

Risk analysis of hemolysis and establishment of prenatal prediction model in newborns due to maternal-fetal ABO blood type incompatibility

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ABSTRACT

This study aims to investigate the impact of blood type, antibody titer, and pregnancy history on the incidence and severity of ABO hemolytic disease of the newborn (HDN). Data from 214 ABO HDN cases (2018–2021) were analyzed using elution tests for diagnosis and transcutaneous bilirubin for severity. The positivity rate was significantly higher in the OA group (86.73%) than in the OB group (53.47%, $P < 0.01$), although differences in bilirubin levels between the two groups were limited. An antibody titer of ≥ 64 was associated with a marked increase in HDN incidence; however no consistent correlation with bilirubin levels was observed. Pregnancy history showed no significant correlation. Logistic regression identified blood type and antibody titer as significant predictors of HDN incidence (odds ratio [OR] = 6.09, $P < 0.01$; OR = 1.001, $P < 0.05$), whereas pregnancy history was not statistically significant (OR = 0.633, $P > 0.05$). The prediction model, expressed as $\text{logit}(P) = \ln[P/(1-P)] = 0.023 + 1.806 \times \text{blood type (1)} + 0.001 \times \text{titer} - 0.457 \times \text{pregnancy history (1)}$, achieved an area under the curve (AUC) of 0.776, indicating moderate predictive accuracy. The study suggests that newborn blood type and maternal antibody titer are key factors for predicting ABO HDN incidence, though not its severity, while pregnancy history has limited relevance. This predictive model provides a moderately accurate tool for risk assessment.

Keywords: ABO blood group incompatibility, hemolytic disease of the newborn, prenatal prediction

INTRODUCTION

Hemolytic disease of the newborn (HDN) due to maternal-fetal blood group incompatibility can be caused by various red blood cell (RBC) antibodies,

with those of the ABO and Rh blood group systems being the most common. In the Han Chinese population, the prevalence of RhD-negative individuals is less than 1%, resulting in HDN due to maternal-fetal blood group incompatibility in clinical practice

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being more frequently associated with the ABO system^[1-3]. Currently, clinical risk assessment of this disease primarily relies on factors such as parental blood types (to predict the newborn's possible blood type), prenatal antibody titers, and the mother's history of blood transfusions and pregnancies^[4-5]. However, the accuracy of single-factor assessments remains uncertain, and there is a lack of high-quality research investigating whether such assessments can effectively predict both the incidence and severity of the disease. This paper presents a cross-sectional retrospective cohort study that collects data on eligible cases of ABO maternal-fetal blood group incompatibility in a local region. It aims to provide a comprehensive analysis system that integrates routine laboratory tests with essential maternal information to predict the risk of HDN onset, including the incidence and severity of hemolysis.

MATERIALS AND METHODS

Source of cases

A total of 789 suspected cases of HDN were initially collected from local medical institutions between 2018 and 2021. Among these, 214 cases with complete information on prenatal prediction of maternal-fetal blood group incompatibility and postnatal neonatal hemolysis testing were selected for analysis. These cases were divided into OA and OB groups. The OA group (mothers with blood type O and newborns with type A) consisted of 113 cases. In this group, maternal age was 28.98 ± 3.42 years; 58 mothers had a history of pregnancy, while 55 did not; gestational age was 272.3 ± 7.7 days; mode of delivery included 43.86% vaginal deliveries and 56.14% cesarean sections; neonatal weight was 3354.61 ± 374.16 g. The OB group (mothers with blood type O and newborns with type B) comprised 101 cases, with a maternal age of 30.54 ± 3.94 years; 61 mothers had a history of pregnancy and 40 did not; gestational age was 272.4 ± 9.3 days; vaginal deliveries accounted for 39.60% and cesarean sections for 60.40%; neonatal weight was 3282.18 ± 437.77 g. No significant differences were observed between the two groups regarding maternal age, pregnancy history, gestational age, mode of delivery, or neonatal birth weight. Only 4 cases had a history of blood transfusion, all of which belonged to the OB group. This project was approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University (Approval No. 219, 2020).

