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Original Research Article

PREVALENCE AND DISTRIBUTION OF RH BLOOD GROUP ANTIGENS AND PHENOTYPES IN BLOOD DONORS AT A TERTIARY CARE INSTITUTE IN WESTERN UTTAR PRADESH: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: The Rh system, most immunogenic after ABO, contributes significantly to alloimmunization when red cells with incompatible antigens are transfused. Determining regional Rh antigen and phenotype frequencies is essential for improving antigen-matched transfusion and reducing alloimmunization risk. The present study was undertaken to observe the frequency distribution and prevalence of Rh antigens and phenotypes in the local blood donor population and to compare the difference between proportions between various groups.

Materials and Methods: This cross-sectional study was conducted at the blood centre of a medical institute in Western Uttar Pradesh. Donor records from April 2017 to March 2022 were retrieved and analysed for the distribution and prevalence of Rh antigens and phenotypes across gender and ABO groups.

Results: Among 54,986 donors (97.64% males, 2.36% females), 97.97% were Rh-D positive. Blood group B was most common (35.67%), followed by O (29.60%), A (24.62%) and AB (10.11%). The e antigen was most prevalent (98.92%), followed by D (97.97%), C (90.62%), c (52.80%), and E (19.60%). Antigen E showed higher prevalence in females ($p = 0.004$). Rh antigen distribution varied significantly across ABO groups ($p < 0.00001$). Rh-D negative donors showed higher c and e frequencies ($p = 0.02$). Fourteen Rh phenotypes were identified, with CCDee being the most common (44.40%), and phenotype CCDEe showed a significant gender difference ($p = 0.032$).

Conclusion: A varied distribution of Rh antigens and phenotypes among donors highlights the need for rational transfusion practices. Antigen-matched transfusions can reduce alloimmunization risks in transfused and pregnant individuals.

Keywords: Rh antigens, Rh phenotypes, Rh-antigen frequency, antigen-matched transfusions, blood donor.

INTRODUCTION

The human Rh blood group system, historically misnamed “Rhesus”, was discovered through the clinical observations of P. Levine and R.E. Stetson in 1939 and the experimental studies of K. Landsteiner and A.S. Wiener in 1939–1940. It is now recognized as a complex system comprising over 60 distinct antigens.[1,2] Of all the Rh antigens, the major clinically significant antigens are D, C, c, E, e.[3] The two genes encoding the Rh antigens are situated on chromosome 1. RHD gene encodes the RhD antigen

while RHCE gene encodes two polypeptides C and E following mRNA splicing leading to eight haplotypes, Dce, dce, DCe, dCe, DcE, dcE, DCE and dCE.[4,5] Varied inheritance of Rh genes in individuals results in different observable expressions of the Rh antigens on the red cell surface.[6]

As the Rh antigens are highly immunogenic, with D being second only to the ABO antigens in clinical significance, alloimmunization through transfusion, feto-maternal haemorrhage during pregnancy, or transplantation, remains a potential risk in individuals exposed to non-self red cell antigens. These antigens are clinically important due to their association with delayed hemolytic transfusion reactions and hemolytic disease of the fetus and newborn, a major cause of perinatal mortality and morbidity.[7,8] Although such adverse clinical outcomes have been frequently reported with anti-D antibodies, alloantibodies against C, c, E, e have also been implicated in several cases.[9-11] Knowledge of the Rh antigen distribution among local donors enables transfusion services to provide appropriate, crossmatched, antigen-negative blood, preventing alloimmunization and its complications in transfusion and pregnancy.

Given the absence of data on the Rh antigen profile of blood donors in the Western Uttar Pradesh of North India, the present study was undertaken to evaluate the frequency distribution and prevalence of Rh antigens (D, C, c, E, e) and their phenotypes across gender and ABO types (A, B, AB and O). Further, comparisons were made between groups to assess frequency distributions and differences in proportions.

MATERIALS AND METHODS

This retrospective cross-sectional observational study was conducted at the Blood Centre, Department of Immuno-haematology and Blood Transfusion, of a tertiary care medical institute in Western Uttar Pradesh, India. Donor records from April 2017 to March 2022 were retrieved from the electronic database. The dataset included 54,986 voluntary and replacement donors typed for Rh antigens D, C, c, E,e.

