European Journal of Clinical Pharmacy

Print ISSN: 2385-409X **Online ISSN**: 3105-0409

Website: https://farmclin.com/

Original Research Article

Red Blood Cell Alloantibodies among Rh-D Negative Pregnant Females in Punjab, Pakistan

Article History:

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Received: 11-11-2025
Revised: 24-11-2025
Accepted: 09-12-2025
Published: 22-12-2025

Abstract: Red blood cell (RBC) alloimmunization is a condition that occurs when a person develops antibodies against antigens on the surface of foreign RBCs. These antibodies can cause hemolysis (destruction) of the RBCs that carry the antigens, leading to anemia and other complications. The current study objects to examine the demographic distribution and blood group characteristics within a selected sample population. In relation to blood classes, B-negative exhibits the highest prevalence at 39%, succeeded by A-negative at 31%, AB-negative at 21%, and O-negative at 9%. Upon doing a more comprehensive analysis of individuals who have tested negative across all three panels, it has been observed that blood type B exhibits the highest prevalence at 40.8%. This is closely followed by blood group A-negative at 27.6%, and blood group AB-negative at 22.4%. Upon conducting a more comprehensive analysis of the blood groups among persons who tested negative for all three panels, it is evident that B-negative is the prevailing blood type, accounting for 40.8% of the cases. Subsequently, A-negative constitutes 27.6% of the cases, while AB-negative comprises 22.4% of the cases. The O-negative blood type exhibits the lowest prevalence, accounting for 9.2% of individuals within this particular grouping. The present study additionally examines the titration values associated with anti-D antibodies, thereby uncovering variances in their concentrations. The titration result of 1:024 implies a very high concentration, whilst the value of 1:4 denotes a comparatively lower concentration. The study of the standard curve highlights that the prevailing titration value is 1:64, indicating a mean concentration of anti-D antibodies in the bloodstream. The outcomes of this study deliver treasured insights into the demographic distribution and blood group characteristics within the sample population. Furthermore, these findings offer a deeper understanding of probable patterns and changes in anti-D antibody concentrations.

Keywords: Demographic composition, Blood group characteristics, Prevalence, Titration values, Clinical implications, pregnant women, Blood type prevalence.

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INTRODUCTION

Red blood cell (RBC) alloimmunization is a condition that occurs when a person develops antibodies against antigens on the surface of foreign RBCs. These antibodies can cause hemolysis (destruction) of the RBCs that carry the antigens, leading to anemia and other complications. Alloimmunization can happen in two main scenarios: transfusion and pregnancy. In transfusion, alloimmunization occurs when a person receives blood from a donor who has different RBC antigens. The most common and clinically significant antigens are those of the ABO and Rh systems, but there are many other minor antigens that can also trigger an immune response. The risk of alloimmunization depends on several factors, such as the frequency and volume of transfusion, the antigenic mismatch between donor and recipient, and the genetic and environmental factors that influence the immune system (1).

In pregnancy, alloimmunization occurs when a mother and her fetus have different RBC antigens, usually inherited from the father. The utmost common source of alloimmunization in pregnancy is Rh incompatibility, which happens when the mother is Rh-negative and the fetus is Rh-positive. This can result in hemolytic disease of the fetus and newborn (HDFN), a serious condition that can cause fetal anemia, hydrops fetalis, jaundice, kernicterus, and even fetal death 3. Other RBC antigens, such as Kell, Duffy, Kidd, and MNS, can also cause HDFN. The analysis of alloimmunization is grounded on the recognition of antibodies in the serum of the transfused or pregnant person. The identification of the specific antigen involved is important for determining the clinical significance and management of the condition. The severity of hemolysis and anemia can be assessed by various methods, such as direct antiglobulin test (DAT), indirect antiglobulin test (IAT), antibody titers, flow cytometry, and fetal blood sampling (2).

The treatment of alloimmunization aims to prevent or reduce hemolysis and anemia, and to avoid complications. In transfusion, this can be achieved by selecting compatible blood products for the recipient, using leukoreduced or washed RBCs, and administering immunosuppressive drugs or intravenous immunoglobulin (IVIG) in some cases 2. In pregnancy, this can be achieved by administering anti-D immunoglobulin to Rh-negative mothers, performing intrauterine transfusions to the fetus if needed, and delivering the baby at an appropriate time 3. The prevention of alloimmunization is based on avoiding unnecessary exposure to foreign RBC antigens. In transfusion, this can be achieved by using restrictive transfusion thresholds, matching blood types for major antigens, and screening for antibodies before transfusion (2). In pregnancy, this can be achieved by administering anti-D immunoglobulin to Rh-negative mothers before or after delivery or any event that may cause fetomaternal hemorrhage, such as abortion, amniocentesis, or trauma (3, 4).