Blood group typing

All blood groups were detected using microcolumn gel cards coated with monoclonal blood group antibodies (DG Diana, Barcelona, Spain), and the results were subsequently confirmed using either the microplate or tube method (Aikang Biotechnology Co., Ltd., Shenzhen, China).

Elution test

In this study, the heat elution method was performed. Packed RBCs were mixed with normal saline at a volume ratio of 1:1, followed by incubation in a 56 °C water bath with shaking for 5–8 minutes. After centrifugation, the resulting supernatant was collected for subsequent testing.

Antibody titer detection

The plasma sample was treated with an equal volume of 2-mercaptoethanol and incubated at 37 °C for 45–60 minutes. After serial dilution with normal saline, 40 µL of each diluted plasma was added to anti-human globulin gel microcolumn cards (DG Diana), followed by 10 µL of 2%–5% RBC suspension. Following incubation at 37 °C for 30–45 minutes, the cards were centrifuged and observed. The antibody titer was recorded as the highest dilution exhibiting agglutination.

Direct antiglobulin test (DAT)

The patients' RBCs were washed three times with physiological saline and then resuspended to prepare a 1% RBC suspension. Subsequently, 50 µL of this suspension was added to the wells of the anti-human globulin gel microcolumn cards (DG Diana). After centrifugation in a dedicated centrifuge, the results were observed.

Transcutaneous bilirubin measurement

The transcutaneous bilirubin meter probe was placed vertically, firmly, and in full contact with the skin at the selected site, avoiding areas with bruising, erythema, vernix caseosa, edema, or other skin abnormalities. Three measurements were taken at the center of the forehead and the center of the sternum, and the mean value was calculated as the final measurement result.

Statistical analysis

A positive result in the elution test served as the laboratory diagnostic criterion for ABO HDN^[6], and hemolysis severity was determined by transcutaneous bilirubin levels. All data were analyzed using SPSS

version 25.0 (IBM, USA). Between-group comparisons were performed using the chi-square test, and means were compared using independent samples *t*-test. Logistic regression was employed to analyze correlations and to develop a predictive model. Model reliability was assessed using receiver operating characteristic (ROC) curve analysis. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

The impact of blood type on the incidence and severity of HDN

Incidence

The positivity rate of the elution test was significantly higher in the OA group (86.73%, 98/113)

than in the OB group (53.47%, 54/101) ($P < 0.01$). The positive rate of the direct antiglobulin test (DAT) was 27.43% (31/113) in the OA group and 19.80% (20/101) in the OB group, with no statistically significant difference ($P > 0.05$).

Severity

The comparison of bilirubin levels between the OA and OB groups with positive elution tests showed that chest bilirubin levels within 24 hours were significantly higher in the OB group ($P < 0.05$). No other significant differences were observed at most other time points for either site. Among DAT-positive cases, both forehead and chest bilirubin levels within 24 hours were significantly higher in the OB group ($P < 0.05$), with no significant differences at other time points ([Table 1](#)).

Table 1 Comparison of transcutaneous bilirubin levels at the forehead and chest over time between OA and OB groups

	Testing time	Bilirubin value of the forehead ($\bar{x} \pm s$) ($\mu\text{mol/L}$)		<i>P</i> -value	Bilirubin value of the chest ($\bar{x} \pm s$) ($\mu\text{mol/L}$)		<i>P</i> -value
		OA	OB		OA	OB	
Positive cases of elution	$h \leq 24$	89.34 ± 32.11	101.29 ± 43.30	0.058	84.61 ± 30.76	96.69 ± 38.36	0.039*
	$24 < h \leq 48$	155.77 ± 36.34	155.46 ± 36.20	0.961	152.38 ± 31.76	151.67 ± 32.32	0.900
	$48 < h \leq 72$	192.91 ± 35.77	185.36 ± 39.44	0.322	193.10 ± 30.39	191.57 ± 33.32	0.814
	$h > 72$	215.48 ± 38.06	199.33 ± 35.10	0.091	206.41 ± 33.97	204.13 ± 29.74	0.787
Positive cases of DAT	$h \leq 24$	97.33 ± 29.16	122.50 ± 43.39	0.018*	92.30 ± 32.55	115.15 ± 30.41	0.016*
	$24 < h \leq 48$	163.63 ± 30.84	167.88 ± 28.77	0.644	161.37 ± 36.61	163.83 ± 30.68	0.816
	$48 < h \leq 72$	187.40 ± 31.95	197.13 ± 36.21	0.489	188.65 ± 36.92	197.25 ± 31.71	0.569
	$h > 72$	204.43 ± 35.74	201.43 ± 28.96	0.850	191.93 ± 33.59	206.43 ± 42.26	0.402

DAT: direct antiglobulin test; *: $P < 0.05$.

