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Research on the Clinical Correlation Between the Detection of Irregular Antibodies in Red Blood Cell Blood Groups and Hemolytic Disease of the Newborn

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Abstract: *Objective*: To explore the clinical correlation between the detection of irregular antibodies in red blood cell blood groups and hemolytic disease of the newborn. *Methods*: This study selected newborns who underwent examinations and were diagnosed with hemolytic disease at our hospital from October 2024 to October 2025 as the research subjects. Based on the severity of their hemolytic disease, the infants were divided into a severe group and a mild group. All the infants underwent detection for irregular antibodies in their red blood cell blood groups. General information, blood types, and irregular antibody test results of the two groups were recorded. Univariate analysis was conducted, and variables with statistical significance from the univariate analysis were included in a multivariate logistic regression analysis to explore the clinical correlation between the detection of irregular antibodies in red blood cell blood groups and hemolytic disease of the newborn. *Results*: Through univariate analysis, it was found that IgG1 and IgG3 subclass antibodies, as well as ABO blood group incompatibility, were statistically significant ($p < 0.05$). When these factors were included in a multivariate logistic regression analysis, it was discovered that IgG1 (OR = 2.461, 95% CI: 1.859–2.709), IgG3 (OR = 2.509, 95% CI: 1.918–2.893), and ABO blood group incompatibility (OR = 2.998, 95% CI: 2.149–3.493) all exhibited a positive correlation with hemolytic disease of the newborn. *Conclusion*: As levels of IgG1, IgG3, and ABO blood group incompatibility increase, the incidence of hemolytic disease of the newborn also rises, warranting clinical attention.

Keywords: Red blood cell blood group; Irregular antibody detection; Newborn; Hemolytic disease; Correlation analysis

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1. Introduction

Hemolytic Disease of the Fetus and Newborn (HDFN) is a common immune-mediated disorder in clinical practice, primarily caused by maternal-fetal blood group incompatibility. IgG antibodies produced in the maternal

body can cross the placenta and enter the newborn's circulation, sensitizing the newborn's red blood cells. The main clinical symptoms include hepatosplenomegaly, hyperbilirubinemia, and, in severe cases, kernicterus and even death [1]. Based on the differences in red blood cell surface antigens within the blood group system, there are certain variations in clinical manifestations. Some studies have indicated that the severity of hemolytic disease is somewhat correlated with the rate of red blood cell destruction [2]. In cases of ABO blood group incompatibility involving O-type mothers and non-O-type infants, the primary reason is that O-type blood can produce antibodies against A and B antigens, although the exact mechanism remains unclear. It is currently widely believed that substances within A and B antigens in the natural environment can stimulate the body, leading to the production of anti-A and anti-B antibodies [3]. ABO blood group antigens are not only present on red blood cells but also widely distributed throughout various tissues in the human body. In clinical practice, the diagnosis and treatment of hemolytic disease of the newborn (HDN) face multiple challenges. Firstly, HDN symptoms caused by ABO blood group incompatibility are usually mild, whereas HDN caused by Rh blood group incompatibility or other rare blood group systems may progress rapidly and require early intervention. Secondly, traditional HDN diagnosis relies on the direct antiglobulin test, free antibody test, and antibody release test; however, these methods have limited sensitivity in detecting low-titer antibodies or antibodies from non-ABO blood group systems [4]. Additionally, while the treatment methods for Hemolytic Disease of the Newborn (HDN) can effectively reduce bilirubin levels, they cannot reverse brain damage that has already occurred. Therefore, early prevention and precise diagnosis become crucial in reducing the harm caused by HDN [5]. Based on this, this study selected newborns who were examined and diagnosed with hemolytic disease in our hospital from October 2024 to October 2025 as the research subjects, and explored the clinical correlation between the detection of irregular antibodies to red blood cell blood groups and neonatal hemolytic disease. The specific report is as follows.

2. Materials and methods

2.1. General information

This study selected a total of 100 newborns who were examined and diagnosed with hemolytic disease in our hospital from October 2024 to October 2025 as the research subjects.

