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DEL phenotype in RhD-negative North Indian blood donors

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Abstract:

BACKGROUND: Rh-DEL type is not detected on routine serology and requires specialized adsorption elution methods which are laborious. Identifying the DEL phenotype in blood donors is important to prevent alloimmunization in transfusion recipients. The present study aimed to determine the frequency of DEL phenotype in RhD-negative North Indian blood donors and correlate the results with Rh Cc/Ee phenotype.

MATERIALS AND METHODS: In this prospective descriptive cross-sectional study, a total of 205 blood donors with historic blood group RhD-negative were enrolled. All samples were subjected to blood grouping using a fully automated immunohematology analyzer and samples that typed as RhD negative by two different anti-D antisera were tested for Weak D. Weak D-negative samples were subjected to adsorption and elution for DEL phenotype. All samples were also tested for extended Rh phenotype for C/c and E/e antigens.

RESULTS: Of the total 11934 donors during the study, 6.2% (n = 743) donors were RhD negative. Of the 205 donors enrolled in the study, two donor samples were serologically weak D positive. None of the remaining 203 donors tested positive for the DEL phenotype. The extended Rh phenotype performed for these donors showed that 6.83% (n = 14) donors were positive for RhC antigen and 1.46% (n = 3) were positive for Rh E antigen. Both weak D-positive donors were also positive for the Rh C antigen.

CONCLUSION: The prevalence of DEL phenotype is low in the Indian population and studies with larger sample sizes are required to determine the effectiveness of routine C/E typing as a strategy to identify DEL-positive individuals.

Keywords:

Adsorption-elution, DEL phenotype, Rh D variants

Introduction

The Rh blood group system is a highly polymorphic blood group system and of all Rh antigens, the D antigen is considered to be the most immunogenic. RhD typing in blood donors is performed serologically using at least two commercially available monoclonal antisera. The RhD variants can be quantitative (Weak D and DEL) or qualitative (Partial D).[1] It is important to test donor blood using a method that can detect weak expression of D antigen as even 0.1 ml of D-positive red cells are capable of stimulating anti-D production in RhD-negative patients.[2] Although most weak or partial D-positive donors are detected using an indirect antiglobulin test, the DEL red cells are not detected by this method and require adsorption and elution or molecular studies.[3]

The frequency of DEL in the Asian population varies in different regions with a reported prevalence of 32% in Taiwan, 30% in Han Chinese, 28% in Japanese, and 17% among Korean D-negative individuals.[4] Such donors have the potential to stimulate anti-D production in the recipient. However, the prevalence of DEL differs according to ethnicity with a frequency as low as

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0.027% in Europeans.[3] Studies from North Indian blood donors have reported the frequency of 0.2%–1.5% DEL phenotype in D-negative individuals using serology;[5,6] while a study from Western India did not find any donor with DEL variant using both serological and molecular testing.[7]

The number of D antigen sites on a normal RhD-positive red blood cell is 10,000–30,000 sites while it is <30 in DEL variant and it is not detected by routine serological tests even after incorporation of antihuman globulin.[3,8] It is important to identify DEL variant in donors as a small amount of D antigen on the red cells has the potential to cause alloimmunization in the recipient.[3]

Authors have reported D alloimmunization in D-negative recipients transfused with DEL-positive blood. Susceptible patients have demonstrated both primary and secondary immune responses.[4] More than 40 alleles have been identified associated with the DEL phenotype.[9] Certain DEL alleles as shown by molecular studies are more likely to cause immunization. *RHD*DEL1* also known as Asian-type DEL is due to a single nucleotide polymorphism found in 98% of East Asian individuals with DEL.[10] It was originally referred as *RHD (K409K)* with a splice site in the exon 9 of RHD gene.[11] A "silent" 1227G>A substitution in *RHD* codon 409 adjacent to the exon 9/intron 9 boundary is responsible; hence it is also referred to as *RHD* (1227G>A).[4,11] RHD**DEL2* (RHD 3G>A) was the second common allele found in China.[12] *RHD*DEL1* allele has been reported from Japan, Korea, and China to stimulate anti-D production in the patients transfused with red blood cells expressing this allele. On the other hand, transfusion recipients with *RHD*DEL1* allele are not at risk of forming anti-D following the transfusion of RhD-positive RBCs. Pregnant women with certain partial or hybrid DEL alleles have the potential to form anti-D. Hence, all such recipients with RhD variants are considered RhD negative and are candidates for Rh immunoglobulin prophylaxis to prevent the risk of hemolytic disease of the fetus and newborn.[4]

