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Antenatal antibody screening irrespective of RH status at a tertiary care hospital: A prospective study

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ABSTRACT

Background: Maternal alloimmunization is still the leading cause of fetal anemia and is responsible for neonatal mortality and morbidity in developing countries. Evidence-based guidelines are essential for implementing antenatal alloantibodies screening in developing countries like India which will help to formulate recommendations and reduce adverse outcomes of Hemolytic disease of fetus and new born.
Aims: To determine the frequency of alloimmunization among in Antenatal women during routine antenatal visits irrespective of Rh status.
Materials and Methods: The prospective study carried out in a tertiary care hospital has enrolled 1000 antenatal women (500 each of Rh-positive and Rh-negative women) attending antenatal clinics and admitted for institutional deliveries, were screened for red cell alloimmunization and association between alloimmunization rate in antenatal women with variables was carried out to determine the clinical significance.
Results: Among 1000 antenatal women enrolled and screened 33 (3.3%) antenatal women were found to be alloimmunized. The prevalence of alloimmunization among Rh-negative women is 5.4% (27/500). While the prevalence of alloimmunization among Rh-positive women is 1.2% (6/500). Majority of the alloimmunized cases were multigravida. 75.7% (25/33) antibodies identified in our study were anti-D antibodies and 24.24% (8/33) were non anti-D antibodies.
Conclusions: Successful implementation of Antenatal antibody screening program requires a coordinated Team approach between the Transfusion medicine, Obstetrics, Radiology and Pediatrics departments. Early screening irrespective of Rh status and effective utilization of RhIg prophylaxis in Rh negative antenatal women is the only solution to reduce fetal, neonatal morbidity and mortality due to alloimmunization.

1. Introduction

Maternal red blood cell (RBC) alloimmunization is still the leading cause of fetal anemia and is responsible for fetal and neonatal morbidity and mortality in developing countries. Maternal alloimmunization occurs as a result of sensitization of women's immune system to foreign erythrocyte surface antigens either due to fetomaternal hemorrhage or transfusion, which in turn stimulates the production of immunoglobulin G (IgG) antibodies. These Ig G type of maternal antibodies can cross the placental barrier and evoke immune mediated destruction of fetal red cell antigens resulting in fetal or neonatal anemia. Early identification of alloimmunization among antenatal women can significantly reduce the morbidity and mortality in the affected neonates. American Association of Blood Banks and British Committee for Standards in Hematology guidelines recommend to screen all the pregnancies irrespective of the Rh status for unexpected antibodies, at the initial visit and follow up with repeat screening at 28

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weeks.[1,2] Several other guidelines from developed countries also recommend that first-trimester screening allows timely interventions for the treatment of HDFN due to non-anti D antibodies.[3,4] However, the spectrum of universal screening of all antenatal women, including D-antigen positive antenatal cases is still debated and controversial.[5,6] Although routine screening is done for Rh-negative cases, the prevalence of other antibodies is less well known with limited studies available from different parts of India. There is also a wide variation in alloimmunization rates between geographic areas according to the available literature from India.[7–12] The prospective study aimed was to determine the frequency of alloimmunization, and specificity of antibodies irrespective of Rh status, among the antenatal women attending a tertiary care hospital.

2. Materials and Methods

A prospective study was carried out in the Department of Transfusion medicine among Rh-negative antenatal women attending antenatal clinics and Rh-positive antenatal women admitted for institutional deliveries between September 2018 to September 2020. Institutional ethical clearance was taken. Informed consent was taken from the study participants before screening for the presence of clinically significant alloantibodies. Demographic details of the antenatal women, obstetric history (still births, abortions, MTP and any history of neonatal jaundice), history of blood transfusion, history of anti Rh D prophylaxis was documented in the screening register before taking the blood samples.

3ml and 4ml of blood samples were collected in an EDTA and a plain test tube respectively, for performing ABO (Cell and Serum grouping) and Rh D grouping, Indirect antiglobulin test and antibody screening using column agglutination technique (Diagnostic Grifols, Inc. Spain) as per blood center standard operating procedures. Samples that were screened positive were further tested for alloantibody identification and titrations. Antibody screening and identification were performed by using a commercial RBC panel (Bio-Rad ID, Inc. Switzerland) with known antigens against patient's serum as per manufacturer's instructions. The extended phenotyping of Rh antigens was performed in these cases specific using Rh phenotyping cards (Bio-Rad ID, Inc. Switzerland). Samples that were screened positive for multiple alloantibodies were stored below -30 C for further immunohematology workup.

2.1. Statistical analysis

The data was analyzed using SPSS version 22.0 statistical software package. Descriptive statistics for categorical variables were expressed as frequencies and percentages. Association between the antenatal women alloimmunized and other parameters like gravid status, Rh D status, obstetric history, abortions history and history of blood transfusion was carried out using Odd's ratio. All statistical analysis was performed at a 5% level of significance and was considered significant if p-value <0.05.

