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Case Series

Significance of red cell alloantibodies other than anti-D during pregnancy and their effect on the newborn: A case series

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ABSTRACT

Background: Contrary to well-established guidelines in developed countries, awareness regarding red cell alloantibodies in antenatal period are lacking in India. Investigating for indirect antiglobulin test (IAT) is mostly limited to the Rh D negative antenatal cases. This case series revisits this vital aspect of maternal and fetal safety. Instances of alloantibody other than anti-D are reported.

Materials and Methods: Study was done in Transfusion Medicine department of a tertiary care hospital in North India during 2019-2020. IAT was performed not during the 1st or 2nd trimesters of pregnancy but as a routine compatibility test during delivery. Patients with positive IAT were further evaluated for the detection of alloantibody by using identification panel red cells. Result: Eight antenatal cases with irregular antibodies other than anti-D during 2019-2020 are described. Antibodies detected per patient were single (three cases of anti-E, one of anti-Fya, one of anti-M) or multiple (two cases of anti-E plus anti-c, one of anti-E plus anti-K). Direct antiglobulin test of four babies born to these mothers was found to be positive, one of whom was still born and rest recovered with medical management. Two other babies had DAT negative and two mothers presented late after still birth. Alloantibody titer indicated in patient with anti-E during mid-pregnancy had titer was undetectable by standard tube technique.

Conclusion: Non anti-D alloantibodies can potentially affect fetus, asserting equal attention as anti-D. IAT should not be missed in pregnancy as it is common to investigations for compatibility as well as for fetal wellbeing assessment.

1. Introduction

Alloantibodies against non-self red cell antigens are triggered most frequently by sensitizing events like transfusion, transplantation and pregnancy.[1,2] Such alloantibodies, also called irregular antibodies not only cause difficulty in finding suitable blood for the patient but also threaten the fetus if it develops or recurs during pregnancy. A recent review article by Gupta et al., reiterates the significance of non-ABO red cell antibodies occurring in pregnancies.[3] Infact, occurrence of more persistent alloantibodies in females has been observed to be predominantly due to pregnancies.[4]

Western literature shows 1 in every 80 pregnant female have alloantibodies which affects every 1 in 300 to 600 live births.[5–7] Developed countries have well-established guidelines for screening for red cell alloantibodies in pregnancy.[8–10] Policies on alloantibody related safety during pregnancy and awareness about it has been lacking in our country beyond anti-D. Red cell antigens other than D like C, c, E, e, Kell, M, N and sometimes Duffy antigens are immunogenic.[11] Antibodies formed against these antigens during pregnancy can be harmful for fetus and cause hemolytic disease of fetus and newborn (HDFN) if not intervened. The problem potentially lingers in the newborn adding to morbidity. Investigation of indirect Coombs test

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(ICT) better known as indirect antiglobulin test (IAT) which looks for alloantibodies in patient's plasma is most often limited to Rh D negative patients. Thus, alloantibodies occurring in Rh D positive pregnant patients are most often missed.

As per pre-transfusion protocols, ABO blood group and screening for alloantibodies in all patients is essential.[1,2] In IAT patients' plasma is screened against commercially available phenotyped red cells to ascertain if any alloantibody is present. The implicated alloantibody is identified by an array of investigations which includes but may not limit to IAT of patient plasma against another extended set of phenotyped red cells. Complex immunohematology (IH) procedures like enzyme treatment of cells, adsorption-elution, polyethylene glycol treatment, etc could be added to identify the single or multiple alloantibodies occurring together in some patients.[12] Some interesting cases of alloantibodies other than anti-D occurring during antenatal or postnatal period, most of which were detected incidentally are presented in this case series.

