ORIGINAL REPORT

Prevalence of clinically significant red blood cell alloantibodies in pregnant women at a large tertiary-care facility

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More than 50 red blood cell (RBC) alloantibodies are known to cause hemolytic disease of the fetus and newborn (HDFN). Although Rh immune globulin (RhIG) prophylaxis has significantly reduced the incidence of pregnancies complicated by anti-D, the need to detect and monitor maternal alloantibodies capable of causing HDFN is still a concern. The prevalence and specificity of these alloantibodies were determined. In this retrospective study, the prevalence and specificities of unexpected RBC alloantibodies known to cause HDFN in pregnant women at a tertiary-care facility during a 5-year period were compiled and analyzed. Patient selection was carried out by computerized search of patient data based on an obstetric location and the presence or history of RBC antibody between January 1, 2007, and December 31, 2011. The information was organized by ABO and D status of the patient, antibody specificity, and transfusion needs. Of the 8894 obstetric patients identified during the 5-year period, 264 (3.0%) had one or more unexpected RBC antibodies. Of these 264 women, 107 (40.5%), or 1.2 percent overall, had an alloantibody known to cause HDFN, with a total of 15 different alloantibodies identified. The most common alloantibody found was anti-E (n = 33), followed by anti-M (n = 26) and anti-D (n = 20). In pregnancies of D– women, the most common clinically significant antibodies found were anti-D (n = 20), anti-C (n = 11), and anti-E (n = 2). In pregnancies of D+ women, the most common antibodies were anti-E (n = 31), anti-M (n = 25), and anti-K (n = 16). A total of eight pregnancies with alloantibodies required intrauterine transfusions with the specificities of anti-D; anti-D,-C (n = 2); anti-D,-C,-E; anti-D,-C,-K; anti-D,-C,-Jk^b; anti-D,-S; and anti-E,-c. At a large academic tertiary-care center, approximately 1 in 83 obstetric patients had one or more RBC alloantibodies capable of causing HDFN. Anti-E, -M, and -D were the most frequent specificities, respectively. ***Immunohematology* 2013;29:127–130.**

Key Words: prevalence, red blood cell alloantibodies, pregnant women

Hemolytic disease of the fetus and newborn (HDFN) results from the destruction of fetal and newborn red blood cells (RBCs) targeted by maternal RBC alloantibodies that have the capability of crossing the placenta and entering the fetal circulation. Directed to inherited paternal RBC antigen(s), these antibodies are able to bind to the corresponding antigens, marking them for destruction by the fetal spleen, resulting in fetal distress. There are more than 50 RBC alloantibodies that have the capability of crossing the placenta and causing HDFN, with anti-D followed by anti-c and anti-K having the highest probability of causing severe HDFN.[1,2]

Although advances have been made in the past 50 years, including the implementation of Rh immune globulin (RhIG) to prevent anti-D HDFN in the 1960s, HDFN caused by anti-D as well as by non-D antibodies still remains a serious concern. Because women can be alloimmunized to RBC antigens through transfusion and pregnancy, it is still necessary to detect and monitor maternal alloantibodies that may put the fetus at risk for HDFN.[3]

The prevalence of alloantibodies in pregnancy has been investigated in multiple studies during the past few years in various countries, such as Sweden, Nigeria, Croatia, and the Netherlands.[1,4–6] A compilation of similar data from the United States is limited. Although the frequency of alloantibodies at our institution would not necessarily reflect the prevalence in the United States, an evaluation of such data at a large hospital with high-risk obstetric patients would help to reiterate the importance of screening for and monitoring those pregnancies that present with antibodies that may put the fetus at risk for HDFN. Therefore, we determined the prevalence and specificity of unexpected maternal RBC alloantibodies associated with HDFN in a large obstetric population at our hospital. The associations between ABO group and D type and the presence of clinically significant RBC alloantibodies associated with HDFN were also determined.

