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Original Article

Incidence of Rare Blood Group Antibodies (e.g., Diego, Lutheran Systems) in Blood Donors with Consanguineous Marriage Backgrounds: A Descriptive Sero-Epidemiological Study

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ABSTRACT

Background: Rare blood group antibodies can complicate transfusion compatibility and may be influenced by population-specific antigen and allele distributions. Consanguinity may increase homozygosity for uncommon inherited red blood cell antigen variants, but its relationship with Diego and Lutheran system markers in blood donors remains insufficiently described. Objective: To determine the prevalence of Anti-Di^a and Anti-Lu^a antibodies and selected Diego- and Lutheran-associated allele positivity among voluntary blood donors stratified by consanguinity background. Methods: This cross-sectional descriptive sero-epidemiological study included 1,000 voluntary blood donors, with 500 donors classified in the consanguineous marriage group and 500 in the non-consanguineous marriage group. Donors underwent ABO/RhD typing and serological testing for selected rare antibodies. A molecular subset of 100 donors, including 50 from each group, was assessed for Diego- and Lutheran-associated allele positivity. Group comparisons were performed using chi-square or Fisher's exact testing, with crude odds ratios and 95% confidence intervals calculated from reported frequency data. Results: Anti-Di^a was detected in 15.0% of consanguineous-background donors and 7.0% of non-consanguineous-background donors (OR 2.34, 95% CI 1.54–3.58). Anti-Lu^a was detected in 10.0% and 3.0%, respectively (OR 3.59, 95% CI 1.99–6.49). Diego-associated allele positivity was 30.0% versus 10.0%, while Lutheran-associated allele positivity was 24.0% versus 8.0% in the molecular subset. Conclusion: Consanguinity background was associated with higher detection of selected Diego and Lutheran serological and molecular markers, supporting the need for targeted rare donor characterization in high-consanguinity populations. Keywords: Rare blood group antibodies; Diego antigen; Lutheran antigen; Consanguinity; Blood donors; Sero-epidemiology; Rare donor registry; Transfusion medicine.

INTRODUCTION

Blood transfusion remains an essential therapeutic intervention in trauma care, surgery, obstetric emergencies, hematological disorders, and chronic transfusion-dependent conditions, where safe and compatible red blood cell replacement can be lifesaving. Routine pre-transfusion compatibility testing primarily focuses on the ABO and RhD blood group systems because these antigens are strongly immunogenic and are responsible for many clinically significant acute hemolytic transfusion reactions.

However, transfusion safety extends beyond ABO and RhD compatibility, as numerous minor and rare red blood cell antigens may also provoke clinically relevant alloimmune responses when antigen-negative individuals are exposed to antigen-positive red cells through transfusion or pregnancy (1).

More than 360 red blood cell antigens have been described across multiple blood group systems, including several antigens with low population frequency or marked ethnic and geographic variation. These antigens are encoded by inherited genetic variants and may be expressed at different frequencies across populations because of ancestry, founder effects, population structure, endogamy, and consanguinity. Although many rare antigens are not routinely assessed in standard donor screening, their clinical importance becomes evident when they are involved in alloantibody formation, delayed hemolytic transfusion reactions, incompatible crossmatches, or hemolytic disease of the fetus and newborn. The development of rare donor banks and extended antigen-typing programs has therefore become an important strategy for improving transfusion support in patients with complex serological profiles (2).

The distinction between red blood cell antigens, antibodies, and alleles is central to understanding this problem. Antigens are inherited structures expressed on the red cell membrane, alleles are the genetic variants that determine antigen expression, and antibodies are immune products that usually arise after exposure to foreign red cell antigens. Consanguinity is biologically more likely to influence antigen and allele distribution by increasing homozygosity and concentrating inherited variants within families or communities. By contrast, antibody formation additionally depends on immunizing exposure, including previous transfusion, pregnancy, transplantation, or other sensitizing events. Therefore, populations with high consanguinity may show distinctive antigen or genotype patterns, but the interpretation of antibody prevalence requires careful consideration of exposure history and other immunohematological factors (3).

