Original Article:

Prevalence of "unexpected antibodies" in the antenatal women attending the Government Maternity Hospital, Tirupati

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ABSTRACT

Background: All antibodies to red cell antigens, other than naturally occurring anti-A and anti-B are considered unexpected. They can be either alloantibodies or auto antibodies. In pregnant women, these antibodies may cross the placenta and cause haemolytic disease of the foetus and newborn (HDFN). Timely detection of such antibodies in antenatal women is essential for early management of HDFN.

Methods: A prospective cross-sectional study was carried out on 2060 multiparous pregnant women attending the Government Maternity Hospital, Tirupati to detect prevalence of unexpected antibodies. The women were grouped and typed for ABO and rhesus (Rh) D antigens by tube method and screened for alloantibodies by column agglutination technology. The medical and detailed obstetric history of these women were reviewed.

Results: The overall prevalence of alloantibodies was 1.1%. There was a statistically significant difference between alloimmunization rates in the Rh D-antigen negative and D-antigen positive women (12.8% versus 0.3%). The antibodies detected in this study were, anti-D (63.8%), anti-D+C (13.7%), anti-C, anti-E, anti-M, anti-Le^a, and anti-Le^b (4.5% each). Anti-D contributed to 77.3% of total alloimmunization in this study.

Conclusions: In spite of the introduction of prophylactic Rh- immunoglobulin, anti-D (77.3%) is still a common antibody identified in the antenatal women of our region. In developing countries like India, universal antenatal antibody screening, though desirable may not be justified at present as the cost and infrastructure required would be immense. However, it is necessary to impose properly formulated protocols to screen at least the pregnant women with adverse obstetric history.

Key words: *Unexpected antibodies, Alloimmunization, Antenatal multigravida, Haemolytic disease of foetus and newborn*

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INTRODUCTION

Blood group antibodies are immunoglobulins that react with antigens on the surface of red blood cells (RBCs). They can either be acquired naturally or through immunization with foreign RBCs.[1] The naturally occurring antibodies are produced in response to the environmental stimulants such as bacteria.[2] Anti-A and anti-B formed in this manner are often referred as 'natural' antibodies; also called 'expected' antibodies, because in adults with a normal immune system, these antibodies are almost present when the corresponding antigens are absent on the red cells. In contrast, all antibodies to red cell antigens other than naturally occurring anti-A and anti-B are considered 'unexpected'. They can be either alloantibodies, directed toward non-ABO system antigens absent on the red cells or autoantibodies directed towards self antigens. The latter may cause auto immune haemolytic anaemia. Close to 300 different blood group alloantibodies have been described.[3] In

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pregnant women, some of these antibodies may cross the placenta and cause haemolytic disease of the foetus and the newborn (HDFN),[4] a condition in which transplacental passage of maternal antibodies results in immune haemolysis of foetal/neonatal RBCs by either anti-A and anti-B or unexpected immune antibodies which develop following sensitizing event like transfusion or pregnancy.

The introduction of postnatal Rh-immunoglobulin immunoprophylaxis in 1970 has reduced the incidence of maternal alloimmunization from 14% to 2%.[5] Subsequently antenatal immunoprophylaxis has also been started which has further reduced it to 0.1%.[5] Besides the anti-D alloantibody, moderate to severe HDFN attributed to antibodies to other antigens of the Rh system like anti-E, anti-C and antigens of other blood group system have been described from Asian countries.[6,7] Despite prophylactic use of Rh immunoglobulins, anti-D remains the most common antibody identified as the major cause of alloimmunization.

In India, antibody screening is done at some of the transfusion centers, and that also in Rh-D negative mothers only. Few studies from New Delhi, Tamil Nadu and Karnataka have reported frequency of these unexpected antibodies as 1.3%, 1.5% and 1.4% respectively.[8-10] No such reports are available from Andhra Pradesh. Timely detection of such antibodies in antenatal women will be essential both for transfusion safety in mother and early management of HDFN.

