

## Phenotypic Profile of Rh and Kell Blood Group Systems Among Rh Negative Blood Donors in Blood Centres of J.L.N. Medical College and Associated Group of Hospitals, Ajmer

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### Abstract

**Background:** The Rh and Kell blood group systems are highly immunogenic and crucial for safe transfusion and for preventing alloimmunization, especially in Rh-negative patients.

**Objectives:** To determine the prevalence of extended Rh (C, c, E, e) and Kell antigens and weak D Antigen among Rh-negative donors in Ajmer, and compare findings with other studies.

**Methods:** An 18-month cross-sectional study included 1,686 Rh D-negative donors. Standard serological techniques were used for ABO, Rh, Kell, and weak D testing. Data were analyzed using chi-square tests

**Results:** e antigen was present in 100% of donors, c in 98.45%, C in 10.37%, and E in 2.01%. The dominant phenotype was dccee (rr) (87.6%). Kell positivity was 1.66%. Weak D prevalence was 0.47% (95% CI: 0.15%–0.80%) and showed a significant association with the dCcee (r'r) phenotype ( $p < 0.001$ ). No significant links were seen with age, gender, or ABO groups.

**Conclusion:** The high dccee (rr) frequency and rare Kell and weak D highlight the need for extended Rh and Kell typing to reduce alloimmunization risks. Local data support improved matching for safer transfusion practices.

**Keywords:** Rh phenotype, Kell antigen, weak D, blood donors, alloimmunization.

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### Introduction

The discovery and classification of blood group systems remain pivotal milestones in transfusion medicine, drastically improving transfusion safety and patient outcomes. The ABO and Rhesus (Rh) blood groups are the most clinically significant, with the Rh system specially the D antigen being highly immunogenic and critically relevant in transfusion reactions and hemolytic disease of the fetus and newborn (HDFN). However advances such as Rh immunoprophylaxis have dramatically reduced HDFN incidence. [1]

The Kell system, second only to Rh in immunogenicity, is also vital for preventing alloimmunization, particularly in multi-transfused

patients and pregnant women. Despite this, transfusions in many settings still rely primarily on ABO and RhD matching, underscoring the need for extended phenotyping. Understanding Rh phenotypes, including weak and partial D variants, is essential for optimizing transfusion strategies, minimizing alloimmunization, and ensuring maternal-fetal safety. Expanding immunohematology testing and donor registries tailored to population-specific antigen frequencies will further enhance transfusion compatibility. [2]

Continued research into the genetic diversity of Rh and other blood group antigens, coupled with improved phenotyping and genotyping, remains

vital to advancing safe and effective transfusion practices worldwide

## Methods

**Study Design and Setting:** This prospective study was conducted at the Blood Centre, Department of Immunohematology and Transfusion Medicine, J.L.N. Medical College and Associated Hospitals, Ajmer, Rajasthan, India, over 18 months (May 2023 – October 2024).

**Participants:** All eligible Rh D-negative voluntary and replacement blood donors were included. Donors were selected per National Blood Transfusion Guidelines. Rh D-positive donors, ineligible donors, and samples inadequate for phenotyping were excluded. The final sample size was 1,686 donors.

**Sample Collection and Testing:** Blood samples were collected in EDTA tubes from diversion pouch at the time of donation. ABO and Rh D typing were performed by slide and tube techniques using monoclonal anti-D reagents. Weak D testing was done using the indirect antiglobulin test with

LISS/Coombs' gel cards. Extended Rh (C, c, E, e) and Kell (K) phenotyping was performed using DiaMed ID-cards with monoclonal antisera. No additional blood was drawn beyond routine collection.

**Statistical Analysis:** Data were analyzed using SPSS (v23) and Primer software. Results are presented in tables and graphs. Chi-square test was used for categorical variables; p-values <0.05 were considered statistically significant. Findings were compared with national and international studies to assess regional variation.

## Results

This study was conducted over a period of 18 months and included a total of 1,686 Rh D-negative voluntary and replacement blood donors at the Department of Immunohematology and Transfusion Medicine, J.L.N. Medical College, Ajmer. The objectives were to assess the distribution of extended Rh (C, c, E, e), Kell (K) antigens, and weak D detection in Rh D- negative individuals.

