The relationship between inflammatory factor expression and blood pressure and urinary protein in the placenta of gestational hypertension rats

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Abstract. The association between inflammatory factor expression and blood pressure with urinary protein in the placenta of pregnant women with pregnancy-induced hypertension (PIH) was investigated to provide a new vision for the clinical prevention and treatment of PIH. Rats were used as animal models and were randomly divided into three groups (control, hypertension and treatment groups) on day 15 of pregnancy with 20 rats in each group. The 10% hypoxia-induced PIH group was induced with administration of an anti-hypertensive drug, and the treatment group was giventreprostinil for one week after the 10% hypoxia-induced PIH. On the 21st day, the experiment was terminated and the placenta was taken to measure the mRNA and protein expression levels of IL-6 and TNF-α, respectively. Pearson's correlation analysis demonstrated the correlation between IL-6 and TNF-α with blood pressure and urinary protein. The blood pressure and urinary protein concentrations in the hypertension group were significantly higher than that in the control group, and the expression levels of IL-6 and TNF-α in the hypertension group were significantly higher ($P<0.05$). The treatment group significantly reduced inflammatory cytokines and blood pressure and urinary protein levels ($P<0.05$). Pearson's correlation analysis showed that IL-6 and TNF-α were positively correlated with blood pressure and urinary protein concentration. The blood pressure and urinary protein concentration in PIH rats and the expression levels of IL-6 and TNF-α were significantly higher, and IL-6 and TNF-α were positively correlated with blood pressure and urine protein concentration.

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Introduction

Pregnancy-induced hypertension (PIH) is a multi-system disease with unknown etiology, including hypertensive disorder complicated pregnancy such as eclampsia, or preeclampsia combined with chronic hypertension (1,2). Symptoms of patients are manifested as facial swelling, leg edema and headache during pregnancy. In addition, complications of PIH patients include eclampsia, placental abruption, oliguria, anuria, blurred vision and hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome (3). Common symptoms of infants born to PIH mothers include intrauterine death (IUD), intrauterine growth retardation (IUGR), perinatal asphyxia, neonatal infection, bleeding, and other complications (4). Advances in modern biochemistry, histology and enzymology research, changes in the maternal body and their effects on the fetus can now be accurately observed (5). However, PIH is the leading cause of maternal and fetal morbidity in developing countries due to poor assessment of the health status of pregnant women. It is reported that more than 60,000 maternal deaths each year are caused by PIH worldwide (6). The mortality rates of infants with a low birth weight in the perinatal and neonatal periods were 10% and 40-50%, respectively (7).

PIH is defined as systolic blood pressure (SBP) >140 mmHg and diastolic blood pressure (DBP) >90 mmHg. According to different scores, it is subdivided into three stages: the mild stage (SBP=140-149 mmHg and DBP=90-99 mmHg), the moderate stage (SBP=150-159 mmHg and DBP=100-109 mmHg) and the severe stage (SBP ≥160 mmHg and DBP ≥110 mmHg), whose symptoms are manifested as hypertension and proteinuria (8). At present, the pathogenesis of PIH is not yet fully elucidated. Previous studies revealed that the possible pathogeneses are shallow placenta accreta, trophoblastic ischemia and hypoxia, vascular endothelial cell injury, immune and inflammatory reaction disorders and thrombosis (9). Of these, the placenta plays a key role in the pathogenesis of PIH (10).

In this study, hypertensive pregnant rats were used as study models to explore the relationship of the expression of inflammatory cytokines with blood pressure and urine proteins in the placenta.

Materials and methods

Experimental animals. A total of 135 Spragne-Dawley rats were purchased from the Hunan Slack Jingda Laboratory Animal Co., Ltd. (Changsha, China), including 60 females and 75 males, aged 2-3 months and weighing approximately 200-250 g. The rats were bred in the specific-pathogen-free (SPF) environment, in which sufficient water and fodder were guaranteed. The temperature was kept constant at 25˚C, the light time was 6 a.m.-6 p.m., the relative humidity was approximately 70%, and the experiment was started after the rats were bred for one week to adapt to the environment. The study was approved by the Ethics Committee of Qilu Hospital of Shandong University (Jinan, China).

