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Two Cases of Anti-D Alloimmunization in D-Negative Thai Patients as a Result of the Asian-Type DEL on Transfused Red Cells

Kanyapon Suksard Komon Luangtrakool Thongbai Rungroung
Sutthisak Chamsai Pradermchai Saetam Kulvara Kittisares
Parichart Permpikul Janejira Kittivorapart

Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

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Abstract

Introduction: DEL is known to be one of the weakest D variants, which can be detected by the adsorption-elution technique or by molecular study. Currently, in Thailand, we do not routinely test for DEL variants serologically or genetically among serologic RhD-negative blood donors. ***Case Presentation:*** We reported 2 cases of alloimmunization after transfused with Rh DEL, *RHD*DEL1* allele, in the Thai population. The first case was a 73-year-old male with anemia who presented with post-cardiac arrest and septic shock. The patient was group B, RhD-negative, and was transfused with RhD-negative red blood cells (RBCs). Antibody screening and identification found that the patient developed anti-D and anti-Mi^a during the admission course. The second case was a 38-year-old woman with pseudomyxoma peritonei who developed anti-D after receiving four units of RhD-negative RBCs during cytoreductive surgery with hyperthermic intraperitoneal chemotherapy. Both patients did not receive anti-D immunoglobulin and had no previous history of anti-D detection. We retrospectively investigated and found two units of *RHD*DEL1* among the RBCs transfused to these patients. ***Discussion:*** Previous reports of several cases of anti-D alloimmunization in RhD-negative recipients transfused by *RHD*DEL1*, an Asian-type DEL, are limited only to East Asia. We first identified 2 patients with anti-D alloimmunization after receiving the *RHD*DEL1* RBCs in the Thai population. This raises concern about Rh DEL screening among D-negative Thai blood donors and whether to remove DEL units from the D-negative inventory to improve patient safety.

Introduction

The DEL phenotype is defined as an extremely weak expression of the D antigen on red blood cells. Serologically, DEL is detectable only by adsorption and elution assay [1, 2]. There is no agglutination in the conventional indirect antiglobulin test on D typing; therefore, most individuals with DEL are classified as D-negative. Among the 45 reported alleles of DEL phenotype [3], the most frequently reported mutation in East Asia is *RHD*DEL1* (c.1227G>A) or Asian-type DEL. This variant represents approximately 17–30% of the negative phenotype D in countries of East Asia [4]. The prevalence of RhD-negative in the Thai population is 0.3% [5] with previously reported DEL phenotypes and *RHD*DEL1* of 37.7% and 33.6% among RhD-negative blood donors [6]. However, this is the first report of anti-D alloimmunization in two recipients in Southeast Asia.

karger@karger.com
www.karger.com/tmh

Correspondence to:
Janejira Kittivorapart, janejira.kii@mahidol.edu

Case Report

The first case was a 73-year-old man who presented with cardiac arrest at the emergency department due to acute kidney injury and metabolic acidosis. Return of spontaneous circulation was achieved after cardiopulmonary resuscitation, and the patient was admitted to the medicine unit. During an approximately 2-month hospital stay, the patient developed pneumonia with severe septic shock and an unknown cause of anemia (hemoglobin 7.0–8.3 g/dL) without an obvious bleeding site. A total of 10 units of group B, RhD-negative red blood cells (RBCs) were transfused on different occasions during his admission. To our surprise, 6 weeks after admission, the patient developed anti-D and anti-Mi^a antibodies, as demonstrated in online supplementary Figure S1 (for all online suppl. material, see https://doi.org/10.1159/000533625). The anti-Mi^a reacted from the room temperature phase to the 37°C phase, but not the indirect antiglobulin phase. It is difficult to know if this antibody was naturally occurring or alloimmunization due to a previous transfusion. We performed the Mi^a antigen typing from the patient's cells and found it to be Mi(a-). Unfortunately, information on the RhCE phenotype of the patient was not available, and he passed recently after anti-D was detected.

