Maternal red blood cell alloantibodies identified in blood samples obtained from Iranian pregnant women: the first population study in Iran: RBC ALLOANTIBODIES AND PREGNANT WOMEN

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Maternal red blood cell alloantibodies identified in blood samples obtained from Iranian pregnant women: the first population study in Iran

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BACKGROUND: The objective was to determine the frequency of occurrence of alloantibodies among pregnant women in Iran.

STUDY DESIGN AND METHODS: This was a prospective cross-sectional study, which was carried out in the immunohematology reference laboratory of the Iranian Blood Transfusion Organization in Tehran, Iran, in 2008 to 2015. Screening and identification of red blood cell (RBC) alloantibodies was done on the sera of 7340 pregnant females using the standard tube method and gel column agglutination technique.

RESULTS: Alloantibodies were identified in the serum of 332 of the 7340 (4.5%) pregnant women. A total of 410 antibodies were detected in 332 positive maternal serum samples with no previous history of blood transfusion. Anti-D was the most common antibody accounting for 70.5% of all the antibodies formed in D– women. The incidence of specific alloimmunization other than Rh group was 14.4%.

CONCLUSION: We concluded that the alloimmunization rate was high in comparison with wide pattern in previous studies. In Iran, like other developing countries, alloimmunization screening tests are performed only to detect anti-D in pregnant D– women. This high rate of alloimmunization, quite possibly, is due to the fact that the majority of blood samples came from pregnant women known to have previous obstetric problems. However, we suggest that RBC antibody screening tests should be extended to all D+ women.

Alloimmunization of red blood cells (RBCs) in pregnant women is still a challenge to clinicians. Maternal immunoglobulin G (IgG) antibodies are the main cause of fetal RBC hemolysis by targeting fetal RBC antigens. Although advances, including the implementation of RhIG to prevent anti-D hemolytic disease of the fetus and newborn (HDFN) in the 1960s, have been made, HDFN caused by anti-D as well as by non-D antibodies is still a serious concern. There are more than 50 RBC alloantibodies that cause HDFN, with anti-D followed by anti-c and anti-K having the highest probability of causing severe HDFN.[1] Despite use of D prophylaxis, anti-D is the most common and severe form of immunization.[2,3]

The prevalence of alloantibodies in pregnancy has been reported in various countries.[1,4-7] A compilation of similar data from Iran is limited. An evaluation of such data from a large and main referral laboratory to which blood samples from pregnant women and thalassemia patients are referred from all over the country would help to reiterate the importance of screening for and

ABBREVIATIONS: HDFN = hemolytic disease of the fetus and newborn; IBTO = Iranian Blood Transfusion Organization.

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monitoring those pregnancies that present with antibodies that may put the fetus at risk for HDFN. Therefore, we aimed to determine the frequency of alloimmunization in pregnant women who were referred to the immunohematology reference laboratory of the Iranian Blood Transfusion Organization (IBTO). Before 2015 there was no national protocol for testing of pregnant women for the detection and identification of RBC antibodies.

MATERIALS AND METHODS

This prospective cross-sectional study was carried out on 7340 pregnant women referred for ABO/D typing and antibody screening during a period of 8 years from 2008 to December 2015 in the immunohematology reference laboratory of the IBTO. The majority of samples were collected from the patients with positive history of multiple unsuccessful pregnancies (at least two times) to identify suspicious alloimmunization, prenatal and postnatal titration evaluation, and better management of possible need for intrauterine transfusion for alloimmunization in their pregnancy. All mothers with only anti-D as a result of RhIG were excluded from the study.

Sample size

Sample size included blood samples from individuals referred to the immunohematology reference laboratory of the IBTO.

Blood samples

ABO and D tests

Six milliliters of a peripheral blood sample was drawn from each individual into a vial containing EDTA. Blood samples were collected under aseptic condition from antecubital vein for determination of blood groups. Initial ABO blood grouping was determined by tube method using commercially prepared antisera, anti-A, anti-B, anti-AB (Iranian Blood Research and Fractionation). Presence of D antigen was determined by anti-D (Iranian Blood Research and Fractionation). For D– weak D test was performed. Repeat ABO and D blood grouping for confirmation of blood group was performed by conventional tube technique as per our standard operating procedure using monoclonal reagents from different commercial companies included Bio-Rad and CE-Immumodiagnostika: anti-A (Bio-Rad), anti-B (Bio-Rad), and anti-D (CE-Immumodiagnostika). Testing for the presence of weak D phenotype was done for all individuals typed as D as per the manufacturer's instructions. Tube technique using 2% to 5% RBC suspension and anti-C, anti-c, anti-E, and anti-e monoclonal antibodies (Diamed AG) as per the manufacturer's instruction was performed for Rh antigen (C, E, c, e) typing. A positive reaction of 3+ to 4+ strength of agglutination indicated the presence of corresponding antigen and absence of agglutination was confirmed macroscopically per the manufacturer's instruction indicating its absence.

