

Prediction of ABO hemolytic disease of the newborn using prenatal quantification of maternal vascular endothelial cadherin and anti-A/anti-B IgG titer in China

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Research Article

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Abstract

Objective To study the value of maternal vascular endothelial cadherin (VE-cadherin) level and anti-A/B IgG titer in the prenatal diagnosis of ABO hemolytic disease.

Methods We conducted a case-control study of blood group O Rh(D) positive mothers according to the occurrence of ABO-HDFN in newborns. Then the level of maternal VE-cadherin and anti-A /B IgG titer of the two groups were compared and analyzed, and their correlation with total bilirubin in children, and their value for the prenatal diagnosis of ABO-HDFN was analyzed.

Results The VE-cadherin level and anti-A/B IgG titer of pregnant women in the ABO-HDFN group were higher than those of the control group ($P < 0.001$). There was a positive correlation between VE-cadherin level and total bilirubin ($r = 0.3520$, $P < 0.05$) in the ABO-HDFN group, but no significant correlation between IgG titer and TBIL ($r = 0.1289$, $P = 0.4605$). The AUC of the VE-cadherin level was 0.762, the sensitivity and the specificity was 80.95%, 63.16%; the AUC, sensitivity and specificity of IgG titer were 0.862, 88.10% and 71.05%, respectively. The AUC, sensitivity and specificity of the combined diagnosis were 0.915, 92.86% and 84.21%, respectively.

Conclusion The level of maternal VE-cadherin and anti-A/B IgG titer have important reference value for the prenatal diagnosis of ABO-HDFN, and they have good joint diagnostic efficacy.

Introduction

Hemolytic disease of the fetus and newborn (HDFN) generally refers to alloimmune hemolytic disease of the fetus or newborn caused by maternal blood group incompatibility. Clinical manifestations include fetal edema, early abortion or stillbirth, neonatal jaundice, anemia, hepatosplenomegaly, nuclear jaundice, or even death[1–2]. Due to the marked decline of maternal-fetal rhesus incompatibility, ABO alloimmunization is now the most prevalent cause of HDFN, which has been reported from time to time in severe cases[3–4].

Maternal blood group alloantibodies of the immunoglobulin- γ (IgG) class, can be transferred across the placenta and destroy fetal cells via the phagocytosis system of the fetus and newborn causing HDFN[5–6]. Therefore, it is necessary to regularly monitor maternal IgG anti-A /B antibody level before delivery. However, there are currently different opinions on the correlation between the severity of neonatal hemolysis and the titer of serum antibodies in pregnant women[7].

The ABO antigens are expressed on the surfaces of red cells but also many other tissues, especially the vascular endothelium[8–9]. Previous studies have confirmed that vascular endothelium was damaged in neonates with ABO-HDFN by measuring vascular Endothelial Microparticles (EMPs), and this injury is related with the occurrence and severity of haemolytic disease[10–11]. Vascular endothelial cadherin(VE-cadherin) is a membrane protein that is the major component of endothelial cell-to-cell adherens junctions[12], which has a key role in the maintenance of vascular integrity, endothelial permeability and

angiogenesis[13]. Therefore, the author wondered whether the concentration of markers of vascular endothelial injury in maternal peripheral blood (such as VE-cadherin) would be affected through placenta during intrauterine onset.

In this study, correlation analysis was conducted on the occurrence and development of ABO-HDFN by detecting maternal peripheral blood VE-cadherin level and IgG anti-A /B titer in late pregnancy, in order to provide theoretical basis for prenatal diagnosis and early prevention and treatment of ABO-HDFN.

Materials And Methods

Participants and blood samples

This study was carried on 80 cases of Rh (D) positive pregnant women who were examined in *Huangdao district people's Hospital in Qingdao*. All of them were negative in irregular antibody screening, and their blood types were different from their husbands. There were 36 pregnant women with A-O blood group, 41 with B-O blood group and 3 with AB-O blood group, aged from 20 to 45 (30.44 ± 5.30) years.

The studied pregnant women were divided into two groups: Controls (n = 38), the healthy controls; ABO-HDFN group (n = 42), whose baby were positive in release test were identified as hemolytic disease, regardless of the results of direct anti-hemolysis test and free-blood test, the pregnant women were assigned to the ABO-HDFN group.

