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IDENTIFICATION OF BS GENE HAPLOTYPES IN INDIVIDUALS WITH FALCIFORM ANEMIA IN MATO GROSSO DO SUL, BRAZIL

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ABSTRACT

Background: Sickle cell anemia is a genetic disease determined by homozygous hemoglobin S. It is marked by clinical variability depending on some factors such as the haplotypes associated with the globin *β*S gene.
Objective: This investigation was undertaken to characterize the haplotypes of the βS gene in people with sickle cell anemia assisted in the state of Mato Grosso do Sul, Brazil.
Material and methods: A cross-sectional study was carried out in 47 blood samples from individuals with sickle cell anemia of both sexes attended at hematology outpatient clinics of two public institutions. DNA was extracted from the leukocytes obtained from the whole blood of those surveyed using the phenol / chloroform method. It was used for the identification of haplotypes by PCR / RFLP. The analyzed variables were sickle cell anemia, haplotypes, sex and age.
Results: Of the 47 blood samples, 26 were from female and 21 from male, with ages ranging from 3 to 63 years (23 ± 12.2 years). In relation to haplotypes, there was predominance of Central African Republic (CAR) or Bantu (69.1%), followed by Benin (21.3%), Atypical (8.5%) and Cameroon (1.1%).
Conclusion: It was verified that the CAR haplotype was the most frequent in the state of Mato Grosso do Sul, corroborating with the data obtained in most of the Brazilian regions.

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INTRODUCTION

Sickle anemia is due to a point mutation (GAG → GTG) in the sixth codon of the β globin gene, leading to the substitution of amino acid glutamic acid for valine in the sixth position of the polypeptide chain.

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Due to such mutations occurs the production of hemoglobin S (Hb S), an anomalous hemoglobin that when deoxygenated can lead to the alteration of its Structure (Steinberg, 1999). The inheritance of the symptomatic forms of Hb S including the homozygous - sickle cell anemia (Hb SS) in some Brazilian regions can be considered as a public health problem (Bonini-Domingos, 2016). The distribution of the β^S globin gene in the Brazilian population is heterogeneous and depends on the negroide or caucasoide. In the North and Northeast regions of Brazil, the prevalence of heterozygote for Hb S

ranges from 6% to 10%, in the South and Southeast regions it ranges from 2% to 3% (Cançado and Jesus, 2007). On the other hand in the Central-West Region of Brazil the variation is 3.1% from hetero zygote (Hb AS) (Naoum, 2000). In the Center-West region, in the state of Mato Grosso do Sul, Brazil, observational studies were performed to detect HbS. In 190,809 children screened for hemoglobinopathies by HPLC in the period from 2000 to 2005, the presence of Hb S was observed in 2.624 (1,38%) of the patients (Holsbach *et al.,* 2008). Studies carried out with 233 blood samples in pregnant women attended at the outpatient gynecological service at the University Hospital of the Federal University of Mato Grosso do Sul,Brazil, obtained the presence of 3% Hb AS (Sakamoto *et al.,* 2012). In addition, a study of 182,398 children screened for hemoglobinopathies by HPLC showed that cases of sickle cell disease have increased in this region, with 33 cases of sickle cell anemia (Torres *et al.,* 2017). Individuals with sickle cell anemia have clinical characteristics influenced by factors that may aggravate the clinical state of the disease. In fact, these factors will depend on the level of fetal hemoglobin contained in the erythrocyte and its haplotype (Steinberg, 2005). In sickle cell anemia different haplotypes were identified for the the β^S globin gene, which depending on the origin of the patient are named according to the geographical location or region of Africa.

