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Blood group genotyping in multi-transfused patients

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ABSTRACT

Background: In chronically transfused patients, the classical hemagglutination assays may be inaccurate in defining the RBC phenotypes of the patients due to previous transfusions.
Design: DNA samples from 39 multi-transfused patients including thalassemia and sickle cell disease were used for red blood cell genotyping. The Rh-Type and KKD-Type (BAGene, BAG Healthcare) were used to determine the polymorphisms associated with antigen expression for RHD, RHCE and Kell, Kidd, Duffy blood group systems, respectively. Results were compared with previously determined phenotyping results for RhD, RhCcEe and Kell by hemagglutination method.v
Results: Nineteen out of the 37(51%) patients had discrepancies between genotyping and phenotyping results in a total of 25 alleles. In 12 patients, the discrepancies had the potential of alloimmunization.
Conclusion: Blood group genotyping has vital importance in transfusion management of chronically transfused patients especially if the patients were not phenotyped before starting the initial transfusions.

1. Introduction

Transfusion management of chronically transfused patients such as thalassemia and sickle cell disease (SCD) is often complicated. Presence of donor's erythrocytes in the circulation from the previous transfusion prevents accurate blood group phenotyping unless the phenotyping is performed before initiation of transfusion. Exposure of these patients with donor erythrocytes carrying foreign antigens increases the risk of alloimmunization. Formation of clinically significant alloantibodies may result in hemolytic transfusion reactions during the subsequent transfusions. The presence of alloantibodies further delays the process, at the blood banks, of finding compatible red blood cell (RBC) units which do not carry the corresponding antigens. Moreover, alloimmunization shortens the survival of the transfused erythrocytes and increases the frequency of transfusion requirement which eventually increases the body iron load.

The alloimmunization rate of 5.3–37% have been reported in various thalassemic populations [1–4]. This rate can be even as high as 43% in SCD [5,6]. Antibodies against antigens from Rh and Kell systems are most frequently involved. Although the transfusion of phenotype matched RBC units for Rh and Kell antigens in addition to ABO and RhD has substantially decreased the risk of alloimmunization, the risk has not been completely eradicated in multi-transfused patients [1,7].

The International Transfusion Society have described more than 300 inherited blood group antigens on the surface of human red cells. The molecular basis of almost all blood group antigens has been determined [8–11]. Blood group diversity results from different mechanisms, most

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frequently single nucleotide polymorphisms (SNPs). Additionally, gene deletions or gene rearrangement with recombination and conversion mechanisms, typically seen in Rh blood group system, may affect the expression of blood group antigens [12]. In Europeans, D-negative phenotype is almost always due to homozygous deletion of RHD gene. On the other hand, in addition to homozygous deletion, various variant D-alleles (e.g. RHDψ, RHD-CE-D^s) may result in lack of D-antigen expression in Africans and Asians [13–16]. Thus, the differentiation between different variant D-alleles is usually possible with only molecular methods [17,18]. Despite the complexity of RHD, C/c and E/e polymorphisms are formed by SNPs at 307T > C (Ser103Pro) and 676C > G (Pro226Ala) of RHCE gene, respectively [19,20]. Additionally, the polymorphisms of the Kell (KEL1/KEL2), Kidd (Jka/Jkb) and Duffy (Fya/Fyb) blood group systems also result from SNPs on their respective genes [21–24].

The main use of blood group genotyping has been RH genotyping of the fetus to prevent the hemolytic disease of fetus and newborn. Blood group genotyping is also helpful in determining the accurate RBC phenotype of alloimmunized patients and patients who are difficult to phenotype due to positive direct antiglobulin test. During the last decade, a number of studies have pointed to the benefit of blood group genotyping and transfusion of genotype-matched RBC units to prevent alloimmunization in chronically transfused patients since discrepancies between the phenotyping and genotyping are frequently detected [25–28]. In this study, multiply transfused patients at a single center have been genotyped for Rh, Kell, Kidd and Duffy blood group antigens by using sequence specific primer-polymerase chain reaction (SSP-PCR) method and the results were compared with the phenotype results previously performed by classical hemagglutination method.

2. Materials and methods

2.1. Patients

We studied DNA samples from 39 multiply transfused patients who agreed to participate in this study by signing an institutional review board (IRB)-approved informed consent. This study was explained to every patient or family who also had ample opportunity to ask questions and receive clear, honest answers. Ankara University Medical School and its IRB comply with the World Medical Association Declaration of Helsinki.

2.2. Control group

Both genotyping and phenotyping studies were also performed on blood samples from 22 healthy blood donors who volunteered to participate in the study by signing an IRB-approved informed consent.

2.3. Agglutination tests

Phenotyping was performed by hemagglutination in gel cards according to the manufacturer instructions (BioVue, ORTHO Clinical Diagnostics, Raritan, NJ).

2.4. DNA preparation

Genomic DNA was obtained according to classical phenol-chloroform method from 9 ml peripheral venous blood samples drawn into polyethylene tubes containing 1 ml 0.5 M EDTA and eluted into 50 μl TE buffer [29]. The DNA concentration of each sample was determined by measurement of optical density at 260 and 280 nm and 50–100 ng DNA was used per PCR reaction.

2.5. SSP-PCR analysis

Sequence Specific Primers (SSPs)-PCR method is based on the fact that primer extension, and hence successful PCR relies on an exact match at the 3′-end of both primers. Therefore, only if the primers entirely match the target sequence amplification is obtained. Rh and Kell, Kidd, Duffy (KKD) genotyping were performed by SSP-PCR kits Rh-Type and KKD-Type, respectively (BAGene, BAG Health Care, Lich, Germany). The kits consist of PCR plates with chambers with prealiquoted and dried reaction mixes containing allele-specific primers, internal control primers and nucleotides. The master mix was prepared with 10× PCR buffer, DNA solution, Taq Polymerase and Aqua dest. PCR amplification parameters were used according to manufacturer instructions. The amplification products were separated by electrophoresis on 2.5% agarose gel. The molecular genetic determination of standard *RHD*/*RHCE* alleles and C^W allele as well as a few *RHD* variants (DVI, DIV type 3, *Cdes*, *RHDY*, *RHD*(W16X), *RHDCE*(8-9)-*D*, *RHD-CE*(3-7)-*D*) and DEL [*RHD*(K409K), *RHD*(M295I), *RHD*(IVS3 + 1-G > A)] was performed. The polymorphisms related to KEL1/KEL2 [T698C (Met198Thr)] of KEL gene, Jka/Jkb [838G > A (Asp280Asn)] of SLC14A1 gene and Fya/Fyb [125G > A (Gly42Asp)] of Duffy (FY) gene were investigated. Homozygous mutation (−67T > C) at the GATA-1 binding point at the promoter region of FY gene (FY^{*-} null01) was also screened [30,31].

3. Results

3.1. Patients

A total of 39 patients (27 male and 12 female) with a median age 19 (range: 1–67) years have been genotyped for RHD/RHCE and KKD blood group systems. Patients' diagnosis' were beta thalassemia major (n:35), SCD (n:2), congenital dyserythropoietic anemia (n:1) and undefined chronic anemia (n:1). The previous phenotyping results for RhD, RhCcEe and Kell1 could be reached in 37 patients. Erythrocyte transfusions were done every 2–4 weeks in thalassemia patients and every 2–3 months in all others. Only 3(7%) patients had formed alloantibodies to various antigens including E, c, K, C^w, Le^a, Le^b .

3.2. Genotype frequencies

The genotype frequencies observed in patients and healthy blood donors were similar and have been shown in Table 1. The most frequent genotypes of the Rh system