All donors were screened and selected according to national guidelines, including counselling, medical examination, and statutory deferral criteria. [12,13] Blood was collected in 350ml or 450ml bags. Fresh blood samples mixed with CPD anticoagulant, collected through the tubing of the blood bag into barcode-labelled sterile, EDTA and plain vials for routine blood grouping, Transfusion transmissible infections (TTIs) screening and antibody screening.

For antigen typing of donor, red cells for ABO and Rh system antigens, a fully automated immunohematology analyser (NEO, USA) was used, using monoclonal antisera and haemagglutination microplates (Immucor, India). Monoclonal antisera Anti-A, Anti-B, Anti-AB were used for ABO typing, Novaclone Anti-D (IgG + IgM) Monoclonal Blend and Anti-D Monoclonal Blend, were used for Rh-D typing. Other Rh antigens were typed using Monoclonal Anti-C, Anti-E, Anti-c, Anti-e antisera.

Data on donor gender, ABO type, Rh-D status, and Rh (C, c, E, e) were compiled in Microsoft Excel and analysed descriptively as frequencies and percentages. Antigen and phenotype prevalence was calculated overall and by gender, ABO group, and Rh-D status [Table 1]. Comparisons between groups were performed using Chi-square (χ^2) and Z-tests, with $p < 0.05$ considered statistically significant.

RESULTS

The present study enrolled 54,986 blood donors, replacement and voluntary, of which 97.64% (n=53,691) were males and 2.36% (n=1,295) were females. Rh-D positive and Rh-D negative blood donors comprised 97.97% (n=53,872) and 2.03% (n=1,114), respectively. Among ABO groups, B was the most common (35.67%, n=19,616), followed by O (29.60%, n=16,274), A (24.62%, n=13,537) and AB (10.11%, n=5,559).

The distribution of Rh antigens in the study population and across genders is presented in Figure 1. Overall, e antigen showed the highest prevalence, followed by D, C, c, and E. The antigen distribution pattern was similar in males and females with no significant gender related difference in overall Rh antigen frequencies ($p = 0.536$). However, E antigen, showed a significantly higher prevalence among female donors compared to males ($Z = 2.0135$, $p = 0.004$).

Figure 2 illustrates the distribution of Rh antigens across ABO groups. Although e antigen remained the most common, followed by D, C, c, and E in all groups, the relative frequencies varied significantly among ABO types ($\chi^2 = 149.16$, $p < 0.00001$). Antigens D and e were most frequent in group O, while c was highest in AB, C in group B, and E in AB donors.

Figure 3 depicts the distribution of Rh antigens with respect to Rh-D status and gender. In both Rh-D positive and negative donors, e was the most frequent antigen, followed by C, c, E in Rh-D positive and c, C, E in Rh-D negative donors. Rh-D negative donors showed significantly higher frequencies of c and e compared with Rh-D positive donors ($p = 0.02$). Gender-wise differences within each Rh-D group were minimal, with only minor variations in the prevalence of C, c, and E antigens.

Table 2 summarizes the overall and gender-wise distribution of Rh phenotypes. Fourteen phenotypes were identified, with CCDee being the most common and ccddEE the rarest. Although minor gender differences were noted for several phenotypes, most variations were small. A statistically significant difference between males and females was observed only for phenotype CCDEe ($Z = –2.1381$, $p = 0.032$).

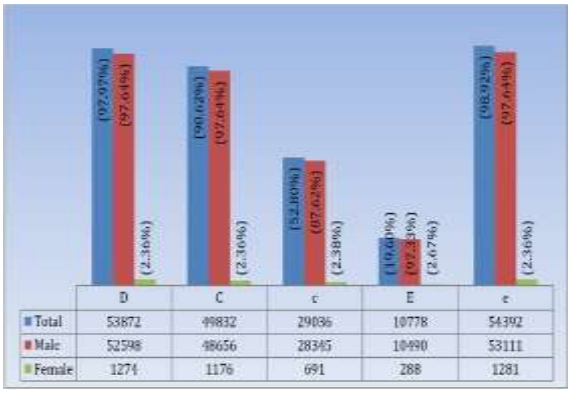

Figure 1: Frequency distribution of Rh antigens among blood donors, total and gender wise

Figure 2: Frequency distribution of Rh antigens with respect to ABO group of blood donors

[Table 3] presents the distribution of Rh phenotypes across ABO groups. Overall, phenotype patterns were comparable among A, B, O, and AB donors, with only small variations in prevalence. A few phenotypes were absent in specific ABO groups, such as ccddEE and CCddEe in groups A and O, and CCddEe in group AB.

