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Original Article

Combined detection of anticardiolipin and anti-β2-glycoprotein 1 antibodies may predict pregnancy outcome

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Abstract: Antiphospholipid syndrome (APS) is a typical non-inflammatory autoimmune disease that is common in young women. It is characterized by the presence of the anti-cardiolipin antibody (ACA) and anti-β2 glycoprotein 1 (anti-β2-GP1) antibody and is associated with a high risk of arteriovenous thrombosis. We measured the expression of ACA and anti-β2-GP1 antibodies in the serum of pregnant women early in their pregnancy, and analyzed the pregnancy outcome of the primigravidas who were positive for both the antibodies, so as to evaluate the efficacy of the combined determination in predicting pregnancy outcome. A total of 102 pregnant women who visited the Hebei General Hospital from January 2014 to December 2017 were enrolled in the study. The serum levels of ACA and anti-β2-GP1 antibodies were determined in all the enrolled pregnant women using the enzyme-linked immunosorbent assay (ELISA) method and the correlation between positive ACA/anti-β2-GP1 antibody and the adverse pregnancy outcomes was analyzed. Meanwhile, the difference in the pregnancy outcomes between patients who were positive for ACA only, for anti-β2-GP1 only and for both of the two antibodies was also investigated. The incidence of adverse pregnancy outcomes of pregnant women who were positive for both the ACA and the anti-β2-GP1 antibodies (48.87%) was higher than that of those positive for ACA only (28.67%) and those positive for anti-β2-GP1 only (36.66%). The positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of the combined determination of the two predictors was 81.75%, 95.84%, 88.37% and 95.92%, respectively. The combined determination of ACA and anti-β2-GP1 antibodies early in pregnancy may predict the occurrence of pregnancy outcome, with superiority over either of the two predictors alone.

Keywords: Anticardiolipin antibody, anti-β2-glycoprotein 1 antibody, pregnancy outcome

Introduction

The antiphospholipid antibodies (aPLs) represent a heterogeneous group of specific autoantibodies produced against anionic phospholipids or phospholipid-protein complexes, and the anticardiolipin antibody (ACA) is a member of this autoantibody family. The presence of these abnormal antibodies may lead to anti-phospholipid syndrome (APS), which is characterized by thrombosis and is associated with various diseases including arteriovenous thrombosis. In addition, APS is a non-inflammatory autoimmune disease. The presence of the aPLs represents a predisposition for thrombosis or adverse pregnancy outcomes such as habitual miscarriage, stillbirth and preterm delivery. The diagnosis largely depends on laboratory findings [1]. Studies have shown that people who tested positive for ACA are prone to various types of arteriovenous thrombosis, including deep vein thrombosis, pulmonary embolism, retinal artery thrombus and placental thrombosis. The diagnostic value of all the available detection methods for aPLs has been well investigated, especially the clinical value of ACA detection for predicating adverse pregnancy outcomes; nevertheless, it has not been clarified whether the method is applicable to all the relevant extrapolation about all the types of adverse pregnancy outcomes.

The ACA and anti-β2-GP1 antibodies are the most commonly used indicators for detecting

aPLs [2]. Studies have revealed that various pregnancy-related disorders can be attributed to ACA. Pregnant women with adverse pregnancy outcomes are often found to have abnormal ACA status, indicating that ACA is closely associated with a series of pathological changes among pregnant women [3-5]. In recent years, a large number of studies have shown that the existence of the anti-β2-GP1 antibody is closely related to recurrent fetal loss. In this study, pregnant women early in their pregnancy who visited our hospital were selected as the research subjects to determine the combined predicative value of ACA and anti-β2-GP1 antibodies in adverse pregnancy outcomes.

Materials and methods

General data

A total of 102 pregnant women who visited the Hebei General Hospital from January 2014 to December 2017 were enrolled in the study. Two ml of fasting blood was collected from each subject in the early morning and it was placed at room temperature for 2 hours; then it was centrifuged at room temperature and the isolated serum was stored in a freezer at -30°C until the assay. The serum levels of ACA and anti-β2-GP1 antibodies were determined in all the enrolled pregnant women using the ELISA method. The subjects were divided into three groups, namely group A, group B and group C, based on the test results of the two antibodies. Subjects in group A were only positive for ACA antibody (ACA-IgM >7 MPLU/mL), those in group B were only positive for anti-β2-GP1 antibody (>90 U/mL) and those in group C were positive for both the ACA and anti-β2-GP1 antibodies. Inclusion criteria: women confirmed with a successful pregnancy; having completed prenatal registration and those who underwent routine prenatal examinations in Shijiazhuang Gynecological & Obstetrical Hospital; gestational age ≤16 weeks; those with complete clinical data and confirmed pregnancy outcomes. Those with a previous history of immune diseases, fetal malformation or fetal hereditary diseases were excluded. The study was approved by the medical ethics committee of Shijiazhuang Gynecological & Obstetrical Hospital. All the subjects provided written informed consent after having been fully informed about the relevant content of the study.

