Logistic regression analysis of key markers of pre-thrombotic state screening in RSA patients

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Research Article

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Abstract

Purpose

To screen the important markers of prethrombotic status (PTS) in patients with recurrent abortion (RSA) and establish a multi-factor Logistic regression model, and to predict the risk of PTS in patients with RSA, so as to provide evidence for the early clinical prevention and treatment of RSA.

Methods

A total of 193 patients with RSA who visited our hospital from December 2019 to May 2021 were selected as the case group and 131 normal pregnant women who visited our hospital during the same period as the control group. The age, body mass index (BMI) and PTS-related markers were compared between the two groups. Including coagulation factor V1691, prothrombin gene (F_{II})20210, plasminogen activator inhibitor-1(PAI-1)675, methylene tetrahydrofolate reductase (MTHFR)677 and 1298, methionine synthase reductase (MTRR)66 gene locus polymorphism, homocysteine (HCY) level, protein C(PC) and protein S(PS) activity. The above markers were screened to establish a multi-factor Logistic regression model, and the predictive value of the model was assessed by ROC curve.

Results

Multivariate Logistic regression analysis showed significant differences in BMI, PAI-1-675 and MTHFR677 between the two groups ($P < 0.05$), indicating that BMI > 24, PAI-1(675) and MTHFR(677) locus polymorphisms were the high risk factors for RSA in women of childbearing age. The results of ROC curve evaluation model indicated that the area under the ROC curve (AUC) was 0.664 (AUC > 0.5), suggesting that the prediction model was reliable.

Conclusion

The statistical model established by testing the high risk factors of BMI, PAI-1(675) and MTHFR(677) and applying multivariate Logistic regression and ROC curve analysis can effectively predict the prethrombotic state of patients with RSA.

Introduction

Recurrent spontaneous abortion (RSA) refers to three or more consecutive spontaneous abortions with the same partner, but most experts believe that two consecutive abortions should be paid attention to and evaluated, because its risk of re-abortion is close to that of three abortions.¹ Recurrent spontaneous abortion has great difficulty in clinical diagnosis and treatment because of complex etiology and lack of clear clinical manifestations. Prethrombotic state (PTS) is a pathological process of dysfunction or

disorder of coagulation and anticoagulant system, fibrinolytic system and anti-fibrinolytic system caused by a variety of factors. PTS is a risk factor for thrombosis. Studies have shown that when the coagulation function is abnormal, the blood flow state of uterine and placenta will change, and local tissues are prone to forming microthrombus, which will lead to placental fibrosis deposition, infarction and eventually lead to various adverse pregnancy outcomes. 2 There is increasing evidence that PTS plays an important role in the pathogenesis of RSA. Brenner believed that PC defect, PS defect, FV and FII mutations, and hyperhomocysteinemia had significant correlation with abortion. 3 Studies have confirmed that genetic polymorphisms of MTHFR, MTRR, FV, FII and PAI-1 can promote thrombosis and induce abortion. 4 Screening the MTHFR and MTRR gene polymorphisms and monitoring the HCY level have important guiding significance for perinatal health care. 5 Therefore, many scholars in home and abroad have proposed to increase the screening of laboratory diagnosis of PTS in RSA patients to prevent the occurrence of abortion. 6 However, there is no clear and uniform standard for the diagnosis of PTS and the screening of markers in RSA patients. In this study, important markers of PTS in RSA patients were screened and a multivariate Logistic regression model was established to predict the risk of PTS in RSA patients.

Materials And Methods

Research design and subjects

A total of 193 cases with two or more spontaneous abortions who visited the Pre-natal Diagnosis Center of the Second Affiliated Hospital of Harbin Medical University from December 2019 to May 2021 were selected as the case group, and 131 cases of normal pregnant women in the same period were selected as the control group. Inclusion criteria: patients with two or more spontaneous abortions were selected as the case group; The control group was selected from the population with no previous pregnancy history or normal pregnancy history. Patients in the two groups had not taken any medicine that affected the observation effect recently. Sign informed consent. Exclusion criteria: (1) Various acute and chronic diseases such as heart, liver, kidney, brain, and pelvis; (2) Chromosome abnormalities existing in both husband and wife; (3) Immune diseases, hematological diseases and endocrine diseases; (4) Malignant diseases; (5) Hysterosalpingography and Doppler ultrasound diagnosis of reproductive anatomy deformity.

