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Red cell alloimmunization among antenatal women attending a tertiary care hospital in south India

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***Background & objectives*: Detection of maternal alloimmunization against red cell antigens is vital in the management of haemolytic disease of the foetus and newborn (HDFN). This study was conducted to measure the presence of allosensitization to blood group antibodies in the antenatal women attending a tertiary care hospital and to observe the proportion of minor blood group antibodies to assess the benefit of screening for the same.**

***Methods*: All antenatal women registered in the hospital between January 2008 and January 2009, were screened for irregular antibodies using a commercial 3-cell antibody screening panel. Antibody identification was performed on samples found positive using a commercial 11 cell-panel.**

***Results*: Screening was performed on 5347 women, 339 (6.34%) of whom were Rh negative. Allosensitization was found in 79 women (1.48%; confidence interval 1.17 -1.84). In 29 of these 79 (37%) women the allo-antibodies could not be identified. In the remaining 50 women, 54 antibodies were characterized. A total of 40 clinically significant antibody specificities were identified among 36 women, of whom four were Rh(D) positive. Allosensitization with clinically significant antibodies was found in 9.43 per cent (confidence interval 6.55-13.06) Rh(D) negative and in 0.08 per cent (confidence interval .02-0.2) Rh(D) positive women. Anti D was the most frequent antibody found in 8.85 per cent Rh(D) negative women. The remaining clinically significant antibodies identified included anti-C, c, E, Jk^a, Jk^b, M and S. In Rh(D) negative women, anti-D and antibodies of the Rh system contributed 83.3 and 94.4 per cent of clinically significant antibodies. However, in Rh(D) positive women, non-Rh antibodies comprised three out of four clinically significant antibodies.**

***Interpretation & conclusions*: The presence of alloimmunization in our study corroborated with data reported from India. The most frequent antibody was anti-D. However, a significant fraction was non-D. Alloimmunization among Rh(D) positive women though low as compared to Rh(D) negative women, included clinically significant antibodies, and most of these were non Rh.**

Key words Alloimmunization - antenatal screening - anti-D - red cell antibodies - Rh(D)

Anti-D occurring in Rh negative women was a major cause for severe haemolytic disease of the foetus and new born (HDFN) world wide until the 1960s[1,2]. Following successful implementation of prophylaxis, changes in birth order and improved quality of medical care, morbidity and mortality due to Rh(D) related HDFN in many countries drastically reduced from 12-13 to 1-2 per cent[3-5]. Meanwhile, other irregular antibodies that were found to cause HDFN, principally anti-c, anti-E, and antibodies to antigens of Kell, Kidd, Duffy and MNS blood group systems, gained prominence[6].

Currently, the availability of wider screening panels has enabled the detection of various minor blood group antibodies, some of which are well known to have relevance in the antenatal setting[2]. In developing countries the financial burden of routine screening for irregular antibodies, has to be weighed against the benefits. We conducted this study to measure the presence of allosensitization to blood group antibodies and the proportion of minor blood group antibodies in the antenatal women attending a tertiary care centre located in south India.

Material & Methods

All antenatal women registered in the department of Obstetrics and Gynecology, Christian Medical College and Hospital, Vellore, Tamil Nadu, India, between January 2008 and January 2009 were included in the study. In our hospital, a blood sample is routinely collected at the booking visit and sent to the blood bank for ABO blood grouping and Rh(D) typing. From January 2008, the blood bank additionally screened each of these samples for irregular antibodies by Indirect Coombs Test (ICT) using a commercial 3-cell antibody screening panel (Surgiscreen; Ortho Clinical Diagnostics Inc., USA). On those samples found to be positive on the screen, antibody identification was performed using a commercial 11-cell antibody identification panel (Resolve Panel A; Ortho Clinical Diagnostics Inc., USA).

The blood samples (5 ml) were collected in vacutainer tubes, allowed to clot, and centrifuged to separate serum from red cells. A 3 per cent red cell suspension in saline was prepared and used for blood grouping and Rh typing[8]. Antibody screening and identification were done using the column agglutination method in Coomb's phase using low ionic strength solution (LISS) enhancer, as per the manufacturer's instructions. Serum samples positive on antibody screening were selectively frozen at -70º C for antibody identification, which was performed on these samples together at a later date.

Results

A total of 5347 pregnant women were screened, of whom 339 (6.34%) were Rh(D) negative. A positive screen was initially obtained in 97 patients. Using the antibody identification panel, 79 of the 97 women showed reactivity, while 18 showed a negative reaction which was reproduced on repeat screening. The presence of blood group allosensitization among the antenatal population was found in 1.48 per cent women (79/5347) (confidence interval 1.17-1.84). Among the 79 alloimmunized women, 33 (41.8%) were Rh(D) positive while 46 (58.2%) were Rh(D) negative.

