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To Determine the Prevalence of Red Cell Alloimmunization among Antenatal Women at Tertiary Care Hospital in Western Rajasthan, India

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Abstract: ***Introduction: Alloimmunization during pregnancy, particularly in women who are multigravida or have a history of adverse obstetric outcomes, poses significant risks, such as Hemolytic Disease of the Fetus and Newborn (HDFN). Alloimmunization occurs when maternal antibodies are formed against fetal red blood cell antigens. Screening for these antibodies is essential, especially for women with previous blood transfusions or complications, as it helps in the prevention and management of HDFN. Despite the importance of screening, systematic antibody screening is not widely implemented in many parts of India, especially for non - Rh antibodies. Aim: To determine the seroprevalence of unexpected alloantibodies in antenatal women attending a tertiary care hospital in Western Rajasthan, India. Objectives: 1) To assess the prevalence of red cell alloimmunization among antenatal women. 2) To identify the specific antibodies in the detected cases of alloimmunization. 3) To determine the antibody titers. Materials and Methods: A prospective, hospital - based cross - sectional study was conducted at the Department of Immunohematology and Transfusion Medicine, Sardar Patel Medical College, Bikaner. The study involved 803 pregnant women who consented to participate. Data were collected using structured questionnaires, and blood samples were analyzed for ABO and Rh blood groups, antibody screening, and identification using automated and gel column agglutination techniques. Antibody titration was performed using the standard tube method. Statistical analysis was performed using SPSS. Results and Observations: The study included 803 pregnant women with an average age of 25.82 years. Rh - positive women accounted for 93.4%, and the most common blood group was B (39.47%). The alloimmunization rate was 0.99%, with a higher rate of 7.54% in Rh - negative women compared to 0.53% in Rh - positive women. The most common antibody identified was Anti - D (50%), followed by Anti - C, Anti - c, Anti - e, and Anti - K (12.5% each). A history of blood transfusions (3.61%) and bad obstetric history (3.48%) were significantly associated with higher alloimmunization rates. Conclusion: The prevalence of red cell alloimmunization in this study was found to be 0.99%, with a notably higher rate among Rh - negative women. Anti - D was the most commonly identified antibody. Targeted screening for alloimmunization, particularly in Rh - negative women and those with a history of blood transfusions or bad obstetric history, is recommended. This could significantly reduce the risk of HDFN and improve maternal and fetal outcomes. However, due to limited resources in developing countries like India, universal antenatal antibody screening may not be feasible, but focused screening for high - risk groups is crucial.***

Keywords: Alloimmunization, Antenatal women, Red cell antibodies, Rh - negative, Rh - ositive, ABO blood group

1. Introduction

Alloimmunization is a critical concern for pregnant women, particularly those who are multigravida or have a history of adverse obstetric outcomes. To minimize the risk of Hemolytic Disease of the Fetus and Newborn (HDFN), pretransfusion testing, including antibody screening and identification, is essential for women of childbearing age. This is particularly important for women who may develop alloantibodies due to previous transfusions or obstetric histories, as these antibodies can complicate transfusion therapy and pretransfusion testing.

The ABO blood group system, discovered by Landsteiner in 1900, was foundational in the development of blood typing. Advances in testing, including the discovery of irregular antibodies and the development of techniques by Coombs et al., have significantly enhanced transfusion safety. Alloimmunization occurs when an incompatible antigen triggers an immune response, leading to the production of antibodies, which can result in complications like HDFN.

The incidence of alloimmunization in pregnant women varies globally, with rates ranging from 0.4% to 8.74%. Factors such as ethnicity, availability of antenatal care, and implementation of anti - D prophylaxis programs influence these rates. In India, systematic antibody screening is performed for Rh - negative women or those with a poor obstetric history, but many regions lack widespread screening for non - Rh antibodies.

HDFN, which can cause significant fetal morbidity and mortality, is largely preventable. Early detection of irregular alloantibodies through screening can enable timely interventions, such as intrauterine transfusion or post - delivery exchange transfusion, to improve outcomes for both mothers and infants. This study was initiated to assess the prevalence and clinical impact of maternal alloantibodies in pregnant women attending the antenatal clinic at our institution, where antibody screening is not routinely conducted.

