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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, originando 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[4-6].

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,

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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 Na_2S2O_4 for each ml of phosphate solution (K_2HPO_4 – 29.66 g; KH_2PO_4 – 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% male. 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**).

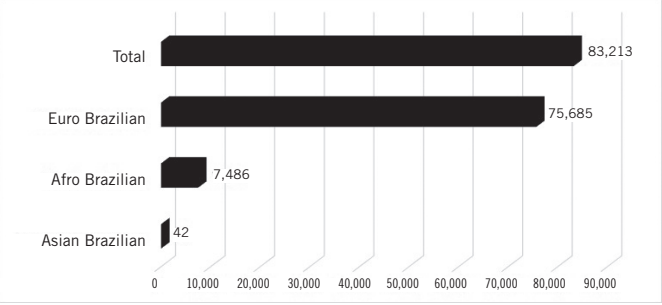

FIGURE 1 – *Distribution graph of blood donors according to ethnic group*

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

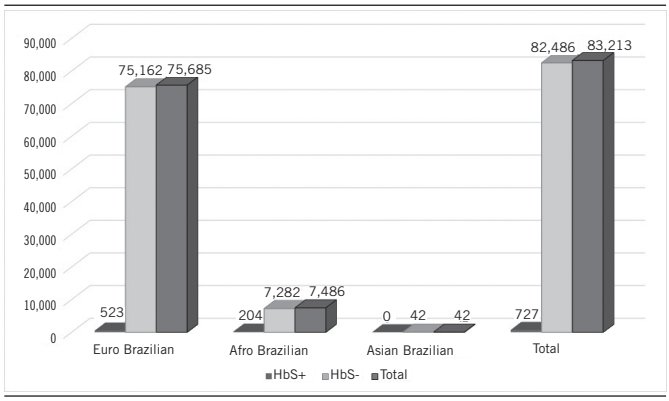

FIGURE 2 – *Distribution graph of HbS among blood donors, grouped according to ethnic group*
HbS+: positive hemoglobin S; HbS-: negative hemoglobin S.; HbS: hemoglobin S.

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, 5].

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).

TABLE – Prevalence of sickle cell trait and SCD in different Brazilian populations.

Region	Population	Trait	SCD	Year	Reference
Eastern Parana – Curitiba (PR)	83,213	727 (0.9%)	--------	2007-2008	Present study
Northwest Parana – Umuarama (PR)	585	15 (2.5%)	--------	2005-2006	Seixas *et al*. (2008)[13]
Distrito Federal (GO)	116,271	3,760 (3.2%)	109 (0.09%)	2004-2006	Diniz *et al*. (2009)[15]
Porto Velho (RO)	25,446	760 (3%)	9 (0.03%)	2003	Siqueira *et al*. (2009)[16]
Piauí (PI)	39	(3.9%)	--------	2007-2008	Soares *et al*. (2009)[17]
Rio Grande do Norte (RN)	320	8 (2.5%)	0	2004-2005	Lima *et al*. (2006)[18]
Paraná (PR)	548,810	8,355 (1.53%)	12 (0.002%)	2002-2004	Watanabe *et al*. (2008)[19]
Vitória (ES)	150	2 (1.33%)	---------	2006	Moraes & Depianti (2008)[20]
Rio de Janeiro (RJ)	99,260	4,663 (4.7%)	83 (0.08%)	2000-2001	Lobo *et al*. (2003)[21]
Sergipe (SE)	1,345	75 (5.6%)	--------	2004-2005	Vivas *et al*. (2006)[22]
Primavera do Oeste – Mato Grosso (MT)	2,708	64 (2.36)	--------	2005-2010	Da Silva *et al*. (2012)[23]
Tocantins (TO)	167	8 (4.8%)	0	2011	Souza *et al*. (2013)[24]

SCD: sickle cell disease.

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[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[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 carriers, since many external factors and habits (sports, altitude, sedentary lifestyle)[21] may be significant in the lives 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

1. Naoum PC. A origem das anemias hereditárias no Brasil. Rev Cienc Hoje. 1984; 3(4): 58-64.

2. Naoum PC. Interferentes eritrocitários e ambientais na anemia falciforme. Rev Bras Hematol Hemoter. 2000; 22(1): 5-22.

3. Galiza Neto GC, Pitombeira MS. Aspectos moleculares da anemia falciforme. J Bras Patol Med Lab. 2003; 39(1): 51-6.

4. Ministério da Saúde. Secretaria de Políticas de Saúde. Manual de doenças mais importantes, por razões étnicas, na população brasileira afro-descendente. Brasília: Ministério da Saúde; 2001.

5. Ramalho AS. As hemoglobinopatias hereditárias: um problema de saúde pública no Brasil. Ribeirão Preto: Ed. Soc. Bras. Genética; 1986.

6. Agência nacional de Vigilância Sanitária. Perfil do doador de sangue brasileiro. 2006.

7. Weatherall D, Hofman K, Rodgers G, Ruffin J, Hrynkow S. A case for developing North-South partnerships for research in sickle cell disease. Blood. 2005; 105(3): 921-3.

