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Assessment of rhesus and kell blood group antigens, phenotypes, and their allelic frequencies in North Indian blood donors

Sangeeta Pahuja, Sonal Jain, Manupriya Nain, Ruchika Goel, Shivali Sehgal, Manjula Jain

Abstract:

BACKGROUND: Prevalence of rhesus (Rh) and Kell antigens in a population vary with race, ethnicity, and geographical location. With advances in immunohematology, non-D antigens, and their corresponding antibodies are increasingly being found to be culprits for alloimmunization.

MATERIALS AND METHODS: Assessment of the phenotype of Rh and Kell blood group antigen in the healthy donor population from North India was done, and estimation of the frequencies of these alleles in our population was performed.

RESULTS: The most common antigen in the North Indian donor population was "e" (95.6%) followed by "C" (89.6%), "c" (57.7%), and "E" (17.29%) in that order. The most prevalent phenotype in the Indian population was found to be "CDe" followed by "CcDe" and "CcDEe." "K" antigen was found to be positive in 1.81% of the population.

DISCUSSION: Knowledge of the Rh antigen profiles in a given population can be very helpful in formulating transfusion guidelines specific to a particular population with an aim to minimize the cost and maximize the benefits. With this aim in mind and considering the problems encountered in developing countries like ours, we conducted Rh and Kell antigen profiling of donors. Comparative analysis with other population studies and implications for transfusion protocols is evaluated.

CONCLUSION: Assessment of Rhesus antigen profile of a particular population is useful to develop cost effective ways of providing maximum benefits of blood transfusion with least resources.

Keywords:

Alleles, kell, phenotypes, rhesus

Introduction

Rhesus (Rh) blood group is one of the most complex and polymorphic blood group systems. Observation by Levine *et al*. that the development of stillborn fetus (hemolytic disease of the newborn [HDN]) was associated with immune reaction to paternal antigens led insights into the complexities of Rh blood group system.[1] More than 150 RhCE and 500 RhD alleles have been documented. Anti-Kell is the most common immune red cell antibody outside ABO and Rh systems and is considered clinically significant, both from point of view of causing severe hemolytic disease of the fetus and newborn and hemolytic transfusion reaction.[2] Of the common Rh and Kell antigens, D is highly immunogenic followed by "K," "c," and "E."

D has been center of the spotlight for many decades while other antigens have remained in the shadows.[2] Several studies have

Department of Immunohematology and Blood Transfusion, Lady Hardinge Medical College, Connaught Place, New Delhi, India

Address for correspondence:
Dr. Sangeeta Pahuja, Blood Bank, Lady Hardinge Medical College and Associated Hospitals, Connaught Place - 110 001, New Delhi, India.
E-mail: sangeeta.pahuja.s@gmail.com

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revealed Rh and Kell antigens to be the major culprits of alloimmunization in multitransfused recipients and in HDN.[3-5]

Prevalence of Rh and Kell antigens in a population varies with race, ethnicity, and geographical location. Phenotypic distribution and allelic frequencies of blood group antigens are one of the major determinants of alloimmunization risks. Phenotypic profiling and assessment of allele frequencies help in evaluating the distribution of antigens in a particular population and at the same time helps in estimating the chances of getting compatible blood units for patients who have developed multiple antibodies.[6] Although large scale studies are available from European and other countries on Rh phenotypic profiling and allele frequencies, only a few studies are available from the subcontinent of India on the prevalence of Rh and Kell antigens.

We undertook this study to assess the phenotype of Rh and Kell blood group antigen in the healthy donor population and to estimate the frequencies of these alleles in the North Indian population.

Materials and Methods

An initial study was conducted in Regional Blood Transfusion Centre, Lady Hardinge Medical College, and Associated SSK and KSC Hospitals which have the combined capacity of 1227 beds. We undertook the pilot study to do Rh profiling (D, C, c, E, e) of 2000 blood donors coming to our blood bank. Donors who hailed from Delhi NCR and neighboring states were included in the study, representing the North Indian population. Immigrant population from other states was excluded.

Simultaneously, partial better match policy (phenotype matching for "c," "E," "K") was initiated for thalassemic patients in our hospital.[3] We retrieved the data of 22,095 North Indian donors over a period of 2 years, who were phenotyped for "c," "E," "K" apart from ABO and D. Therefore, we estimated the "c" and "E" in 24,095 (2000 + 22,095) donors, K in 22,095 donors and "C," "e" antigens in 2000 donors. The study protocol was approved by the Institutional Ethics committee. Consent was taken from donors at the time of donor screening.

