

This group of patients does not represent the bulk of individuals with SC hemoglobinopathy in Salvador-Bahia-Brazil, because of the small number of patients in the sample, perhaps explain with complications such as hepatomegaly, bone or join pain, retinopaty and leg ulcer were infrequent or hardly observed. RBC from these patients with HbSC disease contains comparable amounts of HbS and HbC, only one patient had increased level of HbF (16.7g/dl). Hematological and biochemical profiles of the disease were defined while the patients were in their usual steady state.

Splenomegaly is a commonly described physical finding in children with HbSC disease, other studies in Brazil found 50%[15] and 58%[27] of splenomegaly differing from our study that found 25.4%, this low number may be a special characteristic of the group in study, these authors did not made any association between age and splenomegaly. Rivera-Ruiz[21] found palpable splenomegaly in 34% of patients and was more common in males. Hepatomegaly was found only in 9 (14.3%) patients, differing from Rio de Janeiro a Southernst Brazilian State with 59% of hepatomegaly[15], Jamaica 38%[22] and US with 16%[3].

Proliferative retinopathy is more common and more severe than in sickle cell anemia and progressive loss of vision may have its onset early in the second decade. Nagel et al.[17] found that retinopathy appears in patients between 15 and 30 years old but in our group retinopathy was present in seven (11.1%) patients aged among 26 to 57, four of these presents Ben I haplotype, one Ben II and two CAR II haplotype. It was previously described that the higher Hb and Hct levels in HbSC disease may be responsible for the higher incidence of retinopathy in this disease, but these hematological data were not observed in this study with association to retinopathy. Balo et al.[4] found 84% of retinopathy SC disease. There are few publications concerning retinal complications of hemoglobinopathies.

Bone or join pain was found in 58.7 % of the patients in according with Ballas et al.[3] that found 50% of HbSC patients with painful crisis. Only 8 (17.7%) patients in the present study had some kind of infection, seven of these with respiratory infection and one osteomyelitis. These findings indicate that SC disease is characterized by a wide range of clinical severity, milder than sickle cell anemia.

The symptomatology of this group of SC disease patients seems to be peculiar. Comparing our population with others in the world, this study demonstrates some aspects of HbSC disease in Brazil that were not previous appreciated, and confirmed features described by others investigators in world. But what we are asking is: What is the influence of β^S and β^C globin gene haplotypes in the investigated SC disease group? Does the β^S haplotype is more critical from the clinical features of the HbSC disease or the β^C haplotype is responsible for better clinical of the SC disease? We found that Ben I haplotype had more painful events that Ben II and that other haplotypes. Leg ulcer appears in the CAR I haplotype. Splenomegaly was more frequent in the β^C I haplotype than in β^C II. Thalassemia was more present in Ben I haplotype. Our data suggest that there is an influence of β^S and β^C globin gene haplotypes in

the phenotype of Northeast SC disease patients. We are addressing our study to answer this question or whether chromosomes carrying the βC mutation interact differentially with the common haplotypes associated with β^S gene and affect the clinical features of HbSC disease. Further studies need to be developed to confirm the different association between haplotype and phenotype SC disease patients.

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