Materials and Methods

Data collection and analysis were performed at the Johns Hopkins Hospital in Baltimore, Maryland, and included all women seen in the hospital's obstetrics department between January 1, 2007, and December 31, 2011, who had an ABO and D type and antibody screen performed. Patient selection was carried out by computerized search of patient data based on the parameters of obstetric location and the presence of RBC alloantibody. Only female patients who had a record of,

or presented with, one or more RBC alloantibodies during pregnancy were included in the search. Each patient's ABO and D type and any RBC transfusions given were then compiled from the blood bank database and analyzed. The identification of passive maternal alloantibody in the baby was detected by a positive direct or indirect antiglobulin test. For those babies with passive antibody of maternal origin, the occurrence of transfusion of antigen-negative units was determined.

The methods used to detect the antibodies included routine screening and antibody identification in accordance with AABB guidelines.[2,3] A history of RhIG administration was verified by review of medical records at the time of presentation. All mothers with only anti-D as a result of RhIG were excluded from the study. Patients with demonstrable alloantibodies or a history of demonstrable alloantibodies were included. The antibodies were then evaluated and sorted according to their ability to cause HDFN as outlined in the AABB guidelines for prenatal and perinatal immunohematology.[3]

The total number of antenatal cases was determined using the institution's laboratory information system. All duplicate samples on the same patient and all cancelled and rejected specimens were removed from the total, and only specimens with completed ABO and D types and antibody screens were included. Approximately 9 percent of the women identified ended up delivering elsewhere, but they were included in the analysis.

The association between ABO type and D types and the presence of clinically significant RBC alloantibodies associated with HDFN was determined using χ^2 statistics for proportion and frequency comparisons.

Results

Between January 1, 2007, and December 31, 2011, 8894 different obstetric patients were typed for ABO and D and screened for RBC alloantibodies. Approximately 91 percent of these women delivered at Johns Hopkins Hospital, but all 8,894 women were included in the analysis. During this same period, there were 9,734 deliveries at Johns Hopkins Hospital with approximately 20 percent being one or more repeat deliveries from the same woman.

The percentage of the 8894 women who had clinically significant alloantibodies by group O, A, B, and AB blood types were 1.1 percent, 1.4 percent, 1.2 percent, and 1.5 percent, respectively (Fig. 1). No significant association ($p = 0.64$) was found between the ABO group and the presence of clinically significant RBC alloantibodies associated with HDFN.

Of the 8894 obstetric patients screened, 8147 (91.6%) were D+ (Fig. 2) and 747 (8.4%) were D–. A total of 264 (3.0%) women demonstrated or had a history of one or more RBC antibodies at the time of pregnancy. Of the 264 patients with antibodies, 157 (59.5%) had antibodies typically considered clinically benign in regard to HDFN, and 107 (40.5%), or 1.2 percent overall, had at least one antibody known to cause HDFN.[3] The most frequent antibody identified was anti-E (n = 33), followed by anti-M (n = 26) and anti-D (n = 20). A total of 145 antibodies, with 15 different specificities, that are known to cause HDFN were identified. Of the 107 patients with antibodies known to cause HDFN, 28 (26.2%) had multiple antibody specificities; a breakdown of those antibodies is shown in Table 1.

