The association between thrombin-activated fibrinolytic inhibitor levels and risk of recurrent spontaneous abortion based on anti-thyroid antibodies status: a retrospective case-control study

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Abstract

OBJECTIVE: The objective of this study was to investigate the relationship between plasma levels of thrombin-activated fibrinolytic inhibitor (TAFI) and recurrent spontaneous abortion (RSA) in varying conditions of anti-thyroid antibodies (ATA), and to provide appropriate recommendations for RSA prevention. **Design:** Retrospective case-control study. **Setting:**Department of Obstetrics and Gynecology, Shengjing Hospital, China Medical University. **Sample:** There were 1068 subjects in this retrospective study, including 402 RSA patients and 666 controls. **Methods:** Collected case records from the Department of Obstetrics and Gynecology from January 2020 to March 2022 for comparative analysis between cases and controls. **Main outcome measures:** Patients' socio-demographic factors, plasma TAFI levels, ATA status, including thyroid peroxidase antibodies (Anti-TPO) and thyroglobulin antibodies (Anti-TG), thyroid function, coagulation function, and so on. Multivariate adjusted conditional logistic regression and restricted cubic spline (RCS) models were applied to evaluate odds ratios (ORs) and their 95% confidence intervals (CIs) between plasma TAFI levels and the risk of RSA under different ATA conditions according to the normal reference range of plasma TAFI levels (24-35ug/ml). **Results:** Compared to normal TAFI levels (as reference), low TAFI levels (<24ug/ml) had a significantly increased risk of RSA, which was shown in the Anti-TPO positive group (OR, 2.15; 95% CI, 1.221-3.785; P=0.008), Anti-TPO negative group (OR, 1.429; 95% CI, 1.032-1.98; P = 0.032), and Anti-TG positive group (OR, 2.215; 95% CI, 1.265-3.878; P = 0.005). In addition, the RSC model showed that plasma TAFI levels showed a linear negative dose relationship with the risk of RSA. **Conclusions:** The study indicates that pregnant women with plasma TAFI levels below the normal range, especially those who are ATA positive, are at a higher risk of developing RSA. These findings suggest the need to develop prevention recommendations.

Introduction

Recurrent spontaneous abortion (RSA) is defined as two or more consecutive clinical pregnancy failures that affect 1-5% of women of reproductive age [1]. RSA has a significant negative impact on women's physical and psychological health and is a serious reproductive problem whose incidence has been on the rise in recent years [2]. Currently, RSA is known to have various causes, including genetic, thrombotic, infectious factors, immune and endocrine dysfunction, and anatomical abnormalities of the reproductive tract [3, 4]. Thrombin-activated fibrinolysis inhibitor (TAFI) is the main inhibitor of fibrinolysis, and it plays an important role in RSA due to its role in the coagulation and fibrinolytic system as well as in inflammation [5].

TAFI is a procarboxypeptidase that is synthesized primarily by the liver and is present in the circulation as an inactive zymogen[6]. TAFI can be activated by thrombin, thrombin-thrombin regulatory protein, and plasma proteins. The activated form of TAFI cleaves its C-terminal lysine or arginine residue from the peptide substrate, resulting in partial degradation of fibrin from the C-terminal lysine residue [7, 8]. This mechanism

enables TAFI to exert antifibrinolytic effects [9]. In vivo studies have shown that fibrin degradation products may induce apoptosis in trophoblast cells, leading to embryonic cell death and fetal loss in mice [10]. However, the association of TAFI with RSA is less studied and controversial. A case-control study in Italy provided evidence that high activity TAFI levels are associated with a reduced risk of early recurrent fetal loss [11], while another Dutch case-control study showed that TAFI was not associated with RSA[12].

Additionally, TAFI may play a broad role in the regulation of inflammation. Its activated form inactivates several inflammatory mediators by removing the C-terminal arginine, including bradykinin, bovine toxin C3a and C5a [9, 13]. And inflammation is closely associated with thyroid autoimmunity (TAI), which is defined as the presence of anti-thyroid antibodies (ATA), including thyroid peroxidase antibodies (Anti-TPO) and thyroglobulin antibodies (Anti-TG) [14]. It is generally accepted that Anti-TPO positive women are at higher risk of incidental miscarriage, preterm birth, and postpartum thyroid disease[15, 16]. Some studies have shown that patients with RSA have higher levels of Anti-TPO [17]. Furthermore, it has been shown that TAI is associated with resistance to fibrinolysis ex vivo and C3 plasma levels [18]. Therefore, there may be an association between ATA and TAFI in terms of inflammation.