Aim: To study seroprevalence of unexpected alloantibodies in antenatal females

Objectives:

1) To find out the seroprevalence of alloimmunization in antenatal females.
2) To find out the antibody specificity of detected antibody.
3) To determine the titre of the antibody identified.

2. Materials and Methods

This prospective hospital - based cross - sectional study was conducted at the Department of Immunohematology and Transfusion Medicine, Sardar Patel Medical College, Bikaner, after obtaining approval from the Institutional Ethics Committee and written informed consent from the participants. The study aimed to investigate the seroprevalence of red cell alloantibodies in pregnant women.

Study Design and Sample Size: A descriptive observational study design was used. The sample size was calculated based on a 95% confidence level, assuming a 3.1% seroprevalence of red cell alloantibodies in pregnant women, with an allowable error of 1.2%. A total of 802 maternal venous samples were collected.

Inclusion and Exclusion Criteria: Inclusion criteria: Pregnant women attending the antenatal clinic (ANC) and providing consent.

Exclusion Criteria: Women who received anti - D prophylaxis or blood transfusion during the current pregnancy, and those who did not consent.

Study Tools: A structured pre - tested questionnaire (Performa A, B, and C) was used to collect demographic, clinical, and laboratory data. Obstetric history, clinical examination, ABO and Rh blood group typing, antibody screening, and identification were performed.

Blood Grouping and Antibody Testing:

- **Blood Grouping:** Automated blood grouping was done using the NEO Iris system.
- **Indirect Antiglobulin Test (IAT):** Performed using gel column agglutination technique.
- **Antibody Screening and Identification:** Conducted using Solid Phase Red Cell Adherence (SPRCA) technology, and antibody titration was performed using the standard tube method.

Sample Collection: 3 ml of EDTA blood was drawn from participants during ANC visits. Blood samples were tested for ABO, Rh types, and antibody screening using the NEO Iris automated analyzer. Positive samples were further evaluated for antibody identification and titration.

Statistical Analysis: Data was entered into Microsoft Excel and analyzed using SPSS version 21.

3. Results and Observations (Summarized)

In this study of 803 pregnant females, we analyzed data based on age, blood group, Rh status, gravida status, and clinical history (blood transfusion and bad obstetric history). Key findings include the following:

1) **Patient Demographics:**
- The majority (52.1%) of patients were aged 18 - 25 years, with a mean age of 25.82 years.
- Most participants (93.4%) were Rh (D positive), and the most common blood group was B (39.47%).

2) **Gravida Status and Clinical History:**
- 322 women were primigravida (first pregnancy), and 481 were multigravida (subsequent pregnancies).
- A total of 86 women had a history of blood transfusion (10.7%), and 119 (14.8%) had a bad obstetric history.

3) **Frequency of Alloimmunization:**
- Overall, the alloimmunization rate was 0.99%. Among Rh - negative women, 7.54% were alloimmunized, compared to 0.53% among Rh - positive women.

4) **Antibody Specificities:**
- Anti - D was the most common antibody (50% of cases), followed by Anti - C, Anti - c, Anti - e, and Anti - K (12.5% each).

5) **Clinical Correlations:**
- **Gravida Status and Alloimmunization:** 3 women from the second gravida and 2 from the third and fifth gravidas were alloimmunized.
- **Blood Transfusion History and Alloimmunization:** 3.61% of women with blood transfusions were alloimmunized (statistically significant).
- **Bad Obstetric History and Alloimmunization:** 3.48% of women with a bad obstetric history were alloimmunized (statistically significant).
- **Antibody Titers:**
- Antibody titers ranged from 16 to 128, with Anti - D having the highest titer (64 - 128).

