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Epidemiological, clinical, and severity characterization of sickle cell disease in a population from the Brazilian Amazon

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Abstract
Objective/background: Sickle cell disease (SCD) is a chronic inflammatory condition caused by a point mutation in the *HBB* gene. Here we characterized the clinical presentation of SCD in a population from Amazonas State in northern Brazil, in order to evaluate whether the higher Amerindian ancestry observed in this relatively isolated geographic region would influence the clinical presentation of SCD.
Methods: This was a cross-sectional study characterizing the clinical presentation of SCD patients registered at HEMOAM, Amazon, Brazil. Data were obtained using a structured questionnaire, and by a review of the medical records.
Results: Of the 236 SCD patients listed in the historical records, 122 were included in this study. The median age was 15 years, with a male to female ratio of 52:70. The population was characterized by a high level of socioeconomic vulnerability, with only 2.1% presenting a family income above five minimum wages. Homozygous HbS (SS) was the most prevalent form of SCD (89.7%), and the diagnosis of SCD was performed in the context of complications in 92.3% of patients. The median frequency of vaso-occlusive crisis in the past 12 months was 2 (0–10). Using a validated clinical severity score based on clinical and laboratory data, no significant difference could be observed when compared to other populations.

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Conclusion: Our results represent the first comprehensive characterization of epidemiological, laboratorial, and clinical data of SCD in the region of the Brazilian Amazon. Despite the higher contribution of Amerindian ancestry previously demonstrated in this region, the main clinical characteristics of SCD seem similar to those reported in other populations.

Introduction

Sickle cell disease (SCD) is the most frequent hereditary hemoglobin (Hb) disorder in the world that results from a mutation in the gene coding for beta-globin (*HBB*) [1,2]. The condition is characterized by chronic hemolytic anemia and widespread vaso-occlusive events leading to tissue ischemia and progressive organ failure. Robust evidence gathered in the past decades demonstrates that the immune system, and in particular the innate inflammatory pathways, play a critical role in the pathogenesis of SCD [3,4]. Hence, the clinical presentation and the severity of SCD are also influenced by genes other than *HBB*, particularly those associated with the immune system [5].

SCD was introduced in the American continent through the slave trade, and it is estimated that between 1492 and 1870 about 11,000,000 sub-Saharan Africans were forced to emigrate to the Americas as slaves [6]. Currently, the average prevalence of heterozygotes for the SCD mutation is 2%, rising to 6—10% among blacks, leading to an estimate of 700—1,000 new annual cases of SCD in Brazil [7].

Brazil has a trihybrid population with European, African, and Amerindian roots. Although the country experienced large degrees of ethnic admixture, migration waves to different parts of the country resulted in considerable phylogeographical heterogeneity, with the Amazon region in northern Brazil presenting a larger influence of Amerindian roots compared to other regions of the country [8,9]. Of note, this heterogeneity has been confirmed by analysis of mitochondrial DNA (mtDNA) and DNA polymorphisms [10,11]. Unfortunately, very limited data is available on the impact of this diverse ethnic background on health and disease. The prevalence of heterozygotes for HbS is higher in the north and northeast regions (6% to 10%), and lower in the south and southeast regions (2% to 3%) [9]. However, whether this diverse genetic background affects the clinical presentation of SCD has not been completely described. Hence in the present study, we present a comprehensive description of clinical and laboratory data of a population of SCD patients from the largest state of the Brazilian Amazon.

Materials and methods

Study participants

This was a cross-sectional study including a population of SCD patients from the State of Amazonas in northern Brazil, registered at the Hematology and Hemotherapy Foundation (HEMOAM) in the capital city Manaus. With an area of 1.5 million km^2 (corresponding to 20% of continental USA) and 4 million inhabitants (evenly distributed between Manaus and other small towns), Amazonas is the largest state in Brazil, and HEMOAM is the only institution responsible for providing care for SCD patients in this large geographical area. All patients with a confirmed diagnosis of SCD attending the outpatient clinic of HEMOAM between March 2016 and March 2017 were invited to participate. Patients younger than 2 years were excluded from the analysis, and patients experiencing an acute event were enrolled in their next visit, at least 3 months from the last acute crisis. At the time when the study was initiated, the historical registry of SCD patients of HEMOAM included 236 individuals. All clinical data were collected by one of the investigators (P.C.) who was not involved in the care of these patients, provided by our local hematology staff. The study was performed in accordance with the Declaration of Helsinki and was approved by the institute's research ethical committee (CAAE 48830215.2.0000.0009). All patients or legal guardians provided a written informed consent prior to enrollment in the study.