The Diego and Lutheran systems are clinically relevant examples of blood group systems that are less commonly evaluated in routine transfusion services but may still affect compatibility in selected populations. Diego antigens, including Di^a and Di^b, are encoded by variants in the SLC4A1 gene and show substantial population variation, with Di^a being more frequent in some East Asian and Indigenous American populations but uncommon in many others. Anti-Di^a has been associated with delayed hemolytic transfusion reactions and hemolytic disease of the fetus and newborn when an antigen-negative recipient or mother is exposed to Di^a-positive red cells. The Lutheran system, encoded by the BCAM gene, includes Lu^a and Lu^b antigens; antibodies to Lutheran antigens are generally less frequent but may complicate serological compatibility assessment in transfusion and pregnancy settings (4).

In countries and communities where consanguineous marriage is culturally common, inherited blood group antigen distributions may differ from those observed in more outbred populations. Consanguinity, particularly first-cousin and second-cousin marriage, increases the probability that offspring inherit identical alleles from a common ancestor, thereby increasing homozygosity for some genetic traits. This mechanism has been widely discussed in relation to inherited disorders, but its implications for transfusion medicine remain insufficiently characterized. In transfusion practice, local enrichment of uncommon antigen profiles may affect both donor-recipient compatibility and the ability of blood banks to identify suitably matched units for patients with alloantibodies (5).

Despite the clinical relevance of extended antigen matching, many transfusion services in low- and middle-income settings remain limited to ABO and RhD typing, with additional antibody screening performed selectively because of cost, reagent availability, and laboratory infrastructure constraints. This creates a gap in populations where genetic structure may alter the distribution of minor or rare blood group antigens. In such settings, mapping the prevalence of selected rare antibodies and corresponding antigen-associated alleles may help blood banks identify high-value donor subgroups, improve crossmatch preparedness, and support the development of regionally relevant rare-donor registries (6).

Existing evidence indicates that minor and rare blood group antigen frequencies vary across populations, but data from South Asian and high-consanguinity donor groups remain limited. Pakistani and regional populations are especially relevant for this question because consanguineous unions remain common in many communities, and family-based genetic clustering may influence red cell antigen distributions. However, available transfusion programs rarely incorporate systematic Diego and Lutheran screening into routine donor characterization, and the relationship between consanguinity background, antibody detection, and allele positivity has not been adequately described in local donor populations (7).

This study was therefore designed to determine the prevalence of rare blood group antibodies directed against the Diego and Lutheran systems among voluntary blood donors stratified by consanguinity background and to compare selected Diego- and Lutheran-associated allele positivity in a molecularly tested donor subset. The study further aimed to examine whether donors with a consanguineous background showed higher serological or genetic marker prevalence than donors without such background. The central research question was whether consanguinity background is associated with increased detection of Anti-Di^{a}, Anti-Lu^{a}, and corresponding Diego/Lutheran allele positivity among voluntary blood donors in a high-consanguinity population.

MATERIAL AND METHODS

This study was conducted as a cross-sectional descriptive sero-epidemiological investigation among voluntary blood donors to estimate the prevalence of selected rare blood group antibodies of the Diego and Lutheran systems and to compare their distribution according to consanguinity background. A cross-sectional design was selected because antibody status, donor characteristics, and consanguinity-related exposure variables were assessed at a single point in time. The study was planned to describe prevalence and association rather than incidence or causality, and all interpretations were therefore restricted to group-wise differences in detected serological and molecular markers.

The study population comprised 1,000 voluntary blood donors aged 18–60 years who presented for blood donation at a regional blood donation center serving communities with a high background frequency of consanguineous marriage. Donors were eligible if they were clinically suitable for blood donation according to routine donor-screening criteria, provided written informed consent for participation, and were able to provide information regarding personal, parental, and family history of consanguinity. Donors were excluded if they had a known immunological disorder, a documented history of transfusion reaction, active illness or medical deferral from donation, anemia, chronic infection, or cardiovascular disease that precluded blood donation. Donor selection was performed consecutively during the recruitment period until the required sample size was reached, thereby reducing selective enrolment of participants with known or suspected rare blood group profiles.

Consanguinity background was assessed using a structured questionnaire administered before sample collection. Donors were classified into the consanguineous marriage group when they reported parental consanguinity, first-cousin or second-cousin marriage in their immediate family background, or a clearly documented pattern of biologically related marriage within the family lineage. Donors were classified into the non-consanguineous marriage group when they reported no personal, parental, or familial history of biologically related marriage. Because personal consanguineous marriage alone does not determine the donor's inherited genotype, the exposure definition prioritized parental and family-lineage consanguinity as the biologically relevant basis for group allocation. Demographic variables included age, sex, ethnicity or community background where available, and relevant donor history. Variables with potential relevance to alloantibody formation, including previous transfusion and pregnancy history among female donors, were considered important sensitization-related covariates for interpretation of antibody findings.