8. Bandeira FMCG, Leal MC, Souza RR, Furtado VC, Gomes YM. Diagnóstico da hemoglobina S: análise comparativa do teste de solubilidade com a eletroforese em pH alcalino e ácido no período neonatal. Rev Bras Materno Infantil. 2003; 3(3): 265-70.

9. Naoum PC, Bonini-Domingos CR. Técnicas laboratoriais para identificação das hemoglobinas normais e anormais. In: Naoum PC, editor. Hemoglobinopatias e talassemias. São Paulo: Savier Editora; 1997. p. 144-171.

10. World Health Organization. Sickle cell anemia. Report by the secretariat. Executive Board; 2005. 117 session.

11. Naoum PC, Bonini-Domingos CR. Doença falciforme no Brasil. Origem, genótipo, haplótipos e distibuição geográfica. J Bras Patol Med Lab. 1997; 33(3): 145-53.

12. Paiva RB, Ramalho AS, Cassorla RMS. A anemia falciforme como problema de saúde pública no Brasil. Rev Saude Publica. 1993; 27(1): 54-8.

13. Seixas FAV, Silva CD, Tominaga J, Ferro OC, Nilson LG. Incidence of hemoglobinopathies in Northwest Paraná, Brazil. Rev Bras Hematol Hemoter. 2008; 30(4): 287-91.

14. Ministério da Saúde. Manual de educação em saúde: linha de cuidado em doença falciforme. Brasília: Ministério da Saúde; 2009.

15. Diniz D, Guedes C. Confidencialidade, aconselhamento genético e saúde pública: um estudo de caso sobre o traço falciforme. Cad Saude Publica. 2005; 21(3): 747-55.

16. Siqueira B, Zanotti L, Nogueira A, Maia ACS. Incidência de anemia falciforme, traço falcêmico e perfil hemoglobínico dos casos diagnosticados na triagem neonatal no estado de Rondônia no ano de 2003. Saber Científico. 2009; 2(1): 43-53.

17. Soares LF, Oliveira EH, Lima IB, Bonini-Domingos CR. Hemoglobinas variantes em doadores de sangue do Centro de Hematologia e Hemoterapia do Estado do Piauí (Hemopi): conhecendo o perfil epidemiológico para construir a rede de assistência network. Rev Bras Hematol Hemoter. 2009; 31(6): 471-2.

18. Lima RCF, Castro EFP, Nobrega SM, et al. Triagem de hemoglobinas anormais em crianças e adolescentes. Newslab. 2006; 76: 130-40.

19. Watanabe AM, Pianovski MAD, Neto JZ, Chautard-Freire-Maia EA, Wittig EO. Prevalência da hemoglobina S no estado do Paraná, Brasil, obtida pela triagem neonatal. Cad Saude Publica. 2008; 24(5): 993-1000.

20. Moraes EA, Depianti JB. Triagem da hemoglobina S em pacientes atendidos pelo Marcos Daniel Laboratório em Vitória, ES. Rev Bras Hematol Hemoter. 2008; 30(2): 162-6.

21. Lobo CL, Bueno LM, Moura P, et al. Triagem neonatal para hemoglobinopatias no Rio de Janeiro, Brasil. Rev Panam Salud Publica. 2003; 13(2/3): 154-9.

22. Vivas WLP, Fabbro ALD, Cipolotti R, Hemoglobina S. Heterozigose para hemoglobinopatias em doadores de sangue do Centro de Hemoterapia de Sergipe. Rev Bras Hematol Hemoter. 2006; 28(4): 284-7.

23. Silva RA, Souza AVV, Mendes SDO, Luz GRP. Estudo genético--populacional da doença falciforme a partir de doadores de sangue em primavera do Leste-MT. Biodiversidade. 2012; 11(1): 108-14.

24. Souza LO, Teles AF, Oliveira RJ, et al. Triagem das hemoglobinas S e C e a influência das condições sociais na sua distribuição: um estudo em quatro comunidades quilombolas do Estado do Tocantins. Saude e Soc. 2013; 22(4): 1236-46.

25. Instituto Brasileiro de Geografia e Estatística. Censo Demográfico 2000 [Internet]. Available at: http://www.ibge.gov.br/.

26. Instituto Brasileiro de Geografia e Estatística. Censo Demográfico 2010 [Internet]. Available at: http://www.ibge.gov.br/.

27. Ramalho AS, Magna LA. Aconselhamento genético do paciente com doença falciforme. Rev Bras Hematol Hemoter. 2007; 29(3): 229-32.

28. Silva RB, de Paiva e Ramalho AS. Riscos e benefícios da triagem genética: o traço falciforme como modelo de estudo em uma população brasileira. Cad Saude Publica. 1997; 13(2): 285-94.

29. Prudêncio BCAB, Covas DT, Bonini-Domingos CR. Comparação de metodologia utilizada para a detecção de hemoglobina S (Hb S) em doadores de sangue. Rev Bras Hematol Hemoter. 2000; 22(2): 99-109.

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