All participants signed the study protocol and informed consent for voluntary enrollment. The study was approved by the Institutional Review Board of Second Affiliated Hospital of Harbin Medical University.

Instruments and reagents

NP968 automatic nucleic acid extraction instrument (xi' an Tianlong); G1000 PCR amplification instrument (Hangzhou Bioer); Nanodrop one micro nucleic acid quantitative instrument (Thermo); 3500DX XL Sequencer (ABI); HCY kits (Ortho-clinical diagnostics); PC and PS activity assay kit (Storax, France)

Methodology

The non-anticoagulant blood was collected from the elbow vein in the fasting state. After standing at room temperature for 15 min, the supernatant serum was collected by centrifugation to detect the HCY level, PC and PS activity. The PTS-related genes were amplified by polymerase chain reaction (PCR) and sequenced by Sanger method. The genetic polymorphisms of age, BMI, Fv (1691), F β (20210), PAI-1(675), MTHFR(677), MTHFR(1298), and MTRR(66) sites in the two groups were analyzed and compared. The detection of each item was completed in strict accordance with the experimental operating procedures.

Statistical Analysis

SPSS 25.0 soft ware was used for statistical analysis. Enumeration data were expressed as the percentage of cases, and inter-group comparison was performed using χ^2 test. Age, BMI, PAI-1(675), MTHFR(677), MTHFR(1298), MTRR(66) locus polymorphism, HCY level, and PC and PS defects in the case group and the control group were compared, and the differences were considered to be statistically significant when $P < 0.05$. Independent variables with $P < 0.1$ in the univariate analysis were included in the multivariate logistic regression analysis, and the OR value and 95% CI were calculated. If $P < 0.05$, statistical significance existed. The ROC curve was used to evaluate the multivariate logistic regression analysis model.

Results

Establishment of logistic regression model

A univariate analysis was performed by χ^2 test on age, BMI, PAI-1(675), MTHFR(677), MTHFR(1298), MTRR(66) locus polymorphism, HCY level, and PC and PS defects in the two groups (see Table 1). Risk factors with $P < 0.1$ in univariate analysis were assigned (see Table 2) and included in Logistic multivariate analysis. The construction of multi-factor logistic regression risk prediction model: $0+1+2+...+N$ was converted to risk probability prediction model of recurrent spontaneous abortion: $P =$. The results showed that BMI, PAI-1(675) and MTHFR(677) gene locus polymorphism were the independent risk factors for RSA, and the differences were statistically significant ($P < 0.05$) (see Table 3)

Evaluation of logistic regression model

The binary Logistic regression analysis showed that the model had statistical significance ($p < 0.001$). The predictive variables were obtained according to the Logistic regression model, and the ROC curve of the model was drawn with RSA as the state variable. The results showed that the ROC curve was far from the reference line, indicating that the model was credible. The area under the ROC curve (AUC) of the model for predicting the risk factors related to PTS in RSA patients was 66.4%, the 95% confidence interval (95% CI) was 60.5–72.3%, and the standard error was 0.030. Compared with the area under the curve of 0.5, the difference was statistically significant ($P < 0.001$), as shown in Fig. 1.

Table 1
Univariate analysis of PTS related items of RSA patients [case (%)]

