

Original Article

Clinical study of the relationship between prenatal antibody titer and hemolytic disease of newborn

Ruiling Bao¹, Shan Wang², Qingfeng Yang¹

Departments of ¹Transfusion, ²Clinical, Xinxiang Central Hospital, Xinxiang 453000, Henan, China

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Abstract: Hemolytic disease of newborn (HDN) is caused by the emergence of inappropriate antibodies against fetal blood antigens, which includes ABO and Rh blood group incompatibility. This study investigated the association between prenatal blood group antibody titer and HDN to provide a theoretic basis for clinical practice. ABO and Rh blood group of 7894 pregnant women and their husbands were screened. Patients with blood group incompatibility were chosen for blood antibody test and antibody titer analysis. Umbilical cord blood was tested for HDN. Antibody titers were measured by ELISA and western blot. The association between prenatal blood antibody titer and HDN were analyzed. There were 4742 cases (60.1%) of ABO-incompatible couples, 2574 cases with anti-A/B antibody titer ≥ 64 (54.3%), including AB/O, A/O and B/O. Sixty cases showed fetal-maternal blood group incompatibility (ABO/HDN), suggesting a significant positive correlation between mother blood group antibodies and fetal cord blood bilirubin $> 80 \mu\text{mol/L}$. There were 60 Rh-incompatible couples (0.91%) including 8 cases who showed antibodies positive with increased anti-E and D titers. RH-HDN was confirmed by umbilical blood group test. Logistic regression analyses identified Rh blood group incompatibility (OR = 1.56), anti-E positive (OR = 2.15), anti-D positive (OR = 1.32), anti-A/B IgG titer ≥ 64 (OR = 1.63) as risk factors for HDN ($P < 0.05$). Prenatal blood group antibodies test can predict the relationship between blood group antibody titer and HDN by tracking anti-A/B antibody titers, and thereby prevent the occurrence of fetal RH-HDN.

Keywords: Hemolytic disease of newborn, serology test, ABO blood group system, Rh blood group system, blood group antibodies

Introduction

Hemolytic disease of newborn (HDN) is an auto-immune hemolytic disease caused by fetal-maternal blood group incompatibility which usually occurs in fetuses and newborns [1]. When a fetus or baby's blood group antigen inherited from his father is incompatible with his mother, in other words, the mother lacks such RBC blood group antigen, the fetus or infant's blood group antigen is alloantigen to the mother and flows through the placenta into the mother's body, leading to the production of antibodies by her immune system [2, 3]. When this antibody flows through the placenta into the fetus or newborn, it will bind to the antigen on the red blood cells, resulting in HDN [4, 5]. Clinically, most cases of HDN occur when the mother is Rh-negative O blood type, and the fetus or infant is Rh-negative A or B blood type [6].

HDN is primarily caused by fetal-maternal ABO blood group incompatibility, accounting for 86% of all HDN worldwide [7-9]. In contrast, HDN due to Rh blood group incompatibility accounts for approximately 15% of HDN [10]. Examination of prenatal blood group antibodies is an important clinical method for the prevention of deformed or abnormal birth [11-13]. Currently, the screening of HDN is mainly performed by checking the ABO and Rh blood group antibodies of the couples to detect the incompatibility of their blood type [14]. Also, the presence of Rh-negative antibodies in pregnant women will be examined to detect fetal-maternal blood incompatibility and thereby prevent the occurrence of HDN [15, 16]. A correlation between maternal IgG anti-A or anti-B antibodies and ABO blood group incompatibility in HDN newborns has been suggested [17-19], despite that there is no evidence for a direct link between them.

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Table 1. Parents' ABO blood type, maternal serum anti-A (B) IgG titer, and HDN

Parents' blood type	Mother/infant blood type	Antibody titer ≥ 64	Confirmed HDN	Positive rate (%)
O/A	O/A	468	376	80.5
O/B	O/B	436	306	70.4
O/AB	O/A(B)	106	72	80.0
B/A	B/A	14	8	57.4
A/B	A/B	10	6	60.0
Total		1034	768	74.3

In this study, serum ABO and Rh blood group antibody were both tested to check the fetal-maternal blood compatibility and the possible correlation between HDN and blood group antibody screening was investigated. Furthermore, the risk factors for HDN were analyzed using a logistic regression model. The current study will provide a theoretical basis for the detection, prevention, and treatment of HDN in clinical practice.

Materials and methods

Subjects

This study included a total of 7894 pregnant women who underwent prenatal checkup in the Department of Obstetrics and Gynecology at the Xinxiang Central Hospital from July 2010 to July 2015. The patients (aged 22-40 years old) had no cardiovascular or infectious diseases. The gestational age ranged between 18 and 40 weeks. The study protocol was approved by the Research Ethics Committee of Xinxiang Central Hospital, and all patients signed their informed consent before the commencement of the study.

