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Hemoglobinopathies: peripheral blood analysis from academics of a university in Alfenas - MG

Hemoglobinopatias: investigação em sangue periférico de acadêmicos de uma universidade de Alfenas - MG

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ABSTRACT: Human hemoglobin (Hb) are globular tetramers formed by a combination of two "type α" polypeptide chains with two "type β" chains, the main types of hemoglobin in a healthy adult are Hb A1, Hb A2, and Fetal Hb. Hemoglobinopathies are diseases with mutations affecting globin genes, resulting in structural changes and/or hemoglobin molecular function changes. Hemoglobin S and C are the most frequent to vary among hemoglobin among Brazilians. The goal is to detect abnormal hemoglobin in an academic population of Alfenas-MG. We collected 336 samples of peripheral blood to carry out the research study, which was hemolyzed using saponin and chloroform. Next, the electrophoresis of hemoglobin in alkali pH was performed for qualifying normal and the majority of abnormal hemoglobin. The confirmation of hemoglobin S was conducted by the sickle cell test. 97.62% (n=328) were compatible with hemoglobin AA, and 2.38% (n=8) were consistent with hemoglobin AS (sickle cell trait) from the total analyzed samples. The average prevalence of Hb AS is close to 2% of the entire Brazilian population. Therefore, the results found in the present study confirm those described in Brazilians.

Keywords: Hemoglobinopathies; Hemoglobin S; Sickle cell anemia.

RESUMO: As hemoglobinas (Hb) humanas são tetrâmeros globulares formados pela combinação de duas cadeias polipeptídicas do "tipo α" com duas cadeias do "tipo β", sendo Hb A1, Hb A2 e Hb Fetal os três principais tipos de hemoglobinas no adulto normal. As hemoglobinopatias são doenças causadas por mutações que afetam os genes de globinas, que resultam em alterações estruturais e/ou funcionais das moléculas de hemoglobina. Dentre as hemoglobinas variantes, as mais frequentes na população brasileira são a hemoglobina S e C. O objetivo é detectar hemoglobinas anormais em uma população acadêmica de Alfenas-MG. Para a realização do estudo, foram coletadas 336 amostras de sangue periférico, hemolisadas com saponina e clorofórmio. Em seguida, foi realizada a eletroforese de hemoglobina em pH alcalino para qualificação de hemoglobinas normais e grande parte das anormais. A confirmação da hemoglobina S foi feita pela prova de falcização. Do total de amostras analisadas, 97,62% (n=328) apresentam perfil eletroforético compatível com hemoglobinas AA e 2,38% (n=8) compatível com hemoglobinas AS (traço falciforme). No Brasil, a prevalência média de Hb AS é próxima de 2% na população total. Portanto, os resultados encontrados reafirmam a média encontrada na população brasileira de pessoas com o traço falciforme.

Descritores: Hemoglobinopatias; Hemoglobina S; Anemia falciforme.

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INTRODUCTION

HbA (α2β2) is the primary hemoglobin in a healthy adult in small quantities of HbA2 (α2δ2, <2.5% -3.0%) and HbF (α2γ2, 1% -2%)[1].

Hemoglobinopathies are characterized by the abnormal synthesis of polypeptide chains of the globin constituting different types of hemoglobin of the red globules. There are two primary defects, namely reduced synthesis of the affected globin chain(s) (thalassemias) and structural variations of the hemoglobin (for example, sickle cell syndromes)[1].

The most frequent abnormal variants of hemoglobin in the Brazilian population are S (HbS) and C (HbC) hemoglobin; both originated from Africa, thereby showing the intense participation of Negro traits in the composition of the Brazilian population[2].

Hemoglobinopathies are considered public health problems in many countries, including Brazil, due to their genetic diversity and clinical importance. They are inserted in neonatal screening programs aiming at improving the quality of life of patients through early diagnosis by way of implementation of prophylactic measures and adequate therapeutic procedures[3].

Sickle cell disease is defined as a genetically-inherited hemoglobinopathy characterized by the predominance of the hemoglobin S (HbS) in red blood cells. They undergo alterations in their physical-chemical properties when deoxygenated, resulting in changing the configuration of the red-blood-cell shape into a "sickle" shape[4,5].