Antigen profiling was done using monoclonal IgM antisera (Biorad) against "C," "c," "E," "e" antigens. For determination of D status, two antisera; monoclonal IgM, and monoclonal IgG + IgM were used (Tulip Diagnostics).

The antigens were tested by tube method. For each sample, 5% donor red cell suspension was added to respective antisera, incubated for 15 min and centrifuged for 20 s at 1000 g. The tubes were observed for agglutination, all negative reactions were confirmed microscopically. All D negative samples were tested for weak D using Biorad gel card system (ID-Diaclon Anti-D for weak D). After performing antigen profiling, we assessed the phenotype. We calculated the gene frequencies of "K" and "k" using Hardy–Weinberg equation[2]:

$p^2 + 2pq + q^2 = 1$, $P + q = 1$,

where p and q are gene frequencies of "K" and "k" alleles, respectively.

Results

The most common blood group was B (33.5%) followed by O (31.8%), A (25.2%), and AB (9.5%). Of 24,095 donors, 23,109 (95.90%) were Rh (D) positive and 986 (4.09%) were Rh (D) negative, confirmed by weak D testing.

The most common antigen in North India donor population was "e" (95.6%) followed by "C" (89.6%), "c" (57.7%) and "E" (17.29%) in that order. In the group of "D" positive donors, the frequency of "e" antigen was 95.4%, whereas it was present in 100% of Rh (D) negative donors. "C" was present in only 5% Rh (D) negative donors, but was present in 93.35% of Rh (D) positive donors [Table 1]. Prevalence of "c" and "E" was found to be more variable, "c" was found in 56.04% of Rh (D) positive donors and in 98% of Rh (D) negative donors. "E" was identified as least prevalent antigen (among C, c, E, e) with percent positivity in Rh (D) positive and Rh (D) negative donors being 17.95% and 1.92%, respectively. Concomitant presence of "C" with "D" was noted in 93.35% of donors.

Prevalence of "K" antigen was 1.81% with positivity being 1.84% in Rh D positive donors and 1.21% in Rh D negative donors. The antigenic prevalence of different antigens is given in Table 1.

Phenotype assessment was done for the donors in whom all five antigens were tested. The most frequent phenotype was "CDe," present in 42.2% of the donors followed by "CcDe" (34.5%). Among "D" positive donors also, "CDe" was most commonly found, present in 43.9%,

Table 1: Antigen prevalence

	Percentage of total (%)	Percentage of Rh positive (%)	Percentage of Rh negative (%)
C (*n*=2000)	89.6	93.35	5
c (*n*=24,095)	57.7	56.04	98.0
E (*n*=24,095)	17.29	17.95	1.92
e (*n*=2000)	95.6	95.4	100
K (*n*=22,095)	1.81	1.84	1.21

Rh=Rhesus

closely followed by "CcDe" (35.9%). "cde" accounted for 3.7% of all donations, whereas among "D" negative donors, it was the most common phenotype (92.5%). The phenotypes and their relative frequencies are given in Table 2.

A probable genotype can be predicted after reference to the known chromosome frequencies in the population. The most frequent probable genotype was R^1R^1 (CDe/CDe) followed by R1r (CDe/ce). The most probable and alternative genotypes are given in Table 2.

"K" antigen was found to be positive in 400 samples tested (22,095 donors), making the prevalence of "K" positivity in general population to be 1.81%.

Considering the gene frequencies of "K" and "k" to be *p* and q, respectively, Hardy–Weinberg equation was applied:

$p^2 + 2pq + q^2 = 1$, $p + q = 1$ (p^2 refers to KK, q^2 refers to kk and $2pq$ refers to Kk).

Since "K" antigen was expressed in 1.81% of donors,

$p^2 + 2pq = 0.0181$; $q^2 = 1 - 0.0181 = 0.9819$.

Therefore, $q = 0.9909$.

$p + q = 1$, $P = 1 - 0.9909 = 0.0091$.

Phenotype prevalence of KK, Kk and kk were evaluated by calculating p^2, $2pq$ and q^2 respectively [Table 3].