Exclusion criteria: (1) neonatal hemolysis caused by other blood group systems and other causes (thalassemia, hereditary spherocytosis, deficiency of glucose-6-phosphate dehydrogenase, etc.) ; (2) Co-infection: clinically diagnosed as infection (including sepsis, pneumonia, etc.) or CRP > 10mg/L; (3) neonatal hypoxia: fetal distress in utero, perinatal asphyxia; (4) large head hematoma or severe contusion; (5) maternal smoking or chronic hypertension, diabetes, systemic lupus erythematosus or other autoimmune diseases.

Peripheral venous blood or umbilical cord blood were collected into ethylene diamine tetra-acetic acid (EDTA) (1.2 mg/mL) for haematological profiling. For the irregular antibody screening, direct anti-human globulin test, free antibody test, and antibody release test, chemical analysis and C-reactive protein assays (CRP), clotted samples were obtained and serum was separated by centrifugation for 15 minutes at $1,000 \times g$.

Measurements

100 μ L of maternal plasma was mixed with the same amount of β -mercaptoethanol and incubated at 37°C for 10min. After dilution with normal saline, Red blood cells with ABO reagent of the same blood group as her husband were added. After incubation at 37°C for 30 min, IgG antibody titer of corresponding blood group was determined [14].

The maternal plasma obtained by centrifugation of EDTA anticoagulant specimens was used for enzyme-linked immunosorbent assay, and the OD value was measured at 450nm wavelength with a microplate reader. All operations were performed in accordance with the reagent operating instructions.

Ethical approval

This study was reviewed and approved by the ethics Committee of our hospital. All subjects obtained the consent of pregnant women and their families and voluntarily signed informed consent.

Statistical analysis

The measurement data were tested for normality by KS (D) test, and the indicators of normal distribution were expressed as mean± standard deviation (Mean±SD). Inter-group comparisons were analyzed by independent sample T test, and inter-group comparisons were analyzed by One Way ANOVA method. Indicators with non-normal distribution were represented by median and quartile spacing (IQR), mann-Whitney U test was used for inter-group comparison, and Kruskal-Wallis H test was used for inter-group comparison. Statistical data were expressed as percentage (%), and comparisons between groups were performed by Chi-square test or Fisher's exact probability method. Spearman correlation analysis was used to analyze the correlation between the two factors. Diagnostic efficiency was evaluated by ROC curve. P (probability) <0.05 was accepted as a significant difference in the analyses.

Results

Clinical data of the pregnant women and newborns are shown in Table 1. There was no statistically significant difference between the ABO-HDFN group and the control group in terms of age, gravidity, delivery, birth weight and gender of newborns (P>0.05).

Table 1 Comparison of clinical data between pregnant women and neonates

Variables	ABO-HDFN	Controls	χ^2 /Fisher/t	P value
	n=42%	n=38%		
Maternal age(years)			1.280	0.527
20~29	17(40.48%)	20(52.63%)		
30~39	23(54.76%)	17(44.74%)		
≥40	2(4.76%)	1(2.63%)		
Gravidity			0.782	0.434
≤1	11(26.19%)	13(34.21%)		
≥1	31(73.81%)	25(65.79%)		
Delivery			0.661	0.437
Cesarean	19(45.24%)	20(52.63%)		
Normal	23(54.76%)	18(47.37%)		
Gestational age(weeks)			1.222	0.221
<38	3(7.14%)	6(15.79%)		
≥38	39(92.86%)	32(84.21%)		
Sex of newborn			0.169	0.866
Male	18(42.86%)	17(44.74%)		
Female	24(57.14%)	21(55.26%)		
Neonatal blood type			0.258	0.796
A	20(47.62%)	17(44.74%)		
B	22(52.38%)	21(55.26%)		
Birth weight(g)	3567±450.09	3571.05±418.11	0.045	0.964

Compared with the control group, the VE-Cad of pregnant women was significantly higher in the ABO-HDFN group (2.25 (1.15-5.12) vs. 0.65 (0.21-1.62)) ng/mL, $P < 0.001$), as shown in Figure 1 .

Table2 shows a significantly higher maternal IgG antibody titer in the the ABO-HDFN group than in the control group. There were 41 cases(97.62%) had IgG anti-A/B titer $\geq 1:64$ in the ABO-HDFN group; only 17 cases(44.74%) in the control group $\geq 1:64$.

