Original Article

Molecular Background of RhD-positive and RhD-negative Phenotypes in a Saudi Population

Mohrah A. Alalshaikh, Ammar H. Alsughayir[1], Alyazeed S. Alsaif[2], Sarah A. Ababtain[3], Shaika Y. Aloyouni[3], Khawlah E. Aldilaijan[3], Sahar F. Alsubaie

Department of Clinical Laboratory Sciences, Applied Medical Sciences, King Saud University, [1]King Fahad Medical City, The Saudi Society of Blood and Marrow Transplantation, [2]Transfusion Medicine and Blood Bank Department, King Fahad Medical City, [3]Department of Research, Health Sciences Research Centre, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia

Abstract

Background: The *RHD* gene is one of the most complex blood group genes. The molecular background of the *RHD* gene in RhD-negative and RhD-positive individuals varies within and among different populations. Knowing the molecular basis of the *RHD* gene in a specific population is required to establish effective genotyping methods. While the molecular basis has been revealed in many ethnicities, such as Caucasians and Black Africans, it still requires elucidation in Arabs.

Objectives: The aim of this study was to gain insights into the molecular basis of RhD-positive and RhD-negative phenotypes in Saudi donors.

Materials and Methods: Conventional serological tests were used to determine the Rh phenotypes in 136 Saudi donors by typing D, C, c, E, and e antigens. Multiplex-PCR and Single Specific Primer-PCR were used to detect the presence of exons 3, 4, and 7 and the *hybrid Rhesus box* gene, respectively, in RhD-negative and/or RhD-positive samples.

Results: Of the 136 samples, 70 were RhD positive and 66 were RhD negative. None of the RhD-negative donors had any of the three tested exons, whereas the *hybrid Rhesus box* gene was detected in all, indicating the zygosity status of the *RHD* deletion allele. The *hybrid Rhesus box* gene was detected in 79% of the RhD-positive individuals, suggesting high frequencies of *RHD*-negative haplotypes.

Conclusions: The study findings indicate that Saudis with the RhD-negative phenotype are likely to have an entire *RHD* deletion in the homozygous state. However, a more comprehensive analysis of variant *RHD* alleles in the Saudi population is required to implement effective and dedicated molecular *RHD* typing strategies.

Keywords: Hybrid *rhesus box*, Rh phenotype, *RHD* genotyping, Saudi

Address for correspondence: Mohrah A. Alalshaikh, Almalqua, Riyadh, Saudi Arabia.
E-mail: malalshaikh@ksu.edu.sa

Submitted: 11-Dec-2023 **Revised:** 14-Mar-2024 **Accepted:** 28-Mar-2024 **Published:** 20-Jun-2024

INTRODUCTION

The RhD antigen is one of the most important immunogenic antigens in transfusion, as in alloimmunized patients, an incompatible RhD transfusion can cause a hemolytic transfusion reaction, and in the case of women, an incompatible pregnancy can cause hemolytic disease of newborns. Serological tests are considered standard

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/sjmm
	DOI: 10.4103/sjmms.sjmms_664_23

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Alalshaikh MA, Alsughayir AH, Alsaif AS, Ababtain SA, Aloyouni SY, Aldilaijan KE, *et al*. Molecular background of RhD-positive and RhD-negative phenotypes in a Saudi population. Saudi J Med Med Sci 2024;12:210-5.

RhD detection tests with high accuracy; however, they are not effective under certain conditions.[1] For this reason, genotyping by molecular techniques is required, for example, to determine the *RHD* zygosity in paternal blood, the blood type in frequently transfused patients, or the RhD status for infants of RhD-negative sensitized pregnant women, or to resolve RhD discrepancies between different serological reagents in blood donors.[2-4]

