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Profile of Rh, Kell, Duffy, Kidd, and Diego blood group systems among blood donors in the Southwest region of the Paraná state, Southern Brazil

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ABSTRACT

The aim of this study was to assess the distribution of alleles and genotypes of the blood group systems Rh, Kell, Duffy, Kidd, and Diego in 251 regular blood donors registered in the hemotherapy unit of the Southwestern region of Paraná, Southern Brazil. The frequencies were obtained by direct counting on a spreadsheet program and statistical analyses were conducted in order to compare them with other Brazilian populations using chi-squared with Yates correction on OpenEpi software. The frequencies of *RHD** negative, *RHCE*c/c* and *RHCE*e/e* were higher than expected for the Caucasian population. A difference was also observed for *FY* alleles, *FY*01/FY*01* genotype and *FY*02N.01* -67T/C (GATA Box mutation). Two homozygous individuals were defined as a low frequency phenotype K + k– (*KEL*01.01/KEL*01.01*) and, for Diego blood group system the rare *DI*01* allele was found in ten blood donors, of which one was *DI*01/DI*01* (0.4%). The allele and genotype frequencies of Kidd blood group system were similar to expected to Caucasians. The results showed the direction in which to choose donors, the importance of extended genotyping in adequate blood screening and the existence of rare genotypes in Brazilian regular blood donors.

1. Introduction

Blood transfusion saves lives and improves health, and better selection of blood donors is an important aim of current measures in reducing risks for recipients [1]. The indications for blood transfusions are based on clinical and laboratory parameters, aimed to benefit the patient [2]. Although the safety of blood transfusion has improved dramatically over the past few decades, there are still residual risks of alloimmunization to red blood cell (RBC) antigens [3]. Alloantibodies complicate transfusion therapy, causing immediate or delayed hemolytic transfusion reactions, with an incidence of around one per 7000 RBC units transfused [4].

Currently 35 systems of erythrocyte blood groups have been described according to the International Society of Blood Transfusion (ISBT) (http://www.isbtweb.org). The correct determination of the blood group in hemotherapy is important not only to prevent problems of incompatible transfusions but also to allow better use of blood product units with less frequent phenotypes.

The clinical significance of RBC antigens depends on the incidence of the antigen, which may vary in different ethnic groups [5], on its immunogenicity and on specific clinical

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situations [6,7]. The occurrence of alloantibodies in multitransfused patients stimulated several researchers to determine the frequency of alloimmunization in different populations, taking into account the racial differences among them, diagnosis, age, and risk factors that lead to this alloimmunization [7–9]. The most implicated antibodies in delayed hemolytic transfusion reactions are directed against antigens D, K, E, Fy^a and Jk^a [10].

Because establishing a database of regular blood donor genotype is highly desirable, the aim of this study was to assess the distribution of alleles and genotypes of Rh, Kell, Duffy, Kidd, and Diego blood group systems in regular volunteer blood donors registered in the hemotherapy unit of the Southwestern region of Paraná, Southern Brazil.

2. Methods

2.1. Sample selection

The study was carried out in accordance with the standards recommended by the Ethics Committee on Human Research of the Maringa State University (581/2011), and all subjects provided a written consent in order to participate in the study.

The population included 251 regular blood donors coming from the Francisco Beltrão Blood Center, Southwestern region of the state of Paraná, Southern Brazil, from September 2012 to July 2013. These individuals were voluntary blood donors who present themselves spontaneously to the center of blood donation and are considered loyal donors who hold three blood donations in twelve months. The State law 13964/02 provides cultural benefits to such donors, enabling control of the loyalty of blood donation. This is not a population study and exclusion criteria of samples were not defined and applied (except for those excluded from donating blood – Law 7649/1988 [11] MoH Ordinance 2712/2013); however, each donor was analyzed once. About 15% of blood donors were excluded for health reasons. Blood donor age range went from 18 to 64 years and included all ethnic groups.

2.2. Serologic tests

Red blood cell phenotyping for ABO and Rh was performed by conventional tube technique, and Kell, Duffy and Kidd blood group system phenotyping was performed in a gel card according to the manufacturer's procedure (DIAMED Latino América, SP, Brazil). Red blood cells were not tested with anti-Di^a and anti-Di^b.