2. Materials and Methods

Study was done in tertiary care hospital in North India during 2019-2020. IAT was performed as a routine test for compatibility. It was performed on anti-IgG + anti-C3d antihuman globulin embedded gel cards which are columns containing either gel (Bio-Rad) by column agglutination technique (ID Dia cell I-II-III; Bio-Rad, DiaMed GmbH, Switzerland) or solid phase red cell adherence (SPRCA) technique (Immucor, Inc. Norcross, GA) on an automated platform Echo (Immucor, INC Norcross, GA, USA). Cases which were detected to have IAT positive were evaluated for the irregular antibody by use of gel cards technique using identification panel red cells ID-DiaPanel (Bio-Rad DiaMed GmbH, Switzerland)

3. Cases Report

The eight cases which are mentioned in table I are as follows:

3.1. Case I

A booked case of term pregnancy was admitted for planned caesarean section. One unit of packed red blood cell (PRBC) was arranged by the obstetrician. The IAT was found positive. Specificity of the alloantibody was anti-E. The patient was phenotyped for the Rh and Kell antigens and was 'E' negative. One unit of E antigen negative packed red blood cell (PRBC) compatible on antihuman globulin (AHG) phase was used for transfusion. Transfusion was uneventful for the mother. The newborn had DAT positive (2+) and raised indirect bilirubin by day 3 of life. However, the baby recovered well with phototherapy.

3.2. Case II

A 26 years old female came for follow-up of still birth at term pregnancy which terminated three months back. She had received no transfusions ever. She had one child delivered six years back and no abortions. PRBC was arranged in lieu of low hemoglobin. Her IAT was positive and she was found to be carrying two alloantibodies, anti-E and anti-c in her plasma. Her red cells phenotyped 'E' and 'c' negative. Possibility of hemolytic disease of fetus and newborn (HDFN) in the recently delivered fetus could not be ruled out.

3.3. Case III

A 39-year old female patient was admitted for caesarean section with simultaneous fibroid resection was found to have alloantibody anti-Fy^a during her pre-transfusion investigation. She had received blood transfusion 4-5 years back following hemorroidectomy. She had an abortion 1-2 years back at a gestational age of 6-8 weeks. In the current pregnancy, the baby delivered was DAT positive. The Fy^a antigen status of the baby could not be investigated due to the fact that the red cells were already sensitized thus IAT test for antigen typing of Duffy was not possible serologically. Elution study was missed. The newborn did not require any transfusion and recovered with medical management only. Finding Fy^a antigen negative PRBC was a challenge and required multiple unit crossmatch and typing.

3.4. Case IV

A 19-year old pregnant patient was under follow-up at our hospital. She had an abortion 7 to 8 months prior to her current pregnancy. She received red cell transfusion shortly after the abortion. She was Rh D negative and she had not received any RhIg immunoglobulin in the ongoing pregnancy. The IAT was being seeked to decide the administration of Rh immunoprophylaxis. The IAT tested positive and anti-E alloantibody was detected. Since the pregnancy was continuing, the alloantibody titer estimation was important. The titer of allo anti-E antibody was below detectable limit by the standard tube technique. Ultrasonographic findings of the fetus were within normal limits. The next follow-up was done after 4 weeks which had similar findings. The pregnancy continued till term with an unaffected fetus. DAT of the newborn could not be performed.

3.5. Case V

A case of a postnatal lady, who delivered a still born baby at 28 weeks gestation was referred to TM department to look for alloantibodies in patient's plasma, if any. She had one abortion and two live births. She had received

PRBC transfusions after second delivery. The current day investigations revealed she had multiple alloantibodies, anti-E and anti-K none of which were diagnosed earlier. Baby's sample was not available for evaluation.

3.6. Case VI

The TM department received request for blood arrangement of a newborn baby who had increasing trends of bilirubin. Pediatrician wanted to stay prepared for an exchange transfusion, in case it is required. On investigation, the baby was found to be DAT positive (2+). As a protocol, mother's blood sample was received for crossmatch. She had an abortion in the 1^{st} pregnancy and this baby was her 1^{st} live child. Her investigations showed IAT positive and the alloantibody identified was anti-E. The baby was typed for 'E' antigen by IgM type of antisera and was found to be 'E' antigen positive. An elution study of baby's red cells was missed. The baby however, did not require exchange transfusion and improved with phototherapy and medical management.

