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Diagnostic and Prognostic Value of Deregulated Long Non-Coding RNA Plasmacytoma Variant Translocation 1 in Patients with Gestational Hypertension

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This study aimed to investigate the serum plasmacytoma variant translocation 1 (PVT1) level in pregnant women with gestational hypertension and pre-eclampsia and its diagnostic value for diseases and its influence on pregnancy outcome. Serum PVT1 levels in 72 pregnant women with gestational hypertension, 72 pregnant women with pre-eclampsia and 71 healthy pregnant women were evaluated by RT-qPCR, and the diagnostic significance of PVT1 for gestational hypertension was verified by receiver operator characteristic (ROC) curve. The correlation between PVT1 and clinical indicators were evaluated by Pearson correlation coefficient method. Logistic regression analysis evaluated the influencing factors in the development process of gestational hypertension to pre-eclampsia. The effect of PVT1 level on pregnancy outcome was evaluated by prognostic analysis. Results showed that PVT1 level was down-regulated in gestational hypertension group compared with healthy control group, whereas PVT1 level was down-regulated more significantly in pre-eclampsia group than in gestational hypertension group. ROC curve showed that PVT1 had high diagnostic accuracy for gestational hypertension. Pearson correlation coefficient and multiple linear regression analysis showed that PVT1 was correlated with systolic blood pressure, diastolic blood pressure, interleukin (IL)-6 and tumor necrosis factor-*α*. Logistic regression analysis revealed that IL-6 and PVT1 were the influencing factors of gestational hypertension to pre-eclampsia transition. Moreover, prognostic analysis manifested that the incidence of fetal growth restriction in low PVT1 expression group was significantly higher than that in high PVT1 expression group. The expression level of PVT1 has a high diagnostic accuracy for gestational hypertension, and the low PVT1 expression group is more prone to fetal growth restriction.

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Introduction

Hypertensive disorders of pregnancy (HDP) are a group of diseases including gestational hypertension (GH), pre-eclampsia (PE), pregnancy complicated with chronic hypertension, chronic hypertension complicated with pre-eclampsia (Zhang et al. 2018; Liu et al. 2021). Patients may be accompanied by systemic multiple organ dysfunction, such as renal insufficiency, liver damage, nervous system, and blood system complications (Joo et al. 2021). Without effective control and treatment, severe cases may lead to convulsions, coma, and even maternal and infant death (Wang et al. 2019b). The worldwide incidence of HDP is about 4.6%, which is one of the main reasons that affect the health and mortality of pregnant women and perinatal infants (Shao et al. 2021). According to the study, HDP patients and their offspring have significantly increased incidence of cardiovascular and nervous system diseases compared with normal pregnant women (Hou et al. 2020). Thus, the early diagnosis and timely treatment of this kind of disease is particularly important.

Recently, many studies have suggested that non-coding RNAs may be involved in the pathogenesis of HDP. Long non-coding RNA (lncRNA) is related to the occur-

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rence and development of cancer, cardiovascular diseases, and many diseases by regulating gene expression at transcription level and post-transcription level. It has been reported that a variety of lncRNAs, including CEACAMP8, LOC284100, are abnormally expressed in HDP patients (He et al. 2013; Wang et al. 2018). Ou et al. (2020) found that silencing MALAT1 expression alleviated hypertension symptoms in HDP model rats. Plasmacytoma variant translocation 1 (PVT1), located on chromosome 8q24, was initially identified as an oncogene in solid tumors such as cervical cancer, ovarian cancer, and lung cancer (Li et al. 2020). A previous study showed that the expression level of PVT1 was significantly lower in patients with recurrent spontaneous abortions (RSA) than that of normal pregnancy group (Yang et al. 2020). Wang et al. (2019a) reported that the expression of PVT1 in placenta tissue of gestational diabetes mellitus (GDM) and eclampsia patients was significantly lower than that of normal placenta tissue, and the downregulation of PVT1 significantly inhibited the proliferation and migration of trophoblast cells. Thus, PVT1 does play a certain regulatory role in pregnancy-related diseases.

At present, there are few clinical studies on the effect of PVT1 on GH and PE. This study preliminarily evaluated the clinical diagnostic ability of PVT1 for GH and the influence of PVT1 expression level on the pregnancy outcome of pregnant women with GH and PE by detecting the difference in the expression level of PVT1 in the blood of GH, PE, and healthy pregnant women.

