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Relationship between the prevalence of hemoglobin S and the ethnic background of blood donors in Paraná state

Relação entre prevalência da hemoglobina S e origem étnica de doadores de sangue no estado do Paraná

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ABSTRACT

Introduction: Hemoglobin S (HbS) is one of the most common inherited hematological disorders in humans. In Brazil, the sickle-cell disease (SCD) has significant epidemiological importance due to its prevalence and the morbidity-mortality and, therefore, it has been identified as a matter of public health. Objective: To determine the prevalence of HbS among Asian Brazilian, Afro Brazilian, and Euro Brazilian individuals of a blood bank in Curitiba. Material and method: The study was conducted from January 2008 to December 2009, and included 83,213 donors seen at the Instituto Paranaense de Hemoterapia e Hematologia. Results and discussion: The prevalence of HbS in the studied population was 0.9%, among them, 0% Asian Brazilians, 2.7% Afro Brazilians, and 0.7% Euro Brazilians. There was a positive association, statistically significant for the sickle cell trait in Afro-descendants, with odds ratio (OR) 4.01; confidence interval (CI) 3.42-4.72; and 95% confidence. Conclusion: This study showed higher rates of sickle cell trait in Afro Brazilians, which corroborates data published in other Brazilian regions and states.

Key words: hemoglobin S; blood donors; prevalence.

INTRODUCTION

Sickle cell anemia (SCA) is a disease characterized by a mutation on chromosome 11, which results in substitution of valine for glutamic acid at position 6 of the N-terminus of the beta-globin chain, resulting in an abnormal hemoglobin, originating uma hemoglobina anormal, called hemoglobin S (HbS), which replaces the hemoglobin A (HbA) in affected individuals(1-3). Thus, erythrocytes, whose predominant content is HbS, assume, in hypoxic conditions, a form similar to a sickle, hence the name sickle cell, resulting from the polymerization of hemoglobin S(3).

In Brazil, the disease has a higher incidence among Afro Brazilians, but also occurs in Euro Brazilians; there is a tendency for the disease to reach an increasingly significant portion of the population, due to the high degree of miscegenation in Brazil. In Southeastern Brazil, the average prevalence of heterozygous (carriers) is 2%, this value climbs to around 6%-10% among Afro descendant in Northeastern Brazil(1-3).

In some regions of Africa, 40% of the population has sickle cell trait, and sickle cell disease (SCD) reaches a prevalence of 2%-3% of the population. It is believed that SCD emerged as a nature response to avoid malaria from destroying the population in Africa, in the presence of mutations. With the change, these people started to produce HbS, instead of HbA. It is in this type of hemoglobin that the protozoan Plasmodium sp. enters and multiplies, causing cell destruction. Thus, HbS would be a protective factor for malaria. Due to the forced migration, that is, the slave trade and population movements in search of better living conditions, this mutation has spread worldwide(1-7).

Individuals with sickle cell trait are clinical and hematological healthy and, therefore, able to donate blood. However, the use of this blood is restricted, especially in patients with hemoglobinopathies,
in subjects with severe acidosis, in newborns, and in intrauterine transfusions procedure, due to potential triggers for sickle cell in
the receiver, and also the changes in blood product, as a result of
processing and storage(8).

The Brazilian hemotherapy has achieved a high level of
expertise and technical competence in recent years, in order
to minimize risks and prioritize transfusion quality. Abnormal
hemoglobin detection became mandatory in blood banks
according to RDC nº 153, June 14, 2004.

Despite numerous researches involving the disease and the
sickle cell trait, epidemiological studies, especially in the state of
Paraná, evaluating the frequency of HbS in patients with different
ethnic backgrounds are scarce. Therefore, relevant studies to
ensure the epidemiology of SCD, specifically in this state, prove
to be valuable.

OBJECTIVE

Our objective was to determine the prevalence of HbS in
patients with different ethnic backgrounds, among blood donors
in Curitiba.

MATERIAL AND METHOD

The study was conducted in 83,213 blood donors at the
Instituto Paranaense de Hemoterapia e Hematologia, from
January 2008 to December 2009, which were submitted to blood
collection by venipuncture for examinations for the diagnosis
of sickle cell trait. This study was approved by the Ethics Committee
under number 360.918/2013.

Blood samples were collected by venipuncture, using
ethylenediamine tetraacetic acid (EDTA) anticoagulant for
further performance of blood exams. We collected the data
from the respective donors available in the blood bank system,
such as gender and ethnic background. It is emphasized that
the ethnic component was determined by individual self-
declare.