Assay methods

The serum that was stored in the freezer at -30°C was removed for the assay. The assay was performed as follows: 1) the corresponding serum samples were diluted 100 fold with PBS buffer; 2) the diluted samples were transferred to the corresponding micro-well plates at a volume of 100 ul per well and were incubated in a constant temperature incubator at 37°C for 30 min. Then the solution in the plates was emptied, and the plate bottom was rinsed repeatedly three times with the rinsing solution prepared in advance; 3) a 100 μl volume of enzyme-conjugated solution was added into the microwells and incubated at room temperature for 15 min. The strips were emptied and rinsed repeatedly at least 3 times with the aforesaid rinsing solution; 4) the tetramethylbenzidine (TMB) substrate solution was added to the plates at a volume of 100 μL per well and incubated in a constant temperature incubator at 37°C for 15 min; then the reaction was terminated by the addition of 100 μL termination buffer; 5) the absorbance (OD) was measured at the wavelength of 450 nm using the enzyme-linked immunometric microplate reader and the titers of the ACA and anti-β2-GP1 antibodies were calculated based on the pre-prepared standard curve.

Observational indicators

The adverse pregnancy outcomes of these subjects included pregnancy-induced hypertension syndrome, preterm delivery, placental abruption, stillbirth and fetal growth restriction (FGR). The expression of ACA and anti-β2-GP1 antibodies in the three groups of adverse pregnancy outcomes was determined and compared; meanwhile, the incidence of the three groups of adverse pregnancy outcomes was compared.

Statistical methods

The obtained data was statistically analyzed using SPSS 20.0 software. The measurement data was expressed as mean ± standard deviation (SD), and *t* test was utilized for inter-group comparisons; the enumeration data was expressed as percentage (%) and inter-group comparison was carried out with the chi-square test. The relationship between the levels of ACA and anti-β2-GP1 antibodies and adverse pregnancy outcomes was investigated using the

Table 1. Comparison of general data of subjects in the three groups

Group	Average age (yrs)	Mean gestational age (wks)	Average gravidas (times)	Average paras (times)
A (*n* = 48)	32.12±10.32	11.22±0.51	1.28±0.28	1.43±0.31
B (*n* = 32)	31.28±10.68	12.06±0.62	1.34±0.22	1.38±0.25
C (*n* = 22)	33.36±11.02	11.31±0.63	1.24±0.31	1.19±0.12
F	2.020	1.090	1.020	1.450
P	0.267	0.330	0.364	0.438

Table 2. Expression of ACA (GPLU/mL) in subjects with adverse pregnancy outcomes in the three groups

Group	PIH syndrome*	Preterm delivery	Placental abruption	Stillbirth	FGR
A (*n* = 48)	7.49±1.51	8.37±1.21	9.57±1.11	9.52±1.01	8.42±1.09
B (*n* = 32)	6.36±0.61	5.42±0.41	3.67±0.71	4.12±0.32	6.12±0.17
C (*n* = 22)	9.17±2.47	10.43±0.73	9.41±0.81	0	9.54±0.61

*PIH: Pregnancy-induced hypertension.

Table 3. Expression of anti β2-GP1 antibody (U/mL) in subjects with adverse pregnancy outcomes in the three groups

Group	PIH syndrome*	Preterm delivery	Placental abruption	Stillbirth	FGR
A (*n* = 48)	84.63±1.83	62.12±0.32	72.12±0.02	63.12±0.42	62.12±0.32
B (*n* = 32)	92.63±0.96	99.63±0.47	104.63±0.83	94.63±0.34	109.63±1.67
C (*n* = 22)	94.63±1.02	104.63±0.56	111.63±0.78	0	102.32±2.01

*PIH: Pregnancy-induced hypertension.

Table 4. Comparison of the adverse pregnancy outcomes among subjects in the three groups, n (%)

Group	PIH syndrome*	Preterm delivery	Placental abruption	Stillbirth	FGR	Total	*P* value
A (*n* = 48)	5 (10.67)	2 (3.67)	1 (2.33)	1 (1.33)	5 (9.67)	14 (28.67)*	<0.05
B (*n* = 32)	4 (9.33)	1 (6.33)	1 (3.33)	1 (1.03)	5 (10.67)	12 (36.66)*	<0.05
C (*n* = 22)	4 (16.33)	1 (7.33)	1 (4.33)	0	5 (17.33)	11 (48.87)	

**P*<0.05, compared with group C; PIH: Pregnancy-induced hypertension.

Spearman rank correlation method, and *P*< 0.05 was considered statistically significant. The predictive value of the ACA and anti-β2-GP1 antibodies detection alone or in combination for predicating adverse pregnancy outcomes was analyzed using the multivariate logistic regression model.