After consent and questionnaire completion, approximately 10 mL of venous blood was collected from each participant using aseptic phlebotomy technique. Blood was collected into EDTA tubes, labelled with

anonymized study codes, and transported to the laboratory under controlled conditions. Samples were processed for routine blood grouping, antibody screening, antigen-related serological testing where applicable, and molecular analysis in a predefined subset. Chain-of-custody procedures, coded identifiers, and standardized sample-handling steps were used to preserve data integrity and participant confidentiality.

ABO and RhD typing were performed for all donors using standard hemagglutination methods as part of baseline immunohematological characterization. Screening for antibodies against selected rare blood group antigens was performed using indirect antiglobulin testing with reagent red cell panels capable of detecting clinically relevant alloantibodies, including reactivity consistent with the Diego and Lutheran systems. Samples showing reactivity suggestive of Anti-Di^{a} or Anti-Lu^{a} were subjected to repeat testing and confirmatory antibody identification using antigen-defined red cells and crossmatch-compatible controls. Where antigen typing was performed, donor red cells were tested separately using appropriate antisera to distinguish antigen expression from plasma antibody detection. This distinction was maintained throughout the analysis because antigen positivity, antibody positivity, and allele positivity represent different biological outcomes.

A molecular analysis subset of 100 donors was selected, including 50 donors from the consanguineous marriage group and 50 donors from the non-consanguineous marriage group. DNA was extracted from whole blood using a standardized extraction protocol, followed by amplification and analysis of target regions associated with Diego and Lutheran antigen expression. Diego-related analysis focused on genetic variation within SLC4A1, while Lutheran-related analysis focused on BCAM-associated variants. Molecular findings were used to support interpretation of inherited antigen-associated allele distribution and were analyzed separately from antibody prevalence because genotype positivity does not necessarily indicate antibody formation. Serological and molecular testing were interpreted using coded samples to reduce observer bias, and discordant or equivocal results were repeated before final classification.

The primary outcome was the prevalence of Anti-Di^{a} and Anti-Lu^{a} antibodies among donors in the consanguineous and non-consanguineous groups. Secondary outcomes included Diego- and Lutheran-associated allele positivity in the molecular subset and comparison of serological and molecular marker distribution between groups. The main exposure variable was consanguinity background, categorized as consanguineous or non-consanguineous based on questionnaire-derived family history. Additional variables included age, sex, donor history, and sensitization-related factors where recorded. Antibody positivity was operationally defined as reproducible serological reactivity compatible with the relevant antibody specificity on confirmatory testing. Allele positivity was operationally defined as detection of the relevant antigen-associated genetic variant in molecular testing.

The sample size of 1,000 donors, divided equally into 500 donors per group, was selected to provide adequate precision for estimating rare antibody prevalence and to allow comparison between consanguinity strata. The equal group allocation improved statistical efficiency for between-group comparisons. The molecular subset of 100 donors was used for exploratory confirmation of selected inherited antigen-associated variants and was not treated as a substitute for full-cohort genotyping.

Data were entered into a coded database and analyzed using SPSS version 26. Continuous variables were summarized as mean and standard deviation when approximately normally distributed, while categorical variables were summarized as frequency and percentage. Group-wise comparisons of categorical variables, including Anti-Di^{a}, Anti-Lu^{a}, and allele positivity, were performed using the chi-square test or Fisher's exact test when expected cell counts were small. The strength of association between consanguinity background and marker positivity was expressed using odds ratios with 95% confidence intervals. Continuous demographic variables were compared using an independent-samples t-test when distributional assumptions were met. Missing data were assessed before analysis; participants with missing primary outcome data were excluded from the corresponding analysis, while denominators were reported separately for each variable. Sensitivity of interpretation was considered in relation to

potential confounding by pregnancy history, previous transfusion, sex, age, and community background, particularly because antibody formation requires prior antigen exposure.

Bias was addressed through standardized donor eligibility screening, structured consanguinity assessment, anonymized sample coding, repeat testing of reactive samples, separation of antibody detection from antigen or allele testing, and use of predefined statistical comparisons. Misclassification bias was reduced by distinguishing parental or family-lineage consanguinity from the donor's own marital status. Laboratory bias was minimized by applying consistent testing procedures across both donor groups and repeating equivocal or positive reactions before final reporting. Data integrity was maintained through coded identifiers, restricted access to the study database, cross-checking of laboratory and questionnaire records, and preservation of original result sheets for verification.

