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Maternal-fetal alloimmunization: perinatal outcomes in a reference hospital in Northeastern Brazil

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SUMMARY

OBJECTIVE: To assess the prevalence of maternal alloantibodies in pregnant women at a maternity hospital in northeastern Brazil and describe their perinatal outcomes.

METHODS: A retrospective cohort study reviewed maternal and newborn medical records between January 2017 and October 2018 to assess for the presence of maternal alloantibodies.

RESULTS: The following maternal alloantibodies were found in the 41 cases surveyed: anti-D, 28 cases (45%); anti-C, 7 cases (11%); anti-c, 1 case (1.6%); anti-E, 4 cases (6.4%); anti-Cw, 1 case (1.6%); anti-K, 2 cases (3.2%); anti-Jka, 1 case (1.6%); anti-M, 3 cases (4.8%); anti-Fya, 2 cases (3.2%); anti-Fyb, 1 case (1.6%); anti-Lea, 5 cases (8%); anti-Leb, 3 cases (4.8%); and anti-Dia, 4 cases (6.4%). Anti-D antibodies were the most frequent cause of erythrocyte alloimmunization (80%). Fetal anemia was observed in four pregnancies based on the peak systolic velocity of the middle cerebral artery. In one case, the mother showed anti-M, and anti-Lea alloimmunization, but the direct antiglobulin test results for the newborn were negative, and no unfavorable neonatal outcomes were observed. In one case of a mother with anti-C and anti-D alloimmunization, the neonate showed anti-D antibodies only in the serological panel and required phototherapy. Neonates with plasma antibodies and jaundice requiring phototherapy only had a serological panel with anti-D, anti-C, anti-c, and anti-E antibodies. Intervention was required for 2.5% of pregnant women with positive antibody screens and 81% of newborns with positive direct antiglobulin test results.

CONCLUSION: Despite being a rare condition, maternal alloimmunization by irregular antibodies can result in high perinatal morbidity and mortality.

KEYWORDS: Fetal erythroblastosis. Antibodies. Fetal outcomes.

INTRODUCTION

Maternal alloimmunization during pregnancy occurs when the pregnant woman has an immune response to a fetal erythrocyte antigen inherited by the father, which is not present in the maternal erythrocytes[1]. Although prophylaxis with anti-D immunoglobulin has been recommended by the World Health Organization (WHO) since the 1970s, the most common immunization is still against the Rh-D antigen[2].

Widespread use of anti-D immunoglobulin has significantly reduced cases of maternal Rh-D alloimmunization. However, no prophylactic immunoglobulin exists to prevent the formation of maternal antibodies against other erythrocyte antigens—non-anti-RhD alloimmunization—which also contributes to perinatal morbidity and mortality[3].

Several reported erythrocyte antigens are associated with perinatal hemolytic disease[3]. In addition to the anti-D antibody, those most commonly associated with fetal anemia are anti-c, anti-E, and anti-Kell[4]. Others, such as anti-C, e, Kidd, Duffy, and MNS blood group antibodies, can lead to fetal disease, but more rarely, and usually with mild outcomes[5].

The consequences of alloimmunization during pregnancy depend on antibody type, quantification, and affinity for the corresponding antigen, and can result in pregnancies without fetal impairment until hydrops fetalis or intrauterine fetal death[4]. Perinatal hemolytic disease in the newborn should be suspected when prenatal screening for maternal alloimmunization is positive and/or ultrasound scans show hydrops fetalis or anemia; severe or rapidly progressive hyperbilirubinemia

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when no history of maternal prenatal alloimmunization is observed; or when major anemia or hemolysis is detected in laboratory tests and/or in the case of positive direct antiglobulin test (DAT) results[6].

This study aimed to assess the prevalence of positive antibody screens and the types of clinically significant maternal alloantibodies present in pregnant women at a reference service in Northeast Brazil, as well as the perinatal outcomes of each case.