Discussion

Rh blood group system shows extensive polymorphism and allelic frequencies vary with different populations.[7] Alloimmunization rates (in multi-transfused patients and antenatal care (ANC) patients) and types of antibodies produced vary and are dependent on the heterogeneity of the population as well as the phenotype of that population.

Rh and Kell are known to be the most important culprits for alloimmunization.[8] Limited literature is available on antigen distribution, phenotyping, and assessment of allelic frequencies of Rh and Kell blood groups, particularly from developing countries like India.

Among Rh blood group system, the most frequent antigen in the North India population was found to be "e" closely followed by "C." Our results are in accordance with other studies from North India which have reported high prevalence of "e" antigen.[9-14] In Georgia, the prevalence of "e" antigen has been reported to be 100%.[15] Similarly, in most of the other studies "e" is highly prevalent antigen, being present in >90% of the population.[15-18] "c" and "E" antigens were more variable antigens in our study, accounting for 57.7% and 17.29%, respectively. Our results are similar to other studies from the Indian subcontinent.[9-14]

In our study, the prevalence of "C" antigen was 89.6% which is in accordance with similar studies from India.[9-14] However, the prevalence of "C" antigen has been reported as high as 93% in the population of Asian descent[2] and as low as 2.78% from Nigeria.[18] Antigen prevalence of different populations is tabulated in Table 4.

The most frequent phenotype in our study was found to be "CDe" followed by "CcDe" and "CcDEe." The three together accounted for 87.7% of all donors and 91.35% of Rh D positive donors. In Turkish donors

Table 2: Rh phenotypes and predicted probable genotypes of rhesus positive and rhesus negative donors (*n*=2000)

Rh phenotype	Most likely genotype	Other possible genotypes	Percentage of total	Percentage of Rh positive donors
Rh positive				
CCDee	R1R1	R1 r'	42.2	43.9
CcDee	R1r	R1R0	34.5	35.9
CcDEe	R1R2	R1r''/R2 r'/Rz r	11.0	11.6
ccDEE	R2R2	R2r''	2.7	2.8
ccDEe	R2r	R2R0	2.6	2.7
CcDEE	R2Rz	Rzr''	1.5	1.6
ccDee	R0r	R0R0	1.1	1.1
CCDEE	RzRz	Rz r^y	0.2	0.2
CCDEe	R1Rz	Rz r'	0.2	0.2
Rh phenotype	**Most likely genotype**	**Other possible genotypes**	**Percentage of total**	**Percentage of Rh negative**
Rh negative				
Ccdee	rr		3.7	92.5
CCdee	r'r'		0.1	2.5
Ccdee	r'r		0.1	2.5
ccdEe	r''r		0.1	2.5

Rh=Rhesus

"CcDe" has been reported to be the most common phenotype and "CDe" accounted for 21.8% of donors.[19] In the Bangladeshi population "CcDEe" was found to be the most frequent phenotype,[20] while it was ccDee in the Nigerian population.[17] Our results are in accordance with the data from India.[9,10,13,14] The most probable genotype was R1R1 (CCDDee) similar to results from north India,[9,10,14] while in Turkish donors and the Bangladeshi population, it is reported to be as R^1r and R^1R^2, respectively.[19,20] This further emphasizes the variability in Rh phenotypes of people of different races and geographic locales [Comparative analysis shown in Table 5].

Among non "D" antigens, "K" is known to be the most immunogenic, followed by c and E.[21] Moreover, they are also culprits of alloimunisation in multitransfused patients.[22] In our study, the prevalence of K antigen was 1.81%. Our results are similar to results of Gajjar *et al*. who have reported the prevalence of "K" antigen to be 1.78%. However, these rates are lower than those reported from North India, in which it has been reported as 5.56% and 3.5%.[9,10]

Table 3: Phenotype prevalence of 'K' and 'k'antigens and Allelic frequencies of antigens

Phenotype prevalence of 'K' & 'k'antigens	
Phenotype	**Frequency**
KK	0.00008
Kk	0.01803
kk	0.98188
Allelic frequencies of antigens	
Allele	**Frequency**
K	0.0091
k	0.9909