ABO and RhD blood grouping were performed by conventional tube technique as per our standard operating procedure using monoclonal reagents: anti-A, anti-B, and anti-D (DIAMED/BioRad Latino América S.A., Lagoa Santa-MG, Brazil). Testing for the presence of weak D phenotype was done for all donors typed as negative D. The RhD typing was performed by hemagglutination in a semi-automated microplate (DIAMED/BioRad Latino América S.A., Lagoa Santa-MG, Brazil) using a monoclonal IgM DVI- (TH28) and anti-DVI+ (MS201/MS26 – IgM/IgG). Samples with negative results or ≤2 + were investigated for weak D using anti-D polyclonal Human and LISS/Coombs cards and anti-D monoclonal IgG (MS26), IgM (TH28) and DVI+. RBCs were also Rh phenotyped using monoclonal antibodies against C, c, E and e (MS24, MS201, MS26 and MS80). All reagents were from DIAMED Latino América S.A., Lagoa Santa-MG, Brazil. For red blood cell antigen typing for Kell, Duffy and Kidd blood group systems, red cells from each donor sample were prepared as 0.5% suspension and was performed by column agglutination methodology gel test with polyclonal or monoclonal antibodies (DIAMED/BioRad Latino América S.A., Lagoa Santa-MG, Brazil). The procedure for above antigen typing was done as per the manufacturer's instructions.

2.3. Sample collection and DNA extraction

To extract the DNA, the buffy coat was obtained from 4 mL of peripheral blood collected in EDTA by centrifugation (210 *g* for 15 min). The DNA was extracted using the salting-out method [12] with some modifications previously described in the literature [13]. The concentration and quality of the DNA were analyzed by optical density in a Thermo Scientific Nanodrop 2000® apparatus (Wilmington, USA).

2.4. Genotyping of RHD, RHCE, KEL, FY, SLC14A1 and DI genes

Genotyping of alleles of the Rh blood system was performed according to the protocol previously described in the literature. For the presence of the *RHD* gene and the *RHCE*C/c* alleles, the amplification of the multiplex-PCR (polymerase chain reaction) technique was used [14]. The AS-PCR (allele-specific polymerase chain reaction) technique was used to detect the *RHD*pseudogene* (*RHD*ψ, RHD*04N.01*), a region of 37-bp duplication in the *RHD* gene [15]. For both reactions we used 50 ng of DNA, 50 pmol of each primer, 2 nmol of each dNTP, 0.5 U of Taq DNA polymerase (Invitrogen Life Technologies®, Grand Island, NY, USA) and buffer to make up a final volume of 25 μL. The multiplex-PCR amplification cycles were performed in a System 9700 PCR thermocycler (Applied Biosystems®, Foster City, CA, USA) consisting of: denaturation at 95 °C for 15 minutes and 30 cycles of one minute at 94 °C, one minute at 65 °C and 3.5 minutes at 72 °C followed by a ten minute span cycle at 72 °C. For AS-PCR, the amplification cycles consisted of: denaturation at 95 °C for five minutes and 28 cycles of one minute at 94 °C, one minute at 60 °C and one minute at 72 °C, followed by a seven minute span cycle at 72 °C. The analysis of the obtained PCR products was performed right after electrophoresis in 2% agarose gel, stained with SYBR Green (Invitrogen Life Technologies®, Grand Island, NY, USA) in a micro cube SSP gel system (One Lambda®, San Diego, CA, USA).

*RHCE*E/e, KEL*01.01/KEL*02, FY*01/FY*02, FY*02N.01* (GATA-1 mutation –67T>C), *JK*01/JK*02* (*SLC14A1* gene) and *DI*01/DI*02* genotyping was performed by the RFLP-PCR (restriction fragment length polymorphism–polymerase chain reaction) technique according to protocols previously described in the literature [16,17], with some modifications previously described [18]. The PCR reaction included 50 ng of DNA, 50 pmol of each primer, 2 nmol of each dNTP, 0.5 U of Taq DNA polymerase (Invitrogen Life Technologies®,