Original Research Paper

Volume-8 | Issue-11 | November-2018 | PRINT ISSN No 2249-555X

Pathology

A PROSPECTIVE ONE YEAR STUDY OF FREQUENCY OF RH AND IRREGULAR ANTIBODIES AMONG PREGNANT WOMEN AND ITS IMPLICATIONS

Dr. E. Sabari Priya Department of Transfusion Medicine, MGMCRI, Pillaiyarkuppam, Puducherry - 607403

ABSTRACT

Background: Maternal alloimmunization is triggered by previous incompatible transfusion or fetomaternal haemorrhage. In view of role played by antibodies other than anti-D in development of HDFN (Haemolytic disease of Newborn), it is necessary to screen all antenatal mothers for significant alloantibodies. This prospective study was conducted for 1 year among antenatal mothers.

Methods: Antibody screening was performed among antenatal mothers by Column Agglutination Technology, those mothers in whom antibody was positive were further subjected to antibody identification and followed by estimation of antibody titre

Results: Prevalence of red cell antibodies among antenatal mothers was 0.8%. Anti-D was the most common antibody (50%) identified. Percentage of antibody positive in Rh negative mothers were significantly higher ($p<0.05$) compared to Rh positive mothers

Conclusion: Anti-D is still the commonest antibody detected among antenatal mothers. The occurrence of other irregular antibodies is very low. The scarcity of resources makes universal screening of all antenatal mothers for irregular antibodies not cost effective.

KEYWORDS : Antenatal mothers, irregular antibodies, Rh positive

INTRODUCTION

Haemolytic disease of the fetus and newborn (HDFN) is the destruction of fetal and newborn red cells by maternal alloantibodies specific for inherited paternal red cell alloantigen(1).

Alloimmunization in pregnant women has been studied in different areas of the world with reported frequency ranging from 0.4- 3.1% —2,7. Although anti-D (Rh alloimmunization) was once the major cause of HDFN, the widespread adoption of Rh immunoprophylaxis has resulted in marked reduction of maternal alloimmunization due to Rh D(8). Maternal alloimmunization to other red cell antigens is coming into prominence. Despite this, anti-D (rhesus isoimmunisation) continues to be the commonest cause of death from severe HDFN. More than 50 other red cell antigens have been reported to be associated with HDFN. The most important of irregular red cell antibodies apart from anti-D are directed against Rh (C,E,c,e), Kell (anti-K), Duffy (anti-Fy^a and anti-Fy^b), Kidd (anti-Jk^a and anti-Jk^b) and MNS (anti- M, N, S and s) blood group systems(9).

In view of the role played by the antibodies other than anti-D in the development of HDFN, it may be necessary to screen all antenatal mothers irrespective of whether they are Rh (D) positive or negative to identify clinically significant alloantibodies that might cause HDFN. Most developed countries have guidelines for screening all pregnant women for irregular red cell antibodies.

According to guidelines of British Committee for Standards in Haematology, (BCSH) all pregnant women should be ABO and D antigen typed and screened for the presence of red cell antibodies early in pregnancy and at 28 weeks of gestation (10). However no such guidelines are followed in developing countries like India.

Proper periodic screening helps the physician to identify those mothers with irregular antibodies in whom the fetus is at risk to develop HDFN. It also alerts blood bank personnel about the difficulty in providing blood for those antenatal mothers with irregular antibodies.

The purpose of this study was to evaluate the frequency of maternal alloimmunization to red cell antigens in our population.

MATERIALS AND METHODS

This prospective study was conducted in antenatal mothers attending Outpatient Department in a tertiary care center in South India for one year. The study protocol was approved by the Institute Ethics Committee (IEC) prior to the commencement of the study.

All antenatal mothers in whom antibody screening was performed were included in the study after explaining about the nature of the study. Written informed consent was obtained from antenatal mothers willing to participate in the study. The mother's characteristics and data regarding their antenatal course of pregnancy which included number of pregnancies, period of gestation , type of delivery, maternal- fetal outcome, history of any pregnancy terminations, blood transfusion, ABO group and Rh typing were documented. Immunisation with anti-D, any previous antibody screen done was also noted.

The mother's serum was tested using commercially obtained screening cell panel comprising of three cells containing known blood group antigens. The three cell antibody screen was performed with strict adherence to manufacturer's instructions.