The haplotypes include Bantu or Central African Republic (CAR), Benin, Senegal, Cameroon (CAM) and Arab-Indian (ARAB) (Pagnier *et al.,* 1984; Lapouméroulie *et al.,* 1992). Atypical haplotypes have been described (Srinivas *et al.,* 1988, Zago *et al.,* 2000). Haplotypes are associated with a clinical picture and varying levels of HbF: the Haplotype CAR is associated with decreased levels of HbF (<5%), whose clinical picture is the most severe; the Benin haplotype has medium levels of HbF (5 to 15%) and intermediate clinical course; the Senegal haplotype has high levels of HbF (>15%) and the less severe clinical course of the disease. Those with the Arab-Indian haplotype have high HbF levels and a heterogeneous clinical course (Nagel, 1984; Powars, 1991). Considering the peculiarities highlighted above, the knowledge of the haplotypes of the β^S gene in individuals with sickle cell anemia in Mato Grosso do Sul / Brazil becomes a priority. With the aim of contributing to a better understanding of the clinical and prognostic variability of sickle cell anemia, which brings indicators for therapeutic conduction, this manuscript aims to characterize the haplotypes of the β^S gene in individuals with sickle cell anemia in Mato Grosso do Sul, Brazil.

MATERIALS AND METHODS

In this manuscript, a descriptive cross-sectional study was conducted with 47 individuals with clinical and laboratory diagnosis of sickle cell anemia (SS homozygous form) attended at two Hematology Services of Public Teaching Hospitals of Mato Grosso do Sul (patient confirmed by Hb electrophoresis in pH alkaline and acidic pH). On the other hand, individuals with heterozygosis for Hb S and interaction with other hemoglobinopathies were excluded. The variables investigated were sickle cell anemia, age, sex and haplotypes. This study was approved by the Research Ethics Committee involving human beings of the Federal University of Mato Grosso do Sul, Brazil (favorable opinion Protocol No. 1608). In order to clarify and dispel any doubts, the participants and/or those in charge of the participants were previously informed about the objectives and procedures of the research. Thus, all participants signed the Informed Consent Term. Blood samples (n = 47) obtained from participants with sickle cell anemia were collected in a tube containing the EDTA anticoagulant and screened for hemoglobin (electrophoresis at alkaline and acid pH) at the time of diagnosis confirmation. The phenol / chloroform method was used to extract deoxyribonucleic acid (DNA). Restriction polymorphism analyzes were performed through the amplification of each region of the DNA containing the sites of interest by the Polymerase Chain Reaction (PCR) method. The determination of the haplotypes was performed by PCR followed by restriction analysis. For the S haplotypes six polymorphic sites were analised (5'γ^G-*Xmn* I, γ^G-*Hind* III, γ^A-*Hind* III, ψβ-*Hinc* II, 3'ψ*β*-*Hinc* II, 5'β-*Hinf* I) (Sutton *et al.,* 1989). According to the restriction profile for the polymorphic regions of the Beta-globin (*β*) cluster, it was possible to define the haplotypes β^S. The data obtained were analyzed through the program BioEstat version 5.0. For comparison between haplotypes (independent samples) the Kruskal Wallis test was used, as well as the Student Newman Keuls test. In the statistical analysis, the level of statistical significance considered was $p < 0.05$.

RESULTS

In the present study, 26 (55.3%) of the 47 blood samples from individuals with sickle cell anemia attended at the Public Hematology Services of Mato Grosso do Sul were female. However 21 (44.7%) of individuals were male. The subjects' ages ranged from 3 to 63 years, with a mean of 23 years (± 12.2), without family ties (non-blood relatives), and who had not received blood transfusions in the last 120 days. Of the 47 analyzed samples, 22 (46.8%) were CAR / CAR haplotype, 14 (29.8%) CAR / Benin, 7 (14.9%) CAR / Atp, two Ben / Ben, and one Benin / Cameroon and Benin / Atypical (Table 1). The Senegal and Arab-Indian haplotypes were not identified in this study. According to the 94 chromosomes analyzed, 65 (69.1%) were of the CAR or Bantu haplotype, followed by Benin in 20 (21.3%), Atypical in 8 (8.5%) and one to the haplotype Cameroon (Table 2). The haplotypes include Bantu or Central African Republic (CAR), Benin, Senegal, Cameroon (CAM) and Arab-Indian (ARAB).