The samples were subjected to HbS search, with solubility
test for screening, according to Soares et al. (2009)(8), followed by
confirmation of HbS presence by electrophoresis. The procedure
used was adapted from the method previously described in the
literature(8, 9).

For the solubility test, 200 µl of blood were placed in
each well of a microtiter plate, and then adding 50 ml of the
hemolyzing solution, consisted of 10 mg of Na₂S₂O₃ for
each ml of phosphate solution (K₂HPO₄ = 29.66 g; KH₂PO₄ =
16.89 g; saponin – 1.25 g; 125 ml of distilled water). After
homogenization, 20 ml were removed and applied to Whatman
filter paper nº 6. The test reading was performed with naked
eye, two minutes after application of hemolysate on the filter
paper, resulting in dark center with a clearer halo for positive
testing.

All samples were subjected to alkaline and acid electrophoresis
tests, using agarose gels from the Companhia Equipadora
de Laboratórios Modernos (CELM), because they show good
resolution of the fractions.

RESULTS

From a total of 83,213 donors, 60.8% were female and 39.2%
males. The higher frequency of men was expected because the male
donor rate in Brazilian blood banks is known to be greater than the
female(6). However, it is worth remembering that the gene encoding
HbS does not bind to sex. Regarding the ethnic component, the
highest frequency among donors was Euro Brazilians (90.9%),
followed by Afro Brazilians (9%) and Asian Brazilians (0.05%)
(Figure 1).

![Figure 1](image1.png)

**Figure 1** – Distribution graph of blood donors according to ethnic group

We detected 747 samples positive to HbS solubility and, of
these, 727 were confirmed by electrophoresis, resulting in 20 false
positive for the screening test. Of all donors, 0.9% had sickle cell
trait, of which 0% Asian Brazilian, 72% Euro Brazilians, and 28%
Afro Brazilians (Figure 2).

Despite the predominance of Euro Brazilians donors, the
percentage of individuals who are carriers for HbS in this group
was 0.7% (523/75,685), while in Afro Brazilians this rate was 2.7% (204/7,486).

Comparing the solubility technique with the electrophoresis technique, we observed 99% specificity and 100% sensitivity. To evaluate the association of the ethnic backgrounds with the relative risk for developing the disease, we calculated odds ratio (OR) by grouping: Asian Brazilian with Euro Brazilian and Afro Brazilian. We found OD 4.01 with \( p = 95\% \) and confidence interval (CI) 3.42-4.72, which suggest a positive association between sickle cell trait and Afro Brazilians.

**DISCUSSION**

According to the World Health Organization (WHO), 5% of the world population carries the gene for hemoglobinopathies, and every year about 300,000 children are born with hemoglobinopathies. From these data, 200,000 SCD cases occur Africa\(^{(10)}\).

In Brazil, SCD has significant epidemiological importance because of the prevalence and mortality rates showed, and, therefore, it has been identified as a public health issue\(^{(1, 11, 12)}\). This is the hereditary disease with the highest most prevalence in the country due to increased national miscegenation, with a tendency to achieve increasingly significant portion of the population\(^{(1, 13)}\).

The state of Paraná has an heterogeneous population made up of descendants of many ethnic backgrounds: Poles, Italians, Germans, Ukrainians, Dutch, Spanish, Japanese, and Portuguese, among others\(^{(13)}\), which makes an interesting sample for research on the prevalence of sickle cell trait. It is known, however, that the miscegenation favors the dispersion of abnormal genes, especially those that cause hematological abnormalities, and that, besides the ethnic factor, several environmental factors also influence the sickle cell disease\(^{(1, 2, 11)}\).

Comparing the frequency of HbS in relation to skin color, it was observed that it was higher among Afro Brazilians (2.7%) than among Euro Brazilians (0.7%) individuals. These data corroborate researches conducted by other authors in the state of São Paulo\(^{(1)}\), in this study the authors report that the Afro descendant individuals had a higher frequency of HbS when compared to Euro Brazilians, and that Euro descendants subjects showed significant prevalence of hemoglobin usually more common in Africans. Thus, we can reassert that HbS, albeit being more prevalent in Afro descendant individuals, it must not be regarded as color “specific marker”, since it is also present in white people.

The epidemiology of sickle cell trait in Euro descendant population found in the present study differs from that reported by other authors\(^{(11)}\), which also investigated individuals from the South region, where the HbS prevalence found was 50%. One hypothesis to explain the differences between the prevalence is that the latter study was conducted a decade before the former and, therefore, the prevalence may change over the years. Another relevant issue is the self-declaration for the ethnic background, which may be a bias for comparative analyzes, as this parameter is based on culturally established criteria by the informer and generally includes only the skin color, beyond the fact of the intense miscegenation characteristic of Brazil.