MATERIAL AND METHODS

This study was planned to assess the prevalence of unexpected antibodies in multigravida women attending the antenatal outpatient clinic of Government Maternity Hospital attached to Sri Venkateswara Medical College, Tirupati, Andhra Pradesh. This prospective study was carried out at the Department of Immuno Haematology and Blood Transfusion, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh over a period of one year, from June 2012 to June 2013. Written informed consent was obtained from all the women. Ethical clearance was obtained from the Institutional Ethical Committee.

The study was conducted on 2060 multiparous pregnant women irrespective of their period of gestation and obstetric history. Primigravidae and women who had received anti-D prophylaxis in the current and previous pregnancy were not included in the study. For each patient, name, age, sex, obstetric history, blood group, husband's blood group (wherever possible), and history of blood transfusions were recorded prior to taking the blood samples. Blood samples were collected into 3 mL of ethylene diamine tetraacetic acid (EDTA) vials. All the samples were centrifuged at 3000 rpm for 3 minutes and plasma was separated. Before proceeding to antibody screening, the subject's ABO and Rh group were determined as per the standard operating procedure (SOP) followed in the department.[11] All Rh D-negative samples were subjected to weak-D testing by an indirect antiglobulin test and Rh-D positive and negative results were recorded.

Antibody screening and identification was done using semi automated column agglutination technology in Coombs' phase. A commercially available three cell panel (ID DiaCell I, II, III; Diamed ID micro typing system, DiaMed GmbH, Switzerland) was used for antibody screening procedure in which the subject's plasma was reacted with panel of red cells using low ionic strength saline (LISS) Coombs' gel card (DiaMed GmbH, Switzerland). The cards were incubated at 37 °C for 15 minutes and then centrifuged for 10 minutes. The plasma samples which were positive on antibody screen were frozen at −40 °C for antibody

identification, which was performed at a later date. An extended 11-cell panel was used for antibody identification (DiaMed 11 cell DiaPanel, DiaMed ID microtyping system, DiaMed GmbH, Switzerland).

A review was conducted regarding medical history, obstetric history (including any still births, abortions, medical termination of pregnancy (MTP) and cases of HDFN among siblings) and any past blood transfusions of all the subjects.

Comparison of categorical data between antibody screen positive and negative individuals was done using Chi-square test or Fisher's Exact test as appropriate. Demographic and clinical variables were presented as frequency (%). Incidence is presented as proportions with 95% confidence intervals. All statistical analysis was carried out at 5% level of significance and a p-value <0.05 was considered significant. Statistical analysis was carried out using SPSS version 16, SPSS Inc, Chicago, USA.

RESULTS

Their mean age was 23.8 + 3.3 years, (range 18 - 39 years); 96.5% of them were in the age-group of 18 to 30 years and 3.5% of them were above 31 years. The most common phenotype was O positive (41.9%) followed by B positive (31.8%). There were 1927 (93.5%) Rh-D positive women while 133 (6.5%) were Rh-D negative (Table 1). A total of 25 antibodies were detected in 22 antenatal women, giving the overall prevalence of 1.1%.

Table 1: Frequency of blood groups in 2060 multiparous pregnant woman

Blood group	No.	%
A	422	20.5
B	654	31.8
O	864	41.9
AB	120	5.8
Total	2060	100

Among the 133 who were D antigen-negative, 17 developed antibodies. The alloimmunization rate in this group was 12.8%. Among these 17 antibodies, 14 (82.4%) were anti-D alone. Anti-D in combination with anti-C were observed in 3 out of 17 (17.7%). Among the 1927 Rh-D positive women, 5 developed antibodies, giving an overall prevalence of alloimmunization in Rh D -positive group of 0.3%. The antibodies identified were anti-E, anti-C, anti-M, anti-Le^a and anti-Le^b in each one of them (Table 2). There was a statistically significant difference between alloimmunization rates in the D-antigen negative and D-antigen positive groups (12.8% versus 0.3%, p<0.001).

