The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi

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Background. Universal screening of all antenatal women, including D antigen-positive pregnant ones, is mandatory in most developed countries. However, no guidelines on this issue are available for developing countries such as India. Furthermore, there is limited information on immunisation rates in pregnant women (D antigen-positive and D antigen-negative) from India. We, therefore, studied the prevalence of alloantibodies among multigravida women in India.

Materials and methods. In this prospective study, carried out to detect the prevalence of alloantibodies among multigravida women in India, 3,577 multigravida women attending antenatal clinics were typed for ABO and D antigens and screened for alloantibodies by column agglutination technology. The medical history and detailed obstetric history of these women were reviewed and information recorded on any prior haemolytic disease of the foetus and newborn among siblings and/or blood transfusions.

Results. The overall prevalence of alloantibodies in this study was 1.25%. There was a statistically significant difference between alloimmunisation rates in the D antigen-negative and D antigen-positive groups (10.7% versus 0.12%, respectively). Anti-D antibody contributed to 78.4% of total alloimmunisations in our study.

Discussion. Anti-D was the most common culprit responsible for alloimmunisation. Other alloantibodies found included anti-C, anti-M, anti-S and anti-c. Large-scale population-based studies are required to assess the real magnitude of alloimmunisation in pregnant women in India.

Keywords: alloimmunisation, irregular erythrocyte antibodies, pregnancy.

Introduction

Red cell immunisation during pregnancy is a challenge that continues to task obstetricians and blood transfusionists even 50 years after the introduction of Rhesus (Rh) D prophylaxis. Anti-D prophylaxis had brightened the hopes that haemolytic disease of foetus and newborn (HDFN) due to D antigen incompatibility was in the last throes of life. However, we have reached the 21st century and the burden of alloimmunisation in pregnancy is still on our backs. Apart from the D antigen, other blood group antigens of the Rh system (C, c, E, e, C^w) and other blood group systems have come into limelight. Alloimmunisation in pregnant women has been extensively studied in different areas of the world, with the frequency being found to range from 0.4% to 2.7% worldwide[1-12].

Universal screening of all antenatal women, including D antigen-positive pregnant ones, is highly debated and controversial[4-6,13].

Most developed countries have guidelines for screening all pregnant women for irregular erythrocyte antibodies. According to the guidelines of the British Committee for Standards in Haematology, all pregnant women should be ABO and D antigen typed and screened for the presence of red cell antibodies early in pregnancy and at the 28th week of gestation[13]. According to guidelines in The Netherlands, it has been mandatory since 1998 to screen all pregnant women for the presence of irregular antibodies in the first trimester of pregnancy[8]. However, no such guidelines are followed in developing countries like India. Moreover, published data show wide variation in alloimmunisation rates between different

geographic areas. Lee *et al.*[5] suggested that routine antenatal antibody screening for Chinese women may not be worthwhile except in D antigen-negative subjects or those with a prior history of haemolytic disease of the newborn. Their view is supported by Wu *et al.*[6].

There are limited data available on immunisation rates in pregnant women from India or on the antigens responsible for any immunisation.

It is universally considered that there should be evidence-based guidelines for screening of alloantibodies in pregnant women in developing countries such as India for proper management of child birth.

Materials and methods

This study was planned to assess the prevalence of irregular erythrocyte antibodies and major culprits responsible for alloimmunisation in all multigravida women attending the antenatal clinics of Lady Hardinge Medical College (LHMC) and associated Smt Sucheta Kriplani Hospital and Kalawati Saran Children Hospital. Smt Sucheta Kriplani Hospital is a tertiary care hospital and is referred patients from in and around Delhi for follow up and management during pregnancy (antenatal care) and child birth. This prospective study was carried out at the Regional Blood Transfusion Centre of our hospital over a period of 1.5 years, from June 2008 to December 2009. The study included 3,577 subjects and written consent was obtained from all the women.

The study was conducted on all the multiparous pregnant women irrespective of their period of gestation and obstetric history.

Primigravidas and women who had received anti-D prophylaxis in the current pregnancy were not included in the study. For each patient, name, age, sex, obstetric history, blood group, husband's blood group (wherever possible), history of having received anti-D immunoprophylaxis (in the current pregnancy) and history of blood transfusions were recorded prior to taking the blood samples. Blood samples were collected into EDTA vials and sent to the blood bank.

