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Article / Artigo

Sickle cell disease: a population genetics study based on blood donors in São José dos Campos, São Paulo, Brazil

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*Sickle cell disease is a genetic disease that originated in several different regions, in particular African communities. The disease is present in the Brazilian population. The large number of heterozygotes and the severe clinical symptoms of homozygotes have drawn special attention from the government institutions in Brazil. Since the genetic origin of the disease was elucidated, sickle cell disease has become the focus of an every-growing number of scientific investigations. This investigation was performed to correlate the presence of the sickle cell trait in inhabitants of São José dos Campos, Brazil, with data on immigrants. The study sample consisted of 93,604 blood donors of the Hematology and Hemotherapy Service in São José dos Campos from 2004 to 2008. An analysis of the donors identified 400 heterozygous individuals with the sickle cell trait (Hb S - 0.43%) but no homozygotes. The results are completely different from the national pattern and are strongly supported by local history. Rev. Bras. Hematol. Hemoter. 2010;**32**(4):286-290.*

Keywords: *Hemoglobins; blood donors; hemoglobin SC disease; public health.*

Introduction

First described in 1904 by James Herrick, sickle cell disease is the most common hereditary blood disease in the world.[1] Here in Brazil it was first described in the work of Jesse Accioly in Bahia in 1947.[2] The genetic etiology of this disease has an autosomal recessive pattern due to a point mutation (GAG => GTG) in the beta globin gene of hemoglobin (Hb), resulting in an abnormal hemoglobin called hemoglobin S (Hb S). This mutation leads to the substitution of glutamic acid by valine at position 6 of the beta chain and, consequently, the physical-chemical modification of the whole hemoglobin molecule.[3] Glutamic acid is negatively charged as opposed to valine which is neutral, thus there is a change in the charge of the Hb molecule which results in slower mobility of the Hb in electrophoresis when compared to Hb A.[4]

Due to its physical characteristics under specific conditions, Hb S can polymerize, causing a deformation of red blood cells that take on a sickle shape. Hb S is responsible for vaso-occlusion, and consequently painful episodes and organ damage. Individuals who are heterozygous for Hb S, represented as Hb AS, are asymptomatic. These individuals have no disease, nor do they have abnormalities in the number and shape of red blood cells and are usually only identified in routine checkups.[4] However, there are reports of sudden death and medical complications including hematuria, hyposthenuria, pulmonary embolism and splenic infarction especially when carriers are exposed to extremely low oxygen (O_2) tension conditions such as strenuous physical exertion and depressurization during flights.[5]

Originally from different Asian and African populations,[6] in particular, those from the African

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continent,[7] the mutation that gives rise to Hb S exists in Brazil. Due to a typical feature of the Brazilian population, racial mix, the frequency of mutant alleles is significantly high.[8,9] From 0.1% to 0.3% of the black population is affected by the disease; it is estimated that there are 2 and 10 million Hb S carriers.[10] In the southeast of Brazil, the estimated prevalence of sickle cell trait is 2% of the general population however it is 6% to 10% among blacks.[11] A population-based study in Minas Gerais reported an incidence of one new case of homozygous sickle cell disease in every 2800 births[12] and in Rio de Janeiro the reported incidence is of one new case in every 1196 live births.[10] Clinical manifestations begin in the first year of life. The symptoms vary and can include acute chest syndrome and bacterial infections which are the cause of considerable morbidity and mortality.[13] In Brazil, approximately 78.6% of deaths related to sickle cell disease occur before age 29 years old.[14]

Despite all the technological advances over the last two decades and the implantation of Decree 822/01 of the Ministry of Health on June 6, 2001, which established the National Neonatal Screening Program (PNTN) for sickle cell disease, there is no definitive cure for this genetic alteration. And thus this disease has been the subject of much discussion and planning strategies by the Ministry of Health and by state and municipal Health Departments.[15-17] Together, these organizations seek proposals to adequately treat patients, as well as to help them understand this genetic disease and to provide palliative care in order to reduce suffering. Preventive measures, including newborn screening, educating caregivers and patients, counseling on diet, immunization and prophylaxis with penicillin to prevent pneumococcal infection, and even the transplantation of hematopoietic stem cells contribute to reduce morbidity and mortality and improve quality of life.[18,19] Another aspect addressed by the government and defended by many healthcare professionals is genetic counseling, which, when performed within the proper ethical framework, enables individuals and their families to make informed and balanced decisions about childbearing.[20-22] This is, therefore, one primarily goal of care, which may or may not have preventive consequences.

