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Determination of the Rh/Kell phenotypes in donor as well as patients might be significant to provide phenotype-matched blood to cancer patients: A retrospective analysis from a tertiary care oncology center in North India

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Abstract:

BACKGROUND: Multiple reports are available from different parts of the globe indicating the incidences of alloimmunization and blood transfusion-related reactions, which emphasizes the need for phenotyping and providing antigen-matched safe blood.

AIMS AND OBJECTIVES: This study aims to determine the frequency of Rh and Kell antigens and phenotype for both donors and patients to propose the importance of providing Rh Kell phenotype cross-matched packed red blood cell (RBC) units to minimize the alloimmunization and transfusion reactions.

MATERIALS AND METHODS: Ten thousand blood donors and four thousand patients were investigated between October 2017 and July 2019. Each donor unit was tested for blood grouping, antibody screening, and Rh Kell antigen Phenotyping, and the blood unit was issued after the patient's blood grouping, antibody screening by 3 cell panels, and Rh Kell antigen phenotyping followed by cross-matching with an Rh Kell-matched phenotype RBC unit.

RESULTS: Nine thousand four hundred and fifty-two donors were D positive (94.5%) while 548 tested D negative (5.5%). Overall Rh and K antigens frequencies in donors were: "e" (98%) >"D" (94.5%) >"C" (86.6%) > "c" (57.5%) >"E" (18.8%) >K (0.98%). Among patients, 3762 tested D positive (94.05%), and 238 tested D negative (5.95%). Overall Rh and K antigens frequencies in patients were: "e" (98.5%) >"D" (94.05%) >"C" (90.2%) >"c" (51%) >"E" (18.2%) >K (1.8%).

CONCLUSION: Our study has given us more clarity on the prevalence of major Rh and K antigens in our donor as well as patient populations, highlighting the similarities as well as differences. This variance holds a great significance, since such donor units when transfused into patients may lead to alloimmunization and adverse transfusion reactions. Hence, the determination of Rh and Kell phenotypes and providing phenotype-matched blood will help prevent such events.

Keywords:

Adverse transfusion reactions, alloimmunization, phenotype-matched transfusion, red blood cell antigens, and antibodies

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Introduction

Red blood cell (RBC) transfusion is a life-saving therapy in patients with malignancies.[1] Alloimmunization is a complication of RBC transfusion that worsens with the amount of exposure to allogeneic transfusions and the antigen disparity between donor and recipient. Multiple reports are available from different parts of the globe indicating the incidences of alloimmunization and blood transfusion-related reactions, which emphasizes on the need for phenotyping and providing antigen-safe matched blood.[2,3]

Antibodies to the Rh system are the most implicated in all these reports of alloimmunization.[4,5] Variation in the Rh antigens between donors and patients is significant, as such transfusions may lead to alloimmunization and adverse transfusion reactions. This becomes even more significant in oncology patients, who undergo multiple blood transfusions. These patients can develop alloantibodies against antigens of the minor blood group systems, of which Rh and K are considered the most significant,[6-9] due to their prevalence and immunogenicity. Hence, the determination of Rh phenotypes by complete antigen phenotype matching can help in the selection of the RBC unit which has an antigenic composition the same as that of the recipient. However, it comes with a huge financial burden. Thus, a partial approach for matching/mapping of antigens (such as Rh [D, C, c, E, e] and K) that are mainly responsible for alloimmunization in multi-transfused cases can be taken as described in various studies.[1,3] In a study from myelodysplastic syndromes patients, the rate of alloimmunization was reduced to 11% versus 23% in institutions where prophylactic antigen matching for RhCE and K policy was considered.[10] Thus, "Partial matched blood" to the recipient, can significantly decrease the chances of alloimmunization or a transfusion reaction in an already alloimmunized individual.[11]

As discussed above, oncology patients undergo multiple blood transfusions, and there is an increased risk of the development of alloimmunization in them. Blood transfusion services (BTS) aim to provide adequate and safe blood (free from transmitted infections and compatible with the recipient) to minimize the transfusion reactions. We thus undertook the following randomized study at BTS at Rajiv Gandhi Cancer Institute (RGCI), New Delhi, with an aim to determine the frequency of Rh and Kell antigens and phenotype for both donors and patients to propose the importance of providing Rh Kell phenotype cross-matched packed RBC (PRBC) units to minimize the alloimmunization and transfusion reactions.

