BRIEF REPORT

Screening for DEL phenotype in RhD negative Indians

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Funding information

Council for Scientific and Industrial Research

Background: DEL phenotype represents a very weak form of D variant detected only by adsorption and elution technique. DEL phenotype individuals mistyped as RhD-negative can lead to alloimmunization after transfusion or pregnancy. Molecular techniques have now been used to identify DEL variants. They are commonly encountered in the East Asian population with *RHD*(K409K) being the most frequent allele. *RHD*(M295I) is the most common DEL allele in Caucasians. As there is a paucity of data on DEL phenotype in the Indian population, the study aims to screen RhD negative individuals for two most common DEL mutations.

Material and Methods: EDTA blood was collected from 900 RhD negative individuals. Serological analysis included testing for the five major Rh antigens- C, c, D, E, and e by tube technique. Samples showing negative reaction for the presence of D antigen by Indirect Antiglobulin test were further tested for DEL phenotype by adsorption and elution technique. Molecular analysis involved DNA extraction and testing by PCR-SSP for *RHD*(K409K) and *RHD*(M295I) DEL alleles.

Results: Rh phenotyping showed 153 Rh negative individuals with r'r, ten with r''r and 737 with rr phenotype. All the samples tested negative for RhD antigen by adsorption and elution method. The two common DEL mutations *RHD*(K409K) and *RHD*(M295I) were also not detected in the study population.

Conclusion: The study population showed the absence of the two common DEL alleles, concluding the variant to be rare. A comprehensive study with a larger sample size to look for other DEL mutations should be performed.

KEYWORDS

D variants, DEL phenotype, Indians, polymerase chain reaction using sequence specific primers, RhD negative

1 | INTRODUCTION

The Rh blood group system is one of the most important and complex among the 36 known blood group systems. The RhD antigen is highly immunogenic and has been implicated in transfusion reactions and hemolytic disease of the fetus and newborn.¹ The Rh locus consists of the *RHD* and the *RHCE* gene. The high degree of homology and opposite orientation of the two genes favors the production of numerous Rh variants. Broadly Rh variants are classified as weak D, partial D and DEL.² RhD variants have been documented to cause allo anti-D immunization in Rh negative patients after transfusion and pregnancy. RhD variants should be identified as they are

clinically important to avoid the risk of immunization. Unlike weak D and partial D antigens which may be identified by routine serology, the DEL variants are not identified by Indirect Antiglobulin Test (IAT) and such individuals are typed as D-negative.³ However, reports of primary and secondary immunization of D-negative recipients by DEL positive units necessitate caution in D negative donors and antenatal women.^{4,5}

DEL represents a weakened form of RhD which cannot be detected by conventional serology and requires the use of adsorption elution technique and hence the earlier name D_{el} (D-elute).^{6,7} The antigen density on DEL positive RBCs is very low with number of D sites ranging from 20-40 per cell.⁸ DEL phenotype is reported in

one-third of the East Asian D-negative Chinese Han population and *RHD* 1227G>A (K409K) is the most common mutation identified, hence is also called as the Asia-type *DEL*.^{9,10} This *DEL* phenotype occurs with a reported frequency of 1:110 and 1:9091 in Chinese and German populations, respectively.^{11,12} In mainland China, *RHD*(K409K) is the only reported *DEL* allele. A silent 1227G>A substitution at the exon 9/intron 9 boundary that is the splice site results in the loss of the exon 9 transcript.¹³ The most common *DEL* allele in Caucasians is the *RHD*(M295I) with a frequency of 1:6493 in Europeans.¹² A much higher frequency of 1:272 was found in D negative donors from Croatia.¹⁴ This allele shows a weak D phenotype and a *DEL* phenotype when present in a cDe and CDe haplotype, respectively. Primary and secondary anti-D immunization due to *DEL* K409K allele has been reported, although no report of immunization by M295I allele is available.^{4,15} Currently 40 alleles have been listed in the RhesusBase that have been described to be associated with the *DEL* phenotype. Other mechanisms responsible for the *DEL* phenotype include insertions, deletions, formation of hybrid alleles, stop codons etc.¹⁶ Barring a single report, no data from the Indian population is available with respect to the occurrence of the *DEL* phenotype.¹⁷ India represents a formidable heterogeneous ethnic population and it is important to fill this lacunae. Thus, the two predominant *DEL* alleles were selected for screening the test population.