Blood group antigens are molecular moieties that are located on the surface of erythrocytes. These antigens are able to provoke an immunological reaction if the immune system recognizes them as being from a foreign source. In the wide variety of blood group systems, the ABO and Rh systems stand out as particularly important in certain circumstances, such as during pregnancy and blood transfusions (5). The term "blood compatibility" refers to the capacity of the blood of one human to be safely infused or exchanged with the blood of another individual, without inducing any unfavorable immunological responses in either of the individuals. Testing for blood compatibility entails performing a technique that is intended to determine an individual's blood type and antibody profile. This makes it possible to more easily match the individual with appropriate blood products or donors (6).

The complication known as Hemolytic Disease of the Newborn (HDN), which may also be referred to as Hemolytic Disease of the Fetus and Newborn (HDFN), is one of the most serious side effects of red blood cell alloimmunization. This disorder manifests itself when a mother's blood type is different from that of her developing fetus, which causes the mother's antibodies to pass through the placental barrier and attack the erythrocytes of the developing fetus. This condition is also known as Rh incompatibility. The subsequent repercussions include fetal anemia, jaundice, hydrops fetalis (an abnormal collection of fluid in the tissues of the fetus), and unfortunately fetal mortality. Jaundice is a yellowing of the skin (7).

The presence of Rh incompatibility is widely recognized as the most important contributor to HDN's etiology. An Rh incompatibility occurs in a pregnancy when a woman who is Rh-negative has a fetus that is Rh-positive. Anti-D immunoglobulin can be given to Rh-negative women either before or after delivery, or in reaction to any incident that might give rise to fetomaternal hemorrhage, such as abortion, amniocentesis, or trauma. This harrowing dilemma, however, can be avoided entirely with the use of a preventative measure that involves the injection of anti-D immunoglobulin. In addition, a wide variety of additional blood group antigens, such

as Kell, Duffy, Kidd, and MNS, possess the capacity to influence the growth of HDN (8).

MATERIALS AND METHODS

Study Design

The experimental study was designed at the Department of Medical Laboratory Technology.

Study Setting

The study was conducted at Department of Medical Laboratory Technology, Riphah International University, Lahore.

Duration of the study

The study was done into 8 to 12 months after release of the approval of synopsis from department.

Sample Size

The sample size for that study was estimated to be approximately 100 participants. This number had been carefully determined to ensure that it was representative of the population under investigation and that the study results were statistically meaningful and reliable.

Sampling Techniques

In this study, a convenient sampling technique was employed to select contributors for the research. This approach containsselecting individuals who remain readily available and accessible for data collection, often based on their proximity or ease of recruitment.
BIORAD Panel 3

We began by washing the Biotestcell-3 cells three times with phosphate-buffered saline (PBS). This step ensured the cells were prepared for the antigen detection process. We suspended the cells in PBS to achieve a 2% concentration. This concentration was essential for accurate testing. We took a microtiter plate and added 50µL of the prepared cell suspension to each well. This step was crucial for setting up the testing environment. Next, we added 50 µL of the patient's serum or plasma to each well of the microtiter plate. This introduced the patient's sample into the testing process. We centrifuged the microtiter plate at 1000 rpm for 5 minutes. This step aided in the separation and isolation of components within the wells. We incubated the microtiter plate at a temperature of 37°C for a duration of 30 minutes. This controlled environment allowed for specific reactions to occur. After incubation, we once again centrifuged the microtiter plate at 1000 rpm for 5 minutes. This step further facilitated the identification of reactions. We examined the wells of the microtiter plate carefully for agglutination. The presence of agglutination indicated that the patient had an antibody directed against the antigen present on the red blood cells in that specific well. This panel included cells representing all ABO types, Rh types, Kell types, Duffy types, Kidd types, MNS types, Lewis types, and P types. This comprehensive coverage ensured a thorough antibody screening process. The Biotestcell-3 antigen profile was a valuable and versatile tool for detecting unexpected red blood cell antibodies, making it an essential resource in clinical settings. It was of utmost importance to meticulously follow the provided instructions throughout the procedure to guarantee the accuracy and reliability of the results.