2.1.1. Inclusion criteria

(1) Age at birth ≤ 30 days
(2) Complete clinical data
(3) Completion of three tests for neonatal hemolytic disease with complete data
(4) The family members of the newborns signed informed consent forms, indicating their voluntary participation in this study

2.1.2. Exclusion criteria

(1) Concomitant with other immune diseases
(2) History of immunotherapy
(3) Presence of congenital red blood cell defects

2.2. Methods

2.2.1. Blood group antibody detection

Conduct ABO and RhD blood group typing, and use microcolumn gel test cards for labeling. Add 50 μL of red blood cell suspension into microwells 1–4, and add maternal serum into microwell 5. Add standard A and B red blood cell suspensions. After completion, centrifuge at 900 rpm for 2 minutes, and then interpret the results. Result interpretation: Specific antigen-antibody complexes formed between red blood cell antigens and corresponding antibodies in the microcolumn gel float on the gel surface or within the gel [6].

Subsequently, perform irregular antibody screening with the following specific steps

(1) Label the microcolumn gel test cards
(2) Add 50 μL of a 0.5–0.8% standard O red blood cell suspension into tubes 1–6
(3) Add the serum to be tested into tubes 1–5
(4) After sample addition, place the test cards into a 37°C incubator and immediately centrifuge using a dedicated serological centrifuge at 900 rpm for 2 minutes, then interpret the results [7]
(5) Result interpretation: If tubes 1–5 show negative results, the serum does not contain standard O red blood cell antigen-specific IgG incomplete antibodies.

2.2.2. Research methods

A total of 100 pediatric patients were included in this study and were divided into a milder group and a more severe group based on their conditions, which were regarded as the dependent variables. The independent variables selected were general information, blood type, and irregular antibody test results.

2.3. Statistical methods

In this study, statistical software SPSS 21.00 was utilized for data processing and calculation during data comparison. The chi-square test was employed for measurement data, expressed as (n, %), while the *t*-test was used for count data, expressed as ($\overline{x} \pm s$). Variables with statistical significance identified through univariate analysis were included in multivariate logistic analysis. A calculated result with $p < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Analysis of general information

A total of 100 pediatric patients were included in this study, comprising 56 male patients (56.00%) and 44 female patients (44.00%). The average age in days was (13.24 ± 3.47) d, with an average birth weight of (3.84 ± 1.06) kg and an average birth length of (54.06 ± 8.94) cm. In antibody tests, the mean IgG1 level was (2.64 ± 0.71)%, IgG2 was (80.97 ± 9.06)%, IgG3 was (11.58 ± 3.23)%, and IgG4 was (6.24 ± 1.25)%. There were 17 cases (17.00%) of ABO blood group incompatibility, as detailed in **Table 1**.

Table 1. Analysis of general data results

Variable	Category	Result
Gender [n(%)]	Male	56 (56.00)
	Female	44 (44.00)
Age (days)	Mean ± SD	13.24 ± 3.47
Weight (kg)	Mean ± SD	3.84 ± 1.06
Length (cm)	Mean ± SD	54.06 ± 8.94
IgG1 (%)	Mean ± SD	2.64 ± 0.71
IgG2 (%)	Mean ± SD	80.97 ± 9.06
IgG3 (%)	Mean ± SD	11.58 ± 3.23
IgG4 (%)	Mean ± SD	6.24 ± 1.25
ABO incompatibility [n(%)]	Cases	17 (17.00)

3.2. Analysis of univariate results

In this study, 67 patients were included in the milder group and 33 in the more severe group. Univariate analysis revealed that IgG1 and IgG3 subclass antibodies, as well as ABO blood group incompatibility, were statistically significant ($p < 0.05$). Other variables did not show statistical significance, as detailed in **Table 2**.