Materials and Methods

Study population and sample collection

The study recruited 144 pregnant women with HDP, including 72 GH patients and 72 PE patients. In addition, 71 healthy pregnant women who came to the hospital for prenatal examination during the same period were selected as healthy control (HC) group. The diagnosis of HDP was based on American College of Obstetricians and Gynecologists (2019). Inclusion criteria of GH are: in the late pregnancy, usually more than 20 weeks of gestation, women had systolic blood pressure (SBP) ≥ 140 mmHg and diastolic blood pressure (DBP) ≥ 90 mmHg. Pre-eclampsia (PE) refers to the symptoms of headache, dizziness, nausea, vomiting and epigastric discomfort in pregnant women on the basis of hypertension and proteinuria, which generally occurs after 20 weeks of pregnancy. Inclusion criteria for PE are: in the third trimester, pregnant women developed GH (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg) with proteinuria (urinary protein ≥ 0.3 g/24 h). All the above study subjects were singleton pregnancies. Pregnant women with multiple pregnancies, diabetes, essential hypertension, infectious disease, autoimmune disease, hepatic and renal dysfunction were excluded from this study.

This study followed the Declaration of Helsinki and was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital, Capital Medical University. All subjects voluntarily participated in the study and signed informed consent.

General information and relevant clinical data were collected and recorded, and were summarized in Table 1. Blood pressure was measured with a standard mercury sphygmomanometer. Beckman AU5421 automatic blood biochemical analyzer was used to detect triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Serum levels of interleukin (IL)-6 and tumor necrosis factor (TNF)-α were detected by the commercial human ELISA kit (USCNK Life Science, Wuhan, China).

Fasting venous blood of 5 mL was collected from all subjects in the morning of the next day after enrollment, centrifuged at 3,500 × g for 5 min, and the serum was separated and stored in a −20°C refrigerator for subsequent experiments.

Table 1. Basic clinical information of the subjects.

Measurements	Healthy control (HC) n = 71	Gestational hypertension (GH) n = 72	Pre-eclampsia (PE) n = 72
Age (years)	28.14 ± 2.80	28.38 ± 2.94	28.15 ± 2.71
Pregnancy (week)	35.89 ± 2.00	36.00 ± 1.98	36.25 ± 1.77
BMI (kg/m^2)	25.07 ± 3.18	25.21 ± 2.79	25.97 ± 3.73
TG (mmol/L)	1.24 ± 0.22	1.26 ± 0.29	1.31 ± 0.32
HDL-C (mmol/L)	1.28 ± 0.28	1.21 ± 0.16	1.21 ± 0.22
LDL-C (mmol/L)	2.06 ± 0.45	2.10 ± 0.38	2.16 ± 0.59
SBP (mmHg)	112.93 ± 8.88	146.44 ± 8.66***	149.31 ± 18.25***
DBP (mmHg)	73.42 ± 4.84	93.44 ± 2.03***	94.90 ± 6.24***
IL-6 (ng/ml)	409.48 ± 24.31	608.89 ± 18.64***	616.80 ± 20.10$^{***,\#\#}$
TNF-α (ng/ml)	29.22 ± 4.99	46.12 ± 5.39***	48.24 ± 7.28$^{***,\#\#}$

Data are expressed as n or mean ± SD. $^{***}P < 0.001$, compared with healthy control; $^{\#\#}P < 0.05$, compared with gestational hypertension.

BMI, body mass index; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; IL-6, Interleukin-6; TNF-α, tumor necrosis factor-α.

RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR) assay

RNAs were extracted from serum using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The purity and concentration of RNA were detected by spectrophotometer. The ratio of OD260 to OD280 of RNA with the purity meeting the requirements was 1.9 to 2.0. 300 ng extracted RNAs were reverse transcribed into cDNA using a cDNA synthesis kit (SuperScript II Reverse Transcriptase kit, Qiagen, Germantown, MD, USA). Subsequently, RT-qPCR analysis was conducted by miScript SYBR® Green PCR kit (Qiagen) on the Light Cycler 480 Real-Time PCR system. GAPDH gene was used as the house-keeping gene for normalizing the expression of PVT1. The sequences required for this section are as follows: PVT1: 5'-AGAACTGTCCTTACGTGACC-3' (forward primer) and 5'-AGAGCACCAAGACTGGCTCT-3' (reverse primer). GAPDH: 5'-ACTAGGCGCT CACTGTTCT-3' (forward primer) and 5'-ATCCGTTGACTCCGACCTTC-3' (reverse primer). Each sample was measured in triplicate.

