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Effects of maternal serum IgG anti-A (B) and neonatal direct anti globulin red blood cell antibody expression on the occurrence and development of neonatal ABO haemolysis.

Ming Chen*, Yu Zeng, Xiaohe Chen

Department of Blood Transfusion, the People's Hospital of Ruian, Ruian, China

Abstract

Objectives: To explore the expression of maternal serum IgG anti-A (B) and neonatal direct anti globulin, free antibody and Red Blood Cell (RBC) antibody on the occurrence and development of ABO Haemolytic Disease of the New-born (ABO-HDN) and provide a valuable reference for the early diagnosis of the disease.

Methods: we selected 546 cases of jaundice and hyperbilirubinemia neonates in our hospital from February 2013 to February 2016, tested the maternal serum IgG anti-A (B) titer, neonatal direct anti globulin, free antibody and RBC antibody, calculated the prevalence ratio of ABO haemolytic disease and analysed the effects of these indexes in the occurrence and development of ABO-HDN.

Results: Among 546 cases, 227 cases were diagnosed as ABO haemolytic disease, the prevalence ratio being 41.6%. The prevalence rate of ABO haemolytic disease of group A and group B were not statistically significant (P>0.05). Pearson correlation analysis showed that there was a positive correlation of pregnant women's serum IgG anti-A (B) titer and neonatal ABO haemolytic rate (r=0.883, P<0.01). The serum IgG anti-A (B) titer level of pregnant women was not significantly correlated with the severity of the disease. The difference was not statistically significant (P>0.05). Among all the 227 patients with ABO haemolytic disease, the positive rate of RBC antibody testing was 100.0%, which is higher than that of the free antibody testing 74.9%. The difference was statistically significant (P<0.05) and the positive rate of free antibody testing was also higher than that of direct anti globulin testing 14.9%. The difference was statistically significant (P<0.05).

Conclusion: The combined detection of pregnant women's serum IgG anti-A (B) and neonatal erythrocyte antibody expression is expected to provide evidence for the clinical early diagnosis of ABO-HDN. At the same time, with the increase of the titer of pregnant women's serum IgG anti-A (B), risks of ABO-HDN will gradually increase, which should be paid more attention.

Keywords: IgG anti-A (B), Direct anti globulin, Free antibody, Red blood cell antibody, ABO haemolytic disease of the new born.

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Introduction

The ABO haemolytic disease is a common Haemolytic Disease of the New-born (HDN), which is caused by the blood group incompatibility of mother and foetus. The clinical manifestations usually include jaundice, haemolytic, anaemia, bilirubin encephalopathy, some even have occurrence of oedema, heaptosplenomegaly, ascites and pleural effusion with a high rates of disability and death [1]. Currently, it still lacks definite standards for the prenatal diagnosis and intervention of ABO-HDN. Some scholars believed that it had some effects on the prenatal diagnosis of HDN to test the maternal serum IgG anti-A (B) titer, but other researchers pointed that IgG antibody titer change only had limited directive effects on the clinical preventive treatment [2]. We tried to combine neonatal direct anti globulin, free antibody and RBC antibody to the laboratory testing of ABO-HDN on the basis of the maternal serum IgG anti-A (B) titer testing and some references [3], and achieved satisfactory results.

Materials and Methods

Criteria for selection and exclusion

All the samples were selected from the ABO haemolytic disease neonates in our hospital from February 2013 to February 2016. A sample of infants who have symptoms of jaundice and hyperbilirubinemia and who were single foetuses with no more than 7 days after born and whose maternal dipotassium ethylene diaminetetra-acetate (K_2EDTA) blood samples were well preserved and whose mothers were of blood group O and their spouses non-O blood type were selected for

enrolment in the study. Neonates who were born ≤ 37 weeks gestation to mothers or their blood specimens were ≥ 48 h for testing or their mother was irregular antibody-positive or had transfusion records or liver and kidney dysfunction, blood or immune system diseases were excluded. At last, 546 babies met the criteria were enrolled in the study.

Testing

Diagnosis of ABO haemolytic disease: ABO haemolytic disease was diagnosed as described in literatures such as: hyperbilirubinemia [4], maternal-foetal ABO/Rh incompatibility with direct anti globulin test positive or antibody released test positive.

