Association of serum levels of vascular endothelial growth factor and placental growth factor in early threatened abortion and premature delivery: A case-control study

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Abstract. Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) serve key roles in the regulation of vascular development, revascularization and vasopermeability in the endometrium, decidua and trophoblasts. Furthermore, both VEGF and PlGF are modulators of embryonic vascular development. Thus, the present study aimed to investigate the serum levels of VEGF and PlGF in female patients with early threatened abortion (TA) who experienced preterm delivery. The present case-control study included 130 pregnant patients with or without TA that were admitted to The Maternal and Childcare Hospital of Nantong University from January 2019 to January 2022. Patients were divided into two groups: i) Group A, which included 55 patients diagnosed with TA with slight vaginal bleeding and closed cervical internal os within the first 6-12 weeks of pregnancy; and ii) group B, which included 75 patients with healthy asymptomatic pregnancy. Blood samples were obtained from all patients and VEGF and PlGF levels were examined prior to treatment, and the chi-squared, Student's t-test and two-way ANOVA followed by Bonferroni's post hoc analysis were used to analyze statistical differences between the two patient groups. Results of the present study demonstrated that patients with TA had significantly lower levels of VEGF and PlGF, compared with the controls. In patients with or without TA, the levels of serum PlGF in the preterm delivery group were significantly decreased compared with patients that did not experience preterm delivery. However, there was no significant difference in the levels of VEGF between patients with or without preterm delivery. In addition, lower levels of PlGF, compared with those in patients without TA, may be associated with an increased risk of preterm delivery in patients without early TA.

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Introduction

The World Health Organization defines abortion as the termination of pregnancy before 20 weeks of pregnancy or a fetal birth weight of <500 g (1). Threatened abortion (TA) occurs in 30-40% of pregnancies (2,3), and is diagnosed as a combination of vaginal bleeding, a closed cervix and the presence of the fetal heartbeat (4). The probability of abortion during the first 12 weeks of pregnancy is >80% (5). Following the development of China's Three Child Policy, the number of pregnant women has increased in China, which may increase the incidence of TA (6). Although TA is not directly associated with increased maternal morbidity or mortality, it may be associated with preterm delivery, premature rupture of membranes, low birth weight, fetal growth limit, placental abruption and an increase in cesarean sections (1). Furthermore, TA may have a negative impact on the mental health of patients (7). Notably, preterm delivery is defined as delivery prior to 37 weeks of pregnancy. Preterm delivery accounts for 5-10% of all deliveries worldwide and is associated with ~75% of neonatal mortality and more than 50% of long-term newborn morbidities, including neurological impairments and chronic lung diseases, as well as learning disability and, psychological, behavioral and social troubles (8,9).

The mechanisms underlying TA are complex and pathogenesis may be associated with infection (10), thyroid issues, endocrinopathy (11), genital deformity (12) chromosomal mutations (13), immune system collapse, diabetes, increased maternal age, previous abortion, passive smoking, obesity and a history of drug or alcohol consumption (14,15). At present, research is focused on the potential association between TA and the placenta. The most common source of vaginal bleeding in early pregnancy is the placenta (16). Placental defects may cause the placenta to synthesize and release angiogenic and anti-angiogenic factors in the feto-maternal circulation, such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) (16). VEGF is an angiogenic factor that serves a role in full-term pregnancy,

contributing to implantation and placental growth (17). In addition, PlGF is a member of the VEGF family and acts as a modulator of decidual angiogenesis and a mediator of trophoblast function (18). However, the specific roles of VEGF and PlGF in TA are yet to be fully elucidated. Thus, the present study aimed to determine the serum levels of VEGF and PlGF in patients with early TA who experienced preterm delivery.

Materials and methods

Patients. The present case-control study included a total of 130 pregnant female patients with or without TA and with or without preterm delivery. All patients were admitted to The Maternal and Childcare Hospital of Nantong University (Jiangsu, China) from January 2019-January 2022, and were aged 20-35 years and all cases were singleton pregnancies. Patients were divided into two groups: i) Group A, which included 55 patients diagnosed with TA with slight vaginal bleeding and closed cervical internal os within the first 6-12 weeks of pregnancy; and ii) group B, which included 75 patients with healthy asymptomatic pregnancies. Premature delivery refers to childbirth at 28 weeks of pregnancy but less than 37 weeks. Both groups of patients were evaluated via vaginal examination. The present study was approved by The Medical Ethics Committee of The Maternal and Childcare Hospital of Nantong University (approval no. Y2018036).

The inclusion criteria for the present study were as follows: i) Gestational age, 6-12 weeks; ii) singleton pregnancy; iii) patient age, 20-35 years; iv) termly prenatal visits; v) parity <3; and vi) previous medical abortion due to unexpected pregnancy.