METHODS

A retrospective cohort study was conducted using the medical records from the Hematology and Hemotherapy Center of Ceará. Serological panels were conducted to detect antibodies among the patients of Assis Chateaubriand School Maternity, Universidade Federal do Ceará (UFC), specifically pregnant and postpartum women with positive antibody screens and/or newborns with positive DTA results. This study surveyed 84 serological panels with maternal alloantibodies from January 2017 to October 2018. This study was approved by the Ethics Committee of UFC under No. 34115320.6.0000.5050.

Maternal serological panels were assessed as follows.

1. ABO and RhD typing was performed in tubes, with anti-A, anti-B, anti-D, and Rh control sera (Fresenius) and A1 and B red blood cells (Revercel, Fresenius);
2. Antibody screens were performed using a LISS/Coombs IgG ± C3d gel card centrifuge (Bio-Rad) with DiaCell I-II red cells (Bio-Rad);
3. Polyspecific DAT was performed using a LISS/Coombs gel card centrifuge containing antibodies against human IgG + C3d (Bio-Rad);
4. Erythrocyte phenotyping was performed for C, c, E, e, K, k, Kpa, Kpb, Jka, Jkb, M, N, S, s, Fya, Fyb, P1, Lua, Lub, Lea, and Leb antibodies using an Rh + Kell card and ID-Antigen Profiles I, II, and III (Bio-Rad).

Neonatal serological panels were assessed as follows.

1. ABO, RhD typing, and DAT were performed using Bio-Rad's newborn card;
2. Erythrocyte phenotyping was performed for antigens corresponding to maternal antibodies C, c, E, e, K, k, Kpa, Kpb, Jka, Jkb, M, N, S, s, Fya, Fyb, P1, Lua, Lub, Lea, and Leb using an Rh + Kell card and ID-Antigen Profiles I, II, and III (Bio-Rad);
3. In cases of positive DAT results, eluate testing using the glycine-acid technique was performed with the DiaCidel kit (Bio-Rad), according to the manufacturer's instructions.

The eluate was tested with red blood cells from DiaPanel and DiaPanel P (Bio-Rad) and red blood cells from Revercel diluted at 1% with diluent 2 (Bio-Rad), when the newborn was not O, for testing in a gel card centrifuge. If the eluate tested negative in newborns with DAT results from alloimmunized mothers and the phenotyping of the newborn was positive for the corresponding antigen, the eluate test results were considered a false negative. In these cases, the maternal antibody with specificity for the antigen expressed in the neonate's red blood cells was implicated as a cause of perinatal hemolytic disease.

After the serological panels were analyzed, information was collected from the medical records of 41 pregnant women with maternal alloantibodies, except for those with anti-RhD only, who gave birth at the institution or were followed up in high-risk prenatal care.

RESULTS

A total of 1052 antibody screens were performed in 2017, 40 of which were positive (3.8% prevalence rate). Between January and October 2018, 1339 antibody screens were performed, 20 of which were positive (1.4% prevalence rate), for an overall prevalence of 2.5%.

Of the 60 patients with positive antibody screens between January 2017 and October 2018, 45 medical records were analyzed, with the following prevalence: anti-D 53.3% (24); anti-C 15.5% (7), anti-c 2.2% (1), anti-E 6.7% (3), anti-Cw 2.2% (1), anti-K 4.4 % (2), anti-Jka 2.2% (1), anti-M 6.7% (3), anti-Fya 4.4% (2), anti-Fyb 2.2% (1), Anti-Lea 11.1% (5), anti-Leb 6.7% (3), anti-Dia 6.7% (3).

A survey of 84 alloantibody serological panels (including pregnant women, postpartum women, and newborns) was carried out from January 2014 to October 2018. From these 84 panels, 41 clinical cases of alloimmunization by non-anti-D alloantibodies were analyzed (Table 1). Serological panels of pregnant women, postpartum women, or newborns alloimmunized with anti-D only were discarded, and those with an alloantibody other than anti-D were included in the analysis.

