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Coagulation Status in Women with Missed Abortion

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Abstract

Background: Missed abortion is a special type of spontaneous miscarriage, as well as a complex and multifactorial reproductive health problem that affects people all over the world. The purpose of this study was to assess the status of coagulation function in a large series of reproductive-age women diagnosed as missed abortion. Likewise, we want to explore the association between coagulation and missed abortions, in order to evaluate whether they could be used as early predictive factors for missed abortions.

Methods: A total of 11182 women who suffered from missed abortion from Peking University Third Hospital and 5298 healthy age –matched early pregnancy women were enrolled in our study. Coagulation function test (prothrombin time, activated partial thromboplastin time), fibrinolysis status detection (fibrinogen, D-Dimer), anticoagulation function tests (protein C, protein S and antithrombin III) and lupus anticoagulants (LAC) were examined on an automated coagulation analyzer. In addition, platelet counts were detected by automated hematology analyzer. Platelet aggregation (PAgT) were tested by light transmission aggregometry (LTA). All

tests were taken according to standard operating procedures of the instruments.

Results: Compared with healthy pregnancy, the level of D-Dimer and platelet count were higher, and the antithrombin III (AT-III) activity was lower ($P < 0.05$). 13.1% patients with missed abortion were positive for LAC, and platelet aggregation was increased in 47.4% patients. There were no significant differences in the levels of PT, aPTT, TT, fibrinogen, protein C and protein S between the patients with missed abortion and healthy pregnancy. Moreover, multivariate logistic regression analysis showed that D-Dimer, dRVVT-R, AT-III and PAgT had significant predictive value for missed abortion. **Conclusion:** These findings provide evidence of hypercoagulability in patients with missed abortion. Lupus anticoagulant, PAgT and D-Dimer were the strongest predictors of missed abortion. These data suggest that treatment of the lupus anticoagulant and antiplatelet aggregation could be considered in missed abortion.

Key words: miss abortion, coagulation, anticoagulation, lupus anticoagulant, platelet aggregation, hypercoagulability.

Introduction

Missed abortion is defined as a special type of spontaneous miscarriage without intervention, which is usually detected by ultrasound in the early stage of pregnancy. In this type of miscarriage, there is an arrest of embryonic or fetal development, and the ultrasonic result can find an empty gestational sac or an embryo/fetus without cardiac activity [1, 2]. Missed abortion accounted for about 15% to 20% of spontaneous miscarriage in all clinically recognized pregnancies [3]. Actually, missed abortion is a common gynaecological disease with a high incidence of 3.89 - 14.1%, which brings great physical and psychological pain to women, including endometrial injury, coagulative dysfunction, depression, and anxiety [4]. At present, missed abortion is a complicated problem, various causes have been reported in different studies, including age, genetic abnormalities, endocrinological disorders, immunological dysfunctions, hereditary thrombophilia, infections, environmental factors, apoptosis and oxidative stress [5-8]. A significant association between thrombophilia and miscarriage was reported in the European prospective cohort study on thrombophilia [9]. While inflammatory cytokines are closely related to the pathophysiology of recurrent miscarriage [10,11]. Notably, it is becoming increasingly clear

that inflammation and coagulation are two major host-defense systems that interact with each other [12, 13]. However recurrent miscarriages have been studied in several reports, few reports presented the characteristics of missed abortions.

In the present study, we aimed to assess the status of coagulation function in a large series of reproductive-age women diagnosed with missed abortions. Simultaneously, we want to explore the association between coagulation and missed abortions, in order to evaluate whether they could be used as early predictive factors for missed abortions.

Materials and methods

Subjects

A total of 28918 women diagnosed with missed abortion aged 22 to 48 years were enrolled from outpatients and inpatients of Peking University Third Hospital, from January 2013 to June 2019. All patients met the following inclusion criteria: (1) no history of venous thrombosis or pathological bleeding, hereditary thrombophilia, tumors, diabetes, cardiovascular disease, acute or chronic infection, chronic nephropathy; (2) none of the patients had taken any antiplatelet drugs, steroid hormones, oral contraceptives 3 months prior to the study; (3) Patients with missing results from coagulation tests and complete blood count tests were excluded. Consequently, 17736 cases were excluded, 11182 patients were finally enrolled (Figure 1). 5298 normal pregnancy women at trimester 1(<12weeks of gestation), aged 20 to 45 years were also recruited in our study as controls. All the enrolled pregnant women had complete clinical data, including physical examination, ultrasound examination, urine pregnancy tests and other laboratory examination, met the criteria referred to our previous studies [14, 15]. Besides, the pregnant women with cardiac disease, diabetes, hystero myoma, pregnancy-induced hypertension syndrome, intrahepatic cholestasis of pregnancy were excluded.