Materials and Methods

Study site and design

RGCI and Research Centre (RC) is a dedicated tertiary care Oncology center in Delhi which exclusively caters to all kinds of cancer patients from across and outside the country. Donors at our blood bank are from across India; 85%–90% are from North India such as Delhi NCR, UP, Haryana, Punjab, and Bihar; and 10%–15% from the rest of the country.

This retrospective study was conducted in the Department of Transfusion Medicine RGCI and RC and the data of 10,000 blood donors and 4000 patients (study population) tested between October 2017 and July 2019 was collected. Each donor unit at our blood bank was tested for blood grouping, antibody screening, and Rh Kell antigen phenotyping and issue of blood was carried out after the patient's blood grouping, antibody screening by 3 cell panels, and Rh Kell antigen phenotyping followed by cross-matching with an Rh/K matched phenotype RBC unit, on an automated platform to improve precision and accuracy.

Plasma samples

Three milliliters ethylenediaminetetraacetic acid) (EDTA) blood samples from donors at the time of donation and from the patients at the time of blood request received were collected as per our blood bank policy for Determination of Rh phenotypes & Kell antigen typing. The plasma was separated from the EDTA blood samples by centrifugation at 3000 RPM for about 15 min.

Measurements

The determination of ABO/D, Rh/K antigens were carried out using the Ortho® VISION automated platform (Ortho Clinical Diagnostics, Raritan, USA) using suitable BioVue® cassettes based on Column Agglutination technology. All Rh D-negative reactions were repeated for weak D testing as per the Blood Bank protocol and adhering to the National Guidelines. No weak D samples were detected among the population studied. Each serum sample was tested for ABO/D using the Biovue®ABO-Rh/Reverse grouping cassettes (707100) and Rh/K cassette (707280) (for C, c, E, e, and K antigens). Reverse grouping was performed using Affirmagen® reagent RBCs. Antibody Screening for donors was carried out with Ortho® Pooled Screening cells and patient samples using Surgiscreen® 3 Cell panels.

Data collection and analysis

All results were exported into Excel sheets directly from the hospital laboratory integrated system. The data were then analyzed for donors and patients. Statistical analysis was done using Microsoft Excel Software (Microsoft Redmond, Washington, USA). The frequency

of antigen or phenotype was calculated by totaling the number of donors or patients positive for an antigen or phenotype divided by the total number of donors or patients screened. Results are expressed as a percentage.

Results

Donor (D) and patients (P) data

Out of 10,000 donors studied [Table 1], 9814 were male (98%) and only 186 were female (2%). While out of 4000 patients studied [Table 2], 1722 (43.05%) were female and 2278 (56.95%) were male. Blood grouping (ABO typing) of donors and patients revealed "B" as the most common group at 37% (in donors) and 36.63% (in patients); followed by "O" with a frequency of 32% (in donors) and 30.8% (in patients); with next being "A" at 22% (in donors) and 23.3% (in patients); and finally, the least common being "AB" with frequency of 9% (in donors) and 9.23% (in patients). Bombay Phenotype was also identified in 1 sample.

Table 1: Antigen frequency (%) among the donors in the study: Frequency (%) of ABO, 5-Rh antigens (D, C, c, E, e) and C, c, E, and e in Rh D-positive and D-negative donors

	Percentage
Frequency of ABO (number of donors)	10,000
A	22
B	37
AB	9
O	32
Frequency of the 5 Rh antigens: D, C, c, E, and e (number of donors)	10,000
D	94.50
C	86.60
c	57.50
E	18.80
e	98.50
Frequency of the Rh antigens (C, c, E, and e) in D-positive donors (number of D-positive donors)	9452
C	90.20
c	55.60
E	19.80
e	98.20
Frequency of the Rh antigens (C, c, E, and e) in D negative donors (number of D negative donors)	548
C	24.30
c	90
E	2.60
e	99.80

Table 2: Antigen frequency (%) among the patients in the study: Frequency (%) of ABO, 5-Rh antigens (D, C, c, E, e), and C, c, E, and e in Rh D-positive and D-negative patients