Establishment of PIH rat models. The purchased adult rats were bred in a cage at a ratio of female:male=4:5. Vaginal secretions of female rats were scraped at 9:00 every morning, which were then smeared on glass slides to be observed under a microscope (Olympus Corporation, Tokyo, Japan). If sperms were found, the day would be recorded as day 0 of pregnancy, indicating that all the females were pregnant. These rats were normally bred until day 15 of pregnancy, and then 60 pregnant rats were randomly divided into the normoxia with normal blood pressure group (control group, n=20), the 10% hypoxia-induced PIH group (hypertension group, n=20) and the group in which caudal veins were consecutively administered with an anti-hypertensive drug, Treprostinil for one week after 10% hypoxia-induced PIH (treatment group, n=20).

Data collection. The caudal vein blood pressure of the three groups of pregnant rats was tested on day 15, 18 and 21 of pregnancy with a rat caudal artery non-invasive blood pressure monitor, respectively. Specific operation procedures were carried out. Tails of rats were fixed using rat fixators and heated at a constant temperature, and the pressurized tail cuff and the pulse transducer were sequentially placed at the appropriate position of rat tails to determine the initial pulse level. To measure blood pressure, the rubber balloon was inflated and pressurized, so that the pressure within the pressurized tail cuff was increased until the pulse completely disappeared. Approximately 20 mmHg pressure was further increased, and then slow deflation for decompression was conducted until the pulse signal was restored to the initial level. At this time, systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate were read from the pressure capsule or recording system. Measurement was continuously conducted five times, and the average value was taken as a measurement value, followed by collection of 24 h urine proteins on day 15, 18 and 21 of pregnancy. On day 22 of pregnancy, chloral hydrate was used for anesthesia, and pregnant rats were sacrificed via cervical dislocation. The typical placenta of the two uterine horns was dissected and rinsed with saline, which was then absorbed by the filter paper and stored at -80˚C in a refrigerator for standby application.

Detection of messenger ribonucleic acid (mRNA) levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) by reverse transcription polymerase chain reaction (RT-PCR). Rat placental tissues were removed on day 22 of pregnancy, and the total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The extracted total RNA was dissolved in diethyl pyrocarbonate (DEPC)-treated water. The concentration of RNA was measured at a wavelength of 260 nm using spectrophotometry (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Complementary deoxyribonucleic acid (cDNA) (1 μg) was synthesized using the Takara Reverse Transcription kit (Takara Bio, Inc., Otsu, Japan; cat. no.: 639505, Japan). ReverTra Ace quantitative PCR (qPCR) RT kit (Toyobo, Osaka, Japan; cat. no.: FSQ-101) was used to measure the mRNA level of each index. Reaction conditions were: 50˚C for 10 min; 95˚C for 5 min; 95˚C for 15 sec followed by 60˚C for 30 sec; a total of 40 cycles. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was taken as the internal reference. The relative expression level of each index was calculated as: $2^{-\Delta Cq}$ [ΔCq = Cq (target gene) - Cq (GAPDH)]. Primer sequences of each target gene and internal reference used were: GAPDH (forward: GATGCTGGTGCTGAGTATGTCG; reverse: TGGTGCAGGATGCATTGCTGA); IL-6 (forward: AATCTGCTCTGGTCTTCTGGAG; reverse: GTTGGAT GGTCTTGGTCCTTAG); TNF-α (forward: GACTTTAAGG GTTACCTGGGTTG; reverse: TCACATGCGCCTTGATGT CTG).