This study was approved by the ethics committee of Peking University Third Hospital. Informed consent was obtained from all subjects.

Coagulation function tests

3mL of whole blood were drawn by vein puncture of an antecubital vein and collected into a vacuum tube (Becton Dickinson Medical Devices Co Ltd., Franklin Lakes, New Jersey) containing

1:9 volume of 0.109 mol/L sodium citrate. The blood samples were centrifuged for 10 min at 3000 rpm to obtain platelet-poor plasma avoiding the inclusion of activated platelets. A calibrated automatic coagulation analyzer (ACL TOP®, Instrumentation Laboratory, Spain) was used to measure plasma prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen (Fib), D-Dimer, and the activity of free protein S (fPS), protein C (PC) and antithrombin-III (AT-III).

Lupus anticoagulant detection

Lupus anticoagulant (LAC) detection was performed according to the guidelines of the International Society on Thrombosis and Hemostasis (ISTH). The dilute Russell's viper venom time (dRVVT) and aPTT were examined on an automated coagulation analyzer (ACL TOP®, Instrumentation Laboratory, Spain). These tests were performed according to standard operating procedures (SOP) of the manufacturer. If aPTT was prolonged, a 1:1 mix of patient sample with pooled normal plasma was used to perform mixing study. If results from mixing studies failed to correct, it suggested the possibility of the presence of LAC. The phospholipid dependence was confirmed by positive phospholipid-neutralizing assays for dRVVT screen /confirm ratio. dRVVT screen/confirm ratio > 1.11 was considered positive.

Platelet counts and platelet aggregation test (PAgT)

Whole blood samples were taken from venipuncture into a vacutainer tube containing moderate EDTA at room temperature. Platelet counts were detected by automated hematology analyzer (XN2000, Sysmex, Japan) within 1 h after sample collection. Blood was collected into 3.2% sodium citrate (1:9), which PAgT was assessed by aggregation analyzer (AggRAM, Helena Laboratories, USA). To produce platelet-rich plasma (PRP), the sample tubes were centrifuged at 100 g for 15 min at room temperature (approximately 25 °C). The suitable PRP (450 µL) was removed to dedicated reaction cuvette and kept at room temperature until testing. Platelet-poor plasma (PPP) was made by further centrifugation of PRP, at 2400 g for 20 min. The PPP was aspirated as described for the PRP. The PRP and PPP were incubated for 5 min at 37 °C, respectively. Then PPP tube was inserted into the test hole and set the 100% aggregation rate. Magnetic beads were added into the PPP tube to calibrate the 0% aggregation rate. To measure the aggregation of PRP, 50 µL ADP (20 µM) was added, and the changes of light absorbance was monitored to record the aggregation process.

All methods in our study were carried out in accordance with relevant guidelines and regulations.

Statistical analysis

Statistical analyses were performed by SPSS 20.0 (SPSS, Inc., Chicago, IL, USA) and Prism 5 (GraphPad Software, La Jolla, CA, USA). Continuous variables with normal distribution were expressed as mean $\pm$ standard deviation (SD). According to CLIS document CA28-A3 [13], reference intervals were established using the 2.5th and 97.5th percentile of the distribution. Multivariable Cox regression analysis was used to evaluate the independent predictors of missed abortion. $P < 0.05$ was considered to represent a significant difference.

Results

Characteristics of the subjects included

To investigate characteristics of coagulation status in women with missed abortion, a total of 11182 patients aged 22 to 48 years were enrolled from outpatients and inpatients of Peking University Third Hospital, from January 2013 to June 2019. Their average age was 33.20 ± 7.43 years. While, 5298 controls aged 20 to 45 years were also recruited in our study, and the mean age was 33.00 ± 4.38 years. Serum indices of glucose, liver and kidney function were normal in all participants described in Table 1. There was no significant difference between patients and controls ($P > 0.05$).

Coagulation function screening tests

Our results showed that there were no differences in aPTT, TT and fibrinogen between patients with missed abortion and the healthy controls. APTT were 30.76 ± 2.86 s and 30.99 ± 3.63 s, PT were 10.92 ± 0.64 s and 10.75 ± 0.36 s, TT were 14.04 ± 0.70 s and 14.24 ± 1.49 s, fibrinogen were 2.99 ± 0.39 g/L and 3.076 ± 0.64 g/L in the patients and healthy pregnant women ($P > 0.05$), respectively (Figure 2A and 2B). Obviously, D-Dimer showed an increasing trend in patients with missed abortion, and the difference was statistically significant ($P = 0.021$) (Figure 2C).