Interpretation of results for the tube tests

Positive: RBCs formed a solid complete agglutinate on the bottom of the tube in several medium or small agglutinates. These were graded from 4+ to 1+ and indicated the presence of the corresponding antigens.

Negative: A compact button of cells on the bottom of the tube indicated the absence of the corresponding antigen.

Antibody screening and identification

A homemade available three-cell antigen panel (IBTO minipanel) was used for the antibody screening procedure in which the patient's serum was added to RBCs with and without papain enzyme using low-ionic-strength saline (LISS). IBTO minipanel and antibody screening kit and also selected cells were validated in a 2-year period using commercial CE-marked Diamed three-cell kits. Antibody screening test were performed twice in parallel using IBTO produced kits and Diamed kits. The results were compared and in case of positive results, the 11-cell antibody panel from Diamed was used simultaneously with IBTO 11-cell antibody panel. An IBTO-homemade antibody panel and selected cells were used to exclude and include alloantibodies.

A commercial antiglobulin gel card (Invitrogel AHG Coombs) and standard tube methods (Bio-Rad AHG) were used. The gel cards were incubated at 37°C for 15 minutes and then centrifuged for 10 minutes. On those samples found to be positive on the screening test, antibody identification was performed using a homemade 11-cell antibody identification panel expressing at least 18 US Food and Drug Administration–recommended RBC antigens, that is, D, C, c, E, e, M, N, S, s, P_1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a, and Jk^b in LISS (DiaLISS, Bio-Rad) with and without papain (Bio-Rad). Homozygous expression of Rh, Duffy, Kidd, and MNs antigens were included in the panel.

Clinically significant alloantibodies were defined as those antibodies that potentially could cause RBC destruction based on the reactivity at 37°C and/or antihuman globulin phase. Antibodies were categorized as passive anti-D due to RhIG injections based on three criteria, antibody reaction strength of less than 2+, anti-D titer of equal to or less than 4, and RhIG injection within past 2 to 3 months of testing. We also defined unspecified antibodies as those that are not defined in any of 31 blood group systems.

Statistical analysis

RBC antigen calculation and phenotype frequencies of the various blood group systems was done by totaling the number of individuals positive for a particular antigen phenotype divided by the total number of individuals screened. Results are expressed as percentages.

Ethical considerations

This study was approved by the ethics committee of IBTO and health services. Individuals were asked to sign an informed consent form before blood samples were obtained. All terms of the Helsinki Declaration were considered and the personal information remained anonymous.

RESULTS

During the study period, 7340 pregnant women were typed for ABO and D and screened for RBC alloantibodies. Of them, 332 women with an age range between 21 to 43 years old had positive antibody screen test results with one or more identified alloantibodies.

Blood group A was the most prevalent among patients with positive antibodies. In D+ patients (n = 6305), 68 (20.5%) were alloimmunized and 6237 (89%) were nonalloimmunized versus 264 (79.5%) alloimmunized and 771 (11%) nonalloimmunized in D– donor's (Table 1).

A total of 313 specific antibodies were detected in 332 positive maternal serums females with a total number of 1110 pregnancies. The number of unsuccessful pregnancies ranged between 1 and 14 times with a median of three pregnancies. Alloimmunizations occurred in 3% of women during their first pregnancy. An overall prevalence of alloimmunization including clinically significant and nonsignificant antibodies was 4.5%. A total of 23.6% were unspecified clinically significant, nonsignificant antibodies and antibodies reacted due to the RhIG injection (13.53% were unspecified clinically significant and nonsignificant antibodies and 10.07% were passive anti-D).

Of all 410 antibodies detected in this study, 55 were found in D+ women, giving an overall alloimmunization prevalence of 13.4% in the D+ patients. No anti-D alloantibody was identified in D+ women.

Among the 264 alloimmunized pregnant women in the D– patients, 193 cases developed specific antibodies versus 42 cases of 68 D+ alloimmunized pregnant women (prevalence of specific antibodies alloimmunization of 73.1% vs. 61.8%). Among D– women, despite receiving RhIG, anti-D was the most common antibody, accounting for 70.5% of all the antibodies formed alone in D– women. Within the D– group, the most common maternal dual and triple antibodies were anti-D+C (n = 37), investigation of anti-G was not performed among women with anti-D+C and anti-D+E+C (n = 4), respectively.

Within the whole study group, anti-D alone contributed for 39.9% of all specific antibodies, anti-D in combination with other antibodies was 64.3% of all specific clinically significant antibodies (n = 283), and specific alloimmunization other than Rh group was 14.4%. Table 2 shows all antibodies that were detected in maternal serum.

TABLE 1. Frequency of blood groups in our study population*

Blood group	Alloimmunized women	Nonalloimmunized women
A	123 (37)	2313 (33)
B	90 (27)	1682 (24)
O	85 (25.6)	2453 (35)
AB	34 (10.2)	560 (8)
D+	68 (20.5)	6237 (89)
D–	264 (79.5)	771 (11)

*Data are reported as number (%).