Detection of protein levels of IL-6 and TNF-α in placenta tissues by western blot analysis and immunohistochemistry. Placenta tissues of the rats were cut into pieces and homogenized. Then an appropriate amount of radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime Institute of Biotechnology, Guangzhou, China) was added and homogenized with the mixed solution of 1% cocktail protease (Proteintech Group, Inc., Chicago, IL, USA). After centrifugation at 13,000 x g for 30 min, the supernatant was taken to determine its protein concentration. Protein samples (40 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The total protein membrane was transferred using polyvinylidene fluoride (PVDF), and the bands were incubated using the primary antibodies and anti-rabbit secondary antibodies of IL-6 (1:800; Abcam, Cambridge, UK; cat. no. ab6672) and TNF-α (1:600; Abcam, cat. no. ab6671). Hypersensitive chemiluminescence (Millipore, Billerica, MA, USA) was used to detect the abundance of the target protein under an enhanced chemiluminescence (ECL) system (Millipore). Image Lab software (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to analyze the gray level of the target protein. The relative content of the target protein was the ratio of the gray level of the target protein to the corresponding internal parameter bands. The results were analyzed by independent t-test.

Tissue samples were fixed in 4% neutral formalin for 12 h, dehydrated by gradient ethanol and embedded in paraffin, and then serially sectioned at 4 μm. The slices were dewaxed and hydrated. After 30 min of permeation with 5% Triton and 15 min of microwave-treated antigen retrieval, the slices were incubated with 3% H_2O_2 deionized water for 10 min to

Table I. Measurement results at three time-periods of the average arterial pressure and urine protein in three groups of rats.

	Blood pressure (mmHg)			Urine proteins (mg/ml)		
Group	15 days	18 days	21 days	15 days	18 days	21 days
Control group	100.3±3	101.2±2.5	102.3±2.5	155.3±13.6	165.3±5.1	165.3±5.1
Hypertension group	102.8±2	146.6±2.1[a]	156.6±2.1[a]	166.3±6.9[a]	566.3±7.3[b]	566.3±7.3[b]
Treatment group	106.2±3	103.3±3.2	110.3±3.2	145.3±8.6	153.3±7.9	153.3±7.9
P-value	>0.05	<0.05	<0.05	>0.05	<0.05	<0.05

[a]P<0.05, [b]P<0.01 compared with those in the control group.

block the action of endogenous peroxidase. Subsequently, the slices were dropwise added with 100 μl primary antibodies of IL-6 and TNF-α (diluted at 1:500, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and incubated at 4˚C overnight. Then the slices were washed with phosphate-buffered saline (PBS), dropwise added with horseradish peroxidase-labeled goat anti-rabbit secondary polyclonal antibody (1:1,000; cat. no. ab6721; Abcam), and incubated for 30 min at room temperature, followed by 3,3'-diaminobenzidine (DAB) color development with PBS as a negative control. Four different fields of view of each slice were randomly selected, and the optical density value under each field of view was measured, respectively.

Statistical analysis. Statistical Product and Service and Solutions (SPSS) 10.0 software was used for data analysis. Measurement data were expressed as mean ± SD. ANOVA was used for comparison between multiple groups and the post hoc test was SNK test. Correlation analyses of inflammatory cytokines with blood pressure and urine proteins were conducted using Pearson's correlation analysis. Quantification analysis was performed using an unpaired Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

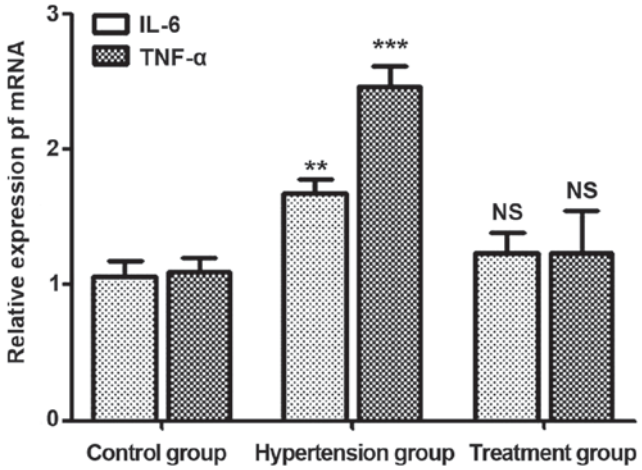

Figure 1. Detection of mRNA levels of TNF-α and IL-6 in placenta tissues of pregnant rats using fluorescence quantitative PCR compared with those in the control group. NS, not significant. **P<0.05; ***p<0.01.