Sickle-cell anemia disease is caused by a change between the amino acids (A – T) in the β gene – adult globin, causing a valine to be substituted by glutamic acid in the sixth position of the β-globin chain. The dimerization of these mutant β-globin chains with α-globin result in the formation of HbS (α2β2S). Homozygous (HbS) patients are the most severe form of sickle cell disease, as the mutant β-globin chains polymerize in deoxygenated red blood cells. That makes them become misshapen and change into a biconcave morphology as a hook/sickle shape. Repetitive cycles of sickling make the HbS molecules change from oxygenated to deoxygenated states, causing fragility of the red blood cells and promoting vaso-occlusion, painful crises, chronic anemia, acute chest syndrome, organ insufficiency, stroke, and eventually death[6].

Sickle cell anemia results in the presence of hemoglobin S responsible for many deaths among children and adults, varying among geographic regions in Brazil. That depends a great deal on the quality of the treatment provided to children. Sickle cell disease kills more children from ages 1 to 5 years old, decreasing after 5 years old to adolescence and then increasing after twenty-years-old. More Negro and mixed-race children die from sickle cell disease than white children. The Ministry of Health estimates 14 people die from undefined *Causa Mortis* in every 100 people. The data from the Ministry of Health reveal that there are hindrances in getting access to healthcare services and insufficiency in preventive diagnosis for genetic diseases, thereby contributing to an even higher incidence in the country[7].

Studies have demonstrated where there has been neonatal screening instituted for hemoglobinopathies and supplying follow-up to patients in specialized centers. Thereby, the overall mortality of these children has been reduced from 80% to 1.8% in those countries[8].

Thus, this study has sought to characterize the hemoglobin profile of a student population in Alfenas - MG and, more specifically, detect the occurrence of abnormal hemoglobin. That is to supply guidance to heterozygote carriers on variant hemoglobin regarding the need for seeking genetic counseling, if they wish to have a child, as there is the possibility of bearing a homozygote child, who may suffer from a severe disease.

MATERIALS AND METHODS

The present study was performed on a population of 336 students, both male and female, independent of race, socioeconomic condition, the ages ranged from 21 to 45 years old, and they were previously counseled on their participation in the research study and signed an informed consent form (ICF).

A skilled professional performed a blood sample collection (3mL), through a venous puncture, utilizing silicon hoses containing the anticoagulant EDTA in a proportion of 1 mg/mL of blood. The collected samples were processed, obtaining hemolyzed blood samples in saponin.

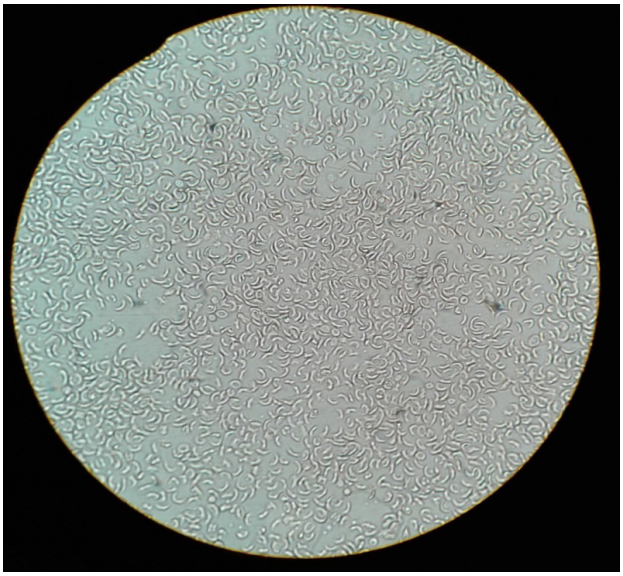
Following that, the samples were submitted to laboratory analysis through hemoglobin electrophoresis in cellulose acetate in an alkaline plug, pH 8,6. The sickling test confirmed the presence of hemoglobin S[9,10,11].

