Critical analysis of two cases of major crossmatch incompatibility in tertiary care hospital in Delhi

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Abstract:
Rh blood group is one of the most complexes of the human blood groups system. RHD gene encodes the D antigen, and the RHCE encodes the C, c, E, and e antigens. Out of these, Rh D antigen is the most immunogenic while e antigen is the least immunogenic. Rh antibodies are produced in Rh-negative individuals following sensitization which occurs either through pregnancy or during blood transfusion. We hereby report two cases of anti-e antibody, both presenting as major crossmatch incompatibility.

Keywords:
Crossmatch incompatibility, hemolysis, immunogenic

Introduction
Rh blood group is one of the most complexes of the 34 human blood groups system as highly polymorphic closely linked genes encode them. Till date, 49 Rh antigens are known, out of which five principle Rh antigens D, C, c, E, and e are responsible for the majority of clinically significant Rh antibodies. Out of these, Rh D antigen is the most immunogenic while e antigen is the least immunogenic. There is paucity of literature regarding the role of Non-anti-D antibodies especially anti-e presenting as hemolytic disease of newborn (HDN), cross match incompatibilities and hemolytic transfusion reaction.

We hereby report two cases of anti-e antibody, both presenting as major crossmatch incompatibility.

Case Reports

Case 1
A 6-month-old male presented to the pediatric emergency at Kalawati Saran Children Hospital with complaints of progressive pallor and yellowish discoloration of sclera for the past 15 days. On physical examination, hepatosplenomegaly was absent. No history of any previous blood transfusion was present. Biochemical parameters showed mild indirect hyperbilirubinemia. Hematological findings showed hemoglobin of 5 g/dl with elevated reticulocyte count (corrected reticulocyte count 3%). We received ethylenediaminetetraacetic acid sample of the patient for requisition of packed red cell for anemia. Patient’s blood group was found to be A+ on forward as well as reverse grouping at room temperature. Multiple units of packed red cells were found incompatible on crossmatch. Hence, the patient was taken up for a complete immunohematological workup. Extended blood grouping (forward as well as reverse grouping) was done at three temperatures (4°C, 22°C, and 37°C). We hereby report two cases of anti-e antibody, both presenting as major crossmatch incompatibility.

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37°C) and the blood group was confirmed to be A+. Direct Coombs test performed using gel card was 4+ [Figure 1a] with positive auto-control (3+). Further direct Coombs test (DCT) profile of the patient showed the presence of IgG [Figure 1b]. Hence, a possibility of autoantibody was considered. Indirect Coombs test was performed. Antibody screening (3-cell panel) using Biorad ID-Diacell I-II-III Asia was positive in all three red cell panels in Liss Coombs phase at 37°C [Figure 1c]. Antibody identification (11-cell) ID Diapanel, Biorad showed variable strength reaction in all red cell panels except 3rd cell, thus confirming an antibody specificity of anti-e. ICT in normal saline phase at 4°C was negative. Further Rh-Kell extended antigen profile of the baby showed the presence of “e” antigen [Figure 1d]. The titers of anti-e done by double dilution by tube method were 8. Based on the above immunohematological findings, a final diagnosis of warm autoantibody with anti “e” specificity was made.

Case 2
A 34-year-old female, G3P2 L2A0 presented to Obstetrics casualty in our hospital in early labor with severe anemia at 37 weeks of gestation. The previous two pregnancies were uneventful normal vaginal deliveries with no history of neonatal anemia/jaundice. No previous history of blood transfusion was present. As the patient was in active labor, the requisition for packed red cells was received at our Regional Blood Transfusion Centre. The blood group of the patient was found to be O+. However, multiple units put for crossmatch turned out to be incompatible. Extended blood group (forward as well as reverse grouping) of the patient confirmed the blood group as O+, and no discrepancy was noticed at any of the three temperatures (4°C, 22°C, and 37°C). DCT as well as auto-control of the patient was negative [Figure 2a]. ICT antibody screening (3-cell panel) showed a positive reaction in all three cells [Figure 2b]. Results of antibody identification panel (11-cell Panel) were consistent with anti-e specificity. Antibody titers of anti-e antibody done by gel card method were 1024. The extended Rh–Kell antigen profile of the mother was negative for “e” antigen [Figure 2c], suggesting the presence of allo anti-e antibody in the mother. The blood group of the husband was AB+, and his extended Rh-Kell grouping showed the presence of “e” antigen [Figure 2d]. Packed red cells which were negative for “e” antigen and compatible with the patient’s sample were issued. Next day, the female delivered a baby boy by normal vaginal delivery. The baby had moderate anemia with hemoglobin levels of 8 g/dL, slightly raised reticulocyte count and indirect hyperbilirubinemia (total bilirubin –11.2 mg/dL, indirect bilirubin – 9.0 mg/dL). Baby’s cord blood sample was received for blood grouping and DCT. The blood group of the baby was found to be A+, with the presence of C, c, E, and e on extended Rh-Kell grouping [Figure 2e]. The DCT was positive with the strength of 3+. Elution studies could not be performed as the quantity of the baby sample was insufficient, and the patient was lost to follow-up.