Anti-D alone was the most common antibody encountered, accounting for 63.7%. Multiple antibodies like anti-D+C were seen in 3/22 (13.7%). Antibodies belonging to the Rh system accounted for 86.4% of overall alloimmunization and remaining 13.6% belong to MNS and Lewis systems (Table 3).

In our study, alloantibodies were found in 5/260 (1.9%) of antenatal women with adverse obstetric history and in 17/1800 (0.94%) of antenatal women without any bad obstetric history (p=0.160) (Table 4).

In our study we observed a statistical significance between alloimmunization and gravida status (Table 5). Out of 2060 mothers, history of blood transfusion was present in 14 (0.7%) women but none had alloantibodies. Prevalence of alloimmunization among pregnant women documented in various published studies[8-10,13-16] and the present study is shown in Table 6.

DISCUSSION

HDFN is a condition caused by maternal antibodies to foetal red cell antigens which cross the placenta and cause haemolysis in foetus. The sensitizing event causing alloimmunization is frequently a previous pregnancy or a transfusion, where the mother

Table 2: Distribution of alloantibodies detected

Antibodies	No. with alloantibodies	Distribution		
		Rh-D positive (n = 1927)	Rh-D negative (n = 133)	p-value
Anti-D	14	-	14	
Anti D + C	3	-	3	
Anti- E	1	1	-	
Anti- C	1	1	-	
Anti- M	1	1	-	
Anti- Le^a	1	1	-	
Anti- Le^b	1	1	-	
Total	22	5 (0.3%)	17 (12.8%)	< 0.001

Table 3: Frequency of alloantibodies according to blood group systems

Antibody type	Sub type	No.	% of total	Total (%)
Rh	Anti-D	14	63.7	86.4
	Anti-D+C	3	13.7	
	Anti-C	1	4.5	
	Anti-E	1	4.5	
MNS	Anti-M	1	4.5	4.5
Lewis	Anti-Le^a	1	4.5	9.1
	Anti-Le^b	1	4.5	

Table 4: Association of adverse obstetric history with alloimmunization

Variable	Antibodies		Significance
	Detected	Not detected	
Adverse obstetric history	5	255	
Present (n = 260)			
Absent (n = 1800)	17	1783	OR = 2.0565 (95% CI = 0.7522-5.6624; p = 0.160)

OR = odds ratio, 95% CI = 95% confidence intervals

Table 5: Antibody formation in relation to gravida status

Gravida status	G_2	G_3	G_4	G_5	G_6	G_7	Total
No.	1698	313	38	8	2	1	2060
Antibody positive	14	4	4	0	0	0	22
%	0.8	1.3	10.5	0	0	0	

P<0.05 (by $\div^2$ test = 34.27, degrees of freedom = 5)

Table.6. Prevalence of alloimmunization among pregnant women in the present study compared to observations in other published studies

Study	Place	Study period	Total No. of women screened	No. of patients with antibodies	No. of antibodies	Overall prevalence	Type of antibodies	Special comments
Koelewijn JM et al[13]	The Netherlands	2008	305,000	1,002	-	1.2% of all, 0.4% of non-RHD patients		First trimester screening enables timely treatment of HDFN caused by antibodies other than anti-D
Al-Ibrahim et al[14]	Saudi Arabia	2008	1,195	42	-	1.9%	52.4% Rh group, 2.4% Kell, 2.4% Kidd, 2.4% Lewis, 2.4% Duffy, 4.8% non-specific, 33.3% auto antibodies	
Lee et al[15]	China	2003	28,303	213	230	0.8%	Clinically significant 0.3%, Anti Mi -57.6% Anti E -19.7%	Routine antenatal antibody screening for Chinese women may not be worthwhile
De Vrijer et al[16]	The Netherlands	1999		65	81	2.7%	Anti-D, anti- Kell, anti-c (non-RHD IEA-1.6% of pregnant women)	First trimester screening is recommended
Shanthala AM et al[9]	Bengaluru	2003-2004	624	9	9	1.4%	Rh D contributed to 88.9% of all the antibodies formed.	
Pahuja S et al[8]	New Delhi	2008-2009	3,577	45	51	1.3%	Rh D contributed to 78.4% 0f all the antibodies formed.	
Varghese J et al[10]	Vellore	2008-2009	5347	79	54	1.5%	Rh D + contributed to 41.8% Rh D – contributed to 58.2%	
Present study	Tirupati	2012-2013	2060	22	25	1.1%	Rh contributed to 86.4% Anti-M-4.5% Anti-Le^a-4.5% Anti-Le^b-4.5%	

