Original Article
Placental growth factor, an index for detection of women with preeclampsia

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Abstract: We aimed to investigate the correlation between placental growth factor (PIGF) expression in placenta of preeclampsia (PE) patients and PE symptoms. Between June 2013 and June 2015, a total of 141 placenta samples were selected from 141 gravidas who delivered in The Second Hospital of Hebei Medical University. Among 141 gravidas, 50 healthy gravidas were regarded as the control group, 45 gravidas with mild PE were the MPE group and 46 gravidas with severe PE were the SPE group. Systolic pressure (SBP), diastolic pressure (DBP) on two occasions after 20 weeks of gestation and birth weight were recorded. PIGF expression and microvessel density (MVD) were detected via enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry, respectively. SBP and DBP in the control, MPE and SPE groups were increased in turn; birth weight, PIGF expression and MVD in the control, MPE and SPE groups were decreased in turn. Correlation analysis showed that PIGF expression was negatively correlated with PE symptoms at peripartum, SBP and DBP, but positively with birth weight and MVD (all *P* < 0.05). Our results demonstrated that PIGF expression was negatively correlated with BP, and positively correlated with birth weight and MVD. Thus, PIGF can sever as a detection index of PE, which provides a direction for diagnosis and treatment of PE.

Keywords: Preeclampsia, placenta, placental growth factor, blood pressure, birth weight, microvessel density

Introduction

Preeclampsia (PE) is a hypertensive, multi-system disease that is characterized by the onset of high blood pressure and proteinuria after 20 gestational weeks. It complicates 3~8% of all pregnancies [1, 2]. Statistics reported that PE is the major cause of maternal and fetal morbidity in the world, and account for 16% of all maternal deaths in developed countries [3, 4]. It is reported that PE derives from the placenta, beginning with invasion of inadequate cytotrophoblast and ending with wide dysfunction of maternal endothelial [5]. Patients with PE may have some clinical symptoms and signs, including proteinuria, high blood pressure, headaches, sudden weight gain, blurred vision, swelling, as well as some fetal complications (stillbirth and growth restriction) [6]. Previous evidence have demonstrated that multiple conditions are related to increased PE risk, such as diabetes mellitus, chronic hypertension, renal disease, multifetal gestations, hydatidiform mole, advanced maternal age and obesity [7, 8]. Besides, some antiangiogenic factors released by the placenta, hypoxia, inflammation, excessive oxidative stress, perturbation of the renin-aldosterone-angiotensin II axis, immune maladaptation, and genetic susceptibility may all participate in the development of PE [9].

Placental growth factor (PIGF), a proangiogenic protein, is a member of the vascular endothelial growth factor (VEGF) family produced by villous syncytiotrophoblast in the placenta [10]. The human *PIGF* gene is located at chromosome 14q24 and formed by 7 exons spanning 13.7 kb, except the upstream and downstream regulatory sequences [11]. Previous evidence found that PIGF has four isoforms, including PIGF-1, PIGF-2, PIGF-3 and PIGF-4 [12]. The biological function of PIGF was activated through the combination of specificity and VEGR-1/Flt-1 receptor to influence the differentiation and maturation of endothelial cells [13]. Previous

Table 1. Baseline characteristics of the control, MPE and SPE groups

Baseline characteristics	The control group (n = 50)	MPE (n = 45)	SPE (n = 46)
Delivery time (wks)	38.50 ± 1.75	37.58 ± 1.78*	35.24 ± 3.21*,#
Age of pregnancy (y)	28.32 ± 4.24	28.07 ± 4.09	27.72 ± 4.39
First delivery	24 (48.00%)	24 (53.33%)	29 (63.04%)
History of abortion	6 (12.00%)	8 (17.78%)	11 (23.91%)
Cesarean	13 (26.00%)	21 (46.67%)	36 (46.67%)*,#
History of preeclampsia	0 (0%)	1 (2.22%)	3 (6.52%)
Diabetes mellitus	3 (6.00%)	7 (15.56%)*	9 (19.57%)*
Chronic hypertension	0 (0%)	5 (11.11%)	9 (19.57%)*
Nephropathy	0 (0%)	2 (4.44%)	4 (8.70%)*
Familial hypertension	4 (8.00%)	7 (15.56%)	15 (32.61%)*
Smoking history	9 (18.00%)	11 (24.44%)	8 (17.39%)
IUGR	5 (10.00%)	8 (17.78%)	17 (36.96%)*,#
Placental abruption	0 (0%)	2 (4.44%)	9 (19.57%)*,#
IUFD	0 (0%)	0 (0%)	3 (6.52%)*
Fetal deat	0 (0%)	0 (0%)	4 (8.70%)*,#
ROM before delivery	0 (0%)	4 (8.89%)*	13 (8.89%)*,#
PPH	1 (2.00%)	2 (4.44%)	11 (23.91%)*,#

IUGR: intrauterine growth retardation; IUFD: intrauterine fetal death; ROM: rupture of fetal membranes; PPH: postpartum hemorrhage; MPE: mild preeclampsia; SPE: severe preeclampsia; wks: weeks; y: year; *: compared with the control group, $P < 0.05$; #: compared with MPE group, $P < 0.05$.

evidence demonstrated that PlGF is involved in endothelial stimulation, wound healing, bone marrow-derived cell activation as well as pathologic angiogenesis [14, 15]. Subsequently, increased PlGF levels were also found in various cell types, such as smooth muscle cells, vascular endothelial cells, retinal pigment epithelial cells, hematopoietic cells, keratinocytes as well as many tumor cells [16]. Compelling clinical studies also revealed that PlGF gave the highest strength of association with PE [17, 18]. However, few study have been carried out to further explore the correlation between PlGF expression and PE symptoms, so the present study was conducted to find out the relationship between PlGF expression in placenta of PE patients and PE symptoms.

