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Blood Group Genotyping Facilitates Transfusion of β-Thalassemia Patients

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We evaluated the usefulness of blood group genotyping as a supplement to hemagglutination to determine the red blood cell (RBC) antigen profile of polytransfused patients with β-thalassemia. We selected 10 alloimmunized patients who were receiving antigen-matched RBCs based on phenotype, and had clinical evidence of delayed hemolytic transfusion reaction. DNA was prepared from blood samples and *RH E/e*, *K1/K2*, *FY A/FY B*, and *JK A/JK B* alleles were determined by PCR-RFLP. *RH D*/non-*D* was determined according to the PCR product size associated with the *RHD* gene sequence in intron 4 and exon 10/3′UTR. *RH C/c* was tested by multiplex PCR. The phenotypes and genotypes of nine of the 10 samples were discrepant. Five of the discrepancies occurred in the Rh system. One sample was phenotyped as Rhcc and genotyped as *RH C/C*, and two samples were phenotyped as RhCc and genotyped as *RH C/C*. Two other samples were phenotyped as RhEe and genotyped as *RH e/e.* Three samples had discrepancies in the Kidd system with phenotype Jk(a + b +) and were genotyped as homozygous for *JK B*. One sample had a discrepancy in the Duffy system: it was phenotyped as Fy(a + b−) and homozygous for *FY B*. Genotyping was very important in determining the true blood groups of many polytransfused patients with β-thalassemia, and it assisted in the identification of suspected alloantibodies and the selection of antigen-negative RBCs for transfusion. J. Clin. Lab. Anal. 16:216–220, 2002.

Key words: genotyping assays; blood group antigens; hemagglutination; transfusion; microchimerism

INTRODUCTION

The incidence of alloimmunization to red blood cell (RBC) antigens other than ABO and D is particularly high in patients with hemoglobinopathies (1), reaching up to 29% in patients with β-thalassemia who have received one or more transfusions (2–4). This high rate occurs due to differences in the frequencies of RBC antigens between white blood-donor populations and patients of other ethnic origins (5). Alloimmunization may cause a variety of problems in long-term medical and transfusional management (3–7). Most of these problems pertain to finding appropriate antigen-negative blood for transfusion to alloimmunized patients.

Programs to prevent alloimmunization to RBC antigens have been designed and implemented to provide antigen-matched RBC transfusions to patients with β-thalassemia, particularly those who are alloimmunized and/or in need of chronic transfusion support (8,9). There is no consensus among different institutions about the selection of antigen-negative units for the prevention of alloimmunization (10–12). In our institution, matching includes (in addition to ABO) phenotyping for Rh, Kell, Duffy, and Kidd prior to transfusion, both as a means of preventing alloimmunization to these RBC antigens, and as part of the antibody identification process.

RBC phenotyping is essential to confirm the identity of suspected alloantibodies and to facilitate the

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identification of antibodies that may be formed in the future. Accurate antigen typing of transfused patients is often a difficult task due to the presence of donor RBCs in the patient's circulation. Thus, in these patients phenotyping can be time-consuming and difficult to interpret. It is also difficult to type cells when a patient's RBCs test positive for direct antiglobulin and no direct agglutinating antibody is available.

DNA technology has led to increased understanding of the molecular basis of many blood group antigens. The majority of blood group polymorphisms are associated with a single point mutation in the gene encoding the protein carrying the blood group antigen (13,14). This knowledge allows the use of DNA testing to predict the blood group antigen profile of an individual, which can then be used to overcome the limitations of hemagglutination assays (15).

Several assays for blood group genotyping of patients have recently been developed with the goal of assessing and reducing the risk of hemolytic disease in newborns (16–19). In a previous study we applied these assays to the genotyping of patients who were receiving chronic transfusion care (20).

The dilemma of using DNA obtained from a transfused patient's white blood cells (WBCs) is that the donor leucocytes contained in the transfused units could, at least theoretically, interfere with genotyping results (21–23). However, studies performed with patient WBC samples have shown that these cells can be used reliably to determine a blood group polymorphism by polymerase chain reaction (PCR)-based assays, even when blood samples from recently transfused patients are used as the source of the DNA (18–20). These studies demonstrate that post-transfusion blood samples can be safely used to genotype blood groups.

This study compared red cell phenotypes with genotypes obtained by PCR-RFLP in 10 alloimunized patients with β-thalassemia. We observed that genotype is more accurate than phenotype for determination of blood groups in polytransfused patients with β-thalassemia. Additionally, we verified that post-transfusion blood can be used as source of DNA for blood group genotyping.

MATERIAL AND METHODS

Samples

Blood samples and buccal epithelial cells were collected from each patient after informed consent was obtained by the health-care physician or nurse specialist.

