

Original Article

The association of AT-III and D-Dimer with unexplained recurrent spontaneous abortion and their diagnostic value for prethrombotic state

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Abstract: Objective: This study aimed to investigate the role of antithrombin III (AT-III) and D-dimer (D-Dimer) in patients with unexplained recurrent spontaneous abortion (URSA); Methods: Sixty pregnant women with URSA (AEP group), 80 non-pregnant women with a history of URSA (ANP group), 50 healthy women in early pregnancy (NEP group) and 50 healthy non-pregnant women (NNP group) were retrospectively enrolled. Their serum AT-III and D-Dimer levels were measured. The patients in the ANP group were divided into three subgroups according to the number of miscarriages: 3 miscarriages (32 cases), 4 miscarriages (22 cases), and >4 miscarriages (26 cases), and the differences in serum AT-III and D-Dimer levels among these subgroups were compared. The patients in the AEP group were monitored for changes in AT-III and D-Dimer levels, and finally the diagnostic value of AT-III and D-Dimer levels were calculated for the prethrombotic state of URSA. Results: (1) AT-III and D-Dimer levels differed significantly among the 4 groups ($P<0.05$); (2) As the number of miscarriages increased, D-Dimer levels elevated and AT-III levels decreased, with significant differences among three subgroups ($P<0.05$); (3) As the treatment proceeded, patients in AEP and ANP groups showed a tendency for a gradual increase in AT-III levels and a significant decrease in D-Dimer levels, with significant differences before and after treatment ($P<0.05$); (4) The diagnostic AUC of AT-III and D-Dimer in the prediction of prethrombotic state of URSA were 0.8922 (95% CI=0.8026-0.9819, $P<0.0001$) and 0.8776 (95% CI=0.7643-0.9909, $P<0.0001$). Conclusion: AT-III and D Dimer levels are closely associated with URSA. The results of this study have preliminarily confirmed the feasibility of applying AT-III and D Dimer to screen the prethrombotic state of URSA, and their clinical application will help provide a reference for determining the cause of miscarriage in the infertile population with URSA and facilitate successful pregnancy.

Keywords: Antithrombin III, D-Dimer, unexplained recurrent spontaneous abortion, patients

Introduction

Unexplained recurrent spontaneous abortion (URSA) is defined as the loss of two or more consecutive pregnancies before the 20th week of gestation [1]. URSA generally refers to abortions that occur in early gestation and, rarely, also in late gestation [2]. With increased work pressure and a fast-paced lifestyle, the incidence of URSA has been increasing yearly, accounting for about 1%-5% of the total number of pregnancy disorders. This disorder can have a serious impact on the physical and mental health, thus it is recommended to carry out interventions for such patients [3, 4].

The pathogenesis of URSA remains unclear, and studies have confirmed that chromosomal

factors, endometrial factors, placental factors, anatomic factors, immune factors, infections, and exposure to adverse environmental factors may induce the development of this disorder [5, 6]. The correlation between the prothrombotic state and URSA has attracted the attention of scholars [7]. It was found that some patients with URSA can have normal pregnancies after aggressive anticoagulation of the thrombus in decidua, placental villi, and umbilical cord blood vessels [8, 9]. However, few studies have explored the underlying mechanism. Both AT-III and D-Dimer are commonly used clinical indicators related to blood coagulation [10]. This study investigated the predictive value of these indicators in patients with URSA by setting up controlled subgroups, aiming to provide a clini-

cal reference for improving clinical outcomes in patients with URSA.

Materials and methods

Baseline data

Sixty pregnant women with URSA (AEP group), 80 non-pregnant women with a history of URSA (ANP group), 50 healthy women in early pregnancy (NEP group) and 50 healthy non-pregnant women (NNP group) from January 2018 to December 2020 were retrospectively enrolled for the study.

Inclusion criteria: (1) patients diagnosed with URSA in AEP and ANP groups [11]; (2) patients with complete clinical data. The study was approved by the ethics committee of Houjie Hospital with the approval number NCT012-57896. Informed consent was waived since this was a retrospective analysis, and all data were anonymous.