Anticoagulation function tests

The results of AT-III, fPS and PC in patients with missed abortion were compared with pregnant women, which are nature crucial physiological anticoagulants in vivo. We found that there were no significant differences of fPS between the two groups. In the patients and controls, fPS was

75.65±23.45% in patients with missed abortion and 74.50±24.29% in healthy pregnant women.

AT-III was 99.32±13.08% in the patients, and it was 109.15±6.18% in the controls. Obviously, AT-III in the patients group was lower than that in the control group, while PC was higher in the group of patients (111.71±21.19%), and the difference was significant ($P<0.05$) (Figure 3A).

Lupus anticoagulant measurements in patients with missed abortion

In this study, screening for lupus anticoagulant(LAC) was performed using the dilute Russell's viper venom time (dRVVT), LA1 screening reagent/LA2 confirmation reagent(dRVVT-R), which normal ratio being 0.92- 1.11, > 1.11 was considered as positive. Among 11182 patients with missed abortion, 1485 were positive for dRVVT tests. However, only one case in the control group was positive for dRVVT, and the difference was statistically significant ($p<0.001$).

Changes in platelet aggregation and platelet counts

In this study, we found that the PAgT values showed an increasing trend in patients with missed abortion and there were significant differences compared with healthy pregnant women. As shown in Figure 3B, in the patients and controls, the PAgT values were 87.70±12.48% and 78.31±8.66%, respectively ($P=0.004$). In addition, the results showed that the level of platelet counts in the patients with missed abortion was significantly higher than that in the control group, platelet counts were $(240\pm59)\times 10^9/L$ and $(212\pm48)\times 10^9/L$, respectively ($P=0.009$) (Figure 3C).

The proportion outside the normal reference intervals of various coagulation related parameters

We calculated the reference intervals of various coagulation function and platelet related parameters for healthy pregnant women at trimester 1 (<12 weeks of gestation). The 2.5th-97.5th percentiles of aPTT, PT, TT, fibrinogen, D-Dimer, AT-III, PC, fPS, dRVVT, PAgT and platelet count were listed in Table 2. According to the reference intervals we established for pregnant women, up to 47.4% patients with missed abortion had PAgT above the normal reference range. While dRVVT-R, platelet count, D-Dimer exceeded their reference intervals by 13.1%, 10.7% and 8.2%, respectively (Figure 4).

Multivariate logistic regression of variables predicting missed abortion

In multivariate logistic regression analysis, PAgT, D-Dimer, dRVVT, AT-III and PC were identified independent predictors for missed abortion (Table 3).

Discussion

Missed abortion is a special type of spontaneous miscarriage and a common gynaecological disease. It has been reported that the incidence of fertile women with missed abortion is 3.89 - 14.1% and which dramatically increased in the last few years [4]. In the present study, we found that the incidence of missed abortion showed a rising trend from 2013 to 2019 of Peking University Third Hospital in Beijing, China. Obviously, more and more women are suffering from this disease. Nevertheless, several reasons have been identified for the failure of pregnancies in missed abortion, such as genetic abnormalities, endocrinological disorders, immunological dysfunctions, hereditary thrombophilia, infections, environmental factors, and oxidative stress [5-7]. However, the exact mechanisms remain unclear.

In this study, we conducted a retrospective analysis of 11182 patients with missed abortion and 5298 normal pregnancy women to evaluate the coagulation status in patients with missed abortions, and assess the predictive effects of coagulation related factors in missed abortions. We found that women with missed abortions have elevated D-Dimer level compared with normal early pregnancy. In addition, PC levels showed an increasing trend in patients with missed abortion, but AT-III levels showed an opposite trend. It is worth mentioning that dRVVT-R, PAgT values and platelet count were higher in patients with missed abortion, compared with the control group. Moreover, PAgT, D-Dimer, dRVVT-R, AT-III and PC retained significant predictive values for missed abortion in multivariate logistic regression analysis. These findings provide evidence of a hypercoagulable status in women with missed abortions. At the same time, it reflects the intimate relationship between coagulation and missed abortions.