Results

Comparisons of the average blood pressure and urine protein in the three groups of rats. At different time points (day 15, 18 and 21 of pregnancy), blood pressure and urine proteins were measured, respectively, and the average value was calculated. As shown in Table I, compared with those in the control group, blood pressure and urine proteins in the hypertension group were significantly increased (P<0.05). However, the treatment group was given anti-hypertensive drugs after inducing hypertension. There were no statistically significant differences in blood pressure and urine proteins at different time points between the control group and the treatment group (P>0.05).

mRNA levels of IL-6 and TNF-α in placenta tissues of pregnant rats. On day 21 of pregnancy, placenta tissues were extracted and ground for RNA extraction. mRNA levels of IL-6 and TNF-α were detected. Results (Fig. 1) showed that mRNA levels of IL-6 and TNF-α in the hypertension group were higher than those in the control group (P<0.05), while there were no statistically significant differences in mRNA levels of IL-6 and TNF-α between the treatment group and the control group (P>0.05).

Protein levels of IL-6 and TNF-α in placenta tissues of pregnant rats. Proteins in placenta tissues were extracted, and protein levels of IL-6 and TNF-α were detected. Results showed that the average protein levels of IL-6 and TNF-α in the hypertension group were higher than those in the control group (P<0.05), while there were no statistically significant differences in protein levels of IL-6 and TNF-α between the treatment and control groups, suggesting that the expression levels of IL-6 and TNF-α are positively correlated with PIH (Fig. 2).

Detection of the expression levels of IL-6 and TNF-α in placenta tissues by immunohistochemistry. Placenta tissues were isolated, fixed and embedded in paraffin. Slices were used to detect the expression levels of IL-6 and TNF-α in placental tissues using immunohistochemistry. As shown in Fig. 3 and Table II, IL-6 and TNF-α were negatively expressed in the control group but positively expressed in the hypertension group at a high degree, and the expression levels of IL-6 and TNF-α in the hypertension group were significantly higher than those in the control group (P<0.05). IL-6 and TNF-α were positively expressed in the treatment group at a