To our knowledge, limited previous research has been conducted to investigate the relationship between plasma levels of TAFI and RSA in varying conditions of ATA. Therefore, the objective of this study is to investigate this association among 1,068 pregnant Chinese women (402 cases and 666 controls) and to explore independent risk factors for RSA in the presence of different conditions of ATA. Furthermore, this research aims to analyze the influence of TAFI and ATA on RSA in terms of inflammation, to provide suitable recommendations for the diagnosis of RSA.

Materials and Methods

Study population and data source

We conducted a retrospective case-control study to investigate the effect of TAFI on RSA in Anti-TPO, Anti-TG negative, and positive pregnant women, using gestational examination data from January 2020 to March 2022 from the Department of Obstetrics and Gynecology, Shengjing Hospital, China Medical University. We defined RSA as the occurrence of two or more miscarriages with the same partner before 24 weeks of gestation. All patients underwent a standardized diagnostic protocol, and those who received a confirmed diagnosis of RSA by the hospital were included in this study. Ultimately, we included 402 patients with RSA and 666 normal pregnant women. The study was approved by the Ethics Committee of Shengjing Hospital, China Medical University.

Exposure and outcome variables

We extracted clinical and laboratory data from the electronic medical records using the case information management system at Shengjing Hospital, China Medical University. These include maternal age, plasma TAFI levels, maximum platelet aggregation rate (including ADP, Arachidonic acid [AA]), coagulation measurements (prothrombin time [PT], prothrombin time activity [PTA], activated partial thromboplastin time [APTT], fibrinogen content [FIB], Thrombin clotting time [TT], D-dimer), blood cell tests (lymphocyte percentage [LYMPH], monocyte percentage [MONO], basophil percentage [BASO], Red cell distribution width -CV value [RDW-CV], Red cell distribution width -SD value [RDW-SD]), thyroid function tests (free triiodothyronine [FT_3], free thyroxine [FT_4], thyroid stimulating hormone [TSH], thyroid peroxidase antibodies [Anti-TPO], thyroglobulin antibody [Anti-TG]), thromboelastography tests (reaction time [R], clotting time [K], Angle, maximum amplitude [MA], coagulation index [CI], estimated percentage of lysis [EPL], lysis after 30 min [LY30]). In this study, we measured plasma TAFI using an ELISA assay with an AU480 fully automated biochemical analyzer from Beckman Coulter, USA, and a TAFI quantitative assay kit from Liaoning Mediatek Biotechnology Co. Normal range of plasma TAFI reference hospital plasma TAFI normal range: 24-35ug/mL.

Statistical tests

Continuous variables were described using mean and standard deviation (SD) or median and interquartile

range (IQR). One-way analyses were performed after the Shapiro-Wilk normality test, and independent samples t-tests and Mann-Whitney U tests were used to compare differences between the two groups for parametric and nonparametric variables, respectively. The threshold of statistical significance was set at a p-value < 0.05. All data analyses were performed using R software (https: www.r-project.org, version 4.1.3) and SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

Stepwise logistic regression is used to analyze the independent risk variables for RSA in each group. To evaluate the relationship between TAFI and RSA, binary logistic regression was used, with the normal reference range of TAFI as a reference. The adjusted confounders were the variables screened by the multifactorial regression analysis.

Unconditional logistic regression models were conducted in stratified analysis by age, coagulation indicators, thyroid function indicators. Restricted cubic spline models with four knots were used to explore the potential association between TAFI and RSA under different antibody conditions. The four knots include the TAFI 1st percentile, 24ug/mL, 35ug/mL, and the TAFI 99th percentile.

Results

Comparison of baseline characteristics of RSA patients and normal pregnant women with different ATA

In the overall population, there were 1068 study subjects, of whom 402 (37.6%) were RSA patients, and 666 (62.4%) were normal pregnant women. Among the study subjects, 253 (23.7%) tested positive for Anti-TPO, and 273 (25.6%) tested positive for Anti-TG, with a higher proportion of individuals testing negative for both antibodies. The correlation between variables and RSA in the overall population is presented in Supplementary Table S1. ADP, AA, R, K, CI, EPL, and LY30 did not show statistical significance, while the remaining variables demonstrated statistical significance.