The Committee for Ethics in Research on Human Beings approved the study at UNIFENAS-Alfenas document number registered as 2.746.378.

RESULTS

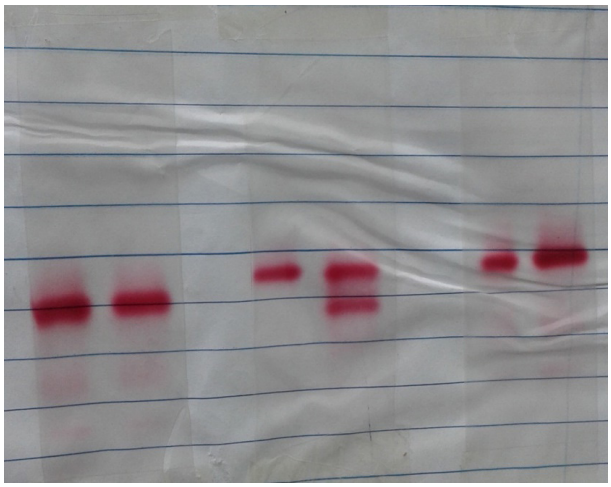
Among the total of 336 samples analyzed in this study, 97.61% (n = 328) presented an electrophoretic profile compatible with types and quantities of hemoglobin considered normal, denominated as AA; 2.38% (n = 8). It showed an electrophoretic pattern consistent with hemoglobin AS. These individuals are carriers of the sickle cell trait and, then to confirm the AS diagnosis, a sickling test was performed (Figure 1), making it possible to visualize drepanocytes (sickle cells), coherent with the preliminary result on hemoglobin electrophoresis, thereby confirming, in its particular cases, the presence of HbS in

specified samples (Figure 2).



Source: Hematology Laboratory, Unifenas, Alfenas.

Figure 1 – Positive Sickling Test utilizing 5% of sodium metabisulfite (x400)



Source: Hematology Laboratory, UNIFENAS.

Figure 2 – Alkaline electrophoresis of hemoglobin in agarose gel. Differentiation of electrophoresis mobility of hemoglobin

DISCUSSION

Among variant hemoglobin (abnormal hemoglobin), with multidisciplinary interest, variant hemoglobin S and C were highlighted. Those two types of abnormal hemoglobin present pathophysiological alterations whose repercussion level is dependent on the constitution of the genotype in these hemoglobin[12].

There is an estimate of around 7 million people who are carriers of some hemoglobin abnormality in Brazil. The most frequent is the sickle cell trait and the hemoglobinopathy C trait that generally are asymptomatic. The breeding of carriers of these defects can originate from the birth of children who suffer from severe anemia, and quite often, blood transfusions are necessary from birth and for the rest of their lives, bringing about cardiac, renal, and hepatic diseases[13]. The purpose of this study has been to determine the prevalence of these variations in the hemoglobin. To provide improved guidance to carriers, to make genetic counseling possible due to the high incidence of abnormalities in hemoglobin and the seriousness of the breeding of these individuals.

In a study conducted by Santiago et al.[14] on volunteers throughout all "Quilombola" communities in Brazil revealed a specific distribution of variants of Hb in all the investigated communities. The hemoglobin profiles among the studied communities showed that 91.45% of the studied population presented (normal) hemoglobin HbAA; 2.63% HbAC; 0.07% HbCC; 4.95% HbAS (sickle cell trait), and 0.31% HbSS (sickle cell disease). The distribution of the variant hemoglobin is related to the colonization process of each region. Due to the typical makeup of the Brazilian population, it can be considered that Africans contributed to the emergence of the main types of hemoglobinopathies. The distribution of variants of Hb was heterogeneous among all the Brazilian states, and Bahia displayed the highest incidence and prevalence of sickle cells, diseases, and other variants of Hb.

