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ORIGINAL ARTICLE

Correlation between Maternal Anti-E Antibody Titer and the Severity of Hemolytic Disease of the Fetus and Newborn

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SUMMARY

Background: **Hemolytic disease of the fetus and newborn (HDFN) caused by anti-E antibodies remains a clinical challenge. The correlation between maternal anti-E antibody titers and HDFN severity, particularly the critical titer threshold for severe clinical outcomes, remains unclear. This study aimed to investigate the correlation between maternal anti-E antibody titers and the severity of HDFN.**
Methods: **Clinical data were retrospectively collected from 55 pregnant women with anti-E antibody and their newborns (June 2020 - May 2024), including general maternal information and diagnostic findings. Anti-E antibody titers were measured, and neonatal outcomes were recorded.**
Results: **Among the 55 pregnant women, anti-E antibody titers ranged from 1:1 to 1:1,024. HDFN occurred in 56.4% (31/55) of cases. Titers ≥ 1:16 were significantly associated with higher HDFN incidence ($\chi^2 = 14.996$, $p < 0.001$). Neonatal indirect bilirubin levels correlated significantly with both HDFN occurrence ($r = 0.589$, $p < 0.05$) and maternal anti-E antibody titers ($r = 0.657$, $p < 0.05$). Neonatal length of hospital stay also showed a positive correlation with maternal antibody titers ($r = 0.798$, $p < 0.05$).**
Conclusions: **A maternal anti-E titer ≥ 1:16 is a critical threshold predictive of HDFN occurrence and severity, providing a valuable marker for early risk stratification and clinical management.**
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KEYWORDS

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INTRODUCTION

Hemolytic Disease of the Fetus and Newborn (HDFN) is an immune-mediated hemolytic disorder in fetuses or newborns caused by maternal-fetal blood group incompatibility. It is characterized by high morbidity during the fetal and neonatal periods. Typical symptoms include severe hemolysis accompanied by serious complications such as liver dysfunction, ascites, fetal edema, congestive heart failure, and acute bilirubin encephalopathy [1,2]. In severe cases, HDFN may lead to miscarriage or stillbirth. Maternal IgG antibodies efficiently bind to fetal red blood cells, triggering complement-

mediated intravascular hemolysis and enhancing phagocytosis by the mononuclear-macrophage system. This process leads to rapidly progressive anemia and hyperbilirubinemia, without treatment, this can cause permanent neurological damage or even death in newborns. The two most common clinical forms result from ABO and Rh blood group incompatibility [3]. Reports indicate that total serum bilirubin peaks in Rh-HDFN are 30 - 50% higher than in ABO-HDFN. Furthermore, the rate of exchange transfusion is significantly higher in Rh-HDFN patients (68.6%) compared to those with ABO-HDFN (12.3%) [4]. A survey of 2,241 patients identified anti-E (15.39%) as the second most common antibody causing HDFN, after anti-D (39.81%). However, the widespread application of Rh (D) immunoprophylaxis has made Rh (D)-mediated HDFN preventable and controllable, significantly reducing its prevalence. Another study of 42,517 hospitalized Han Chinese patients identified anti-E antibodies in 41.0% (87/212) of Rh-positive individuals, significantly higher than the rates of anti-D (21.2%, 45/212) or other specific antigen antibodies. This pattern was also observed in a retrospective Wuhan study (2006 - 2016), which showed that 94.48% of 344 unexpected antibodies causing HDFN belonged to the Rh system, with anti-E being the second most prevalent antibody after anti-D [5].

Although anti-E induced HDFN carries significant clinical importance, a research gap persists regarding the correlation between maternal anti-E antibody titers and HDFN severity. This retrospective cohort study aims to: 1) investigate the correlation between maternal anti-E antibody titers and the severity of hemolytic disease in fetuses and newborns, and 2) identify the critical anti-E titer threshold of severe HDFN, supporting its use in early risk stratification and clinical management.

MATERIALS AND METHODS

Materials

We retrospectively collected clinical data from 55 pregnant women who underwent anti-E antibody titer testing between June 2020 and May 2024, along with their corresponding fetuses and newborns.

