

ORIGINAL ARTICLE

Frequency of Red Cell Alloantibodies in Pregnant Women of North West Pakistan

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ABSTRACT

Objective: The objective of this study was to determine the frequency of red cell alloantibodies in pregnant women of North West Pakistan.

Study Design: It was a descriptive study.

Place and Duration of Study. This study was conducted in one year November 2012 to October 2013 at the Haematology Department, Army Medical College, National University of Sciences and Technology (NUST) in collaboration with Department of Gynaecology and Obstetrics, Military Hospital, Rawalpindi.

Materials and Methods: A total of 600 samples were studied and it was a non probability convenience sampling. Pregnant females of any age, parity and gestational age were included in the study and women with known autoimmune diseases (SLE, Rheumatoid Arthritis) were excluded. Data was collected through specifically designed proforma and was analysed by using SPSS version 20. Descriptive statistics were used to describe the data. Frequency and percentages were calculated for qualitative variables like blood group and alloantibodies. Mean and standard deviation were calculated for quantitative variables like age, gestational age and parity. Chi-square test was applied to find an association between all categorical variables. p-value <0.05 was considered significant.

Results: The frequency of alloantibodies in pregnant women in this study was 0.5% (3/600). Prevalence of alloimmunization specifically in Rh- negative blood group was 5.5% (3/54). All the antibodies detected were anti-D antibodies.

Conclusion: Rh D antibodies are the only frequent antibodies in majority of pregnant women with Rh negative blood group. So the practice of routine antenatal antibody screening for every pregnant woman should be avoided.

Key Words: *Antibody Formation, Blood Group Antigens, Pregnancy, Pakistan.*

Introduction

Red blood cell alloantibodies are the unexpected immune- antibodies found other than the naturally occurring antibodies in the body, produced in response to the introduction of red cells possessing antigens that the subject lacks, as in cases of pregnancy, transfusion, transplantation or injection of any immunogenic material.^{1,2} In pregnancy alloantibodies appear when fetal RBCs carrying a paternal antigen that is foreign to the mother, enters the maternal circulation and this incompatibility of blood groups between the mother and fetus can lead to Alloimmune Haemolytic Disease of the

Newborn.³

Antibody screening is done in pregnancy to identify these pregnancies which are at risk of fetal and neonatal hemolytic disease resulting from clinically significant maternal alloantibodies.⁴ Despite the introduction of Rh Ig this ABO and Rh incompatibility is still the major cause of Haemolytic disease of the newborn in developing countries raising the importance of antibody screening in all antenatal women irrespective of their blood phenotype.⁵

Studies have shown variation in frequency of alloantibodies among different countries.^{6,7} In Asia although Anti-D antibodies are the most frequent but the difference lies in the frequency of rest of the clinically significant antibodies.^{8,9}

Developed countries like UK and Netherlands do have their own guidelines for the antibody screening in pregnant women.^{10,11} In Pakistan only few case reports and prospective studies reveal the presence of some rare alloantibodies like Anti-Rh17, Anti-Rhnull and Anti-Kell antibodies.^{12,13,14} These cases do raise the importance of such studies in order to know the prevalence of alloantibodies among Pakistani

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women which can enable us to set our own guidelines for the antenatal antibody screening.

Materials and Methods

It was a descriptive study and was conducted in the Hematology Department, Army Medical College, National University of sciences and Technology (NUST) in collaboration with Department of Gynaecology and Obstetrics, Military Hospital, Rawalpindi. It was completed in one year from Nov 2012 to Oct 2013. It was a Non Probability Convenience Sampling.

A total of 600 pregnant females were recruited from the out-patient department of Gynaecology and Obstetrics who came for routine antenatal checkups and were advised routine blood tests. All pregnant women were included irrespective of their age, parity and gestational age. Women with known autoimmune diseases were excluded. History was taken from the females according to the structured questionnaire. Their age ranged from 19 to 40 years. Majority of females were in their third trimester and were primigravida. They were asked about their gravida status, transfusion experience and especially the blood group of their husbands. Out of 600 women only 321(53.5%) were aware of their husband's blood group. Obstetric history was also taken and those who gave history of one or more abortions were considered to have bad obstetric history.

Permission from the hospital ethical committee was taken. Informed consents were obtained from all the patients.

5.5 ml of Venous blood was withdrawn from antecubital vein using 10 ml syringe and was collected in two separate tubes. 2.5 ml blood was for the tube containing EDTA for ABO and Rh grouping and the other 3 ml blood was left to clot in the plain tube for antibody screening and identification. Sample of each patient was given a laboratory number and record was maintained. Each sample was then analysed.