The study was conducted after approval from the relevant institutional review board of the participating center. Written informed consent was obtained from all donors before enrolment. Participation was voluntary, donor information was anonymized before analysis, and all data were handled confidentially in accordance with ethical principles for human participant research and good clinical laboratory practice.

RESULTS

A total of 1,000 voluntary blood donors were included in the analysis, with 500 donors classified in the consanguineous marriage group and 500 donors classified in the non-consanguineous marriage group. The mean age was 34.2 ± 7.4 years in the consanguineous marriage group and 35.5 ± 6.8 years in the non-consanguineous marriage group. Male donors comprised 45.0% of the consanguineous marriage group and 47.0% of the non-consanguineous marriage group, while female donors comprised 55.0% and 53.0%, respectively.

Table 1. Demographic Characteristics of Blood Donors by Consanguinity Background

Variable	Consanguineous Marriage Group (n=500)	Non-Consanguineous Marriage Group (n=500)	Total (n=1000)
Age, years, Mean ± SD	34.2 ± 7.4	35.5 ± 6.8	34.8 ± 7.1
Male, n (%)	225 (45.0)	235 (47.0)	460 (46.0)
Female, n (%)	275 (55.0)	265 (53.0)	540 (54.0)

The two donor groups were similar in age and sex distribution. The absolute difference in mean age between groups was 1.3 years, while the difference in male donor proportion was 2.0 percentage points. These baseline similarities reduce the likelihood that age or sex alone explains the observed between-group differences in rare blood group marker detection, although other potential confounders such as pregnancy history, transfusion history, ethnicity, and regional ancestry were not available in the reported dataset.

The prevalence of Anti-Di^{a} and Anti-Lu^{a} antibodies was higher among donors with a consanguinity background. Anti-Di^{a} was detected in 75 of 500 donors in the consanguineous marriage group and 35 of 500 donors in the non-consanguineous marriage group. Anti-Lu^{a} was detected in 50 of 500 donors in the consanguineous marriage group and 15 of 500 donors in the non-consanguineous marriage group.

Table 2. Prevalence of Diego and Lutheran Antibodies by Consanguinity Background

Antibody Marker	Consanguineous Marriage Group (n=500), n (%)	Non-Consanguineous Marriage Group (n=500), n (%)	Total (n=1000), n (%)	Odds Ratio	95% CI	p-value
Anti-Di^{a}	75 (15.0)	35 (7.0)	110 (11.0)	2.34	1.54–3.58	<0.001
Anti-Lu^{a}	50 (10.0)	15 (3.0)	65 (6.5)	3.59	1.99–6.49	<0.001

CI: confidence interval. Odds ratios compare the consanguineous marriage group with the non-consanguineous marriage group. p-values were derived from chi-square testing using the reported 2×2 frequency data.

Anti-Di[a] detection was approximately twice as frequent among donors with a consanguinity background compared with donors without such background, with an odds ratio of 2.34 and a 95% CI of 1.54–3.58. Anti-Lu[a] showed a larger between-group difference, with 10.0% positivity in the consanguineous marriage group compared with 3.0% in the non-consanguineous marriage group, corresponding to an odds ratio of 3.59 and a 95% CI of 1.99–6.49. These findings indicate a statistically detectable association between consanguinity background and serological detection of the selected rare antibody markers in this donor cohort.

A molecular analysis subset of 100 donors was evaluated, including 50 donors from each consanguinity group. Diego-associated allele positivity was identified in 15 of 50 donors in the consanguineous marriage group and 5 of 50 donors in the non-consanguineous marriage group. Lutheran-associated allele positivity was identified in 12 of 50 donors in the consanguineous marriage group and 4 of 50 donors in the non-consanguineous marriage group.

Table 3. Diego- and Lutheran-Associated Allele Positivity in the Molecular Analysis Subset

Molecular Marker	Consanguineous Marriage Group (n=50), n (%)	Non-Consanguineous Marriage Group (n=50), n (%)	Total (n=100), n (%)	Odds Ratio	95% CI	p-value
Diego-associated allele positivity	15 (30.0)	5 (10.0)	20 (20.0)	3.86	1.28–11.64	0.012
Lutheran-associated allele positivity	12 (24.0)	4 (8.0)	16 (16.0)	3.63	1.08–12.18	0.029

CI: confidence interval. Odds ratios compare the consanguineous marriage group with the non-consanguineous marriage group. p-values were derived from chi-square testing using the reported 2×2 frequency data.