Sickle cell anemia is the most prevalent hereditary disease in Brazil, affecting from 0.1 to 0.3% of the population (predominantly Negro), with the trend to increasingly impact more significant portions of Brazilians, arising from the high degree of miscegenation present in this country. Population studies have demonstrated an increasing presence of hemoglobin S in white individuals[15]. The average prevalence of Hb AS is around 2% of the total population. However, when considering only black people, the average incidence is about 5%, including substantial regional differences. For example, in the states of Bahia and Rio de Janeiro, the prevalence is over 5%. In contrast, in the states of Paraná and Santa Catarina, it is lower than 2%[12]. Estimates indicate that 5 to 6% of the population carry the Hemoglobina S gene (HbS) and the incidence is around 700 - 1000 new cases each year[16]. Among white people, the average prevalence of Hb AS varies from 0.5 to 1.3%, thereby evidencing the miscegenation representation of the Brazilian population[17].

Araújo[18], for example, concluded that among the 1,940 blood samples from the umbilical cord of newborns arising from three public maternity hospitals in the city of Natal, the sickle cell trait (genotype AS) was the most prevalent. That represents 1.5% (29 newborns), compared to sickle cell anemia (genotype SS) that corresponded to

0.05% (1 newborn).

It is estimated that there are around two million carriers of the Hb S gene in Brazil and from 25 to 50 thousand people have the homozygotic form (Hb SS) denominated as sickle cell disease based on the data from the National Neonatal Screening Program (NNSP) [19].

Cançado and Jesus[20] researched data from the Ministry of Health, enabling quantitative analysis of the distribution of the HbS gene in Brazil. There is a higher concentration of heterozygotic individuals (AS) based on the analysis of the frequency of the S gene in different regions of the country, especially where there was accentuated traffic of African slaves.

Martinset al.[21], in a study on 151 patients, demonstrated the morbimortality of sickle cell anemia. Among these, the average age was 17.7 years old, and 52.4% were female. There were 11 deaths, with an average age of 33.5 years old, only one was under 10 years old, and the most frequent cause was the insufficiency of multiple organs. That study showed a shorter life expectancy for carriers of the sickle cell disease.

Felix et al.[22] in their research, reports epidemiological and social aspects of sickle cell disease (SCD) on a study on 47 sickle cell disease patients. Their ages were equal to or over 18 years old, 78.7% are black, 17% mixed race, and 4.3% white, the highest prevalence, is among the female gender (59.6%). Sickle cell disease reflects negatively on occupational activities, and the quality of life of patients was confirmed in this study.

According to the Ministry of Health[23], in Brazil, due to the large contingent of African population uprooted from their origins for enslaved work, the sickle cell disease is part of a group of diseases and relevant problems affecting the black population. For that reason, SCD was included in the initiatives of the National Policy of Integral Attention to the Health of the Negro Population and in articles 187/188 of the MS/GM Decree # 2,048, dated September 3, 2009, that regulates SUS (Brazil's Universal Healthcare System). According to the State Neonatal Screening Programs, Brazilian states have displayed a higher incidence of born-alive babies diagnosed with the disease, and sickle cell traits were in the states of Bahia and Rio de Janeiro, followed by Pernambuco and Maranhão.

The Ministry of Health, in March 2018, expanded the age range for recommending bone marrow transplant for the treatment of sickle cell disease – a genetic disease mainly affecting the Negro population. Now, age is no longer a restrictive criterion for this transplant, which is the only method for curing the disease. In 2015, 1,145 new cases of the disease were diagnosed in the SUS Neonatal Screening Program[24].

Since the genetic trait for hemoglobinopathy determination can be silent, but it manifests in the subsequent generation as homozygosis, thereby making awareness essential to the heterozygote population of Hb S and other hemoglobinopathies. That is related to the risks of having a child stricken by these severe diseases and the implications regarding its health, family, and society at large.

Hemoglobin S presents physiopathological alterations whose degrees of repercussion to the carrier are dependent on the constitution of the genotype. The diagnosis of the pathology can be performed from a prenatal to an adult age with good anamnesis of the patient, verifying the contribution from the family through the presence of heterozygosis in the genitors[25].

CONCLUSION

The results from this study make it possible to conclude that the incidence of variant S hemoglobin from a heterozygote inheritance, characterized by the sickle cell trait present in approximately 2% of the university population in Alfenas, MG, similar to what is found in the entire Brazilian population.

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