Exclusion criteria: (1) patients with genital tract abnormalities; (2) patients with history of underlying disease prior to pregnancy; (3) patients with concurrent thyroid hormone abnormalities and positive thyroid antibodies; (4) patients with positive intravaginal mycoplasma, urealyticum and chlamydia; (5) patients with karyotype abnormalities in both spouses; (6) patients with history of concurrent mental illness; (7) patients received anticoagulant or fibrinolytic drugs within the last two months.

Intervention methods

Laboratory tests: Fasting blood samples were collected from elbow veins of patients in four groups in the early morning, anticoagulated with 0.11 mol/L sodium citrate, centrifuged for 10 min, and the plasma was stored at -80°C. After collection, the samples were sent for detection. The levels of AT-III and D-Dimer were measured by enzyme-linked immunosorbent assay (ELISA) using Sysmex CA1500 automatic coagulation analyzer (Sysmex Corporation, Japan). All the kits were purchased from Dade Behring GmbH (Marburg, Germany). The operation was implemented strictly in accordance with the kit instructions. Each indicator was tested 3 consecutive times and the average value was taken as the final result.

Clinical interventions: Patients in AEP and ANP groups were treated with a combination of Chinese and western medical interventions, including aspirin enteric-coated tablets (Bayer Healthcare Company Limited, Beijing, China, Approval No. J20171021), progesterone injection (Tianjin Kingyork Pharmaceuticals Co., Ltd., Tianjin, China, Approval No. H12020534), estradiol and dydrogesterone Tablets (Abbott Biologicals B.V., Weesp, The Netherlands, Approval No. H20150346). All of the above drugs were administered continuously until 12 weeks of gestation, accompanied by subcutaneous injection of low-molecular heparin calcium q.d. 4100 IU/time around the umbilicus until 16 weeks of gestation. Patients were also treated by Chinese patent medicine, Gushen Antai pill (Beijing Boran Pharmaceutical Co., Ltd., Beijing, China, Approval No. Z20030144) composed of radix-polygoni multiflori, radix rehmanniae praeparata, cistanche salsa, radix dipsaci, uncaria, semen cuscutae, rhizoma atractylodis macrocephalae, radix scutellariae, and radix paeoniae lactiflorae.

Outcome measurement: (1) Differences in plasma AT-III and D-Dimer levels among individuals in AEP, ANP, NEP and NNP groups; (2) Differences in maternal AT-III and D-Dimer levels among women in ANP with different numbers of miscarriages; (3) Changes in plasma AT-III and D-Dimer levels after clinical interventions in AEP and ANP groups; (4) Calculation of the diagnostic AUC values of plasma AT-III and D-Dimer for URSA by drawing ROC curves.

Statistical methods

The collected data were analyzed using SPSS 22.0. The counted data expressed as rate (%) were examined using chi-square test. The measured data expressed as ($\bar{x} \pm s$) were examined by t-test. Comparison among multiple groups was conducted using ANOVA with post hoc test. ROC curve method was adopted to calculate the diagnostic value. $P < 0.05$ was considered as a significant difference [12].

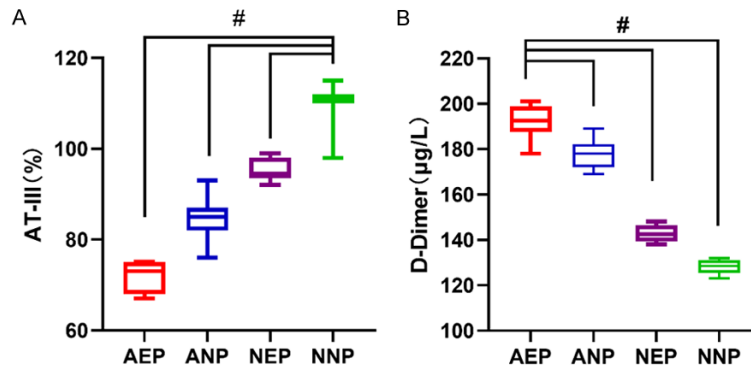
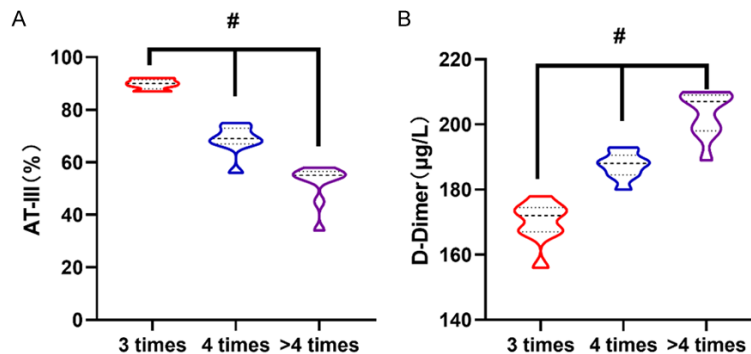
Results

