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ORIGINAL ARTICLE

Frequency of Red Cell Alloantibodies in Pregnant Females of Navsari District: An Experience that Favours Inclusion of Screening for Irregular Erythrocyte Antibody in Routine Antenatal Testing Profile

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Abstract

Background Alloimmunisation due to irregular erythrocyte antibodies is a recognised cause of hemolytic disease of the fetus and newborn (HDFN). Prior knowledge of red cell alloimmunisation in pregnant females guides the obstetrician to monitor the foetus for HDFN and if required for appropriated intervention. As limited data are available on prevalence of red cell alloimmunisation in pregnant females in India, the current study was carried out to know the prevalence of red cell alloimmunisation in pregnant females coming at our laboratory.

Methods Screening for irregular erythrocyte antibodies was performed in 1960 pregnant females after obtaining informed consent between June 2015 and June 2016. MatrixTM screening and identification reagent red cells from Tulip Diagnostics (P) Ltd were used, and column agglutination technique was employed as a method for the test.

Results Twenty antibodies (all of single specificity) were detected in 1960 samples giving a prevalence rate of alloimmunisation of 1.02%. Out of the 20 antibodies, 18 were identified to be anti-D, 1 was anti-c and 1 antibody was anti-H. The results obtained were then compared with those reported in the literature.

Conclusion Red cell alloimmunisation is not uncommonly observed in pregnant females; the information gained can help the obstetrician to identify high-risk cases to timely

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start antenatal and post-natal treatment. Obstetricians should request screening for irregular red cell antibody desirably in all pregnant females; however, if limiting factors are there, it should be done at least in select group of pregnant females having bad obstetric history.

Keywords Alloimmunisation · Antenatal · RBC antibody · HDFN · Antibody screening

Introduction

Ultimate desire of any pregnant female is to give birth to a healthy baby at term; however, many clinical conditions hinder this desire and are associated with foetal–perinatal morbidity and mortality. Hemolytic disease of the fetus and newborn (HDFN) is one of such conditions in which the lifespan of the foetal and neonatal red blood cells (RBCs) is shortened due to maternal alloantibodies against the RBC antigens inherited from the father. Severe HDFN may cause foetal death or can result in hydrops and jaundice, leading to kernicterus and permanent cerebral damage or death of the neonate, while the only clinical sign of mild HDFN is mild neonatal jaundice which is often treated with phototherapy alone. Maternal alloimmunisation against red cell antigens is a prerequisite for this condition to develop. The implicated antibodies could be naturally occurring (anti-A, anti-B) or immune antibodies which develop following a sensitising event such as transfusion or pregnancy [1].

The introduction of anti-D prophylaxis has greatly reduced the frequency of HDFN due to immune anti-D; however, this antibody still remains the most important cause of HDFN. The number of irregular antibodies reported during pregnancy has, however, increased, in part because of greater use of blood transfusion in the obstetric population. Besides anti-D, moderate to severe HDFN cases attributed to other alloantibodies have been reported from Asian countries in the last decade [2–8].

Alloimmunisation in antenatal pregnant females have been extensively studied worldwide and has been reported to be in the range of 0.4 to 2.7% [9]; however, limited data on red cell alloimmunisation amongst pregnant females are available from India. Prior knowledge of red cell alloimmunisation in pregnant female guides the obstetrician to monitor the foetus for HDFN and if required for appropriate intervention. A study was carried out at our clinical laboratory with the aim of knowing the prevalence of red cell alloimmunisation and identifying the specificity of the antibody in antenatal females of Navsari District coming for routine antenatal laboratory investigations.

Materials and Methods

Antibody screening for red cell alloantibodies was performed on a total of 1960 pregnant females at Bhanumati Clinical Laboratory, Navsari, between June 2015 and June 2016 after taking their informed consent. No specific criteria were applied for selecting the cases, and all pregnant females coming for the first time at our laboratory were screened irrespective of their trimester status, parity or Rh status. Samples that turned out to be positive for the presence of red cell alloantibody were further processed for identification of the antibody. 4 millilitre of blood was collected in each of K2 ethylenediaminetetra acetic acid and plain vacutainer tubes. Matrix^{TM} Gel System Erygen-AS 0.8% and Matrix^{TM} Gel System Erygen-ID 0.8% reagent red cells from Tulip Diagnostics (P) Ltd (Fig. 1) were used, respectively, for irregular red cell alloantibody detection and identification.