During prenatal care, fetal anemia was detected in four pregnancies by measuring the mean peak systolic velocity of the middle cerebral artery (MCA-PSV). In case 1, the mother was alloimmunized with anti-M antibodies, and in case 8 by anti-Lea and anti-Leb antibodies; however, in both cases, the newborns' DAT results were negative and no unfavorable neonatal outcomes were observed.

In case 12, the mother was alloimmunized with anti-C and anti-D antibodies, but the newborn's serological panel revealed

Table 1. Types of maternal alloantibodies and perinatal outcomes for 41 alloimmunized pregnant women.

Case	Maternal alloantibodies	Gestational complications related to the presence of maternal alloantibodies	Newborn's DAT result	Newborn alloantibodies	Perinatal outcomes related to the presence of maternal alloantibodies
1	Anti-M	Fetal anemia	NEG	–	–
2	Anti-c	–	POS	Anti-c	Phototherapy
3	Anti-Lea	–	NEG	–	–
4	Anti-Leb	–	NEG	–	–
5	Anti-C, anti-D	–	POS	Anti-C, anti-D*	Transfusion
6	Anti-Lea	–	NEG	–	–
7	Anti-Lea	–	NEG	–	–
8	Anti-Lea, anti-Leb	Fetal anemia	NEG	–	–
9	Anti-K	–	NEG	–	–
10	Anti-Fya, anti-Dia	–	POS	Anti-Fya*	–
11	Anti-Leb	–	NEG	–	–
12	Anti-C, anti-D	Fetal anemia	POS	Anti-D	Phototherapy
13	Anti-C, anti-D, anti-E, anti-Dia	–	POS	Anti-D, anti-E	Phototherapy, exchange transfusion
14	Anti-E	–	POS	Anti-E	Phototherapy
15	Anti-C, anti-D	–	POS	Anti-D	Phototherapy
16	Anti-c, anti-E	–	POS	Anti-c	Phototherapy
17	Anti-Dia, anti-E	–	NEG	–	–
18	Anti-Cw	–	POS	Anti-Cw	Phototherapy
19	Anti-Dia	–	NEG	–	–
20	Anti-M	–	Not performed	–	Fetal death
21	Anti-Jka	–	POS	Anti-Jka	–
22	Anti-Lea	–	NEG	–	–
23	Anti-M	–	NEG	–	–
24	Anti-Fy3	–	POS	Not possible to identify antibody	Phototherapy
25	Anti-E	–	NEG	–	–
26	Anti-D, anti-C, anti-K	–	NEG	–	–
27	Anti-D, anti-C	–	NEG	–	–
28	Anti-C	–	POS	Anti-C	Phototherapy
29	Anti-M	–	NEG	–	–
30	Anti-Dia	–	NEG	–	–
31	Anti-M	–	NEG	–	–
32	Anti-M	–	NEG	–	–
33	Anti-f	–	POS	Anti-f	Phototherapy
34	Anti-M	–	NEG	–	–
35	Anti-Fya, anti-G	Fetal anemia	POS	Anti-Fya, anti-G	Phototherapy, transfusion, exchange transfusion
36	Anti-D, anti-C	–	POS	Anti-D	Phototherapy
37	Anti-E, anti-c	–	POS	Anti-c	Phototherapy
38	Anti-Fy3	–	POS	Anti-Fy3*	-
39	Anti-D, anti-C, anti-K, anti-E, anti-S	–	POS	N/A	Phototherapy
40	Anti-E, anti-Fyb	–	NEG	–	–
41	Anti-K	–	NEG	–	–

DAT: direct antiglobulin test; N/A: not available; NEG: negative; POS: positive.
*Not identified by the eluate technique.

anti-D antibodies only, with phototherapy being required. In case 35, the mother was alloimmunized with anti-Fya and anti-G antibodies, and the same antibodies were detected in the newborn, resulting in unfavorable neonatal outcomes requiring phototherapy, blood transfusion, and exchange transfusion.