Blood Samples

We studied peripheral blood samples from 10 alloimmunized patients with β-thalassemia who received transfusions of RBC units matched for antigens in the Rh, Kell, Duffy, and Kidd blood group systems at Hemocentro (Unicamp, Campinas, Brazil). One patient had anti-K, two patients had anti-E, three patients had anti-c, one patient had anti-Fy^a, and three patients had anti-Jk^a. These patients had been transfused on at least three occasions and their blood samples had been phenotyped at the time of each transfusion. The most recent blood sample from each patient was also genotyped for *RH*, *KEL*, *JK*, and *FY*.

Buccal Epithelial Cells

Buccal epithelial cell samples were obtained from the patients who had phenotype/genotype discrepancies. We obtained buccal epithelial cells by swiping the mouth mucosa with a cotton wool swab.

Agglutination Tests

Phenotypes were determined by hemagglutination in gel cards (Diamed AG, Morat, Switzerland) using two different commercial sources of anti-sera (Gamma Biologicals, Houston, TX; Diamed AG).

DNA Preparation

DNA was extracted from blood samples using the Easy DNA Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. DNA extraction from buccal epithelial cells contained in the cotton wool swabs were extracted using the Easy DNA Kit according to the manufacturer's protocol #3.

PCR Amplification

The primers and amplification conditions used were as described in previous works (15,17). Briefly, PCR was performed with 100–200 ng of DNA, 50 pmole of each primer, 2 nmole of each dNTP, 1.0 U *Taq* DNA polymerase, and buffer in a final volume of 50 μl. The PCR analysis for the presence of *RHD* was performed in two genomic regions, intron 4 and exon 10, as previously described (15,18,20). For intron 4, three primers (RHI41, RHI42, and RHI43) yielded a product of 115 bp for *RHD* and 236 bp for *RHCE*. For exon 10, a common 5′ primer (EX10F) was used for both *RHD* and *RHCE*. When paired with the RHD-specific 3′-untranslated region (UT) primer (RHD3UT), it produced a product of 245 bp, and when paired with the RHCE-specific 3′-UTR (RHCE3UT), it yielded a product of 160 bp.

Table 1. Phenotyping and genotyping results for RH, K1/K2, JK A/JK B, and FY A/FY B on samples from 10 alloimmunized patients with β-thalassemia

Genotype	Phenotype			
Rh System	RhD+	RhD−		
RHD+/RHCE+	9	0		
RHD−/RHCE+	0	1		
	RhEE	RhEe	Rhee	
RH E/E	0	0	0	
RH E/e	0	0	0	
RH e/e	0	2	8	
	RhCC	RhCc	Rhcc	
RH C/C	1	2	1	
RH C/c	0	5	0	
RH c/c	0	0	1	
Kell System	K+k+	K−k+		
K1/K2	0	0		
K2/K2	0	10		
Kidd System	Jk(a+b−)	Jk(a+b+)	Jk(a−b+)	
JK A/JK A	2	0	0	
JK A/JK B	0	1	0	
JK B/JK B	0	3	4	
Duffy System	Fy(a+b−)	Fy(a+b+)	Fy(a−b+)	Fy(a−b−)
FY A/FY A	2	0	0	0
FY A/FY B	0	4	0	0
FY B/FY B	1	0	3	0

Multiplex PCR

RH C/c genotyping was performed by a multiplex assay that detects the presence of D, differentiates *RH C/c*, and identifies *RHDΨ* (24).

RFLP Analysis

PCR amplified products were digested overnight with the appropriate restriction enzymes (18,20) (MBI Fermentas, Amherst, NY; New England Biolab, Beverly, MA), in a final volume of 20 µl using 10 µl of amplified product and enzyme in 1× buffer according to the manufacturer's instructions. *Mnl* I enzyme was used to determine *RH E/e* polymorphism. The enzymes *Bsm* I, *Mnl* I, and *Ban* I were used to determine *KEL 1/KEL 2* (698C>T), *JK A/JK B* (838A>G), and *FY A/FY B* (125 G>A), respectively.

RESULTS

Correlation Between Phenotype and Genotype of the 10 Alloimmunized Patients

The phenotype and genotype results from the patients with β-thalassemia are shown in Table 1. Nine of the 10 patients had phenotype/genotype discrepancies.

Rh System

Presence or absence of *RHD*

Complete agreement between phenotype and genotype was observed for RhD. Of the 10 samples, eight were both phenotyped and genotyped as RhD-positive (with amplified product from both *RHD* and *RHCE*), and two samples were phenotyped and genotyped as RhD-negative (with amplified product from *RHCE* but not from *RHD*).

RH E/e

There was agreement between phenotypes and genotypes in eight of the 10 samples. Two discrepant samples were phenotyped as RhE/e and genotyped as *RH e/e*. These two samples had anti-E in their serum.

RH C/c

Seven of the 10 samples had concordant phenotypes and genotypes. One discrepant sample was phenotyped as Rhcc and genotyped as *RH C/C*, and there was anti-c in the serum. The two other samples were phenotyped as RhC/c and genotyped as *RH C/C*, and there was anti-c in the serum.