Differences in baseline data

Patients in AEP, ANP, NEP, and NNP did not differ significantly ($P > 0.05$) in terms of age, wei-

Table 1. Comparative baseline data ($\bar{x} \pm s$)

Baseline data	AEP	ANP	NEP	NNP	F	P
Mean age (years)	28.19 \pm 2.11	28.21 \pm 1.98	28.44 \pm 1.22	28.21 \pm 1.89	0.054	0.957
Average weight (kg)	70.29 \pm 2.39	70.32 \pm 2.29	70.11 \pm 2.11	69.98 \pm 2.98	0.072	0.944
Average height (cm)	168.28 \pm 10.22	169.11 \pm 9.98	168.22 \pm 8.29	167.98 \pm 9.01	0.451	0.654
Average BMI (kg/m ²)	22.11 \pm 1.23	21.98 \pm 1.54	21.89 \pm 1.45	21.99 \pm 1.76	0.983	0.718

**Figure 1.** Differences in AT-III and D-Dimer levels among different groups. The NNP group had the highest levels of AT-III (A) and the lowest levels of D-Dimer (B). #P<0.05.**Figure 2.** Differences in AT-III and D-Dimer levels in URSA patients with different numbers of miscarriages. As the number of miscarriages increased, blood levels of D-Dimer (B) increased and AT-III (A) decreased (P<0.05). The differences between any two groups were statistically significant (P<0.05). #P<0.05.

ght, height, and BMI, suggesting that the four groups were comparable (Table 1).

Differences in AT-III and D-Dimer levels

Women in the NNP group had the highest AT-III levels among all four groups, followed by NEP, ANP and AEP groups, and the differences in AT-III levels among all groups were significant (P<0.05). Women in the AEP group had the highest D-Dimer levels, followed by ANP, NEP

and NNP groups, with significant differences in D-Dimer levels among all groups (P<0.05) (Figure 1).

AT-III and D-Dimer levels in URSA patients with different numbers of miscarriages

Women in the ANP group were divided into three sub-groups according to number of miscarriages: the group with 3 miscarriages (32 patients), the group with 4 miscarriages (22 patients), and the group with >4 miscarriages (26 patients). A comparison between groups showed that as the number of miscarriages increased, D-Dimer levels increased and AT-III levels decreased, with significant differences among the 3 groups (P<0.05). Pairwise comparison showed that the differences in the above two indicators between any two groups were significant (P<0.05) (Figure 2).

Changes in AT-III and D-Dimer levels in AEP group during treatment

Patients in AEP group were treated with routine therapy, and their AT-III and D-Dimer levels at 3, 6 and 9 months of gestation were recorded. Results showed that with the prolongation of pregnancy, the AT-III levels in AEP group tended to gradually increase, and D-Dimer levels tended to decrease significantly (P<0.05). AT-III and D-Dimer levels at 9 months of gestation differ significantly from those at 3 months and 6 months of gestation (Figure 3).