There is an association between the DEL phenotype and the Rh C antigen. Certain ethnic groups that type as D negative on serology with red blood cells expressing the C or E antigen are more likely to have the DEL phenotype. DEL has been reportedly associated with C/E-positive phenotype in many populations. The correlation of Rh C/E positive with DEL can be of help as the former is cost-efficient, easier to perform and can help in identifying probable DEL phenotype.[13]

Based on these reports, the strategy of using cost-effective and quick methods of screening D-negative blood donors by C and E typing could be of paramount importance in identifying DEL phenotype. The aim of the study was to determine the frequency of DEL phenotype in RhD-negative North Indian blood donors and to correlate the results of the DEL phenotype with RhCc/Ee phenotype. The study will help to formulate strategies to be adopted for RhD-negative blood donors to prevent alloimmunization in transfusion recipients.

Materials and Methods

Study design

This prospective descriptive cross-sectional study was conducted in the department of transfusion medicine of a tertiary care center. A total of 205 blood donors from Chandigarh, Punjab, and Haryana comprising mainly Hindu and Sikh communities with historic blood group RhD negative were enrolled in the study. The duration of the study was 9 months. The study was approved by the research and ethics committee of the institution and informed consent was obtained from the donors. All blood donors were screened as per the standard donor eligibility criteria prescribed. Whole blood was collected from blood donors in blood donation camps or in-house.

Donor testing

RhD-negative donors were randomly selected using convenient sampling. The donors were enrolled in the study only based on their known and confirmed RhD-negative status and informed written consent was obtained before blood collection. Donor whole blood samples were collected in EDTA vacutainers for blood grouping and screening for infectious disease markers. All samples were subjected to blood grouping using a fully automated immunohematology analyzer Neoiris (Immucor, USA) and samples that typed as RhD negative by two different anti-D antisera-Novaclone Anti-D IgM + IgG (Immucor Inc., USA) with D175 IgM and D415 IgG clones and anti-D series 5 monoclonal blend (Immucor Inc., USA) were further tested for Weak D using a blend of IgG and IgM anti-D reagent (Tulip Diagnostics, India) by column agglutination technique (Biorad, Switzerland). Weak D-negative samples were subjected to adsorption and elution to screen for the DEL phenotype. All samples were also tested for extended Rh phenotype for C/c and E/e antigens using the same platform Neoiris (Immucor, USA) and monoclonal antisera. Direct antiglobulin test and autologous control were performed using the column agglutination technique (Biorad, Switzerland) to rule out false positivity in all weak D/DEL-positive samples.

Adsorption elution testing

The adsorption elution procedure was standardized in the laboratory with appropriate controls to detect

DEL-positive donor before using for the study purpose. For adsorption done on all RhD-negative weak D-negative samples, 100 µl of washed red cells were mixed with an equal volume of anti-D monoclonal IgG (Eryclone Anti-D Rho, IgG, Tulip Diagnostics, Goa, India) in a test tube and the mixture was incubated at 37°C for an hour in a serological water bath. After incubation, the cells were washed 6–8 times with normal saline and the supernatant of the last wash was preserved for testing at a later stage. Subsequently, gentle heat elution of packed red cells at 45°C–56°C for 8–10 min was performed to elute any anti-D adsorbed onto the red cells. On centrifugation, the recovered eluate was tested in parallel with the last wash supernatant against O RhD-positive cells and O RhD-negative control cells using LISS Coombs cards (IgG and C3d) by column agglutination method (Biorad, Switzerland). Results were interpreted only if agglutination with the last wash was negative when tested with O RhD-positive cells and negative control cells. Agglutination with eluate was to be considered as DEL phenotype positive and no agglutination as true RhD negative.[14]

Statistical analysis

The sample size was calculated based on the expected prevalence of DEL reported in a previous study.[6] The following formula was used for calculating the sample size $n = Z^2 P (1 - P)/d^2$, where n is the sample size, Z is the statistic corresponding to the level of confidence (95%), *P* is expected prevalence and d is precision (2%).