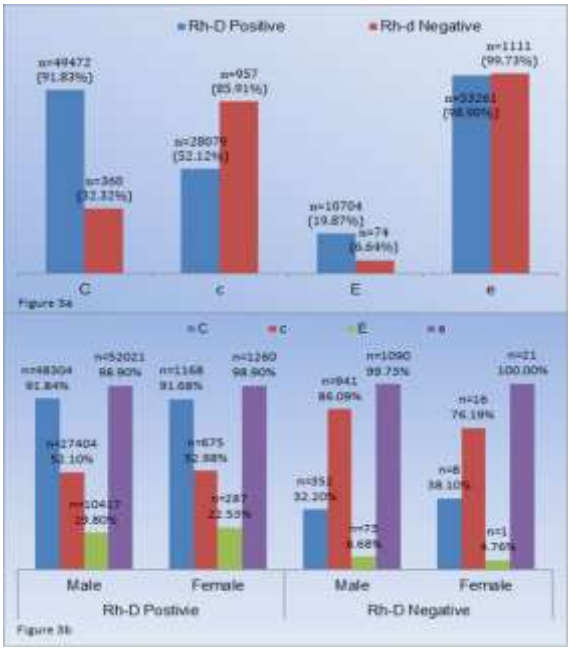

Figure 3: 3a Prevalence of C, c, E, e Rh antigens with respect to Rh-D status of blood donors. 3b Prevalence of other Rh antigen among Rh-D positive and D Negative donors with respect to gender.

Table 1: Formulae for calculating prevalence

Type of Prevalence	Formula for calculating prevalence
Prevalence of ABO, Rh types in Blood Donors	$\frac{\text{Total number of specific ABO type donors}}{\text{Total number of blood donors}} \times 100$
Total Prevalence of Rh antigen (D, C, c, E, e) in blood donor	$\frac{\text{Total number of specific Rh type donors}}{\text{Total number of blood donors}} \times 100$
Prevalence of Rh antigens/ phenotypes with respect to gender	$\frac{\text{Total number of specific Rh Antigen/ Phenotype in a specific gender}}{\text{Total number of Blood Donors in the specific gender}} \times 100$
Prevalence of Rh antigen with respect to ABO type	$\frac{\text{Total number of specific Rh Antigen/ Phenotype in a specific ABO type}}{\text{Total number of Blood Donors inthe specific ABO type}} \times 100$
Prevalence of Other Rh antigen with respect to Rh- D	$\frac{\text{Total number of other specific Rh Antigen/ Phenotype in a specific Rh - D type}}{\text{Total number of Blood Donors in the specific Rh - D type}} \times 100$

Table 2: Frequency distribution & prevalence of Rh Phenotypes with respect to gender

S. No.	Rh Phenotype (Fisher and Race)	Total		Male		Female	
		n	Prev.	n	Prev.	n	Prev.
1.	CCDee	25316	46.04%	24741	46.08%	575	44.40%
2.	CcDee	16576	30.15%	16195	30.16%	381	29.42%
3.	CcDEe	7103	12.92%	6915	12.88%	188	14.52%
4.	ccDee	1276	2.32%	1245	2.32%	31	2.39%
5.	ccDEe	2533	4.61%	2472	4.60%	61	4.71%
6.	ccDEE	591	1.07%	577	1.07%	14	1.08%
7.	CCDEe	477	0.87%	453	0.84%	24	1.85%
8.	CCddee	153	0.28%	148	0.28%	5	0.39%
9.	Ccddee	154	0.28%	151	0.28%	3	0.23%
10.	CcddEe	49	0.09%	49	0.09%	0	0.00%
11.	ccddee	733	1.33%	721	1.34%	12	0.93%
12.	ccddEe	18	0.03%	17	0.03%	1	0.08%
13.	ccddEE	3	0.01%	3	0.01%	0	0.00%
14.	CCddEe	4	0.01%	4	0.01%	0	0.00%

Table 3: Prevalence & Frequency distribution of Rh phenotypes with respect to blood group (A, B, O, AB)