Epidemiological data show that in Brazil the birth rate of children with SCD per year is 1/1,000 per live births\(^{(14)}\). In the state of Paraná, this ratio is 1:13,500 for children born with SCD and 1:65 for sickle cell trait children\(^{(14)}\). The prevalence of sickle cell trait and SCD in the Brazilian regions are described in **Table**.

In this study, from the total number of individuals with sickle cell trait \((n = 727)\), we observed 72% \((n = 523)\) Euro Brazilians and 28% \((n = 204)\) Afro Brazilians. One hypothesis for the above data is the fact that the number of Afro Brazilians individuals in our sample is much lower (1.3%) when compared with the number of Euro Brazilians individuals (90.9%). This, however, reflects the Paraná population, whose the majority of inhabitants are white, representing 77.2% and 70.3% of the population in the demographics census of 2000 and 2010, respectively\(^{(25, 26)}\).

The data reported here show that anemia and sickle cell trait should not be used as ethnic identification criteria, since they can be present in Euro Brazilians individuals (Figures 1 and 2).
According to the Normas Técnicas do Ministério da Saúde (NTMS) (Decree 1376 of 19/11/1993), the HbS detection is recommended due to its significant prevalence (25%-45%) in the Brazilian population. Given this fact, the chance of finding a receiver with sickle cell trait in blood donors individuals in Brazil is great, which would decrease the effectiveness of the transfusion, because the number of abnormal cells from the donor would cause, even in receivers with the hemoglobin A (HbAA), an inefficient transfusion, thereby not fulfilling its role, to replace the number of red blood cells.

Population screening of asymptomatic heterozygotes for genetic counseling is a controversial procedure, because it involves the risk of labeling, discrimination, stigmatization, loss of self-esteem, and invasion of privacy. However, some authors support this procedure, when carried out by professionals with adequate training\textsuperscript{(15, 27, 28)}.

Comparing the HbS solubility technique with the technique used as the confirmatory test (electrophoresis) we found 2.75\% ($n = 20$) of false positive cases, showing 99\% specificity and 100\% sensitivity. Other authors report that false positive results for the solubility test can be seen in conditions such as: polycythemia, multiple myeloma, hemoglobin Bart’s, hemoglobin C (HbC), recent transfusion, chronic renal failure, and excess blood in the reaction; false negative results can occur if the hemoglobin level is lower than 7 g\%, when HbS is present in small quantities (in neonates and after transfusion), if there is deterioration and inactivity of reagents, or due to the inadequate amount of blood used in testing\textsuperscript{(29)}.

Due to the high prevalence of HbS in our country, this screening enables clarifications, not only for reproductive orientation, but also applicable knowledge in other aspects of life of individuals with sickle cell trait or SCD.

**CONCLUSION**

This study showed higher rates of sickle cell trait in Afro Brazilians, which confirms data published in other Brazilian regions and states. Therefore, taking into account the miscegenation and the prevalence of hemoglobinopathies in Brazil, as well as the scarcity of epidemiological studies showing the prevalence of such diseases in the Paraná population, it is clear the importance of further research in this regard, as well as the implementation of more screening centers to detect these genetic changes countrywide, in order to allow a better understanding of this inherited conditions.
RESUMO

Introdução: A hemoglobina S (HbS) é uma das alterações hematológicas hereditárias de maior frequência na espécie humana. No Brasil, a anemia falciforme (AF) tem importância epidemiológica significativa em virtude da prevalência e da morbimortalidade que apresenta e por isso tem sido apontada como uma questão de saúde pública. Objetivo: Determinar a prevalência de HbS entre indivíduos de origem asiática, afro e euro-brasileira de um banco de sangue de Curitiba. Material e método: O estudo foi realizado no período de janeiro de 2008 a dezembro de 2009, no qual foram incluídos 83,213 doadores atendidos no Instituto Paranaense de Hemoterapia e Hematologia. Resultados e discussão: A prevalência geral de HbS na população estudada foi de 0,9%, sendo 0% entre asiático-brasileiros, 2,7% entre afro-brasileiros e 0,7% entre euro-brasileiros. Houve associação positiva, significativa estatisticamente, para o traço falcêmico nos afrodescendentes, com odds ratio (OR) de 4,01 e intervalo de confiança (IC) 3,42-4,72, com 95% de confiança. Conclusão: O presente trabalho demonstrou maiores índices de traço falcêmico em afro-brasileiros, o que corrobora dados publicados em outras regiões e estados brasileiros.

Unitermos: hemoglobina S; doadores de sangue; prevalência.

REFERENCES