Effect of PVT1 expression level on pregnancy outcome

Subjects in the GH and PE groups were combined into one group, and pregnant women were divided into PVT1 high expression group and PVT1 low expression group according to the mean value of PVT1 expression level, and fetal growth restriction, fetal distress, neonatal asphyxia, neonatal death, and other special events occurred during delivery of each group were recorded.

Statistical analysis

Statistical data were analyzed using GraphPad Prism 6.0 and SPSS 22.0. All data were expressed as mean ± standard deviation (SD). Differences between two groups were evaluated by student t-test, and differences among multiple groups were analyzed by one-way analysis of variance (ANOVA). Receiver operator characteristic (ROC) curve was established to assess the diagnostic value of PVT1 for GH. Pearson correlation coefficient method was performed to evaluate the correlation between PVT1 level and clinical indicators in pregnant women with GH and PE. A multiple regression analysis was then used to assess the independent association between these clinical indicators and PVT1 levels. Logistic regression analysis assessed the value of different variables in the progression of GH to PE.

Results

Demographic and clinical data

Basic clinical information of all subjects in this study is shown in Table 1. There were no significant differences in age, pregnancy, BMI, TG, HDL-C, and LDL-C ($P > 0.05$) among HC, GH and PE groups. In addition, the levels of SBP, DBP, IL-6 and TNF-α in GH and PE groups were significantly up-regulated compared with HC group ($P < 0.001$). Notably, IL-6 and TNF-α levels were dramatically higher in the PE group than in the GH group ($P < 0.01$).

Expression level of serum PVT1 in all groups

As can be seen from Fig. 1, the expression of PVT1 showed a significant downregulation trend in the GH group compared with the HC group ($P < 0.001$). Interestingly, we also found that the level of PVT1 was lower in the PE group than in GH group ($P < 0.001$).

The diagnostic value of serum PVT1 for GH

As shown in Fig. 2, the ROC curve illustrated that serum PVT1 had a good specificity for distinguishing GH patients from the HC group. When the cut-off value was

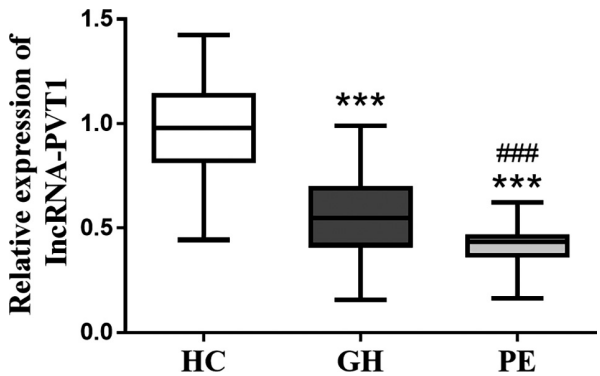

Fig. 1. The expression level of serum long non-coding RNA PVT1 (lncRNA PVT1) in all subjects.
The expression of PVT1 was significantly lower in the gestational hypertension group (GH) and much lower in the pre-eclampsia group (PE) than in the healthy control group (HC). $^{***}P < 0.001$ vs. HC, $^{\#\#\#}P < 0.001$ vs. GH. Data are represented as box plots charts (boxes show means and upper and lower quartiles of the data and whiskers indicate minimum and maximum values).

