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A Cross Sectional Study of Seroprevalence of RBC Alloantibodies in Antenatal Females

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Abstract

Background: As HDFN is preventable cause leads to fetal morbidity and mortality. HDFN due to alloantibodies other than IgG anti-D can be treated with intrauterine transfusion during antenatal period and with exchange transfusion after delivery. Hence, if all pregnant women whether Rh(D) positive and Rh(D) negative are screened for irregular IgG alloantibodies during antenatal period and followed subsequently if any antibodies are present, it may helps in deciding importance of antibody screening during antenatal period, its clinical outcome, mode of intervention to save life of fetus and new born.

Methods: In this prospective study, all pregnant females attending the antenatal clinic (ANC) of Department of Obstetrics and Gynaecology (OBG), at Swai Man Singh Medical College and Attached Hospitals, Jaipur were included. The study was undertaken after approval of protocol by Institutional Ethics Committee and after obtaining written informed consent from the patients enrolled in the study. The number of pregnant women attending antenatal clinic per year is around 45000 at our institute. 3012 such women were included this study with 'high risk pregnancy' i.e. previous history of multiple spontaneous abortion, previous history of still birth, previous history of blood transfusion, multiple pregnancies, any surgical intervention, Rh D negative females without any Anti D immuno-prophylaxis etc. were included in the study.

Results: Out of 3012 pregnant females included in the study, 24 (0.8 %) females had 3 cell antibody screening results positive and thus alloimmunized to red cell antigens. All these 24 females having significant clinical history and including multigravida which was statistically significant according to Chi square test (p=0.005).

Conclusion: Overall frequency of maternal alloimmunization was 0.8% (24/3012). In high risk group (i.e. multigravida, history of blood transfusion, bad obstetric history) it was 1.1%.

Keywords: Alloimmunization, Blood group, Anti D immuno-prophylaxis.

Introduction

Transfusion of blood and blood products are valuable health resource in many clinical conditions such as thalassemic, anaemic, leukemic, transplant patients. Transfusion and bad obstetric history in females may develop alloantibodies which can significantly complicate transfusion therapy and results in difficulties in pre transfusion testing.

Alloimmunization is a great matter of concern with pregnant females who are multigravida and having bad obstetric history. Pretransfusion testing in each and every women of child bearing age group is essential along with antibody screening and identification to minimize risk of Hemolytic disease of fetus and newborn (HDFN).[1]

Basic pathogenesis of HDFN is transplacental passage of maternal IgG antibodies against paternally derived antigens[2] on fetal red blood cell and through blood transfusion[3], these antibodies destroy fetal red cell in utero as well as after birth. The clinical spectrum of the disease is quite variable. Disease develops in intrauterine life and as well as in newborns. Clinically HDFN is characterized by mild hemolytic anemia, hyperbilirubinemia, hepatomegaly, or manifest as severe anemia with hydrops and death of the fetus. Naturally occurring ABO antibodies and alloantibodies directed against Rhesus (Rh) antigens and other minor blood group antigens may cause HDFN.

According to literature, Rh (D) antigen is most immunogenic[4], most common cause of alloimmunization in Rh (D) negative women. Introduction of routine antenatal and postnatal prophylaxis with anti-D immunoglobulins, has declined rate of alloimmunization less than 0.2%.[5] Other red blood cell antigens Rh antigens i.e. C ,c, E, e, non Rh antigens namely Kell, MNS, Duffy (Fy) , Kidd (jk) have emerged as an important cause of HDFN in new borns after red cell alloimmunization.[6] Anti-K, anti-c, anti-Fy^a and anti-E all cause most severe form of HDFN followed by Anti-D.[7] Irregular alloantibodies developed against these minor blood groups and other Rh antigens are problematic since no such prophylactic immunoglobulins are available to prevent HDFN. Screening for these antibodies is a regular part of antenatal workup in the western countries, but no such protocol exists in developing countries.

According to BCHS guideline an evidence based recommendation for application of blood grouping and red cell antibody testing in pregnant females to predict, the potential for, and where possible, prevent, haemolytic disease of fetus and newborn (HDFN). Blood group and antibody status of a pregnant woman should be tested as early as female attends ANC clinic and at 28 weeks of gestation to identify ABO group and D status and to detect red cell antibodies having potential to be clinically significant. In India most of antenatal females are from rural areas where no immuno-prophylaxis for Rh-D negative females nor antibody screening is done.