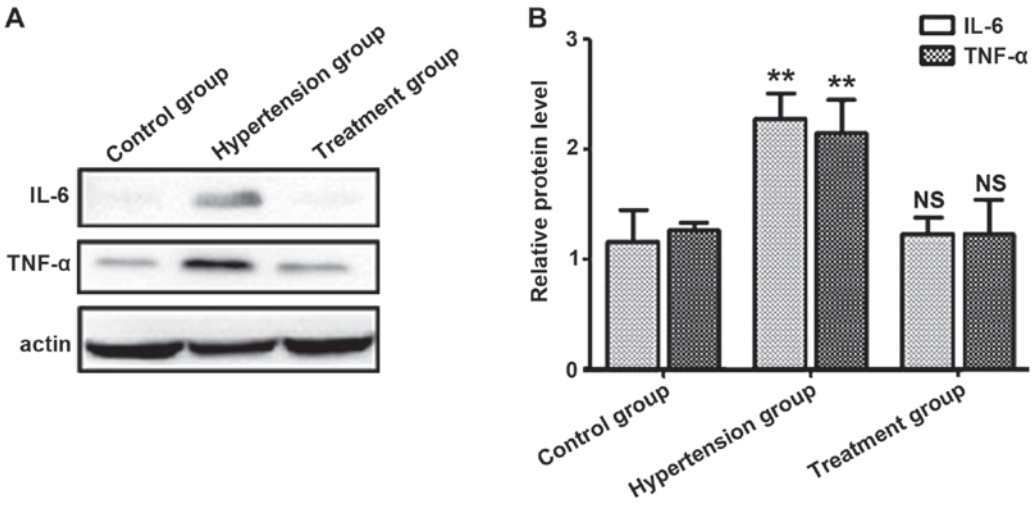

Figure 2. Detection of protein levels of IL-6 and TNF-α in placenta tissues of pregnant rats using western blot analysis. NS, not significant. **P<0.05. (A) Representative of western blots of IL-6 and TNF-α protein expression. (B) Quantitative analysis of the results of western blot analysis.

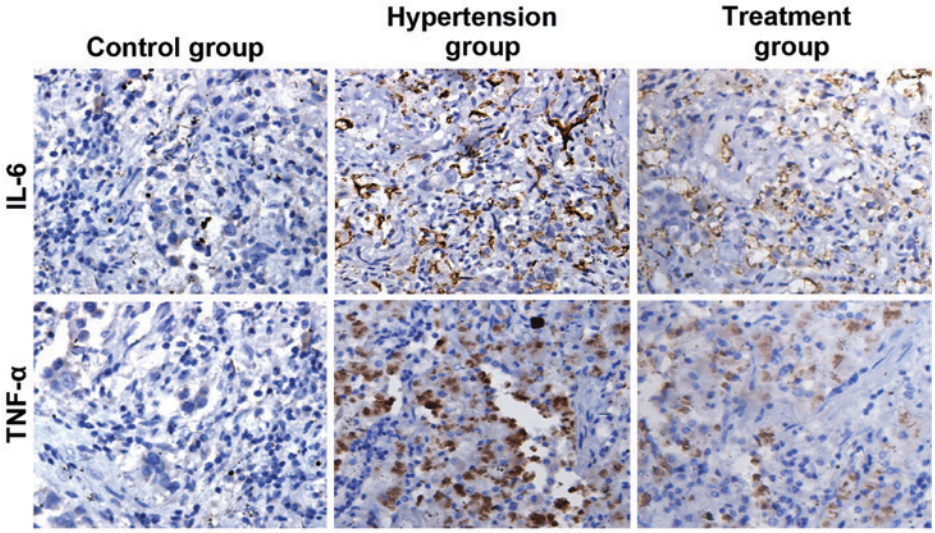

Figure 3. Detection of the expression levels of IL-6 and TNF-α by immunohistochemistry.

Table II. Comparison of the optical density values of IL-6 and TNF-α in placenta tissues among the three groups.

Target	Control group	Hypertension group	Treatment group
IL-6	35.6±12.8	282.1±57.5[a]	66.33[b]
TNF-α	39.9±13.1	346.5±49.6[a]	56.28[b]

[a]P<0.01 compared with those in the control group; [b]P<0.01 compared with those in the hypertension group.

low degree, and the expression levels of IL-6 and TNF-α in the treatment group were significantly lower than those in the hypertension group (P<0.05).

Correlation analyses of IL-6 level in placenta tissues with blood pressure and urine proteins. Placental proteins were extracted, and the expression level of IL-6 proteins was detected. The correlation analysis of the expression level of IL-6 with the corresponding blood pressure of rats was conducted. Pearson's correlation analysis was used to analyze the correlation between IL-6 and blood pressure, and between IL-6 level and urine proteins. Results showed that the IL-6 protein level was positively correlated with blood pressure and urine proteins (Fig. 4).

Correlation analysis of TNF-α level in placenta tissues with blood pressure and urine proteins. Placental proteins were extracted, and the expression level of TNF-α proteins was detected. The correlation analysis of the expression level of TNF-α with the corresponding blood pressure of rats was conducted. Pearson's correlation analysis was used to analyze the correlation between TNF-α and blood pressure, and between TNF-α level and urine proteins. Results revealed that the TNF-α protein level was positively correlated with blood pressure and urine proteins (Fig. 5).

