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Frequency of red blood cell genotypes in multi-transfused patients and blood donors from Minas Gerais, Southeast Brazil

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ABSTRACT

Background and objectives: The frequency of red blood cell (RBC) antigens in Brazil varies due to differences in the ethnic groups in different regions; however, these studies have not been performed in Minas Gerais, where African admixture is more prevalent in comparison with other states. Due to these facts, this study aimed to determine the frequency of RBC genotypes on Rh, Kell, Duffy and Kidd systems in blood donors and multi-transfused patients from Minas Gerais, Southeast Brazil.
Methods: Blood samples were collected from 170 donors and 117 patients with different diagnosis and at least three RBC transfusions. DNA was extracted from leukocytes and genotyped by PCR-SSP, Multiplex or RFLP to alleles of the referred systems. The results were compared by the Chi-Square test, with a significance level of 5%.
Results: The most frequent genotypes were: *RHD+, RHCE*ce/RHCE*ce, KEL*2/KEL*2, FY*B-67T/FY*B-67T* and *JK*A/JK*B. FY*B-67C/FY*B-67C, RHD*Ψ* and *JK*A/JK*A* genotypes were more prevalent in sickle cell disease (SCD) patients than in donors. Many differences in RBC genotype frequencies were observed in comparison with studies from other states and countries.
Conclusion: The results reinforce the importance of determining RBC genotypes of blood donors and patients in different regions of Brazil and the world, improving the transfusion safety of individuals requiring chronic RBC transfusions, especially those with SCD, due to ethnic differences in relation to donors.

1. Introduction

Red blood cell (RBC) antigens are structures that may induce an immune response, with risk of hemolytic transfusion reactions [1–4]. Their discovery, since the first half of the 20th century, was considered one of the most important advances in medicine [5]. Currently, more than 300 antigens were discovered and organized in 36 blood group systems, being ABO, Rh, Kell, Duffy, Kidd and MNS the most clinically relevant [1]. Their frequencies vary as for the ethnicity [1,6–10] and, due to the considerable differences with relation to the ethnic groups in the Brazilian states, the frequency of these antigens is also quite variable in the country [11].

According to the last census performed in Brazil (2010), the percentages of Caucasians, Browns and Blacks in São Paulo, Paraná, Santa Catarina and Bahia are, respectively, 63.91%, 29.11% and 5.52%; 70.32%, 25.09% and 3.17%; 83.97%, 12.41% and 2.94%; 22.19%, 59.16% and 17.10% [12]. In Minas Gerais, these percentages are 45.39% of Caucasians, 44.28% of Browns and 9.22% of Blacks [12] and despite the difference of the ethnic profile in relation to the other localities, no study on frequency of RBC genotypes has been performed although these frequencies have been reported out in São Paulo [13–15], Paraná [16,17], Santa Catarina [11] and Bahia [18].

This fact, associated with the importance of RBC genotyping to reduce the risks of alloimmunization and hemolytic transfusion reactions in patients receiving regular and chronic RBC transfusions, let us to search and determine the frequency of RBC genotypes on Rh, Kell, Duffy and Kidd systems in a population of blood donors and multi-transfused patients from Minas Gerais,

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Southeast Brazil and compare the genotype frequencies between donors and patients and with those found in other Brazilian regions and countries.

2. Materials and methods

2.1. Study subjects

A total of 170 blood donors matched for gender and with a median of six previous donations (1-80) at the Hemocentro Regional de Uberaba (HRU)/Fundação Hemominas and 117 patients, 63 with sickle cell disease (SCD), 30 with oncohematological diseases, 15 with chronic renal failure (CRF), four with aplastic anemia, three with beta-major thalassemia and two with erythroid aplasia who received at least three RBC transfusions at the HRU and/or Hospital de Clínicas/Universidade Federal do Triângulo Mineiro (HC/UFTM) were included in the study after signing an informed consent.

2.2. Ethical principles

The study was performed in accordance with the Declaration of Helsinki for experiments involving humans and was approved by the Research Ethics Committees of UFTM (2226) and Fundação Hemominas (341).

2.3. Patient and donor informations

Clinical and epidemiological data were collected through informations given for each donor or patient and from the archives and medical records of the HRU and/or HC/UFTM. To identify the ethnicity, the criteria used by IBGE (Brazilian Institute of Geography and Statistics) were adopted, which classifies the individuals in Whites (Caucasians), Blacks (Negroes), Browns ("Pardos"; mixed people, neither Caucasians nor Blacks), Orientals and Indigenous, according to a questionnaire applied to them [19]; a method considered effective, since 96% of the responders self-classified properly [20]. In addition, we compared the informed classification with that observed through the morphological characteristics (skin color, skull and face format, type of hair etc.). The ethnicity of the ancestors (parents and grandparents) was also collected through informations given by each individual.