Table 1 and Table 2 demonstrate the baseline characteristics of RSA patients versus normal pregnant women. In terms of plasma TAFI levels, all groups, regardless of the status of antibodies, exhibited significantly lower levels in RSA patients compared to normal pregnant women with statistical significance. The median TAFI level in normal pregnant women was approximately 25 ug/mL, which was about 2 ug/mL higher than that of RSA patients. Interestingly, the Anti-TPO negative group had higher levels of plasma TAFI compared to the Anti-TPO positive group in both cases and controls.

Regarding the age of the study population, the results of both tables indicated that RSA patients were older than normal pregnant women. However, statistically significant differences were observed only in the Anti-TPO negative and Anti-TG positive groups. With regards to the platelet aggregation function indicators (ADP and AA), Only AA showed statistically significant in the Anti-TG negative group.

With regard to coagulation, the results were similar across antibody states. In the Anti-TPO negative and Anti-TG negative groups, all coagulation parameters (PT, PTA, APTT, FIB, TT, DD) showed statistically significant differences.

Concerning blood cell tests and thyroid function, the results of the analysis were the same in the two negative groups, and the analysis of the results was similar in the two positive groups. In both negative groups, except for MONO, all blood cell tests (LYMPH, MONO, BASO, RDW-CV, RDW-SD) and thyroid function indicators (FT_3, FT_4, TSH) showed statistically significant differences. In both positive groups, LYMPH showed statistical differences in both groups. BASO showed statistical differences only in the Anti-TPO positive group, while FT_3 showed statistical differences only in the Anti-TG positive group.

On the aspect of thromboelastography indexes, the results of the analysis of the two positive groups were the same as those of the two negative groups. Only LY30 was statistically different in the two positive groups. In both the Anti-TPO negative and Anti-TG negative groups, Angle and MA showed statistically significant differences.

Multifactorial logistic analysis of independent risk factors for RSA

The factors independently associated with RSA in different groups are shown in Table 3. In the overall population, TAFI, DD, and RDW-CV variables were shown to be protective factors, all other variables were considered risk factors. The regression analysis results showed that the β-coefficient of plasma TAFI level was -0.04 [odds ratio (OR) 0.961; 95% CI, 0.936-0.987; P = 0.004].

In the Anti-TPO positive and Anti-TG positive populations, relatively few factors were independently associated with RSA. In the Anti-TPO positive group, TAFI (OR, 0.943; 95% CI, 0.896-0.992; P = 0.025], LYMPH (OR. 1.034; 95% CI, 1.003-1.066; P =0.03), and LY30 (OR, 0.162; 95% CI, 0.035-0.738; P=0.019) were independent predictors of RSA. In the Anti-TG positive group, TAFI (OR, 0.937; 95% CI, 0.884-0.992; P = 0.027), age (OR, 1.133; 95% CI, 1.057-1.215; P < 0.001), Fib (OR, 0.593; 95% CI, 0.393-0.893; P = 0.012). FT_3(OR, 2.205;95% CI, 1.307-3.722; P=0.003), and LY30(OR, 0.433;95% CI, 0.238-0.788; P=0.012) were independent predictors of RSA. In the Anti-TPO negative group, TAFI (OR, 0.965; 95% CI, 0.935-0.996; P = 0.028), and age (OR, 1.067; 95% CI, 1.026-1.11; P = 0.001) were independent predictors of RSA. It is worth noting that TAFI was shown to be an independent protective factor for RSA in all four groups, except in the Anti-TG negative group.

Correlation between plasma TAFI levels and risk of RSA

Table 4 shows the correlations between plasma TAFI levels explored in the binary logistic regression model and the risk of RSA. The total population was divided into three groups according to the normal range of plasma TAFI levels: normal levels of plasma TAFI were defined as 24-35ug/ml, TAFI¡24ug/mL, TAFI¿35ug/mL. The adjusted covariates for each group were the variables that were statistically significant by logistic regression. Compared with TAFI levels of 24-35ug/ml. In the Anti-TPO positive group, the risk of RSA was significantly increased in those with TAFI <24 (OR, 2.15; 95% CI, 1.221-3.785; P=0.008). This result was also seen in the Anti-TPO negative group and the Anti-TG positive group. In the Anti-TPO negative group, the risk of RSA was increased in those with TAFI <24 (OR, 1.429; 95% CI, 1.032-1.98; P=0.032); in the Anti-TG positive group, the risk of RSA was significantly increased in those with TAFI <24 (OR, 2.215; 95% CI, 1.265-3.878; P=0.005). It is important to note that in the Anti-TPO negative group, the OR for the increased risk of RSA in the TAFI <24 population was smaller than in the two-positive group.