**Table 1: Prevalence of Rh Antigens, Kell and Weak D**

Antigen	Number	Percentage
e	1686	100%
c	1660	98.45%
C	175	10.37%
E	34	02.01%
KELL	28	01.66%
WEAK D	8	0.47%

The e antigen was universally present in all Rh D-negative donors (100%).

The c antigen showed high prevalence (98.45%), confirming its frequent occurrence in this population. The C (10.37%) and E (2.01%) antigens were relatively infrequent. The Kell

antigen (1.66%) was detected in a small proportion of donors, consistent with its known rarity and immunological significance.

Weak D antigen was detected in 8 donors (0.47%), underscoring the importance of detecting weak D for proper classification and transfusion decisions.

**Table 2: Prevalence of Rh phenotype**

Rh ANTIGENS	D-C-E-c+e+	D-C+E-c+e+	D-C-E+c+e+	D-C+E-c-e+
PHENOTYPE	dccee (rr)	dCcee (r'r)	dccEe (r'r')	dCCee(r'r')
Possible GENOTYPE	dce/dce	dCe/dce	dcE/dce	dCe/dCe
Our results	1477(87.60%)	149(8.83%)	34(2.01%)	26(1.54%)

The most prevalent Rh phenotype was dccee (rr) at 87.60%. The dCcee (r'r) phenotype was observed in 8.83% of donors. Rare phenotypes such as dccEe (r'r') and dCCee (r'r') were seen in 2.01% and 1.54%, respectively.

## Distribution of Donors by Age Group

A total of 1686 donors were included in the study. The age-wise distribution was as follows:

- 18–29 years: 751 donors (44.54%)
- 30–39 years: 611 donors (36.23%)

- 40–49 years: 259 donors (15.36%)
- 50–65 years: 65 donors (3.85%)

## Interpretation

The majority of donors (44.54%) belonged to the 18–29 years age group. A progressive decline in donor frequency was observed with increasing age.

The donor population was predominantly young adults, indicating better participation in blood donation among younger age groups, with reduced contribution from older individuals.

**Table 3: Distribution of Rh Phenotypes among Different Age Groups**

	Age			
	18-29 Years	30-39 Years	40-49 Years	50-65 Years
<b>dccee (rr) 1477</b>	654(44.27%)	541(36.62%)	219(14.82%)	63(4.26%)
<b>dCcee (r'r) 149</b>	70(46.97%)	52(34.89%)	26(17.44%)	1(0.67%)
<b>dccEe (r''r) 34</b>	16(47.05%)	10(29.41%)	7(20.58%)	1(2.94%)
<b>dCCee (r'r') 10</b>	10(38.46%)	9(34.61%)	7(26.92%)	0(0%)

Among Rh-negative donors, the dccee (rr) phenotype was the most prevalent, as expected. The dCcee (r'r), dccEe (r''r), and dCCee (r'r') phenotypes occurred less frequently. A slight variation in phenotype frequency was seen across age groups, but this was not statistically significant ( $\chi^2 = 7.94$ ,  $df = 9$ ,  $p = 0.54$ ). Overall, Rh phenotype distribution remained stable across ages, indicating no meaningful age-related trend.

**Table 4: Distribution of donors on gender basis**

Sex	No. of donors	Percentage
Male	1641	97.34%
Female	45	02.66%
Total	1686	100%

**Rh Phenotype Distribution by Gender**

A total of 1686 donors were analyzed, comprising 1640 males and 46 females.

The distribution of Rh phenotypes was as follows:

- dccee (rr) was the most common phenotype, observed in 1477 donors, including 1436 males and 41 females.
- dCcee (r'r) was found in 149 donors, with 146 males and 3 females.

- dccEe (r''r) was present in 34 donors, including 33 males and 1 female.
- dCCee (r'r') was observed in 26 donors, with 25 males and 1 female.

The donor pool was predominantly male. Among both genders, dccee (rr) was the most frequent phenotype. Statistical analysis showed no significant difference in Rh phenotype distribution between males and females ( $\chi^2 = 0.4023$ ;  $p = 0.9398$ ).

