



THE PREVALENCE AND CLINICAL SIGNIFICANCE OF IRREGULAR ANTIBODIES OTHER THAN ABO IN RH NEGATIVE PREGNANT WOMEN IN TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: Routine pre transfusion testing consists of ABO and Rh typing, antibody screening and compatibility testing. The purpose of the antibody screen is to detect red blood cell antibodies other than Anti-A or Anti-B. These antibodies are called 'unexpected' because only 0.3 to 2% of the general population have positive antibody screen. Objective: This study was undertaken to know the prevalence of irregular antibodies in Rh negative pregnant women and also to analyse the clinical significance of these antibodies in the form of neonatal outcome. Materials and method: A Prospective study was conducted on the study population from September 2011 to February 2013. During this period all the antenatal cases reporting in the department of Obstetrics and Gynaecology, Safdarjung hospital, New Delhi were typed for ABO and D antigen. Out of these antenatal cases 500 Rh negative pregnant women were included in the study and were screened for red blood cell alloantibodies by performing Indirect Coomb's test (ICT). The specificity of the antibody was identified by further testing of those samples that gave positive reaction on initial screening. All these Rh negative antenatal cases were followed up and their neonates were examined for the evidence of hemolysis in the form of anaemia, jaundice, splenomegaly.

Results: This study noted the prevalence of irregular red cell antibodies in 4.2% of pregnant women. Anti-D is the most common Antibody identified, accounting for 66.7%. Anti-C and anti-D together accounted for 23.9%. Anti-Kell and Anti-Jkb were identified in 0.4%. Incidence of neonatal anemia is significantly higher in babies born to mothers with RBC antibodies. Conclusion: The prevalence of irregular Red Cell Antibodies in Rh negative women is 4.2%.

KEYWORDS : Blood groups, ABO, Rh.

INTRODUCTION

The human red blood cell (RBC) membrane is complex and contains a variety of blood group antigens, the most clinically significant being the ABO and the Rh system.¹ Maternal alloimmunization occurs when a woman's immune system is sensitized to foreign erythrocytes surface antigens, stimulating the production of immunoglobulin G (Ig G) antibodies.^{2,3} The presence of red cell alloantibodies during pregnancy can result in severe obstetric complications ranging from new-born child's haemolytic disease to fetal death by anaemia.⁴

The red blood cell (RBC) alloantibodies other than naturally occurring anti-A or anti-B are called unexpected RBC alloantibodies and can be found in 0.3-38% of subjects.^{5,6} Immunization to RBC antigens may result from pregnancy, transfusion, transplantation or from injection with immunogenic material.⁷

Before the discovery of the Rhesus immunoglobulin (Rh IG), HDFN due to anti D was a significant cause of perinatal mortality.⁸ The introduction of Rh D typing and anti-D immunoprophylaxis has resulted in a decline in the frequency of anti-D and that of hemolytic disease of the newborn due to anti-D. However, alloimmunization due to other red cell antigens such as Kell, C and E, continues to be important.⁹ Antibodies such as anti-C, anti-Fy^a, anti-MNS and anti-Jk^a are also known to cause fetal and neonatal hemolytic disease.¹⁰ Alloimmunisation in pregnant women has been extensively studied in different areas of the world, with the frequency being found to range from 0.4% to 2.7% worldwide.¹¹

This study was undertaken to measure the frequency of these antibodies and to assess the clinical outcome of pregnancies in

women visiting Obstetrics and Gynecology department of a tertiary care hospital, who might have clinically significant antibodies other than anti-ABO.

AIMS AND OBJECTIVES

1. To determine the frequency and clinical significance of irregular antibodies other than ABO in Rh negative pregnant women associated with hemolytic disease of the newborn.
2. To study the distribution of ABO blood group in Rh negative pregnant women.
3. To identify the Red blood cell antigens other than ABO responsible for irregular antibodies production and thereby resulting in hemolytic disease in the newborn of Rh negative pregnant women.

MATERIALS AND METHOD

This prospective study was conducted in the Department of Pathology and the Department of Transfusion medicine in collaboration with the Department of Obstetrics and Gynaecology and the Department of Paediatrics, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, over a period of one and a half years. During this period all the antenatal cases (irrespective of gestational age, gravida status or anti-D immunoprophylaxis status) reporting in the department of Obstetrics and Gynaecology, were typed for ABO and D antigen using DIAGAST Erythrocytes Magnetised Technology (E.M.Technology). Their obstetric and transfusion histories, history of jaundice in previous baby, history of immunoprophylaxis for anti-D, history of amniocentesis and other relevant histories were obtained through structured questionnaires.

Sample size: 500 Rh negative pregnant women were included in the study. All the cases were followed up and their neonates were examined for the evidence of hemolysis in the form of anaemia, jaundice, splenomegaly. The blood sample taken from the cord blood or from the peripheral vein of the neonate was examined for blood group, hemoglobin, bilirubin and irregular antibodies (By Direct Antiglobulin Test, using E.M.Technology). Those mothers who did not complete the follow up were excluded from the study. After taking relevant histories, blood samples from pregnant women were collected. Written or oral informed consent was obtained from all the participants. Five milliliters (5ml) of whole blood was drawn through venepuncture. 2ml of whole blood was dispensed into EDTA tube and was used for ABO and Rhesus grouping. The other 3ml of blood was allowed to clot in a plain vial, centrifuged at 2500-3000 r.p.m. for 3min and the serum separated into a separate plain tube with a cap. ABO and Rh blood grouping was done by DIAGAST Erythrocytes Magnetised.