As shown in Fig1, the multivariate adjusted spline regression model showed a linear negative dose response relationship between TAFI and RSA in all subgroups, with P values for nonlinearity all greater than 0.05. Of note, in the two positive subgroups, OR value was approximately less than 1 when TAFI levels were within the normal reference range of 24-35 ug/mL. However, when TAFI levels were below 24 ug/mL, the OR value increased and was higher than that in the two negative subgroups. On the other hand, when TAFI levels were above 35 ug/mL, an increasing TAFI level was found to be associated with a higher risk of RSA, but the trend is not obvious. This trend was not observed in the two negative subgroups.

The impact of TAFI on RSA in specific subgroups.

Similar results were observed in the stratified analysis by age, BMI, PT, PTA, APTT, TT, DD, FT_3, FT_4, TSH, Anti-Tpo, and Anti-TG. As shown in Figure 2, The correlation between TAFI and the risk of RSA is more significant in participants with age>32, PT>10.9s, PTA[?]109%, APTT >32.1s, TT [?]16.2s, DD [?]90ug/ml, FT_3>4.42pmol/L, FT_4 [?]13.08pmol/L, TSH >1.64uIU/mL, and Anti-TPO negative.

Discussion:

Main Findings

Previous studies have shown a link between TAFI and RSA, but there has been controversy. These studies tend to focus on the potential effects of TAFI on RSA in terms of coagulation and fibrinolysis. And the presence of ATA in the body can lead to inflammatory reactions, which has caught our attention. Therefore, we conducted a retrospective case-control study to explore the effect of TAFI levels on RSA under different conditions of ATA, and found that when the plasma TAFI level is below the normal range of TAFI, there is a high risk of RSA in the Anti-TPO positive, Anti-TPO negative, and Anti-TG positive populations.

There is a dose-response relationship between plasma TAFI levels and the risk of RSA, which is significantly associated.

Interpretation

A possible explanation for our findings is that TAFI is involved in regulating the immune response and complement-mediated vascular inflammation. High TAFI levels suppress the inflammatory response in vivo and reduce the incidence of miscarriage. TAFI regulates the levels of inflammatory mediators such as C3a, C5a, bradykinin, and bone bridging proteins and suppresses inflammation in vivo by inactivating inflammatory mediators[19, 20]. For instance, it has been shown that there is a significant negative correlation between serum TAFI and C3a levels[21]. Some investigators have identified a role for TAFI in inflammation, and their results suggest that TAFI plays an important role during pneumococcal meningitis, which is likely to be mediated through inhibition of the complement system, and influences the occurrence of inflammation [22]. The presence of ATA in the body leads to an inflammatory and immune response, and a recent meta-analysis suggests that they may increase the risk of pregnancy failure, although the exact mechanism is not yet known[23]. ATA can cause thyroid cell damage through activation of the complement system and cytotoxicity[24], which are associated with the outcome of pregnancy failure. It is reasonable to speculate that TAFI may exert a protective effect on RSA by inhibiting the inflammation caused by ATA. In the study population of two positive groups, there were more inflammatory mediators, and when TAFI was at a low level (<24ug/mL), the anti-inflammatory ability was weaker, which presented as a risk factor.

It is worth noting that when the plasma TAFI level is lower than the normal range of TAFI, the risk of RSA in the Anti-TPO negative population is also increased, but the OR value of 1.429 is significantly smaller than that of the two positive groups. We speculate that this is related to coagulation and fibrinolysis system disorders. A possible explanation for this result is that a low fibrinolytic state may be beneficial for pregnancy. The fibrinolytic system plays an important role in helping with placental implantation, fetal growth, and development during pregnancy [25]. An animal experiment had shown that degradation products of fibrinogen can cause apoptosis of mouse trophoblast cells, leading to fetal loss [10]. Reducing the activation of TAFI can enhance the degradation of fibrinogen, thereby increasing fibrinogen degradation products. Therefore, low levels of TAFI can increase the risk of RSA.