Table 1. Individuals with sickle cell anemia according to genotypes. Hematology Clinic of the HU / UFMS and HRMS (n = 47)

Genotypes	n	%
*CAR/CAR	22	46,8
CAR/BEN	14	29,8
CAR/ATP	7	14,9
BEN/BEN	2	4,3
BEN/ATP	1	2,1
BEN/CAM	1	2,1
Total	47	100,0

*Central African Republic (CAR), Benin (BEN), Cameroon (CA

Table 2. Individuals with sickle cell anemia according to haplotypes. Hematology Clinic of the HU / UFMS and HRMS (n = 47)

Haplotypes	n	%
CAR	65	69.1
Benin	20	21.3
Atypical	8	8.5
Cameroon	1	1.1
Total	94	100.0

*Central African Republic (CAR)

DISCUSSION

The population studied is predominantly women and young adults living in Mato Grosso do Sul, but they are accompanied by two hospitals in the public network of Mato Grosso do Sul, Brazil. In this research, the distribution of haplotypes shows that CAR (69.1%) was the most prevalent than the Benin haplotype (21.3%). A study carried out in the city Ribeirão Preto showed a predominance of the CAR haplotype (66.2%) in relation to Benin (23%) in individuals bearing the β^S gene (Zago *et al.,* 1992). This prevalence was also observed in individuals with sickle cell anemia in São Paulo (Gonçalves *et al.,* 1994; Figueiredo *et al.,* 1996). In Rio de Janeiro the CAR haplotype (54%) was more frequent (44.6%) than Benin (Fleury, 2007). On the other hand, in studies conducted in Rio de Janeiro, the Bantu haplotype followed by Benin were predominant and confirmed historical facts of the arrival of the β^S gene for Brazil (Silva Filho *et al.,* 2010). The frequency of βS haplotypes in patients in Rio showed the high frequency of Bantu and Benin haplotypes, confirming the ethnic origin of Western and South-Central African in the Brazilian population (Okumura *et al.,* 2013). In the Northeastern region of Brazil, there was a predominance of CAR haplotype (79.1%) in the state of Pernambuco (Bezerra *et al.,* 2007), 66.2% in the Fortaleza capital was CAR type, 22% Benin (Silva *et al.,* 2009). In addition, a study carried out in Rio Grande do Norte showed the presence of 75.5% of CAR haplotypes (Cabral *et al.,* 2010).

In the state of Bahia, the most frequent haplotype was CAR / Benin (51.2%), followed by Benin / Benin (28.8%). Study found 10.9% of Senegal haplotypes in Belém, which is the northern region of Brazil (Cardoso and Guerreiro, 2006). A study developed in a quilombo population in the Ribeira Valley in São Paulo found 8.1% of this haplotype (Mello Auricchio, 2007). Senegal and Arab-Indian haplotypes were not observed in the present study sample. Similar results were found in other Brazilian regions (Bezerra *et al.,* 2007; Silva *et al.,* 2009; Sillva Filho *et al.,* 2010). Study found 10.9% of Senegal haplotypes in Belém, which is the northern region of Brazil (Cardoso and Guerreiro, 2006). A study developed in a quilombo population in the Ribeira Valley in São Paulo found 8.1% of this haplotype (Mello Auricchio, 2007). In a study carried out in Amapá, the following haplotypes were present: Bantu haplotype (61.2%), Benin haplotype (26.6%) and Senegal haplotype (12.2%). However, it was observed absence of atypical Cameroon and Saudi (Castelo *et al.,* 2014). The results presented in this manuscript corroborate with studies carried out in other Brazilian regions that show diversity of ethnic origins and different frequencies of haplotypes (Castelo *et al.,* 2014).

Conclusion

From the study carried out in this manuscript involving individuals with sickle cell anemia in clinical care in Mato Grosso do Sul, 69.1% had a CAR haplotype, 21.3% from Benin, 8.5% from Atypical and 1.1% from Cameroon. The characterization of the haplotypes identified in participants with sickle cell anemia in Campo Grande, MS, Brazil, contributes to the prognosis and clinical follow - up of the disease. In addition, it will provide elements for a database that will help to understand the ethnic composition of the population of our state, since the founders came from different Brazilian regions and many of them descended from slaves.

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