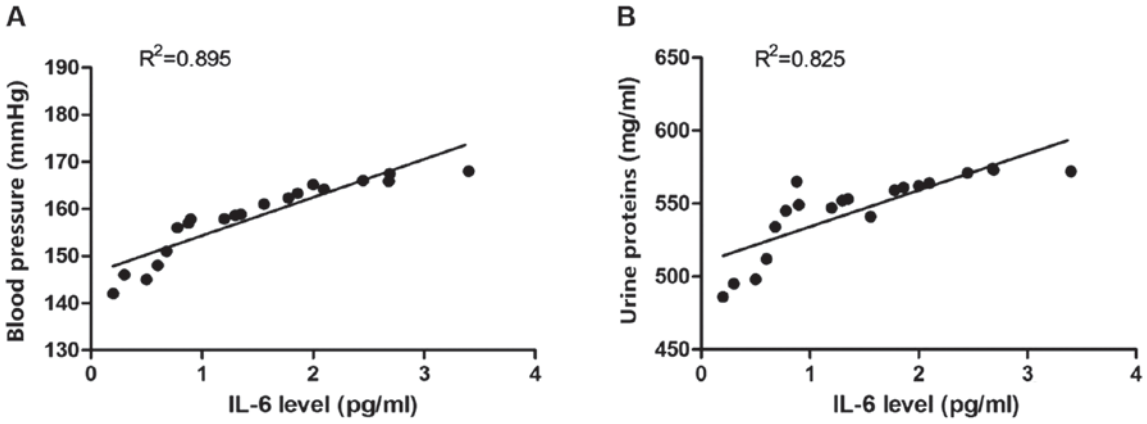

Figure 4. Correlation analyses of IL-6 level in placenta tissues with blood pressure and urine proteins. Placental IL-6 level of the hypoxia-induced PIH rat model is positively correlated with blood pressure (A) and urine proteins (B). R^2 values are 0.895 and 0.825, respectively, and Pearson's correlation analysis was used.

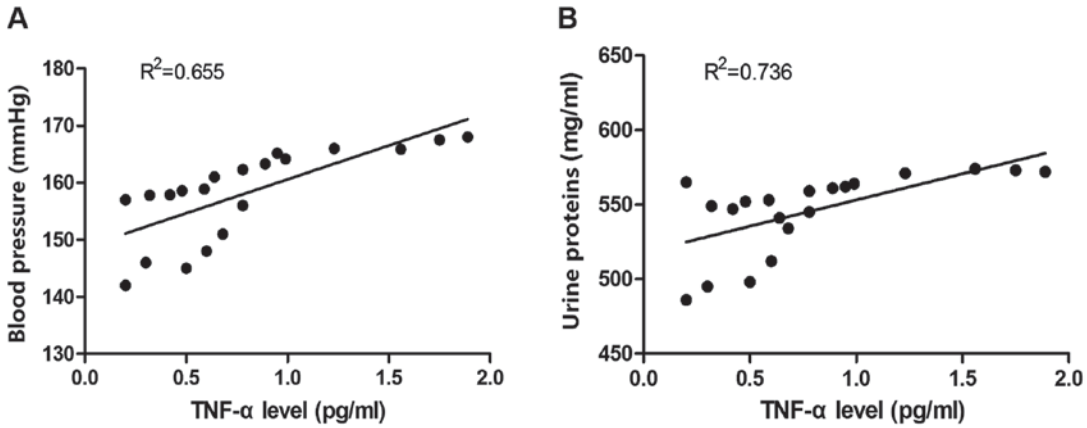

Figure 5. Correlation analyses of TNF-α level in placenta tissues with blood pressure and urine proteins. Placental TNF-α level of the hypoxia-induced PIH rat model is positively correlated with blood pressure (A) and urine proteins (B). R^2 values are 0.655 and 0.736, respectively, and Pearson's correlation analysis was used.