The BIORAD-11 panel procedure

The procedure for utilizing the Biotestcell-I11 Plus panel involved several steps. Firstly, the Biotestcell-I11 Plus cells underwent three washes with phosphate-buffered saline (PBS). These cells were then suspended in PBS at a 2% concentration. Subsequently, 50µL of this cell suspension was added to each well of a microtiter plate, along with 25µL of the patient's serum. The microtiter plate was then centrifuged at 1000rpm for 5 minutes. Following this, the plate was incubated at 37°C for 30 minutes and once again centrifuged at 1000rpm for 5 minutes. The final step involved examining the wells of the microtiter plate for agglutination. The presence of agglutination indicated the existence of an antibody against the specific antigen on the red blood cells in that particular well (9, 10).

Sample Selection

Inclusion Criteria

This study included pregnant individuals who were RhD negative and the age range for inclusion was between 18 and 45 years.

Exclusion Criteria

Females who were not pregnant and individuals who were Rh positive were excluded from participation in this study. This also applied to male participants, who were not within the scope of this research.

Data Collection Procedure

Data was collected through a pre-designed Performa from RhD negative pregnant females. Initially, the participants consent was obtained, and they were informed about the study's objectives. Subsequently, demographic information, including names, ages, education levels, and other pertinent personal details, was recorded for each participant. A cross-sectional survey was conducted utilizing a convenient sampling technique. The researcher visited various laboratories and hospitals in Lahore to collect the data. SPSS software was employed for data analysis, and references were cited using EndNote X9.

RESULTS

Prevalence of Blood Groups in study group

Table 1: Prevalence of Blood Groups in study group

Blood Groups	n=100	%
A -ve	31	31
B -ve	39	39
AB -ve	21	21
O -ve	9	9

****-ve (Negative),**

Table 2: Statistical characteristics of Blood Groups in study group

Blood Groups	Mean	SD
A -ve	25	12.4
B -ve	25	12.4
AB -ve	25	12.4
O -ve	25	12.4

-ve (Negative), SD (standard deviation)

Prevalence of Three Panel in study group

Table 3: Evaluating the Prevalence of Three Panels within the Study group

Blood Groups	Three Panel Negative (n)	Three Panel Negative (%)
A -ve	21	27.6
B -ve	31	40.7
AB -ve	17	22.3
O -ve	7	9.2

Prevalence of Eleven Panel in study group

Table 4: Evaluating the Prevalence of Elven Panels within the Study group

Blood Group	Eleven Panel Positive (n)	Eleven Panel Positive (%)
A -ve	10	13.1
B -ve	8	9.5
AB -ve	4	5.2
O -ve	2	2.6

DISCUSSION

The table provides a detailed overview of age groups, frequencies, and percentages in the sample. The smallest age group, 18-21, has a frequency of 25, representing 25% of the total sample. Conversely, the largest age group, 34+, has a frequency of 3, making up 3% of the sample. A significant majority (61%) falls within the age range of 22-29, with only 15% aged 30 or older. The age group 26-29 stands out with the highest frequency (36) and percentage (36%), while the 34+ age group has the lowest frequency (3%). Comparisons between age groups highlight interesting patterns. The 22-25 and 18-21 age groups show similar frequencies and percentages. However, the 30-33 age group has less than half the frequency and percentage of the 26-29 age group.

Notably, differences between frequencies in adjacent age groups vary from 1 to 24, indicating a non-constant distribution pattern across the age spectrum. The study also reveals that the percentage of patients who test positive for all three panels differs across different blood types. The percentage of three-panel-positive individuals is largest among those with the A-negative blood group (10/31, or 32.3%). The lowest percentage is reported in those with an O-negative blood type; just 22.2% of this group are positive on all three panels. Eight out of thirty-nine (20.5%) patients with blood type B and four out of twenty-one (19%) with blood type AB are three-panel positive.

In 2018, Afzal and colleagues studied in Karachi the prevalence of ABO and Rh blood groups among the local populace. In the study, researchers tested the blood types of 1,000 individuals from various parts of Karachi. The percentage of people with blood types B+ and O-negative is higher in Karachi than the global average, whereas the percentage of people with blood types A+ and O+ is lower. This could be a result of the geographical and historical diversity of the Karachi populace (11).

According to Mehmood et al., Nine hundred out of a thousand samples (90%), according to the results, were Rh-positive. The remaining 100 samples were Rh-negative. Incompatibility between a donor and a receiver in the Rh blood group system can lead to transfusion reactions or hemolytic illness of the infant (12).

According to the Shah et al., B+ is the most frequent blood group in Karachi, followed by O+, A+, and O-negative. The research also found that Rh+ is the most frequent blood type in Karachi, followed by Rh-(13).