was exposed to the relevant antigen. HDFN due to alloimmunization shows wide spectrum of severity; some may have only mild jaundice on first day of life, but rapid fall of haemoglobin than other newborn infants. In others jaundice develops more rapidly, unless treated by exchange transfusion may lead to kernicterus and permanent brain damage. With a still more severe haemolytic process, profound anaemia develops and the infant may die *in utero* at any time from about seventh week of gestation onwards.[4]

In the present study, unexpected antibodies were detected in 22/2060 women (1.1%) of which 20 were found to have antibodies, capable of causing such HDFN. The presence of alloimmunization (1.1%) in our study correlates fairly well with other studies,[8,10] though a little less comparatively. In our study 133 (6.5%) women were Rh D negative (Table 1). Similar incidence has been observed in reports from South India, 6.4% in Vellore, Tamil Nadu,[10] comparatively, Rh-D negative phenotype is slightly higher (11%) in one North Indian study.[8]

The alloimmunization rate in the Rh-D positive women is 0.3% in our study; this is in accordance with one report[12] where a rate of 0.2%; but our rate is comparatively higher than the 0.12% observed from New Delhi[8]. This may be due to higher prevalence of Rh-D positive women in our study than in their study[8] (93.5% Vs. 89%). The allosensitization observed in Rh-D negative women in our study was 12.8%; compared to the other studies from India, this is higher as shown in (Table 6). This may be attributed to the better access of health care services in other places and non adoption of the immunoprophylaxis by our study population.

In our study, antibodies other than anti-D identified were anti-D+C (13.75%), anti-C, anti-E, anti-M, anti Le^a and anti-Le^b (4.5% each). There are reports of detection of alloantibodies other than anti-D in 14% of the subjects in whom they studied. Anti-C and anti-E were the most common antibodies reported.[13] Similar reports of alloimmunization due to antigens other than D antigen have been reported.[17] In another study[18] alloantibodies were evident in 17 of the 500 (3.4%) pregnant women and the specificity of the antibodies was as follows: anti-C 1.2%, anti-E 0.6%, anti Js^b 0.6%, and anti-K 1%. No anti-D was identified despite 8.6% of the study population being Rh-D negative.[18]

Of the other antigens of the Rh system, anti-E is frequently encountered, often second or third in frequency to anti-Kell and anti-D.[19] A case of HDFN due to anti-E alloantibody to Rh-D positive mother has been reported.[20] Anti-E alloimmunization is associated with mild to moderate HDFN.[19,21] In our study one anti-E antibody (4.54%) was identified in a third gravida mother.

In our study we identified one anti-C (4.5%) in Rh-D positive mother. Severe hydrops has been reported in an infant of Rh-D positive mother due to anti-C antibody diagnosed antenatally.[22] A case of HDFN due to anti-C in Rh-D positive mother has also been reported.[23] Similar incidence has been seen in another study also.[10]

We have not observed any of these antibodies except anti-M (4.5%) of the other types of antibodies that are occasionally associated with HDFN (anti Jk^a, Jk^b, S, etc.,). Anti-M antibody can cause immediate, delayed type of transfusion reaction[24, 25] and HDFN. Though rare, sometimes these IgM type of antibodies can be reactive at 37 ^oC. HDFN due to this antibody had been reported.[26] In a study[10] 8% of these antibodies have been observed; among these anti-M was 1.3% which is similar to our study. A case report of HDFN by anti-M has also been published.[27]