E.M. Technology (Erythrocytes Magnetised Technology):

The E.M. Technology is a new technology based on the magnetization of red cells.

The magnetic force, by attracting the red cells to the bottom of the wells enhances the process of haemagglutination. It uses the DIAGAST experience of Microplates technique with 96 U shape wells microplates, predispensed reagents for forward typing, precoated Anti Human Globulin(AHG) for indirect antiglobulin test. It is provided with internal quality controls. The grouping and phenotyping tests were based on the principle of haemagglutination.

The procedure is summarised as-

- IgM antibodies+ antigens of the red cells
- Forward type-monoclonal IgM antisera+red cells of the patient
- Reverse type-IgM antibodies of the patient(anti-A and/or -B)+known red cells(A1, B or A1, A2, B, O)

Positive reaction induces a specific agglutination but Negative reaction does not induce any specific agglutination.

Antibody screening and identification was done by the Indirect Antiglobulin Test(IAT) using E.M.Technology-based on the principle of solid phase AHG coated wells, which are IgG specific for antibodies and identification test are used. Three magnetised cell panel are used for antibody screening and for identification 11 cell panel are used. Positive reaction is shown by the formation of a layer

in the well, and negative reaction is seen as a dot in the center of the well.

For Neonatal testing, 2.5 ml of blood sample was taken from the cord blood or from the peripheral vein of the neonate. Out of this 1.5 ml blood was collected in plain vial and was tested for bilirubin and remaining 1 ml was collected in EDTA vial and were tested for blood group, hemoglobin and presence of irregular antibodies by Direct Antiglobulin Test. The Direct Antiglobulin Test was based on the principle of solid phase. In this 2 different AHGs are coated in the wells:1 anti-IgG in the first well and 1 anti-C3d in the second well. A third well is used for the negative control. Formation of a layer in the well indicates positive result and formation of a dot in the center of the well indicates negative result.

Statistical analysis of the data was done according to the standard procedures followed and by using other relevant tests like, CHI-SQUARE test, p Value <0.05 was taken as statistically significant.

OBSERVATIONS AND RESULTS

The demographic profile of the study cases was as follows.

The age of the mothers ranged from 19-37 years. Majority of the patients (n=251) were in the age range of 20-24yrs followed by (n=34) in 25-29 yrs age group

The commonest blood group identified was B neg. (36%) followed by O neg. (28%) and A neg. (26%).The least common blood group was AB negative (10%).

Out of 500 pregnant women majority (48%) were primi gravida, 6% were grand multigravida (no. of pregnancy 4 or more) and rest (46%) were in between (no. of pregnancy between 1-4). History of blood transfusion was present in one case. History of immunoprophylaxis with anti-D was present in 22% (n=108) of mothers while it was unknown in 2% of cases.

The study showed that 21 (4.2%) were positive for red blood cell antibodies and 479 (95.8%) had negative Red cell antibody screen. The prevalence of irregular red cell antibody was 4.2% in our study. Anti-D (66.7%) is the most common Antibody identified in women with routine antibody screening. Anti-Jkb (4.7%) was identified in one woman and Anti-Kell in one woman. Anti-D was present along with Anti-C in 5 women. There did not exist any significant relationship between ICT positivity and Blood Transfusion in the present study. The important results in mother have been summarized in the Table 1.

Table 1 : Results in mother

1) Age distribution	Years	Number	Percentage %
	<20	15	3%
	20-24	251	50%
	25-29	171	34%
	30-34	57	12%
	35 and above	6	1%
	Total	500	100%
2) ABO grouping	Blood group	Number	
	A neg.	132	
	AB neg.	49	
	B neg.	180	
	O neg.	139	
	Total	500	
3) Gravida	No. of pregnancy	No. of patients	
	1	241	
	4 or more	29	
	Rest	230	
	Total	500	

4) Blood transfusion history	Previous blood transfusion	No. of patients
	Absent	493
	Present	7
	Total	500
5) Immunoprophylaxis	Immunoprophylaxis	No. of patients
	Absent	382
	Present	108
	Unknown	10
	Total	500

Table 1 : Results in mother (Contd.)

6) ICT	Result of ICT	No. of patients	Percentage %		
	Negative	479	95.8%		
	Result of ICT	No. of p	Percentage %		
	Negative	479	95.8%		
	Positive	21	4.2%		
	Total	500	100%		
7) RBC antibodies	Antibody	Frequency	Percentage%		
	Anti-D	14	66.7%		
	Anti-C+Anti-D	5	23.9%		
	Anti-Jkb	1	4.7%		
	Anti-Kell	1	4.7%		
	Total	21	100%		
8) BT Vs ICT	Blood transfusion	ICT		P value=0.576	
		Neg	Pos		Total
	Absent	472	21		493
	Present	7	0		7
	Total	479	21		500

Fetal Out come in the studied group was as follows. Out of 500 women who were included in the study, 475 women delivered live born, 3 still born children and 22 had abortion. There were 2 women with abortion in the RBC Antibody positive group.