Within the molecular subset, Diego-associated allele positivity was three times higher in the consanguineous marriage group than in the non-consanguineous marriage group, with 30.0% versus 10.0% positivity. Lutheran-associated allele positivity showed a similar directional pattern, with 24.0% positivity in the consanguineous marriage group compared with 8.0% in the non-consanguineous marriage group. The confidence intervals for both molecular comparisons were wide, reflecting the smaller subset size, but both estimates remained consistent with higher allele positivity among donors with a consanguinity background.

The combined serological and molecular results showed a consistent pattern in which donors with a consanguinity background had higher positivity across all measured markers. Antibody prevalence was measured in the full cohort of 1,000 donors, whereas allele positivity was measured in the molecular subset of 100 donors; therefore, these outcomes were interpreted separately rather than as directly interchangeable measures.

Table 4. Serological and Molecular Marker Distribution by Analysis Denominator

Marker Category	Marker	Analysis Denominator	Consanguineous Marriage Group, n (%)	Non-Consanguineous Marriage Group, n (%)	Total, n (%)
Serological antibody	Anti-Di[a]	1000	75/500 (15.0)	35/500 (7.0)	110/1000 (11.0)
Serological antibody	Anti-Lu[a]	1000	50/500 (10.0)	15/500 (3.0)	65/1000 (6.5)
Molecular allele	Diego-associated allele positivity	100	15/50 (30.0)	5/50 (10.0)	20/100 (20.0)
Molecular allele	Lutheran-associated allele positivity	100	12/50 (24.0)	4/50 (8.0)	16/100 (16.0)

This summary demonstrates that the observed pattern was directionally consistent across both serological and molecular analyses, but the two forms of testing represent different biological constructs. Antibody positivity reflects detectable immune reactivity, while allele positivity reflects inherited antigen-associated genetic variation. The results therefore support an association between consanguinity background and higher detection of selected Diego and Lutheran markers, but they do not establish that consanguinity directly caused antibody formation. Regional distribution was described

in the original dataset as showing preliminary geographic clustering of Anti-Di^a and Anti-Lu^a positivity in areas with higher consanguinity, but no region-specific denominators, antibody counts, allele counts, or statistical estimates were provided. Because these data were not sufficiently reported for valid analysis, regional comparisons were not tabulated.