Discussion

Hypoxia induction method was used to establish the model of PIH in this study, and the expression levels of blood pressure, urine proteins and inflammatory cytokines in placenta tissues were further measured in order to analyze the correlation of the expression of inflammatory cytokines in placenta tissue with blood pressure and urine proteins. The main purpose of this study was to provide a scientific basis and a new study perspective for the prevention of PIH in the first trimester of pregnancy.

PIH, especially preeclampsia (PE), is considered a multifactorial disease. There are many theories about PIH pathogenesis, and their common feature is that the placenta plays a key role (11). The 'two-stage model' theory holds that placental implantation, vascularization or dysfunction and the role of maternal factors can lead to PIH (12). Factors associated with PIH pathophysiology include cardiovascular adaptations and vasoconstriction, genetic predisposition, poor immune tolerance of the placenta to maternal tissues, platelet activation and vascular endothelial dysfunction (13). In addition, a study has shown that the co-existing PIH metabolic disorders can lead to endothelial dysfunction, hyperlipidemia and insulin resistance, which are associated with PIH (14). Sgambati *et al* found that compared with those with normal pregnancy, the reduction of the level of the angiogenic factor [vascular endothelial growth factor (VEGF)] in umbilical cord blood of patients with PIH is inhibited, and the ratio of angiopoietin 1/pro-angiogenin 2 is significantly reduced (15). Abnormalities in immune factors such as autoantibodies, oxidative stress and natural killer (NK) cells can lead to placental dysfunction and impaired placental perfusion. The latter, as a stimulus for the placenta releasing anti-angiogenesis and inflammatory mediators, will eventually lead to endothelial dysfunction and organ damage (16). The increased numbers of activated monocytes and macrophages in the endometrium of PIH patients and the increased production of antioxidants, redox factors and reactive oxygen species (ROS) lead to vascular endothelial dysfunction in PIH patients (17). A study has shown that NK cell function of patients with early-onset severe preeclampsia is associated with the production of cytokines (18).

According to clinical reports, the possible pathogeneses of PIH patients include the excessive inflammation reactions of the matrix on the uterine placenta, a large amount of cytokines released by the placenta, and the production of various inflammatory cytokines such as TNF-α and IL-6 in immunoactivated endothelial cells and lymphocytes in blood circulation, which

promote various immune responses. Endothelial cells interact with inflammatory cells in the body and promote the development of hypertension during pregnancy (19,20).

In this study, hypoxia-induced PIH model was applied to measure blood pressure and the concentration of 24 h urine proteins in each group on day 15, 18 and 21 of pregnancy, respectively. On day 21 of pregnancy, the experiment was terminated, the placenta was taken, the mRNA and protein expression levels of IL-6 and TNF-α were measured, respectively, and the expression level of tissues was detected by immunohistochemistry. Compared with those in the control group, the expression levels of IL-6 and TNF-α in the PIH group were significantly increased, and immunohistochemistry results revealed that the expression levels of IL-6 and TNF-α were also significantly increased, proving that inflammatory cytokines, IL-6 and TNF-α are positively correlated with blood pressure and the concentration of urine proteins.

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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