Blood group was determined by forward and reverse blood grouping technique using commercially prepared blood grouping reagents (Biotec) and freshly prepared pooled red cells. During Rh blood typing, indirect antiglobulin test was performed on all negative results in order to confirm the weak D phenotype before reporting the sample as Rh

positive or negative. But we were not able to notice the presence of any weak D antigen among 54 Rh negative blood samples.

All samples irrespective of their blood group were screened for antibodies using 3 cell panel (Diamed) by performing Indirect Antiglobulin Test (IAT). The samples which showed positive results on screening were identified using 11 cell panel (Diacell). Microscopy was done on all negative samples in all stages of IAT.

Data was entered on a specifically designed proforma and was analysed using SPSS version 20. Descriptive statistics were used to describe the data. Frequency and percentages were calculated for qualitative variables like blood group, alloantibodies. Mean and standard deviation (SD) were calculated for quantitative variables like age, gestational age, parity. Chi-square test was applied to find an association between all categorical variables. p-value <0.05 was considered significant.

Results

A total of 600 women were screened for red cell alloantibodies. Age range of patients included in the study was 19 years to 40 years. Maximum number of patients 351(58.5%) presented in their third trimester. (Fig1).

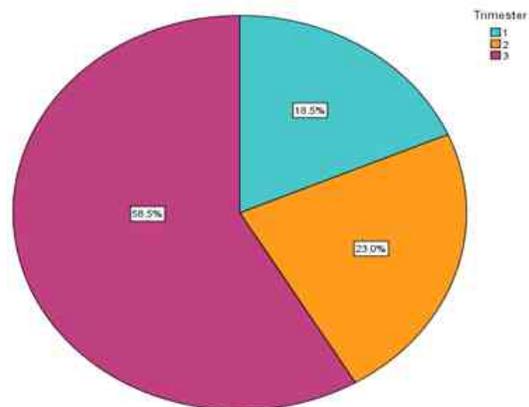


Fig 1: Presentation of Study Group in Various Trimesters

Previous transfusion history was given by 123(20.5%) women. Regarding the major blood group systems, Figure II shows the frequency of major blood group systems

There were 546 (91%) D antigen positive and 54(9%) D antigen negative women in the study group.

Among the study group there were 348(58%) primigravida and 252(42%) multigravida females.

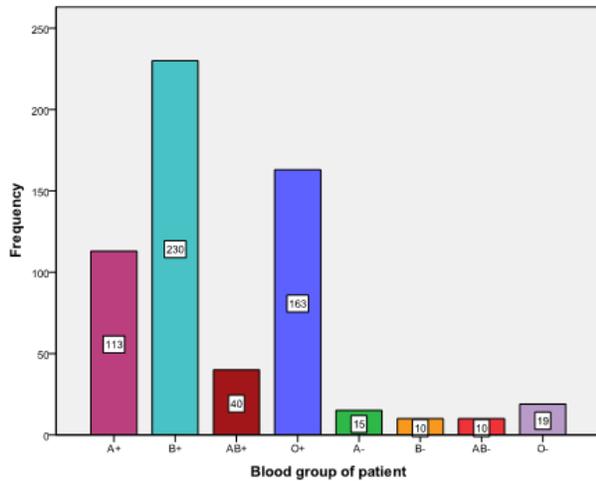


Fig 2: Frequency of Different Blood Groups among Study Group

Out of 348 primigravida 37(10.6%) were found to be D antigen-negative and the rest of 17 D antigen negative were multigravida (Table I).

Table I: Rh Phenotypes of Primigravida and Multigravida Females

Rh D Antigen	Frequency (%)	Primigravida (348)	Multigravida (252)
Positive	546(91%)	311(89.4%)	235(93.3%)
Negative	54 (9%)	37(10.6%)	17(6.7%)

A total of 3 irregular antibodies were identified in their blood samples, showing an overall frequency of alloantibodies as 0.5% (3/600). Within the whole study group (n=600), anti-D was the only detected antibody, accounting for 100% of all the allantibodies. The husband's blood group was found to be D antigen-positive in all. Among the 54 women in the D antigen-negative group, 3 developed antibodies, so the prevalence of alloimmunization in this group was 5.5% (3/54). No antibody was detected among the D antigen-positive group, showing an association of Rh D antigen with antibody formation (p<0.001). An association is seen between antibody formation and the blood phenotype, as all the three women who were anti-D antibody positive belong to A negative blood phenotype. In this study, alloantibodies were found in 1.2% (3/249) of multigravida females and in 0% (0/348) of antenatal women who were primigravida showing statistically significant association between multigravida status and alloimmunization rate (p<0.041). As alloantibodies were found in 2.7% (3/112) of antenatal females with an adverse

obstetric history and in 0% (0/485) of antenatal women without an adverse obstetric history hence this study also shows a statistically significant association between adverse obstetric history and alloimmunisation rate (p<0.001) (Table II).