Data were compiled using MS Excel. Descriptive statistics were obtained for all study variables. Categorical variables were reported as counts and percentages. Continuous data were analyzed either in the form of its mean and standard deviation or the form of its median and interquartile range (IQR), as per the requirement.

Results

During the study from July 2021 to March 2022, a total of 11934 donors donated blood which included 10,001 (83.8%) voluntary donors and 1933 (16.2%) replacement donors. Of the 11934 blood donors, 96.9% (*n* = 11568) were male and 3.1% (*n* = 366) were female. The percentage of RhD-positive donors was 93.8% (*n* = 11191) while 6.2% (*n* = 743) of donors were RhD negative. A total of 205 RhD-negative donors who were already aware of their RhD-negative status and gave consent were enrolled in the study. The mean age of the donors included in the study was 32.86 ± 9.7 years (range 18–61 years) and the median age was 31 years (IQR 15). Only 5.4% (*n* = 11) of the study subjects comprised female donors. The characteristics of the study population are shown in Table 1. Of the 205 donors, two donors were Weak D positive who had donated earlier as RhD negative and were not aware of their weak D status. None of the remaining 203 donors included in the study tested positive for the DEL phenotype. The 95% confidence interval (CI) for the proportion of DEL positive was (0, 0.018;) using the Poisson distribution based on sample size. Further workup was done on the two donors that tested positive for Weak D. The auto control and DAT were negative and both the donors were positive for the Rh C antigen. The probable Rh phenotype of donors is shown in Table 2. The prevalence of Rh C antigen was 6.83%, and Rh E antigen was 1.46% while c and e were positive in all 205 donors (100%).

Discussion

The majority of transfusion medicine laboratories rely on serological typing for Rh antigens in routine practice. Rh D variants such as weak D, partial D, and DEL phenotype are not differentiated using serology and need specialized procedures. While an indirect antiglobulin test may detect weak D, it is not possible to differentiate it from the partial D variant for which molecular methods are required. The DEL phenotype can be detected serologically using adsorption and elution techniques; however, confirmation of the alleles requires molecular analysis. A higher frequency of DEL phenotype has been reported in individuals of Asian ancestry.[3] Blood donors with DEL phenotype are capable of sensitizing RhD-negative recipients to the D antigen. Immunization following DEL-positive transfusion can be considered a potential adverse effect following transfusion which may be underreported due to the lack of active surveillance of recipients.

Table 1: Characteristics of study population

Donor characteristics	*n*	Percentage
Gender		
Male	194	94.6
Female	11	5.4
Type		
Voluntary donors	166	80.9
Replacement donors	39	19.1
Blood group		
O negative	62	30.2
A negative	51	24.9
B negative	74	36.1
AB negative	16	7.8
DEL positive (95% CI)	0	0 (0–0.018)
Weak D positive (95% CI)	2	0.01 (0.002–0.034)

CI=Confidence interval

Table 2: Probable phenotypes of donors

Probable phenotype	*n*	Percentage
rr (dce/dce)	188	91.7
r'r (dCe/dce) (Including two Weak D positive)	14	6.83
r"r (dcE/dce)	3	1.46

The overall prevalence of RhD negative in our population was 6.2% and the prevalence of DEL phenotype in RhD-negative blood donors was 0% (95% CI [0,0.018]). Studies across the globe have reported varied prevalence of DEL phenotype with a higher prevalence in Asian donors. The most prevalent DEL phenotype is due to the RHD (K409K) allele in the East Asian population which is more prevalent in China;[15] however, molecular characterization has not been adequately studied in India. The present study was conducted on North Indian blood donors. Previous studies from north India have reported a serological prevalence of 0.2%–1.5%[5,6] while in another study from Western India, all samples were negative for DEL phenotype using adsorption elution and molecular testing for two common *DEL* alleles, *RHD* (K409K) and *RHD* (M295I).[7] A comparison of data from various studies in different populations is depicted in Table 3. Besides the difference in the population, other possible reasons could be the type of reagents and platform used for D typing and the difference in elution methods. Serology was the major method employed while one study also performed molecular testing.