The impact of prenatal antibody titers on the incidence and severity of HDN

Incidence

The cases were categorized into three titer groups: 8–32, 64–256, and ≥ 512 . The elution test positivity rate was significantly lower in the 8–32 titer group compared with the higher titer groups, both in the combined analysis and in the separate analyses of the OA and OB groups ($P < 0.05$). There was no significant difference between the 64–256 and ≥ 512 titer groups. For DAT, the positivity rate increased significantly with higher titers in the combined analysis and in the OA group ($P < 0.05$). In the OB group, only the ≥ 512 titer group exhibited a significantly higher DAT positivity rate than the 8–32 titer group.

Severity

The results indicated that, in cases with a positive OA elution test, overall significant differences were observed among titer groups for chest transcutaneous bilirubin levels measured after 72 hours ($P = 0.032$), with the 8–32 titer group being significantly higher than the ≥ 512 group ($P = 0.032$). No significant

overall differences were observed across the remaining time periods and titer groups. However, pairwise comparisons revealed that within 24 hours, the forehead transcutaneous bilirubin in the 64–256 titer group was significantly higher than that in the 8–32 titer group at the same site ($P = 0.032$). The results for the OB group were more complex. No overall significant differences were observed among titer groups for chest transcutaneous bilirubin at 24–48 hours or forehead transcutaneous bilirubin at > 72 hours. However, for the remaining time periods, overall significant differences were found among titer groups for both forehead and chest bilirubin values. Pairwise comparisons showed that within 24 hours, bilirubin levels on the forehead and chest in the ≥ 512 titer group were significantly higher than those in the 64–256 titer group ($P = 0.010$ and $P = 0.024$, respectively). At 24–48 hours, forehead bilirubin in the ≥ 512 titer group was significantly higher than that in the 64–256 titer group ($P = 0.021$). At 48–72 hours, forehead bilirubin in the ≥ 512 titer group was significantly higher than that in the other two titer groups ($P = 0.011$ and $P = 0.014$, respectively), while

chest bilirubin in the 64–256 titer group was significantly lower than that in the other two titer groups ($P = 0.015$ and $P = 0.019$, respectively). At > 72 hours, chest bilirubin in the 8–32 titer group was significantly higher than that in the ≥ 512 titer group

($P = 0.009$). Although significant differences in bilirubin levels at the same site were observed among certain titer groups at specific time points, no clear pattern was found between titer and bilirubin levels ([Table 2](#)).

Table 2 Comparison of bilirubin levels at different time points among different titer groups in elution test-positive cases

Titer group	Bilirubin value of the forehead ($\bar{x} \pm s$) (μmol/L)				<i>P</i> -value	Bilirubin value of the chest ($\bar{x} \pm s$) (μmol/L)				<i>P</i> -value
	8–32	64–256	≥ 512			8–32	64–256	≥ 512		
OA	≤ 24 h	79.48 ± 32.83	96.46 ± 30.67	87.31 ± 35.46	0.144	80.28 ± 34.56	86.25 ± 25.85	87.91 ± 36.49	0.637	
	24–48 h	145.56 ± 35.69	161.46 ± 37.34	155.93 ± 35.99	0.233	144.76 ± 32.35	154.10 ± 28.82	158.41 ± 35.57	0.253	
	48–72 h	189.09 ± 31.78	200.27 ± 36.86	180.69 ± 36.96	0.104	194.09 ± 26.93	194.33 ± 30.19	192.35 ± 30.82	0.957	
	> 72 h	219.14 ± 43.65	213.42 ± 43.11	213.16 ± 40.72	0.825	218.50 ± 37.81	202.26 ± 32.40	195.92 ± 29.71	0.031	
OB	≤ 24 h	102.69 ± 29.65	93.07 ± 44.64	130.55 ± 32.65	< 0.01	98.23 ± 33.19	89.71 ± 43.79	117.36 ± 17.73	< 0.01	
	24–48 h	160.62 ± 31.73	148.37 ± 32.40	176.30 ± 29.44	< 0.01	159.62 ± 26.54	144.15 ± 34.40	161.20 ± 31.00	0.066	
	48–72 h	186.80 ± 33.92	174.90 ± 32.94	231.00 ± 21.58	< 0.01	206.70 ± 34.76	176.68 ± 27.40	211.17 ± 28.91	< 0.01	
	> 72 h	203.67 ± 9.95	200.94 ± 47.05	213.00 ± 35.34	0.307	216.00 ± 13.73	206.27 ± 39.32	197.00 ± 35.38	0.022	