RHD is a very complex gene, with >800 known alleles, 33% of which are considered weak D, and 28% of which are associated with the RhD-negative phenotype.[5] The *RHD* gene encodes the RhD protein, which spans the red blood cell (RBC) membrane 12 times. The frequency of the RhD antigen varies in different populations, presenting in about 85% of Caucasians and Arabs and at greater frequency in other populations, such as Black Africans and Indians.[6-8] Although individuals are categorized as having either a D-positive or D-negative phenotype, variants of the RhD antigen exist as a result of variations in the encoded gene. These RhD variants can be quantitatively different from the wild-type RhD antigen and include weak D types, which are characterized by smaller numbers of RhD copies on RBCs, and partial D types, which show variations in the RhD epitopes. Individuals with the D-negative phenotype have different genetic backgrounds, as most D-negative Caucasians have an entire deletion in the *RHD* gene, while most RhD-negative Black Africans have the *RHDΨ* gene.[9]

The prevalence of blood group alleles differs significantly between different ethnic groups; therefore, knowing the frequency of alleles in a specific population is pivotal in establishing appropriate molecular typing methods. In Saudi Arabia, the frequency of RhD antigen has been reported to be around 90%.[10,11] However, in the Saudi population, the molecular backgrounds of RhD-positive and negative phenotypes have not been elucidated. The aim of this study was to gain insights into the genetic background of the RhD-negative phenotype in the Saudi population and to determine the homozygosity of the *RHD* gene among RhD-positive Saudi individuals.

MATERIALS AND METHODS

Donors

EDTA blood samples were collected from 70 RhD-positive and 66 RhD-negative randomly selected Saudi donors at a tertiary care hospital in Riyadh, Saudi Arabia. All procedures of the study were performed in compliance with relevant laws and institutional guidelines and were approved by the Ethics Committee of King Fahad Medical City, Riyadh. Informed consents were obtained from the donors.

Phenotype determination

The antigenic phenotype (D, C, E, c, e) of the samples was determined serologically using the column agglutination technique performed by a blood typing instrument (IH-1000; Bio-Rad, Hercules, CA, USA). The monoclonal antibody reagents (Bio-Rad, Hercules, CA, USA) were as follows: anti-D (RH1, clones ESD-1M + 175-2), anti-C (RH2, clones MS-273 + P3x25513G8), anti-c (RH4, clone 951), anti-E (RH3, clones MS-80 + MS-258), and anti-e (RH5, clones MS-62+ P3GD512). The frequency of each phenotype was calculated based on the total observed Rh-positive or Rh-negative phenotypes. The most probable genotype was selected based on the most expected genotype in the Saudi population.[12]

DNA extraction

Genomic DNA was extracted from the blood samples using QIAamp DNA mini kits (Qiagen, Hilden, Germany) and a DAN extraction instrument (QIAcube; Qiagen, Hilden, Germany). The concentration of extracted DNA was determined using a Nanodrop 2000 instrument (Thermo Scientific), and all DNA samples were stored at −80°C.

Multiplex PCR

Multiplex PCR was used to detect RHDψ and some other possible RhD-negative molecular backgrounds in 66 RhD-negative samples. This was done by amplifying *RHD* exons 3, 4, and 7 with RHD sequence-specific primers specific for their 3' ends [Table 1].[13,14] The multiplex PCR conditions were slightly modified from those described by Maaskant-van Wijk *et al*.[13] and Ouchari *et al*.[15] Each 50-μL PCR reaction mixture contained 175 ng of DNA, the primer concentrations were 0.1 μM, and PCR master mix (DreamTaq Green PCR Master Mix, Thermo Scientific, NH, USA) was used. To avoid false negative results, a 429 bp fragment generated from the growth hormone gene was used as an internal control [Table 1]. Each cycling condition consisted of an initial denaturation at 94°C for 2 min, followed by 32 cycles performed at 94°C for 3 min, 60 for 1 min, and 72°C for 45 s, and a final cycle at 72°C to complete extension by using a thermal cycler (Veriti, Applied Biosystems, Thermo Fisher Scientific, USA). The products were electrophoresed on 3% agarose gels and visualized by ethidium bromide staining.