Rh	A		B		O		AB	
Phenotype	n	Pre.	n	Pre.	n	Pre.	n	Pre.
CCDee	6142	45.37%	9197	46.89%	7588	46.63%	2389	42.98%
CcDee	4018	29.68%	5880	29.98%	4985	30.63%	1693	30.46%
CcDEe	1795	13.26%	2456	12.52%	2025	12.44%	827	14.88%
ccDee	332	2.45%	443	2.26%	375	2.30%	126	2.27%
ccDEe	664	4.91%	855	4.36%	764	4.69%	250	4.50%
ccDEE	149	1.10%	210	1.07%	168	1.03%	64	1.15%
CCDEe	161	1.19%	190	0.97%	51	0.31%	75	1.35%
CCddee	39	0.29%	48	0.24%	44	0.27%	22	0.40%
Ccddee	29	0.21%	54	0.28%	49	0.30%	22	0.40%
CcddEe	11	0.08%	19	0.10%	13	0.08%	6	0.11%
ccddee	192	1.42%	251	1.28%	207	1.27%	83	1.49%
ccddEe	5	0.04%	7	0.04%	5	0.03%	1	0.02%
ccddEE	0	0.00%	2	0.01%	0	0.00%	1	0.02%
CCddEe	0	0.00%	4	0.02%	0	0.00%	0	0.00%

DISCUSSION

Differences in genetic diversity across populations lead to substantial variation in red blood cell antigen expression among ethnic groups,[14] and even mild variability within the same population across different regions.[15–18] In a setting without routine molecular testing, red cell phenotyping remains essential to prevent accidental exposure to foreign antigens that may cause alloimmunization or hemolytic disease of the fetus and newborn.[1] The present study was undertaken to derive region-specific data on the distribution of Rh antigens, phenotypes, and probable genotypes among blood donors in Western Uttar Pradesh, with the aim of supporting antigen-matched transfusion in at risk individuals.

Gender distribution of blood donors: Male donors outnumbered females(97.64% vs 2.36%), similar to studies from Maharashtra by Sarkar et al.[15](96.00 % vs 4.00%), New Delhi by Makroo et al.[16](96.01% vs 3.99%), Chennai by Chitra et al.[17] (94.00% vs 6.00%), New Delhi by Mangwana et al.[19] (97.26% vs 2.74%), New Delhi by Pathak et al.[20] (98.00% vs 2.00%) and Patna by Ranjan et al.[21](97.96% vs 2.03%). A slightly more female representation was observed from Kolkata by Basu et al.[18] (88.87% vs 11.12%), New Delhi by Agarwal et al.[22] (87.40% vs 12.60%) and Bengaluru by Dharshan et al.[23] (90.40% vs 9.60%).The predominance of males in hospital-based centres was attributed to their role as accompanying attendants and higher deferral rates among females. In contrast, studies from Bangladesh and Pakistan reported a nearly equal gender distribution.[24,25]

Rh antigens: Total Prevalence

e antigen: The prevalence of e antigen in the present study (98.92%) was similar to high frequencies (>98%) reported from across India, including Maharashtra by Sarkar et al.[15]), New Delhi by Makroo et al.[16], Mangwana et al.[19], and Pathak et al.[20]), Gujarat by Shah et al.[26]) and Tamil Nadu by Chitra et al.[17] Slightly higher prevalence was observed in Gujarat by Gajjar et al.[27] and Davad et al.[28]) (99.07% and 99.67%, respectively) and in Bihar(99.00%) by Ranjan et al.[21]) A lower prevalence of 77.30% was observed by Dharshan et al.[23] in Bengaluru. The consistently high frequency of the e antigen in most regions minimizes the risk of alloimmunization to anti-e.

D antigen: The frequency of the Rh-D antigen in Indian blood donors is high, with most studies reporting frequencies above 90%in various regions such as New Delhi, Maharashtra, West Bengal, Andhra Pradesh, Gujarat and Karnataka. In the present study, Rh D-positive donors comprised 97.97%, similar to observations by Basu et al.[18] (96.60%), Birader et al.[29] (96.20%) and Shah et al.[26] (96.2%).Slightly lower D positive rates were reported by Sarkar et al.[15] (92.27%), Davad et al.[28] (92.20%) and Makroo et al. (92.70%).[16]In the present study, Rh D-negative donors constituted 3.05% of all blood donors, which was lower than the prevalence rates reported by Gundrajukuppan et al.[6] (5.9%), Makroo et al.[16] (6.30%), Dharshan et al.[23] (6.10%) and Davad et al.[28] (7.80%).