Many Indian reports in which HDFN due to non-Rh (D) antibodies have been described in Rh(D) positive mothers.[8-10] First pregnancy is usually unaffected as Rh antigen in Rh(D) negative females sensitize and produce IgG antibodies in mother and all subsequent pregnancies are affected if fetus inherit paternal antigens. In Rh (D) positive females having blood transfusion history or multigravida may develop alloantibodies.[2]

As HDFN is preventable cause leads to fetal morbidity and mortality. HDFN due to alloantibodies other than IgG anti-D can be treated with intrauterine transfusion during antenatal period and with exchange transfusion

after delivery. Hence, if all pregnant women whether Rh(D) positive and Rh(D) negative are screened for irregular IgG alloantibodies during antenatal period and followed subsequently if any antibodies are present, it may helps in deciding importance of antibody screening during antenatal period, its clinical outcome, mode of intervention to save life of fetus and new born.

Material and Methods

In this prospective study, all pregnant females attending the antenatal clinic (ANC) of Department of Obstetrics and Gynaecology (OBG), at Swai Man Singh Medical College and Attached Hospitals, Jaipur were included. The study was undertaken after approval of protocol by Institutional Ethics Committee and after obtaining written informed consent from the patients enrolled in the study. The number of pregnant women attending antenatal clinic per year is around 45000 at our institute. 3012 such women were included this study with 'high risk pregnancy' i.e. previous history of multiple spontaneous abortion, previous history of still birth, previous history of blood transfusion, multiple pregnancies, any surgical intervention, Rh D negative females without any Anti D immuno-prophylaxis etc. were included in the study. The blood samples from these women were tested for ABO and Rh (D) type and were also tested for the presence of any irregular antibodies by fully automated immunohematological analyser-NEO Iris (a trademark of immucor). Then antibody specificity and titer were also determined.

Study Design

This was a hospital based Cross -Sectional study conducted in Department of Immunohematology and Transfusion Medicine at Swai Man Singh Medical College, Jaipur.

Study Type: Descriptive type of observational study.

Sample Size

- Sample size is calculated at 95% confidence level assuming 2% seroprevalence of Red cell alloantibodies in pregnant women as per result of reference study.
- At absolute allowable error of 0.5% in prevalence, minimum 3012 blood samples of eligible females required as sample size of present study. 3012 maternal venous samples were collected.
- Detailed obstetric history including last menstrual period (LMP), expected date of delivery (EDD), period of gestation (POG) at which sampling was done, gravid status [primigravida (PGR) or multigravida (MGR)], clinical examination were recorded as per the attached proforma (Proforma-1).
- Blood grouping was performed using fully automated analyser-NEO iris.
- IAT (indirect antiglobulin test) and DAT (direct antiglobulin test) were performed using column agglutination technique.
- Antibody screening and identification were done using Solid Phase Red Cell Adherence (SPRCA) capture technology.
- Antibody titer was determined using standard tube technique.[52]

Data analysis

Statistical analysis was performed with the SPSS, version 21 for Windows statistical software package (SPSS inc., Chicago, IL, USA). The Categorical data was presented as numbers (percent) and were compared among groups using Chi square test. The quantitative data was presented as mean and standard deviation and were compared by students t-test. Probability was considered to be significant if less than 0.05.

Results

In this study a total 3012 pregnant females were included according to their gravida status, previous blood transfusion, bad obstetric history and Rh-negative females without Anti-D immunoprophylaxis. Indirect antiglobulin Test (IAT) was performed to detect sensitization against various red cell antigen. Results were interpreted in relation with past history and gravida status.

Further 3 cell screening and antibody identification were carried out using SPRCA (Solid Phase Red Cell Adherence) method. Clinical and laboratory findings were correlated with antibody specificity and titres of corresponding antibody.

Table 1: Age Distribution

Age group	Number of Cases	Percentage
18-25	1301	43.19
26-35	1584	52.58
>35	127	4.21
Total	3012	100.00
Mean±SD Age	26.95±4.49	

The study population belonged to child bearing age group ranging from 18 to 45 years of age. The mean age was 26.95 years with SD of 4.49 years.

Table 2: ABO and Rh Blood group distribution

ABO	Rh (D Positive)	Rh (D Negative)	Total
A	662	43	705
B	975	98	1073
O	841	88	929
AB	281	24	305
Total	2759	253	3012
P value	0.090 Non significant		

In statistical analysis, 705 (23.40%) were of group A (Rh positive 662 and Rh negative 43), 1073 (35.62%) were of group B(Rh positive 975 and Rh negative 98), 929 (30.84%) were of group O (Rh positive 841 and Rh negative 88), 305 (10.12%) were of group AB (Rh positive 281 and Rh negative 24).

Table 3: Distribution according to Rh

	Number	Percentage
Rh (D Positive)	2759	91.60
Rh (D Negative)	253	8.40
Total	3012	100.00

Rh distribution among study population was Rh(D) Positive 91.6% and Rh Negative was 8.4% respectively.

Table 4: Distribution of Rh antigen

Pheno	Rh (D Positive)	Rh (D Negative)	Total	% positive cases	% negative cases
C	2504	17	2521	83.69%	16.31%
c	1613	250	1863	61.85%	38.15%
E	583	3	586	19.45%	80.55%
e	2738	252	2990	99.26%	0.74%

In study population C 83.69%, c-61.85%, E-19.45%, e-99.26% was Rh antigen distribution other than D.