The impact of pregnancy history on the incidence and severity of HDN

Incidence

No significant difference in elution test positivity was found between groups with and without a history of pregnancy either in the combined analysis or in the separate analyses of the OA and OB groups. For DAT, a significantly higher positive rate was observed in the OA group with a history of pregnancy

compared to those without such a history (37.93% vs. 18.18%, $P = 0.023$). No such difference was observed in the OB group.

Severity

In the OA group, bilirubin levels at both sites were significantly higher in the group without a history of pregnancy during the first 48 hours. In the OB group, no significant differences in bilirubin levels were observed between those with and without a history of pregnancy at any time point ([Table 3](#)).

Table 3 Comparison of bilirubin levels in cases with and without a pregnancy history

		Bilirubin value of the forehead ($\bar{x} \pm s$) ($\mu\text{mol/L}$)			Bilirubin value of the chest ($\bar{x} \pm s$) ($\mu\text{mol/L}$)		
		With a pregnancy history	Without a pregnancy history	<i>P</i> -value	With a pregnancy history	Without a pregnancy history	<i>P</i> -value
OA	≤ 24 h	82.44 $\pm$ 33.22	97.11 $\pm$ 29.46	0.026*	76.06 $\pm$ 33.46	94.91 $\pm$ 23.95	0.002**
	24–48 h	144.38 $\pm$ 35.16	169.18 $\pm$ 33.42	0.001*	144.50 $\pm$ 32.69	163.07 $\pm$ 27.86	0.004*
	48–72 h	188.53 $\pm$ 39.35	199.14 $\pm$ 30.31	0.229	188.30 $\pm$ 31.87	199.90 $\pm$ 28.04	0.122
	> 72 h	209.25 $\pm$ 33.37	226.38 $\pm$ 44.16	0.153	201.18 $\pm$ 30.81	215.56 $\pm$ 38.21	0.180
OB	≤ 24 h	95.44 $\pm$ 42.24	110.65 $\pm$ 44.42	0.221	92.38 $\pm$ 42.09	103.60 $\pm$ 31.25	0.309
	24–48 h	156.85 $\pm$ 36.39	152.76 $\pm$ 36.79	0.710	151.34 $\pm$ 34.88	152.29 $\pm$ 27.88	0.923
	48–72 h	189.56 $\pm$ 41.79	174.91 $\pm$ 33.20	0.312	191.46 $\pm$ 36.96	190.55 $\pm$ 26.21	0.942
	> 72 h	201.81 $\pm$ 40.08	194.38 $\pm$ 23.71	0.635	210.67 $\pm$ 28.58	191.88 $\pm$ 29.71	0.153

*: $P < 0.05$; **: $P < 0.01$.

Establishment of the predictive diagnostic model

Logistic regression analysis confirmed that blood type (odds ratio [OR] = 6.09, 95% confidence interval [CI], $P < 0.01$) and antibody titer (OR = 1.001, 95% CI, $P < 0.05$) were significant predictors of HDN incidence, whereas pregnancy history was not (OR = 0.633, 95% CI, $P > 0.05$). The derived prediction formula is as follows: $\text{logit}(P) = \ln[P/(1-P)] = 0.023 + 1.806 \times \text{blood type (1)} + 0.001 \times \text{titer} - 0.457 \times \text{pregnancy history (1)}$ (blood type A = 1, B =

0; pregnancy history = 1, no pregnancy history = 0).