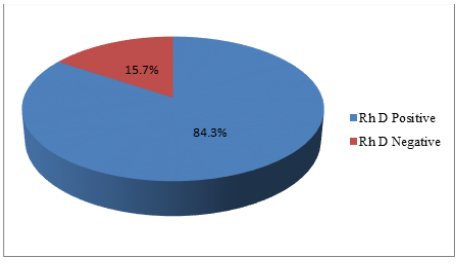
Those serum samples found to be positive on screening were further subjected to identification of alloantibodies using commercially available 11 cell identification panel. The serum of antenatal mothers in whom antibody was identified, was further subjected for titre determination. Baby's cord blood of antibody positive mother's was tested for Direct Antiglobulin Test (DAT).

Analyses were carried out doing both descriptive and international statistics. Data were expressed using descriptive statistics such as frequencies and percentage. The association between the presence of antibodies and other study parameters such as abortions, gravidity status, gestational age and blood transfusions were carried by using chi-square tests. All statistical analysis was carried out at 5% level of significance and p value <0.05 was considered as significant.

RESULTS

During the study period, antibody screening was done on 1350 antenatal mothers attending Obstetrics and Gynaecology outpatient department on a particular day of the week for one year. Group B was the most prevalent 568 (42.1%) and one mother (0.1%) with Bombay group (Oh) was identified. Among the 1350 antenatal mothers, 1138(84.3%) were Rh D positive and 212(15.7%) were Rh D negative (Figure 1).

Figure 1: Rh D typing Distribution



The gravidity status among antenatal mothers ranged from 1 to 11. Among them 700 mothers (51.9%) were mutigravida and 650 (48.1%) were primigravida.

Among the 1350 antenatal mothers, four (0.3%) had a past history of

blood transfusion and 1346 mothers (99.7%) had no history of transfusion. Among the 212 Rh negative antenatal mothers, 185 (87.3%) of antenatal mothers had received postnatal anti-D and 27 (12.7%) had not received anti-D. Out of the total 1350 antenatal mothers, antibody screen was positive in 10 mothers. The prevalence of red cell antibodies among the antenatal mothers was 0.8% (Table 1).

Table 1: Distribution of red cell antibody screening

Antibody screen	Number	Percentage
Positive	10	0.8%
Negative	1340	99.3%

Anti-D (50%) was the most common antibody identified among the antenatal mothers. Anti-D along with Anti-C (30%) was the second most common antibody identified. Anti-M (10%) and Anti H (10%) were identified in one woman each (Table 2)

Table 2: Frequencies of various red cell antibodies among antenatal mothers

Antibody	Number	Percentage
Anti D	5	50
Anti D + Anti C	3	30
Anti M	1	10
Anti H	1	10

It was found that among the 212 Rh negative mothers 9 (4.3%) were found to be positive for antibody and among 1138 Rh positive mothers only 1 (0.1%) was found to be positive for antibody. This shows that the percentage of antibody positive in **Rh negative mothers were significantly higher ($p<0.05$) compared to Rh positive mothers (Table 3)**

Table 3: Positive antibody screen and Rh typing

Rh status	Antibody screen		Percentage
	Positive	**Negative**	
Rh D positive (n=1138)	1	1137	0.1
Rh D negative (n=212)	9	203	4.3

The distribution of antibodies among gestational age of mothers, shows that no antibodies were identified in mothers with gestational age of less than 12, eight antibodies were identified in mothers with gestational age of 12 to 28 weeks and two antibodies were identified in women between 29 to 40 weeks of gestation.

It shows that 8 out of 161 mothers with history of abortions were reported with RBC antibody (5%) against 2 out of 539 mothers without history of abortions (0.37%). This indicates that the RBC antibody was found to be significantly higher ($p<0.05$) among mothers with history of abortions than without history of abortions (Table 4)

Table 4: Relationship between abortion and antibody screen

Antibody screen	History of abortions *		Total	p value
	No	**Yes**		
Positive	2	8	10	$p< 0.001$
Negative	537	153	690	
Total	539	161	700	

*Excluding the primi gravida

Out of the four mothers who gave history of transfusion, two had antibodies (50%), whereas out of 1346 mothers without transfusion, only 8 (0.6%) had antibodies. More mothers with history of transfusion had antibodies $p<0.05$.

Out of the 10 mothers who tested antibody positive, follow up was available for seven mothers. Direct antiglobulin test (DAT) was performed on seven babies of antibody positive mothers. Out of which six babies had a positive DAT. DAT was negative in one baby whose mother had a positive antibody screen for anti M.

