ORIGINAL REPORT

Genotyping for Dombrock blood group alleles in Northern Pakistani blood donors

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Genotyping can be used to identify rare blood group antigens and to solve suspected blood group discrepancies, particularly when serologic methods are limited. Unfortunately, only a few such studies have been performed in Pakistan. The present study was conducted to determine the frequency of Dombrock blood group alleles by genotyping samples from blood donors from the north of Pakistan. Blood samples were taken with consent from 300 blood donors; DNA was extracted and tested for *DO*01* and *DO*02* alleles by sequence-specific primer polymerase chain reaction (PCR-SSP), followed by gel electrophoresis. Allele frequencies were calculated. The observed and expected genotype frequencies were compared using the χ^2 test. The allele frequencies for *DO*01* and *DO*02* were 0.40 and 0.60, respectively. Genotype frequencies were in Hardy-Weinberg equilibrium. This study in Pakistani blood donors provides Dombrock blood group allele frequencies by PCR-SSP. This approach is efficient and economical and can be applied in developing countries. The findings can contribute to the development of in-house red blood cell panels, identification of rare blood types, and establishment of a national rare blood donor program. ***Immunohematology*** **2021;37:113–117. DOI: 10.21307/immunohematology-2021-016.**

Key Words: Dombrock, red blood cell antigens, sequence-specific primer polymerase chain reaction (PCR-SSP), genotyping

The study of erythrocyte antigens is advancing as a vigorous field of research, particularly after the development of molecular testing methods. The International Society of Blood Transfusion (ISBT) has described more than 300 blood group antigens[1,2] belonging to 43 blood group systems (www.isbtweb.org). Human blood group genes manifest polymorphism, and their products are immunogenic.[3] Antibodies against red blood cell (RBC) antigens can lead to reduction in life span of the transfused RBCs, hemolytic transfusion reactions, and hemolytic disease of the fetus and newborn.[4]

Commercially available RBC antibody identification panels are generally typed for ABO, Rh, Kell, Kidd, MNS, Duffy, Lewis, and Lutheran blood group antigens.[5] Serologic reagents for extended blood typing are costly, available in limited quantities, and nonexistent for some blood group systems like Dombrock.[5,6] Genotyping can be done to predict rare antigens or antigens that cannot be serologically tested, to resolve blood group discrepancies, to help alloantibody identification, and to screen for matched donors, among other applications. In theory, all RBC antigens with recognized DNA polymorphisms can be genotyped because, in contrast with antisera, there is no scarcity of reagents for molecular testing.[7] DNA-based methods have made it possible to ascertain a greater number of RBC antigens than by serology alone.[8] Worldwide, many transfusion centers have adopted extensive RBC antigen typing to provide antigen-negative blood to patients with corresponding antibodies and for better matching of blood group antigens between donors and recipients to reduce the risk of alloimmunization.[9] Nevertheless, the polymorphism of Dombrock blood group antigens in Pakistan has not yet been reported.

The Dombrock blood group system was first described in 1965, when an unknown antibody was found in the blood of Mrs. Dombrock after she received a blood transfusion. Since then, many studies have exposed its complexity.[10] The Dombrock system has been given the ISBT number 014. It encompasses 10 antigens including Do^a (DO1) and Do^b (DO2) encoded by *DO*01* and *DO*02,* respectively.[11]

Blood group phenotype can be predicted from genotype by understanding the molecular basis of the blood group genes.[12] DNA-based methods are envisaged as useful tools in transfusion medicine and are being widely used for predicting blood group antigens. Molecular methods can now be used to establish the prevalence of various blood group antigens.[13] With this background, the present study evaluated the frequency of Dombrock alleles in Northern Pakistani blood donors using a cost-effective molecular protocol.

Materials and Methods

This cross-sectional analytical study was performed at the Hematology Department of the Army Medical College in collaboration with the Armed Forces Institute of Transfusion (Rawalpindi, Pakistan) from January to December 2019. Approval for this study was received from the ethics review

board and the institutional review board. A total of 300 blood donors were included in this study after providing informed consent. Donor information was recorded on an internal log, which was kept confidential. Additional data were analyzed including age, gender, ethnic group, and ABO/D typings.