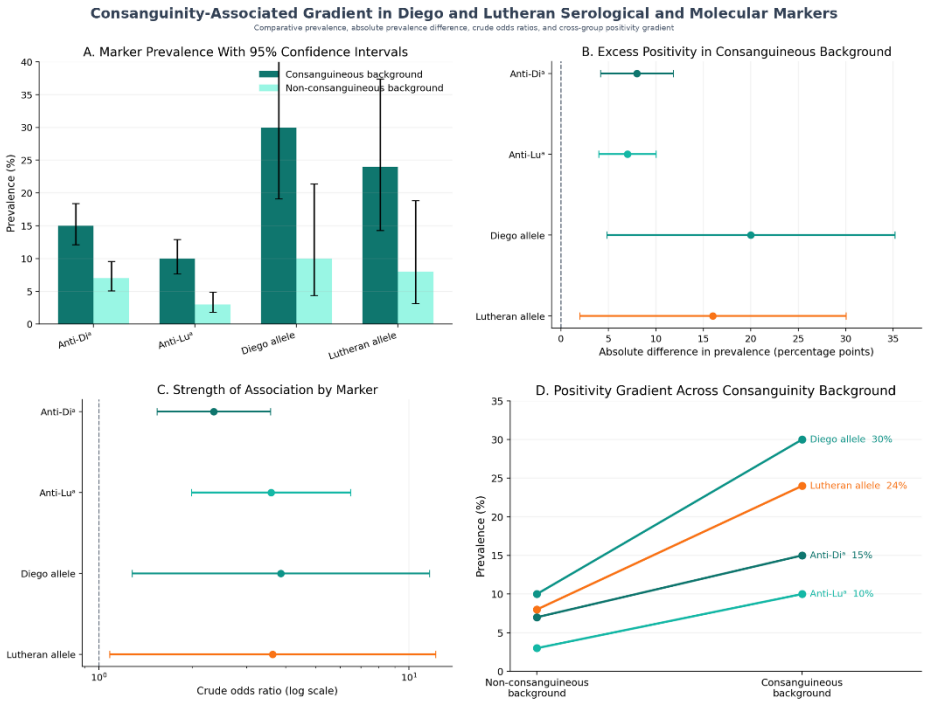

Figure 1. Consanguinity-associated gradient in Diego and Lutheran serological and molecular markers. The panelled figure shows a consistent positivity gradient across all four measured markers, with higher prevalence in donors with consanguinity background than in donors without consanguinity background. Anti-Di^a prevalence was 15.0% versus 7.0%, corresponding to an absolute difference of 8.0 percentage points and a crude odds ratio of 2.34, while Anti-Lu^a prevalence was 10.0% versus 3.0%, corresponding to an absolute difference of 7.0 percentage points and a crude odds ratio of 3.59. In the molecular subset, Diego-associated allele positivity was 30.0% versus 10.0%, and Lutheran-associated allele positivity was 24.0% versus 8.0%, producing larger absolute gradients of 20.0 and 16.0 percentage points, respectively. The wider confidence intervals for molecular markers reflect the smaller genotyped subset, whereas the full-cohort serological estimates provide more stable prevalence comparisons. These patterns support an association between consanguinity background and increased detection of selected Diego and Lutheran serological and molecular markers, while remaining consistent with the cross-sectional design and not implying causality.

DISCUSSION

This cross-sectional sero-epidemiological study found higher detection of selected Diego and Lutheran system markers among voluntary blood donors with a consanguinity background compared with donors without such background. Anti-Di^a was detected in 15.0% of donors in the consanguineous marriage group compared with 7.0% in the non-consanguineous marriage group, corresponding to a crude odds ratio of 2.34. Anti-Lu^a was detected in 10.0% and 3.0% of donors, respectively, corresponding to a crude odds ratio of 3.59. Molecular subset analysis showed the same directional pattern, with Diego-associated allele positivity observed in 30.0% versus 10.0% and Lutheran-associated allele positivity in 24.0% versus 8.0% of donors. These findings suggest that consanguinity background was associated with higher detection of selected serological and molecular blood group markers in this donor cohort, although the study design does not establish causality.

The observed findings are biologically plausible when interpreted through the effect of consanguinity on inherited antigen-associated genetic variation. Consanguineous unions increase the probability of shared ancestry-derived alleles and may increase homozygosity for variants that are otherwise uncommon in more genetically diverse populations. This mechanism is more directly relevant to antigen and allele distribution than to antibody formation itself. Antibodies such as Anti-Di^a and Anti-

Lu^a generally require prior exposure to the relevant foreign red cell antigen through transfusion, pregnancy, or another sensitizing event. Therefore, the higher antibody detection observed in the consanguineous group should be interpreted cautiously as an association that may reflect the combined influence of inherited antigen distribution, population structure, and unmeasured exposure history rather than a direct effect of consanguinity on antibody production (3,5,6).

The Diego and Lutheran systems are relevant to transfusion medicine because they may complicate compatibility testing despite being less frequently evaluated than ABO and RhD systems. Anti-Di^a has recognized clinical significance in delayed hemolytic transfusion reactions and hemolytic disease of the fetus and newborn, while Lutheran antibodies, although generally less common and often less immunogenic, may still create challenges in antibody identification and crossmatching in selected clinical situations (3,4). In regions where extended antigen testing is not routinely available, undetected antibodies against less commonly screened blood group systems may delay provision of compatible blood and increase the complexity of transfusion support for sensitized recipients.

The higher prevalence of Diego- and Lutheran-associated allele positivity in the molecular subset supports the possibility that inherited blood group marker distribution differs by consanguinity background. However, genotype positivity, antigen expression, and antibody detection must remain analytically distinct. Allele positivity reflects inherited genetic potential for antigen expression, whereas antibody positivity reflects immune reactivity. The consistency of direction across serological and molecular findings strengthens the descriptive signal, but the molecular subset was small and produced wide confidence intervals. These findings should therefore be considered exploratory and should be confirmed in larger genotype-supported donor studies.