3. Results

1000 antenatal women who enrolled for the study were categorized into 500 each of Rh positive and Rh-negative mothers. Their blood samples were collected and screened for red cell alloantibodies. The median age of the study group was 25 years (ranging from 18-45 years). Majority (82.6%) of antenatal women were between 18-30 years age. The gravida status among antenatal women ranged from 1 to 8, of which 332 (33.2%) cases were primigravida and 668 (66.8%) cases were multi gravida.

Most common blood group among the study population was 'O' group followed by B, A and AB blood group. The distribution of blood groups among Rh-positive and Rh-negative antenatal women is shown in Table 1.

Table 1: Distribution of ABO and Rh (D) blood group system

ABO	Rh (D positive) n= 500	Rh (D Negative) n=500
A	101 (20.2%)	90 (18%)
B	157 (31.4%)	165 (33%)
O	215 (43.0%)	210 (42%)
AB	27 (5.4%)	35 (7.0)
Total	500	500

In our study 287 (28.7%) antenatal women had bad obstetric history, 223(22.3%) had a history of prior abortion and 31(3.1%) had a history of prior blood transfusion. When compared to Rh-positive mothers, 125/500 (25%) had a prior history of abortion and 26/500(5.2%) had transfusion history in Rh-negative mothers. Overall Bad obstetric history was found in 162/500 (32.4%) Rh-negative antenatal women.

A total of 41 antibodies were identified during the study period in 33/1000 (3.3%) antenatal women. Alloimmunization rate was found to be 1.2% (6/500) among Rh-positive women and 5.4% (27/500) among Rh-negative women. A statistically significant correlation was found between alloimmunization and Rh-negative antenatal women, p<0.0001. Distribution and frequencies of alloantibodies detected are shown in Table 2.

Among alloimmunized women, majority (97%) were found to be Multigravida. A significant correlation was found between alloimmunization rate and increasing gravida status. Alloimmunization rates among women with bad obstetric History was found to be 5.6% (16/271) when compared to 2.4% (17/696) in women with no such history. Further, the rate of alloimmunization among antenatal women with bad obstetric history and history of blood transfusion was found to be statistically significant.

Table 2: Distribution and frequencies of Alloantibodies detected

Antibody Type	Antibody specificity	No of alloantibodies detected	D antigen Negative cases	D antigen Positive cases	Percentage of total Antibodies detected
Rh	Anti D	18	18 (66.66%)	-	78.78%
	Anti D and Anti C	14	7 (25.92%)	-	
	Anti c	1	-	1 (16.66%)	
MNS	Anti M	4	2 (7.40%)	2 (33.33%)	12.12%
Lewis	Anti Leb	2	-	2 (33.33%)	9.09%
	Anti-Lea and Anti Leb	2	-	1 (16.66%)	
	Total	41	27	6	

However, rate of alloimmunization was not statistically significant in relation to the history of abortion. Association of alloimmunization with different variables is shown in the Table 3.

4. Discussion

The alloimmunization rates reported among the antenatal women from various studies in India ranged from 1.02% to 3.60% when compared to 0.5% to 6% in the western studies.[4,13–17] This variation may be due to heterogeneity of population involved, prevalence of genotype in study population, Rh blood group enrolled, parity, birth rates, screening protocols and techniques involved for antibody screening. Further most of these cases are reported from tertiary care hospitals or Medical colleges with facilities for immunohematology, obstetric and pediatric care.

Our study found the alloimmunization rates among Rh-positive and Rh-negative women. The prevalence of alloimmunization among Rh-negative women was 5.4% (27/500) when compared to 1.2% (6/500) in Rh positive women. Results are in concordance with those of Das et al. who conducted a study from one of the tertiary care hospital in South India.[11] Several studies across India reported an alloimmunization rate of 4.42- 29.03% among Rh negative mothers and 0.08 to 2.2% among Rh positive mothers which was similar to our study (Table 4). This heterogeneity is due to infrequent antenatal visits, remote hilly areas, non-availability of antibody screening facilities, no universal screening of all antenatal mothers and immunoprophylaxis. As per National family health survey-IV, 42.1% women of Telangana state had regular antenatal care compared to 21% women across India.[18]

Our study reported anti D antibodies in 25/33 (75.46%) and non anti D antibodies in 8/33(24.24%) of the study participants. Similar to other studies across India, our study also reports that, despite immunoprophylaxis anti D antibodies still are the major causes of alloimmunization.[7–11] This is evidenced by the fact that 21/25 of antenatal women with anti D antibodies received immunoprophylaxis during previous pregnancies. Bowman et al in his study on failures of Rh immunoglobulin prophylaxis reported a residual risk of Rh immunization of 0.24% to 0.31% in Rh positive pregnancy.[19] Out of many possible causes, inappropriate Rh Immunoglobulin administration (i.e., dosing, timeline according to recommendations) occult fetomaternal hemorrhage that occurs before antenatal Rh Ig administration (28weeks) and lack of its quantification are leading causes to failure of immunoprophylaxis.[20]

Our study reported a higher rate of alloimmunization in antenatal mothers with high order gravid status. Further there was a statistically significant association found between alloimmunization with bad obstetric history and transfusion history. This association is in concordance with several other studies.[7,10,11] 5.4% [12/223] of antenatal mothers in our study who had history of prior abortions were found to be alloimmunized. Although few studies have reported risk of alloimmunization after spontaneous and therapeutic abortions, our study did not show any significant difference with history of abortion and alloimmunization rate among antenatal women.[19,21]