Single specific primer PCR

Single specific primer (SSP)-PCR was used to detect the *hybrid Rhesus box* gene, which presents as a result of *RHD* gene deletion, to determine the molecular background of 66 RhD-negative samples and the RhD homozygote status of 70 RhD-positive samples. A 2778 bp fragment amplified from this gene can be detected in both RhD-positive individuals

Table 1: Primers used to detect *RhD* exons 3, 4, and 7 and the hybrid Rhesus box gene

Specificity	Primer	Primer direction	Sequence	Product size
Exon 3	R364	Forward	5′TCGGTGCTGATCTCAGTGGA3′	111
	R474M	Reverse	5′ACTGATGACCATCCTCATGT3′	
Exon 4	R496	Forward	5′CACATGAACATGATGCACA3′	126
	R621	Reverse	5′CAAACTGGGTATCGTTGCTG3′	
Exon 7	R973	Forward	5′AGCTCCATCATGGGCTACAA3′	96
	R1086	Reverse	5′ATTGCCGGCTCCGACGGTATC3′	
Hybrid and upstream rhesus box	U1-s	Forward	5′TGAGCCTATAAAATCCAAAGCAAGTTAG3′	2778
Hybrid and downstream rhesus box	Rn31	Reverse	5′CCTTTTTTTGTTTGTTTTTGGCGGTGC3′	
Growth hormone	HGHF	Forward	5′GCCTTCCCAACCATTCCCTTA3′	429
	HGHR	Reverse	5′TCACGGATTTCTGTTGTGTTTC3′	

with a hemizygous *RHD* gene and RhD-negative individuals. This fragment was amplified using the Hybrid and upstream Rhesus box primer and Hybrid and downstream Rhesus box primer [Table 1]. The PCR primers and conditions were similar to those described by Khosroshahi *et al.*[16] As in the multiplex experiment, a band generated from growth hormone was used as an internal control. Each 25-μL PCR reaction mixture contained 175 ng of sample DNA, and the final concentration of the primers was 0.4 μM. Amplification was performed in a thermal cycler (Veriti, Applied Biosystems). After initial denaturation at 95°C for 10 min, 35 cycles were carried out using the following sequence: denaturation at 92°C for 20 s, annealing at 64°C for 30 s, extension at 68°C for 3 min, and one cycle at 72°C for 5 min to complete the extension. The products were electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining.

RESULTS

Rh phenotypes

Serological tests were performed using conventional methods routinely conducted for blood donors. Of the 136 samples, 70 were RhD positive and 66 were RhD negative. No weak D results were observed among the serologically tested RhD-positive samples. The Rh phenotype frequencies were calculated based on the number of donors [Table 2]. Most of the RhD-positive individuals had DCce (34.3%) or DCe (25.7%) phenotypes, whereas around 94% of the RhD-negative individuals had the ce phenotype.

RhD exon analysis

In this study, we adopted a multiplex PCR method that used *RHD*-specific exon 3, 4, and 7 primers to screen *RHD* alleles in the RhD-negative Saudi population. This method can detect many inactive *RHD* alleles, such as those resulting from inactive mutations in different *RHD* exons. A 429 bp fragment of the growth hormone gene was used as an internal control. None of the 66 RhD-negative samples showed any of the three examined exons [Figure 1].

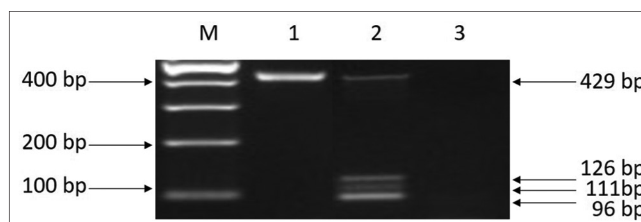

Figure 1: PCR-multiplex analysis for exons 3, 4, and 7. Lane 1: Standard RhD-negative; Lane 2: RhD-positive sample; Lane 3: negative control. The 429 bp fragment of the growth hormone gene is used as an internal control. M: Molecular marker 100 bp

Hybrid rhesus box analysis

A genomic segment of the hybrid *Rhesus box* gene was amplified by the PCR-SSP technique, and the gene was detected in all 66 of the tested RhD-negative samples [Figure 2]. The zygosity of the *RHD* gene in RhD-positive individuals was determined, depending on the results of the hybrid *Rhesus box* gene. The presence of the hybrid *Rhesus box* gene indicates hemizygosity of the *RHD* gene, whereas its absence indicates homogeneity. Among the 70 tested RhD-positive samples, 21% (n = 15) had a homozygous and 79% (n = 55) had a hemizygous *RHD* gene.