Blood group	Frequency (*n*=4000), *n* (%)	D positive (*n*=3762) (%)	D negative (*n*=238) (%)
A	933 (23.33)		
B	1465 (36.62)		
AB	369 (9.22)		
O	1233* (30.83)		
D	3762 (94.05)		
C	3608 (90.2)	93.4	39.5
c	2039 (51)	49.7	71.8
E	727 (18.2)	19.1	4.2
e	3941 (98.5)	98.5	99.2

*1 example of Bombay Phenotype Oh was found

All the 10,000 donors and 4000 patients were also subjected to Rh D testing and all D-negative donors were further confirmed by performing the mandatory D weak (Du) test. Of the 10,000 donors analyzed, 9452 (94.5%) were D positive while 548 (5.5%) tested D negative. There was no weak D case detected [Table 1]. Of the 4000 patients analyzed, 3762 (94.05%) were D positive while 238 (5.95%) tested D negative [Table 2].

The prevalence of the other 5 Rh antigens tested in the donors and patient's population is shown in Tables 1 and 2, respectively. Table 1 shows that in the donor population, the frequency of "e" antigen was highest at 98.5%, followed by "D" at 94.5%, then "C" at 86.6%, "c" at 57.5% and "E" at 18.8%. Table 2 shows the frequency of these Rh antigens in patients; the "e" antigen was most common at 98.5%, followed by "D" at 94.05%, then "C" at 90.2%, "c" at 51% and "E" at 18.2%.

We have also analyzed the antigen frequency of C, c, E, and e antigens in the presence and absence of D antigen in the study population of donors and patients [Tables 1 and 2]. We observed the presence of C antigen at a frequency of 24.3% in Rh D-negative donors. Our data show the presence of the C antigen at almost 40% in Rh D-negative patients. We also observed a decrease in the prevalence of c antigen [Table 1].

Table 3 describes the Rh Phenotype frequencies found in blood donors and patients. In our study, 14 probable phenotypes were found. In donors, the most common was DCe/DCe (R1R1: 41.87%), followed by DCe/dce (R1r: 30.39%); while in patients, DCe/DCe (R1R1: 41.87%) was most common, followed by DCe/dce (R1r: 30.39%). Four rare phenotypes, (r'r" [0.04%], r"r [0.09%], r"r" [0.01%] and R^ZR^Z [0.01%]) in donors and 03 phenotypes (r'r [0.03%], r"r" [0.08%] and r^Yr^Y[0.03%]) in patients. The data also show that the prevalence of K antigen was 0.98% in donors and 1.8% in the patient population.

Discussion

Oncology patients undergo multiple blood transfusions and thus are at an increased risk of the development of

Table 3: Rh phenotype frequencies found in blood donors and patients in this study

Nomenclature		Percentage prevalence of phenotypes of patients	Percentage prevalence of phenotypes of donors
Fisher-race	Weiner		
DCCee	R^1R^1	46.73	41.87
DCcee	R^1r	27.93	30.39
DCcEe	R^1R^2	12.58	12.83
DCCEe	R^1R^Z	0.23	0.13
Dccee	R^0r	1.48	3.58
DccEe	R^2r	3.70	1.51
DccEE	R^2R^2	1.03	1.51
DCCEE	R^ZR^Z	0.40	0.01
ddccee	rr	0.13	4.05
dCcee	r'r	0.03	0.74
dCCee	r'r'	3.45	0.55
dCcEe	r'r"	0.60	0.04
dccEe	r"r	1.65	0.09
dccEE	r"r"	0.08	0.01
dCCEE	r^Yr^Y	0.03	-

alloimmunization. We thus undertook the following randomized study at Rajiv Gandhi Cancer Institute, New Delhi, with an aim to determine the frequency of Rh and Kell antigens and phenotype for both donors and patients in the region. With this we aim to emphasize the importance of the use of Rh/K phenotype cross-matched PRBC units to minimize the alloimmunization and transfusion reactions.