Combined Tables:

Category	Subcategory	Number of Cases	Percentage
Age Distribution	18 - 25 years	418	52.1%
	26 - 35 years	355	44.2%
	>35 years	30	3.7%
Blood Group & Rh Status	**Mean±SD Age**	25.82±4.42	
	A (Rh Positive)	160	21.42%
	B (Rh Positive)	295	39.47%
	O (Rh Positive)	233	31.13%
	AB (Rh Positive)	62	7.97%
	Rh (D Positive)	750	93.4%
	Rh (D Negative)	53	6.6%
Gravida Status	Primigravida	322	40.1%
	Multigravida	481	59.9%
History of Blood Transfusion	Present	86	10.7%
Bad Obstetric History	Present	119	14.8%

Alloimmunization Frequency	Positive (3 - cell)	8	0.99%
	Rh (D Positive)	4	0.53%
	Rh (D Negative)	4	7.54%
Antibody Specificities	Anti - D	4	50%
	Anti - C	1	12.5%
	Anti - c	1	12.5%
	Anti - e	1	12.5%
	Anti - K	1	12.5%
Gravida Status & Alloimmunization	2nd Gravida	3	
	3rd Gravida	2	
	5th Gravida	2	
	6th Gravida	1	
Blood Transfusion & Alloimmunization	No History	712	0.70%
	Present	83	3.61%
Bad Obstetric History & Alloimmunization	No History	680	0.59%
	Present	115	3.48%
Antibody Titers	Anti - D	64 - 128	
	Anti - C	32	
	Anti - e	64	
	Anti - c	32	
	Anti - K	16	

This summary consolidates the demographic data, clinical history, alloimmunization rates, antibody specificities, and clinical correlations in a compact and organized table.

4. Discussion

Rh isoimmunization, a manageable issue in high - socioeconomic countries, remains a concern in India, especially in middle - and lower - class populations. Despite Rh immunoprophylaxis programs, alloimmunization rates are still high, and more research and structured guidelines are needed to improve antenatal care, particularly in Rh D positive women. While policies are in place, implementation varies across India, and a more comprehensive approach is needed to reduce the burden of Hemolytic Disease of the Fetus and Newborn (HDFN).

The prevalence of alloimmunization in the present study was 0.99%, similar to other Indian studies (0.5–5.4%). RhD antibodies accounted for 50% of alloimmunizations, and the rate was higher in Rh - negative women (7.54%) than in Rh - positive women (0.53%). A history of blood transfusions, bad obstetric history (BOH), and multiparity were associated with higher alloimmunization rates. Anti - D remains the most common antibody, though non - anti - D antibodies such as Anti - C, Anti - K, Anti - e, and Anti - c were also observed. Anti - D titers in the study ranged from 64 - 128, highlighting its clinical significance.

Given these findings, antibody screening should focus on Rh - negative women and those with BOH to reduce alloimmunization rates. Awareness campaigns about Rh isoimmunization and anti - D prophylaxis should be prioritized, especially in rural India. Additionally, antigen phenotyping of fathers and advanced immunohematological techniques could help in managing alloimmunized pregnancies more effectively.

Comparisons with previous studies show regional variations in antibody prevalence and emphasize the importance of localized research to improve transfusion practices and prevent alloimmunization. While this study has limitations, such as representing only hospital patients, it provides valuable insights into the prevalence and management of alloimmunization in Indian pregnancies.

5. Summary and Conclusion (Shortened)

This study examined 803 pregnant females, focusing on those with a history of blood transfusion, bad obstetric history (BOH), or multigravida status. The key findings include:

1) **Alloimmunization Rate**: The overall frequency was 0.99%, with 8 out of 803 cases.
2) **Demographics**: The study population had an average age of 25.82 years, with 40.09% being primigravida and 59.90% multigravida.
3) **Blood Group Distribution**: The most common ABO blood group was B (39.47%), followed by O (31.12%), A (21.41%), and AB (7.97%).
4) **Rh Distribution**: Rh - positive was 93.4%, while Rh - negative was 6.6%.
5) **Prevalence in Subgroups**: Alloimmunization was higher in those with a history of blood transfusions (3.6%), BOH (3.4%), and multigravida status. The rate was 7.54% in Rh - negative and 0.53% in Rh - positive women.
6) **Antibody Findings**: The most common antibody was Anti - D (50%), followed by Anti - c, Anti - C, Anti - K, and Anti - e (12.5% each). The antibody titers ranged from 16 to 512.

Conclusion

In developing countries like India, universal antenatal antibody screening is not currently feasible due to cost and infrastructure limitations. However, targeted screening for women with BOH and anemia correction can reduce alloimmunization risks. Classifying pregnancies based on risk factors for fetal anemia will aid in better pregnancy management and improve outcomes. Pregnancies with high antibody titers may require intensive care and follow - up.

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