Variables		Controlgroup (n=131)	Case group (n=193)	χ^2 value	P value
Age (years old)	≤ 35	101(77.1)	139(72.0)	1.0480	0.3060
	> 35	30(22.9)	54(28.0)		
BMI(kg/m ²)	≤ 24	120(91.6)	148(76.7)	12.1489	0.0005
	> 24	11(8.40)	45(23.3)		
PAI-1(675)	5G/5G	63(48.1)	63(32.6)	8.2189	0.0164
	4G/5G	46(35.1)	82(42.5)		
	4G/4G	22(16.8)	48(24.9)		
MTHFR(677)	CC	44(33.6)	79(40.9)	5.2892	0.0710
	CT	46(35.1)	75(38.9)		
	TT	41(31.3)	39(20.2)		
MTHF(1298)	AA	109(83.2)	165(85.5)	4.4681	0.1071
	AC	19(14.5)	28(14.5)		
	CC	3(2.29)			
MTRR(66)	AA	45(34.4)	44(22.8)	8.7112	0.0128
	AG	47(35.9)	100(51.8)		
	GG	39(29.8)	49(25.4)		
HCY	Negative	98(74.8)	131(67.9)	1.8102	0.1785
	Positive	33(25.2)	62(32.1)		
PC Defect	No	127(96.9)	192(99.5)	3.3012	0.0692
	Yes	4(3.05)	1(0.52)		
PS Defect	No	112(85.5)	151(78.2)	2.6897	0.1010
	Yes	19(14.5)	42(21.8)		

Table 2
Assignment table of PTS related factors of RSA patients

Variables	Assignment
BMI(kg/m ²)	≤24=1; >24=1
PAI-1(675)	5G/5G=0;4G/5G=1 >4G/4G=2
MTHFR(677)	CC=0;CT=1 >TT=2
MTRR(66)	AA=0;AC=1 >CC=2
PC defect	No=0;Yes=1

Table 3
Logistic multivariate analysis of PTS related items in RSA patients

Variables	β	SE	wald	P Value	OR(95%CI)
BMI	1.1829	0.3628	10.6283	0.0011	3.264(1.603-6.646)
PAI-1(675)	0.4106	0.1885	4.7464	0.0294	1.508(1.042-2.182)
MTHFR(677)	0.2233	0.1013	4.8591	0.0275	1.250(1.025-1.525)
MTRR(66)	0.1111	0.1616	0.4730	0.4916	1.118(0.814-1.534)
PC defect	-2.1430	1.1434	3.5130	0.0609	0.117(0.012-1.103)

Discussion

RSA patients are often accompanied by a tendency of thrombosis. To study the effect of PTS on the pathogenesis of RSA may be of great significance for the early prevention and intervention of RSA. However, thrombotic diseases often lack obvious clinical symptoms and signs before their occurrence or in the early stage of their formation, so prediction and early diagnosis are rather difficult. Logistics regression analysis is a generalized linear regression analysis model, which can be used to explore the risk factors of disease occurrence and predict the probability of disease occurrence according to the risk factors. Some scholars have applied it to the etiology research of RSA.⁷ In this study, multivariate Logistic regression was used to assess the influence of important markers of PTS on the occurrence of RSA. The ROC curve evaluation model showed good simulation degree. It was finally concluded that BMI, PAI-1(675) and MTHFR(677) gene locus polymorphism were risk factors for RSA.

PTS-related genetic markers in patients with RSA

The most common pathogenesis of hereditary PTS in western populations is FV mutation, followed by F II mutation. The mutated FV can not only continue to express procoagulant activity, but also decrease the cleavage ability of activated protein C, thereby generating activated protein C resistance, manifested as

PTS8. The research by Poort et al has shown that the G-A conversion of nucleotide 20210 at the 3' untranslated region of coagulation F II can increase the plasma prothrombin level of the body, 9 thus causing venous thromboembolic diseases. In this study, no F V 1691 or F II 20210 polymorphic mutation was found in the tested women, 10 suggesting that Chinese women with RSA have rare F V 1691 or F II 20210 polymorphic mutations in prethrombotic state. This may be due to the uneven distribution of PTS across ethnic groups and regions.

PAI-1 is the main inhibitor of plasma fibrinolytic system and the main substance that inhibits fibrinolytic activity in the blood circulation. The increased level of PAI-1 leads to the decreased fibrinolytic activity and enhanced coagulation process. It is one of the risk factors for thrombotic diseases. Sheppard believed that PAI-1 expression products increased the deposition of vascular fibrin in endometrium during early pregnancy, and decreased uterine and placental blood flow, resulting in abortion.¹⁴ Glueck et al found that the PAI-1 (-675) 4g/5g polymorphism was the susceptible genotype for unexplained recurrent spontaneous abortion and had a strong independent correlation with adverse pregnancy outcomes.¹⁵ In this study, we found that PAI-1(675) was closely related to RSA in a multi-factor regression analysis ($P < 0.05$; OR=1.508), indicating that the PAI-1(675) locus polymorphism was an independent risk factor for RSA.