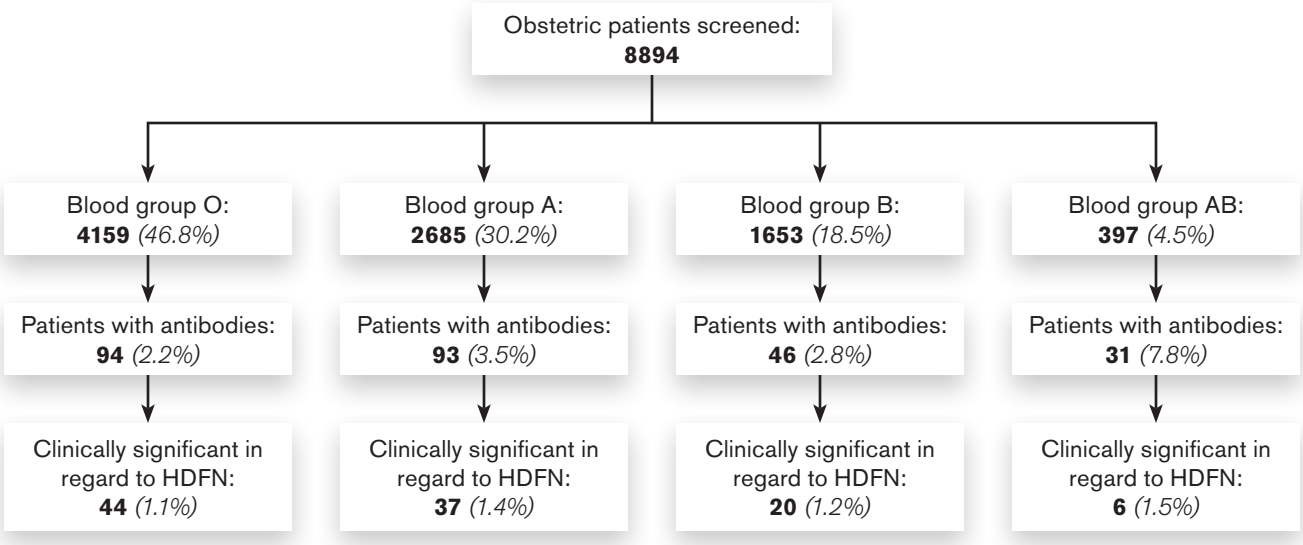

Fig. 1 Distribution of obstetric patients with red blood cell antibodies between January 1, 2007, and December 31, 2011, by ABO group. HDFN = hemolytic disease of the fetus and newborn.

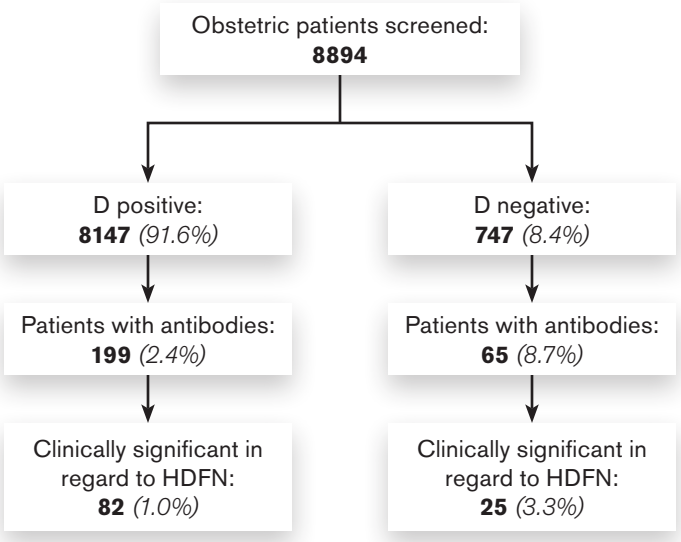

Fig. 2 Distribution of obstetric patients with red blood cell antibodies between January 1, 2007, and December 31, 2011, by D type. HDFN = hemolytic disease of the fetus and newborn.

Of the 8147 D+ women, 82 (1.0%) had one or more alloantibodies capable of causing HDFN compared with 25 (3.3%) of the 747 D– women ($p < 0.0001$). Of the 82 D+ pregnant women with antibodies, anti-E (n = 31), anti-M (n = 25), and anti-K (n = 16) were the most frequent. Anti-D was seen in 20 of the 25 pregnancies in D– women with antibodies, followed by anti-C (n = 11) and anti-E (n = 2).

Of those obstetric cases identified in the study with antibodies known to cause HDFN, 14 women required RBC transfusions during their pregnancy or at delivery because of sickle cell disease (n = 5) or postpartum hemorrhage (n = 9). Eight women required one or more intrauterine transfusions (IUTs). The antibody specificities requiring an IUT were anti-D; anti-D,-C (n = 2); anti-D,-C,-E; anti-D,-C,-K; anti-D,-C,-Jk^b; anti-D,-S; and anti-E,-c. The most frequent antibody was anti-D, in seven of the eight pregnancies requiring an IUT.

Approximately 91 percent, or 7457, of the pregnant women in this study delivered at our facility. Of those infants born at our institution, 24, or 0.3 percent, were identified as having passive alloantibody of maternal origin. Anti-D (n = 17), with or without other antibody specificities, was the most common. Of those infants with passive alloantibody of maternal origin, five infants received transfusion of antigen-negative RBCs within 24 hours of birth. All five infants had passively acquired anti-D, with four of five infants demonstrating one to two additional alloantibodies (anti-S, anti-C, anti- K, or anti-Jk^b).