All the samples were centrifuged at 2,000 rpm for 5 minutes and plasma separated immediately and stored at –20 °C until the tests were performed to detect antibodies. ABO blood grouping and D typing were performed for each patient and their husbands (wherever possible) using the tube method according to the Regional Blood Transfusion Centre's Standard Operating Procedures. All 'D' negative samples by tube method were confirmed for weak D by an indirect antiglobulin test and subsequently by column agglutination technology (Diamed gel card method, Diamed Switzerland).

A commercially available three-cell antigen panel (ID Diacell I, II, III; Diamed ID microtyping system, Diamed Switzerland) was used for the antibody screening procedure in which the patient's serum was reacted with red cells using low ionic strength saline (LISS) Coombs' gel card (with and without papain). The cards were incubated at 37 °C for 15 minutes and then centrifuged for 10 minutes. If the antibody screen with the three-cell antigen panel was positive, an extended 11-cell panel was used for antibody identification in LISS with and without enzyme (DiaMed 11 cell diapanel).

A review was conducted of the medical history, obstetric history (including any still births, abortions, medical terminations of pregnancy and cases of HDFN among siblings) and any past blood transfusions of the alloimmunised patients.

Statistical analyses were carried out using SPSS ver.13 software (SPSS, Chicago, USA).

Results

During the study period, 3,577 multigravida women were screened for the presence of alloantibodies. With regards to the major blood group systems (ABO and Rh), the most common phenotype was B positive. There were 3,183 D antigen-positive women (88.9%) and 394 D antigen-negative ones (11.0%) (Table I). A total of 51 antibodies were detected in 45 patients, giving an overall prevalence of alloimmunisation of 1.2% (45/3,577).

Among the 394 women in the D antigen-negative group, 41 developed antibodies, so the prevalence of alloimmunisation in this group was 10.4% (Table II).

Six patients had two types of antibodies; hence 47 antibodies were detected in these 394 patients.

Within the D antigen-negative group, 40/47 (85.1%) of the antibodies were anti-D (alone or in combination with anti C), 6/47 (12.8%) were anti-C (in combination with anti-D) and 1/47 (12.8%) was anti-M.

Table I - Frequency of blood groups in our study population (n=3,544).

Blood group	N. of women	%
A	912	25.49
B	1,330	37.18
O	982	27.45
AB	353	9.86
Total	3,577	
D antigen-positive	3,183	88.98
D antigen-negative	394	11.02

Table II - Association of D antigen with alloimmunisation.

	Antibodies not detected	Antibodies detected
D antigen-positive	3,179	4 (0.125%)
D antigen-negative	353	41 (10.4%)

Of all 51 antibodies detected in this study, four were found in D antigen-positive women, giving an overall prevalence of alloimmunisation in the D antigen-positive group of 0.12% (4/3,183). There was one case each of anti-M, anti-c, anti-S and anti-K (Table III).

Table III - Distribution of alloantibodies detected.

Antibodies (n=45)	N. of patients with alloantibodies	Distribution		Adverse obstetric history
		D antigen-positive women	D antigen-negative women	
Anti-D	34	-	34	22 patients
Anti-C and anti-D	6	-	6	4 patients
Anti-M	2	1	1	2 patients
Anti-c	1	1	-	Nil
Anti-S	1	1	-	Nil
Anti-K	1	1	-	Nil

Within the whole study group (n=3,577), anti-D was the most common antibody, accounting for 78.4% of all the antibodies formed (either alone or in combination). Multiple antibodies (dual) were present in 13.3% (6/45) patients. The most common combination in our study was anti-C and anti-D (shown to be two different antibodies by selective adsorption studies). Antibodies belonging to the Rh system accounted for 92.2% of overall alloimmunisation and Rh and Kell (together) accounted for 94.1% of alloantibodies in our study group (Table IV).

Table IV - Frequency of alloantibodies according to blood group systems.

Antibody type	Subtype	Number	Percentage of total	Total
Rh	Anti-D	40	78.43%	
	Anti-C	6	11.76%	92.15%
	Anti-c	1	1.96%	
Kell	Anti-K	1	1.96%	1.96%
MNS	Anti-M	2	3.92%	5.8%
	Anti-S	1	1.96%	
Total		51 (in 45 patients)		

In our study, alloantibodies were found in 5.5% (28/509) of antenatal females with an adverse obstetric history and in 0.5% (16/3,068) of antenatal women without an adverse obstetric history ($p<0.001$) (Table V).