In one case, fetal death occurred at 25 weeks of gestation in a primigravida without comorbidities, alloimmunized with anti-M antibodies, but the newborn's DAT was negative. Anti-K was present in maternal blood in cases 9, 26, and 41, but the newborn's DAT was negative, since all neonates were K negative.

Of the 41 alloimmunized pregnant women described in Table 1, 18 had newborns with positive DAT. Only 3 of these 18 newborns, which is equivalent to a percentage of 16.7%, did not need any type of intervention during pregnancy or postpartum. The other 83.3% required phototherapy, blood transfusion, or exchange transfusion. Of the 32 pregnant women alloimmunized with non-anti-D antibodies only, 11 had newborns with positive DAT, of which 9 newborns required at least phototherapy.

DISCUSSION

Among the alloimmunized pregnant women reviewed in this study, the prevalence of anti-D antibodies was much higher than that of other types, supporting previous findings that the most frequent cause of erythrocyte alloimmunization is anti-D (80%)[7]. Also highly prevalent were antibodies that have demonstrated an important association with severe hemolytic disease, such as anti-E and anti-K[4].

In case 8, we have the presence of maternal antibodies anti-Lea and Anti-Leb, but the newborns' DAT results were negative. Because the erythrocyte antigens Lea and Leb are poorly developed at birth, these antibodies are not associated with the development of perinatal hemolytic disease, and pregnant women with anti-Lea and anti-Leb alloimmunization do not require follow-up[8].

Anti-Fya and anti-G were detected in the newborn in case 35, resulting in phototherapy, blood transfusion, and exchange transfusion. The Duffy blood group system consists of the five antigens, and the most important are Fya and Fyb. Although anti-Fyb antibodies are unrelated to perinatal hemolytic disease, anti-Fya antibodies do show an association[3]. Antigen G is usually present when antigens D and/or C are also present, and absent when both are absent[9]. In initial serological tests, anti-G antibodies can mimic anti-D and anti-C. Anti-G alloimmunization can also be associated with perinatal hemolytic disease, must be identified, and also requires follow-up during pregnancy[10,11].

Newborns who had detectable antibodies in the plasma and developed jaundice requiring phototherapy only had a panel including those antibodies with the most clinical significance: anti-D, anti-C, anti-c, and anti-E. Anti-K, despite being one of the non-anti-RhD antibodies most strongly associated with perinatal hemolytic disease, was present in maternal plasma but did not lead to neonatal DAT positivity or any unfavorable fetal or neonatal outcomes, since all neonates were K negative[4].

The MNS blood group system consists of several erythrocyte antigens, but anti-M, -N, -S, -s, and -U antibodies are most strongly associated with perinatal hemolytic disease[3]. In this study, fetal death occurred in a primigravida alloimmunized with anti-M antibodies, but the newborn's DAT was negative. In a case reported by Lin et al.[12], a newborn with severe hemolytic disease caused by anti-M alloimmunization initially presented negative DAT results. Yasuda et al.[13] found a rate of up to 79% negative direct Coombs results in cases of MN incompatibility.

With the spread of anti-D immunoglobulin prophylaxis since the 1960s, maternal alloimmunization rates have decreased[3], but non-anti-RhD antibodies, such as anti-c, anti-E, and anti-Kell, have also been identified as causes of perinatal hemolytic disease, usually with mild to moderate presentations. Despite the predominance of anti-D antibodies among the alloimmunized patients in this study (53.3%), other types of antibodies also showed significant prevalence rates.

This study has an important limitation due to the lack of data on fetal genotyping and some neonatal phenotyping. These tests are important to confirm whether the maternal alloantibody is clinically relevant to the fetus or newborn.