Table 1. Antibody specificities in pregnant women with clinically significant antibodies between January 1, 2007, and December 31, 2011.

Antibody specificity	# of pregnancies in D+ women with antibody specificity	# of pregnancies in D– women with antibody specificity
D		8
D+C		6
D+C+E		2
D+C+K		1
D+C+Jk^b		1
D+S		1
D+M		1
C		1
C+E	1	
C+e	1	
C+K	1	
C+K+S	1	
C+S	1	
C+K+Fy^a+M+S	1	
E	21	
E+c	5	
E+c+K	1	
E+K	1	
E+K+Kp^a	1	
E+S	1	
c	1	
e	1	
K	9	1
K+C^w	1	
Fy^a	1	1
Jk^a	3	1
M	24	
N	2	1
S	1	
U	3	

Discussion

This study revealed that of the 8894 pregnant women screened, the percentage of women who demonstrated or had a history of an RBC alloantibody at the time of pregnancy was 3.0 percent, with 1.2 percent of women having at least one alloantibody known to cause HDFN. The most frequent alloantibody found in patients with at least one antibody known to cause HDFN was anti-E (30.8%), followed by anti-M (24.3%) and anti-D (18.7%). In one study evaluating the prevalence of antibodies in Dutch women, anti-E was also found to be the most common antibody detected.[7]

Nearly half (46.8%) of these women typed as blood group O, followed by 30.2 percent, 18.5 percent, and 4.5 percent

typing as blood groups A, B, and AB, respectively, with 91.6 percent typing as D+. These percentages are similar to those in a black non-Hispanic population in the United States as reported by Garratty et al., in which 50.2 percent, 25.8 percent, 19.7 percent, and 4.3 percent typed as blood groups O, A, B, and AB respectively, with 92.9 percent typing as D+.[8] These findings at our institution are most likely attributable to the fact that approximately 62.5 percent of the patients who deliver at our hospital are African American and 4.9 percent are Asian American.

In our study, 1.0 percent of D+ women had one or more alloantibodies known to cause HDFN compared with 3.3 percent of D– women. The most common alloantibodies in D+ women with at least one antibody known to cause HDFN were anti-E (37.8%), anti-M (30.5%), and anti-K (19.5%); in D– women they were anti-D (80%), anti-C (44.0%), and anti-E (8%).

Five infants required transfusion of antigen-negative units within 24 hours of birth, and eight women required at least one IUT. Anti-D was implicated in seven of eight (87.5%) of the IUT cases, which is comparable to the 85 percent of IUT cases in which it was implicated in a previous study,[9] although the numbers are small. Nearly all the cases requiring transfusion in which HDFN was implicated involved Rh antibodies and multiple antibody specificities, which complicated both the identification and monitoring of those antibodies and the providing of antigen-negative units.

Although rare, there have been reports of anti-M causing HDFN.[10] For this reason, in this study all pregnancies with anti-M were considered to have the potential to cause HDFN. There were no cases in this study in which an IUT was required because of anti-M.

The most common alloantibody known to cause HDFN found in pregnancy in this study was anti-E, followed by anti-M and anti-D. Despite efforts to eliminate HDFN caused by anti-D, that alloantibody remains the third most common one found in pregnant women and the most common antibody found in D– pregnant women at our institution. The manner in which D alloimmunization occurred in these patients was beyond the scope of our study.

It is possible that the 1.2 percent prevalence of RBC alloantibody clinically significant for HDFN in our obstetrics population is higher than that found in the general obstetrics population in the United States. Given that Johns Hopkins Hospital is a tertiary-care center, it is likely that a proportion of obstetric patients were referred from other hospitals because they were known to be alloimmunized and thus at high risk, resulting in a higher prevalence. Also, the number of women and babies requiring transfusion in this report is likely an underestimate, as approximately 9 percent of the 8894 pregnant women screened delivered elsewhere. Nevertheless, these findings help to reinforce the importance of educating pregnant women and physicians concerning the risks of HDFN and the need to detect and monitor alloantibodies, not only anti-D but also other alloantibodies known to cause HDFN in pregnant women.

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