Kell, Kidd, and Duffy Systems

Kell

There was complete agreement between phenotype and genotype results for K. One patient had anti-K in his serum.

Kidd

In the Kidd system, phenotype/genotype did not correlate in three of the 10 samples: they were phenotyped as Jk(a+b+) and genotyped as *JK B/JK B*. These three samples had anti-Jk^a in the serum.

Duffy

One sample had a discrepancy in the Duffy system typing: it was phenotyped as Fy(a+b−) and genotyped as *FY B/FY B*. This sample had anti-Fy^a in the serum.

Genotypes Results Obtained on DNA From Buccal Cells and Peripheral Blood From the Nine Patients With Phenotype/Genotype Discrepancies

In order to demonstrate the absence of microchimerism, the DNA from buccal cells of the nine patients with discrepant phenotype/genotype results was tested by PCR-RFLP. The results were identical to those obtained

when tests were performed using DNA from peripheral blood samples.

DISCUSSION

This study shows the relevance of performing molecular analysis to determine blood group in transfusion-dependent patients, such as those with β-thalassemia. By employing PCR-RFLP assays we have shown that mistyping occurs when hemagglutination is performed to determine blood groups in patients who have been recently transfused with multiple units of donor RBCs, because it is difficult to distinguish between donor and patient cells (20). As observed in the correlation of the genotype/phenotype results, discrepancies were found in nine of the 10 alloimmunized transfused patients studied. Five of the discrepancies occurred in the Rh system. One sample was phenotyped as Rhcc and genotyped as *RH C/C*, and two samples were phenotyped as RhCc and genotyped as *RH/CC*.

Two other samples were phenotyped as RhEe and genotyped as *RH e/e*. These five patients had been transfused with phenotypically matched RBCs and had developed alloantibodies (not autoantibodies) to the detectable phenotype.

We confirmed previous study observations that DNA prepared from blood samples collected from a patient who has recently received a transfusion can be used for blood-group genotyping without risk of detecting microchimerism (18–20).

Our study also identified one discrepant sample in the Duffy system. The hemagglutination result was Fy(a + b−) and the genotype result was *FY B/FY B*. Accordingly, DNA samples obtained from buccal epithelial cells confirmed the genotype obtained from the blood sample. The phenotype performed in segments of the transfused units further confirmed that the Fy(a + b−) reflected the donor's blood type. This patient had anti-Fy^a in his serum. Three samples also presented a discrepancy in the Kidd type. All three samples phenotyped as Jk(a + b +) were genotyped as *JK B/JK B*. The absence of *JK A* was confirmed by genotyping DNA samples obtained from buccal epithelial cells, and by performing phenotyping in segments of the transfused units, it was demonstrated that the Jk(a +) reactivity was due to donor RBCs in the circulation of the patients. These three patients have been immunized for Jk^a antigen because they were receiving antigen-matched RBC units based on the phenotyping interpretation.

One patient who presented anti-K in her serum, but had concordant phenotype/genotype results, was sensitized by pregnancy.

The nine alloimmunized patients previously typed differently by hemagglutination had clinical evidence of delayed hemolytic transfusion reaction. They benefited from receiving antigen-matched RBCs based on genotype, as assessed by better in vivo RBC survival rates and diminished frequency of transfusions.

As previously discussed, the seriousness of the alloimmunization problem has led to recommendations that patients with β-thalassemia be transfused with blood of donors whose RBC antigens are more closely matched to those of the recipients (5,8–10). However, accurate antigen typing in transfused patients is a major problem due to the presence of donor RBCs in the patients' circulation. Based on these and previous results (20), and under the test conditions we have established, we recommend the addition of blood-group genotyping for transfused patients to provide antigen-matched RBC transfusions.

It is important to remember that PCR-based assays are prone to different types of errors than those observed with hemagglutination assays. For instance, contamination with amplified products may lead to false-positive test results. In addition, the identification of a particular genotype does not necessarily mean that the antigen will be expressed on the RBC membrane. This complexity is exemplified by the existence of genes that silence mutations in locations other than that being analyzed (e.g., point mutation in the GATA box), by genes that are silenced by a gene-encoding protein with a modifying effect (e.g., Rh_{mod} and Rh_{null}), and by the detection or lack of detection of a hybrid gene (25–28).

Alternatives to hemagglutination tests to determine a patient's antigen profile should be considered for alloimmunized patients with β-thalassemia who need repeated transfusion therapy. In conclusion, blood-group genotyping facilitates transfusion of β-thalassemia patients by allowing the determination of the true blood-group genotype, and by assisting in the identification of suspected alloantibodies and the selection of antigen-negative RBCs for transfusion.

As automated procedures attain higher and faster throughput at lower cost, blood group genotyping is likely to become more widespread. We believe that the use of PCR technology will become more widespread in the next few years as a means of overcoming the limitations of hemagglutination in transfusions.

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