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ORIGINAL ARTICLE

Antibody screening in the general population: A single center experience in Karachi.

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ABSTRACT… Objective: To evaluate the prevalence and spectrum of red cell antibodies in the general population of Karachi. **Study Design:** Cross-sectional Descriptive study. **Setting:** Department of Blood Bank Chughtai Lab, Karachi. **Methods:** April 2024 to April 2025. **Methods:** A total of 999 samples were recruited for antibody screening. Individuals of all age groups and both genders were included. From each participant, approximately 7 mL of venous blood was drawn using a vacutainer and collected into two separate tubes. Descriptive statistics were used to summarize the findings (using SPSS v24.0). **Results:** 999 samples were screened for red cell antibodies, of which 77 (7.7%) tested positive. The majority of positive cases were females (93.5%), with a median age of 28.5 years compared to 26 years in males. Anti-D was the most common alloantibody (32.5%), followed by weak positive reactions (16.9%), auto antibody-positive cases (14.3%), and combinations such as anti-D with anti-C (9.1%) and anti-E (9.1%). Less frequent antibodies included anti-c, anti-K, anti-M, anti-C, anti-Jka, and anti-Fya. A significant association was observed between gender and the type of antibody detected (p = 0.016). **Conclusion:** The prevalence of red cell alloantibodies in the subset population of Karachi was 7.7 %, with a marked predominance among females. Anti-D emerged as the most common antibody, followed by other Rh and non-Rh specificities.

Key words: Alloantibodies, Allo-immunization, Anti-D Antibodies, Blood Group Antigens.

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INTRODUCTION

The International Society of Blood Transfusion recognized over 300 different blood group antigens, of which 29 belong to distinct blood group antigens.[1] Red cell antibodies typically develop as a result of blood transfusions or pregnancy. The alloantibodies are formed as a result of a natural immune system response when the mother's blood group is different from the fetal blood group. This can lead to complications such as hemolytic disease of the newborn if not properly managed.[2] Understanding these blood group antigens and their corresponding antibodies is crucial for ensuring safe blood transfusions and successful pregnancies. These alloantibodies are mostly IgG in nature that can cross the placenta; they can cause hemolytic disease of the fetus and newborn.[3] When maternal red cells cross the placenta, they may cause hemolysis of fetal red cells, resulting in hemolytic disease of the fetus and newborn (HDFN).[4]

The symptoms of fetal or newborn present with fetal anemia, hyperbilirubinemia, hydrops fetalis, and intrauterine death.[5] The clinically significant antibodies formed against the red cell antigens that lead to HDFN, acute or chronic hemolytic transfusion reaction, and reduced lifespan of erythrocytes.[6] The most common alloantibodies are ABO, Rh, and Kell. Other antibodies include Duffy (fya, Kidd (Jka, Jkb), MNS (M, N, S, s), Lewis (Le), Lutheran (Lu), therefore 98% of HDFN are related to Rh and ABO discordancy, while 2% are due to irregular antibodies. The significant Rh antigens include D, C, c, E, e.[7]

Among all antibodies, the Rh antibodies are basic and important. RhD causes severe HDFN, and non-D-type antigens may also mediate moderate to severe Hematological Transfusion Reaction (HTR) and HDFN. In 1970 the immunoprophylaxis against anti-D was introduced which resulted in a radically reduced number of anti-D alloimmunization from 16-17 % to 1-2%.[8]

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Since no immunoprophylaxis has been introduced against non-D antibodies to date; these irregular antibodies are still problematic. These irregular antibodies can lead to complications in blood transfusions and pregnancy, necessitating careful monitoring and management.[9]