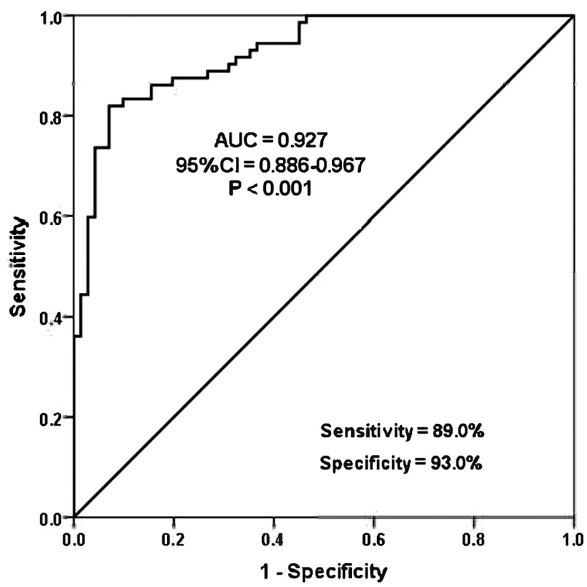

Fig. 2. Receiver operator characteristic (ROC) analysis of long non-coding RNA PVT1 (lncRNA PVT1).
PVT1 had the ability to distinguish GH patients from healthy controls (HC) (AUC = 0.927, sensitivity = 89.0%, specificity = 93.0%).

0.881, the area under the curve (AUC) was 0.927, 95% confidence interval (CI) was 0.886 to 0.967, and the sensitivity and specificity of PVT1 were 89.0% and 93.0%, respectively.

Association between serum PVT1 and clinical indicators

As shown in Table 2, Pearson correlation coefficient analysis showed that serum PVT1 levels were negatively associated with SBP ($r = -0.683$, $P < 0.001$), DBP ($r = -0.499$, $P < 0.001$), IL-6 ($r = -0.563$, $P < 0.001$), and TNF-α ($r = -0.686$, $P < 0.001$). Besides, in the multiple linear regression model, only SBP, DBP, IL-6 and TNF-α were significantly associated with PVT1 among many clinical indicators as independent variables ($P < 0.001$).

Independent influence of various indicators on the development of GH to PE

As illustrated in Table 3, logistics regression analysis showed that both TNF-α (OR = 0.411, 95% CI = 0.187-0.905, $P = 0.027$) and PVT1 (OR = 0.185, 95% CI = 0.082-0.417, $P < 0.001$) were the independent influencing factors in the progression from GH to PE.

Effect of PVT1 on pregnancy outcome

Table 4 showed the statistics of pregnancy-specific events in 144 cases. From the results, it is not difficult to see that the occurrence of gestational hypertension does have a negative impact on the pregnancy outcome. Fetal growth restriction, fetal distress, neonatal asphyxia, and neonatal death occurred to varying degrees in both high and

Table 2. Correlation analysis between PVT1 and various indicators.

Characteristics	Bivariate correlation		Multiple linear regression			
	r	*P*-value	Coefficient	Standard error	t	*P*-value
Age (years)	0.075	0.372	0.005	0.003	1.728	0.086
Pregnancy (week)	− 0.115	0.169	− 0.007	0.005	− 1.494	0.138
BMI (kg/m^2)	0.095	0.256	− 0.003	0.003	− 1.025	0.307
TG (mmol/L)	− 0.001	0.995	− 0.001	0.028	− 0.018	0.985
HDL-C (mmol/L)	0.031	0.712	− 0.024	0.043	− 0.547	0.586
LDL-C (mmol/L)	− 0.034	0.687	0.034	0.017	1.944	0.054
SBP (mmHg)	− 0.683	< 0.001	− 0.012	0.002	− 6.770	< 0.001
DBP (mmHg)	− 0.499	< 0.001	0.030	0.005	5.812	< 0.001
IL-6 (ng/ml)	− 0.563	< 0.001	− 0.002	0	− 5.269	< 0.001
TNF-α (ng/ml)	− 0.686	< 0.001	− 0.011	0.002	− 4.548	< 0.001

BMI, body mass index; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; IL-6, Interleukin 6; TNF-α, tumor necrosis factor-α.

Table 3. Relationship between different variables and the development of gestational hypertension into pre-eclampsia.

Characteristics	OR	95% CI	*P*-value
Age (years)	0.603	0.271-1.342	0.215
Pregnancy (week)	0.565	0.250-1.277	0.170
BMI (kg/m^2)	0.526	0.240-0.152	0.108
TG (mmol/L)	0.466	0.214-1.012	0.054
HDL-C (mmol/L)	1.378	0.632-3.003	0.420
LDL-C (mmol/L)	0.765	0.331-1.771	0.532
SBP (mmHg)	0.448	0.197-1.017	0.055
DBP(mmHg)	0.490	0.216-1.110	0.087
IL-6 (ng/ml)	0.778	0.331-1.827	0.564
TNF-α (ng/ml)	0.411	0.187-0.905	0.027
LncRNA PVT1	0.185	0.082-0.417	< 0.001

OR, odds ratio; CI, confidence interval; BMI, body mass index; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; IL-6, Interleukin 6; TNF-α, tumor necrosis factor-α; LncRNA PVT1, long non-coding RNA plasmacytoma variant translocation 1.