All units transfused into this patient were retrospectively investigated: nine of the ten donors had genotypically negative RhD using the multiplex sequence-specific primer (PCR-SSP) of *RHD* at intron 4, exon 7, and exon 10 [7]. The only unit positive for this multiplex PCR was then further tested for the *RHD*DEL1* allele using the PCR-SSP technique and was found to be positive for this variant (shown in online suppl. Fig. S2). The PCR-SSP method we utilized involved amplifying the extracted genomic DNA using the forward and reverse primers, along with the PCR conditions described in the previous literature [8]. In brief, the thermocycler was set as follows: denaturation at 94°C for 5 min, then 35 cycles of 30 s at 94°C, 40 s at 68°C, and 30 s at 72°C. The obtained 348 bp PCR products indicate the *RHD*DEL1* (c.1227G>A). The 629 bp growth hormone gene was used as an internal control.

The donor had the D–C+c+E–e+ phenotype. The duration from transfusion of the DEL unit (November 8, 2021) to anti-D detection (December 16, 2021) was 38 days; during this period, periodic antibody screening was performed and remained negative, including the test on December 2, 2021 (24 days after transfusion). The patient did not receive platelet products or Rh immunoglobulin during this admission. His last transfusion before this event was 5 years ago at a provincial hospital. We then retested the affected donor on the next visit and found similar genetic results. We coated donor RBCs with anti-D and conducted flow cytometry to quantify the D antigen [9]. Our findings revealed 22 D antigen sites on the donor RBCs, which confirmed the donor's DEL status.

The second case was a 38-year-old woman who developed anti-D after receiving four units of RBCs in a cytoreductive surgery with subtotal colectomy and hyperthermic intraperitoneal chemotherapy (HIPEC) procedure to treat pseudomyxoma peritonei. She had two RhD-positive children (more than 10 years ago) with an unknown history of Rh immunoglobulin prophylaxis in both pregnancies, and her last transfusion was 6 months earlier during a surgery. She was referred to our institute for further management. Her blood group typing was group A, D–C+c+E–e+, K–, and negative antibody screening. Two months earlier, the patient had undergone cytoreductive surgery with HIPEC and had received four units of group A, RhD-negative RBCs. After the follow-up imaging study pointed out the remaining tumors, the surgeons decided to set up another operation for tumor removal and requested six units of group A, RhD-negative RBCs. This time, 35 days after receiving RBC transfusions, her type and screen testing were positive for anti-D with the titer of 1:1,024. The patient did not receive any blood transfusions or Rh immunoglobulin after the first procedure. We reviewed the four negative RhD units transfused into this patient and detected one DEL unit. The donor serological test showed DEL by the adsorption and elution method with the D–C+c+E–e+ phenotype. The genotyping study of this donor demonstrated positive for D with the *RHD*DEL1* allele by PCR-SSP. Quantification of the D antigen by flow cytometry showed 32 D antigen sites on donor RBCs.