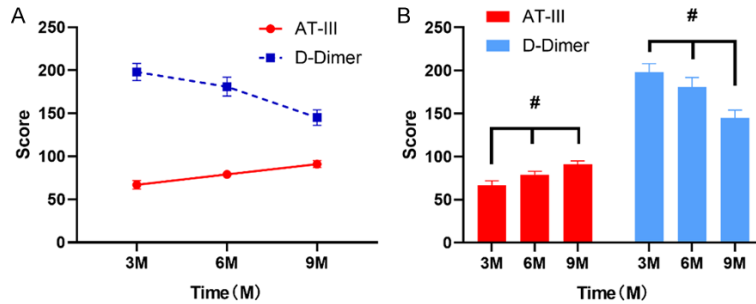


Figure 3. Changes in AT-III and D-Dimer levels in the AEP group during treatment. Comparison showed that with the prolongation of pregnancy, patients in the AEP group showed a tendency for a gradual increase in AT-III levels and a decrease in D-Dimer levels (A), and the differences in AT-III and D-Dimer levels at 9 months of gestation were significantly different when compared with those at 3 months and 6 months of gestation (B). # $P<0.05$.

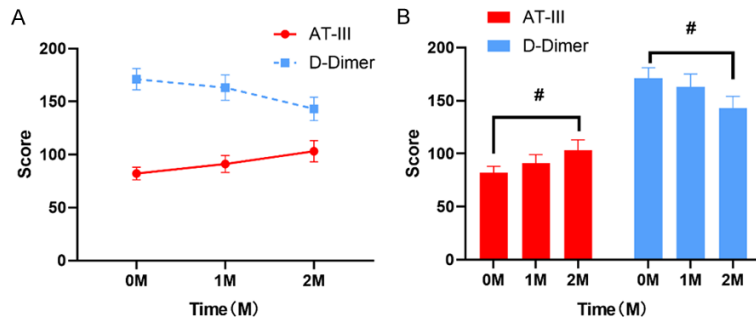


Figure 4. Changes in AT-III and D-Dimer levels in the ANP group during treatment. As the intervention proceeded, patients in the ANP group showed a tendency for gradual increase in AT-III levels and a decrease in D-Dimer levels, with significantly higher AT-III and significantly lower D-Dimer levels at 2 months of treatment compared to pre-treatment ($P<0.05$). # $P<0.05$.

Table 2. Diagnostic value of plasma AT-III and D-Dimer for the prethrombotic state of URSA

Indicator	AUC	Optimal critical value	Standard error SE	P	95% CI
AT-III	0.8922	92.62%	0.046	<0.0001	0.8026-0.9818
D-Dimer	0.8776	151.02 mg/L	0.058	<0.0001	0.7643-0.9909

Changes in AT-III and D-Dimer levels during treatment in the ANP group

Patients in the ANP group were treated clinically, and were followed up to record changes in their AT-III and D-Dimer levels before treatment, at 1 month and 2 months of treatment. Results showed that patients in the ANP group showed a gradual increase in AT-III levels and a significant decrease in D-Dimer levels over time. Compared to those before treatment, AT-III was significantly higher and D-Dimer lev-

els were lower at 2 months of treatment ($P<0.05$) (Figure 4).

Diagnostic value of plasma AT-III and D-Dimer levels for prethrombotic state of URSA

The diagnostic ROC curves of plasma AT-III and D-Dimer in the prethrombotic state of URSA were plotted separately and their AUCs were calculated, which showed that the diagnostic AUC of AT-III in the prethrombotic state of URSA was 0.8922 (95% CI=0.8026-0.9819, $P<0.0001$) and that of D-Dimer was 0.8776 (95% CI=0.7643-0.9909, $P<0.0001$) (Table 2; Figure 5).

Discussion

URSA is a clinically refractory infertility [13]. The incidence of URSA is increasing significantly yearly with changes in lifestyle [14]. The fetus as a semi-allograft during normal pregnancy is not subject to maternal rejection and only leads to the onset of maternal immune tolerance. When the maternal-embryonic immune tolerance mechanism fails, it results in immune-strike abortion [15, 16], which is the prevailing view on the pathogenesis of URSA.