When a foreign antigen is exposed, the recipient develops an immune reaction against the antigen. The immune system produces the alloantibodies against these antigens especially in multiparous females who have one or more prior childbirths.[10] The alloimmunization increases with parity, blood transfusion, and complicated obstetric history, Other factors include trans-placental leakage of feto-maternal blood, feto-maternal hemorrhage, error in transfusion and inadequate screening of mother's blood.[11] The pre-transfusion antibody screening of patient samples before cross-matching is an essential part of compatibility testing in many countries. Providing safe blood transfusions does not imply only testing for infections but also protection from hemolytic transfusion reactions resulting from alloimmunization.[12] Regular screening protocols for the detection of unexpected immune antibodies could improve blood safety and prevent hemolytic reactions.[13]

Pakistan is one of the most populous countries in the, is home to a diverse population with a wide range of ethnic backgrounds. This diversity is particularly evident in provinces like Khyber Pakhtunkhwa and urban centers such as Karachi. Historically, Pakistan has experienced multiple waves of migration, including large-scale influxes from neighboring Afghanistan due to prolonged conflict, as well as from India during the 1947 Partition, and, to a lesser extent, from Iran.[14] These migrations have introduced a wide spectrum of genetic backgrounds into the population, significantly influencing the immunohematologic landscape.[15]

In particular, the admixture of ethnic groups and the high prevalence of consanguineous marriages have contributed to an increased frequency of hereditary blood disorders such as thalassaemia.[16] Alloimmunization risk is heightened due to mismatched transfusions, especially when donor and recipient populations differ in red cell antigen profiles. In this context, red cell antibody screening becomes critically important in identifying clinically significant alloantibodies prior to transfusion, ensuring both safety and efficacy.[17]

Our single-center study in Karachi, a city representing a microcosm of Pakistan's ethnic and genetic diversity aims to explore the frequency and spectrum of red cell alloantibodies in the general population, providing insights for improving transfusion protocols in similarly heterogeneous settings. The limited literature on alloimmunization in general population in Pakistan, majority of blood bank in Pakistan solely use random cross-matching of available inventory units to provide patients with appropriate blood. Therefore, in the absence of a local donor database, providing packed red blood cells that are phenotypically matched could be difficult.

The objective of this study was to evaluate the prevalence and spectrum of red cell antibodies in the general population.

METHODS

This cross-sectional descriptive study was conducted in the Department of Blood Bank Chughtai Lab, Karachi, after obtaining approval from the Institutional Review and Ethics Board (IREB). (CIP/IRB/1285) The study was carried out over a one-year period, from April 2024 to April 2025. A total of 999 samples were recruited for antibody screening. Individuals of all age groups and both genders were included.

From each participant, approximately 7 mL of venous blood was drawn using a vacutainer and collected into two separate tubes. About 3 mL of blood was taken into an EDTA tube for ABO, Rh D blood grouping, and other minor blood group phenotype while 4 mL was collected in a yellow top Gel tube and allowed to clot for antibody screening and identification. Each sample was assigned a unique laboratory barcoded number, and proper documentation was maintained.

Initially, antibody screening was performed, followed by ABO and Rh D blood grouping. In case of a positive screening result, antibody identification was

performed. The ABO and Rh Typing were performed by LORNE Laboratories. The column agglutination gel card method was used for antibody screening, employing BIORAD ID-DiaCell I, II, and III screening panels to detect red cell alloantibodies. Samples showing positive results for alloantibodies were further tested using the ID-Panel 11-cell system, in accordance with the manufacturer's guidelines.

On the day of testing, internal quality control was ensured using both positive and negative controls. Additionally, red cell phenotyping was performed after antibody identification to confirm the presence of the corresponding antibody.

Data were recorded on a structured proforma and analyzed using Microsoft Excel and SPSS version 24. Descriptive statistics were used to summarize the findings. Frequencies and percentages were calculated for categorical variables such as blood group and the presence of alloantibodies, while means and standard deviations (SD) were calculated for continuous variables such as age and gender. The Chi-square test was applied to assess associations between categorical variables, and a p-value of less than 0.05 was considered statistically significant.