DISCUSSION

This is the first report of a study on RBC alloantibodies during pregnancy in Iranian pregnant women who were referred to the immunohematology reference laboratory at the IBTO. This study revealed that the alloimmunization rate was 4.5%, which was high in comparison with wide pattern in previous studies.[2,3,8] This high rate of alloimmunization, quite possibly, is due to the fact that the majority of blood samples came from pregnant women known to have previous obstetric problems. It should be mentioned that comparison of the results of different studies with each other is problematic, because of differences in population selection and heterogeneity, laboratory methods, and national blood transfusion practices.[3,6]

In the studies by Smith and colleagues[9] and Garratty and colleagues,[10] blood group O was the most common while in a recent study, blood group A was the most prevalent among patients with positive antibodies. In our study, the rate of antibody alloimmunization in the D– group was 25%, which is different to a study by Karim and coworkers.[3] This high rate is probably due to the nature of the services provided by our laboratory as a referral center and so data from nonreferral laboratories might be different from data provided by the recent study.

In the D+ group, the alloimmunization rate was 0.9%, but the total number of women with a positive antibody screen test was 17.6%. This significant allosensitization rate in D+ women shows that a routine screening program should be encouraged despite the negative opinion about the cost and benefit of this test.

However, we suggest that for a better prenatal immunohematologic care, antibody screening should be extended routinely to all D+ women at least once during their pregnancy. Koelewijn and colleagues[6] and De Vrijer and colleagues[11] discussed that antibody screening in the first trimester of pregnancy should be encouraged to

TABLE 2. Frequency of alloantibodies according to blood group

Antibody specificity	Total number (%) of women	D+ women	D– women
Anti-D	125 (37.6)	0	125
Anti-D + anti-C	37 (11.1)	0	37
Anti-D + anti-E	7 (2.1)	0	7
Anti-D + anti-E + anti-C	4 (1.2)	0	4
Anti-D + anti-K	2 (0.6)	0	2
Anti-D + anti-C + anti-S	2 (0.6)	0	2
Anti-D+ anti-M	1 (0.3)	0	1
Anti-D + anti-s	1 (0.3)	0	1
Anti-D + anti-C + anti- Jk^a	1 (0.3)	0	1
Anti-D + anti-C + anti- Jk^b	1 (0.3)	0	1
Anti-D + anti-C + anti- Le^a	1 (0.3)	0	1
Anti-E + anti-c	6 (1.8)	5	1
Anti-E	5 (1.5)	5	0
Anti-c	3 (0.9)	3	0
Anti-K	3 (0.9)	3	0
Anti-E + anti-c + anti- Jk^b	2 (0.6)	2	0
Anti-C	2 (0.6)	1	1
Anti-E + anti-Kp^a	1 (0.3)	1	0
Anti-E + anti-Le^b	1 (0.3)	1	0
Anti-e	1 (0.3)	1	0
Anti-M	10 (0.3)	7	3
Anti-P1	5 (1.5)	3	2
Anti-Jk^b	1 (0.3)	1	0
Anti-M + anti-c	1 (0.3)	1	0
Anti-S	1 (0.3)	1	0
Anti-Le^a	2 (0.6)	2	0
Anti-Le^b	8 (2.4)	4	4
Anti-c + anti-Jk^b	1 (0.3)	1	0
Unspecified antibodies	45 (13.55)	26	19

decrease fetal hemolysis and they recommended antibody screening in the first trimester of pregnancy.

Today, with the use of wider screening panels, it is possible to detect various other irregular antibodies that have been found to cause fetal hemolysis.[12] We used a minipanel antibody (IBTO3cells) to detect alloantibodies. The rate of antibody alloimmunization other than Rh was 14.4%, which is higher than studies performed by Karim and coworkers[3] and Pujol and coworkers.[13]

In Iran, like other developing countries, during pregnancy alloimmunization screening tests are performed just on D– women to detect anti-D. Despite the academic teaching offered in the text to the obstetrics and gynecology specialists, there is no national guideline to follow for detecting other unexpected RBC alloantibodies, but in developing countries there is a guideline for screening all pregnant women for irregular RBC antibodies. Also, in North America and several European countries, there are guidelines to recommend D prophylaxis for all D– pregnant women unless the father of the fetus is D–.[14] According to our national policy for RhIG to prevent alloimmunization to D antigen, antepartum and postpartum Rh immunoprophylaxis, which is available in all centers in Iran and is covered by universal health insurance at affordable prices, is administered at 28 weeks of pregnancy, but the patient's improper management during pregnancy by not screening for the possible risk of any alloimmunizations during multiparous pregnancy, the absence of the correct test to the determine exact number of RhIG doses needed for injection in a D– mother with a D+ child, and lack of follow-up for post–RhIG injection effectiveness are all important issues that we think need to be considered in further studies. It is not known if individuals with multiple antibodies were alloimmunized after first or subsequent pregnancies. It is recommended to study the clinical relevance of the non-D antibodies. It is also recommended to perform such a study in nonreferral laboratories.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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