Table 4. Correlation between lncRNA PVT1 expression and pregnancy outcome.

Pregnancy outcome	Cases (n = 144)	lncRNA PVT1 expression		*P*
		Low (n = 87)	High (n = 57)	
Fetal growth restriction				
Yes		12	2	0.042
No		75	55	
Fetal distress				
Yes		10	3	0.09
No		77	54	
Neonatal asphyxia				
Yes		4	1	0.362
No		83	56	
Neonatal death				
Yes		2	0	0.249
No		85	57	

low PVT1 expression group. Notably, in this study, we found that fetal growth restriction was significantly more likely to occur in the low PVT1 expression group ($P < 0.05$).

Discussion

HDP is a special disease occurring during pregnancy, mainly characterized by edema, proteinuria, and hypertension (Hansson et al. 2015). In severe cases, diffuse intravascular coagulation, placental abruption, heart failure, cerebral hemorrhage, coma, convulsion and even death may occur (Cao et al. 2019). At present, the etiology and pathogenesis of HDP have not been clearly defined, and its diagnosis depends on clinical manifestations and laboratory indicators. There is a lack of reliable and early diagnosis methods in the stages of pregnancy, which cannot achieve the purpose of early intervention. Therefore, it is very important to improve the prediction ability of HDP in clinical diagnosis for improving maternal and infant outcomes and prognosis. Currently, there are many commonly used prenatal screening indicators of HDP, including height and weight of pregnant women, color Doppler ultrasound of uterine artery, urinary protein and mean arterial pressure, but the predictive value of each indicator varies greatly (Wang et al. 2021; Wei et al. 2021). Blood pressure is the golden standard for evaluating HDP. However, due to individual differences of HDP pregnant women before 20 weeks of pregnancy, some pregnant women's blood pressure did not increase much or even fluctuate in the early stage, which affected the judgment of medical workers to a certain extent. Color Doppler ultrasound of uterine artery and urinary protein are both auxiliary indicators, which have low predictive value when used alone, so they often need to be used together. Overall, there is still a lack of a specific clinical predictor.

More and more research on diseases is focused on the field of genetics, and the role of non-coding RNAs in pregnancy-related diseases is gradually revealed (Gonzalez Plaza 2020). In this study, we found that the expression of lncRNA PVT1 in serum of HDP patients was abnormal, which was down-regulated in GH group and further declined in PE group. Our data preliminarily showed that PVT1 has a high degree of accuracy in distinguishing the GH group from the healthy group, which demonstrated the clinical value of PVT1 as a diagnostic biomarker. PVT1 has previously been studied as a cancer gene and has been found to play a regulatory role in pregnancy-related diseases in humans in recent decades. PVT1 expression has been reported to be reduced in patients with severe preeclampsia, and this differential expression makes PVT1 a potential prognostic marker (Xu et al. 2018). In the study of pregnancy complications, Qin et al. (2019) found that PVT1 expression was down-regulated in villus samples of spontaneous abortion compared with induced abortion, and they found in vitro that PVT1 may play a regulatory role in disease by regulating the PVT1/miR-424 /eIF5A pathway. The above data supported the results of our study and further verified the abnormal expression of PVT1 in HDP.