Results

Comparison of general data between the three groups

A total of 90 subjects, all primigravida, were selected and divided into three groups. The general data, including average age, average gestational age, average gravida and average para did not differ in patients who were positive for ACA, anti-β2-GP1 and patients who were positive for both ACA and anti-β2-GP1 antibodies (*P*>0.05), as shown in **Table 1**.

Expression of ACA and anti β2-GP1 antibodies in subjects with direct adverse pregnancy outcomes in the three groups

The expression of ACA and anti β2-GP1 antibodies in the three groups of subjects with direct adverse pregnancy outcomes was determined using the ELISA assay method (**Tables 2**, **3**); meanwhile, the inter-group comparison of the incidences of adverse pregnancy outcomes was performed between patients who were positive for ACA only (group A), those who were positive for anti β2-GP1 only (group B) and those who were positive ACA and anti β2-GP1 antibodies concurrently (group C), as shown in **Table 4**. The results showed that the incidence

Table 5. Relationship between ACA level and adverse pregnancy outcomes

Outcome of pregnancy	*R*	*P*
Pregnancy-induced hypertension syndrome	0.125	0.046
Preterm delivery	0.634	0.007
Placental abruption	0.567	0.015
Stillbirth	0.678	0.034
FGR	0.897	0.025

Table 6. Relationship between anti β2-GP1 antibody level and adverse pregnancy outcomes

Outcome of pregnancy	*R*	*P*
Pregnancy-induced hypertension syndrome	0.345	0.037
Preterm delivery	0.434	0.015
Placental abruption	0.374	0.045
Stillbirth	0.598	0.043
FGR	0.579	0.035

Table 7. The predictive values of each indicator (%)

Indicator	Positive predictive value	Negative predictive value	Sensitivity	Specificity
A	66.78	80.91	73.87	82.35
B	75.67	82.21	75.97	84.44
C	81.75	95.84	88.37	95.92

of adverse pregnancy outcomes in the three groups was 28.67%, 36.66% and 48.87%, respectively ($P<0.05$).

Relationship between ACA level and adverse pregnancy outcomes

The relationship between ACA level and the incidence of adverse pregnancy outcomes was evaluated using the Spearman correlation coefficient (r) (**Table 5**). The results showed that the ACA level was positively correlated with the incidence of pregnancy-induced hypertension syndrome ($r = 0.125$), habitual miscarriage ($r = 0.634$), stillbirth ($r = 0.567$), preterm delivery ($r = 0.678$) and FGR ($r = 0.897$) ($P<0.05$).

Relationship between anti β2-GP1 antibody level and adverse pregnancy outcomes

The relationship between anti β2-GP1 antibody level and the incidence of adverse pregnancy outcomes was evaluated using the Spearman correlation coefficient (r) (**Table 6**). The results showed that the anti β2-GP1 antibody level was positively correlated with the incidence of pregnancy-induced hypertension syndrome ($r = 0.345$), habitual miscarriage ($r = 0.434$), stillbirth ($r = 0.367$), preterm delivery ($r = 0.598$) and FGR ($r = 0.579$) ($P<0.05$).

The predictive value of detection of each single indicator and combined detection in predicting adverse pregnancy outcomes

Positivity for ACA (ACA-IgM >7 MPLU/mL) and anti-β2-GP1 antibodies (>90 U/mL) were used in combination as the threshold for predication of adverse pregnancy outcomes, and we found that 22 patients of the 102 pregnant women enrolled in the study were considered as having reached the threshold; of them, 17 developed pregnancy-induced hypertension syndrome. Based on the results of the statistical analysis, the positive predictive value, negative predictive value, sensitivity and specificity of the combined detection of the two antibodies was 81.75%, 95.84%, 88.37% and 95.92%, respectively, which were all significantly higher than those of any of the single detection; hence, it was concluded that this threshold could be adopted to predict adverse pregnancy outcomes (**Table 7**).