Table 4: Comparative analysis of antigen frequencies of different populations

	C (%)	c (%)	E (%)	e (%)
Present study	89.6	57.7	17.29	95.6
Asian[2]	93	47	39	96
White[2]	68	80	29	98
Blacks[2]	27	96	22	98
Palestine[6]	69	81	38	97
North India (Chandigarh)[9]	84.76	52.82	17.9	98.3
North India (Delhi)[10]	87	58	20	98
West India (Gujarat)[11]	91	50.5	16.5	100
East India (West Bengal)[12]	88.4	47.9	17.4	98
South India[13]	88	54.9	18.8	98.4
West India (Gujarat)[14]	88.77	55.89	17.88	99.07
Georgia[15]	61±4.8	89±3.2	23±4.2	100±0
Kalba[16]	73.2	71	21	97.3
Nigeria (2003)[17]	17.7	99.8	20.5	98.7
Nigeria (2005)[18]	2.78	100	18.89	95.56

Table 5: Comparative analysis of most common rhesus phenotypes of different populations

	Weiner	Fisher race
Present study	R1R1	CCDee
Caucasians[2]	R1r	CcDEe
Blacks[2]	R0r	CcDee
North India (Chandigarh)[9]	R1R1	CCDee
North India (Delhi)[10]	R1R1	CCDee
Nigeria[17]	R0r	CcDee
Turkish[19]	R1r	CcDe
Bangladeshi[20]	R1R2	CcDEe

It has been amply emphasized that antigen frequency and variability affect immunization rates. "c" antigen was found in 56.04% of D +ve donors, this implies that nearly 44% our D +ve patients would be "c" negative and there are >50% chances of them receiving "c" positive blood. Considering that "c" is the most immunogenic Rh antigen after "D," there are chances that "c" negative patient would be sensitized to "c" antigen if given unmatched blood. Hence, antigen frequency along with high immunogenicity of "c" could probably explain the occurrence of anti-c as a common antibody in multitransfused alloimmunized recipients.[3-5] The "E" antigen is a low-frequency antigen but is highly immunogenic and anti-E has been reported as one of the most frequent antibodies in multitransfused thalassemics from India.[3-5] On the other hand, high-frequency antigens, "C" and "e" are less immunogenic and are responsible for only mild cases of HDN.

The antigen prevalence of "K" antigen was 1.81%. This implies that 98.2% of donors are "K" negative. Therefore, for "K"; 98.2% of K negative recipient have 1.8% chances of getting "K" positive blood. This has significance when we have to devise the policy for transfusing patients of multitransfused group like thalassemia. This is in keeping with the alloimmunization results from the study on thalassemic children registered in our hospital. "c," "E," "K" together accounted for 60% of all antibodies formed, whereas, we did not find any C or e antibody. Our results were in concordance with other studies.[3-5]

Maternal sensitization to fetal red cell antigens resulting in, sometimes fatal, HDN is a challenge for both clinicians and transfusion medicine specialists. Previous studies have shown that prior red cell transfusion is the main risk factor for non "D" Rh immunization.[23] Since no immunoprophylaxis for these non "D" Rh antigens is available, its primary prevention by giving phenotypically matched blood to all women <45 years of age can prevent major morbidities. Anti "c" and "E" and "K" again hold the dubious distinction of being common culprits of HDN after "D." Considering the antigen profile of our population, giving "c" and "E" and "K" matched blood to all girls <45 years of age can prevent majority of the alloimmunizations.

As highlighted before, 93.35% of our "D" positive donors are also positive for "C" antigen, whereas only 5% of

"D" negative donors had "C" antigen. This implies 95% of Rh "D" negative mothers are also "C" negative, whereas their Rh "D" positive husbands have 93.35% chances of husband being "C" positive. Hence, they have high chances of being sensitized with "C" antigen along with "D" antigen. We have come across cases of anti-D with anti-C/G and are not very infrequent in Delhi. Anti-C is known to cause HDN. Thus, while screening Rh "D" negative mothers for irregular antibodies, due consideration should be given to anti-C along with anti-D and complete antibody identification panel should be put up in all the cases. This is of further relevance when treatment modalities like exchange transfusion are being considered, when child requires to be transfused with both D and C negative blood.

Conclusions

Knowledge of the Rh antigen profile of a particular population may be helpful in devising more cost effective ways of providing maximum benefits with least resources. However, extensive studies need to be done in this field so that treatment decisions can be taken in a more scientific, population specific, and a more cost-effective way.

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Conflicts of interest
There are no conflicts of interest.

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