In our study, the analysis of clinical data revealed that abnormal blood pressure and inflammatory indicators were associated with PVT1 in the case group, and PVT1 was also found to be an important independent factor in the transition from GH to PE. Additionally, low levels of serum PVT1 have been associated with adverse pregnancy outcomes. Some scholars believe that there is a slight inflammatory reaction in pregnancy itself, which will not cause any symptoms of the pregnant women (Jabbour et al. 2009). Redman et al. (1999) confirmed that the number of activated white blood cells in the blood of HDP patients was much higher than that in normal inflammatory response, so they believed that the vascular endothelial damage induced by HDP was only a part of the systemic inflammatory response. Moreover, Borzychowski et al. (2006) successfully established an animal model of GH by injecting endo-

toxin into mice and confirmed the correlation between inflammation and GH. And they also observed higher levels of inflammatory factors in the serum or placenta in women with PE than in pregnant women with normal blood pressure (Borzychowski et al. 2006). Not only can PE cause heart, liver, brain, and kidney failure, but it can also lead to premature abortion or stillbirth (Hsieh-Lo et al. 2020). If GH cannot be controlled timely and effectively, it is easy to develop into severe PE, which is a serious threat to the life safety of both the pregnant women and the fetus (Li et al. 2021).

At present, although the research on the pathogenesis of HDP is deepening, the specific pathogenesis has not been fully clarified. There are many drugs for the prevention and treatment of HDP in clinic, but there is still a lack of drugs with definite efficacy and good safety. Therefore, research on the pathogenesis of HDP should be continuously strengthened, and effective drug intervention should be actively implemented to better improve the pregnancy outcomes of HDP patients and ensure the safety of mothers and infants. In summary, combined with clinical data, this study revealed that the decreased expression of PVT1 is closely related to the pathophysiological processes of GH and PE. The decrease of PVT1 not only shows high accuracy in the diagnosis of GH, but also provides reference value for predicting adverse pregnancy outcomes.

Conflict of Interest

The authors declare no conflict of interest.

References

American College of Obstetricians and Gynecologists (2019) ACOG Practice Bulletin No. 202: gestational hypertension and preeclampsia. *Obstet. Gynecol.*, **133**, 1.

Borzychowski, A.M., Sargent, I.L. & Redman, C.W. (2006) Inflammation and pre-eclampsia. *Semin. Fetal Neonatal. Med.*, **11**, 309-316.

Cao, Y., Wang, M., Yuan, Y., Li, C., Bai, Q. & Li, M. (2019) Arterial blood gas and acid-base balance in patients with pregnancy-induced hypertension syndrome. *Exp. Ther. Med.*, **17**, 349-353.

Gonzalez Plaza, J.J. (2020) Small RNAs as fundamental players in the transference of information during bacterial infectious diseases. *Front. Mol. Biosci.*, **7**, 101.

Hansson, S.R., Naav, A. & Erlandsson, L. (2015) Oxidative stress in preeclampsia and the role of free fetal hemoglobin. *Front. Physiol.*, **5**, 516.

He, X., He, Y., Xi, B., Zheng, J., Zeng, X., Cai, Q., Ouyang, Y., Wang, C., Zhou, X., Huang, H., Deng, W., Xin, S., Huang, Q. & Liu, H. (2013) LncRNAs expression in preeclampsia placenta reveals the potential role of LncRNAs contributing to preeclampsia pathogenesis. *PLoS One*, **8**, e81437.

Hou, Y., Yun, L., Zhang, L., Lin, J. & Xu, R. (2020) A risk factor-based predictive model for new-onset hypertension during pregnancy in Chinese Han women. *BMC Cardiovasc. Disord.*, **20**, 155.

Hsieh-Lo, M., Castillo-Herrera, G. & Mojica, L. (2020) Black bean anthocyanin-rich extract from supercritical and pressurized extraction increased in vitro antidiabetic potential, while having similar storage stability. *Foods*, **9**, 655.

Jabbour, H.N., Sales, K.J., Catalano, R.D. & Norman, J.E. (2009) Inflammatory pathways in female reproductive health and disease. *Reproduction*, **138**, 903-919.

Joo, E.H., Kim, Y.R., Kim, N., Jung, J.E., Han, S.H. & Cho, H.Y. (2021) Effect of endogenic and exogenic oxidative stress triggers on adverse pregnancy outcomes: preeclampsia, fetal growth restriction, gestational diabetes mellitus and preterm birth. *Int. J. Mol. Sci.*, **22**, 10122.

Li, B., Guo, L.H., Ban, Z.Q., Liu, L., Luo, L. & Cui, T.Y. (2020) Upregulation of lncRNA plasmacytoma variant translocation 1 predicts poor prognosis in patients with muscle-invasive bladder cancer. *Medicine* (*Baltimore*), **99**, e21059.