Maternal serum IgG anti-A (B) titer: We treated 200 μl maternal serum by adding 200 μl 2-mercaptoethanol and put the mixture at 37˚C water bath for 30 min. The serum after treatment were added to each tube by the double-dilution method with a titer of 1:16, 1:32, 1:64, 1:128, 1:512, 1:1024, 1:2048 respectively. The samples within a titer of 1:64 and 1:2048 were placed in labelled anti-human globulin cards, added 1% erythrocyte suspension of the pregnant women's spouses or of the same blood group to them, then incubated for 15min at 37˚C environment, centrifuged 5 min for observation. The results are positive when the red cell clots were located within the gel or on the gel surface and negative when they were at the bottom of the gel; the reference value for positive is ≥ 1:64 [5].

Direct anti globulin: We took 3 ml neonatal anticoagulant, separated the plasma, diluted with saline and formulated 1% erythrocyte suspension. 50 μl suspension was placed in the HDN test card, centrifuged 2 min at 900 r/min and then 3 min 1500 r/min. When there was visible agglutination, the result was positive and negative on the contrary [6,7].

Free antibody: 300 μl neonatal anticoagulant was taken and put in three tubes by adding group-A, group-B, group-O reagents respectively and 50 μl RBC in each tube, then incubated for 30 min at 37˚C environment washed with saline and got the supernatant. 100 μl anti-human globulin reagent was added to each tube and centrifuged the mixture at 3400 r/min for 15 sec, the result was observed by the criteria for direct anti globulin.

RBC antibody: We prepared 150 μl neonatal serum and 150 μl RBC dispersion solution, placed them in 3 tubes respectively, 1% group-A erythrocyte suspension was added to No. 1 and No.4 tubes, while 1% group-B erythrocyte suspension was added to No. 2 and No. 5 tubes and 1% group-O erythrocyte suspension was added to No. 3 and No. 6 tubes, and put the mixture at 37˚C water bath for 15 min, centrifuged and observed the result with the same criteria as direct anti globulin.

Statistical analysis

Statistical analysis was conducted by SPSS18.0. For count data, we used n% to express and category variables were compared using χ^2 analysis. Measurement data was expressed by x ± s. Continuous variables with a normal distribution were compared by t-test, in others M(Q1, Q3) were used and compared using Wilconx. Significance was defined as a p value<0.05.

Results

Diagnosis

Among 546 cases, 227 cases were diagnosed as ABO haemolytic disease, the prevalence ratio was 41.6%. The prevalence ratio of ABO haemolytic disease of A blood type and B blood type were not statistically significant (P>0.05, Table 1).

Table 1. *Prevalence ratio of ABO haemolytic disease of blood group A and B.*

Blood group ratio (%)	Cases	ABO haemolytic disease (n)	Prevalence
Group A	231	92	39.8
Group B	315	135	42.9
Total	546	227	41.6

Maternal serum IgG anti-A (B) titer testing results

With the increase of maternal serum IgG anti-A (B) titer, the ratio of ABO haemolytic disease neonates gradually escalated. Pearson correlation analysis showed that there was a positive correlation between maternal serum IgG anti-A (B) titer and neonatal ABO haemolytic incidence (r=0.883, P<0.01) (Table 2).

Maternal serum IgG anti-A (B) titer and severity of the disease

The maternal serum titer of IgG anti-A (B) were not significantly correlated with the severity of the disease, the difference was not statistically significant (P>0.05, Table 3).

Table 2. *Comparison of maternal serum IgG anti-A (B) titer and neonatal ABO haemolytic incidence.*

Titer	Pregnant women		ABO haemolytic disease new-borns	
	Cases (n)	Ratio (%)	Cases (n)	Ratio (%)
1:16	0	0.00	0	0.0
1:32	83	15.2	1	1.2
1:64	97	17.8	4	4.1
1:128	115	21.1	33	28.7
1:256	91	16.7	47	51.7
1:512	74	13.6	63	85.1
1:1024	49	8.9	44	89.8

1:2048	37	6.8	35	94.6
Total	546	100	227	41.6

Table 3. *Correlation of maternal serum titer of IgG anti-A (B) and severity of the disease.*

Phase	Cases	Mild	Moderate	Severe
<1:64	1	1 (100.0)	0	0
1:64	4	2 (50.0)	2 (50.0)	0
1:128	33	17 (51.5)	16 (48.5)	0
>1:128	189	95 (50.3)	94 (49.7)	0
Total	227	115 (50.7)	112 (49.3)	0

Notes: Compared with pre-operation, *P<0.05; compared with control group, $^{\#}$P<0.05

Testing results of ABO haemolytic disease new-borns

Among all the 227 new-borns with ABO haemolytic disease, the positive rate of RBC antibody testing was 100.0%, which is higher than that of the free antibody testing 74.9%. The difference was statistically significant (P<0.05) and the positive rate of free antibody testing was also higher than that of direct anti globulin testing 14.9%. The difference was statistically significant (P<0.05, Table 4).