DISCUSSION

The molecular background of different blood group systems varies among individuals of different ethnicities, despite the possible similarity in the distribution of blood group phenotypes, and this is similar for the *RHD* gene. The molecular background of the *RHD* gene also varies between different individuals within a population; therefore, knowledge of the nature and distribution of variant *RHD* alleles is important in a population. This knowledge is particularly essential for healthcare providers, as it allows them to implement efficient genotyping to guide transfusion practice, especially for transfusion-dependent patients, and obstetric management.[9,17,18]

In the Saudi population, the molecular determination of the RhD-negative phenotype is unknown; therefore, we

Table 2: The frequency of the Rh phenotypes

RhD status	Phenotype	Observation number (%)	The most probable genotype
RhD-positive (*n*=70)	DCcEe	6 (8.6)	R1R2
	DcEe	11 (15.7)	R2r
	DCce	24 (34.3)	R1r
	Dce	10 (14.3)	R0r
	DCe	18 (25.7)	R1R1
	DcE	1 (1.4)	R2R2
RhD-negative (*n*=66)	cEe	2 (3)	rr"
	ce	62 (93.9)	rr
	Cce	2 (3)	r'r

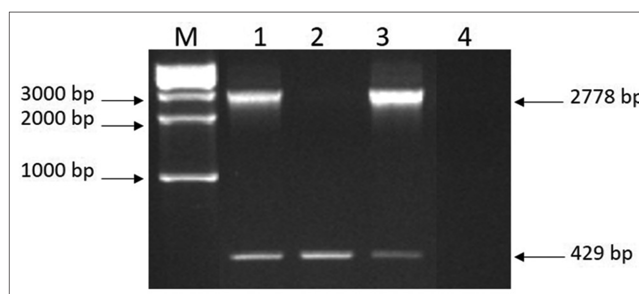

Figure 2: PCR-SSP for hybrid Rhesus box gene. The amplified 2778 bp fragment indicates the presence of the Rhesus box gene. Lane 1: RhD-positive sample hemizygous for the *RHD* gene; Lane 2: RhD-positive sample homozygous for the *RHD* gene; Lane 3: RhD-negative sample; Lane 4: negative control. The 429 bp fragment of the growth hormone gene used as an internal control M: Molecular marker 1 kb

aimed to explore it as a preliminary step to aid in identifying the molecular basis of the *RHD* gene, and subsequently, in establishing a Saudi blood group genotype database.

The PCR-SSP analysis performed on the 66 RhD-negative samples revealed that all these individuals had at least one copy of the hybrid *Rhesus box* gene. However, the RhD-negative phenotype can arise from a heterozygous combination of different alleles associated with this phenotype. Currently, more than 80 *RHD* alleles are known to accompany a null or RhD-negative phenotype.[18] Therefore, in addition to using PCR-SSP to detect the presence of the hybrid *Rhesus box* gene, multiplex PCR was used to detect the presence of three RhD exons. In this technique, specific primer sets were used to detect *RHD* exons 3, 4, and 7, as these could be present in RhD-negative phenotype individuals with inactive *RHD* alleles. These silencing alleles can result from the presence of insertion or inactivated mutations in one of the *RHD* 10 exons, such as the *RHDΨ* or *RHD*01EL.01*. The *RHDΨ* allele is the most common RhD-negative allele in individuals of African descent. This gene has different molecular bases, including insertion of a 37 bp segment in intron 3 (which generates a premature translation termination codon), missense mutations in exon 5, and a nonsense mutation in exon 6.[9] The presence of the *RHD*01EL.01* mutation, which results from an abnormal splicing event in exon 9, was common in the RhD-negative Thai population, accounting for around 10% of those individuals.[19] However, none of the three tested exons were detected in any of our RhD-negative Saudi samples, indicating that these individuals were likely to be homozygous for the *RHD* gene deletion. This result is similar to those found in RhD-negative individuals from Caucasian, Indian, and Iranian populations.[16,20] Nevertheless, as only *RHD* exons 3, 4, and 7 were tested in the RhD-negative samples, the possibility remains that some of the samples may have a nonactive hybrid *RHD* gene rather than a homozygous *RHD* deletion allele, as both would give negative results by the technique used here. Moreover, in this study, the *RHD* gene was investigated by means of PCR techniques, which could fail in detecting some *RHD* negative alleles that could be detected by using gene sequencing methods.