Table V - Association of adverse obstetric history with alloimmunisation.

	Antibodies detected	Antibodies not detected
Adverse obstetric history present (n=509)	28	481
Adverse obstetric history absent (n=3,068)	16	3,052

P<0.001, odd's ratio=11.10 (95% confidence interval=5.75-21.64)

An adverse obstetric history (any history of stillbirth, abortion or medical termination of pregnancy) was present in 65% of patients with anti-D (26/40) and in 66.7% of patients with combined anti-C and anti-D (4/6). A history of blood transfusions was present in 2.2% (1/45) women with alloantibodies and in 1.4% (49/3,577) of all antenatal women.

Out of a total of 40 D antigen-negative women with anti-D, the husband's blood group could be confirmed in only 26 cases and was found to be D antigen-positive in all. Among the non-anti-D group, the husbands of four women had the corresponding

positive antigen on their red cells (anti-C=2, anti-c=1 and anti-K=1).

The data relating to antibody formation and the number of pregnancies are presented in Table VI.

Table VI - Antibody formation in relation to gravida status.

Gravida status	II	III	IV	V	Total
Total cases	2,432	1,017	100	28	3,577
Antibody positive	23	15	4	2	44
%	0.94	1.47	4	7.14	

$p<0.001$ (by χ^2 test=16.49, degrees of freedom=3)

Discussion

Antenatal services in India are fragmented and not uniform and there is a limited amount of published data on alloimmunisation rates among pregnant women in India. Although guidelines for screening have been laid down by the Drug Controller General, India[14], screening for alloantibodies is being done primarily for Rh D-negative women or patients presenting with an adverse obstetric history. In this study we found an overall alloimmunisation rate in pregnant women of 1.25%. Table VII shows a comparison of the published world rates of alloimmunisation in pregnancy. Koelewijn *et al.*[1], in their study to assess the efficacy of a universal antibody screening programme for pregnant females, found a total alloimmunisation rate of 1.2%. They detected alloantibodies other than anti-D of more than one specificity in 14% of index pregnancies, with anti-C and anti-E being most common.

Al-Ibrahim *et al.*[2] found a 2.0% alloimmunisation rate while Howard *et al.*[10] detected clinically significant antibodies among 1.0% of all pregnant women. In contrast, Gottvall *et al*.[3] found an alloimmunisation rate of 0.4% in all pregnancies with clinically significant alloimmunisation in 0.16% of pregnancies. The alloimmunisation rate recorded by De Vrijer *et al.*[9] among 2392 women was 2.71%.

In our study, the alloimmunisation rate in the D antigen-negative group was 10.4%. In the literature, there is a wide variation in alloimmunisation rates among Rh-negative women. Lurie *et al*.[4] found a low alloimmunisation rate of only 0.9% in Israel whereas Al-Ibrahim *et al.*[2] found a higher rate of 7.1% in Saudi Arabia. Salola *et al.*[12] recorded an alloimmunisation rate of 2.98% in Rh-negative women. The rate of alloimmunisation in Rh-negative women in our study is much higher than that in western studies. This can be attributed to the lack of implementation of standardised and universal anti-D immunoprophylaxis in India. Anti-D does therefore continue to the main culprit responsible for alloimmunisation in our country, accounting for 78.4% of all alloantibodies in our study. Our results are in concordance with the results of several other studies. Gottvall *et al.*[3] found that anti-D was the cause of alloimmunisation in 60% of cases (Table VII). Lenkiewicz *et al.*[15] and Howard *et al.*[10] found that anti-D was responsible for 45.5% and 41%, respectively, of cases of significant immunisation. In these studies, anti-D was the leading offender despite immunoprophylaxis.

The alloimmunisation rate within the Rh-positive group in our study was 0.12%. This is in accordance with the findings of several other studies, such as those by Lurie *et al.*[4] and Adenijii *et al.*[16], who reported alloimmunisation rates among Rh-positive women of 0.2% and 0.15%, respectively.