Reagents and equipments

Sodium citrate was purchased from sigma. Anti-ABO IgG and anti-Rh antibody were purchased from Santa Cruz Biotech. (Santa Cruz, CA, USA). Blood test was performed using the Beckman Coulter LH 750/LH 755 automated blood analyzer. Blood antibody screening was performed by the Shanghai Blood Biomedical Co. Umbilical blood bilirubin levels were analyzed using an Olympus AU600 automatic biochemical analyzer. Microcolumn gel and equipment were purchased from Dexia Bioinstrumentation Co.

Collection of blood samples

Blood (5 ml) was drawn from each couple and mixed with the anticoagulant, sodium citrate. The blood sample was divided into two equal portions. One was used for the detection of blood group antibodies, the other for blood test [20].

Serum antibody detection

Microcolumn gel technology was used for the detection of serum titer of Rh antibody and ABO antibody [21]. For those couples with Rh blood incompatibility (Rh-positive/Rh-negative) and ABO blood incompatibility (B/A, A/B, B/O, A/O, and AB/O), serum ABO antibody titer and Rh antibody titer from pregnant women must be analyzed [22]. The result will be designated positive if Rh blood incompatibility or anti-A/B titer ≥ 64 .

Detection of serum antibodies in newborns with HDN

Detection of serum antibodies for newborns with HDN was performed according to the conventional method [23, 24]. Umbilical blood was collected, and three serological parameters of HDN were analyzed using a commercial kit.

Determination of indirect bilirubin

Determination of indirect bilirubin was performed as previously described [25]. Serum samples were collected. Sufficient amount of heme oxygenase were added, mixed, and kept at room temperature for 30 min to convert the bilirubin to biliverdin. The absorption values were measured before and after the reaction using a spectrometer at the wavelength of 490 nm.

Serological test

Serological test of newborns was performed using a conventional method [26], and HDN was diagnosed based on serum antibody dilution, anti-human globulin test, and free antibody determination [27].

Data analysis

Data were expressed as mean \pm standard error and analyzed by SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Pairwise comparison between two different groups was compared by ANOVA. Correlation analysis was performed by Spearman test [28]. The risk factors for HDN were ana-

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Table 2. Serum anti-A (B) IgG titers of ABO blood incompatible couples

Mother/father blood type	Cases	0-32	64-128	256-512	1024	Antibody titer (80%)
O/A	1410	322	864	210	14	42.2
O/B	1330	288	840	192	10	40.5
O/AB	458	78	282	90	8	14.8
B/A	622	588	30	4	0	1.3
A/B	592	562	28	2	0	1.2
A/AB	158	158	0	0	0	0.0
B/AB	172	172	0	0	0	0.0
Total	4742	2168	2044	498	32	100.0

Table 3. Newborn umbilical blood indirect bilirubin levels and maternal serum anti-A (B) IgG titer

Titer	Bilirubin (mmol/L)	Total				
	15	15-30	30-60	60-100	100	
64	36	90	118	64	18	326
128	10	68	60	40	16	194
256	4	10	30	24	18	86
512	2	4	16	26	30	79
Total	52	172	224	154	82	684

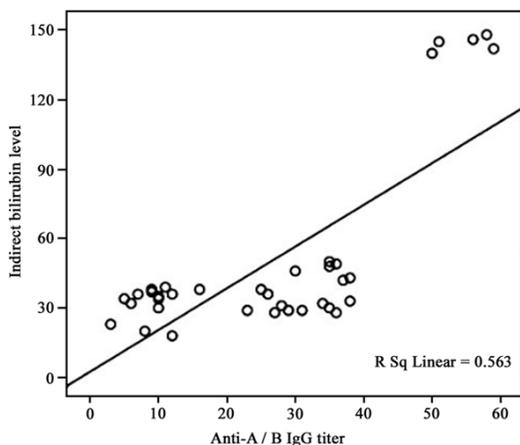


Figure 1. Correlation between maternal anti-A/B IgG titer and newborn's indirect bilirubin levels.

lyzed using a logistic regression model. $P < 0.05$ was considered statistically significant.

Results

ABO blood type, anti-A/B IgG titer in pregnant women, and HDN

In this study, a total of 1034 pregnant women who delivered in the Departments of Obstetrics

and Gynecology at our hospital showed ABO blood incompatibility with anti-A/B IgG antibody titer greater than 64 (**Table 1**). Further studies detected fetal-maternal blood incompatibility which indicated the occurrence of HDN in 768 cases (74.3%) (**Table 1**).

Anti-A/B IgG titer in ABO incompatible pregnant women

Among the 7894 pregnant women selected in this study, 4742 (60.1%) pregnant women showed ABO blood incompatibility. A total of 2574 cases with antibody titer greater than 64 (54.3%), including 30 case (1.4%) of B/A (husband/wife), 34 cases (1.3%) of A/B, 1088 cases (42.2%) of A/O, 1042 cases (40.5%) of B/O, and 380 cases (14.8%) of AB/O (**Table 2**).