Discussion
In the Rh blood group system, RHD gene encodes the D antigen, and the RHCE encodes the C, c, E, and e antigens. Rh antibodies are produced in Rh-negative individuals following sensitization which occurs either through pregnancy or during blood transfusion. Majority of these antibodies are IgG type and cause extravascular hemolysis though few cases of IgM type antibody are also known.

Giblett estimated e antigen to be 1/125th times as antigenic as Rh (D), which itself elicits an antibody
response in only about 70% of individuals lacking the antigen.\textsuperscript{[1]} Thus, the observed incidence of anti-e HDN is much lower than expected.

Hemolytic disease of the fetus and newborn (HDFN) caused by anti-D Rh antibody is well documented, but limited studies emphasize the role of other non-anti-D Rh antibodies with very few case reports regarding anti-e. The incidence of the development of anti-e in antenatal females varies from 0.016% to 1.7%.\textsuperscript{[2,3]} Hanzlick and Senhauser in 1979 reported a case of subclinical HDFN (mild anemia, jaundice, and slight hepatosplenomegaly) in a 28-year-old G5P4 Caucasian female. The e antigen was absent in the pregnant female while the husband and the baby showed heterozygous expression of e antigen.\textsuperscript{[4]} The findings of the e antigen expression were similar to our case (Case 2) in which the e antigen negative mother was sensitized to paternal e antigen inherited by the baby. Farber and Vawter, Wiener et al. and Blajchman and Gordon described moderate-to-severe HDN in antenatal patients with anti-e.\textsuperscript{[5‑7]}

Autoantibodies also sometimes show antibody specificity with anti-E being the most common. However, few clinically significant autoantibodies can also mimic anti-e specificity as reported by Datta et al. in 2015 in a 12-year-old child of chronic immune thrombocytopenia presenting with severe anemia and features of intravascular hemolysis with homozygous expression of e antigen.\textsuperscript{[8]} These immunohematological findings were similar to our case 1 except that the child had heterozygous expression of e antigen with mild features of hemolysis.

Anti-e is an IgG type of Rh antibody, which is active at 37°C it can lead to major cross-match incompatibility. The presence of anti-e in the samples often leads to crossmatch incompatibilities. As e antigen is present in 98% of Caucasians and 96% of the Asian population, so it is often a challenge to find an e antigen negative blood unit for the recipient having anti-e antibody. This often interferes with timely issuance of compatible blood products.

**Conclusion**

In spite of the low frequency of anti-e Rh antibody in our population, it is a clinically significant IgG antibody. It can cause difficulties during major cross-matching and mild-to-severe HDFN in antenatal patients. In our Indian scenario, routine extended Rh-Kell phenotyping is not performed in all the blood banks. Hence, we are not having organized donor registry system for a particular phenotype, which interferes with timely issuance of compatible blood units negative for high prevalent antigens.

To summarize, we suggest that extended Rh-Kell phenotyping should be routinely performed in all the donor units as well as antenatal patients to further reduce the risk of allo/autoimmunization, HDFN, and strengthen our donor registry system.

**Declaration of patient consent**
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**
There are no conflicts of interest.

**References**

1. Giblett ER. A critique of the theoretical hazard of inter vs. intra-racial transfusion. Transfusion 1961;1:233-8.
2. Smith BD, Haber JM, Queenan JT. Irregular antibodies in pregnant women. Obstet Gynecol 1967;29:118-24.
3. Bushnell LN, Levine P, Mcgee R, Robinson E, Stroup M. A summary of atypical antibodies, rare genotypes, and ABO hemolytic disease encountered in a one year survey. Blood 1956;11:1097-117.
4. Hanzlick RL, Senhauser DA. Subclinical hemolytic disease of the newborn due to anti-e. Am J Clin Pathol 1979;72:76-9.
5. Farber S, Vawter G. Clinical pathological conference, the children’s hospital medical center, Boston, Mass. J Pediatr 1960;57:281-6.
6. Wiener AS, Brancato GJ, Wexler IB. Problems in the management of erythroblastosis fetalis. III. Further observations on cases with unusual serologic and clinical findings. Exp Med Surg 1964;22:1-2.
7. Blajchman MA, Gordon H. Successful pregnancy in a patient with Hodgkin’s disease complicated by warm autoimmune hemolytic anemia and anti-e alloimmunization. Transfusion 1972;12:276-9.
8. Datta SS, Reddy M, Basu S. Warm autoimmune hemolytic anemia with mimicking anti-e specificity causing intravascular hemolysis in a chronic ITP patient. Transfus Apher Sci 2015;53:205-7.