Genomic DNA of the study participants was extracted from their EDTA-anticoagulated blood samples using the QIAamp DNA kit (Qiagen, Hilden, Germany). Donor samples were tested for *DO*01* and *DO*02* alleles by sequence-specific primer polymerase chain reaction (PCR-SSP). Each PCR tube contained 10 µL PCR buffer with 1.5 mmol/L $MgCl_2$, 0.4 mmol/L of each of the four deoxyribonucleotide triphosphates, 1 µL allele-specific forward primer, reverse primer plus human growth hormone (HGH) primer (Gene Link, Elmsford, NY), and 0.1 U of 5 U/µL Taq DNA polymerase (Thermo Fisher Scientific, Waltham, MA). DNA was added at a concentration of 2 µL per tube. Primer specifics are given in Table 1.

PCR was performed by using a Gene Amp PCR 2700 thermal cycler (Applied Biosystems, Foster City, CA) under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 10 cycles of denaturation for 10 seconds at 94°C and 1 minute of extension at 65°C, and finally 25 cycles of 30 seconds denaturation at 94°C, 1 minute annealing at 61°C, and extension for 30 seconds at 72°C. The protocol ended with a final elongation step at 72°C for 5 minutes.

As a measure of quality control, 20 DNA samples randomly selected from the study participants were repeatedly tested with a concordance level of 100 percent. An internal control of HGH and a negative control or blank was run with each PCR. Internal quality controls derived from the DNA of the test samples were run alongside the PCR batches. The PCR was repeated whenever the results were ambiguous for any allele. All PCR results were evaluated by experienced professionals.

Polyacrylamide gel electrophoresis was done by adding 0.4 µL of tracking dye (bromophenol blue and xylene cyanol) to the amplified product and loading it into 6 percent polyacrylamide gel. The gels were run at 200 volts for 30 minutes and stained with silver nitrate, formaldehyde, and sodium hydroxide solutions to visualize the bands of amplified DNA.

Statistical Analysis and Allele Frequency Calculation

The genotype frequencies of *DO*01* and *DO*02* were obtained by direct counting from Microsoft Excel spreadsheets, and the allele frequencies were calculated by the following formula: allele frequency = allele numbers/[2 × (sample number)].

Hardy-Weinberg equilibrium status was determined using the Hardy-Weinberg equation: $p^2 + 2pq + q^2 = 1$, where p represents the frequency of the dominant allele, q is the frequency of the recessive allele, p^2 is the percentage of the homozygous dominant individuals, q^2 represents the frequency of the homozygous recessive individuals, and $2pq$ is the percentage of the heterozygous individuals.

The expected and observed genotype frequencies were compared by using Pearson's χ^2 test: χ^2 = (observed value − expected value)^2/expected value. The Pearson's χ^2 test was also used to evaluate the differences in frequencies among Pakistanis and other populations of the world. The p value was designated as significant when it was <0.05. The study data were analyzed using Statistical Package for the Social Sciences (SPSS), version 22 (IBM, Armonk, NY).

Results

Donor ages ranged from 18 to 63 years (mean 29.9 ± 7.6). There was only one female donor (0.3%), and the rest were male (99.7%). Among the 300 donors, 92 (30.7%) were blood group O, 83 (27.7%) were blood group A, 90 (30%) were blood group B, and 35 (11.6%) were blood group AB. Regarding their D status, 263 (87.7%) were D+ and 37 (12.3%) were D−.