A total of 54 antibodies were identified among 50 women, while 29 of 79 (37%) gave an inconclusive pattern. The distribution of antibodies obtained is shown in the Table.

Of the 50 women whose antibodies were characterized, 32 (64%), were found to have antibodies commonly associated with HDFN (anti- D, c, E, e, C, K), four (8%) had antibodies that are occasionally associated with HDFN (anti- Jk^a, Jk^b, S, M) and 14 (28%) had antibodies which are not known to cause HDFN (Le^a, Le^b)[2]. Among the 36 women with clinically significant antibodies, four were Rh positive and 32 were Rh negative. Hence the presence of clinically significant alloimmunization among Rh(D)

Table. Distribution of antibodies among total allosensitized women (n=79), Rh(D) positive (n=33) and Rh(D) negative women (n=46)

Antibody specificity	Total number of women (%); N=79	Rh positive women N=33	Rh negative women; N=46
Anti-D	27 (34.2)	-	27 (58.7)
Anti-D + anti-C	3 (3.8)	-	3 (6.5)
Anti-c	1 (1.3)	1 (3.0)	-
Anti-E + anti-K	1 (1.3)	-	1 (2.2)
Anti-Jk^a	1 (1.3)	1 (3.0)	-
Anti-Jk^b	1 (1.3)	1(3.0)	-
Anti-M	1 (1.3)	-	1 (2.2)
Anti-S	1 (1.3)	1 (3.0)	-
Anti-Le^a	8 (10.1)	5 (15.2)	3 (6.5)
Anti-Le^b	6 (7.6)	6 (18.2)	-
Unidentified	29 (36.7)	18 (54.5)	11 (23.9)

negative and Rh(D) positive women was 9.43 per cent (confidence interval 6.55-13.06) and 0.08 per cent (confidence interval .02-0.2), respectively.

Overall, 40 of the 54 antibodies that were characterized, were clinically significant, of which 30 (75%) were anti-D, 35 (87.5%) belonged to the Rh blood group system, and five (12.5%) were others. Among Rh(D) negative women, anti-D and antibodies of the Rh system contributed 83.3 and 94.4 per cent of clinically significant antibodies, respectively. However, in Rh positive women, non-Rh antibodies comprised three out of four clinically significant antibodies.

Discussion

HDFN is a condition caused by maternal antibodies to foetal red cell antigens, which cross the placenta and cause haemolysis. The antibodies can be natural or immune. In the latter case, the sensitizing event is frequently a previous pregnancy or a transfusion, where the mother was exposed to the relevant antigen.

In developing countries, antenatal screening is generally targeted solely at detection of anti-D. Moreover, the applicability of western guidelines, and the utility of antibody screening panels developed within western populations are not well established. The issue of whether routine antibody screening in Rh positive women is warranted, especially in developing countries has also been debated[7,9].

The presence of alloimmunization in 1.48 per cent women in our study and the general profile of clinically significant antibodies correlated with other studies from India[10,11]. It is possible that some antibodies in our study were missed by the absence of routine third trimester screening. Studies have shown that first trimester screening alone can miss a significant fraction of clinically significant antibodies[12,13]. In addition, our study included only hospital attendees, and is not representative of the prevalence of anti D among a large number of Indian women who do not have access to obstetric care.

Given the low occurrence of allosensitization among Rh(D) positive women, a routine screening programme may not be feasible, as perhaps one out of approximately 1250 Rh(D) positive women would have clinically significant antibodies. However, we suggest that where facilities for management of an allosensitized pregnancy are accessible, the option of screening should be extended to Rh(D) positive women.

Our study found non-D antibodies to constitute a significant proportion of clinically relevant antibodies. In a Croatian study, clinically significant non-D antibodies produced HDFN in approximately 55 per cent of alloimmunized pregnancies, and severe HDFN, defined by perinantal transfusion requirement or death, in approximately 25 per cent[14]. Prevalent screening methods using random O positive pooled cells or cells phenotyped for Rh alone thus ignore a significant component of sensitization. Non-Rh antibodies contributed to 75 per cent of the clinically significant antibodies in Rh(D) positive women, implying the need in this group to use screening panels that are not restricted to Rh but incorporate a wider range of clinically significant antigens.

The antibody identification panel used in our study was not framed to identify anti-Mi, which was reported to be the most frequent irregular antibody in a study from China[15]. Whether this or/and some other population specific antigen can account for the large proportion of unidentified antibodies in our study needs further evaluation. Antibodies that have been reported to cause HDFN in the Indian population include anti-c, anti Jk^b, anti E and anti M[16-18]. However, there are possibly others which remain unreported, or unidentified owing to limitations in facilities for their identification.

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