Table 2 Distribution of antibody titers in pregnant women and the number of children

	≤1:32	1:64	1:128	1:256	≥1:512	Chi-square	P value
ABO-HDFN	1	4	6	14	17	41.25	<0.0001
Control	21	9	4	2	2		

Futhermore, a significant positive correlation was found between VE-Cad level and neonatal total bilirubin ($r=0.3520$, $P<0.05$), but there was no significant correlation between IgG antibody titer and total bilirubin in newborns ($r=0.1289$, $P=0.4605$), as shown in Figure 2.

The ROC curve analysis of the diagnostic value of VE-Cad level and IgG antibody titer showed that the area under the curve (AUC) of VE-Cad level was 0.762, the cut-off value (cut-off) was 1.008ng/mL, the sensitivity was 80.95%, and the specificity was 63.16%; the AUC of the IgG antibody titer was 0.862, the cut-off value was 1:64, and the sensitivity was 88.10% , the specificity was 71.05%; the AUC of the two combined diagnosis was 0.915, the sensitivity and the specificity was 92.86% and 84.21%,respectively(Fig.3,Table3).

Table 3 AUC of VE-Cad level, IgG antibody titer and combination factor

	AUC	Standard deviation ^a	P value ^b	95%CI	
				Lower limit	Upper limit
VE-Cad	0.762	0.054	<0.0001	0.656	0.867
IgG titer	0.862	0.042	<0.0001	0.781	0.944
combination factor	0.915	0.034	<0.0001	0.849	0.982

Note: a. Non-parametric assumption; b. Null hypothesis: true area = 0.5.

Discussion

ABO type fetal hemolytic disease of the newborn (ABO-HDFN) refers to a series of symptoms caused by the presence of IgG antibodies against fetal red blood cell antigens in the mother's body due to the incompatibility of maternal and infant blood types, and the combination of antibodies with the target antigens in the fetus through the placenta. Studies have shown that the earlier the discovery and diagnosis of HDFN, the better its therapeutic effect [15], but the prenatal diagnosis of ABO-HDFN has always been a difficult problem for obstetricians and blood transfusion workers.

The maternal IgG anti A /B titer has a great predictive effect on predicting the development of HDFN in children. Clinically, the maternal serum antibody titer of 1:64 is usually taken as the critical value [16]. If the titer is $\geq 1:64$, it indicates the possibility of the occurrence of ABO-HDFN in infants. In this study, 41 cases (97.62%) of 42 patients in the ABO-HDFN group had antibody titer $\geq 1:64$, which was consistent with relevant literature reports [17], but there was also 1 case with a titer $\leq 1:32$, indicating that

hyperbilirubinemia children with low maternal antibody titer cannot be ignored clinically; while in the control group, those with antibody titer $\geq 1:64$ were as high as 44.74%. Among them, 2 cases of antibody titer $\geq 1:512$ did not develop HDFN, and there was no significant correlation between IgG anti-A/B titer and neonatal TBIL ($P>0.05$), which may be related to factors such as antibody subtype, blood group substance content, placental effect and other factors [18].

ABO blood group antigens are tissue blood group antigens, which are widely present in human tissues and organs, and are strongly expressed on the surface of endothelial cells. Studies have reported [19] that CD144+ microsomes can be used as a marker of vascular endothelial injury in children with ABO-HDFN, and the level of vascular endothelial microsomes (EMPs) in children was significantly correlated with vascular dysfunction and disease severity. It can be seen that the anti-A and anti-B antibodies of the mother not only bind to the corresponding antigens on the red blood cell surface of the child to cause hemolysis, but also bind to the target antigens on the endothelial cell surface to cause vascular endothelial damage [10]. Vascular endothelial cadherin (VE-Cad) is a major structural molecule that constitutes the adhesion and connection between vascular endothelial cells. It regulates the stability of vascular endothelial barrier by interacting with connexin and actin cytoskeleton protein, and is an indispensable substance for vascular development and permeability regulation [20]. In this study, the VE-Cad level of pregnant women in the third trimester of pregnancy was detected and analyzed. It was found that the VE-Cad of pregnant women in the ABO-HDFN group was increased, and the difference was statistically significant ($P<0.0001$), and there was a significant positive correlation between the VE-Cad level of pregnant women and the total bilirubin level of newborns ($r=0.6869$, $P<0.0001$). It is speculated that the VE-Cad level of pregnant women in the third trimester of pregnancy can be used as an indicator to detect ABO-HDFN.