3.7. Case VII

A lady in her third term pregnancy was admitted for delivery. She also had received blood transfusion during her previous deliveries. She was found to be IAT positive. The further investigation found her to be having two alloantibodies i.e anti-c and anti-E. However, the fetus was healthy. The baby delivered did not require any intervention.

3.8. Case VIII

A lady with bad obstetrics outcomes in two subsequent pregnancies was referred for IAT test to TM department. She had never received any blood transfusion nor had any abortion. On further work-up, the implicated alloantibody found was anti-M. The DAT of the still born 2^{nd} baby was found to be 2+ positive. The still born child appeared to have hydrops clinically as per the obstetrician. Elution studies were done on the baby's sample. Anti-M antibody could be eluted, thereby adding strength to the diagnosis of HDFN.

4. Discussion and Conclusion

The case series attempts to draw readers' attention towards potentially problematic alloantibodies which interfere in ante and postnatal periods irrespective of the Rh D status of patient. Every Rh D positive patient solicits as much attention on her IAT status as of an Rh D negative case, as highlighted in this case series. Alloantibodies reported here belong to Rh, Kell, Duffy and MNS blood group systems. Most were incidentally detected during pre-transfusion testing done at the time of delivery whereas one of the non anti-D alloantibody was detected during routine IAT of a Rh D negative antenatal case, as a usual practice. Interestingly, three cases (II, V and VII) had multiple alloantibodies. The fetal red cells were affected as confirmed by DAT of newborn in four cases. In two cases, the fetuses escaped HDFN which could possibly be because the fetus did not inherit corresponding antigen on their red cells. Two mothers presented late after still birth, during follow up for transfusions thus samples of baby could not be evaluated. Blood transfusion is arranged for many institutional deliveries in our country whether blood is utilized or not. The same set of investigations i.e IAT and alloantibody identification (if IAT is positive) are essential to provide safe PRBC for the mother. Then why should the IAT be restricted to Rh D negative cases or high risk pregnancies? The true incidence of alloantibody in pregnancy in India is still elusive as it stays uninvestigated in majority of pregnancies.

Several other studies conducted in India corroborate to findings in this case series and have seen alloantibodies against Rh, Kell, Kidd, MNS, Lewis blood group system in antenatal and postnatal cases.[13–16] Some studies recommend routine antibody screen in all antenatal cases.[17,18] However, some other studies by Gothwal et al. and Suresh et al. suggest that routine antibody screen should restrict to patients with bad obstetric history.[19,20] Though the screening done on a pregnant patient at first visit is a matter of debate in the country, the test done at 28 weeks or thereafter should not be of much argument as the same IAT test is essential component of compatibility test for red cell transfusions. PRBC transfusion is not an uncommon practice in obstetrics.[21]

Majority of the patients reported here had anti-E alloantibody. Finding antigen 'E' negative, 'K' negative red cell units for such patients is not tough as the incidence of 'E' antigen is nearly 20% and 'K' antigen is around 2% in blood donors.[22–24] However, incidence of 'Fy^a' negative donors and 'M' negative donors is relatively less i.e around 12-40% and 12-24% respectively.[23,24] Finding appropriate red cell units in such circumstances takes time and efforts. Anti-K antibodies affect the erythropoiesis process as Kell antigen is expressed not only on mature red cells but also in the precursor red cell thereby making the anemia of fetus more pronounced.[1,2] Though the mechanism of red cell destruction due to anti-M antibodies is not exactly known, studies have seen fetal anemia due to red cell destruction as well as suppression of precursor cells.[25] Two of the patients reported here had anti-K and anti-M. Both patients had still born babies. Features of hydrops were clear in the still born baby of case VIII who had anti-M. However, such history was missed from the other patient.