The molecular basis of RhD-negative phenotype is varied. In Caucasians, the most common RhD-negative allele is *RHD*01N.01,* which has a deletion in all 10 exons of the *RHD* gene.[21] By contrast, in persons of African descent, the most common negative allele is *RHD*08N.01*, the *RHDΨ* gene.[9] In Arabs, very scanty molecular analysis information is available for *RHD* alleles associated with the RhD-negative phenotype. The RhesusBase identifies three variant *RHD* alleles in Arabs, one of which is the *RHD*01N.53*, which results from a single nucleotide mutation in exon 9, and is associated with the RhD-negative phenotype.[22] A study conducted in the Tunisian population showed that the vast majority of RhD-negative individuals have *RHD* deletions, while around 2% have aberrant *RHD* alleles.[15] Similarly, most of the RhD-negative Kuwaiti donors had *RHD* deletion and the number of RhD-negative hypered alleles were detected in around 0.6% of Kuwaiti donors including RHD-CE (3–7)-D, RHD-CE (3–9)-D, and DIIIa/DIIIa-CE (4–7)-D.[23] The molecular background of RhD-negative Omani population revealed that around 7% of RhD-negative individuals had hybrid *RHD* alleles or *RHDΨ* gene.[24]

The Rh phenotype results in our Saudi population showed that most of the RhD-positive phenotypes were DCce (34.3%) or DCe (25.7%), whereas the vast majority (94%) of RhD-negative individuals had a ce phenotype. These results are consistent with other studies conducted in the Saudi population and some other Arab populations such as Emirati, Omani, and Egyptian.[8,25-28] No serologically missing weak D samples were detected in the present study, suggesting that the current serological tests are effective in the detection of weak D samples, and the risk of alloimmunization by weak D donor samples is

expected to be low. However, as the sample size of this study was small and the data were collected from a single donation center, further investigation of the efficacy of the current serological tests is needed on a larger scale and involving multiple local donation centers. Moreover, as most of the aberrant *RHD* genes in RhD-negative individuals were associated with the presence of C or/and E antigens,[29] inclusion of a large proportion of this population would also be warranted.

The PCR-SSP analysis showed that around 80% of the RhD-positive samples had the *Rhesus box* gene, indicating hemizygosity of the *RHD* gene in most of the RhD-positive phenotype individuals. Therefore, the haplotype frequencies of alleles with an inactive *RHD* gene in Saudis with the RhD-positive phenotype seem to be greater than previously estimated.[12,30] This inconsistency may result from the use of different methods, as the previous study calculated the haplotype frequencies using an estimation method that depended on the findings of the serology phenotyping tests, whereas our current study employed molecular methods. Nevertheless, as this study only investigated the *RHD* gene, the molecular bases of both the *RHD* and *RHCE* genes should be investigated to provide more accurate results.

As the sample size of this study is small and only *RHD* gene was investigated, a more comprehensive overview of variant *RHD* alleles is still required for the Saudi population at a greater level to aid in the implementation of efficient genotyping strategies, as knowledge of the molecular background of the *RHD* gene and allele distributions is necessary on a national basis. While molecular data are available for Caucasians, East Asians, and Black Africans, equivalent data have barely been documented in Arabs, indicating a need for a comprehensive overview of variant *RHD* alleles.