This outcome is supported by several studies on TAFI levels and the risk of RSA. Several European case-control studies provide evidence that high levels of TAFI may be associated with a decreased risk of RSA[11, 26, 27]. These findings have important implications for the prevention and management of RSA. However, there are studies in the Netherlands and Turkey that show no association between the level of TAFI and RSA [12, 28]. In contrast, a retrospective case-control study conducted in Spain showed that patients with RSA had statistically significantly higher TAFI antigen levels compared to other groups [29]. The observed differences in the risk of RSA associated with TAFI levels may be attributed to several reasons. Firstly, varying definitions of RSA exist across studies. Some studies define RSA as three or more consecutive pregnancy losses[11, 28, 29], whereas other studies define RSA as two or more consecutive pregnancy losses. When different definitions are used to diagnose or study RSA, it can result in different populations being included in the study, thereby reducing the reliability of the comparative results. Secondly, different studies have used different methods to measure TAFI levels. Some studies used a colorimetric assay[11, 12], and the enzyme-linked immunosorbent assay (ELISA) method is used in most studies. It is worth mentioning that the different sensitivity of ELISA for various isoforms of TAFI can lead to large differences in TAFI levels among studies [30, 31]. Lastly, TAFI levels may vary by geographic region. Despite the differing conclusions of these studies, our dose-response correlation further supports the notion that increased plasma TAFI levels reduce the risk of RSA in our study population.

In addition, The magnitude of the associations between TAFI and RSA was different in subgroups by age, coagulation indicators, and thyroid function indicators. Age and thyroid function were reported as potential influencing factors of TAFI levels. Specifically, a study indicated a positive correlation between TAFI levels and age [32]. Furthermore, TSH levels were identified as predictors of TAFI levels, and fibrinolytic activity was reduced in patients with impaired thyroid function [33, 34].

Strengths and Limitations

To our knowledge, the present study is the first to examine the relationship between TAFI and the risk of RSA based on the ATA profile. However, this study has several limitations worth mentioning. First, as a single-center study, there are some bias in testing TAFI and other indicators only in patients from a single study center. Second, all participants in this study were from Liaoning province, China, and extrapolation of our results to other populations with different demographic characteristics or to other regions should be done with caution. Third, confounding due to unknown or unmeasured factors, such as maternal medication use during pregnancy, cannot be excluded. Last, our study did not measure inflammatory mediators levels to further validate. More large cohort studies are needed in the future to further investigate and validate our results, and to study whether inflammatory mediators play a key role in RSA. In addition, the pathological mechanisms by which low TAFI levels lead to an increased risk of RSA need to be further investigated.

Conclusions: In summary, our study suggests that pregnant women with plasma TAFI levels below the normal range, especially in ATA-positive populations, are at higher risk for RSA. Therefore, pregnant women with low TAFI levels and ATA-positive should be vigilant for the occurrence of RSA, which has made a certain contribution to the prevention of RSA.

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

Authors' roles

Concept or design: PM, WH, YW, JD; **Acquisition of data:** PM, WH, YW; **Analysis or interpretation of data:** PM, WH, PZ, GZ, YY, JG; **Drafting of the manuscript:** PM, WH, JD; **Critical revision of the manuscript for important intellectual content:** PM, WH, PZ, GZ, YY, JG, YW, JD. All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity. All authors read and approved the final manuscript

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Conflict of interest

None declared.

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Figure and table legends

Fig 1 Multivariable-adjusted association of TAFI with risk of RSA by restricted cubic regression. The shaded area represents a 95% confidence interval. The solid blue line symbolizes the odds ratio (OR), and the red vertical line indicates the TAFI value corresponding to an OR of 1. Adjustment of covariates is based on variables with statistically significant effects in the multivariate logistic regression analysis of each group.

Fig 2 Unconditional logistic regression models were conducted in stratified analysis by age (median), BMI (median), PT (median), PTA (median), APTT (median), TT (median), DD (median), FT3 (median), FT4 (mean), TSH (median), Anti-TPO (negative or positive), Anti-TG (negative or positive). All models were adjusted for Age, PT, DD, LYMPH, RDW-CV, FT3, FT4, TSH, and Angle, except for the stratifying variables per se.

Table 1 Basic characteristics of pregnant women in the case group and control group (Anti-TPO positive and Anti-TPO negative groups).

Table 2 Basic characteristics of pregnant women in the case group and control group (Anti-TG positive and Anti-TG negative groups).

Table 3 Multifactorial logistic analysis of independent risk factors for RSA in different groups.

Table 4 Correlation between plasma TAFI levels and risk of RSA under different groups (normal range of TAFI as reference).

Table S1 Correlation of variables with RSA in the overall population.

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