XQJ and SX established PIH rat models. MRT performed PCR. YYM was responsible for western blot analysis and immunohistochemistry. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Qilu Hospital of Shandong University (Jinan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sibai BM: Diagnosis and management of gestational hypertension and preeclampsia. Obstet Gynecol 102: 181-192, 2003.
2. Buchbinder A, Sibai BM, Caritis S, Macpherson C, Hauth J, Lindheimer MD, Klebanoff M, Vandorsten P, Landon M, Paul R, *et al*; National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units: Adverse perinatal outcomes are significantly higher in severe gestational hypertension than in mild preeclampsia. Am J Obstet Gynecol 186: 66-71, 2002.
3. Barton JR, O'brien JM, Bergauer NK, Jacques DL and Sibai BM: Mild gestational hypertension remote from term: Progression and outcome. Am J Obstet Gynecol 184: 979-983, 2001.
4. Salonen Ros H, Lichtenstein P, Lipworth L and Cnattingius S: Genetic effects on the liability of developing pre-eclampsia and gestational hypertension. Am J Med Genet 91: 256-260, 2000.
5. Naeye RL and Friedman EA: Causes of perinatal death associated with gestational hypertension and proteinuria. Am J Obstet Gynecol 133: 8-10, 1979.
6. Koopmans CM, Bijlenga D, Groen H, Vijgen SM, Aarnoudse JG, Bekedam DJ, van den Berg PP, de Boer K, Burggraaff JM, Bloemenkamp KW, *et al*; HYPITAT study group: Induction of labour versus expectant monitoring for gestational hypertension or mild pre-eclampsia after 36 weeks' gestation (HYPITAT): A multicentre, open-label randomised controlled trial. Lancet 374: 979-988, 2009.
7. Ros HS, Cnattingius S and Lipworth L: Comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study. Am J Epidemiol 147: 1062-1070, 1998.
8. Hermida RC, Ayala DE, Mojón A, Fernández JR, Alonso I, Silva I, Ucieda R and Iglesias M: Blood pressure patterns in normal pregnancy, gestational hypertension, and preeclampsia. Hypertension 36: 149-158, 2000.
9. Vambergue A, Nuttens MC, Goeusse P, Biausque S, Lepeut M and Fontaine P: Pregnancy induced hypertension in women with gestational carbohydrate intolerance: The diagest study. Eur J Obstet Gynecol Reprod Biol 102: 31-35, 2002.
10. Redman CWG and Sargent IL: Pre-eclampsia, the placenta and the maternal systemic inflammatory response - A review. Placenta 24 (Suppl A): S21-S27, 2003.
11. Roberts JM and Redman CWG: Pre-eclampsia: More than pregnancy-induced hypertension. Lancet 341: 1447-1451, 1993.
12. Majumdar S, Dasguptha H, Bhattacharya K and Bhattacharya A: A study of placenta in normal and hypertensive pregnancies. J Anat Soc India 54: 1-9, 2005. http://medind.nic.in/jae/t05/i2/jaet05i2p34.pdf.
13. Xiong X, Demianczuk NN, Saunders LD, Wang FL and Fraser WD: Impact of preeclampsia and gestational hypertension on birth weight by gestational age. Am J Epidemiol 155: 203-209, 2002.
14. Seely EW and Solomon CG: Insulin resistance and its potential role in pregnancy-induced hypertension. J Clin Endocrinol Metab 88: 2393-2398, 2003.
15. Sgambati E, Marini M, Zappoli Thyrion GD, Parretti E, Mello G, Orlando C, Simi L, Tricarico C, Gheri G and Brizzi E: VEGF expression in the placenta from pregnancies complicated by hypertensive disorders. BJOG 111: 564-570, 2004.
16. Peraçoli JC, Rudge MVC and Peraçoli MTS: Tumor necrosis factor-alpha in gestation and puerperium of women with gestational hypertension and pre-eclampsia. Am J Reprod Immunol 57: 177-185, 2007.
17. LaMarca BD, Ryan MJ, Gilbert JS, Murphy SR and Granger JP: Inflammatory cytokines in the pathophysiology of hypertension during preeclampsia. Curr Hypertens Rep 9: 480-485, 2007.
18. Sargent IL, Borzychowski AM and Redman CWG: NK cells and human pregnancy - An inflammatory view. Trends Immunol 27: 399-404, 2006.
19. Hermida RC, Ayala DE, Mojón A, Fernández JR, Silva I, Ucieda R and Iglesias M: Blood pressure excess for the early identification of gestational hypertension and preeclampsia. Hypertension 31: 83-89, 1998.
20. Borzychowski AM, Sargent IL and Redman CWG: Inflammation and pre-eclampsia. In: Seminars in Fetal and Neonatal Medicine. Vol. 11. WB Saunders, Philadelphia, pp309-316, 2006.