Patients were excluded from the present study due to the following criteria: i) Patient age, <20 or >35 years; ii) reproductive tract malformation; iii) multiple gestations; iv) chronic systemic illness or acute infectious disease; v) exposure to high levels of alcohol or nicotine; and vi) a history of natural abortion or any pregnancy complications, such as gestation-mediated hypertension, gestational diabetes mellitus, intrauterine growth limit, intrauterine mortality, preterm delivery, early rupture of membranes or any previous medical history, such as asthma, antiphospholipid antibody syndrome, thyroid dysfunction, tuberculosis and other chronic health disorders.

ELISA. A 3 ml peripheral blood sample was obtained from each participant and stored in EDTA (1.5 mg/ml). Blood samples were separated via centrifugation at 670.8 x g for 10 min at room temperature, and subsequently stored at -80˚C. Levels of VEGF (catalog no. ZK-2291; Zhenke Biology) and PlGF (catalog no. ZK-2017; Zhenke Biology) were quantified using commercially available ELISA kits.

Statistical analysis. Statistical analysis was carried out using SPSS software (version 20.0; IBM Corp.). Differences between groups were analyzed using the chi-squared test, unpaired Student's t-tests and two-way ANOVA followed by Bonferroni's post hoc analysis and presented as mean ± standard deviation, whereas counting data were examined using a chi-squared test and presented as percentages. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient information and preterm delivery rates of patients with or without TA. There were not statistically significant differences in the maternal age, BMI prior to pregnancy, BMI at study enrollment, gravidity or parity between patients with TA and patients with healthy pregnancies (Table I). The preterm birth rate in patients with TA was 21.8% and was 12.0% in patients with healthy pregnancies. Notably, there were no significant differences between the two groups of patients (Table I).

Comparison of VEGF levels (pg/ml) in patients with or without TA and preterm delivery. Patients with TA had significantly lower VEGF levels compared with patients with healthy pregnancies (Table I). There were no significant differences in the levels of VEGF between patients with and without preterm delivery in the patients with or without TA (Table II). No interaction effect was revealed between the two main effects (TA and preterm delivery).

Comparison of PlGF levels (pg/ml) in patients with or without TA and preterm delivery. The levels of PlGF were significantly lower in patients with TA compared with patients with healthy pregnancies (Table I). In patients with or without TA, the levels of serum PlGF in the preterm delivery group were significantly decreased compared with the group that did not experience preterm delivery (Table III). A statistically significant interaction effect between the two main effects was demonstrated (F=8.45, P<0.01) and further cross-comparisons were performed. In the case of preterm delivery, the simple effect of TA was statistically significant (F=7.38, P<0.05). In the case of no preterm delivery, the simple effect of TA was statistically significant (F=176.10, P<0.01). In the case of TA, the simple effect of preterm delivery was not statistically significant (F=2.14, P>0.05). In the case of no TA, the simple effect of preterm delivery was statistically significant (F=27.99, P<0.01).

Discussion

VEGF serves a role in the physiological or pathological anti-placental formation and f previous studies have reported that VEGF is involved in placental angiogenesis (19,20). In the present study, the mean serum level of VEGF in patients with TA was 62.15±10.87 pg/ml, compared with 84.39±10.00 pg/ml in patients with healthy pregnancies. These results demonstrated that the levels of VEGF were lower in patients with TA. Furthermore, the mean serum level of VEGF in patients that experienced preterm delivery was 63.43±4.40 pg/ml, compared with 61.79±12.10 pg/ml in patients that did not experience preterm delivery. The mean serum level of VEGF in patients with healthy pregnancies that experienced preterm delivery was 84.60±10.46 pg/ml, compared with 84.37±10.02 pg/ml in patients that did not experience preterm delivery. Therefore, there was no significant difference in the levels of VEGF

Table I. Patient information and preterm delivery rates and VEGF, PIGF levels of patients with or without TA.

Patient information	Patients with TA (n=55)	Patients without TA (n=75)	P-value
Age, years	29.35±3.23	28.71±3.31	>0.05
BMI before pregnancy, kg/m^2	21.50±2.10	21.39±1.87	>0.05
Enrolled pregnancy BMI, kg/m^2	27.88±3.15	28.68±3.00	>0.05
Gravidity	1.67±0.75	1.59±0.74	>0.05
Parity	0.38±0.68	0.39±0.64	>0.05
Preterm labor, n (%)			
Yes	12 (21.8%)	9 (12.0%)	>0.05
No	43 (78.2%)	66 (88.0%)	>0.05
VEGF levels, pg/ml	62.15±10.87	84.39±10.00	<0.01
PlGF levels, pg/ml	39.71±20.70	110.88±37.76	<0.01

Data are expressed as mean ± standard deviation. TA, threatened abortion.

Table II. Comparison of VEGF levels (pg/ml) in patients with or without TA and preterm delivery.