Table 2. Analysis of univariate results

Variable	Category	Mild group (n = 67)	Severe group (n = 33)	Statistical value (χ^2/t)	*p*-value
Gender [n]	Male (n = 56)	36	20	0.424	0.515
	Female (n = 44)	31	13		
Age (days)	Mean ± SD	13.50 ± 3.45	13.13 ± 3.51	0.501	0.617
Weight (kg)	Mean ± SD	3.88 ± 1.08	3.83 ± 1.05	0.220	0.827
Length (cm)	Mean ± SD	54.01 ± 9.03	54.16 ± 9.01	0.078	0.938
IgG1 (%)	Mean ± SD	2.52 ± 0.69	3.26 ± 0.67	5.091	< 0.001
IgG2 (%)	Mean ± SD	78.29 ± 7.93	83.67 ± 8.04	3.176	0.002
IgG3 (%)	Mean ± SD	11.54 ± 3.26	11.59 ± 3.29	0.043	0.966
IgG4 (%)	Mean ± SD	6.26 ± 1.23	6.23 ± 1.20	0.116	0.908
ABO incompatibility [n(%)]	Cases (n = 17)	7	10	6.178	0.013

3.3. Multivariate logistic regression analysis

Variables that were statistically significant in the above univariate analysis were included in the multivariate logistic regression analysis, specifically incorporating three variables: IgG1, IgG2, and ABO blood group incompatibility. The multivariate analysis revealed that IgG1 (OR = 2.461, 95%CI: 1.859–2.709), IgG3 (OR = 2.509, 95%CI: 1.918–2.893), and ABO blood group incompatibility (OR = 2.998, 95%CI: 2.149–3.493) all exhibited a positive correlation with hemolytic disease of the newborn (HDN). The specific data are presented in **Table 3**.

Table 3. Multivariate logistic regression analysis

Variable	β	S.E.	*p*-value	OR	95% CI
IgG1	0.90	0.87	< 0.05	2.461	1.859–2.709
IgG2	0.93	0.92	< 0.05	2.509	1.918–2.893
ABO incompatibility	0.97	0.94	< 0.05	2.998	2.149–3.493

4. Discussion

The detection of irregular antibodies to red blood cell blood groups is a crucial step in preventing hemolytic disease of the newborn. Irregular antibodies refer to blood group antibodies other than anti-A and anti-B, primarily induced by exposure to foreign red blood cells through events such as blood transfusion, pregnancy, or transplantation [8]. These antibodies can cross the placenta into the fetal bloodstream, bind to antigens on the surface of fetal red blood cells, and trigger immune-mediated hemolytic reactions. Prenatal screening for irregular antibodies can identify in advance whether antibodies that may cause HDN are present in the pregnant woman's body [9,10]. For antibody-positive pregnant women, the risk of fetal hemolysis can be assessed through regular monitoring of antibody titers and fetal ultrasound examinations. When necessary, intrauterine transfusion or early termination of pregnancy can be performed [11]. Irregular antibody testing is also an important screening item before blood transfusion. If a pregnant woman requires a blood transfusion, this testing can prevent the transfusion of red blood cells containing corresponding antigens, thereby avoiding hemolytic transfusion reactions [12]. Rh blood group incompatibility is the second most common cause of hemolytic disease of the newborn (HDN), with HDN caused by anti-D antibodies typically being more severe, leading to fetal anemia, edema, jaundice, and even stillbirth or neonatal bilirubin encephalopathy [13–15].

Based on this, this study selected newborns who underwent examinations and were diagnosed with hemolytic disease at our hospital from October 2024 to October 2025 as the research subjects. Through univariate analysis, it was found that IgG1 and IgG3 subclass antibodies, as well as ABO blood group incompatibility, were statistically significant ($p < 0.05$). Subsequently, multivariate logistic regression analysis revealed that IgG1, IgG3, and ABO blood group incompatibility were all positively correlated with neonatal hemolytic disease.

5. Conclusion

In summary, as a significant complication during the perinatal period, hemolytic disease of the newborn (HDN) is closely related to the production of irregular antibodies against maternal red blood cell blood groups. This study systematically analyzed the clinical value of irregular antibody detection in the prevention, diagnosis, and treatment of HDN, revealing the correlation between antibody types, titers, and the severity of HDN, thereby providing a scientific basis for optimizing clinical management strategies. In the future, with advancements in detection technologies and deeper global collaboration, it is essential to reduce the risk of neonatal mortality and long-term neurodevelopmental impairments, safeguarding the health of every newborn.

Disclosure statement

The authors declare no conflict of interest.

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