Clinical and laboratory data

All clinical data were collected by one of the investigators (P.C.) who was not involved in the care of these patients, provided by our local hematology staff, addressing socio-economic and clinical variables, with a duration of approximately 20—30 minutes per patient. When necessary, information was also obtained from a review of the medical records. Laboratory data were obtained from the medical records of HEMOAM. The most recent results collected at steady-state were considered for all parameters, except for parameters derived from Hb electrophoresis.

Calculation of the SCD severity score

Considering the challenges of comparatively evaluating the severity of SCD between different studies, a validated severity score was used [12]. The score considers clinical and laboratory data and was calculated using an online web tool available at http://www.bu.edu/sicklecell/downloads/Projects, yielding a score that ranges from 0 to 1. This value was then used to define the severity of SCD (stratified as mild, intermediate, or high), according to criteria established in the original publication for each of three different age groups: 2—17 years; 18—40 years; and >40 years. [12]

Statistical analysis

Data are presented as average, median, standard deviation, and range, as indicated in each table or figure.

The comparison between distinct groups was performed using the *t* test or analysis of variance test for continuous variables, or the chi-square test for categorical variables. Correlations were assessed by the Spearman or Pearson correlation tests, according to the data distribution. For the evaluation of the association between SCD severity and clinical characteristics, patients were stratified into two groups according to the severity score: mild and intermediate/high severity scores. Statistical analyses were performed using the GraphPad (San Diego, California, USA) Prism 7.0. A p value < 0.05 was considered as statistically significant.

Results

In total, 122 patients were enrolled in this study. The median age of the patient studied was 15 years, ranging from 2 years to 54 years, and the male to female ratio was 52:70. Although the majority of patients (85/122; 69.6%) lived within 20 km of the treatment center, a significant proportion (22/122; 18.2%) had to travel more than 200 km, mainly using river navigation. As far as ethnicity (defined by self-declaration) is concerned, the majority of the study population was defined as mulatto (72%). Overall, the study population was characterized by a high level of socioeconomic vulnerability, with only 2.1% presenting a family income above five minimum wages (Table 1).

Homozygous HbS (SS) was the most prevalent (89.7%) form of SCD. In the study population, the diagnosis of SCD was performed in the context of complications in 92.3% (n = 96) of patients, with only seven of 122 patients being identified by neonatal screening. The cumulative frequency of SCD complications were as follows: priapism: 32.4%; leg ulcers: 15.4%; splenic sequestration: 41.9%; and stroke: 13.5%. The median frequency of vaso-occlusive crisis in the past 12 months was 2 (0—10) (Table 2).

Laboratory parameters are shown in Fig. 1. We also evaluated the correlation between hemolytic markers and classical severity laboratory parameters in SCD. Significant, yet mild or moderate correlations (p < 0.05) were observed between the reticulocyte count and the platelet count (R = 0.24; p = 0.0079); reticulocyte count and lactate dehydrogenase (R = 0.32; p = 0.0013); reticulocyte count and Hb (R = 0.45; p = 0.0001); and reticulocyte count and leukocytes (R = 0.20; p = 0.022) (Fig. 1).

With regard to the treatment of SCD, hydroxyurea was used by 61.5% of patients at the time of study and a lifetime history of at least one blood transfusion was reported by 82.6% of patients. In addition, 83.9% of the patients diagnosed prior to the age of 5 years reported receiving prophylactic penicillin therapy in their childhood (Table 3).

The mean SCD severity score was 0.34 (0.046—0.89) in patients younger than 18 years, 0.63 (0.20—0.96) in patients between 18 years and 40 years, and 0.80 (0.71—0.97) in

Table 1 Demographic and socio-economic characteristics of patients.

Parameters	
Patient age (y), median (range)	15 (2—54)
Patient gender (*n*), male/female	52/70
Time from diagnosis (y), median (range)	2 (0—53)
Cases diagnosed within 1 y of study initiation	09 (7.3)
Patients previously on follow-up	113 (92.7)
Ethnic background (*n* = 122)	
Mulatto	89 (72.0)
Black	13 (10.6)
White	20 (17.4)
Distance from the treatment center (km) (*n* = 122)	
<20	85 (69.6)
20—200	15 (12.2)
>200	22 (18.2)
Formal education (*n* = 122)	
Day care/preschool	9 (7.4)
Primary education	53 (43.4)
Secondary education	44 (36.1)
Higher education	4 (3.3)
No information	12 (9.8)
Household income (in minimum wages) (*n* = 107)	
<1	10 (9.04)
1—5	94 (87.8)
>5	3 (2.8)
Use of government social benefits (*n* = 122)	
Yes	64 (5 2.45)

Note. Data are presented as *n* (%). y = year.