According to Abbas and colleagues, there appears to be some uniformity in blood group distribution throughout Karachi. The results of the study have important ramifications for blood transfusion practices in Pakistan. Given that the O-positive blood group is so common, it is crucial to keep a large supply of O-positive blood on hand to meet any potential transfusion needs. The need of maintaining an O-negative blood reserve, especially in advance of emergencies, is further emphasized by the fact that O-negative blood is a universal donor type (14).

The study provides important information about blood group distribution in Punjab Pakistan, and the surrounding area. This data has the potential to improve blood transfusion tactics on a local and national level, leading to better blood banking procedures all over the country.

A third antigen, the Rh factor or the Rh antigen, is present on the surface of the red blood cells and is measured by the elven panel positive value, which may be found in the third column of the blood group table. Whether a person has a positive or negative blood group is determined by this antigen. Roughly two-thirds of the population has Rh-positive blood because their red blood cells carry the Rh antigen. One-third of the population lacks the Rh antigen and is therefore blood group negative. The percentage of Rh-positive red blood cells in a sample is represented by the positive number on the elven panel. If the value is higher, then more cells are Rh-positive (15, 16).

The majority of samples proved negative for the three antigens (C, E, and K) analyzed by the three-panel negative and eleven-panel positive tests. When there is an incompatibility between a donor and a receiver, transfusion reactions or infant hemolytic illness might occur because of the Rh blood group system. We tested 76 samples negative for all three antigens. The remaining 24 samples were three panels positive, implying that they possessed at least one of the three antigens.

Only a small percentage of samples tested positive for all eleven antibodies (anti-A, anti-B, anti-D, anti-C, anti-E, anti-K, anti-Fya, anti-Fyb, anti-Jka, anti-Jkb, and anti-M) on the eleven-panel test. Transfusion reactions or newborn hemolytic illness can occur when ABO and Rh blood group antibodies are incompatible between a donor and a receiver. According to the data in the table, 24% of the samples (out of a total of 100) tested positive for the presence of at least one of the eleven antibodies. The remaining 76 samples tested negative for all eleven antibodies (eleven panel negative).

Blood group test results, as well as the results of the three-panel and eleven-panel positive tests, are displayed in the table 4.5. The results of the three-panel negative test and the eleven-panel positive test for samples of the same blood type are shown in the table below. Out of 31 A-negative samples, 21 tested negative on three panels and 10 tested positive on eleven. This means that the absence of the three antigens and the eleven antibodies cannot be determined just by the A-negative blood group. The other blood groups are the same.

Since people of Iranian and Arab descent are more likely to have TPN blood groups, this possibly explains why TPN blood groups are more common in Pakistan. Rh-negative blood types, which are more common among Pakistani communities, are also more common in TPN blood groups (17).

The study's results could affect how blood is transfused in Pakistan. Since TPN transfusions are more prevalent than TPP ones, it's crucial to stock up

on TPN blood. Because it can be transfused into patients with TPN blood who have also developed antibodies against one or more of the red blood cell antigens, TPP blood is still an important component of the blood transfusion system (18).

CONCLUSION

The results of this study offer significant contributions to our understanding of the demographic makeup and blood group attributes of the population under investigation. The data reveals a significant concentration of persons within the 22-29 age bracket, accounting for 61% of the overall sample. In contrast, individuals aged 30 and beyond represent a mere 15% of the total population. Comparisons across different age groups reveal notable patterns, notably when examining the 30-33 age group, which demonstrates a much lower frequency and percentage compared to the 26-29 age group. The analysis of blood groups indicates that the B-negative blood type exhibits the highest prevalence, constituting 39% of the overall population. Subsequently, the A-negative blood group accounts for 31% of the population, while the AB-negative blood group represents 21%. In contrast, O-negative blood type exhibits the lowest prevalence, constituting a mere 9% of the overall population. Among the subset of persons who test negative for all three panels, the B-negative blood type stands out as the most frequently observed, underscoring its high prevalence.

The examination of titration data pertaining to anti-D antibodies reveals disparities in concentration levels. The titration result of 1:024 indicates a significantly elevated concentration, whereas the titration value of 1:64 is indicative of a more typical concentration. The titration value of 1:4, which is the lowest seen, indicates a somewhat lower concentration. This finding suggests that there is variability in the concentrations of anti-D antibodies within the sample. The aforementioned findings jointly contribute to a comprehensive understanding of the demographic and blood group characteristics within the population under study. The examination of age distribution and blood group variances, as well as the analysis of anti-D antibody concentrations, provide a basis for further investigation and prospective ramifications in the realms of healthcare and clinical practice.

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