In our study, two donors were serologically Weak D positive and both donors were also positive for the Rh C antigen. The prevalence of weak D varies between 0.2% and 1% in different populations.[17] We could not perform molecular testing due to the lack of availability at our center. We consider this as a limitation in our study as molecular testing methods have been shown to be more sensitive in detecting D variants. We may have missed some DEL-positive donors without red cell genotyping as the cutoff between weak D, DEL and true D negative is not well-defined using serology alone.

DEL has been shown to be associated with C-positive and E-positive individuals who are D negative. Authors have considered this an effective tool to differentiate D-negative individuals into DEL positive.[13] This can be considered an easier and cost-effective strategy to screen RhD-negative individuals for C and E antigen expression. The frequency of C and E antigen positivity in our RhD-negative donors was 6.83% and 1.46%, respectively, but none of the donors was DEL positive. In a study from India, the authors analyzed the genotype of 171 D-negative blood donors with C or E antigen positive on serology. They found *RHD* whole gene deletion in 69% and nonfunctional, negative hybrid genes in 31% samples while four samples showed single-nucleotide variations. No DEL positive was reported in this study.[18] In a study from the east Asian population, a high association between *RHD***DEL1* allele and C antigen (99%–100%) has been seen.[4] Literature reports have demonstrated a 100%-positive predictive value of typing C and E negative individuals as RhD negative.[13] In a study from Thailand, the authors observed that RhC-positive phenotype along with anti-D adsorption/elution test and RHD 1227A allele SSP-PCR technique is an effective method for distinguishing true RhD negative from DEL phenotype.[19] In a recent study from Korea, RhCE phenotyping was recommended as a screening method for serologically D-negative Asian donors in laboratories where molecular typing is not available.[20] The serological method has been suggested in centers where molecular testing is not feasible. However, seeing the very low prevalence of DEL positivity in India, we do not consider it useful as it can lead to an added burden on cost and workforce. In a recent review from China, the authors concluded that red cell units from blood donors with any DEL allele should be considered DEL positive but not RhD positive due to the scarcity of D-negative donors. They proposed that such units can still be considered RhD negative for most transfusion purposes except for pregnant women, children, and women in childbearing age group.[21]

Our study had a few limitations. It was a single center study conducted on a small sample size and molecular testing was not included due to lack of necessary infrastructure. Future multicenter studies with donors from varied populations are needed to know the exact prevalence of DEL in blood donors and correlate with extended Rh phenotype.

Conclusion

The prevalence of DEL in the north Indian population is low and routine testing for DEL is not recommended. However, studies on molecular testing of D-negative

Table 3: Comparison of DEL and C/E phenotype association in different studies

Authors	Country	Frequency of DEL in D negative (95% CI)	Association with C/E	Method
Wah *et al*.[16]	Myanmar	15.8 (0.115–0.211)	All 35 DEL positive were C positive	Serological
Kim *et al*.[13]	Korea	14.5 (0.114–0.166)	All 98 DEL positive were C positive	Serological and molecular
Samir *et al*.[6]	North India	1.5 (0.005–0.043)	Of 3 DEL positive, 2 were C positive and one E positive	Serological
Chaudhary *et al*.[5]	North India	0.2 (0.0005–0.007)	Both DEL positive were C positive	Serological
Kulkarni *et al*.[7]	Western India	0 (0–0.004)	-	Serological and molecular
Present study		0 (0–0.018)	DEL positive - 0 Weak D positive - 2 (both C positive, E negative)	Serological

CI of studies has been calculated based on sample size and positive DEL samples reported using serology. CI=Confidence interval

individuals can be employed in different regions of the country with a larger sample size to get a clear picture of the D variants and their implications among the diverse population of India.

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Conflicts of interest

There are no conflicts of interest.

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