Our center receives patients from all over India, however, they receive blood transfusions from donors who are largely from the Northern part of the country. Therefore, any significant differences between the Rh antigenicity of the donors and patients would be extremely important as these would impact the quality of blood transfusions given. We thus evaluated the frequency of Rh and Kell antigens and phenotype for both donors and patients in our center and further to get a more comprehensive view of the Indian population, we compared our findings with other published studies from different parts of the country [Supplementary Table 1]. The trend of frequencies of Rh antigens in donors matches with the findings of other studies for North India.[6-9] However, the trend of the Rh frequency results from our patients matches the findings of our previous study. It is noteworthy that our patient data is a new finding as there is no previous publication to show the same.

Prevalence of Rh antigens

The prevalence of the other 05 Rh antigens tested in the donors matched the trends seen in the Indian population, with a little variance in absolute numbers from region to region observed within the country.[8,9,12,13] Our results show that the prevalence of D antigen in the study population is 94.5% and is comparable to that found in the other regions of the country [ranging from 92.25% to 94.1%, Supplementary Table 1]. Similar trends were observed for the prevalence of C, c, E, and e antigens when compared to the other regions of the country.[6-9,14-16]

We also analyzed the antigen frequency of C, c, E, and e antigens in the presence and absence of D antigen in the study population of donors and patients [Tables 1 and 2]. Our data is comparable to the published data from the other regions of the country.[8,9,12,13] However, our study observed a notable increase in the prevalence of C antigen in the absence of D antigen when compared to the data from South India. The presence of C antigen at a frequency of 24.3% in Rh D negative donors in our study varies from the available data.[8,9,12,13] Kahar and Patel[17] reported a 16.67% prevalence of C antigen in D-negative donors while Pachaury *et al*.[18] reported a frequency of 7% for C antigen in the D Negative population used in their study. In D-negative patients, the prevalence of C is at 39.5% with a corresponding decrease in the prevalence of c antigen. It is also important to note that while 98% of the donors are males, the patient data includes 43.05% females. This variance is of great significance since the Rh/K phenotype of patients or donors before transfusion is currently not practiced. However, a more detailed study is required to establish whether the increased prevalence of C antigen in D negative population can be attributed to the female gender. We could not find more data on female population to confirm or rule out this possibility.

Prevalence of Rh phenotypes and their frequencies

A comparison of the donor and patient data from our study shows significant Rh phenotype variations between the two populations. Our study found the prevalence of R1R1 and R1r as the most common phenotype like other studies from across the country [Supplementary Table 2]. However, there were some regional differences in the observed frequencies such as R1R2 shows a higher prevalence in the North as compared to the West while the South falls in between. Furthermore, within the 3 studies done in the North region, there were variations found in the R0r, R2r, and R2R2 phenotypes. Moreover, among Rh D negatives, rr was at variance between North and South as compared to West. A key point here is that if donors and patients are not phenotyped, it can lead to an increase in Rh alloimmunization with c antigen, given the practice of randomly transfusing Rh-Negative blood, since rr is the most common phenotype among donors.

In addition, about 0.7%–0.75% of donor unit find their way into discard if they do not get transfused to

K-positive patients. In our study, 0.98% of donors and 1.98% of patients tested positive for the K antigen. Thus, knowing the percentage of K-positive patients allows for some utilization of K-positive donor units.

Through our study, we are proposing an Rh Kell antigen mapping of donors and patients to prevent the complications of alloimmunization and better utilization of the blood units. The objective of this study was not only to gather data about the prevalence of the five important antigens of the Rh system namely D, C, c, E, e, and K from the Kell system, well known for their immunogenicity and ability to cause alloimmunization; but also to emphasize the significance and utility of providing Rh/K matched (partially matched) units of blood to the recipients.

Conclusion

Oncology patients who undergo multiple blood transfusions develop alloantibodies against antigens of the minor blood group systems, of which Rh and K are considered the most significant, due to their prevalence and immunogenicity. Multiple reports are available from different parts of the globe indicating the incidences of alloimmunization and blood transfusion-related reactions, which emphasizes on the need for phenotyping and providing antigen-matched safe blood. Antibodies to the Rh system are the most implicated in all these reports of alloimmunization. This study has significance for centers like ours who get patients from all over India, however, get donors who are largely from a given region. Our study has given us more clarity on the prevalence of major Rh antigens not only in our donors but also in our patient populations, highlighting the similarities as well as differences. Therefore, any significant differences between the Rh antigenicity of the donors and patients would be extremely important as these would impact the quality of blood transfusions given. It is noteworthy that our patient data is a new finding as there is no previous publication to show the same. With establishing a difference in the Rh/Kell phenotype in the cancer patients, we have thus highlighted the possibility of the significance of determination of Rh/Kell phenotypes, and providing phenotype-matched blood will help prevent such events in multi-transfused.