In our study, we found a statistically significant correlation between frequency of alloimmunisation and adverse obstetric history ($p<0.001$, odds ratio=11.10, 95% confidence interval=5.75-21.64), which means that the odds of an antibody-positive women having an adverse obstetric history were more than 10 times higher than women who were antibody negative. The gravida status of women also showed a statistically significant, positive correlation with alloantibody formation. There are limited published data, particularly from India and South East Asia, on such correlations.

It is difficult to compare the results of different studies because of the heterogeneity of populations involved, varied screening protocols, variation in the definition of clinically significant antibodies and difference in the techniques used for antibody identification.

Despite prophylactic use of Rh immunoglobulins, anti-D is still a common antibody identified as the major cause of alloimmunisation. Koelewijn *et al.*[1] found that the prevalence of alloantibodies other than anti-D is 0.38%. They emphasised that HDFN caused by antibodies other than anti-D occurred in 7-8 cases per 100,000 pregnancies. Without a

Table VII - Prevalence of alloimmunisation among pregnant women: a review of the literature.

Authors of study (Place)	Year	Total n. of women screened	N. of patients with antibodies	N. of antibodies	Overall prevalence	Type of antibodies	Special comments
Koelewijn JM *et al.*[1] (The Netherlands)	2008	305,000	1,002	-	1.232% of all, 0.328% of non RHD patients		First trimester screening enables timely treatment of HDFN caused by antibodies other than anti-D.
Al-Ibrahim *et al.*[2] (Saudi Arabia)	2008	1,195	42	_	1.92%	52.38% Rh group, 2.38% Kell, 2.38% Kidd, 2.38% Lewis, 2.38% Duffy, 4.76% non-specific, 33.33% autoantibodies.	
Gottvall *et al.*[3] (Sweden)	2008	78,145	316	376	0.4%	0.16% symptomatic Anti D-60%, Fya-10%,c-7%, K-4%	
Lurie *et al.*[4] (Tel Aviv, Israel)	2003	1,265	2	2	RHD positive women 0.2%; RHD negative women 0.9%		Routine antibody screen in Rh positive women is not warranted.
Lee *et al.*[5] (China)	2003	28,303	213	230	0.79%	Clinically significant 0.27%, Anti Mi -57.6% Anti E -19.7%	Routine antenatal antibody screening for Chinese women may not be worthwhile
Chandrasekar *et al.*[7] (Ireland)	2001	34,913	186	_	0.53%	99-Rh group other than D, 87-non Rh group.	
De Vrijer *et al.*[9] (The Netherlands)	1999	2,392	65	81	2.71%	Anti-D, anti- Kell, anti-c (non-RHD IEA-1.6% of pregnant women)	First trimester screening is recommended
Howard *et al.*[10] (Liverpool, UK)	1998	22,264	244	244	1%	100-anti D, 144-non RHD	-
Filbey *et al.*[11] (Sweden)	1995	11,350	629	821	0.57%	Clinically significant -0.24%	
Present study (Delhi, India)	2008-2009	3,577	45	51	1.25%	RhD contributed to 78.4% of all the antibodies formed	

universal antibody screening programme for red cell alloantibodies in the first trimester of pregnancy, there would be approximately two foetal deaths due to severe intrauterine anaemia in 100,000 pregnancies (in which intrauterine transfusion could have been beneficial).

Lurie *et al.*[4] have suggested that antibody screening is not warranted from a cost-clinical benefit perspective. Lee *et al.*[5] supported the view that routine antenatal antibody screening for Chinese women may not be worthwhile. Moreover, they found different specificities of antibodies compared to those reported for western countries, with anti-Mi being the most frequently encountered antibody. However, long-term extensive studies have not been done to assess the severity of problem of alloimmunisation in pregnancy, the clinical significance of these non-D antibodies and their impact on outcome and interventional modalities in the Indian population.

Based on the fact that anti-D accounted for 78.4% of all alloantibodies, we need to focus more on anti-D immunoprophylaxis. In our study, there was a glaring, statistically significant difference between alloimmunisation rates in Rh D-negative versus Rh D-positive group (10.7% versus 0.125%; $p<0.001$). Moreover, follow-up and treatment facilities for antibodies other than anti-D are not available in most of centres across India. However, large-scale studies on pregnant women need to be done in order to collect sufficient evidence to be able to formulate guidelines regarding testing and interventional modalities for alloimmunisation in pregnancy.

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