2 | MATERIALS AND METHODS

EDTA-anticoagulated peripheral whole blood was collected from RhD negative individuals from Mumbai after informed consent. Samples found to be D-negative on direct agglutination at IgM phase with an anti-D blend (Diagast, Loos, France) were further tested by IAT at the IgG phase by the tube technique, and those showing negative results were included in the study. Thus, samples of 900 Rh negative individuals were subjected to adsorption and elution test to screen for *DEL* phenotype variants. Test packed RBCs were incubated with an equal volume of anti-D (human polyclonal anti-D prepared inhouse) at 37°C for one hour. After thorough washing an eluate was prepared using the ether elution technique.¹³ The eluates and last-wash of supernatant (which was used as a negative control) were tested by the tube technique. Rh phenotyping was performed in all samples using monoclonal anti-D, C, c, E, and anti-e reagents (Diagast, Loos, France) by tube technique following manufacturer's instructions.

2.1 | Molecular analysis

DNA was extracted from 900 RhD negative samples by phenol-chloroform method. These DNA were screened for two common *DEL* alleles *RHD*(K409K) and *RHD*(M295I) by polymerase chain reaction using sequence specific primers (PCR-SSP). PCR primers and conditions used were as described previously.^{10,13} To confirm the PCR-SSP results, sequencing for *RHD* exons 6 and 9 was performed as described by Fichou et al.¹⁸

3 | RESULTS

Rh phenotyping showed 153 Rh negative individuals with r'r (Cde/cde) phenotype and ten with r'r (cdE/cde) phenotype and the remaining were rr (cde/cde). On testing by adsorption elution all samples were found to be negative for *DEL* phenotype. None of the samples were positive for the two *DEL* alleles, *RHD*(K409K) and *RHD*(M295I). PCR-SSP and sequencing results were concordant.

4 | DISCUSSION

The *RH* gene is highly polymorphic and shows variation with different ethnic groups. The *DEL* phenotype was reported in the early 1980s by Okubo et al.⁶ when samples negative for the D antigen by IAT were found to be positive by adsorption elution. The importance of identification of *DEL* variants in the antenatal RhD negative women cannot be undermined. Even after the introduction of the Rh prophylaxis some proportion of RhD negative women still get immunized. Even low titer anti-D immunization caused by *DEL* positive RBCs negates the effect of the Rh prophylaxis making it futile. *DEL* phenotype blood donors reported as RhD negative when transfused to D negative recipients are known to cause alloimmunization.^{4,15}

DEL phenotype in D-negative donors has been studied in different populations. *DEL* variants have not been reported in Africans, but have a frequency as high as 1:5 in East Asians and 1:350 to 1:2000 among Europeans.¹⁹ Around 5% Indians are RhD negative.²⁰ Incidence of partial D has been found to be 0.15% whereas incidence of weak D has been reported as 0.3%-1%.^{21,22} Molecular analysis of *DEL* phenotype has not been studied in the Indian population. A small study on 200 RhD negative samples from North India has reported incidence of *DEL* positive donors as 1.5% by adsorption elution method.¹⁷ The mutations resulting in the said *DEL* phenotype were not elucidated. No *DEL* variants by adsorption elution have been detected in our study population which included RhD negative individuals from western region of India. This difference probably reflects the ethnic variation in Indian population.

As serologically *DEL* positive individuals were not identified, DNA based assays were used to detect two common *DEL* alleles *RHD*(K409K) and *RHD*(M295I). The purpose of the screening was to determine whether any of these variants were missed by serology as there are some reports of *DEL* positive alleles which were only detected by PCR assays but not by adsorption elution method.²³ PCR-SSP also showed the absence of the *RHD*(K409K) allele and the *RHD*(M295I) allele in our study population. Other *DEL* variants in study samples cannot be ruled out since molecular analysis was not done for the same. Thus, this study concludes the absence of two common of *DEL* variants in the RhD negative individuals from western India.

ACKNOWLEDGMENTS

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How to cite this article: Kulkarni S, Parchure DS, Gopalkrishnan V, Madkaikar M. Screening for DEL phenotype in RhD negative Indians. *J Clin Lab Anal*. 2018;32:e22288.
<https://doi.org/10.1002/jcla.22288>