Table II: Association between Antibody Formation and Rh Phenotype (p<0.001), Gravida Status (p<0.041), Adverse Obstetric History (p<0.001)

Patient Characteristics	Antibody Formation	
	Present	Absent
RhD positive	0(0%)	546(100%)
RhD negative	3(5.5%)	51(94.5%)
Primigravida	0(0%)	348(100%)
Multigravida	3(1.2%)	249(98.8%)
Adverse obstetric history	3(2.7%)	112(97.3%)
Normal obstetric History	0(0%)	485(100%)

Discussion

This study with 0.5% frequency of red cell alloantibodies shows that throughout the world this frequency varies. A comparison of different study results are shown in Table III.

In this study frequency is less as compared to the study carried out in Iran(4.5%) and Southern Pakistan(1.8%).^{15,16} Reason behind it is that firstly the sample size in this study is smaller which increases the chances of missing rare antibodies which are reported in different case reports and secondly majority of females are primigravida in this study.

It is also evident from this study that multigravida status, bad obstetric history and Rh-negative phenotype are main risk factors for antibody formation which supports the studies carried out in India and Malaysia.^{9,17} In contrast to these studies, a Nigerian study reveals comparatively higher alloimmunization rate of Rh positive phenotype when compared to Rh negative phenotype as 13 out of 17 detected antibodies were found in sera of Rh positive females.⁴

Anti D antibodies are the most frequently seen in this study like Europe, Arab and Asia with China as an exception showing anti E and anti Mi antibodies more common than anti D antibodies.^{8,18} Recent studies carried out in America and Australia also show anti E antibody more prevalent than anti D antibodies.^{19,20} So these antibodies other than anti-D antibodies are the reason behind persistence of this hemolytic disease even after the introduction of Rhlg. The different red cell alloantibodies reported to

cause haemolytic disease worldwide are anti Co (a), anti Rh 17, anti Diego, anti Kell , anti c, anti Cw, anti-Jk(b) and anti Kpa.^{21-,29} Keeping in view the evidence of presence of these rare alloantibodies in Pakistani women each and every blood group was screened for red cell alloantibody but none of these were found.

Many developed countries have formulated their own antenatal antibody screening guidelines in order to decrease the disease incidence. These countries include UK, Netherlands, Sweden, Australia and Newzealand.^{10,11,30,31} Studies conducted in China suggested that routine antenatal antibody screening of every Chinese pregnant woman is not beneficial except those who are D antigen-negative or those having a previous history of haemolytic disease of the newborn.^{8,18} Guidelines for screening have also been laid down by the Drug Controller General, India (Drugs and Cosmetics Act., 1989). In 2007, a case was reported in India wherein two women of Rh (D) positive phenotype were found to be positive for alloantibodies has promulgated the need for antibody screening in Rh (D) positive women as well.³²

Unlike these countries, Pakistan lacks the availability of proper antenatal antibody screening guidelines. This study can be helpful in formulating these guidelines by suggesting that regular antibody screening of each and every pregnant woman is not necessary and can be a burden on economy so it should remain limited to females with Rh negative phenotype. Females who have bad obstetric history, are multigravida should also be considered important candidates for antibody screening.

There is a need of carrying this study in all regions of Pakistan with large sample size so that we could be able to identify population which is at increased risk of developing haemolytic disease of newborn.

Table III: Frequency of Red Cell Alloimmunization

Country	Author	Year	Frequency	Most Frequent Antibody
Malaysia	Suria AA, et al ¹⁷	2012	1.3%	Anti-D
India	Pahuja S, et al ⁹	2011	1.25%	Anti-D