C antigen: Prevalence of Rh C antigen in various parts of the country has been reported between 81.70% and 96.50%. The prevalence observed in the present study was 90.62%, comparable to findings from New Delhi (89.56%) by Makroo et al.[16], Gujarat (88.77%) by Gajjar et al.[27] and 89.40% by Shah et al.[26]).Slightly lower rates were reported from Surat (81.74%) by Kahar et al.[32] Bengaluru (82.50%) by Dharshan et al.[23] and Tamil Nadu (84.00%) by Chitra et al.[17])Regional differences in genetic inheritance may explain some variation. However, variability in anti-C antisera performance can also contribute. Anti-C derived from DCE/dce cells may show weaker reactions due to fewer C antigenic sites, leading to potential false-negative serological results. Genotyping is therefore recommended for more accurate determination of antigen prevalence.[33]

c Antigen: In the present study, the prevalence of the c antigen was 52.80%, which falls within the range reported across India. Earlier studies have demonstrated substantial regional variation, with frequencies ranging from 49.30% in Bengaluru (Dharshan et al).[23]to 67.00% in Tamil Nadu (Chitra et al).[17] Other reported frequencies include 50.47% from Patna (Ranjan et al.)[21], 54.90% from Andhra

Pradesh (Gundrajukuppan et al.)[6], 54.80% from Gujarat (Shah et al.)[26], 57.50% from New Delhi (Pathak et al.)[20] and 62.00% from Jammu (Gorkha et al.)[31] These variations likely reflect regional genetic diversity among donor populations.

E Antigen: In the current study the prevalence of E antigen was19.60%. Earlier studies showed regional variability, with frequencies ranging from 15.90% to 36.20%. The highest prevalence was reported from Bengaluru (36.20%) by Dharshan et al.[23] followed by Tamil Nadu (25.00%),by Chitra et al.[17] Reports from New Delhi showed frequencies of 19.85%, 19.66%, and 18.80% (Makroo et al., Mangwana et al., and Pathak et al., respectively).[16,19,20] Similar findings include 19.40% from Jammu (Gorkha et al.)[31],18.80% from Andhra Pradesh (Gundrajukuppan et al),[6] 18.60% and 17.08% from Gujarat by Shah et al. and Gajjar et al., respectively[26,27], with the lowest frequency reported from Patna (15.90%) by Ranjan et al.[21]This variability likely reflects underlying genetic and demographic diversity across regions. Multicentric studies with larger, ethnically representative donor cohorts would help to establish a national baseline data for Rh antigen distribution.

India and bordering countries like Pakistan and Bangladesh exhibit a similar prevalence pattern for Rh antigens, D, C (e>D>C); however, differences were observed in the distribution pattern of E and c antigens. A study from Pakistan (Karachi, Tariq et al.),[25] reported c (66.50%) as the least prevalent antigen (e > D > C > E > c), whereas other previous Indian studies and the present study consistently identify E as the least common antigen (e > D > C > c > E).

Rh antigens with respect to Rh D status

In this study, e was the most prevalent antigen in both males and females (99.31% and 99.45%, respectively) irrespective of Rh-D status, similar to the findings of Sarkar et al.[15]The prevalence of C among D-positive(91.83%) and D-negative donors (32.32%) was comparable to observations from Chitra et al.[17], Pathak et al.[20]and Kahar et al.[32] A strong association of c and e antigens with Rh D-negative status was also noted (prevalence 85.91% and 99.73%, respectively), similar to reports by Makroo et al.[16], Chitra et al.[17]and Lamba et al.[30]

Rh antigens with respect to gender

In the present study, prevalence of E antigen was more in females than in males (22.24% vs 19.54%), while the frequencies of other Rh antigens were comparable between genders. The overall antigen prevalence was similar in both groups. Indian studies have not reported gender specific Rh antigen distribution, these findings cannot be directly compared, necessitating further studies with larger female donor representation.

Rh antigens with respect to ABO type

This study explored Rh antigen distribution in relation to ABO blood groups, unlike most Indian studies. The e antigen showed uniformly high prevalence (98.9%) across all ABO types and D antigen varied minimally (97.57%–98.05%). For both these antigens, prevalence followed the same overall order, O > B > A > AB with only minor variation in percentages. The prevalence order of C, c and E were B > O > AB > A, AB > A > O > B and AB > A > B > O, respectively.