**Table 5: Rh Phenotype Distribution across ABO Blood Groups**

	B NEGATIVE	O NEGATIVE	A NEGATIVE	AB NEGATIVE
<b>dccee (rr) 1477</b>	524	489	315	149
<b>dCcee (r'r) 149</b>	67	40	28	14
<b>dccEe (r''r) 34</b>	9	11	9	5
<b>dCCee (r'r') 26</b>	14	8	2	2
<b>TOTAL</b>	614	548	354	170

B-negative was most common (36.4%), followed by O-negative (32.5%), A-negative (21%), and AB-negative (10.1%). No significant association between ABO blood group and Rh phenotype ( $p = 0.20$ ) suggests that the ABO distribution is independent of Rh phenotypic patterns in this population.

**Table 6: Rh Phenotype Distribution across KELL Positive and KELL Negative Donors**

Rh Phenotype	K+ (28)	K- (1558)
dccee (rr) – 1477	25	1452
dCcee (r'r) – 149	3	146
dccEe (r''r) – 34	0	34
dCCee (r'r') – 26	0	26

Overall Kell Antigen Prevalence Out of the total individuals studied, 28 (1.66%) were K+ (Kell positive) and 1558 (98.34%) were K- (Kell negative).

This confirms that the Kell antigen is relatively rare in this population, which aligns with known prevalence patterns in most populations.

**Distribution across Rh-Negative Phenotypes:**

- dccee (rr) phenotype: Largest group with 1477 individuals, of which 25 (1.69%) were

- K+ and 1452 (98.31%) were K-.
- dCcee (r'r) phenotype: 149 individuals, with 3 (2.01%) K+ and 146 (97.99%) K-.
- dccEe (r''r) and dCCee (r'r') phenotypes: No K+ cases detected, indicating complete absence of Kell antigen in these less common phenotypes.

**Statistical Association (Chi-Square Test):**

- Chi-square statistic ( $\chi^2$ ) = 1.0944,  $p = 0.778423$ .

- Since  $p > 0.05$ , there is no statistically significant association between Rh phenotype and Kell antigen expression.
- This indicates that in this study population, the distribution of the K antigen is independent of Rh phenotype.

#### Distribution of Weak D across Rh Phenotypes

A total of 1686 Rh-negative donors were analyzed for the presence of Weak D. Among them, 8 donors (0.47%) were Weak D positive, while 1678 donors were Weak D negative.

#### Phenotype-wise distribution:

- dceee (rr): 3 Weak D positive, 1474 negative (Total: 1477)
- dCcee (r'r): 4 Weak D positive, 145 negative (Total: 149)
- dccEe (r''r): 0 Weak D positive, 34 negative (Total: 34)
- dCCee (r'r'): 1 Weak D positive, 25 negative (Total: 26)

#### Prevalence of Weak D

The overall prevalence of Weak D was calculated as:  $(8 / 1686) \times 100 = 0.47\%$

The 95% Confidence Interval (Wilson Score) ranged from 0.15% to 0.80%, indicating a low but definite presence of Weak D in the studied population.

#### Statistical Analysis

- Chi-square ( $\chi^2$ ) = 24.13
- p-value = 0.0000336 ( $3.36 \times 10^{-5}$ )

This indicates a statistically significant association between Rh phenotype and Weak D expression.

#### Interpretation

The dCcee (r'r) phenotype showed a disproportionately higher number of Weak D cases (4 observed vs. ~0.72 expected), suggesting a strong association with Weak D expression.

This significant variation implies that:

- Certain Rh phenotypes (especially dCcee) are more likely to harbor Weak D
- These groups may require focused Weak D testing

#### Clinical Significance

Accurate detection of Weak D is essential to:

- Prevent alloimmunization
- Avoid hemolytic transfusion reactions
- Reduce risk of Hemolytic Disease of the Fetus and Newborn

Weak D prevalence was low (0.47%) but showed a significant association with Rh phenotype, particularly dCcee (r'r), highlighting the need for

targeted screening strategies to ensure safe transfusion practices.

This study analyzed Rh phenotypes based on the presence or absence of five major antigens (D, C, E, c, e) and their associated haplotypes using the Fisher-Race system (dce, dCe, etc.).

#### Most Common Phenotype:

- D- C- E- c+ e+ (dce/dce; rr): Found in 87.6% of Rh-negative donors. This genotype reflects the absence of D, C, and E, with presence of c and e antigens— the typical Rh-negative profile.