Alloantibody titer estimation is done against appropriate cells and should ideally be repeated every fortnight till 28^{th} week of gestation and every weekly thereafter.[9] It is often correlated with the fetal middle cerebral artery (MCA) flow Doppler. The critical titer level is defined at

around 16 to 32 for anti-D antibody which is the most common alloantibody found in pregnancy.[26] For all other alloantibodies, an increase in two fold titer levels estimated sequentially warrants attention.[9] Koelewijn et al, described various alloantibodies in pregnancies including those that were seen in cases reported here.[27] They also researched the clinically significant titers of various alloantibodies. They found that critical titer of 16 stands good for majority of alloantibodies. Since all the alloantibodies reported here were detected only at the time of delivery, titration was not indicated. The anti-E antibody which was detected in the Rh D negative patient i.e case IV had anti-E titer not detectable by conventional tube technique at anti-IgG plus anti-C3d antihuman globulin phase.

In India, guidelines for screening the pregnant patients of Rh D negative status is in place, however clarity for Rh D positive females is lacking. Larger studies are needed to ascertain the true incidence of alloimmunization in preganancy. Advanced IH investigations essential for correct treatment in alloimmunized patients are limited to TM centers of tertiary care hospitals. In a resource limited country like India where majority of the blood centres lack such facilities, the best practice would be timely identification of positive case of IAT and referral to hospitals with expert obstetricians, fetal medicine experts and TM specialists to provide optimum care to the pregnant female, the fetus and stay prepared for the newborn.

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6. Conflict of Interest

Author disclose that there is no conflict of interest.