MTHFR and MTRR are the two most important genes in folic acid metabolism. The mutation at site 677 of MTHFR gene and the mutation at site 66 of MTRR gene at site 1298 make the serum HCY level too high and have a direct toxic effect on the embryo, which can damage the vascular endothelium, stimulate the proliferation of vascular smooth muscle cells, and lead to the imbalance of coagulation and fibrinolysis in the body.¹⁶ The results of this study suggested a correlation between the MTHFR(677) locus polymorphism and RSA ($P < 0.05$), but no correlation was found between the MTHFR(1298) locus polymorphism and RSA ($P > 0.05$), which was consistent with the results of a meta-analysis.¹⁷

PTS related coagulation markers in RSA patients

The most common genetic factor in the Han population is congenital anticoagulant protein deficiency, including protein C, protein S and antithrombin, mainly PC or PS, and antithrombin deficiency is rare.¹¹ Because the levels of PC and PS fluctuate significantly during pregnancy, it is more reliable to monitor the nonpregnant levels in women. ¹² In this study, the χ^2 test showed that both PC defects and PS defects had no correlation with RSA ($P > 0.05$). However, the positive rate of PS defects in the case group (21.8%) was significantly higher than the incidence rate of PS defects in the control group (14.5%), which might be related to the insufficient sample size in this study. Therefore, the correlation between PC and PS defects and RSA needs further large sample data analysis.

Some studies have suggested that the increase of HCY is closely related to RSA, and its mechanism may be related to coagulation abnormalities. The main reason for the increase of HCY is folic acid deficiency. After standardized folic acid intervention, the metabolism of folic acid can be regulated to reduce the level of HCY. ^{18,19} In this study, no correlation was found between HCY and RSA, which might be related to the increased publicity of the national policy of free folic acid distribution, the increased awareness of

pre-pregnancy health care for women of childbearing age in the region, and the adequate supplementation of folic acid during the pre-pregnancy period.

Some studies have suggested that the increase of BMI is related to the increased risk of spontaneous abortion,¹³ and its molecular mechanism may be that excessive insulin acts on the ovarian theca cells through insulin receptors to affect the embryo implantation. Logistic regression analysis in this study showed that the OR value of BMI was 3.264, which meant that for every 1 kg/m² increase in body mass index after other factors were controlled, the probability of illness was 3.264 times the original one. Therefore, controlling reasonable BMI might reduce the risk of illness of RSA.

Conclusion

BMI ≥ 24 , PAI-1(675) and MTHFR(677) gene locus polymorphism in patients with RSA are independent risk factors for multiple spontaneous abortions. In the process of clinical work, we should improve the alertness to these patients and carry out early prevention and treatment. The shortcoming of this study was that the included sample size was small, and no validation group was set to further verify the accuracy of the model. In the future, large-sample, multi-center research was required. In addition, the screening of PTS-related markers in this study is not comprehensive, and joint monitoring of coagulation function and platelet aggregation function is required in the future. In conclusion, the multivariate Logistic regression model that can effectively predict the prethrombotic state of RSA patients assessed by ROC curve has been established in this study, which will provide new reference for clinicians to prevent and treat RSA among women of childbearing age.

Declarations

Author contributions

Ning Liu wrote manuscript. Chao Wang, Yu Liu and Ruijing Wang collected data. Yuhong Zhang, Ting Zhang and Tingting Tian analyzed the patient data. Liu Meimei approved and revised the manuscript. All authors contributed to the article and approved the submitted version.

Compliance with ethical standards

Conflict of interest

Ning Liu, Chao Wang, Yu Liu, Ruijing Wang, Yuhong Zhang, Ting Zhang, Tingting Tian and Meimei Liu declare that they have no conflict of interests.

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Figures

Figure 1

ROC curve of Logistic regression model