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Conflicts of interest
There are no conflicts of interest.

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Supplementary Table 1: Comparison of antigen frequency (%) among the donors in the study to the previously published studies: Frequency (%) of ABO, 5-Rh antigens (D, C, c, E, e) and C, c, E, and e in Rh D-positive and D-negative donors

Author	Present study	Chandra *et al.*	Sai Prasad *et al.*	Basu *et al.*	Raja *et al.*
Year	2020	2012	2018	2017	2016
Region	North India	North India	South India	East India	West India
ABO frequency found (%)					
Number of donors	10,000	23,320	43,839	1528	40,732
A	22	21.50	6.98	25	24.35
B	37	34.84	42.61	34	34.43
AB	9	13.91	4.80	9	8.94
O	32	29.75	45.59	32	32.26
Author	**Present study**	**Makroo *et al.***	**Thakral *et al.***	**Gundrajukuppam *et al.***	**Philip *et al.***
Year	2020	2014	2010	2016	2013
Region	North India	North India	North India	South India	West India
Frequency of the 5 Rh antigens: D, C, c, E, and e (%)					
Number of donors	10,000	51,857	1240	1000	10,133
D	94.5	92.7	93.39	94.1	92.25
C	86.6	89.55	84.76	88	87.55
c	57.5	58.64	52.82	54.9	51.06
E	18.8	19.85	17.90	18.8	26.55
e	98.5	98.80	98.30	98.4	98.42
Frequency of the Rh antigens (C, c, E, and e) in D-positive donors (%)					
Number of D positive donors	9452	48,071	1158	941	9348
C	90.2	93.9	90.15	92.5	90
c	55.6	55.5	49.48	52.1	-
E	19.8	21.3	18.9	19.4	-
e	98.2	98.7	98.1	98.3	94.6
Frequency of the Rh antigens (C, c, E, and e) in D-negative donors (%)					
Number of D-negative donors	548	3786	82	59	785
C	24.3	33.7	8.54	15.25	-
c	90	99.25	100	100	97.8
E	2.6	1.8	3.66	8.47	-
e	99.8	99.86	100	100	99.5

Supplementary Table 2: Comparison of Rh phenotype frequencies found in blood donors and patients in this study and earlier findings

Nomenclature		Present study, 2020 (North India)		Percentage prevalence of phenotypes of blood donors in other studies			
Fisher-race	Weiner	Percentage prevalence of phenotypes of patients	Percentage prevalence of phenotypes of donors	Makroo *et al.*, 2014 (North India)	Thakral *et al.*, 2010 (North India)	Gundrajukuppam *et al.*, 2016 (South India)	Philip *et al.*,* 2013 (West India)
DCCee	R^1R^1	46.73	41.87	40.95	43.8	43.4	35.2
DCcee	R^1r	27.93	30.39	30.91	30	31.2	30.7
DCcEe	R^1R^2	12.58	12.83	14.54	8.22	10.7	8.1
DCCEe	R^1R^Z	0.23	0.13	0.32	-	1.3	-
Dccee	R^0r	1.48	3.58	1.15	0.97	1.2	2.2
DccEe	R^2r	3.70	1.51	3.69	8.95	0.5	5.9
DccEE	R^2R^2	1.03	1.51	0.78	1.45	0.7	0.7
DCCEE	R^ZR^Z	0.40	0.01	0.002	-	0.4	-
ddccee	rr	0.13	4.05	4.76	5.81	4.7	0.3
dCcee	r’r	0.03	0.74	2.32	0.56	0.6	2.5
dCCee	r’r’	3.45	0.55	0.05	-	0.1	-
dCcEe	r’r”	0.60	0.04	0.075	-	0.2	-
dccEe	r”r	1.65	0.09	0.05	0.24	0.3	Rare
dccEE	r”r”	0.08	0.01	0.004	-	-	-
dCCEE	r^Yr^Y	0.03	-	-	-	-	-

*Rh phenotypes are presumed as genotype data of the Indian population is not known