Saudi Arabia	Nabeel, S and Bodangi ³³	2011	1.8%	Anti-D
China	Lee CK, et al ⁸	2003	0.79%	Anti-E and Anti-Mi
Taiwan	Wu KH, et al ¹⁸	2003	0.01%	Anti-E and Anti-Mi
Nigeria	Jeremiah ZA, et al ⁴	2011	3.4%	No Anti-D antibody found
Zimbabwe	Cakana, A.Z and Ngwenya, L. ³⁴	2000	1.7%	No Anti-D antibody found
Canada	Heddle NM, et al ³⁵	1993	5.4%	Anti-D
Tunisia	Rekik T, et al ³⁶	2012	3.6%	Anti-D
Turkey	Gunduz E, et al ³⁷	2010	1.21%	Anti-D
Greece	Foudoulaki-Paparizos L, et al ³⁸	2013	0.66%	Anti-D
Sweden	Filbey D, et al ³⁰	1995	0.57%	Anti-D
North west Pakistan	Present Study	2013	0.5%	Anti-D
America	Smith HM et al ¹⁹	2013	3%	Anti-E
Australia	Paul M et al ²⁰	2015	0.73%	Anti-E
Iran	Shahverdi E et al ¹⁵	2017	4.5%	Anti-D
Southern Pakistan	Karim F, et al ¹⁶	2015	1.8%	Anti-D

Conclusion

Rh D antibodies are the only frequent antibodies in majority of pregnant women with Rh negative blood group. However, keeping in view the absence of non-Rh D antibody in our setup, a guideline can be formulated about introduction of routine antenatal red cell alloantibody screening for just the women having Rh-negative phenotype. However in order to lower the risk of Haemolytic disease of the newborn, Rh – positive women with bad obstetric history and

increased gravida status can also be selected for alloantibody screening test.

REFERENCES

- Hoff brand AV. Blood Transfusion. In: Essential Haematology. 6th Ed. UK: Black well publishing limited, Oxford. 2011; pp. 397-412.
- Jeremiah ZA, Mordi A. Evaluation of the clinical utility of maternal alloantibody screening as a surrogate to antiglobulin crossmatch procedures in resource limited settings. *International Journal of Blood Transfusion and Immunohematology*. 2011; 1: 1-6.
- Cohen DW. Hemolytic disease of the newborn: RBC alloantibodies in pregnancy and associated serologic issues. 2012.
- Jeremiah ZA, Mordi A, Buser FI, Adias TC. Frequencies of maternal red blood cell alloantibodies in Port Harcourt, Nigeria. *Asian J Transfus Sci*. 2011; 5: 39-41.
- Basu S, Kaur R, Kaur G. Hemolytic disease of the fetus and newborn: Current trends and perspectives. *Asian J Transfus Sci*. 2011; 5: 3-7.
- Hassan MA, Al Gahtani FH, Gader AM. The frequency of alloantibodies in pregnant and multi-transfused patients – a comparative study between the ID-Micro Typing Gel System and the conventional tube method. *Journal of Applied Haematology*. 2011; 3: 234-42.
- Natukunda B, Mugenyi G, Brand A, Schonewille H. Maternal red blood cell alloimmunisation in south western Uganda. *Transfus Med*. 2011; 21: 262-6.
- Lee CK, Ma ES, Tang M, Lam CC, Lin CK, Chan LC. Prevalence and specificity of clinically significant red cell alloantibodies in Chinese women during pregnancy- a review of cases from 1997 to 2001. *Transfus Med*. 2003; 13: 227-31.
- Pahuja S, Gupta SK, Pujani M, Jain M. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. *Blood Transfus*. 2011; 9: 388-93.
- British Committee for standards in Haematology Guidelines for blood grouping and red cell antibody testing during pregnancy. 2008.
- Semmekrot BA, de Man AJ, Boekkooi PF, Van Dijk BA. Irregular blood group antibodies during pregnancy: screening is mandatory. *Ned Tijdschr Geneesk*. 1999; 143: 1449-52.
- Salamat N, Bhatti FA, Hussain A. Anti-Rh17 (Anti-Hr0): a rare Diagnostic and Management Problem. *J Pak Med Assoc*. 2004; 54: 215-8.
- Qureshi A, Salman M, Moiz B. Rhnull: a rare blood group phenotype. *J Pak Med Assoc*. 2010; 60: 960-1.
- Anwar M, Ali N, Khattak MF, Raashid Y, Karamat KA. A case for comprehensive antenatal screening for blood group antibodies. *J Pak Med Assoc*. 1999; 49: 246-8.
- Shahverdi E, Moghaddam M, Gorzin F. Maternal red blood cell alloantibodies identified in blood samples obtained from Iranian pregnant women: the first population study in Iran. *Transfusion*. 2017; 57: 97-101.
- Karim F, Moiz B, Kamran N. Risk of maternal alloimmunization in Southern Pakistan - a study in a cohort of 1000 pregnant women. *Transfus Apher Sci*. 2015; 52: 99-102.