Li, P., Zhao, J., Gao, P. & Qu, H. (2021) Clinical evaluation of Pinggan Yiqi Yangshen recipe combined with labetalol hydrochloride and magnesium sulfate in the treatment of PIH. *Evid. Based Complement. Alternat. Med.*, **2021**, 3135043.

Liu, Y., Ren, M., Bi, X., Fu, Y., Jing, X., Zhang, H., Cao, B. & Wang, C. (2021) A systematic review on the application of vascular endothelial growth factors in preeclampsia. *Ann. Palliat. Med.*, **10**, 9259-9266.

Ou, M., Zhao, H., Ji, G., Zhao, X. & Zhang, Q. (2020) Long noncoding RNA MALAT1 contributes to pregnancy-induced hypertension development by enhancing oxidative stress and inflammation through the regulation of the miR-150-5p/ET-1 axis. *FASEB J.*, **34**, 6070-6085.

Qin, X., Chen, Y., Chen, S., Liu, X., Zeng, W., Tian, F., Lin, Y. & Fan, C. (2019) Plasmacytoma variant translocation 1 (PVT1) regulates trophoblast viability, proliferation, and migration and is downregulated in spontaneous abortion. *Am. J. Reprod. Immunol.*, **81**, e13048.

Redman, C.W., Sacks, G.P. & Sargent, I.L. (1999) Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am. J. Obstet. Gynecol.*, **180**, 499-506.

Shao, H., Gao, S., Dai, D., Zhao, X., Hua, Y. & Yu, H. (2021) The association of antenatal D-dimer and fibrinogen with postpartum hemorrhage and intrauterine growth restriction in preeclampsia. *BMC Pregnancy Childbirth*, **21**, 605.

Wang, Q., Lu, X., Li, C., Zhang, W., Lv, Y., Wang, L., Wu, L., Meng, L., Fan, Y., Ding, H., Long, W. & Lv, M. (2019a) Down-regulated long non-coding RNA PVT1 contributes to gestational diabetes mellitus and preeclampsia via regulation of human trophoblast cells. *Biomed. Pharmacother.*, **120**, 109501.

Wang, X., Chen, Y., Du, L., Li, X., Li, X. & Chen, D. (2018) Evaluation of circulating placenta-related long noncoding RNAs as potential biomarkers for preeclampsia. *Exp. Ther. Med.*, **15**, 4309-4317.

Wang, Y., Wu, N. & Shen, H. (2021) A review of research progress of pregnancy with twins with preeclampsia. *Risk Manag. Healthc. Policy*, **14**, 1999-2010.

Wang, Y., Zhang, X., Han, Y., Yan, F. & Wu, R. (2019b) Efficacy of combined medication of nifedipine and magnesium sulfate on gestational hypertension and the effect on PAPP-A, VEGF, NO, Hcy and vWF. *Saudi J. Biol. Sci.*, **26**, 2043-2047.

Wei, Z., Mu, M., Li, M., Li, J. & Cui, Y. (2021) Color Doppler ultrasound detection of hemodynamic changes in pregnant women with GDM and analysis of their influence on pregnancy outcomes. *Am. J. Transl. Res.*, **13**, 3330-3336.

Xu, L., Meng, X., Xu, N., Fu, W., Tan, H., Zhang, L., Zhou, Q., Qian, J., Tu, S., Li, X., Lao, Y. & Xu, H. (2018) Gambogenic acid inhibits fibroblast growth factor receptor signaling pathway in erlotinib-resistant non-small-cell lung cancer and suppresses patient-derived xenograft growth. *Cell Death Dis.*, **9**, 262.

Yang, D., Ding, J., Wang, Y., Yuan, M., Xian, S., Zhang, L., Liu, S., Dai, F., Wang, F., Zheng, Y., Zhao, X., Liao, S. & Cheng, Y. (2020) YY1-PVT1 affects trophoblast invasion and adhesion by regulating mTOR pathway-mediated autophagy. *J. Cell. Physiol.*, **235**, 6637-6646.

Zhang, H.X., Zhao, Y.Y. & Wang, Y.Q. (2018) Analysis of the

characteristics of pregnancy and delivery before and after implementation of the two-child policy. *Chin. Med. J.* (*Engl.*), **131**, 37-42.