Discussion

The prevalence of DEL in RhD-negative blood donors in the Asian population is relatively high compared to Caucasians [4, 10]. However, routine serologic methods applied in clinical tests would type DEL as D-negative and would be transfused into D-negative recipients. The alloimmunization of anti-D from DEL donors in Asia has been reported previously [11–16], as summarized in Table 1, particularly by the Asian DEL allele. This allele was identified to have a complete RhD-positive epitope pattern [17, 18]. Anti-D is one of the clinically significant antibodies that can cause major hemolytic transfusion reactions and severe hemolytic disease of the fetus and newborn. In our cases, 2 RhD-negative patients were transfused with DEL, *RHD*DEL1* allele, and developed the D antibody. Focusing on the first case, the duration between alloimmunization was between 24 and 38 days after DEL blood transfusion. This would postulate that this patient developed a primary immune response caused by donors of the DEL phenotype with the *RHD*DEL1* allele. While for the second case, we do not have data on antibody testing in between to know the exact onset of anti-D alloimmunization. However, the very high anti-D titer on day 35 would suggest that this could be due to a secondary immune response rather than a primary response. Previous reports of anti-D alloimmunization from Asian-type DEL were mostly secondary immunizations, except for one Chinese recipient who reported having a primary immune response indicated by the onset of antibody development on day 22 [14]. We also show the scanty D antigen sites on both donor RBCs by flow cytometry. Therefore, this study supports that DEL can cause primary anti-D alloimmunization despite the small amount of D antigens on the RBC. This is the first study of the Southeast Asian population of anti-D alloimmunization from donors with DEL phenotype. There is only a limitated study on DEL in this region. The serological study in Myanmar showed a prevalence of 15.8% of the DEL phenotype among RhD-negative blood donors [19]. Therefore, further studies of DEL in the Southeast Asia area are warranted. A recent study by Nuchnoi and colleagues has suggested a cost-effective method for Asian-type DEL screening in Thai blood donors [20]. The current study

Table 1. Summary of previous reports of anti-D alloimmunization in Asia by DEL red blood cells

Case	Recipient	Age, years	Sex	Blood group	DEL exposure, units	DEL allele	Alloimmunization	Prior transfusion/ pregnancy	Reference
1	Japanese	67	F	B, D–	2 from 59	*RHD*DEL1*	Secondary	D+ transfusion 40 years	Yasuda et al. [11] (2005)
2	Taiwanese	64	M	D–	2 from 6	n/a	n/a	n/a	Chen et al. [12] (2006)
3	Taiwanese	73	M	AB, D–	4 from 6	n/a	Secondary? (day 6)	n/a	Chen et al. [12] (2006)
4	Korean	68	M	O, D–	1 from 4	*RHD*DEL1*	Secondary? (day 9)	None	Kim et al. [13] (2009)
5	Chinese	33	F	B, D–	1	*RHD*DEL1*	Secondary	Pregnancy	Shao et al. [14] (2012)
6	Chinese	45	M	D–	1 from 2	*RHD*DEL1*	Secondary	D+ transfusion 20 years	Shao et al. [14] (2012)
7	Chinese	68	M	D–	2	*RHD*DEL1*	Primary (day 22)	none	Shao et al. [14] (2012)
8	Russian (Korean donor)	64	M	A, D–	2	*RHD*DEL1*	Primary? (day 5–7)	none	Yang et al. [15] (2015)
9	Chinese	44	F	D–	1 from 4	*RHD*DEL1*	Secondary (day 4)	Pregnancy	Wen et al. [16] (2022)
10	Thai	73	M	B, D–	1 from 10	*RHD*DEL1*	Primary (day 24–38)	None	This study
11	Thai	38	F	A, D–	1 from 4	*RHD*DEL1*	Secondary? (day 35)	Pregnancy	This study

n/a, no available data; ?, did not have sufficient information to interpret.

would support the benefit of this practice, removing the DEL units from the D-negative inventory to improve patient safety. Whereas patients and pregnant women with Asian-type DEL should, probably, be treated as RhD-positive individuals without concern for D alloimmunization [21].

Statement of Ethics

Patients or their legal representatives have provided their written informed consent to gain access to and use of confidential and personal medical information used to write the manuscript and publish the manuscript in a scientific journal. No samplings or tests have been conducted for this article or manuscript. This study protocol was reviewed and approved by the Siriraj Institutional Review Board (SIRB), approval number [Si 262/2023].

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Kanyapon Suksard and Thongbai Rungroung were contributed to the serological testing of the samples. Komon Luangtrakool and Pradermchai Saetam performed the molecular assays. Sutthisak Chamsai performed flow cytometry for quantifying antigen site. Janejira Kittivorapart wrote the manuscript. Janejira Kittivorapart, Kulvara Kittsares, and Parichart Permpikul designed the experiments and critically examined the manuscript.

Data Availability Statement

All the data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

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