The study cohort included pregnant women, fetuses, and newborns meeting the following criteria: absence of gestational comorbidities or organ dysfunction; no prior transfusion history, with indirect antiglobulin test (IAT) confirmed positive results; fetuses and newborns exhibiting no intrauterine distress or other hematologic diseases.

Data Collection

Maternal data: We collected antibody screening and antibody identification results, and anti-E titer levels. Pregnancy outcomes for all 55 women were followed up.

Neonatal data: We recorded free and eluted antibody test results from umbilical cord blood. Additionally, we collected serum indirect bilirubin levels and length of hospital stay.

Methods

Neonatal ABO and Rh blood type determination

Neonatal red blood cells (RBCs) were diluted into RBC suspensions. These suspensions were then reacted separately with monoclonal anti-A, anti-B, and anti-D reagents.

Irregular antibody screening and identification

The patient's plasma was incubated with both antibody screening cells and antibody identification panel cells to detect and identify irregular antibodies.

Antibody titer detection

1) Pregnant woman's plasma was mixed with an equal volume of 2-Me (2-mercaptoethanol) and incubated at 37℃ for 60 minutes to denature IgM antibodies.

2) Serial two-fold dilutions of the treated plasma were prepared, ranging from 1:1 to 1:2,048.

3) Each dilution was tested against E antigen-positive RBCs using an anti-human globulin (AHG) gel card. The antibody titer was defined as the highest dilution yielding a 2+ agglutination reaction.

Laboratory evaluation for hemolytic disease of the fetus and newborn

1) Neonatal DAT:

Newborn's red blood cells were added directly to the AHG gel card.

2) Neonatal free antibody test:

Neonatal plasma was incubated with reagent RBCs antigen-matched to the suspected maternal antibody.

3) Elution Test:

a) Neonatal RBCs were washed and resuspended in an equal volume of saline.
b) The suspension was vortexed vigorously for 1 minute at 56℃ and then incubated for 9 minutes.
c) The incubated RBC suspension was centrifuged to obtain the eluate (supernatant).
d) The eluate was tested against reagent RBCs antigen-matched to the suspected maternal an-tibody using the AHG gel card.

Interpretation criteria for HDFN test results are provided in Table 1.

RESULTS

Antibody titer distribution and HDFN risk threshold

Among the 55 pregnant women, anti-E antibody titers ranged from 1:1 to 1:1,024. HDFN incidence rates across titer levels are presented in Table 2. A significant correlation was observed between maternal antibody titer levels and HDFN occurrence ($\chi^2 = 14.996$, $p < 0.001$). The number of HDFN cases was substantially higher when maternal titers were ≥ 1:16 compared to titers < 1:16 (Table 3), indicating that an antibody titer

Table 1. Diagnostic criteria for hemolytic disease of the fetus and newborn.

DAT	Free antibody test	Eluate antibody detection	Results interpretation
-	-	-	HDFN excluded
-	+	-	suspicious HDFN
+	-	+	confirmed HDFN
-	+	+	confirmed HDFN
+	+	+	confirmed HDFN

Table 2. HDFN incidence rates stratified by maternal antibody titers.

Titer (1:n)	1	2	8	16	32	64	128	256	512	1,024	Total
Number of cases	3	4	7	10	6	9	4	4	4	1	55
HDFN cases, n (%)	0 (0.0)	0 (0.0)	2 (6.5)	4 (13.0)	5 (16.1)	6 (19.3)	3 (9.7)	4 (13.0)	5 (16.1)	1 (3.2)	31 (56.4)

Table 3. Association between maternal antibody titer levels and HDFN development (n = 55).

Titer	Non-HDFN cases	HDFN cases	Total
< 16	14	3	17
≥ 16	10	28	38
χ^2			14.996
p-value			< 0.001

Table 4. Association between neonatal indirect bilirubin levels and HDFN development (n = 55).

	Value	95% CI	p-value
Pearson's r	0.589	0.382 - 0.738	< 0.05

CI Confidence Interval.

Table 5. Correlation of neonatal indirect bilirubin levels with maternal anti-E antibody titers (n = 55).