CONCLUSION

The current findings provide insights into the molecular determination of RhD-negative and RhD-positive phenotypes in Saudis. Most Saudi individuals with the RhD-negative phenotype are likely to have an entire *RHD* deletion in the homozygous state similar to that found in Caucasians and Arabs. Moreover, the *RHD* deletion allele is present in most RhD-positive individuals.

Ethical considerations

The study was approved by the Ethics Committee of King Fahad Medical City (Ref. no.: H-01-R012/21-104E; date: April 2021), Riyadh, Saudi Arabia. All donors provided written consent. The study adhered to the principles of the Declaration of Helsinki, 2013.

Peer review

This article was peer-reviewed by two independent and anonymous reviewers.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: M.A.A. and S.F.A.; Methodology: S.F.A. and S.Y.A.; Data analysis: A.H.A., A.S.A., K.E.A, and S.A.A.; Writing–original draft preparation: M.A.A.; Writing – review and editing: A.H.A., S.Y.A, and S.F.A.; Supervision: M.A.A.

All authors have read and agreed to the published version of the manuscript.

Financial support and sponsorship

This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting (Project number: PNURSP2024R713), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gammon R, Conceicao M, Benitez N, Bright F, Counts K, Resto C, *et al.* Comparison of RhD Typing Results by Serology and Molecular Methods. Lab Med. 2023;54(2):190–2.
2. Ramsey G, Park YA, Eder AF, Bobr A, Karafin MS, Karp JK, *et al.* Obstetric and newborn weak D-phenotype RBC testing and Rh immune globulin management recommendations: lessons from a blinded specimen-testing survey of 81 transfusion services. Arch Pathol Lab Med. 2023;147(1):71–8.
3. Silva TCS, Cruz BR, Costa SS, Chiba AK, Barros MMO, Langhi DM, *et al.* RHD and RHCE molecular analysis in weak D blood donors and in patients with Rh antibodies against their own corresponding Rh antigen. Blood Transfus. 2020;18(4):295.
4. Yang H, Llewellyn A, Walker R, Harden M, Saramago P, Griffin S, *et al.* High-throughput, non-invasive prenatal testing for fetal rhesus D status in RhD-negative women: a systematic review and meta-analysis. BMC Med. 2019;17:1–10.
5. Floch A, Téletchéa S, Tournamille C, de Brevern AG, Pirenne F. A review of the literature organized into a new database: RHeference. Transfus Med Rev 2021;35:70-7.
6. Basu D, Datta SS, Montemayor C, Bhattacharya P, Mukherjee K, Flegel WA. ABO, rhesus, and kell antigens, alleles, and haplotypes in West Bengal, India. Transfus Med Hemother 2018;45:62-6.
7. Adewoyin AS, Lee GM, Adeyemo TA, Awodu OA. Rh and Kell blood group antigen prevalence in a multi-ethnic cohort in Nigeria: Implications for local transfusion service. Immunohematology 2018;34:61-5. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29989421. [Last accessed on 2019 Jun 14].