Umbilical blood indirect bilirubin of newborns with HDN and anti-A/B IgG titer of pregnant women

To investigate the possible correlation between maternal serum antibody titers and HDN, umbilical blood indirect bilirubin of newborns with HDN was measured (**Table 3**). Further Spearman correlation test suggested a positive correlation between prenatal serum antibody titer and neonatal umbilical blood indirect bilirubin level (correlation coefficient = 0.563, **Figure 1**), suggesting that the occurrence of HDN was positively correlated with maternal serum antibody titer.

Maternal anti-A/B IgG titer, couple's Rh blood type, and HDN

The number of cases of Rh blood incompatibility, positive maternal anti-A/B IgG titer and HDN was summarized (**Table 4**). Out of the 7894 couples, there were 60 cases of Rh blood incompatibility (0.91%). A total of 8 cases (13.8%) were antibody positive, of which 2 (0.03%) showed anti-E positive with antibody titer of 32, and 6 (0.09%) were anti-D positive with antibody titer above 256.

Risk factors for HDN

The risk factors for HDN were analyzed by a logistic regression model, in which hemolytic

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Table 4. Maternal serum anti-A/B IgG titer, Rh blood type, and HDN

Cases	Rh blood incompatibility (n/%)	Antibody positive (n/%)	Anti-E positive (n/%)	Anti-D positive (n/%)
7894	60/0.91%	8/13.8%	2/0.03%	6/0.09%

Table 5. Logistic regression analyses of risk factors for HDN

Variable	B value	S.E.	χ^2 value	P value	OR value	95% CI
ABO type	0.563	0.471	1.479	0.056	1.24	0.78~1.71
Rh type	0.748	0.613	4.526	0.014	1.56	1.02~1.84
Anti-E antibody	0.526	0.404	10.328	< 0.001	2.15	1.66~3.31
Anti-D antibody	0.947	0.823	9.238	< 0.001	1.32	1.12~1.83
Anti-A/B IgG titer	0.604	0.528	5.542	0.004	1.63	1.50~2.62

was defined as the dependent variable (healthy = 0, diseased = 1), and ABO blood group (compatible = 0, incompatible = 1), Rh blood group (compatible = 0, incompatible = 1), anti-E antibody (negative = 0, positive = 1), anti-D antibody (negative = 0, positive = 1), anti-A/B IgG antibody titer (< 64 = 0, \geq 64 = 1) were designated as independent variables. Based on the multiple regression model ($F = 18.175$, $P < 0.001$), Rh blood group incompatibility (OR = 1.56, $P < 0.05$), anti-E positive (OR = 2.15, $P < 0.001$), anti-D positive (OR = 1.32, $P < 0.001$), anti-A/B IgG titer \geq 64 (OR = 1.63, $P < 0.05$) were identified as risk factors for HDN (Table 5).

Discussion

In this study, the association between prenatal blood group antibody titer and HDN was investigated. It was found that 4742 out of 7894 (60.1%) pregnant women were ABO-incompatible. Among these, 54.3% (2574/4742) had an anti-A/B IgG titer above 64. A total of 1034 pregnant women showed ABO blood incompatible and anti-A/B IgG titer greater than 80. Further post-natal test detected 60 cases of fetal-maternal blood group incompatibility (ABO/HDN), which is consistent with a previous study in which the proportion of fetal-maternal blood group incompatibility and Rh-negative is quite low [3, 4]. Moreover, the umbilical indirect bilirubin level was found to be positively correlated with mother's blood group antibody titer. Among the 7894 pregnant women, a total of 60 cases (0.91%) of Rh incompatible couple were

found, including 8 cases of positive blood group antibodies with increased anti-E and D titer which were confirmed as RH-HDN by blood test. Further logistic regression analyses identified Rh blood group incompatibility, anti-E positive, anti-D positive, and anti-A/B IgG titer \geq 64 as risk factors for HDN. These results have suggested that the screening of prenatal maternal blood group antibodies can be used to predict the risk for fetal RH-HDN and thereby to prevent the occurrence of the disease.

Serological detection of antibody titers can only measure IgM-type irregular antibody and ABO blood type match, whereas polybrene and micro-column gel can be used to detect IgG-type irregular antibody titer. The simple and convenient micro-column gel method has become a part of the conventional serological crossmatch procedure in some hospitals in Europe, America and China [10]. Nevertheless, several technical pitfalls currently remain in the serological tests of antibody titer, such as too many test items, time-consuming operation, experimental errors due to human factors, etc.

There are several drawbacks in the current study. For instance, the number of subjects is small. Future studies on a larger sample size shall be performed to validate our findings. Moreover, the blood group antibody screening is laborious and might not be available in some clinical laboratories, which might hinder its wide application as a method to detect the occurrence of HDN.

In conclusion, our study has suggested that prenatal maternal blood group antibodies screening can predict the relationship between blood group antibody titer and HDN by tracking anti-A/B antibody titers, and thereby prevent the occurrence of fetal RH-HDN.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shan Wang, Department of Clinical, Xinxiang Central Hospital, Xinxiang 453000, Henan, China. Tel: +86-0373-

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2034452; Fax: +86-0373-2034452; E-mail: shanwangd@sina.com

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