The result of DCT was positive in 2% of babies.

There is a significant relation between Results of ICT and Results of Baby Affected at 5 percent level of significance. Out of 14 baby affected 4 were having mothers with negative ICT which indicates some other causes than incompatibility.

There is a significant relation between results of DCT and results of Baby Affected at 5 percent level of significance. Out of 14 babies affected; 6 were having negative DCT which indicates some other causes than incompatibility. Photo-therapy was given to 37% of babies born to mothers who are RBC antibody positive versus 24 % of the mother who were RBC Antibody negative. This is statistically significant.

Exchange transfusion was given to 10.5% of babies born to mothers who are RBC antibody positive versus 0.2% of the mothers who were RBC Antibody negative. This was statistically significant

Table 2: Results in baby

1) DCT	Result of DCT	No. of patients			
	Negative	492			
	Positive	8			
	Total	500			
2) Neonatal outcome Vs RBC Ab Screen	Neonatal outcome	RBC Ab+ve No.	RBC Ab+ve Percentage%	RBC Ab neg. No.	RBC Ab neg. Percentage%
	Live birth	19	90.5	456	95.2
	Still born	0	0	3	0.6
	Abortion	2	9.5	20	4.2
	Total	21	100	479	100%
3) Phototherapy vs RBC Ab screen	Phototherapy	RBC Ab neg. No.	Percentage%	RBC Ab+ve No.	Percentage%
	No	346	76%	12	63%
	Yes	110	24%	7	37%
	Total	456	100%	19	100%

Table 2: Results in baby (Contd.)

4) Exchange transfusion Vs Ab screen	Exchange transfusion	RBC Ab neg. No.	Percentage%	RBC Ab+ve No.	Percentage%
	No	455	99.8%	17	89.5%
	Yes	1	0.2%	2	10.5%
	Total	456	100%	19	100%

DISCUSSION

The incidence of alloimmunization varies greatly among populations. There have been studies which have reported the

significance and incidence of irregular antibodies in pregnant women.^{12,13,14,15,16,17,18}

Although the clinical significance of Rhesus (D) immunization is well known and the evaluation and treatment of such patients is well defined, the management of patients with antibodies to unusual blood group antigen is much less clear.¹⁹ The present study was conducted to see the prevalence of red blood cell antibody in pregnant women. In our study, the age of pregnant women ranged from 19-37 years with majority of the patients (n=251) in the age range of 20-24 yrs. The Commonest blood group of mothers identified in our study was B neg. (36%). This distribution was different from one study as the inclusion criteria were different in that study.⁷ In this study, history of blood transfusion was present in 1% case. This is in contrast to other studies.⁷ The reason for this could

be younger age of mothers in the present study who had no history of blood transfusion.

Most of the mothers (80%) gave no history of previous abortion, 15% gave history of 1 abortion in the past, 4% had history of 2 abortions in the past while 1% had 3 or more abortions previously. This finding was similar to other studies. The present study demonstrates the similar incidence of red cell antibodies with that of other quoted studies. The incidence of red cell antibodies in the present study is 4.2%. The prevalence of irregular antibodies in 6 series is as given below in the table 3.

Table 3 : Comparative study of red cell antibodies

Place	Reference:	Year	No. of Patients	Incidence of Red cell Abs		
				Total	Anti-D	Others
Minnesota	Polesky ¹⁶	1967	43,000	5.7	4.7	1.0
Newyork	Queenan ¹⁸	1969	18,378	3.1	2.1	1.3
Australia	Pepperell ²⁰	1977	72,138	2.0	1.5	0.5
Sweden	Filbey ²¹	1995	1,10,765	0.72	0.48	0.26
Israel	Lurie.S ²²	2003	1265	1.1	0.9	0.2
New Delhi	Present study	2013	500	4.2	3.8	0.4

Limitation of the present study:

The present study was limited by the fact that majority of the women were primigravidae showing no previous history of blood transfusion or any operative procedures done before, thus limiting the number of antibody positive cases. Also, in the present study only 500 Rh negative women were included. Further studies with large number of patients and considering other relevant inclusion criteria can be done to reveal new facts in this field.

CONCLUSION

The prevalence of irregular Red Cell Antibodies in Rh negative women was found to be 4.2% in the present study. Anti-D was the most common antibody identified in RBC Antibodies positive women accounting for 66.7%. Anti-Kell and Anti-Jkb were identified in 0.4%. The most common blood group observed was B-negative. The most common blood group observed in RBC antibody positive screen was A- negative.

Incidence of neonatal anemia, positive DCT and high Cord bilirubin was found in babies born to RBC antibody positive women. Phototherapy and Exchange- transfusion was given to significantly higher number of babies born to mothers with RBC antibody.

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