Out of five mothers who tested positive for anti-D, follow up was available for two mothers. One mother had a titer of 1:16 and her neonate had jaundice which was successfully treated with phototherapy. The other mother who had a titer of 1:512 delivered a stillborn.

Out of the three mothers who tested antibody positive for anti-D+anti-C and follow up was available, one mother had a titer of 1:16 and her neonate had jaundice which was successfully treated with phototherapy. The other mother who had a titer of 1:128 delivered a stillborn. Another mother who had titer of 1:256 received antenatal steroids, neonate had jaundice which was successfully treated with phototherapy and intravenous immunoglobulins.

One mother who typed positive for anti-M had titer of 1 in 16 and follow up was available, delivered a baby with neonatal jaundice which was treated with phototherapy. Another mother who typed positive for anti-H in whom anti-H titer could not be performed, delivered a baby showing all signs of hemolysis.

DISCUSSION

Red cell immunisation during pregnancy is a challenge that continues to task obstetricians and blood transfusionists even many years after the introduction of Rhesus (Rh) D prophylaxis (11). The introduction of prophylactic anti-D immunoglobulin for (RhD) negative women carrying Rh (D) positive fetus has reduced the number of deaths from RhD haemolytic disease of the newborn(3) .

The successful management of RhD disease has brought attention to atypical or irregular erythrocyte antigens and their antibodies (12) . Maternal alloimmunization to other red cell antigens in the Rhesus, Kell and other blood groups currently cannot be routinely prevented and they also cause HDFN(13).

Proper periodic screening of antenatal mothers for irregular antibodies can alert the physician to a potential problem with haemolytic disease. It also helps the laboratory personnel to identify those antenatal mothers with irregular antibodies and who may require blood transfusion(14).

The prevalence of Rh D negative blood group among the studied mothers was 15.7% (Figure 1). In similar studies conducted in literature reported Rh (D) negativity of 11%to 14%[6,11]. Rh (D) negativity in present study is much higher when compared to other studies.

Rh immunoprophylaxis was received only in 87.3% mothers indicating that some proportion of mothers are still at increased risk of developing antibodies against Rh D antigen . The practice of administering 300µg of rhesus immunoglobulin to Rh negative women around 28 weeks of gestation has been reported to reduce the incidence of maternal alloimmunization from 2 to 0.l(15). No antenatal anti-D is antenatally given in our hospital.

In the present study overall alloimmunization rate among the antenatal mothers was 0.8%. This was similar to other studies described in the Western countries which showed alloimmunization rate of 1.2-2% (6, 11).

Among the RhD negative mothers, alloimmunization rate in the present study was 4.3%. There is a wide variation in alloimmunization among Rh negative women in literature which ranged from 0.9% to 10.4 %(6, 11, 16, 17). Among the Rh positive group, alloimmunization rate in the present study was 0.1%. This is similar to other studies which reported alloimmunization rate 0.12% to 0.2 % (11, 17, 18).

Screening and identification of red cell alloantibodies among 1350 antenatal mothers identified 10 antibody positive mothers. Anti-D was the most common antibody identified accounting for 50% of alloantibodies in our study, similar to other studies in literature which ranged from 20% to 60% (3, 12, 19). The next common antibody identified was anti-D+anti-C contributing to 30% of overall alloimmunization in our study.

In the present study, overall alloimmunization due to Rh system accounted for 80% of immunization, similar to other studies in literature which reported immunization of 52.4% to 92.2% (6, 11).

The present study shows that despite the use of RhIg, anti-D is still the most common antibody identified. There was statistically significant difference between alloimmunization rates in Rh D negative versus RhD positive mothers (4.3% versus 0.1%).

It is difficult to compare the results of different studies because of the heterogeneity of populations involved, varied screening protocols and difference in the techniques used for antibody identification(11).

Although HDFN due to irregular antibodies have been reported in literature, screening for all patients in our population for irregular

antibodies is not justified from cost benefit point of view. So it is not necessary to screen for Rh positive women in our population.

LIMITATIONS

The present study has the following limitations

- It was a one-time screening done on antenatal mothers presenting at different weeks of gestation.
- Sample size is small.
- Duration is not sufficient to examine the full clinical impact of a rare condition such as irregular antibodies which does not occur in a regular pattern.