This study evaluated the diagnostic efficacy of pregnant women's VE-Cad level and IgG antibody titer. The AUC of VE-Cad level for ABO-HDFN diagnosis was 0.762, and the sensitivity and specificity were 80.95% and 63.16%, respectively; The AUC of IgG antibody titer was 0.862, the sensitivity and specificity were 88.10% and 71.05%, respectively. The AUC of the combined diagnosis was 0.915, the sensitivity increased to 92.86%, and the specificity was up to 84.21%. It shows that the combined determination of VE-Cad level and IgG antibody titer can improve the sensitivity rate of ABO-HDFN prenatal diagnosis, which is of great significance for the early diagnosis of ABO-HDFN.

Conclusion

The VE-Cad level and IgG antibody titer of pregnant women in the ABO-HDFN group in the third trimester of pregnancy are both increased. The combined detection of VE-Cad level and IgG anti-A/B antibody titer can improve the diagnostic sensitivity and facilitate clinical practice application and operation which play an important role in prenatal diagnosis of ABO-HDFN.

Declarations

Author contributions DJ:Project development, Data analysis, Manuscript writing. JD:Project development. SS:Project development,Data Collection. XD:Data Collection. SL:Data Collection PL:supervision.

Conflict of interest The authors have stated explicitly that there are no conflict of interest in connection with this article. The authors alone are responsible for the content and writing of the paper.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Huangdao District People's Hospital.

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Figures

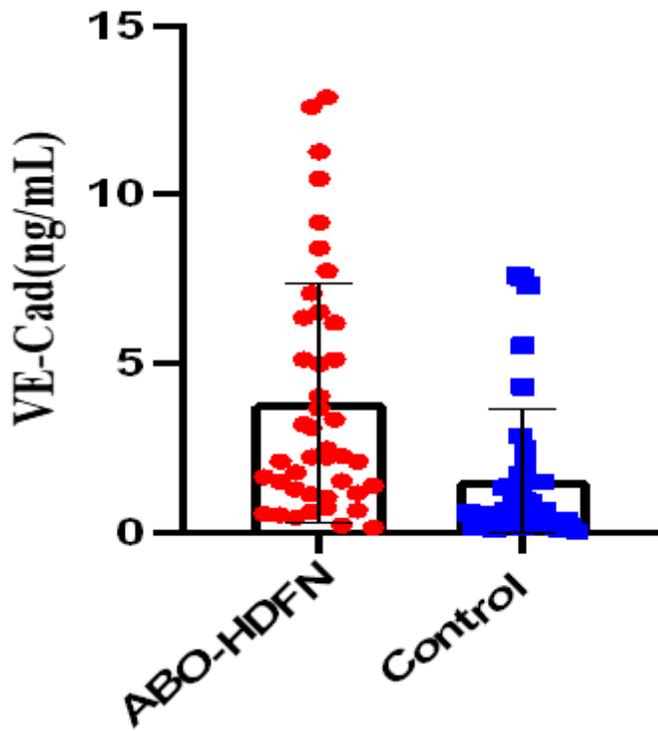


Figure 1

Comparison of VE-Cad levels of pregnant women in the ABO-HDFN group and the control group.