The ROC curve for this model yielded an area under the curve (AUC) of 0.776, indicating moderate predictive accuracy for disease incidence ([Table 4](#), [Fig. 1](#)).

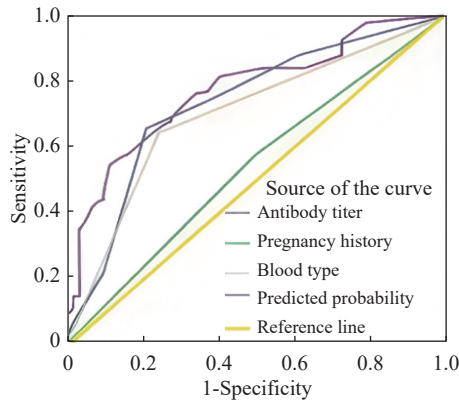
DISCUSSION

This study confirmed that newborn blood type is significantly associated with the incidence of ABO HDN, with the OA combination posing a higher risk

Table 4 Parameters of ROC curve

Variable	Area	Standard error ^a	Asymptotic significance ^b	Lower limit	Upper limit
Titer	0.735	0.039	0.000	0.659	0.811
Pregnancy history	0.539	0.044	0.365	0.454	0.625
Blood group	0.701	0.039	0.000	0.625	0.778
Predictive probability	0.776	0.033	0.000	0.710	0.841

a: under nonparametric assumptions; b: null hypothesis: true area = 0.5.

**Fig. 1** Receiver operating characteristic (ROC) curve

than OB. This is likely attributable to the heightened immunogenicity of the A antigen. However, blood type did not demonstrate a consistent correlation with the hemolysis severity, as measured by bilirubin levels. Similarly, a prenatal antibody titer of ≥ 64 was a significant threshold for increased disease incidence, but higher titers did not reliably predict more severe hemolysis. The dissociation between incidence and severity suggests that the destruction of sensitized RBCs is also influenced by other factors, such as phagocytic cell activity, IgG subclasses, Fc receptor polymorphisms, and complement activity^[7-13], which are not routinely assessed clinically.

Contrary to common expectation, a history of pregnancy did not significantly correlate with the overall incidence of ABO HDN in our cohort. One possible explanation is that the pregnancy process, in fact, increases opportunities for immune stimulation. In HDN related to blood types such as Rh, where antibodies are primarily immune-mediated, the relationship between pregnancy and the onset of hemolysis may be more pronounced. In contrast, antibodies in the ABO blood group system are mostly natural antibodies (including IgG anti-A and anti-B in individuals with type O blood). Therefore, the correlation between pregnancy history and the incidence of hemolysis induced by the ABO blood group system may be less significant. Interestingly, in the OA group, a history of pregnancy was associated with a higher DAT positivity rate; however, bilirubin levels during the first 48 hours were actually lower in

this group than in those without a pregnancy history. This paradoxical finding warrants further investigation and suggests that the role of pregnancy history in ABO HDN is complex and may be modulated by other biological factors.

The predictive model developed in this study, which incorporates blood type and antibody titer, provides a moderately accurate tool for evaluating the risk of ABO HDN incidence. However, accurately predicting the severity of hemolysis is challenging due to the multifactorial nature of RBC destruction. Owing to the limited number of cases with a history of blood transfusion, the study was unable to explore the correlation between transfusion history and the risk of neonatal hemolysis caused by ABO maternal-fetal blood group incompatibility. Future research should prioritize the integration of additional biomarkers to improve the prediction of disease incidence and severity.

Author contributions

Conceptualization, Hua Lu; Methodology, Hua Lu and Yi Zhu; Investigation, Hua Lu, Qi Gao, Luoquan Zhang, Chao Wan, and Yijun Hou; Software, Xiaomin Xu; Data Curation, Hua Lu, Xiaomin Xu, Jun Yang, and Feng Liu; Writing – Original Draft Preparation, Hua Lu; Writing – Review & Editing, Hua Lu; Project Administration, Hua Lu.

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University (Approval No. 219, 2020).

Informed consent statement

Since this study involved only the analysis of relevant test results from enrolled patients and did not involve any private patient information, the Institutional Ethics Committee approved a waiver of patient consent.

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Declaration of competing interest

The authors declared no conflict of interests.

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