	Value	95% CI	p-value
Pearson's r	0.657	0.465 - 0.849	< 0.05

CI Confidence Interval.

Table 6. Correlation between maternal anti-E antibody titers and neonatal length of hospital stay (n = 55).

	Value	95% CI	p-value
Pearson's r	**0.798**	**0.682 - 0.878**	**< 0.05**

CI Confidence Interval.

≥ 1:16 represents a critical threshold for increased HDFN risk.

Correlation between neonatal indirect bilirubin levels and HDFN development

A significant positive correlation was demonstrated between neonatal indirect bilirubin levels and HDFN occurrence ($r = 0.589$, $p < 0.05$), with detailed results presented in Table 4.

Correlation between neonatal indirect bilirubin levels and maternal anti-E antibody titer

A significant positive correlation was observed between neonatal indirect bilirubin levels and maternal anti-E antibody titers ($r = 0.657$, $p < 0.05$), with detailed results presented in Table 5.

Correlation between maternal anti-E antibody titers and neonatal length of hospital stay

A significant positive correlation was observed between maternal anti-E antibody titers and neonatal length of hospital stay ($r = 0.798$, $p < 0.05$), with detailed results presented in Table 6.

DISCUSSION

HDFN is primarily caused by ABO blood type antibodies and irregular antibodies [6]. While ABO-mediated HDFN typically presents with milder symptoms and demonstrates no clear correlation between the antibody titers and clinical severity [7]. HDFN due to irregular antibodies often causes more severe disease due to stronger antigen immunogenicity [8]. Maternal IgG antibodies can efficiently bind to fetal red blood cells, triggering complement-mediated intravascular hemolysis and enhanced phagocytosis by the mononuclear-phagocyte system; this process destroys neonatal red blood cells, leading to rapidly progressive anemia and hyperbilirubinemia. When indirect bilirubin levels in the neonatal serum exceed the liver's metabolic capacity, the bilirubin can enter cardiomyocytes, disrupting their normal metabolism and function. Ultimately, this can result in serious complications such as myocardial injury and even death [9].

Research shows that anti-E antibody has become the primary antibody causing HDFN within the Rh blood group system. It accounts for 38.6% of unexpected antibodies, and 62.3% of cases involving anti-E are delayed in treatment due to failure in detection during initial screening [5,10]. The results of this study demonstrate that a maternal anti-E antibody titer exceeding 1:16 is associated with a significantly increased incidence of HDFN. Additionally, neonatal indirect bilirubin levels and length of hospital stay showed a significant positive correlation with maternal anti-E antibody titers. This suggests that anti-E antibodies may lead to more severe clinical outcomes by accelerating erythrocyte destruction and increasing bilirubin metabolic burden.

The *Royal College of Obstetricians and Gynaecologists* (RCOG) guideline for optimal management of pregnant women with red cell antibodies explicitly recommends blood group typing and antibody screening for all pregnant women during early pregnancy and at 28 weeks of gestation. To mitigate neonatal morbidity, a comprehensive assessment of HDFN risk should be performed for the current pregnancy and a multiple disciplinary team (MDT) approach should be established. This MDT, involving collaboration among obstetrics, ultrasonography, transfusion medicine, and neonatology departments, should implement holistic management spanning preconception, prenatal, intrapartum, and postpartum care to prevent HDFN. Clinical management should prioritize prevention anemia and complications associated with hyperbilirubinemia. Current primary therapeutic interventions include intensive phototherapy, intravenous immunoglobulin (IVIG) infusion, and exchange transfusion [11].

This study demonstrates a close correlation between maternal anti-E antibody titers and both the incidence and severity of HDFN. A critical anti-E antibody titer of 1:16 serves as a predictive biological threshold for HDFN occurrence, indicating a significantly elevated risk of adverse perinatal outcomes. Quantitative measurement of maternal anti-E antibody titers provides a crucial laboratory basis for early warning and severity stratification of HDFN. This approach holds significant clinical value for optimizing antenatal monitoring strategies and enhancing perinatal care.

Declaration of Interest:

The authors declare that they have no conflict of interest.

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