2.4. Samples and DNA preparation

Ten milliliters of blood samples of each donor and patient were collected in EDTA. DNA was extracted from leukocytes by FlexiGene kit (Qiagen), following the manufacture's recommendations.

2.5. RBC genotyping

RBC genotyping was performed by PCR (Polymerase Chain Reaction) assays previouslypublished [16,17,21]. Analysis of the *RHD*Ψ* (RHD Pseudogene) allele was performed by PCR-SSP (Sequence-specific primers) while for *RHD and RHCE*C/c* genotyping we used a PCR-Multiplex. *RHCE*E/e, KEL*1/2, FY*A/B, GATA-67t/c and JK*A/B* alleles were determined by PCR-RFLP (Restriction Fragment Length Polymorphism). Briefly, PCR was performed with 100–200 ng of DNA, 50 pmols of each primer, 2 nmols of each dNTP, 1.0 U Taq DNA polymerase and buffer in a final volume of 30 to 50 μL (microliters). Amplified products were analyzed by electrophoresis in 1.5% agarose gel in Tris-Borate EDTA buffer (TBE). To differentiate *RHCE*E/e, KEL*1/2, FY*A/B, GATA-67t/c and JK*A/B* alleles, PCR amplified products were digested overnight with the appropriate restriction enzymes (MnlI, BsmI, BanI and StyI – New England Biolabs, Ipswich, MA, USA), in a final volume of 20 μL (microliters) using 10 μL (microliters) of amplified product, according to enzyme manufacturer's instructions. The RFLP analyses were performed after electrophoresis in agarose in TBE.

2.6. Statistical analysis

Categorical variables were expressed as absolute numbers and proportions. The chi-square (χ^2) test of homogeneity was used to determine the difference between RBC genotype frequencies of groups/populations, using SigmaStat 3.5 (Systat Software Inc. – San Jose, CA, USA). A p value less than 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the population studied

Of the 170 donors, 50% were men and 50% were women; of the 117 patients, the percentages were, respectively, 47.01% and 52.99%. As for the ethnicity of the donors, 42.99% were Caucasians, 41.18% Browns and 15.88% Blacks; while in the patients, these percentages were, respectively: 27.35%, 43.59% and 29.06%. Median age of the donors was 34 (18–64) and of the patients was 20 (2–84).

3.2. RBC genotype frequencies in the population studied

In donors and patients, the most frequent RBC genotypes for each system were: *RHD+*, *RHD*Ψ* neg., *RHCE*ce/RHCE*ce* (Rh); *KEL*2/KEL*2* (Kell); *FY*B/FY*B*, *GATA-67t/t* (Duffy) and *JK*A/JK*B* (Kidd). Statistical significant difference between the groups was observed only in GATA mutation, with a higher prevalence of *-67c/c* genotype in the patients. However, considering only those with SCD, significant differences also occurred with relation to *RHD*Ψ* and *JK*A/JK*A* genotypes, while *GATA-67t/t* and *FY*A/FY*B* were more frequent in donors (Table 1).

As for the gender, a statistical difference was observed only for the *GATA-67c/c* genotype in the donors, with a higher frequency in men (Table 2).

Considering the ethnicity, among all the 287 individuals, *RHD-*, *FY*A/FY*A*, *GATA-67t/t* and *JK*B/JK*B* genotypes were more prevalent in Caucasians, *RHCE*CC* and *FY*A-67T/FY*B-67C* in Browns and *RHD+*, *RHCE*cc*, *FY*B-67C/FY*B-67C* and *JK*A/JK*A* in Blacks (Table 3).

3.3. Comparison of RBC genotype frequencies found in this study with other Brazilian studies

Among the donors, we observed higher frequencies of *RHCE*cc*, *KEL*2/KEL*2*, *GATA-67t/c* and *JK*A/JK*A* genotypes and lower of *RHCE*Cc*, *KEL*1/KEL*2*, *GATA-67t/t* and *JK*A/JK*B* genotypes compared to studies performed in São Paulo [14,15]. We also verified that *GATA-67t/c* and *GATA-67c/c* were more prevalent in comparison to Paraná [16]; a higher frequency of *FY*B/FY*B*, *GATA-67t/c* and *GATA-67c/c* in relation to Santa Catarina [11] and lower of *GATA-67c/c* compared to Bahia [18]. In all these cases, the differences were significant. In addition, the *JK*A/JK*A* genotype was more prevalent in our study and *JK*B/JK*B* in Paraná [16], while *FY*B/FY*B* was more frequent in relation to São Paulo [14]; and in these comparisons, there was a trend of statistical significance.

As for the patients, comparing our RBC genotype frequencies to those found in other states, *GATA-67c/c* was more frequent in the present study and *GATA-67t/t* in Paraná [17]; while *RHCE*cc* and *GATA-67c/c* were more prevalent in our study and *GATA-67t/t* in Santa Catarina [11]. In all these cases, there were statistically significant differences.

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