Semi-automated column agglutination technology in Coomb's phase using low ionic strength solution enhancer as per the manufacturer's instructions was employed for detection and identification of red cell alloantibodies using Matrix Gel System by Tulip Diagnostics (P) Ltd (Fig. 2).

All the 1960 pregnant females were also typed to know their Rh D antigen status using anti-D(Rho)(IgM) from Tulip Diagnostics (P) Ltd and anti-D(IgM) Monoclonal antibody from J. Mitra & Co. Pvt. Ltd. following the policy of two anti-D antisera usage for labelling a sample to be Rh D negative. Conventional tube technique was used for determining the Rh D status. The results obtained from our study were then compared with similar studies reported in the literature.

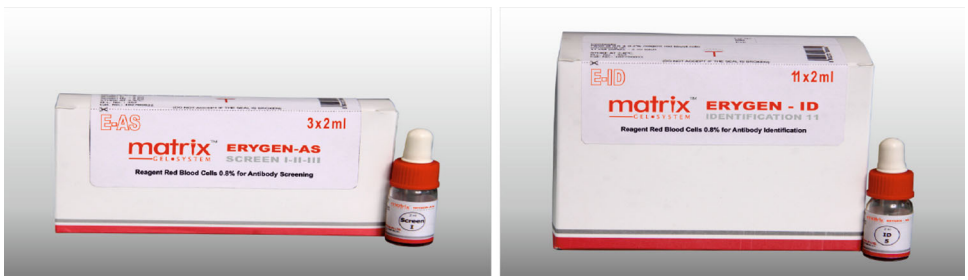

Fig. 1 Reagent red cells used for detection and identification of red cell alloantibodies

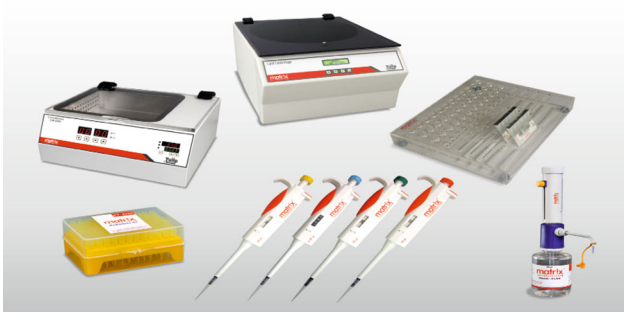

Fig. 2 Equipments used for red cell alloantibody detection and identification

Results

Out of the 1960 antenatal females included in the present study, 62 were Rh D negative and 1898 were Rh D positive. A total of 20 alloantibodies (all of single specificity) were identified in these 1960 samples amounting to prevalence of 1.02%. Out of these 20 antibodies, 18 were anti-D, 1 was anti-c and 1 was anti-H. Eighteen anti-D were identified in 62 Rh D-negative mothers leading to 29% prevalence. The findings of the present study are represented in Table 1.

Discussion

Hemolytic disease of the fetus and newborn (HDFN) is caused by maternal alloimmunisation against red blood cell antigens. In severe cases, HDFN may lead to foetal anaemia with a risk of foetal death and to severe forms of neonatal hyperbilirubinemia with a risk of kernicterus. The overall incidence of haemolytic disease of the newborn varies in different places ranging from as low as 7.2/10,000 births to as high as 14.4/10,000 births [10].

Red blood cell antibody screening programmes are aimed to detect maternal alloimmunisation early in pregnancy to facilitate the identification of high-risk cases to timely start antenatal and post-natal treatment [11].

The International Society of Blood Transfusion now recognises 304 blood group antigens, most of which belong to one of the 36 genetically discrete blood group systems. Antibodies to many of these 304 antigens have the potential to cause HDFN, and they are therefore clinically significant. The order of frequency of HDFN, after the forms due to Rh D incompatibility and ABO incompatibility, is those caused by incompatibility for the c antigen, Kell antigen and the antigens of Duffy system [12].

Prevalence of irregular erythrocyte antibody in pregnant females reported in various studies as shown in Table 2 ranges from 0.89 to 5.98%. In present study also, the prevalence of red cell alloantibody is 1.02% and is in accordance with the prevalence reported from different parts of world.