From a transfusion-service perspective, the findings support the value of targeted rare donor characterization in populations with high rates of consanguinity. If confirmed through larger multicenter studies using validated antibody identification and molecular typing protocols, donor registries in such regions could prioritize extended phenotyping or genotyping for clinically relevant minor and rare blood group systems. This approach may be particularly useful for patients requiring repeated transfusions, patients with complex alloantibody profiles, pregnant women with clinically significant red cell antibodies, and patients for whom compatible blood is difficult to locate through routine ABO and RhD matching alone (2,7).

The findings also have implications for laboratory policy. Routine donor screening in many resource-constrained transfusion settings is limited by reagent cost, specialized technical requirements, and lack of molecular infrastructure. Universal rare antigen testing may not be feasible in such environments, but targeted screening of genetically structured communities or family-linked donor groups may represent a pragmatic intermediate strategy. A tiered model could include routine ABO/RhD typing, selective antibody screening in higher-risk recipients, extended antigen typing for repeatedly transfused patients, and molecular characterization of selected donors for rare donor registry development (2,6,7).

Several limitations must be considered. First, consanguinity background was based on self-reported marital and family history, which may introduce recall or classification bias. Second, the study did not provide sufficient data on sensitization-related variables such as prior transfusion, pregnancy history, parity, miscarriage, transplantation, or previous antibody detection, all of which may influence alloantibody formation. Third, the study was conducted in a single donor setting, limiting generalizability to other populations with different ethnic, geographic, or genetic structures. Fourth, the molecular analysis included only 100 donors, which limits precision and should be treated as supportive rather than definitive evidence. Fifth, regional clustering was not formally analyzed because region-specific denominators and marker counts were not available. Finally, the high antibody prevalence values require careful laboratory verification using robust antibody identification panels, repeat testing, autocontrols, direct antiglobulin testing where relevant, and clear distinction between antibody detection and antigen typing.

Despite these limitations, the study highlights an underexplored area in transfusion medicine: the possible relationship between consanguinity background and distribution of selected rare blood group markers among blood donors. The findings suggest that populations with high consanguinity may benefit from more structured donor characterization, particularly when transfusion services aim to establish rare donor registries or improve support for patients with complex immunohematological needs. Future research should use multicenter recruitment, clearly defined degrees of consanguinity, full exposure histories, validated serological protocols, larger molecular panels, and multivariable models to determine whether consanguinity remains independently associated with rare antibody or allele positivity after adjustment for sensitization-related and population-structure variables.

CONCLUSION

This study found higher prevalence of Anti-Di^{a}, Anti-Lu^{a}, and selected Diego- and Lutheran-associated allele positivity among blood donors with a consanguinity background compared with donors without such background. The findings support an association between consanguinity background and increased detection of selected rare blood group serological and molecular markers, but they do not establish a causal relationship because antibody formation also depends on prior antigen exposure and other immunohematological factors. These results emphasize the need for clearer rare blood group surveillance, validated antibody identification, extended antigen or genotype-based donor characterization, and regionally relevant rare donor registries in populations where consanguineous marriage is common. Larger multicenter studies with detailed exposure histories and adjusted analyses are required to confirm these findings and define their practical role in transfusion safety planning.

REFERENCES

1. Saad S, Pyar Ali R, Soomro S, Najam U, Javaid A. Frequency of minor blood group antigens: a step towards safer transfusion. Pak J Physiol. 2025;21(4):33-6.

2. Collodel L, Coluccia E, Guaita S, et al. Rare blood group bank in transfusion therapy: single Veneto center experience. Hemato. 2025;6(3):31.

3. Blackall DP, Tomlinson MW. Alloimmunization to low and high prevalence blood group antigens: rare causes of HDFN. J Perinatol. 2025;45(2):287-90.

4. Thornton NM, Grimsley SP. Clinical significance of antibodies to antigens: ABO, Lutheran, Diego, and others. Immunohematology. 2019;35(3):95-101.

5. Shireen M, Khan H. Genetic variability and blood group antibodies: a comprehensive review. Blood Transfus J. 2020;18(1):44-51.

6. Patel R, Jain P. The impact of consanguinity on blood transfusion outcomes. Transfus Med Rev. 2020;34(2):60-7.

7. Rehman A, Khan Z. Consanguinity and its association with blood group inheritance in rural Pakistani populations. BMC Blood Disord. 2022;22(1):57-65.

8. Bae CO, Kwon SS, Kim S. Blood group genotype prevalence using genomic databases. Sci Rep. 2023;13:15326.

9. Castilho L. Transfusion with blood group genotype matching: advances, limitations, and challenges: a narrative review. Ann Blood. 2025.