CONCLUSION

- Anti-D is still the commonest antibody detected among antenatal mothers.
- The occurrence of other irregular antibodies is very low.
- Even among those with irregular antibodies, alloimmunization caused severe HDFN only in about half the women.

The scarcity of resources makes the universal screening of all antenatal mothers for irregular antibodies **not** cost effective.

ACKNOWLEDGEMENT:

Late Dr.S.P Subbiah, Additional Medical Superintendent, Head, Department Of Transfusion Medicine, JIPMER, Puducherry

REFERENCES

1. Kennedy M. Perinatal Issues in Transfusion Practice. In: Roback JD, Combs MR, Grossman BJ, Hillyer CD; editors. Technical Manual. 16th ed. Bethesda: American Association of Blood Banks; 2008: 625-637.
2. Queenan JT, Smith BD, Haber JM, Jeffrey J, Gadow HC. Irregular antibodies in the obstetric patient. Obstet Gynecol. 1969 Dec; 34(6):767–71.
3. Howard H, Martlew V, McFadyen I, Clarke C, Duguid J, Bromilow I, et al. Consequences for fetus and neonate of maternal red cell allo-immunisation. Arch. Dis. Child. Fetal Neonatal Ed. 1998 Jan;78(1):F62–66.
4. Chandrasekar A, Morris KG, Tubman TR, Tharma S, McClelland WM. The clinical outcome of non-RhD antibody affected pregnancies in Northern Ireland. Ulster Med J. 2001 Nov;70(2):89–94.
5. Gottvall T, Selbing A, Hildén JO. Evaluation of a new Swedish protocol for alloimmunization screening during pregnancy. Acta Obstet Gynecol Scand. 1993 Aug;72(6):434–8.
6. Al-Ibrahim NA, AlSaeed AH. Red Blood Cell Alloimmunization among Saudi Pregnant Women in the United Province of Saudi Arabia. Kuwait Med J. 2008;40(2):116–23.
7. Koelewijn JM, Vrijkotte TGM, van der Schoot CE, Bonsel GJ, de Haas M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: a population study in the Netherlands. Transfusion. 2008 May;48(5):941–52.
8. Petz L, Garatty G. Hemolytic Disease of the Fetus and Newborn. In: Petz, Garatty; editors. Immune Hemolytic Anemia. 2nd ed. Philadelphia: Churchill Livingstone; 2004: 517-539.
9. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med. 2008 Aug;13(4):207–14.
10. Gooch A, Parker J, Wray J, Qureshi H. Guideline for blood grouping and antibody testing in pregnancy. Transfus Med. 2007 Aug;17(4):252–62.
11. Pahuja S, Gupta SK, Pujani M, Jain M. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. Blood Transfus. 2011 Oct;9(4):388–93.
12. Geifman-Holtzman O, Wojtowycz M, Kosmas E, Artal R. Female alloimmunization with antibodies known to cause hemolytic disease. Obstet Gynecol. 1997 Feb;89(2):272–5.
13. Eder A, Manno C. Alloimmune Hemolytic Disease of the Fetus and Newborn, In:Greer J,Foerster J,Rodgers G,ParaskevasF,GladerJ; editors. Wintrobe's Clinical Haematology.12th ed. Philadelphia : Lippincott, 2009:978-997.
14. Weinstein L. Irregular antibodies causing hemolytic disease of the newborn. Obstet Gynecol Surv. 1976 Aug;31(8):581–91.
15. Bowman JM. The prevention of Rh immunization. Transfus Med Rev. 1988 Sep;2(3):129–50.
16. Solola A, Sibai B, Mason JM. Irregular antibodies: an assessment of routine prenatal screening. Obstet Gynecol. 1983 Jan;61(1):25–30.
17. Lurie S, Eliezer E, Piper I, Woliovitch I. Is antibody screening in Rh (D)-positive pregnant women necessary? J. Matern. Fetal. Neonatal. Med. 2003 Dec;14(6):404–6.
18. Adeniji AA, Fuller I, Dale T, Lindow SW. Should we continue screening rhesus D positive women for the development of atypical antibodies in late pregnancy? J. Matern. Fetal. Neonatal. Med. 2007 Jan;20(1):59–61.
19. Gottvall T, Filbey D. Alloimmunization in pregnancy during the years 1992-2005 in the Central West region of Sweden. Acta Obstet Gynecol Scand. 2008;87(8):843–8.