Table 1. Sequence and specifications of primers used in polymerase chain reaction testing

Gene	Allele	Orientation	Sequence	Time (seconds)	Final concentration (µmol/L)	Product size, (bp)
Dombrock	*DO*01*	Forward	ATTCGATTTGGCCAATTCCTT	51.9	0.2	210
		Reverse	TGACCTCAACTGCAACCAGTT	55.9	0.2	
	*DO*02*	Forward	ATTCGATTTGGCCAATTCCTC	53.9	0.5	209
		Reverse	GACCTCAACTGCAACCAGTC	57.5	0.5	
HGH		Control	GCCTTCCCAACCATTCCCTTA	57.3	0.1	429
		Control	TCACGGATTTCTGTTGTGTTT	53.6	0.1	

Primers acquired from Gene Link, Elmsford, NY.
bp = base pairs; HGH = human growth hormone.

The donors were of different ethnicities: 178 Punjabis (59.3%), 69 Pathans (23%), 12 Sindhis (4%), 2 Balochis (0.7%), and 18 Kashmiris (6%); Hazarawals, Bultis, and Mohajirs collectively represented 21 (7%).

The donors were genotyped for *DO*1* and *DO*2* alleles. The allele frequencies in these donors were calculated as a whole and also in different ethnic groups; results are shown in Tables 2 and 3, respectively.

Table 2. Frequencies of *DO*1* and *DO*2* alleles in Northern Pakistani blood donors

Blood group	Genotype	Observed value	Expected value	Allele frequency
Dombrock	*DO*01/DO*01*	16.3	15.6	0.40
	*DO*02/DO*02*	37.3	36.6	0.60
	*DO*01/DO*02*	46.3	47.8	

Table 3. Allele frequencies of *DO*1* and *DO*2* within different ethnic groups in Pakistan

Allele	Punjabis	Pathans	Sindhis	Balochis	Kashmiris	Others
*DO*01*	0.41	0.41	0.25	0.25	0.44	0.31
*DO*02*	0.59	0.59	0.75	0.75	0.56	0.69

The distribution of genotype frequencies for all analyzed genes was in Hardy-Weinberg equilibrium, without significant differences in the observed and expected frequencies ($p > 0.05$). The genotype distribution of the Dombrock blood group system is summarized below.

Dombrock

The *DO*01/DO*01* genotype was observed in 49 samples (16.3%); *DO*01/DO*02* was observed in 139 samples (46.3%), and *DO*02/DO*02* was observed in 112 samples (37.3%). The allele frequencies of *DO*01* and *DO*02* were 0.40 and 0.60, respectively.

Among the ethnic groups of Pakistan, *DO*01* frequency ranged from 0.25 to 0.44, the highest being in Kashmiris, followed by Punjabis and Pathans, and the lowest in Sindhis and Balochis. *DO*02* frequency varied from 0.56 to 0.75, where Sindhis and Balochis were at the top and Kashmiris were at the bottom, although the ethnic groups were not significantly differently represented. The allele frequencies of both *DO*01* and *DO*02* agreed with the Hardy-Weinberg equilibrium for all major ethnic groups.

Discussion

Genotyping was performed to determine the gene frequencies of *DO*1* and *DO*2* alleles of the Dombrock blood group system in the Pakistani population. It is vital for blood banks to know blood group antigen prevalence and genetic polymorphisms in their population to handle complex serologic problems and ensure provision of blood in special situations. Many PCR-based studies have been conducted to determine the blood group antigen prevalence in individuals of European or African descent, but little information is available on this subject from Pakistan. To our knowledge, this is the first study to report the frequency of *DO*01* and *DO*02* alleles in a Pakistani population.

Pakistan is a country inhabited by people of various cultures and ethnicities. The ethnic groups include Punjabis, Pathans, Sindhis, Balochis, Kashmiris, and others like Hazarawals, Bultis, and Mohajirs. *DO*01* and *DO*02* are prevalent in the Pakistani population. The *DO* gene frequencies of Pakistani blood donors from the major ethnic groups were not significantly different from each other.

The *DO*01* and *DO*02* allele frequencies of the Pakistani population are compared with some other populations in Table 4. Although our population has a different distribution of frequencies of Dombrock alleles as compared with these races, the differences were not found to be statistically significant (i.e., p values >0.05 [data not shown]).