A significant association between thrombophilia and miscarriage was reported in the European prospective cohort study on thrombophilia [9]. The successful outcome of pregnancy depends on the development of the placental cycle. Abnormal placental vascular system can cause various pregnancy disorders such as preeclampsia and intrauterine fetal death [16]. It was proposed that endothelial cells, monocytes, and platelets activated by antiphospholipid antibodies to induce a procoagulant state, which may lead to thrombosis in the placenta and subsequent fetal loss [17,18]. In our study, we found the similar result that the platelet counts were elevated among women with missed abortion. In addition, the proportion of patients with positive dRVVT-R was 13.1%, which suggested that lupus anticoagulant might be positive. Meantime, we found that the

value of PAgT in the patients group is significant higher than the control group. In the study of Karen Flood et al. [19], they showed that patients with unexplained recurrent miscarriage have significantly increased platelet aggregation in response to arachidonic acid. In another study, it discovered that antiphospholipid antibodies played an important role in recurrent miscarriages [20]. However, we failed to obtain the data of antiphospholipid antibodies from all patients with missed abortion. In the further, we will explore the relationship between these antibody levels and missed abortion through a prospective study.

Several reports have suggested a potential association between inherited thrombophilia and certain gestational pathologies. The common heritable thrombophilias markers, such as protein C, protein S, and AT-III, have recently been confirmed to be associated with an increased risk of unexplained pregnancy loss [21]. Studies had reported that heritable deficiencies of protein C and protein S were significantly associated with gestational abnormalities [22,23]. In the study of Ying Wang et al. [24], they also found that compared with the control group, the levels of PC and PS and AT-III showed a statistically significant decrease in the pregnant women with recurrent early miscarriage. The similar observation has been reported previously that a strong association of protein S deficiency with recurrent spontaneous abortion [25,26]. It is generally known that AT-III, fPS and PC are all nature anticoagulants in vivo. AT-III is an important physiological inhibitor of thrombin of the coagulation cascade, which has two important binding sites, one that binds to thrombin and the other one binds to heparin. Likewise, AT-III can inhibit the activated serine proteases such as FXa, FIXa, FXIa, and FXIIa [27, 28]. PS and PC are also two crucial physiological vitamin K-dependent protein anticoagulants. PS is a co-factor of PC, and can bind to activated protein C (APC) localized on the platelet phospholipid surface results in attenuating the effect of FXa on FVa in the coagulation cascade, thereby significantly inhibiting coagulation and fibrin formation [29, 30]. In our study, the mean values of PS were similar between the two groups. On the contrary, the mean values of PC showed an increasing trend in patients with missed abortion. But the AT-III level was significantly lower in the patients group compared with the control group, which suggested that the production of clotting enzymes increased. It has been reported that the measurement of AT-III generally represents the best performance of the congenital thrombophilia assays in principle, however, test performance for PC and PS tends to be a little inconsistent [27]. D-Dimer is a very sensitive marker of fibrinolytic activity following the

formation of fibrin, which is widely used to rule out thromboembolism in clinical practice [31]. The D-Dimer level in the patients group was significantly higher than that in healthy pregnancy women. The elevated levels of D-Dimer may be explained by localized increases in fibrin formation. Therefore, we speculated that more local microthrombosis might be formed in pregnant women with missed abortion.

In conclusion, the changes of biomarkers related to coagulation system, fibrinolysis system and anticoagulation system in women with missed abortion supported that a hypercoagulable state in women with missed abortions. And our results suggested the potential relationship between coagulation function and missed abortions. It was possible that these coagulation associated indicators were not sufficient as diagnostic markers, but a crucial role of a systemic hypercoagulable state in the pathogenesis of the disease cannot be excluded. We believe that the use of anticoagulant therapy for the patients with missed abortions will be encouraging and this preliminary study will stimulate further research in the future.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Peking University Third Hospital (029-01), and the study conducted in accordance with the declaration of Helsinki. Informed consent was obtained from all subjects.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used for this study are available from the corresponding author upon request.

Competing interests

All the authors declared no competing interests. All the authors listed have approved the manuscript that is enclosed.

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Authors' contributions

Shuo Yang, Dandan Chen, Junxiong Wang contributed equally to this work. Shuo Yang and Liyan Cui were responsible for the conception. Shuo Yang and Dandan Chen were responsible for conducted the study and writing of the manuscript. Junxiong Wang was responsible for the data analysis. Rui Qiao was responsible for the interpretation of data. All the authors read and approved the final manuscript.

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Figures

Figure 1

Selection of patients of the study

Figure 2

Coagulation function screening tests (aPTT, PT, TT, fibrinogen, D-Dimer) in patients with missed abortion and healthy pregnant women *P < 0.05 between the patients with missed abortion and healthy pregnant women

Figure 3

Changes in anticoagulation function, platelet aggregation and platelet counts in patients with missed abortion compared with healthy pregnant women *P < 0.05 between the patients with missed abortion and healthy pregnant women

Figure 4

Distribution of D-Dimer, AT-III, dRVVT-R, PAgT and platelet counts in patients with missed abortion according to the reference intervals established by early pregnant women.