Ethnic variation of Rh antigens

Rh antigen frequencies differ markedly across ethnic groups—E>c>D>C>e in Blacks; D>e>C>c> E in Asians; and e>D>c>C > E in Caucasians.[1,34] The present study was consistent with previous Indian data demonstrating the pattern e > D > C > c > E.[16,17,20,23,26]Populations with greater ethnic admixture have a higher tendency toward alloimmunization, as seen in Dutch datasets showing large numbers of clinically significant antibodies, leading to recommendations for extended phenotype-matched transfusion.[35]

Rh Phenotypes

A review of multiple studies indicates that both sample size and heterogeneity of population influence the number of Rh phenotypes detected. Larger cohorts, such as those from New Delhi (Makroo et al. 51,857 donors),[16] and present study (54,986donors) reported 15 and 14 phenotypes, respectively. Similar findings from Delhi were noted by Mangwana et al. [19], (13 phenotypes; 24,745 donors) and Pathak et al.[20] (14 phenotypes; 10,000 donors).

In contrast, smaller studies generally reported fewer phenotypes, such as Chitra et al.[17] in Chennai (8 phenotypes; 100 donors) and Kahar et al. [32] in Surat (10 phenotypes; 115 donors). However, studies with moderate sample sizes- Dharshan et al. [23] in Bengaluru (1,000 donors), Gajjar et al. [7] in Gujarat (1,670 donors), and Basu et al,[18] in Kolkata (1,528 donors) identified 23, 15, and 16 phenotypes, respectively, suggesting greater genetic diversity in these regions. This pattern aligns with demographic data indicating that states such as Gujarat and West Bengal exhibit greater ethnic and genetic heterogeneity, partly due to interstate migration for marriage and livelihood.[36] Such diversity may widen Rh antigenic and phenotypic variation, potentially increasing the risk of alloimmunization in these populations.

Rh Phenotypes with respect to gender

Data on gender wise distribution of Rh phenotypes in India is limited, making inter-study comparison difficult. In this study, 14 phenotypes were observed in males and 11 in females, a difference attributable to the markedly lower female representation (male:female = 47:1). Phenotypes not detected among females included CcddEe, ccddEE and CCddEe, while phenotypes such as CcDEe, ccDEE, CCDEe, ccddee and ccddEe showed slightly higher proportional representation in females [Table 3]. Greater female donor participation in future studies would allow more reliable assessment of phenotype and probable genotype distribution.

The range of phenotypes identified in this study was similar to those reported by Makrooet al.[16] and Basu et al.[18] However, Basu et al.[18] did not observe CcddEe, Ccddee or ccddEE, while Makroo et al.[16] did not report CCddEe. Phenotypes not observed in the present study included CCDEE, CcddEe, CCddEE and CcDEE.

Rh phenotypes with respect to ABO type

In this study, Rh phenotype distribution showed only mild variation across ABO blood groups, and none of these differences were statistically significant ($p > 0.05$). CCDee was the most common phenotype in all ABO groups, followed by CcDee and CcDEe, each showing uniform distribution across A, B, O and AB donors [Table 4]. Less frequent phenotypes such as ccDee, ccDEe, ccDEE and CCDEe were observed in low proportions across all groups, with latter two more common in AB donors. Rare phenotypes like CCddee, Ccddee, ccddee, CcddEe, ccddEe, ccddEE and CcddEe occurred at very low frequencies with first three more common in AB donors and remaining exhibiting no consistent ABO specific pattern.

Overall, while some phenotypes appeared marginally more common in AB donors and others slightly lower in group B, no phenotype showed a strong ABO-linked predominance. Comparable data from other Indian regions are lacking, limiting inter-study comparison. Nevertheless, the observed differences, though statistically insignificant, likely reflect underlying regional genetic diversity, influenced by ethnicity, migration and social–marital patterns rather than any true biological association between ABO type and Rh phenotype.

CONCLUSION

Determining the frequency of Rh blood group antigens and phenotypes in the local donor population provides valuable baseline data for developing a phenol typed donor directory. This facilitates timely provision of antigen-negative compatible blood to patients with clinically significant alloantibodies. Knowledge of antigen prevalence also helps estimate the probability of finding compatible units, optimizes crossmatching, and supports targeted antigen typing in multi-transfused, antenatal, and postnatal patients. The observed variation in Rh antigen frequencies and phenotypic expressions among blood donors underscores the need for a more rational, antigen-matched transfusion approach to enhance blood safety.

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