Table 4. Allele frequencies of *DO*1* and *DO*2* in different global populations

Ethnicity	*DO*01*	*DO*02*
Pakistani	0.40	0.60
Chinese†	0.21	0.99
South Asia†	0.62	0.84
Southeast Asia†	0.33	0.94
Filipino†	0.23	0.98
Japanese†	0.25	0.99
Korean†	0.21	0.99
Native American†	0.59	0.86
European/Caucasian‡	0.67	0.82

†Delaney et al.[13]
‡Reid.[23]

The Dombrock system is clinically significant, and DO1 and DO2 are immunogenic. RBC typing for DO1, and particularly for DO2, is challenging because Dombrock antibodies are frequently found in sera that contain additional

antibodies, deteriorate on storage, and are usually weakly reactive.[14] Anti-DO1 and anti-DO2 have been implicated in immediate and delayed hemolytic transfusion reactions.[15]

The frequency of *DO*01* allele in our study was found to be 39.5 percent. It is lower in people of Chinese, Filipino, Japanese, and Korean descent, and it is higher in people of American, European, and South Asian descent.[13] Another study reported the frequency of *DO*01* to be 24 percent in Japanese, 55 percent in African, and 67 percent in European populations.[16]

The *DO*02* allele frequency in the Pakistani population was found to be 60.5 percent. It is relatively lower than that of Japanese (99.7%), Chinese (98.7%), Korean (98.7%), American (85.8%), and European (82%) populations.[13] According to another report, the frequency of *DO*01* and *DO*02* alleles in individuals of African descent is 27 and 73 percent, respectively.[17] A Chinese study reported the gene frequency of *DO*01* and *DO*02* alleles to be 0.13 and 0.87, respectively.[18] Belsito et al.[19] carried out genotyping of blood donors in Italy and showed that the most common Dombrock phenotype was DO:1,2 (48.9%), followed by DO:–1,2 (29.3%) and DO:1,–2 (21.8%). This finding illustrates the great dissimilarities in gene frequencies of the Dombrock system among different regions and ethnicities.

Knowing the distribution of various blood group antigens in different populations is essential in transfusion medicine because donor–recipient mismatch at the antigenic level leads to the formation of alloantibodies,[1] which can have life-threatening consequences. There are noticeable variations in the prevalence of blood group antigens among geologically and historically distinct populations.[20] Hence, determination of the prevalence of RBC antigens at a subpopulation level is essential, particularly in a multiethnic country like Pakistan.

Compared with classic serologic testing methods, molecular methods have several advantages.[12] Serologic procedures are costly, and various antisera are not available commercially,[21] whereas DNA-based methods are quicker and attain a higher throughput at a lower cost.[22] Genotyping can aid in donor screening for both common and rare blood group antigens,[8] thereby increasing the probability of providing antigen-specific blood products to patients.[22] RBCs with a defined antigen profile can be used for manufacturing antibody identification panels.

To perform a molecular study, we had to consider several factors such as finances; time taken per test; throughput, sensitivity, and specificity of the tests; and equipment availability. Several high-throughput DNA platforms are marketed—for example, Blood Chip, HEA Bead Chip, Luminex XMAP, and so forth—but they are very costly[21] and are thus inappropriate for a country with insufficient resources like Pakistan. Therefore, we chose PCR-SSP for our study. Although PCR-SSP is sensitive and accurate, high-throughput blood group genotyping platforms would be needed in the future for large-scale screening.

Furthermore, we had a sample size of 300 blood donors, whereas to establish a national rare donor program, large-scale screening of blood donors is required. Nonetheless, our study provides a baseline that may be useful for subsequent RBC genotyping studies in the country.

Conclusions

This study in Pakistani blood donors, the first of its kind, reports on Dombrock blood group allele frequencies acquired by the PCR-SSP method. This approach is not only efficient, but also cost-effective, and can be used in resource-constrained environments like ours. The study findings can contribute to the production of in-house RBC antibody identification panels, recognition of rare blood types, and the establishment of a rare blood donor program in Pakistan.

Disclaimer

This article is extracted from author SAJ's masters research thesis.

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