A focus of scientific research since the elucidation of its genetic basis,[2] the study of sickle cell anemia can be considered an inexhaustible subject;[23] the considerable number of academic publications that deal with sickle cell anemia as the central theme is growing annually. In Brazil, there are numerous articles, abstracts and theses on the local problem of sickle cell disease. However, official data on the distribution of Hb S carriers is primarily divulged through healthcare programs controlled by municipal, state and federal governments with epidemiological studies of sickle cell disease being restricted to monthly-released data from the government healthcare system on authorization for hospitalization.[10] Within this context and to contribute to the understanding of sickle cell anemia, this study aims to analyze the genetic profile of the population living in the city of Sao Jose dos Campos, located in the state of Sao Paulo, in respect to the incidence of sickle cell anemia, using data from a blood bank in the city. An ethnic correlation of blood donors in respect to the migration process to the region is also presented.

Methods

This study was conducted using data on 93,604 voluntary blood donors from 2004 to 2008 at a Hematology and Hemotherapy Service in Sao Jose dos Campos, São Paulo. The ages of the donors ranged from 18 to 65 years. Venous blood samples (4.5 mL) were collected in vacutainers® using 1.5 mg/mL EDTA (ethylenediamine tetraacetic acid) as an anticoagulant.[24] Hematological data were determined using the HemoCue® Blood Hemoglobin analyzer. Initial screening was performed by cellulose acetate electrophoresis at 240V for 20 minutes at pH 8.6 with tris-borate hemolysate prepared from whole blood and 1.0% saponin. To confirm the results, electrophoresis using citrate agar gel at an acid pH was performed.[25] Based on data obtained from the hematology service, an analysis of the genetic profile of the city's population was performed employing the Hardy-Weinberg equilibrium.[26,27] This study was approved by the Research Ethics Committee of the Universidade do Vale do Paraiba, (Protocol # H125/CEP/2009).

Results

Of the 93,604 blood donors tested, 400 were carriers of the sickle cell trait (0.43%) but none had sickle cell disease. Of those with the sickle cell trait, 56%, 24% and 20% said they were white, black and mulatto, respectively. No individuals said they were Asians. Analysis of the data confirms the genetic nature of this anomaly, as the city of Sao Jose dos Campos was colonized by immigrants from different regions of the world during different stages of the city's historical-social development, but particularly by white Europeans.[28]

Based on the percentage of subjects who had the sickle cell trait among blood donors, the frequencies of the Hb A and Hb S alleles were calculated in relation to the population dynamics as postulated by the Hardy-Weinberg equilibrium theorem.[26,27] Between 2004 and 2008, the Hb A allele frequency was higher than that of the Hb S mutant (Table 1) in this population sample. In addition, only a small variation in allele frequencies was observed during the period in question, suggesting that no large migratory shifts, which may favor a high fluctuation of allele frequencies, took place in the region over the last twenty years.

Table 1. Hb A and Hb S allele frequencies in blood donors in São José dos Campos

Year	Donors (n)	Sickle cell trait carriers (n)	Hb A allele frequency	Hb S allele frequency
2004	18751	98	0.9974	0.0026
2005	18455	64	0.9983	0.0017
2006	18189	76	0.9979	0.0021
2007	19145	84	0.9978	0.0022
2008	19064	78	0.998	0.002

Considering the large number of blood donors analyzed and the percentage of Hb AS individuals (0.43%), the allele frequency of Hb S (0.0025) was calculated for this population. Furthermore, based on statistical simulations of the Brazilian Institute of Geography and Statistics (IBGE) on the population of São José dos Campos in 2009 (615,871 inhabitants – available at http://www.ibge.gov.br/home/estatistica/populacao/estimativa2009/estimativa.shtm), it was possible to calculate the probable number of individuals with sickle cell anemia (Hb SS) in this population in 2009, that is, 3.8 individuals. The low rate of sickle cell disease in this population shows why sickle cell anemia is not considered a local public health issue unlike much of southeastern Brazil and other regions of the country.[9]

Discussion and Conclusion

Located in the central region of Vale do Paraíba, the city of Sao Jose dos Campos is the seventh largest city in the state of Sao Paulo, with a solid economy due to local industrial and aerospace development (http://en.wikipedia.org/wiki/S%C3%A3o_Jos%C3%A9_dos_Campos). The region was originally settled by Jesuit missionaries and subsequently governed within the hereditary captaincy system and so descendants of white Europeans have always been important in the region. A significant presence of black Africans is noted in the region only in the second half of the nineteenth century with the expansion of coffee and cotton plantations and the abolition of slavery; but few of these migrants settled. Several administrative measures were adopted in an attempt to 'beautify' the city during its history with oppression of the poor, sick and black ex-slaves [29] who gradually distanced themselves from the city.