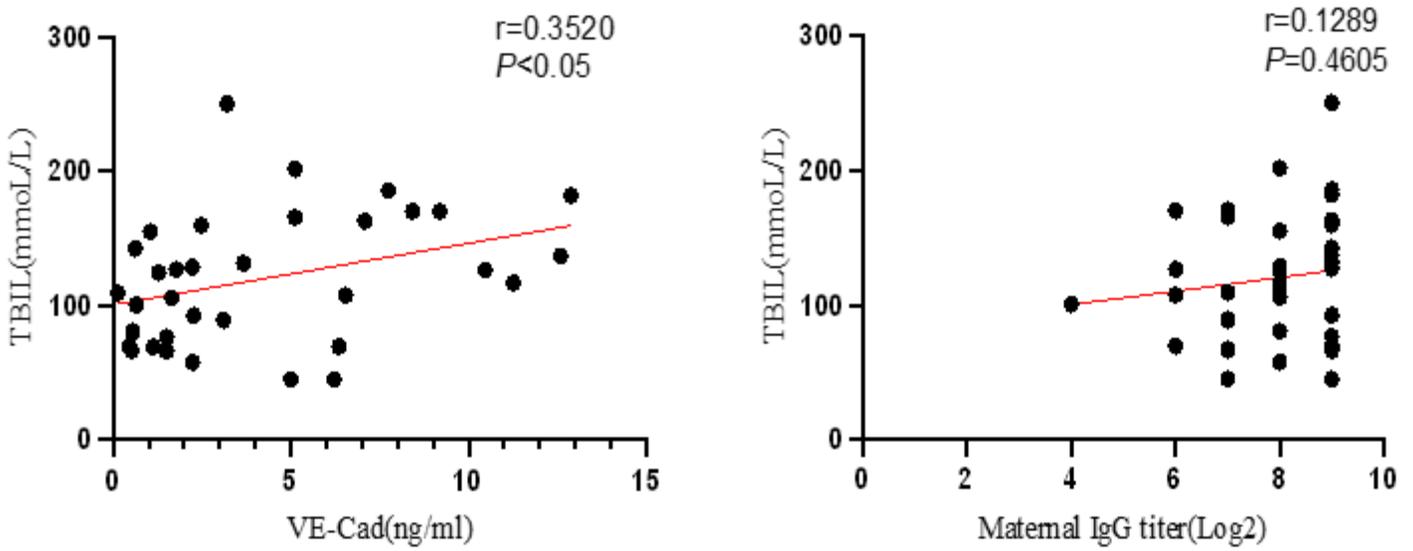


Figure 2

Correlation analysis of VE-Cad level, IgG antibody titer and neonatal TBIL

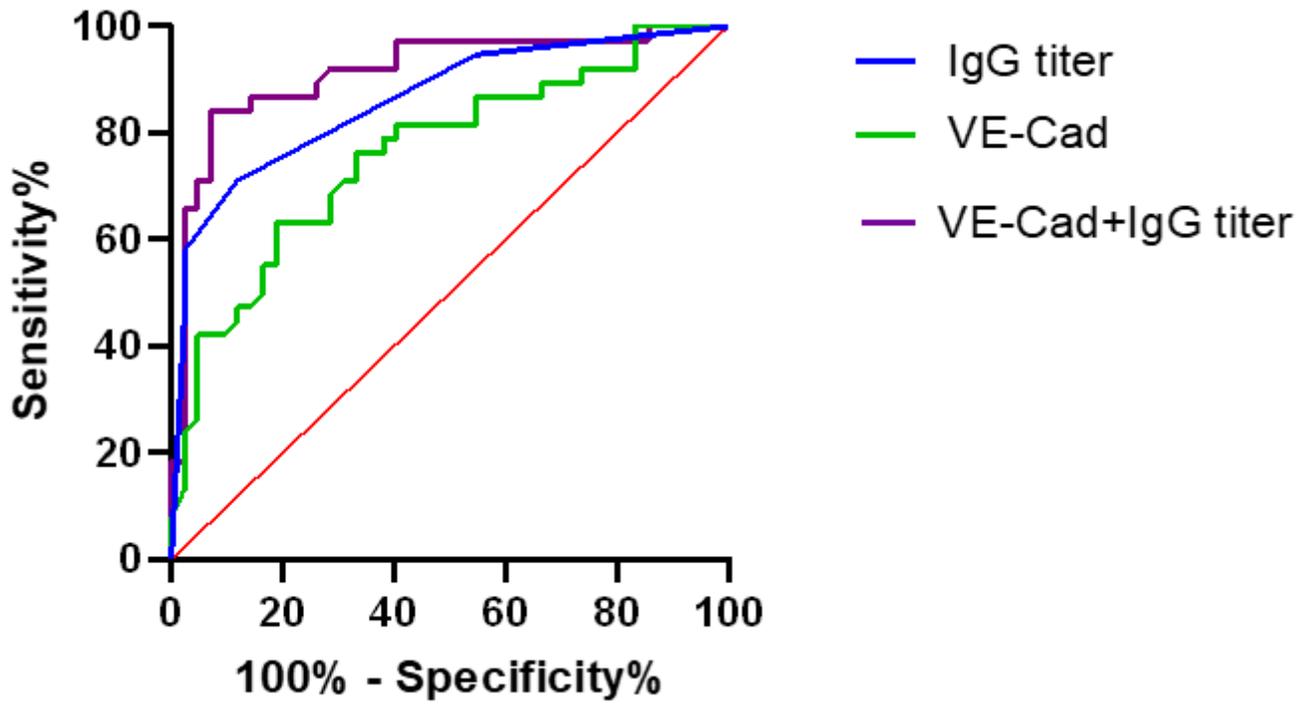


Figure 3

ROC curve analysis of VE-Cad level and IgG antibody titer alone and combined diagnostic value.