Table 2 Clinical characteristics of patients.

Parameters	*n* = 122
SCD type	111 (90.9)
SS	8 (6.57)
SC	1 (0.89)
Sβ thalassemia	2 (1.64)
Age at diagnosis (y)	
Up to 1	34 (30.4)
1—10	61 (54.8)
11—20	7 (6.3)
21—40	9 (7.7)
>41	1 (0.8)
Context of diagnosis	
Due to complications of SCD	96 (92.3)
Due to familiar history	1 (1.0)
Neonatal screening	7 (6.7)
Frequency of complications	
VOC in the past 12 mo, median (range)	2 (0—10)
Priapism[a]	12/52 (23.07)
Cholelithiasis[a]	36/112 (32.1)
Leg ulcers[a]	17/110 (15.4)
Splenic sequestration[a]	44/105 (41.9)
Avascular necrosis[a]	17/109 (15.5)
Stroke[a]	15/111 (13.5)

Note. Data are presented as *n* (%) or *n*/total (%). SCD = sickle cell disease; VOC = vaso-occlusive crisis.
[a] Only patients with complete information.

patients older than 40 years. The proportion of mild, intermediate, and high severity in the study population was 57.5%, 33.5% and 9%, respectively (Fig. 2).

Based on this classification, we evaluated the association of patient's characteristics with SCD severity scores (Table 4). None of the tested factors were associated with a more severe phenotype.

Discussion

Although SCD is a monogenic condition, its clinical presentation is heterogeneous and known to be influenced by polymorphisms in several other genetic modifiers, including those that regulate the inflammatory response [1,13]. Accordingly, pathways such as angiogenesis, hemostasis, leukocyte recruitment, and adhesion have all been shown to play a role in the pathogenesis of SCD [3]. Since these pathways include several genes that have been submitted to intense evolutionary pressure, it is fair to hypothesize that differences in the ethnic background of a population could influence the presentation of SCD [14]. Here we present a comprehensive description of the clinical characteristics of SCD in a mixed population from Brazil characterized by a higher Amerindian ancestry.

Brazil is a country with continental dimensions with a highly miscegenated population. The relative collaboration of European, African, and Amerindian ancestry varies in these populations as shown by genomic data [15]. SCD is present in all of these populations. Estimates suggest the existence of more than 2 million carriers of the HbS gene in Brazil, and more than 30,000 individuals living with SCD, making this the most common genetic disorder in the country [16,17]. However, most studies describing the clinical characteristics of SCD patients in Brazil and in other countries did not include a significant proportion of patients with Amerindian ancestry.

With an area of 1.5 million km^2 (corresponding to 20% of continental USA) and 4 million inhabitants (evenly distributed between Manaus and other small towns), Amazonas is the largest state in Brazil, with 98% of its territory coverage by native forests. The absence of paved roads connecting it to other regions of Brazil results in a relative isolation from other regions of the country both in terms of cultural characteristics and genetic background. Of note, the importance of the relative contribution of different genetic backgrounds for the study of SCD has been recently highlighted by a study using genomic tools that demonstrated these differences in Brazil [10]. In addition, a study addressing the distribution of HbS haplotypes in the same geographic area of the present study also supports this heterogeneity [18]. Therefore, the study of the clinical characteristics of SCD in this relatively isolated area offers an attractive opportunity to evaluate the influence of genetic background on the manifestations of SCD.

In this context, the main result of our study was the demonstration that the frequency of SCD complications and laboratory alterations was similar to other populations. In particular, the median frequency of vaso-occlusive crisis in the past 12 months reported by our patients (two