According to the census of 2000, 78.6% of residents in the city of Sao Jose dos Campos were white (http://en.wikipedia.org/wiki/S%C3%A3o_Jos%C3%A9_dos_Campos), many individuals reject the classification of Black or African descent when discussing ethnical background.[7] Even so, the findings of this study confirm the influence of the historical setting on population dynamics and the genetic profile of the population. Although sickle cell anemia can not be considered a local public health problem because of the low allele frequencies of Hb S estimated in the population of São José dos Campos, the contemporary Brazilian society has the clear characteristic of racial mixing [7,30,31,32] with the flow of genes between individuals of different ethnic backgrounds within the population. Thus, over the years, the percentage of individuals with sickle cell disease may change in the local population. Due to this pattern of racial mixing, heterozygotes need to be aware of their genetic condition and the possibility that they will have children with sickle cell disease and all the implications linked to this. Hence, public healthcare policies should be educative and effective.

Comparatively, the percentage of sickle cell trait carriers (0.43%) in Sao Jose dos Campos is close to the results for some other cities in the state of São Paulo[33,36] but differs significantly to rates reported for other states that had high influxes of black Africans, including Bahia[35] and Rio Grande do Norte,[36] in the northeast of the country and Rio de Janeiro[37] and Minas Gerais[38] in the southeast (Table 2). This reinforces the ethnic character of sickle cell disease and its correlation to the history of Brazilian colonization. However, the lack of data about the slave trade to Brazil and the total number of immigrants who entered the country is hindering a more accurate genetic analysis about the genetics of populations and historical correlations. Even in the local colonization that occurred in Sao Jose dos Campos, inconsistent data exist on the main migratory routes of the region. More sophisticated and hence more time consuming studies, as in the case of the investigation of haplotypes of the population, are identifying some of the gaps of migration records in Brazil.[39] It is expected that soon, this kind of analysis will improve our understanding of the genetic structure of the population resulting from the local history and thereby assist in the diagnosis, prevention and treatment of ethnically-related genetic diseases.

Table 2. Distribution of sickle cell trait carriers[5] in different regions of Brazil

	Sao Paulo[33]	Sao Paulo[34]	Bahia[35]	Rio Grande do Norte[36]	Rio de Janeiro[37]	Minas Gerais[38]
Hb AS	0.6	0.76	9.81	2.22	3.24	2.48

Resumo

A anemia falciforme é uma doença genética com origem multicêntrica, predominantemente em comunidades africanas, e está presente na população brasileira. A alta frequência de heterozigotos e a gravidade clínica dos homozigotos em nossa população vêm sendo alvo de políticas públicas adotadas pelo Ministério da Saúde e outras instituições governamentais no intuito de dispender cuidado especial ao portador. Foco das investigações científicas desde o

*esclarecimento de sua base genética, o estudo da anemia falciforme pode ser considerado um tema inesgotável, visto o considerável número de publicações acadêmicas que se reportam à anemia falciforme como temática central e que crescem anualmente. Dentro desse contexto, e no intuito de contribuir com a informação científica sobre a anemia falciforme, o presente estudo tem por objetivo avaliar a comunidade de São José dos Campos, SP, quanto à presença do traço falcêmico, correlacionando os dados obtidos com o histórico do povoamento local. O estudo foi realizado em 93.604 doadores voluntários de sangue, entre os anos de 2004 e 2008 no Serviço de Hematologia e Hemoterapia de São José dos Campos. Dos 93.604 doadores analisados, encontramos 400 portadores heterozigotos do traço falcêmico Hb S (0,43%); nenhum indivíduo homozigoto foi identificado. Esse índice discrepante dos índices nacionais fundamenta-se na história local. Rev. Bras. Hematol. Hemoter. 2010; **32**(4):286-290.*

Palavras-chave: *Hemoglobinas; doadores de sangue; doença da Hemoglobina SC ; saúde pública.*

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