RESULTS

Out of the 999 samples screened, 77 (7.7%) tested positive for red cell alloantibodies. The study population was predominantly female i.e. 93.5% (n=72) while the remaining were males. The median age of the female and male participants was 28.5 years (25.0-32.8 years) and 26.0 years (8.5-45.0 years) respectively.

The most frequently identified alloantibodies were anti-D (25, 32.5%), followed by equal number of cases of anti-E and combination of anti-D and anti-C (7, 9.1%). A breakdown of the detected alloantibodies is presented in Table-I.

The distribution of the antibodies based on gender is displayed in Figure-1. A statistically significant association was observed between the gender and the type of antibody detected (p-value: 0.016).

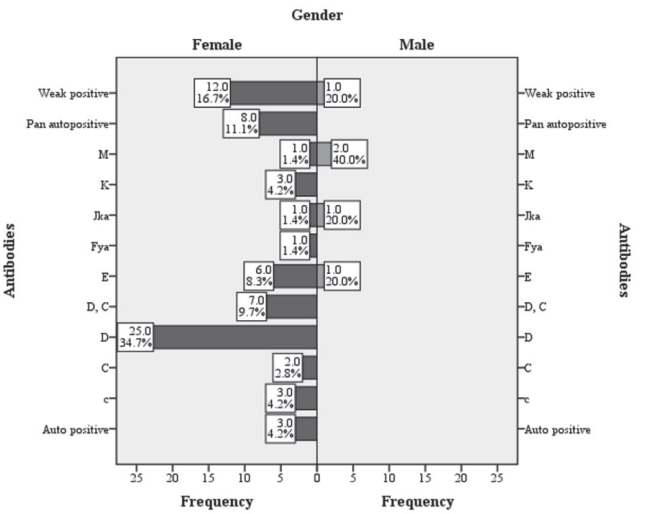
TABLE-I

Frequency of antibodies observed in the study population

Antibody	Frequency, n (%)
Anti-D	25 (32.5)
Weak positive	13 (16.9)
Auto-positive	11 (14.3)
D, C	7 (9.1)
E	7 (9.1)
c	3 (3.9)
K	3 (3.9)
M	3 (3.9)
C	2 (2.6)
Jka	2 (2.6)
Fya	1 (1.3)

FIGURE-1

Gender-wise distribution of the detected antibodies



DISCUSSION

In this single-center study from Karachi, 999 samples were screened for red cell alloantibodies, of which 77 (7.7%) tested positive. The majority of positive cases were females (93.5%), with a median age of 28.5 years compared to 26 years in males. Anti-D was the most common alloantibody (32.5%), followed by weak positive reactions (16.9%), auto antibody positive cases (14.3%), and combinations such as anti-D with anti-C (9.1%) and anti-E (9.1%). Less frequent antibodies included anti-c, anti-K, anti-M, anti-C, anti-Jka, and anti-Fya. A significant association was observed between gender and the type of antibody detected (p = 0.016).

The seroprevalence of alloantibodies (7.7 %) reported in our study has been reported higher than the generally reported prevalence in published literature (around 0.4 to 3 %) in antenatal population.[18] The elevated prevalence in our research probably shows differences in the sampling and population cohort (pre-dominantly female), bias of single-center etc. Anti-D was found to be prevalent in our study (in around 32.5 % of samples). This is in line with the identified anti-D antibodies reported in studies.[19] Nonetheless, the proportion of anti-D observed in our study is relatively lower than other researches.[20]

Detection of other anti-bodies such as anti-C, anti-E, anti-K, anti-M, Jka and Fya etc. shows a mirroring pattern observed in various antenatal and transfusion studies, wherein non-Rh D antibodies and Kell as well as other antibodies that are clinically significant have been reported regularly, even though at lower incidences than anti-D.[21] Similar observations were reported in our study as well. Auto antibody-positive and weakly positive antibodies were reported in notable proportions (14.3 % and 16.9 %, respectively) have also been observed in published literature but in lower frequencies. More commonly, such antibodies were found as part of screening, multi-system disease, autoimmune disease and in history of prior transfusions.[22]