According to the literature, anti-D alloantibody was and is the antibody most frequently responsible for HDFN; nearly 80% of the cases of HDFN are due to anti-D [12]. Barring a few studies shown in Table 2, most of the studies show that anti-D is the most frequently encountered alloantibody amongst all the alloantibodies identified. In our study also, anti-D was the most commonly (18/20, 90%) identified alloantibody. There are various reasons for the continued occurrence of HDFN due to anti-D alloantibody [12]: (1) the possible development of anti-D immunisation during a pregnancy as a result of an occult foetal–maternal haemorrhage(FMH), usually after the 28th week of gestation, which affects about 1% of Rh D-negative mothers of a Rh D-positive foetus; (2) lack of administration of immunoprophylaxis (IP); (3) ineffective IP because the amount administered was not sufficient for the volume of the FMH; (4) possible errors in the typing of the pregnant woman, puerpera or neonate; and (5) possible errors in the transfusion treatment of females of childbearing potential (transfusion of red blood cell concentrates with mismatched Rh D antigen).

If other antibodies of Rh blood group system are clubbed with anti-D, then antibodies of Rh system become the most common antibodies as evident by the details of Table 2. The present study also had antibodies of the Rh system as the predominant alloantibodies (19/20, 95%).

As Rh immunisation decreases due to Rh prevention programmes, other alloimmune antibodies have become more important as a cause of HDFN; moreover, prophylactic immune globulin is not available to prevent these cases. The prevalence of anti-D sensitised pregnancies reported in Western countries is 1 in 1000 and the prevalence of red cell antibodies other than anti-D with the potency to induce HDFN is about 1 in 500 pregnancies

Table 1 Results of the present study

Total no. of antenatal females screened in the study	Rh D-positive antenatal females in the study	Rh D-negative antenatal females in the study	Total number of antibody-positive cases in the study	Different antibody specificities identified in the study	Prevalence of anti-D in Rh-negative mothers
1960	1898 (96.83%)	62 (3.16%)	20 (1.02%)	Anti-D (18), anti-c (1) and anti-H (1)	18/62 (29.03%)

Table 2 Prevalence of irregular erythrocyte antibody in pregnant females reported in various studies

Study and country of study	Year of publication of the study	Total no. of pregnant females screened	No. of pregnant females with irregular erythrocyte antibodies	Prevalence of irregular antibody (%)
Jeremiah et al. (Nigeria) [10]	2011	500	17	3.40
Pahuja et al. (India) [13]	2011	3577	45	1.25
Devi et al. (India) [14]	2011	624	9	1.44
Foudoulaki-Paparizos et al. (Greece) [15]	2013	4368	39	0.89
Jophy Varghese et al. (India) [16]	2013	5347	79	1.48
Hassan et al. (Malaysia) [17]	2014	5163	51	0.99
Velvoka et al. (Macedonia) [18]	2014	14,858	216	1.45
Karim et al. (Pakistan) [19]	2014	1000	18	1.80
Suresh et al. (India) [20]	2015	2060	25	1.21
Present study (India)	2016	1960	20	1.02

[11]. Different studies carried out worldwide (Table 3) also reveal that out of all the antibodies detected in pregnant females about 11–65% of the alloantibodies belong to specificities other than Rh system. In the present study, only one antibody outside the Rh system was identified (1/20, 5%).

The objectives of routine blood grouping and antibody testing in pregnancy are: (1) to identify Rh D-negative women who would then require anti-D immunoglobulin prophylaxis; (2) to detect and identify red blood cell antibodies; (3) to identify pregnancies at risk of foetal and neonatal haemolytic disease resulting from clinically significant maternal antibodies crossing the placenta and entering the foetal circulation; and (4) to identify antibodies which may be relevant to the safe provision of blood should it be required for transfusion. *When clinically significant red blood cell antibodies are present during pregnancy, follow-up antibody testing is necessary to*: (1) identify a foetus that may require treatment before term; (2) predict which infants might require treatment and should be monitored closely after birth; and (3) detect and identify new antibodies, as those who develop one antibody are more likely to develop additional antibodies. *If an antibody is confirmed and is of clinical significance to the foetus, the antibody will be quantified or titrated and follow-up tests performed*. The follow-up investigations are: (1) monitoring maternal red blood cell antibody levels; (2) identifying possible additional antibodies; (3) red blood cell phenotyping and genotyping of the father when necessary; and (4) foetal genotyping if required.