The other antibodies observed in our study, anti-Le^a (4.5%), anti-Le^b (4.5%) are not known to cause HDFN.[28] In one study[10]10.1% and 7.6% of the antibodies were observed to be anti-Le^a and Le^b respectively.[10]

In a prospective study[9] carried out on 624 antenatal cases, red cell antibody screening was positive in 9 (1.4%) of the 624 cases. These were identified as anti-D antibodies (n = 6, 66%), Anti-D with anti-C antibodies (n = 2, 22%), and anti-M antibody (n = 1, 11%).[9] In our study, we identified 3 (13.6%) antenatal women with a combination of anti D and anti C; out of which two caused a HDFN. Anti C alone is rare; usually it is associated with either anti D or anti E.[28]

A statistically significant correlation has been reported between the rate of alloimmunization and adverse obstetric history and also with the gravid status of the women.[8] We also observed a higher prevalence of antibodies in women with adverse obstetric history (Table 4). History of blood transfusions was present in 14/2060 (0.7%) women; but none of them had any alloantibodies. This is in contrast to other studies where the association between alloimmunization and blood transfusion was reported.[8,29,30] This could be due to small number of population who had transfusion history.

In the present study in analyzing the foetal outcome in 22 antibody positive mothers, we were able to follow up only 9 antenatal women and the rest were lost to our follow up. Among these 9 antenatal women, four delivered babies with features of HDFN; all had serum bilirubin levels greater than 32 mg/dL. These were treated postnatally in neonatal intensive care unit with phototherapy and double volume exchange transfusion with compatible blood, and was discharged in stable condition. The antibody specificity being anti-D + C in two antenatal women and anti-D alone in two antenatal women. The remaining 5 antenatal women delivered babies with no features of HDFN; the antibody specificity in these women were anti-D in three, anti-M in one and anti-Le^b in one antenatal woman.

According to National Family Health Survey-2,[31] in India only 65.4% of pregnant women receive at least one antenatal checkup. The proportion of women availing antenatal care in the state of Andhra Pradesh has been reported to be 96% and the corresponding figure for Chittoor district has been 97.7%.[32] In our study population, the antenatal women who developed anti-D (77.3%) did not have institutional antenatal care in the previous pregnancies. The required dose of immunoglobulin following delivery and abortion is given only in institutions and hospitals attached to medical colleges. This care is not extended to the primary health care centers. This could be one of the reasons for the development of such antibodies in our study population.

In developing countries like India, antenatal screening is generally targeted solely at detection of anti-D in Rh negative mothers and routine antenatal antibody screening is done for Rh-D negative mothers only,[33] but some of the reports from India have described alloantibodies in Rh-D positive women also.[8,10,32] In our study 0.4% of alloantibodies were observed in Rh-D positive women (Table 2). Our study included both Rh positive (93.5%) and Rh negative (6.5%) women. Hence antibody screening of both Rh positive and negative women is necessary.

In spite of the introduction of prophylactic Rh-immunoglobulin, anti-D (77.3%) still remains the most common antibody identified in the antenatal women of our region. With a potential risk for HDFN there is a need for the implementation of standardized universal anti-D immuno prophylaxis.

As the other Rh and non-Rh group of antibodies were also identified, routine antibody screening and identification is recommended for all antenatal women. Accessibility of the antenatal services and blood bank facilities are to be made available to all women in reproductive age group, to prevent the risk of HDFN and for the safe transfusion of mother.

In developing countries like India, universal antenatal antibody screening, though desirable may not be justified at present as the cost and infrastructure required would be immense. However, it is recommended to impose properly formulated protocols to screen at least the pregnant women with adverse obstetric history. It is also essential to update the facilities available at the government blood banks in order to decrease the occurrence of preventable perinatal morbidity and mortality due to HDFN.

The screening of antibodies was done only once, irrespective of gestational period in the present study. So there is a possibility of missing of some of the antibodies occurring at a later gestational period. The blood group of the spouse could not be recorded, even in the Rh-D negative women, so that the exact degree of alloimmunization among Rh negative women could not be ascertained.

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