Strong predominance of females as observed in our study (93.5 %) is in line with other researches, most likely due to the fact that many researchers screen pre-dominantly pregnant females thereby demonstrating higher presentation of females and higher rates of allo-immunization linked with obstetrical causes (such as miscarriage, pregnancy states and inadequate prophylaxis).[23] A direct comparison with greater proportion of males is often limited, similarly observed in this study as well.[24]

This study has several limitations. Being a single-center analysis, the findings may not be fully generalizable to the wider population of Karachi or Pakistan. The study population was predominantly female, which could have introduced selection bias and limited gender-based comparisons. The cross-sectional design only provides prevalence data and does not assess risk factors such as parity, transfusion history, or prior exposure to sensitizing events. Additionally, antibody identification was based on available screening methods, and weaker or rare alloantibodies may have been missed.

Future studies should be multi-centered with larger, more balanced populations to better represent the general community. Incorporating detailed clinical and obstetric histories would allow identification of risk factors for alloimmunization. Strengthening routine antenatal antibody screening and ensuring universal access to anti-D immunoprophylaxis should be prioritized.

CONCLUSION

The prevalence of red cell alloantibodies in the subset population of Karachi was 7.7 %, with a marked predominance among females. Anti-D emerged as the most common antibody, followed by other Rh and non-Rh specificities. The significant association between gender and antibody type underscores the role of obstetric sensitization and transfusion practices in alloimmunization. These findings emphasize importance of routine antenatal antibody screening, effective implementation of anti-D immunoprophylaxis, and consideration of extended antigen matching to enhance transfusion safety and reduce alloimmunization-related complications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Gassner C, Castilho L, Chen Q, Clausen FB, Denomme GA, Flegel WA, et al. **International society of blood transfusion working party on red cell immunogenetics and blood group terminology: Report of basel and three virtual business meetings—update on blood group systems.** Vox Sang. 2022 Nov; 117(11):1332-44.
2. Moise KJ Jr, Abels EA. **Management of red cell alloimmunization in pregnancy.** Obstet Gynecol. 2022 May 5; 10-97.
3. Nahendran P, Budin SB, Saat NZM, Yusop MF, Yazid TNT, Anuar NNM. **Haemolytic disease of the newborn-related red blood cell alloantibodies.** J Teknol. 2022; 85(1):1-9.