				TA main effect		Preterm delivery main effect	
Patient group	Preterm delivery	No preterm delivery	P-value	F-value	P-value	F-value	P-value
TA	63.43±4.40	61.79±12.10	>0.05	75.23	<0.01	0.14	>0.05
No TA	84.60±10.46	84.37±10.02	>0.05				
P-value	<0.01	<0.01					

Data are expressed as mean ± standard deviation. TA, threatened abortion.

Table III. Comparison of PlGF levels (pg/ml) in patients with or without TA and preterm delivery.

				TA main effect		Preterm delivery main effect	
Patient outcome	TA	No TA	P-value	F-value	P-value	F-value	P-value
Preterm delivery	28.99±17.89	63.39±19.58	<0.01	61.79	<0.01	23.86	<0.01
No preterm delivery	42.70±20.64	117.36±34.95	<0.01				
P-value	<0.01	<0.01					

Data are expressed as mean ± standard deviation. TA, threatened abortion.

between patients that experienced preterm delivery and those that did not.

PlGF is a dimer glycoprotein that is mainly released by the placenta. PlGF is associated with angiogenesis and serves a role in the growth and differentiation of trophoblasts. The circulating level of PlGF increases during pregnancy, and this serves a role in the progression and maturation of the placental vascular system (18). In the first three months of a healthy pregnancy, the levels of PlGF are low. After this time, levels of PlGF subsequently increase and plateau at ~30 weeks of pregnancy prior to decreasing again (21). Results of the present study demonstrated that the mean serum level of PlGF in patients with TA was 39.71±20.70 pg/ml, compared with 110.88±37.76 pg/ml in patients with healthy pregnancies. These results demonstrated that serum levels of PlGF were significantly reduced in patients with TA. Furthermore, the mean serum level of PlGF in patients with TA that experienced preterm delivery was 28.99±17.89 pg/ml, compared with 42.70±20.64 pg/ml in patients with TA that did not experience preterm delivery. In addition, the mean serum level of PlGF in patients with healthy pregnancies that experienced preterm delivery was 63.39±19.58 pg/ml, compared with 117.36±34.95 pg/ml in patients with healthy pregnancies that did not experience

preterm delivery. Collectively, these results demonstrated that the levels of PlGF in patients without TA were lower in patients that experienced preterm delivery, compared with patients that did not.

The prediction of TA is complex, as predictive methods are unreliable (22) and the associated underlying mechanisms remain to be fully elucidated. A number of previous studies have focused on the association between human chorionic gonadotropin and estrogen in TA (23,24). However, the present study aimed to determine the association between the serum levels of VEGF and PlGF in patients with early TA that experienced preterm delivery. Notably, the results of previous studies are inconsistent (25-29). Eskicioglu *et al* (25) investigated the roles of numerous angiogenic factors in pregnancy losses that occurred during the first trimester, and revealed that the expression levels of VEGF were lower in patients that experienced pregnancy loss compared with the women that did not experience pregnancy loss during the first trimester. Furthermore, Muttukrishna *et al* (26) demonstrated that the levels of PlGF were reduced in patients with TA that experienced miscarriage, compared with patients with TA that experienced full-term pregnancy. In addition, patients with TA that experienced miscarriage had reduced levels of PlGF compared with asymptomatic gestational patients. In a case-control study by Dev *et al* (27), pregnant patients were divided into three groups according to gestational age (6-10, 11-15 and 16-20 weeks). The serum levels of VEGF and PlGF were markedly decreased in patients with TA, compared with patients with healthy pregnancies. However, there were no differences in the levels of serum VEGF and PlGF between patients with different gestational ages. Hussein *et al* (28) revealed that the concentration of PlGF was reduced in patients that experienced preterm delivery compared with women without preterm delivery. Thus, results of the previous study (28) highlighted that reduced expression levels of PlGF may act as a potential biomarker for the prediction of preterm delivery. However, Keskin *et al* (29) revealed that serum levels of VEGF-A were markedly increased in patients with TA, compared with patients with healthy pregnancies without TA. However, there were no differences in the levels of PlGF between the two groups.

In conclusion, the results of the present study demonstrated that patients with TA had reduced levels of VEGF and PlGF compared with patients without TA. In addition, patients that experienced preterm delivery had reduced levels of PlGF compared with patients that did not experience preterm delivery. However, the sample size of the present study was small and geographical factors could have influenced the findings. Predicting and preventing TA remains a challenge for clinicians; thus, further investigations into the mechanisms underlying TA are required. In addition, VEGF and PlGF could potentially be used as biomarkers for preterm delivery in patients with TA.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PZ conceptualized the study and methodology, performed data analysis and investigations and drafted the manuscript. YJ analyzed and visualized data and wrote and edited the manuscript. XH interpretated and visualized data, reviewed and edited the manuscript, supervised the project and conducted project administration. PZ and YJ confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of the Maternal and Childcare Hospital of Nantong University (approval no. Y2018036). All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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