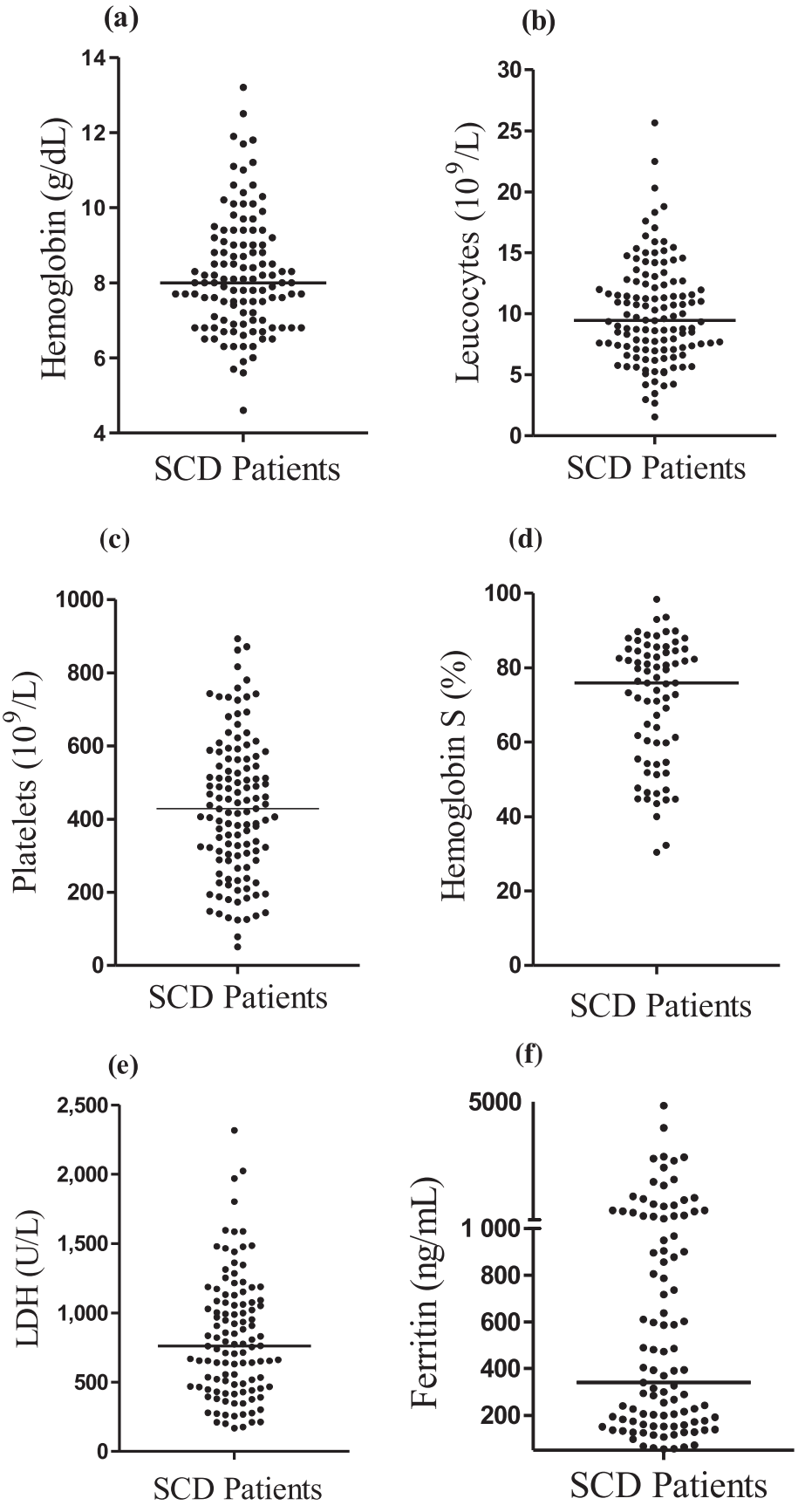

Fig. 1 Laboratory parameters of patients with sickle cell disease (SCD). Bars indicate median values. Hemoglobin, leucocytes, platelets, hemoglobin S, lactate dehydrogenase (LDH), ferritin.

Table 3 Access and treatment strategies of sickle cell disease (SCD) in the study population.

Treatment strategies	
Prophylactic use of penicillin (n = 71[a])	
Yes	57 (83.9)
No	9 (10.3)
No information	5 (5.8)
Transfusions (n = 122)	
Yes	99 (82.6)
No	10 (8.1)
No information	13 (9.3)
Hydroxyurea (n = 122)	
Yes	75 (61.5)
No	36 (29.5)
No information	11 (9.0)

[a] Note. Data are presented as n (%).

episodes) was similar to rates documented in a recent randomized clinical trial (2.89 episodes) [19], and somewhat higher than the frequencies reported in the Cooperative Study of Sickle Cell Disease (CSSCD) (1.39 episodes) [19,20]. While the cumulative frequency of stroke in our population (13.5%) is higher than the frequency reported in the CSSCD study (3.8%) [21], it is similar to the rate reported in a small cross-sectional study in other adult populations living in Cameroon [22] of a stroke prevalence of 13%.