CONCLUSION

To avoid adverse perinatal outcomes, it is critical to reinforce the use of immunoprophylaxis for anti-D alloimmunization in pregnant women. Additionally, given the association of non-anti-D antibodies with the development of perinatal hemolytic disease, it may be warranted to request an antibody screen (indirect Coombs's test) for all pregnant women, even those who are D-positive, and DAT (direct Coombs's test) for newborns whose mothers have an alloantibody other than anti-D, to diagnose non-anti-D maternal alloimmunization.

AUTHORS' CONTRIBUTIONS

URD: Data curation. **DMB**: Methodology. **EAJ**: Writing – original draft. **GT**: Formal Analysis. **DMB**: Formal Analysis. **FHCC**: Investigation, Supervision.

REFERENCES

1. Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ Jr, et al. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. N Engl J Med. 2000;342(1):9-14. https://doi.org/10.1056/NEJM200001063420102
2. Moise KJ Jr, Argoti PS. Management and prevention of red cell alloimmunization in pregnancy: a systematic review. Obstet Gynecol. 2012;120(5):1132-9. https://doi.org/10.1097/aog.0b013e31826d7dc1
3. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med. 2008;13(4):207-14. https://doi.org/10.1016/j.siny.2008.02.007
4. Ghesquière L, Garabedian C, Coulon C, Verpillat P, Rakza T, Wibaut B, et al. Management of red blood cell alloimmunization in pregnancy. J Gynecol Obstet Hum Reprod. 2018;47(5):197-204. https://doi.org/10.1016/j.jogoh.2018.02.001
5. Babinszki A, Berkowitz RL. Haemolytic disease of the newborn caused by anti-C, anti-E and anti-Fya antibodies: report of five cases. Prenat Diagn. 1999;19(6):533-6. https://doi.org/10.1002/(sici)1097-0223(199906)19:6<533::aid-pd570>3.0.co;2-5
6. Murray NA, Roberts IA. Haemolytic disease of the newborn. Arch Dis Child Fetal Neonatal Ed. 2007;92(2):F83-8. https://doi.org/10.1136/adc.2005.076794
7. Lindenburg IT, Smits-Wintjens VE, van Klink JM, Verduin E, van Kamp IL, Walther FJ, et al. Long-term neurodevelopmental outcome after intrauterine transfusion for hemolytic disease of the fetus/newborn: the LOTUS study. Am J Obstet Gynecol. 2012;206(2):141.e1-8. https://doi.org/10.1016/j.ajog.2011.09.024
8. Kennedy MS, Delaney M, Scrape S. Perinatal issues in transfusion practice. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, editors. Technical manual. 18th ed. Bethesda: AABB; 2014. p. 561-70.
9. Allen FH Jr, Tippett PA. A new Rh blood type which reveals the Rh antigen G. Vox Sang. 1958;3(5):321-30. https://doi.org/10.1111/j.1423-0410.1958.tb04013.x
10. Yousuf R, Mustafa AN, Ho SL, Tang YL, Leong CF. Anti-G with concomitant anti-C and anti-D: a case report in a pregnant woman. Asian J Transfus Sci. 2017;11(1):62-4. https://doi.org/10.4103/0973-6247.200770
11. Palfi M, Gunnarsson C. The frequency of anti-C + anti-G in the absence of anti-D in alloimmunized pregnancies. Transfus Med. 2001;11(3):207-10. https://doi.org/10.1046/j.1365-3148.2001.00306.x
12. Lin L, Huang L, Lou G, Luo Y, Fang Q. Prenatal treatment of severe fetal hemolytic disease due to anti-M alloimmunization by serial intrauterine transfusions. Taiwan J Obstet Gynecol. 2017;56(3):379-81. https://doi.org/10.1016/j.tjog.2017.04.022
13. 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. https://doi.org/10.1016/j.tmrv.2013.10.002