However, new evidence has found that approximately 66%

of URSA mothers have at least one inherited (or acquired) abnormality of hemostasis, which is referred to as the prethrombotic state [17], a state of pathologic coagulation-anticoagulation imbalance induced by many factors including platelets, the coagulation-fibrinolytic system, and blood rheology. Mothers in a prethrombotic state develop placental thrombosis which causes decreased placental perfusion, ultimately affecting the exchange of materials between the mother and fetus and inducing the occurrence of spontaneous abortion [18]. This

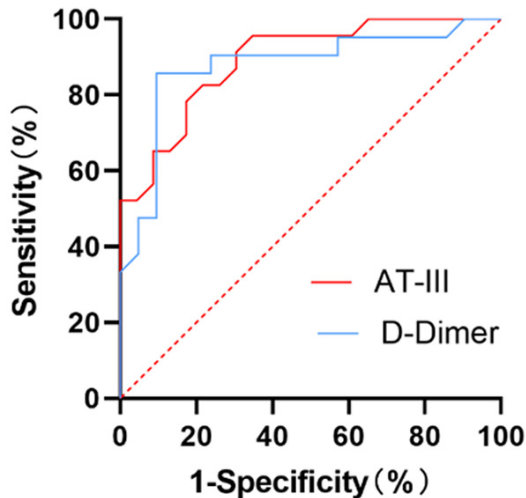


Figure 5. Diagnostic values of plasma AT-III and D-Dimer for the prethrombotic state of URSA. The diagnostic AUC of AT-III for the prethrombotic state of URSA was 0.8922 (95% CI=0.8026-0.9819, $P<0.0001$) and the diagnostic AUC of D-Dimer for the prethrombotic state of URSA was 0.8776 (95% CI=0.7643-0.9909, $P<0.0001$).

viewpoint has also been verified in several clinical studies. A yearly increasing risk of coagulation abnormalities and early puerperal embolism was observed in pregnant women, which may be directly related to the yearly increase in the incidence of URSA [19].

In this study, two coagulation-related indicators, AT-III and D-Dimer, for prediction of URSA were investigated. The results showed that the NNP group had the highest AT-III levels among the four groups, followed by NEP, ANP and AEP groups. Women in the AEP group had the highest D-Dimer levels, followed by ANP, NEP and NNP groups, with significant differences in the above indicators between any two groups. This suggests, on the one hand, that patients with URSA do have abnormal coagulation, but on the other hand, that the pregnancy partially affects coagulation function, which is similar to the findings of other studies [20]. It has been confirmed that pregnant women are susceptible to thrombosis, evidenced by elevated levels of coagulation factors and prothrombin. Meanwhile, their anticoagulants are reduced. With the increase in gestational weeks, the levels of coagulation factors get further increased, followed by prothrombin activation, and a decreased level of fibrinogen activator, contributing to the state of hypercoagulation. This mechanism can accelerate the repair and regeneration of the endometrium following delivery, and

it can reduce the incidence of postpartum hemorrhage [21, 22], which is evidenced in this study where AT-III and D-Dimer levels were altered in AEP and ANP groups after receiving the intervention.

To further investigate the association between AT-III and D-Dimer levels and URSA, we divided the patients in ANP group into three subgroups according to the number of miscarriages and performed a comparison among the groups. Results showed that as the number of miscarriages increased, the D-Dimer levels increased and AT-III levels decreased, and the differences among the three subgroups were statistically significant. We believed that the blood in patients with URSA is in an abnormally hypercoagulable state, which is the main cause of ischemia and hypoxia in embryonic tissue and thus miscarriage outcome in patients [23]. The results suggest a possible role of AT-III and D-Dimer as indicators for assessing the risks of miscarriage in URSA. The diagnostic value of AT-III and D-Dimer for prethrombotic status in patients with URSA was also analyzed and the results showed that their AUCs were 0.8922 and 0.8776, respectively, suggesting good feasibility.

In conclusion, AT-III and D-Dimer are closely related to URSA, and the above indicators are useful for screening of a prethrombotic state in patients with URSA, exhibiting a high diagnostic value. The novelty of this study is that the feasibility of AT-III and D-Dimer for predictive analysis of URSA was demonstrated by conducting multiple comparisons. The shortcomings of this study are the relatively homogeneous source of included patients and the lack of long-term follow-up as well as the lack of analysis of the effects of AT-III and D-Dimer on neonatal outcomes, which should be improved.

Disclosure of conflict of interest

None.

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