However, the comparison of the SCD severity across different populations cannot be safely performed by the individual analysis of these parameters. To overcome this limitation, we also calculated a validated SCD severity score. Again, the results obtained in our population are similar to those reported in studies performed in Brazil and in the USA [12,23].

The calculation of this severity score also allowed us to classify our patients in three severity groups, and to evaluate the impact of several variables assumed to influence the severity of SCD in the clinical evolution of our patients. In particular, we were able to objectively evaluate whether socio-economic and demographic variables are associated with SCD. Interestingly, none of the socio-economic factors intuitively associated with SCD severity such as distance from the treatment center, household income, or reliance on governmental social programs were associated with disease severity. We hypothesize that the high vulnerability of our population, illustrated by the small range of household income, coupled with a relatively low sample size (the p value of this analysis was 1.00) precluded the demonstration of such effect.

In terms of access to diagnosis and treatment, we observed that a high proportion of patients were diagnosed

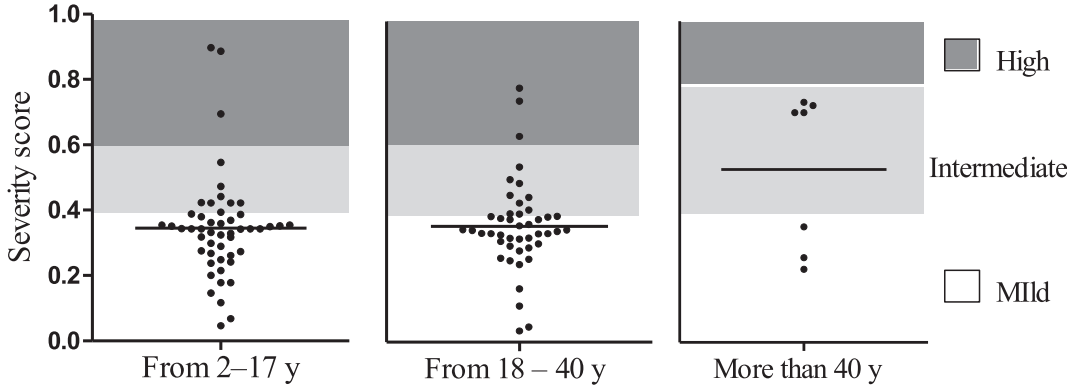

Fig. 2 Distribution of sickle cell disease (SCD) severity scores among different age groups. Bars indicate mean values and background colors indicate different severity strata. *Note*. y = year.

Table 4 Association between patient characteristics and sickle cell disease SCD severity scores.

Patient characteristic					Intermediate/high[a] (n)	
	Mild (n)		OR	p		
Yes	No				Yes	No
Living > 200 km from treatment center	7	53	15	47	0.41 (0.15—1.10)	0.09
Household income < 2 minimum wages)	37	23	39	23	0.94 (0.45—1.97)	1.00
Use of governmental benefits	34	26	31	31	1.30 (0.64—2.66)	0.47
Hydroxyurea use	44	16	39	23	1.62 (0.75—3.50)	0.24
Predominant ethnic background-black	13	47	6	56	2.58 (0.91—7.32)	0.008
Predominant ethnic background-mulatto	35	25	40	22	0.7 (0.37—1.59)	0.57
Predominant ethnic background-White/Caucasian	3	57	8	54	0.35 (0.08—1.41)	0.20

in the context of clinical complications of SCD. In addition, more than 80% of patients had a history of blood transfusions, which is also higher than other cohorts, demonstrating the need for a more rational use of this resource. In contrast, access to two of the cornerstones of SCD treatment was high, with 75 (61.5%) of patients under hydroxyurea therapy, and 57 (83.9%) of patients with a history of penicillin prophylaxis.

The main limitations of our study are those inherent to retrospective studies and involve recall bias and underreporting of clinical data. Although the use of structured interviews and a systematic collection of data from the medical records limit the impact of these biases, readers should consider these limitations when interpreting our data.

In conclusion, our study demonstrated that patients with SCD from the largest state of the Brazilian Amazon have clinical presentation similar to other populations from Brazil and other countries, supporting the preponderance of HbS genotype and classical genetic modifiers [1,18], over other inherited and acquired traits. Considering the recent advances in the field including the feasibility of hydroxyurea use in children [1], advances on gene therapy [23], hematopoietic stem cell transplants [24], and emerging therapies for these patients, understanding the characteristics of SCD in different populations is important to plan how these therapies should be implemented to improve their lives.

Declaration of Competing Interest

The authors declare that they have no conflicts of interests.

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