Table 4. *Testing results of ABO haemolytic disease new-born's.*

Blood group (mother-neonate)	Cases	Direct anti globulin		Free antibody		Red cell antibody	
		Positive (n)	Positive rate (%)	Positive (n)	Positive rate (%)	Positive (n)	Positive rate (%)
O-A	92	15	16.3$^{*\#}$	67	72.8	92	100
O-B	135	19	14.1$^{*\#}$	103	76.3$^{\#}$	135	100
Total	227	34	14.9$^{*\#}$	170	74.9$^{\#}$	227	100

Notes: Compare with free antibody, *P<0.05; Compared with red cell antibody,$^{\#}$P<0.05

Discussion

The ABO-HDN is an immune haemolytic disease of new-borns caused by ABO blood group antibodies, especially among blood group A or B new-borns born to blood group O mothers. As one of the commonest haemolytic diseases of new-borns in China, the process of ABO haemolysis is comparatively mild; some severe neonates may have mild or moderate degrees of haemolysis [8]. Severe cases like oedema, kernicterus or dead foetus are not usual. However, early diagnose and treatment can also prevent permanent neurological developmental disorders or even death [9].

The main pathogenesis for ABO-HDN is the blood group incompatibility of mother and foetus. Foetal red blood cells enter maternal body through the placenta causing blood group antigen stimulation and then inducing passive sensitization and antigen clearance. After foetal red cells were coated by maternal IgG blood group antibodies, a series of antigen-antibody reaction gradually induced clinical symptoms [10]. Maternal serum IgG anti-A (B) titer was regarded as one of the important indicators for early prediction of ABO haemolytic disease of the new born and cited as one of prenatal diagnostic items. Our research results showed that with the increase of maternal serum IgG anti-A (B) titer, the ratio of ABO haemolytic disease neonates gradually escalated, which was also proved by the correlation analysis. This indicated that early monitoring of serum IgG anti-A (B) titers has a positive significance for the prediction of neonatal ABO haemolytic disease. Nevertheless, it was pointed out by Arora et al. [11] that red blood cell surface antigen, IgG antibody typing, placenta density and other factors can also lead to changes in titer, and it is complex and costs high to test IgG antibody subtypes in clinic [11], the reference value is limited by simply applying total IgG titer to predict ABO haemolytic disease of the new born.

In order to further promote predictive diagnosis of ABO-HDN, we conducted this research by selecting direct anti globulin, free antibody and RBC antibody and the result demonstrated that RBC antibody was the most sensitive to ABO haemolytic diagnosis with a positive rate of 100.0%, the next one was free antibody, the positive rate was 74.9% and the positive rate of direct anti globulin was only 14.9% [12]. The first main reason may be that most neonates have a low density of anti-A (B) antigen and insufficient number of antibodies led to the weak positive or negative testing result of direct anti globulin [13]. Secondly, free antibody has a positive significance for early detection of neonatal ABO haemolysis because it can reflect whether neonatal serum exist other antibodies or IgG anti A (B) which do not match with red blood cells besides ABO [14]. Thirdly, RBC antibody testing is released by heating the sensitized red blood cells, which can directly detect antibodies who do not match with neonatal serum. So it is recommended that we can apply the combination of maternal serum IgG anti-A (B) titer and RBC antibody testing to clinical practice to improve the efficiency of neonatal ABO haemolytic diagnosis.

Our study also showed that there was no evident correlation between maternal serum IgG anti-A (B) titer and severity of the disease. This was in accordance to the research of Tufekci et al. [15]. Pregnancy IgG titer level cannot serve as the standard for judging the prognosis of foetus [16]. In order to make an early diagnosis of ABO-HDN, we should also pay

attention to the regular inspection of blood routine, blood group, specific antibody and blood coagulation mechanism on the basis of IgG titer and RBC antibody testing.

In conclusion, it has some significance to test maternal serum IgG anti-A (B), neonatal direct anti-globulin, free antibody and RBC antibody for early diagnosis of neonatal ABO haemolytic diagnosis. Maternal serum IgG anti-A (B) and RBC antibody has high reference values. For pregnant women and new-borns that have abnormalities in these two indicators, we should have an early confirmed diagnosis and take actively preventive and treatment measures to reduce the risk of neonatal ABO haemolytic disease and improve population quality.

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*Correspondence to

Ming Chen

Department of Blood Transfusion

The People's Hospital of Ruian

China