Discussion

The mechanism behind the presence of ACA in the serum of pregnant women experiencing miscarriage, is currently believed to be largely attributed to structural changes in the phospholipids in the cell membrane that stimulate excessive production of the ACA [6-8]. Consequently, the incidence of miscarriage was increased in patients who tested positive for ACA. Although some pregnant women may have normal pregnancy despite of the positivity for ACA [9, 10], the probability of miscarriage in pregnant women who tested positive for both of the antibodies may be 2 to 4 times higher than that those with the presence of either of the two antibodies alone. In some patients who tested positive for both the antibodies but were not affected by the occurrence of placental microcirculation thrombosis during pregnancy, half of

them were found to develop thrombosis within 3 to 10 years after their delivery, during the follow-up visits specifically scheduled for patients who were positive for ACA, and approximately 10% of them developed lupus erythematosus [11-13]. Despite the salvage treatments they have received, FGR was not resolved in most of the cases after the treatments. The above phenomenon indicated that production of ACA is a spontaneous response made by the body against the autoimmune diseases. For patients with persistently positive ACA, cardiac valve replacement may be considered if a decline in cardiac valve function has been detected. In patients with progressive coronary atherosclerosis who are found to be persistently positive for ACA, the morbidity of multiple and multi-lacunar infarctions may need to be elevated. Accordingly, the positive status of ACA represents the autoimmune response of the body, and it is of great importance for providing guidance for clinical practice.

Studies have shown that people who tested positive for ACA are affected by various types of arteriovenous thrombosis, including deep vein thrombosis, pulmonary embolism, retinal artery thrombus and placental thrombosis. Thrombogenesis in the presence of ACA reflects not only the dysfunction of a certain organ, but an autoimmune disorder. Studies have revealed that various pregnancy-related disorders can be attributed to the presence of ACA. Pregnant women with adverse pregnancy outcomes are often found to have abnormal ACA status, which indicates that ACA is closely associated with a series of pathological changes among pregnant women. In this study, the predicative value of the combined detection of ACA and anti-β2-GP1 antibodies, components of the aPLs, was evaluated based on the analysis of the correlation of the combined detection of the aPLs with the pregnancy outcome in pregnant women.

Studies have showed that the ACA titer was closely related to the thrombogenesis caused by the autoimmune responses. In pregnant women who were positive for aPLs, thrombosis may be formed in the placental blood vessels, which will in turn lead to placental dysfunction, and eventually lead to fetal death [14]. The pathological changes may vary from person to person, but they can include infarction and thrombus formation in the vessels of the uterine and the placenta, perivillous fibrin deposition (MFD), or even chronic inflammatory lesions. In addition, when the aPLs status is positive, the risk of developing thrombosis and vascular infarctions in the uterus or placenta will be greatly increased, which has been supported by clinical experience [15]. For pregnant women with positive aPLs, the incidence of fetal loss may be significantly reduced with anticoagulation therapy [16, 17]. It has been reported in the literature that the morbidity of adverse pregnancy outcomes of aPLs-positive pregnant women who received no treatment to address the condition was as high as 90%, while the success rate of pregnancy in pregnant women after anticoagulation treatment could reach 80% [18, 19]. Some further clinical studies have suggested the correlation between the aPLs and the formation of thrombosis. The pathogenesis was believed to be the aPLs related destruction of the anti-coagulation barrier formed by the annexin V, as in most cases, this protein level in the placentas was found to be declined in the pregnant women with adverse pregnancy outcomes. This finding supports the conclusion that the microcirculation thrombosis in the placenta is the cause of adverse pregnancy outcomes [20, 21]. In this study, we measured the serum levels of ACA and anti β2-GP1 antibodies of 102 primigravidas using the ELISA method, and found that the incidence of adverse pregnancy outcomes in the primigravidas who were positive for both ACA and anti β2-GP1 antibodies (48.87%) was higher than that of the ACA-positive only patients (28.67%) and anti β2-GP1 antibody positive only patients (36.66%). The predicative value of combined determination of the two antibodies was superior over either of the single detections for predicating adverse pregnancy outcomes. A limitation of this study is that ELISA is the standard method for testing indicators, which requires the collection of blood samples be fresh, and relevant antibody analysis be provided in a timely manner. Therefore, it is particularly important to analyze the results in combination with the clinical outcomes. In the case that the clinical manifestations and test results do not match, the specimens must be repeatedly checked so that positive results are not missed.

In summary, the combined detection of ACA and anti β2-GP1 antibodies can be used to

screen out pregnant women with potential adverse pregnancy outcomes, hence it has been proven to be a useful tool to guide clinical practice. Preventive measures shall be implemented in accordance with the detection results to minimize the risk of adverse pregnancy outcomes, enhance therapeutic safety, so as to reduce the economic burdens for patients, their family and the society.

Disclosure of conflict of interest

None.

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