Considering red cell alloimmunisation as a recognised and an important cause of HDFN, most developed countries have recommendations for screening all pregnant women irrespective of their Rh D status for irregular erythrocyte antibodies [9, 21–23]; however, such recommendations are not included in the existing guidelines in developing countries such as India [24]. *The reasons for non-inclusion of red cell alloantibody in the routine testing protocols for pregnant females in developing countries may be*: (1) lack of infrastructure and technical expertise for erythrocyte alloantibody screening; (2) reliability of the test; (3) cost involved; and (4) lack of infrastructure and facilities to perform follow-up testing and also to manage such pregnancies. In the last few years with the establishment of Department of Immunohematology and Blood Transfusion (IHBT) in many medical colleges and large hospitals, the infrastructure and technical expertise are readily available for antibody detection and identification. With the increasing use of column agglutination technique for immunohematology testing, the reliability of the test results has increased and the sensitivity and specificity of antibody screening programme for detecting severe HDFN associated with antibodies other than anti-D have markedly improved. Before a few years, the reagent red cells for antibody detection and identification were to be imported and the reagent red cells came from Caucasian population, leading to logistics problems; recently, Indian companies have started supplying these reagent red cells at affordable cost, taking care of availability, shelf life and transportation of reagent red cells. In the past decade, non-invasive monitoring of high-risk cases by laboratory testing, including foetal antigen typing with cell free foetal DNA from maternal plasma, followed if necessary by middle cerebral artery peak systolic velocity doppler ultrasonography to judge the presence of foetal anaemia, has replaced invasive procedures for monitoring foetal haemolysis and

Table 3 Proportion of anti-D alloantibody, all Rh specificity alloantibodies and alloantibodies of other specificities identified in various studies

Study and country of study	No. of pregnant females with only anti-D out of the total pregnant females having irregular antibodies		No. of pregnant females with all Rh specificities out of the total pregnant females having irregular antibodies		No. of pregnant females having antibody specificities other than Rh out of the total pregnant females having irregular antibodies	
Jeremiah et al. (Nigeria) [10]	0/17	00.00%	09/17	52.94%	8/17	47.00%
Pahuja et al. (India) [13]	40/45	88.88%	40/45	88.88%	5/45	11.11%
Devi et al. (India) [14]	8/9	88.88%	8/9	88.88%	1/9	11.11%
Foudoulaki-Paparizos et al. (Greece) [15]	8/39	20.51%	18/39	46.15%	21/39	53.84%
Varghese et al. (India) [16]	30/79	37.97%	31/79	39.24%	48/79	60.75%
Hassan et al. (Malaysia) [17]	3/30	10.00%	17/30	56.66%	13/30	43.33%
Velvoka et al. (Macedonia) [18]	132/216	61.11%	164/216	75.92%	52/216	24.07%
Karim et al. (Pakistan) [19]	4/20	20.00%	7/20	35.00%	13/20	65.00%
Altuntas et al. (Turkey) [9]	48/65	73.84%	52/65	80.00%	13/65	20.00%
Suresh et al. (India) [20]	17/25	68.00%	19/25	76.00%	06/25	24.00%
Present study (India)	18/20	90.00%	19/20	95.00%	1/20	05.00%

anaemia [11]. Many centres of excellence with facilities to diagnose, monitor and manage pregnancies with red cell alloimmunisation having results comparable with the best centres in the world exist in India, and hence, early referral to specialised centres with expertise of specialised intensive foetal monitoring for early diagnosis of foetal anaemia and intrauterine foetal blood transfusion offers optimal perinatal outcome.

With the reported prevalence of irregular red cell antibodies in pregnant females worldwide of 0.89–5.98% and in Indian pregnant females of 1.21–1.48% (present study having prevalence of 1.02%)—refer Table 1—there is an obvious reason to include screening for red cell alloantibody in routine tests requested for pregnant females attending obstetrics clinics. Universal antenatal screening for red cell antibodies is desirable [15, 19], but if there are limiting factors for doing this test, selective screening must be done at least in pregnant women with adverse obstetric history [20].

Conclusion

Red cell alloimmunisation by clinically significant antibodies is a recognised cause of HDFN and can lead to foetal anaemia with disastrous consequences. Screening for red cell alloantibody in pregnant females is a prerequisite to take the benefits of advancements in foetal surveillance and treatment allowing successful outcomes for the affected foetuses. Testing for red cell alloantibody deserves to be included in routine test protocols for pregnant females; obstetricians should start requesting this particular screening test and professional bodies such as Federation of Obstetric and Gynaecological Societies of India need to formulate national guidelines for screening for red cell alloantibody in pregnant females to substantially and sustainably reduce newborn deaths and disability due to HDFN.

Compliance with Ethical Standards

Conflict of interest Dr. Manoj Kahar declares that he has no conflict of interest and has not received any grants for the present study.

Ethical Statement Informed consent was obtained from all patients for being included in the study. All procedures for the study were in accordance with established ethical standards. No identifying information of any patient is included in this article.

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