- Suria AA, Nurudiyana MN, Huik May L, Alex LCS, Noornabillah R, Hud MA, et al. Red Cell Antibody Screening in Pregnancy: A Preliminary Insight. *Med & Health*. 2012; 7: 41-6.
- Wu KH, Chu SL, Chang JG, Shih MC, Peng CT. Haemolytic disease of the newborn due to maternal irregular antibodies in the Chinese population in Taiwan. 2003; 13: 311-4.
- Smith HM, Shirey RS, Thoman SK, Jackson JB. Prevalence of clinically significant red cell alloantibodies in pregnantwomen at a large tertiary-care facility. *Immunohematology*. 2013; 29: 127-30.
- Pal M, Williams B. Prevalence of maternal red cell alloimmunisation: a population study from Queensland, Australia. *Pathology*. 2015; 47: 151-5.
- Michalewska B, Wielgos M, Zupanska B, Bartkowiak R. Anti-Co (a) implicated in severe haemolytic disease of the foetus and newborn. *Transfus Med*. 2008; 18: 71-3.
- Brumit MC, Carnahan GE, Stubbs JR, Storry JR, Reid ME. Moderate hemolytic disease of the newborn (HDN) due to anti-Rh17 produced by a black female with an e variant phenotype. *Immunohematology*. 2002; 18: 40-2.
- Haspl HZ, Marunic BS, Susanj TE, Tomicic M, Stipanovic VK. Anti-Diego a red blood cell alloantibody as a possible cause of anemia in a 3-week-old infant. *Arch Med Res*. 2003; 34: 149-51.
- Tasic M, Milosevic J, Stefanovic M, Janosevic RD, Krstic M, Antic V. Kell immunization--a case report. *Med Pregl*. 2009; 62: 369-72.
- Singla S, Kumar S, Roy KK, Sharma JB, Kachhawa G. Severe hydrops in the infant of a Rhesus D-positive mother due to anti-c antibodies diagnosed antenatally: a case report. *Journal of Medical Case Reports*. 2010; 4: 57.
- Chu HP, Kanagalingam D, Chan DK. Severe intrauterine hemolysis due to anti-C (w). *Am J Perinatol*. 2007; 24: 623-6.
- Kim WD, Lee YH. A fatal case of severe hemolytic disease of newborn associated with anti-Jk(b). *J Korean Med Sci*. 2006; 21: 151-4.
- Costamagna L, Barbarini M, Viarengo GL, Pagani A, Isernia D, Salvaneschi L. A case of hemolytic disease of the newborn due to anti-Kpa. *Immunohematology*. 1997; 13: 61-2.
- Jensen KG. Haemolytic Disease of the Newborn Caused by Anti-Kpa. *Vox Sanguinis*. 1962; 7: 476-8.
- Filbey D, Hanson U, Weststrom G. The prevalence of red cell antibodies in pregnancy correlated to the outcome in the newborn: a 12 year study in central Sweden. *Acta Obstet Gynaecol Scand*. 1995; 74: 687-92.
- ANZBT Scientific Subcommittee. Guidelines for blood grouping and antibody screening in the antenatal and perinatal setting 2nd Ed., Australia: Australian & New Zealand Society of Blood Transfusion Inc, Sydney. 2004.
- Thakral B, Agrawal SK, Dhawan HK, Saluja K, Dutta S, Marwaha N. First report from India of haemolytic disease of the newborn by anti c and anti E in Rh (D) positive mothers. *Haematology*. 2007; 12: 377-80.
- Nabeel S, Bondagi. Rhesus alloimmunization in pregnancy. A tertiary care center experience in the Western region of

- Saudi Arabia. Saudi Medical Journal. 2011; 32: 1039-45.
34. Cakana AZ, Ngwenya L. Is antenatal antibody screening worthwhile in the Zimbabwean population? Cent Afr J Med. 2000; 46: 38-41.
 35. Heddle NM, Klama L, Frassetto R, O'Hoski P, Leaman B. A retrospective study to determine the risk of red cell alloimmunization and transfusion during pregnancy. Transfusion. 1993; 33: 217-20.
 36. Rekik T, Amor BI, Louati N, Rekik H, Menif H, Gargouri J. Irregular antibodies testing during pregnancy in Tunisia: study about 5369 women. Transfus Clin Biol. 2012; 19: 64-73.
 37. Gunduz E, Akay OM, Teke HU, Gulbas Z. Incidence of red-cell alloimmunization due to non-anti-D antibodies during pregnancy: an experience from Turkey. Transfus Apher Sci. 2010; 43: 261-3.
 38. Pappas S, Valsami S, Bournas N, Tsantes A, Grapsas P, Mantzios G, et al. Alloimmunisation during pregnancy in Greece: need for nationwide HDFN prevention programme. Transfus Med. 2013; 23: 254-9.
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