Table 5: Rh phenotype distribution according to blood group

Rh phenotype	Rh (D Positive)	Rh (D Negative)	Total	% of Phenotype
CCee (A1)	1136	4	1140	37.84%
ccEe (B1)	177	0	177	5.87%
ccee (C1)	57	235	292	9.7%
CcEe (D1)	379	1	380	12.6%
ccEE (E1)	20	1	21	0.7%
Ccee (F1)	981	12	993	32.96%
CCEe (G1)	8	0	8	0.3%
NTD	1	0	1	0.03%
Total	2759	253	3012	100%

Table 6: Frequency of alloimmunization in pregnant females

Status	Rh(D Positive)	Rh (D Negative)	Total
Positive (3 cell screening)	10	14	24
Negative (3 cell screening)	2749	239	2988
Total	2759	253	3012
P value	<0.001 Significant		

Out of 3012 pregnant females included in the study, 24 (0.8 %) females had 3 cell antibody screening results positive and thus alloimmunized to red cell antigens. All these 24 females having significant clinical history and including multigravida which was statistically significant according to Chi square test (p=0.005).

Thus the frequency of alloimmunization in pregnant females with significant clinical history and multigravida females was 1.1% (24 out of 2151 pregnant females)

Discussion

Haemolytic disease of Fetus and new born (HDFN) is common cause of neonatal mortality. Basic pathogenesis of HDFN is transplacental passage of maternal IgG antibodies against paternally derived antigens on fetal red blood cells (RBCs). Anti-bodies developed after maternal sensitization are called alloantibodies. Sensitization may occur due to previous pregnancy, blood transfusion any surgical procedure or invasive procedure as amniocentesis. Alloantibodies ABO, Rh including (Anti-D and other Rh antibodies), Non- Rh antibodies as Kell, MNSs, Duffy, Kidd may cause severe HDFN which may be life threating condition and requires urgent medical management.

This was a prospective study that included 3012 pregnant females. Blood samples were collected as female came to attend ANC (Antenatal Clinic). In our study the subjects enrolled were all pregnant females attending Antenatal clinic, multigravida, having blood transfusion, had any surgical procedure, Rh negative females without any history of Anti-D Immunoprophylaxis.

In our study prevalence of Rh antigen was C 83.69%, c 61.85%, E 19.45%, e 99.26%.

Prevalence pattern of Rh antigen in north Indian donors by Thakral et. al. in sample size of 1240 was C 84.75%, c 52.82%, E 18%, e 98.3% respectively.[11]

In an another study by Makroo et. al.(2010) in their study population of 51,857 blood donors, they found the frequency of Rh antigen as C 89.55% , E 19.85%, c 58.64%, e 98.80%.[12]

Pattern of Rh antigen prevalence in our study was in concordance with above two study.

In our study antibody screening was done to evaluate the prevalence of alloimmunization in pregnant females. 24 out of 3012 pregnant females had RBC alloantibodies and giving overall maternal alloimmunization rate of 0.8%.

Various studies had described antenatal antibody screening in different demographic areas. In a study carried out at Department of Transfusion Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow by Chaudhary R et al. in 2017[13] among 172 Rh D negative pregnant females, 57 (33%) pregnant females were IAT positive.

In a study assessing prevalence of alloantibodies in RhD positive and Rh negative females Anderson et al. found prevalence of 2.5% in total 2612 females.[14]

In another study conducted by Heddle et al. in 17,568 pregnant females 58 cases were detected for alloantibodies which were potentially significant. In both studies no such alloantibodies were formed during gestation period which may result in HDFN.[15]

The prevalence rate of alloimmunization in western countries was ranging from 0.15% to 1.1%[7].

In an Indian study conducted in Bangalore[16] rate of alloimmunization in non-Rh D females was 0.48%

Two such studies conducted in Africa in Uganda[17] and Nigeria[18], prevalence rate was 1.7% and 3.4% respectively. This high prevalence may be due to selection of high-risk population like multigravida in their study.

Rate of antibody frequency in present study was in accordance with the Indian studies.

This could be due to lacking of facility at health care centre for antibody screening, identification, and extended phenotype even at tertiary care hospital for antenatal females.

To prevent alloimmunization , extended phenotype of both mother and father should be done. If transfusion is needed in females of child bearing age group extended phenotypically match and antigen negative blood and components should be transfused.

Advanced immunohematological centers free fetal DNA detection and molecular phenotyping has been started to overcome this problem.

Conclusion

Overall frequency of maternal alloimmunization was 0.8% (24/3012). In high risk group (i.e. multigravida, history of blood transfusion, bad obstetric history) it was 1.1%.

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