4. Christensen RD, Bahr TM, Ilstrup SJ, Dizon-Townson DS. **Alloimmune hemolytic disease of the fetus and newborn: genetics, structure, and function of the commonly involved erythrocyte antigens.** J Perinatol. 2023 Dec; 43(12):1459-67.
5. de Oliveira Rodrigues MM, Mattos D, Almeida S, Fiegenbaum M. **Hemolytic disease of the fetus and newborn—a perspective of immunohematology.** Hematol Transfus Cell Ther. 2024 Nov; 46(Suppl 5):S246-57.
6. Yu D, Ling LE, Krumme AA, Tjoa ML, Moise KJ Jr. **Live birth prevalence of hemolytic disease of the fetus and newborn in the United States from 1996 to 2010.** AJOG Glob Rep. 2023 May; 3(2):100203.
7. Sugrue RP, Moise KJ, Federspiel JJ, Abels E, Louie JZ, Chen Z, et al. **Maternal red blood cell alloimmunization prevalence in the United States.** Blood Adv. 2024 Aug 27; 8(16):4311-9.
8. Ares SM, Nardozza LM, Araujo E, Santana EF. **Non-RhD alloimmunization in pregnancy: An updated review.** Rev Bras Ginecol Obstet. 2024 May 20; 46: e-rbgo22.
9. Petroff MG, Nguyen SL, Ahn SH. **Fetal-placental antigens and the maternal immune system: Reproductive immunology comes of age.** Immunol Rev. 2022 Jul; 308(1):25-39.
10. Sikora J, Roback JD. **Obstetric transfusion practice. In: Rossi's Principles of Transfusion Medicine.** 5th ed. Wiley; 2022; 373-80.
11. Wapukha BZ. **Red blood cell alloimmunization and associated risk factors among transfused cancer patients at Moi Teaching and Referral Hospital, Kenya [dissertation].** Kisii: Kisii University; 2022.
12. Luhar RK, Shah RJ, Harimoorthy V. **Antibody screening and identification in voluntary blood donors—need of the hour.** Glob J Transfus Med. 2022 Jan 1; 7(1):47-50.
13. Saba N, Waheed U, Khan MN, Saeed M, Khan TH, Wazeer A, et al. **First report of phenotype prevalence of five major Rh blood group antigens in the blood donor population of Peshawar, Pakistan.** J Islamabad Med Dent Coll. 2023; 12(4):286-91.
14. Khan IA, Fatima M, Imran A, Malik NA, Chughtai AS. **Frequency of common Rh antigens among volunteer blood donors: A single centre study.** Pak J Med Sci. 2023; 39(2):123-7.
15. Aziz A, Tehreem H, Aslam R, Javed MU, Ali MS, Riaz A, et al. **Consanguineous marriages and thalassemia major in Pakistan: A cross-sectional study on awareness and prevalence.** Am J Med Sci Pharm Res. 2024 Jul 29; 6(07):49-61.
16. Aldurayhim MS. **Frequency of alloimmunization in patients on regular blood transfusion in Riyadh, Saudi Arabia: A multicenter retrospective study [master's thesis].** Riyadh: Alfaisal University; 2023.
17. Moinuddin I, Fletcher C, Millward P. **Prevalence and specificity of clinically significant red cell alloantibodies in pregnant women—a study from a tertiary care hospital in Southeast Michigan.** J Blood Med. 2019; 10:283-8.
18. Kahar M. **Frequency of red cell alloantibodies in pregnant females of Navsari district: an experience that favours inclusion of screening for irregular erythrocyte antibody in routine antenatal testing profile.** J Obstet Gynecol India. 2018 Aug; 68(4):300-5.
19. Saboor M, Ahmed S. **Prevalence and specificity of red cell alloantibodies in un-transfused multiparous women.** Clin Lab. 2021 Jul 1; 67(4).
20. Ghaffar S, Uddin N, Yazdani MS, Khan SA, Ghaffar S, Haq H. **Screening and identification of red cell alloantibodies in multiparous women: An institution-based study.** Pak Armed Forces Med J. 2019 Aug; 31(4):748.
21. Bajwa H, Alam M, Rathore MA, Yazdani MS. **Rh alloantibodies in Rh D negative blood group pregnant women—a regional transfusion centre study.** System. 2022; 6:7.
22. Devi KM, Nepram L, Hajong R, Bhattacharyya D. **Prevalence and specificity of red blood cell alloantibodies in patients in a tertiary care center in Meghalaya, India.** Cureus. 2025 Jun 19; 17(6):e86347.
23. Contelli HS, de Oliveira MC, Ido AA, Francalanci EM, da Cunha Terra PO, Ribeiro Filho E, et al. **Assessment of erythrocyte alloimmunization among patients treated at a Brazilian university hospital.** Hematol Transfus Cell Ther. 2024 Nov 1; 46:S128-35.
24. Nathani KJ, Patel J, Modi P, Pandya A, Wadhwani S, Jarag M. **Prevalence of red cell alloantibodies and autoantibodies in patients and donors attending a tertiary care hospital in South Gujarat.** Int J Contemp Med Res. 2021; 8(3):C1-5.

AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Kanwal Shafiq:** Data collection.
2	**Syeda Kanwal Zehra Zaidi:** Data collection.
3	**Munazza Rashid:** Data analysis.
4	**Ghazal Irfan:** Writing.