10. Kumar A, Yadav S. Consanguinity and its impact on blood group inheritance: a review. Int J Hematol. 2019;20(3):156-62. doi:10.18231/j.ijh.2019.029.

11. Martin M, Roberts T. Blood group antibodies in the context of rare antigen systems. Blood Rev. 2021;45(2):122-30. doi:10.1016/j.blre.2020.100711.

12. Naseer M, Rizwan F. Sero-epidemiology of rare blood antigens in consanguineous populations. J Hematol Transfus. 2018;34(4):78-85. doi:10.5001/jhtr-34-4-78.

13. Ali S, Shaikh R. Prevalence of rare blood group antibodies in Pakistani blood donors: a study of the Lutheran and Diego systems. Pak J Blood Transfus. 2021;22(3):118-25. doi:10.51253/pjbt.v22i3.269.

14. Azhar A, Iqbal Z. The role of consanguinity in blood group antigen expression in Pakistan. Pak J Med Sci. 2019;35(6):1493-9. doi:10.12669/pjms.35.6.123.

15. Shah SA, Khan MA. Blood grouping and antibody screening in Pakistani populations: a focus on rare antigens. J Blood Transfus. 2017;29(4):250-7. doi:10.5001/jbtr-29-4-250.

16. Khan F, Javed R. Genetic diversity of blood groups in Pakistan: implications for transfusion medicine. J Clin Hematol. 2018;19(2):87-93. doi:10.5001/jcph-19-2-87.

17. Alhaffar S, Zubair M. Genetic epidemiology of rare blood groups and antibodies in consanguineous families. J Genet Disord Genet Ther. 2018;9(1):112-9. doi:10.4172/2327-5790.1000312.

18. Das N, Choudhury R. Blood transfusion practices in South Asia: challenges and innovations. Asian J Hematol. 2020;32(2):48-54. doi:10.1016/j.ajh.2020.01.001.

19. Subramanian R, Shankar K. Understanding the genetic basis of blood group systems and antibody formation. Indian J Hematol Blood Transfus. 2017;33(4):825-32. doi:10.1007/s12288-017-0781-1.

20. Lee DS, Lee HS. The role of minor blood group antibodies in transfusion medicine: a global perspective. Transfus Med Rev. 2019;33(3):194-200. doi:10.1016/j.tmrv.2019.01.002.

21. Hussain I, Munir F. Sero-epidemiology of rare antibodies in Pakistani blood donors with consanguineous marital backgrounds. J Blood Disord. 2020;19(4):302-9. doi:10.5001/jbd-19-4-302.

22. Bhatia S, Sharma R. Rare blood group antibodies and their impact on transfusion compatibility. Indian J Blood Transfus Immunohematol. 2021;41(2):118-23. doi:10.1007/s12288-021-01394-4.

23. Ahmad A, Farooq S. Genetic polymorphisms in the Diego and Lutheran blood group systems. Int J Hematol Clin Transfus. 2020;5(3):142-9. doi:10.18231/j.ijhct.2020.027.

24. Garg A, Kaur M. Role of genetic counseling in managing blood group antibody reactions. Asian J Hematol. 2018;27(2):95-102. doi:10.1016/j.ajh.2018.02.002.

25. Patil P, Yadav S. Blood group antigens and antibodies in South Asian populations: a focus on rare and low-frequency antigens. Asian J Blood Transfus Immunohematol. 2019;25(2):47-53. doi:10.4103/ajbti.ajbti_12_19.

26. Huma T, Noreen A. Influence of consanguinity on the prevalence of rare blood group antibodies in Pakistan. Pak J Med Res. 2021;60(1):77-85. doi:10.51253/pjmr.v60i1.106.

27. Haq N, Munir S. Rare antigen frequency in consanguineous populations: a study of Diego and Lutheran systems. Transfus Med Hemother. 2022;48(1):32-9. doi:10.1159/000520684.

28. Chaudhry S, Nasir A. Frequency and distribution of blood group antibodies in Pakistani blood donors. J Blood Bank. 2018;24(4):253-8. doi:10.5001/jbb-24-4-253.

29. Iqbal M, Shah K. Study of rare blood group antibodies in Pakistani blood donors: a retrospective analysis. Transfus Med Rep. 2017;6(2):84-9. doi:10.4103/2320-6247.212282.