#### Less Common Phenotypes:

- D- C+ E- c+ e+ (dCe/dce; r'r): 8.83%; indicates the presence of C along with c and e, and absence of D and E.
- D- C- E+ c+ e+ (dCE/dce; r'r): 2.01%; rare, with E antigen present but no D or C.
- D- C+ E- c- e+ (dCe/dCe; r'r'): 1.54%; an uncommon homozygous genotype with C and e only.

#### Key Insight:

- Phenotypes expressing the C antigen are far less frequent than dce/dce, underscoring the predominance of the rr type in this Rh-negative group.
- These findings highlight the importance of extended Rh typing for transfusion compatibility and alloimmunization prevention, particularly in Rh-negative patients who may form antibodies to C or E antigens.

#### Discussion

Identifying antigen frequencies is crucial in transfusion medicine to minimize the risk of alloimmunization and hemolytic disease of the fetus and newborn (HDFN). Among the 43 ISBT-recognized blood group systems, the Rh and Kell systems remain the most clinically significant due to their high immunogenicity and clinical impact. [3] In our study, out of 1686 donors, 97.34% were male and 2.66% were female. This gender pattern is consistent with reports by Gundrajukuppam et al. [4], Mangwana et al. [5], and Lamba et al. [6], who also found male donors to constitute over 94% of their donor populations, citing sociocultural factors that limit female participation.

Our data highlight the extensive polymorphism of the Rh system. The e antigen was present in 100% of donors, which is in line with findings from Thakral et al. [7], Subramanian et al. [8], and Pahuja et al. [9], who also reported universal or near-universal e antigen prevalence among Rh-negative populations in India. The c antigen was found in 98.45%, comparable to Divjot Singh, [10]

Pahuja et al., and Gundrajukuppam et al., who reported c frequencies between 98–100%.

In contrast, the C antigen was found in 10.37%, aligning with Divjot Singh (10%) and Pahuja et al. (5%), but lower than Gundrajukuppam et al. (15.25%). The E antigen (2.01%) also matches trends in studies like Thakral et al. (0%) and Pahuja et al. (1.92%), confirming its status as the least prevalent but highly immunogenic Rh antigen.

The dccee (rr) phenotype was most frequent (87.60%) — a finding consistent with studies by Nanu & Thapliyal [11] (89.8%), Pahuja et al. (92.5%), and Rajeswari Subramaniyan et al. (93.3%). The dCcee (r'r) phenotype occurred in 8.83%, comparable to Nanu & Thapliyal (8.48%) but lower than Siransy Bogui et al. (19.57%). The rare phenotypes dccEe (r'r) and dCCee (r'r') remain uncommon, as confirmed by studies like Agarwal et al. [12], who found them absent or negligible.

The Kell antigen was present in 1.66%, consistent with studies by Garg & Singh [13], Kahar & Patel [14], and Pahuja et al., who all reported Kell frequencies between 1–2% in Indian donors. This is significantly lower than in European populations — for example, Akre [15] and Makroo et al. [16] reported higher rates (up to 5–9%) in France and Germany. Such differences highlight the ethnic variability of Kell distribution.

Weak D was observed in 0.47% of Rh-negative donors (95% CI: 0.15%–0.80%), similar to Indian data by Devi G et al. [17] (0.43%) and Pratima K et al. (18) (0.58%). In contrast, higher rates have been reported internationally, such as Cruz BR et al. [19] in Brazil (0.8%) and Githiomi et al. [20] in Kenya (2%). A significant association ( $p < 0.001$ ) was found between Weak D and the dCcee (r'r) phenotype, echoing the pattern noted by Opoku-Okrah et al. [21] in Ghana, who also suggested a phenotype link with Weak D expression.

Clinical Relevance of this phenotypic mapping confirms the predominance of the dccee (rr) type and the universal presence of the e antigen, consistent with prior regional and global studies. It highlights the importance of extended phenotyping, particularly for patients with chronic transfusion needs, such as those with thalassemia and sickle cell disease, as emphasized by Akre, who documented high alloimmunization rates in multi-transfused patients.

Similarly, the low but significant presence of Kell and Weak D underscores the necessity for routine Kell typing and Weak D genotyping, as recommended by Makroo et al. and Pahuja et al., to reduce transfusion complications and prevent HDFN.

## Conclusion

Our findings align closely with other Indian and international studies, confirming known patterns while adding region-specific data for Ajmer. Maintaining well-characterized donor registries with extended Rh and Kell typing will help minimize alloimmunization and improve transfusion safety.

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