8. Elsayid M, Al Qahtani FS, Al Qarni AM, Almajed F, Al Saqri F, Qureshi S. Determination of the frequency of the most immunogenic Rhesus antigens among Saudi donors in King Abdulaziz medical city – Riyadh. J Nat Sci Biol Med 2017;8:56-9.
9. Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, *et al*. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. Blood 2000;95:12-8.
10. Alzahrani FM, Shaikh SS, Rasheed MA. Frequency of ABO-Rhesus blood groups in the Western region of Saudi Arabia. J King Abdulaziz Univ Med Sci 2018;25:9-13.
11. Altayar MA, Jalal MM, Kabrah A, Qashqari FS, Jalal NA, Faidah H, *et al*. Prevalence and association of transfusion transmitted infections with ABO and Rh blood groups among blood donors in the Western region of Saudi Arabia: A 7-year retrospective analysis. Medicina (Kaunas) 2022;58:857.
12. Alalshaikh M, Almalki Y, Hasanato R, Almomen A, Alsughayir A, Alabdullateef A, *et al*. Frequency of Rh and K antigens in blood donors in Riyadh. Hematol Transfus Cell Ther 2022;44:555-9.
13. Maaskant-van Wijk PA, Faas BH, de Ruijter JA, Overbeeke MA, von dem Borne AE, van Rhenen DJ, *et al*. Genotyping of RHD by multiplex polymerase chain reaction analysis of six RHD-specific exons. Transfusion 1998;38:1015-21.
14. Perco P, Shao CP, Mayr WR, Panzer S, Legler TJ. Testing for the D zygosity with three different methods revealed altered Rhesus boxes and a new weak D type. Transfusion 2003;43:335-9.
15. Ouchari M, Jemni-Yaacoub S, Chakroun T, Abdelkefi S, Houissa B, Hmida S. RHD alleles in the Tunisian population. Asian J Transfus Sci 2013;7:119-24.
16. Khosroshahi BN, Oodi A, Namjou S, Gholamali T, Amirizadeh N. RHD genotyping by molecular analysis of hybrid rhesus box in RhD-negative blood donors from Iran. Indian J Hematol Blood Transfus 2019;35:119-24.
17. Trucco Boggione C, Nogués N, González-Santesteban C, Mufarrege N, Lujan Brajovich M, Mattaloni SM, *et al*. Characterization of RHD locus polymorphism in D negative and D variant donors from Northwestern Argentina. Transfusion. 2019;59(10):3236–42.
18. Keller MA. RH genetic variation and the impact for typing and personalized transfusion strategies: A narrative review. Ann Blood 2023;8:18.
19. Thongbut J, Raud L, Férec C, Promwong C, Nuchnoi P, Fichou Y. Comprehensive molecular analysis of serologically D-negative and weak/partial D phenotype in Thai blood donors. Transfus Med Hemother 2020;47:54-60.
20. Khetan D, Shukla JS, Chaudhary RK. Molecular basis of RhD-negative phenotype in North Indian blood donor population. Indian J Med Res 2022;155:286-92.
21. Wagner FF, Flegel WA. RHD gene deletion occurred in the Rhesus box. Blood 2000;95:3662-8.
22. Garcia F, Rodriguez MA, Goldman M, Azcarate MN, Rodriguez MI, Muniz-Diaz E, *et al*. New RHD variant alleles. Transfusion 2015;55:427-9.
23. Ameen R, Al Shemmari S, Harris S, Teramura G, Delaney M. Classification of major and minor blood group antigens in the Kuwaiti Arab population. Transfus Apher Sci 2020;59:102748.
24. Al-lawati MA. Molecular Background of Serological D Negative Phenotype in the Omani Population [dissertation], Liverpool John Moores University; 2020.
25. Owaidah AY, Naffaa NM, Alumran A, Alzahrani F. Phenotype frequencies of major blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) among blood donors in the Eastern region of Saudi Arabia. J Blood Med 2020;11:59-65.
26. Swelem O, Goubran F, Younis S, Kamel N. ABO, RH phenotypes and kell blood groups frequencies in an Egyptian population. Hematol Transfus Int J 2018;6:70-3.
27. Al-Riyami AZ, Al-Marhoobi A, Al-Hosni S, Al Mahrooqi S, Schmidt M, O'Brien S, *et al*. Prevalence of red blood cell major blood group antigens and phenotypes among Omani blood donors. Oman Med J 2019;34:496-503.
28. Sajwani FH, Amer AM, Khamis FM, AlShamsi SR. Prevalence of major blood group antigens in blood donors at a main donation center in United Arab Emirates. Immunohematology 2021;37:171-7.
29. Floch A. Molecular genetics of the Rh blood group system: Alleles and antibodies – A narrative review. Ann Blood 2021;6:29.
30. Halawani AJ, Arjan AH. ABO, RH, and KEL1 antigens, phenotypes and haplotypes in Southwestern Saudi Arabia. Clin Lab 2021;67:1-5.