References

1. Veenhof MB, Roosmalen J, Brand A. Obstetric transfusion practice. In: Simon TL, McCullough J, Snyder EL, Solheim BG, Strauss RG, editors. Principles of transfusion medicine. Oxford: Wiley-Blackwell; 2016. p. 507–34.
2. Storry JR. Other Blood Group Systems and Antigens. In: Fung M, Grossman BJ, Hillyer CD, Westhoff CM, editors. Technical Manual. Bethesda, MD: American Association of Blood Banks; 2014. p. 337–63.
3. Gupta GK, Balbuena-Merle R, Hendrickson JE, Tormey CA. Immunohematologic aspects of alloimmunization and alloantibody detection: A focus on pregnancy and hemolytic disease of the fetus and newborn. *Transfus Apher Sci*. 2020;59(5):102946.
4. Castleman JS, Kilby MD. Red cell alloimmunization: A 2020 update. *Prenat Diagn*. 2020;40(9):1099–1108.
5. Smith HM, Shirey RS, Thoman SK, Jackson JB. Prevalence of clinically significant red blood cell alloantibodies in pregnant women at a large tertiary-care facility. *Immunohematology*. 2013;29(4):127–30.
6. Markham KB, Rossi KQ, Nagaraja HN, Shaughnessy O, O'Shaughnessy RW. Hemolytic disease of the fetus and newborn due to multiple maternal antibodies. *Am J Obstet Gynecol*. 2015;213(1):61–8.
7. Koelewijn JM, Vrijkotte TG, Schoot C, Bonsel GJ, DeHaas M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: a population study in the Netherlands. *Transfusion*. 2008;48(5):941–52.
8. Milkins C, Berryman J, Cantwell C, Elliott C, Haggas R, Jones J. Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. British Committee for Standards in Haematology. *Transfus Med*. 2012;23(1):3–35.
9. The Management of Women with Red Cell Antibodies During Pregnancy. (Green-Top Guidelines no. 65) ; 2014. Available from: https://shorturl.at/psKP4.
10. Patient Blood Management Guidelines: Module 5 – Obstetrics and Maternity. Canberra, Australia: National Blood Authority; 2015. Available from: https://ranzcog.edu.au/wp-content/uploads/2022/05/Patient-Blood-Management-Guidelines-Module-5-obstetrics-and-maternity.pdf.
11. White J, Qureshi H, Massey E, Needs M, Byrne G, Daniels G, et al. British Committee for Standards in Haematology. Guideline for blood grouping and red cell antibody testing in pregnancy. *Transfus Med*. 2016;26(4):246–63.
12. Walker PS, Hamilton JR. Other Blood group systems and antigens. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, editors. Technical manual. Bethesda: American Association of Blood Banks; 2014. p. 391–424.
13. Sidhu M, Bala R, Akhtar N, Sawhney V. Prevalence, Specificity and Titration of Red Cell Alloantibodies in Multiparous Antenatal Females at a Tertiary Care Centre from North India. *Indian J Hematol Blood Transfus*. 2016;32(3):307–11.
14. Varghese J, Chacko MP, Rajaiah M, Daniel D. Red cell alloimmunization among antenatal women attending a tertiary care hospital in south India. *Indian J Med Res*. 2013;138(1):68–71.
15. Pahuja S, Gupta SK, Pujani M, Jain M. The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. *Blood Transfus*. 2011;9(4):388–93.
16. Basu S, Kaur R, Kaur G. Hemolytic disease of the fetus and newborn: Current trends and perspectives. *Asian J Transfus Sci*. 2011;5(1):3–7.
17. Patel J, Shukla R, Gupte S. Red cell alloimmunization in multitransfused patients and multiparous women. *Indian J Hematol Blood Transfus*. 2009;25(2):49–52.
18. Dholakiya SK, Bharadva S, Vachhani JH, Upadhyay BS. Red cell alloimmunization among antenatal women attending tertiary care center. *Asian J Transfus Sci*. 2021;15(1):52–6.
19. Gothwal M, Singh P, Bajpai A, Garg N, Yadav G, Sharma C. Red cell alloimmunization in pregnancy: a study from a premier tertiary care centre of Western India. *Obstet Gynecol Sci*. 2022;66(2):84–93.
20. Suresh B, Babu KVS, Arun R, Jothibai DS, Bharathi T. Prevalence of "unexpected antibodies" in the antenatal women attending the Government maternity hospital, Tirupati. *J Clin Sci Res*. 2015;4(1):22–30.
21. Kandasamy D, Selvarajan AK, Jeyakumar JD. Outcome of audit and education on blood transfusion practice in obstetrics setting. *Hematol Transfus Cell Ther*. 2022;44(2):151–5.
22. Kahar MA, Patel RD. Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in blood donors of south Gujarat, India. *Asian J Transfus Sci*. 2014;8(1):51–5.
23. Thakral B, Saluja K, Sharma RR, and NM. Phenotype frequencies of blood groups systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in north Indian blood donors. *Transfus Apher Sci*. 2010;43:17–22.
24. Makroo RN, Bhatia A, Gupta R, Phillip J. Prevalence of Rh, Duffy, Kell, Kidd & MNSs blood group antigens in the Indian blood donor population. *Indian J Med Res*. 2013;137(3):521–6.
25. Yasuda H, Ohto H, Nollet KE, Kawabata K, Saito S, Yagi Y, et al. Hemolytic disease of the fetus and newborn with late-onset anemia due to anti-M: a case report and review of the Japanese literature. *Transfus Med Rev*. 2014;28(1):1–6.
26. Mcbain RD, Crowther CA, Middleton P. Anti-D administration in pregnancy for preventing Rhesus alloimmunisation. *Cochrane Database Syst Rev*. 2015;2015(9):CD000020.

27. Koelewijn JM, Slootweg YM, Folman C, Kamp ILV, Oepkes D, Haas MD. Diagnostic value of laboratory monitoring to predict severe hemolytic disease of the fetus and newborn in non-D and non-K-alloimmunized pregnancies. *Transfusion*. 2020;60(2):391–9.

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