Majority of the alloantibodies detected in our study are of Rh blood group system which are implicated in fetus and neonatal morbidity and mortality. Dual antibody specificities were found in 21.21% (7/33) antenatal mothers in this blood group system. Our study also reported non-Rh antibodies of MNS and Lewis blood group system. Although rarely these are implicated in HDN, no major adverse outcomes were noticed in the antenatal women. However, as these IgG antibodies are capable of crossing the placenta, causing HDN and prolonged anemia, antenatal women should be followed up during antenatal visits with foetal monitoring.[22–25] Distribution of alloantibodies with fetus and neonatal outcome and clinical management is shown in Table 5.

With an estimated worldwide prevalence of 276 per 100,000 live births, much higher Rh disease prevalence was reported in our study. Rh disease due to higher-quality perinatal-neonatal care in developed countries has reduced prevalence to 2.5 per 100,000 live births.[26,27] Thus, in low- and middle-income countries, it is imperative to form a national guideline and include antenatal screening as a part

Table 3: Association of alloimmunization with different variables

D- antigen			
	Antibodies detected (n=33)	**Antibodies not detected (n=967)**	**Significance**
Rh D-Positive (n=500)	6 (18.18%)	494 (51.08%)	p-value: 0.0007
Rh D- Negative (n=500)	27 (81.81%)	473(48.91%)	OR: 4.6998
Gravida Status			
Primigravida (n= 332)	1 (3.03%)	331 (34.22%)	p-value: 0.005
Multi gravida (n = 668)	32 (96.96%)	636(34.22%)	OR: 16.6541
Obstetric History			
Good Obstetric History (n=713)	17 (51.51%)	696 (71.97%)	p-value: 0.01 OR: 2.4172
Bad Obstetric History (n=287)	16 (48.48%)	271(28.02%)	
Abortion History			
Abortion History present (n=223)	11 (33.33%)	212 (21.92%)	p-value: 0.1263 OR: 1.7807
Abortion History absent (n=777)	22 (66.66%)	755 (78.07%)	
Transfusion History			
Transfusion History present (n=33)	4 (12.12%)	27 (2.79.8%)	p-value: 0.005 OR: 4.8020
Transfusion History absent (n=969)	29 (87.87%)	940 (97.20%)	

Table 4: Prevalence of alloimmunization among antenatal women: Review of literature

Studies from India (Place)	**Total No. of antenatal women screened**	**Total No of Rh D-antigen positive women**	**Prevalence among Rh D-Positive women**	**Total No of Rh D-antigen negative women**	**Prevalence among Rh D-negative women**
Mahapatra et al 2020 (Odisha)	362	136	2.20%	226	4.42%
Pahuja et al 2011 (New Delhi)	3577	3183	0.12%	394	10.40%
Varghese et al 2013 (Vellore)	5347	5008	0.08%	339	9.43%
Suresh et al 2015 (Tirupati)	2060	1927	0.30%	133	12.80%
Sidhu et al 2016 (Jammu Kashmir)	750	693	0.45%	57	21.06%
Das S et al 2020 (Karnataka)	2336	1826	1.10%	510	6.90%
Present Study (Telangana)	1000	500	1.20%	500	5.40%

of initial antenatal visits along with blood grouping which will reduce the actual global burden of Rh disease and keep a check on non-Rh-D alloimmunization in antenatal women.

5. Conclusion

Anti D antibody is still a common antibody in developing countries causing alloimmunization despite anti D immunoprophylaxis. Due to limited data available on immunization rates in antenatal women or on the antigens responsible for alloimmunization, evidence-based guidelines for screening of alloantibodies in antenatal women in developing countries is essential which will help for prevention of Hemolytic disease of Fetus and Newborn. Successful implementation of screening in antenatal women for alloimmunization is an integrated approach between the Obstetrics, Transfusion medicine, Radiology and Pediatrics departments. Thus, a process should be developed, checklist made and at-risk antenatal women should be followed up from the first antenatal visit to reduce the frequency of alloimmunization. Early screening and effective utilization

Table 5: Distribution of alloantibodies, their effect and clinical management

Antibody specificity	No of cases	Titer	Outcome and management			
			HDFN	IUD	Transfusion support	Phototherapy/ IVIG
Anti D	18	4 -256	12	nil	nil	12
Anti D and Anti C	7	4 -1024	6	1	3	5
Anti c	1	64	1	1	nil	nil
Anti M	4	2	nil	nil	nil	nil
Anti Leb	2	-	nil	nil	nil	nil
Anti-Lea and Anti Leb	1	-	nil	nil	nil	nil
Total	33		19	2	3	17

HDFN: Hemolytic disease of the fetus and newborn, IUD: Intrauterine death, IVIG: Intravenous immunoglobulin

of RhIg prophylaxis in Rh negative antenatal women is the only solution to reduce fetal, neonatal morbidity and mortality. Our study recommends universal antibody screening for all antenatal women.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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