Material and methods

Ethical statement

This study was approved by the Ethical Committee of The Second Hospital of Hebei Medical University. Written informed consents were acquired from all study subjects at the time of hospitalization to undergo diagnostic and therapeutic procedures. This study complied with the guidelines and principles of the Declaration of Helsinki [19].

Study subjects

Between June 2013 and June 2015, a total of 141 placenta samples were gathered from 141 gravidas who delivered in The Second Hospital of Hebei Medical University. Among 141 gravidas, 50 healthy gravidas were regarded as the control group, 45 gravidas with mild PE were the MPE group and 46 gravidas with severe PE were the SPE group. The inclusion criteria of preeclampsia (PE) patients were: gravidas with systolic pressure (SBP) ≥ 140 mmHg or diastolic pressure (DBP) ≥ 90 mmHg on two measurements ≥ 4 h apart after 20 weeks of pregnancy, and urinary protein ≥ 0.3 g or protein/creatinine ≥ 0.3 mg/dL. The SPE group included gravidas with SPE had the features of SBP or DBP ≥ 160/110 mmHg on two measurements ≥ 4 h apart after 20 weeks of pregnancy [10]. Besides, gravidas with one of the following features were also regarded as SPE: blindness, syncope, persistent epigastric pain, concentration of serum transaminase was two times higher than normal value. Baseline characteristics and clinical data of all subjects before and after delivery were also recorded, including delivery time, age of pregnancy, number of pregnancies, history of abortion, cesarean or not, intrauterine growth retardation (IUGR), placental abruption, intrauterine fetal death (IUFD), fetal death, rupture of fetal membranes (ROM) before delivery, postpartum hemorrhage (PPH), blood pressure (SBP and DBP) and birth weight [10, 20].

Enzyme-linked immunosorbent assay (ELISA)

Non-calcification area in the center of placenta samples was selected, followed by 4 times Phosphate Buffered Saline (PBS) washing for
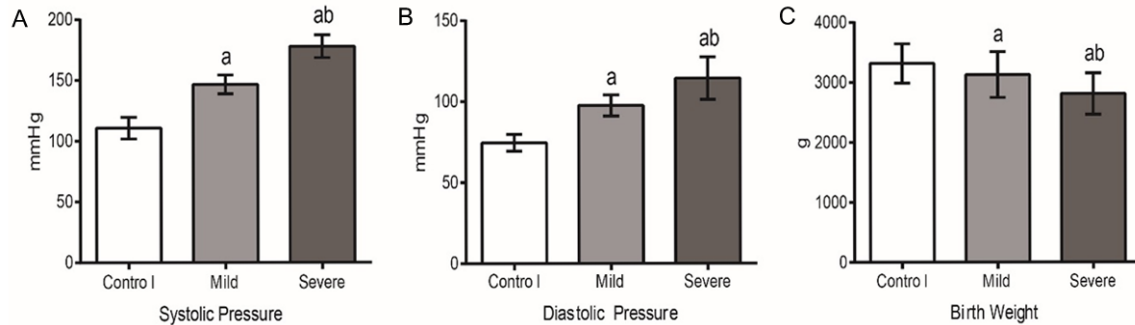
Figure 1. Statistical results of BP and birth weight (BP: Blood pressure; MPE: Mild preeclampsia; A: Systolic pressure; B: Diastolic pressure; C: Birth weight; Control: The control group; Mild: The mild preeclampsia group; Severe: The severe preeclampsia group; a: Compared with the control group, $P < 0.05$; b: Compared with MPE group, $P < 0.05$).
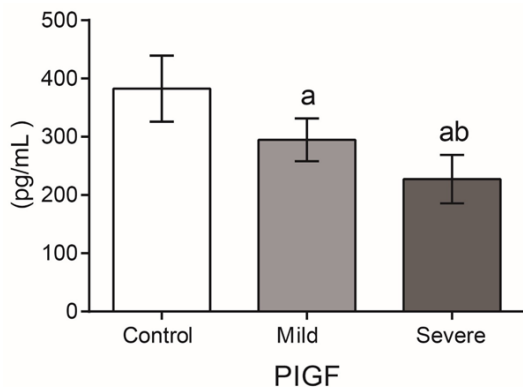
Figure 2. Result of PIGF detection (PIGF: Placental growth factor; Control: The control group; Mild: The mild preeclampsia group; Severe: The severe preeclampsia group; A: Compared with the control group, $P < 0.05$; B: Compared with MPE group, $P < 0.05$).

removing blood. Then, the tissues were froze in liquid nitrogen and stored at -80°C. During protein extraction, radioimmunoprecipitation assay (RIPA) lysis buffer (Gibco) and protease inhibitor (Sigma, St. Louis, USA) were added in tissues for homogenate, followed with 12000 × g centrifugation at 4°C for 10 min. And then, supernatant was collected for protein sample which was detected via PLGFELISA kits (R&D Systems, Minneapolis, MN) [10].
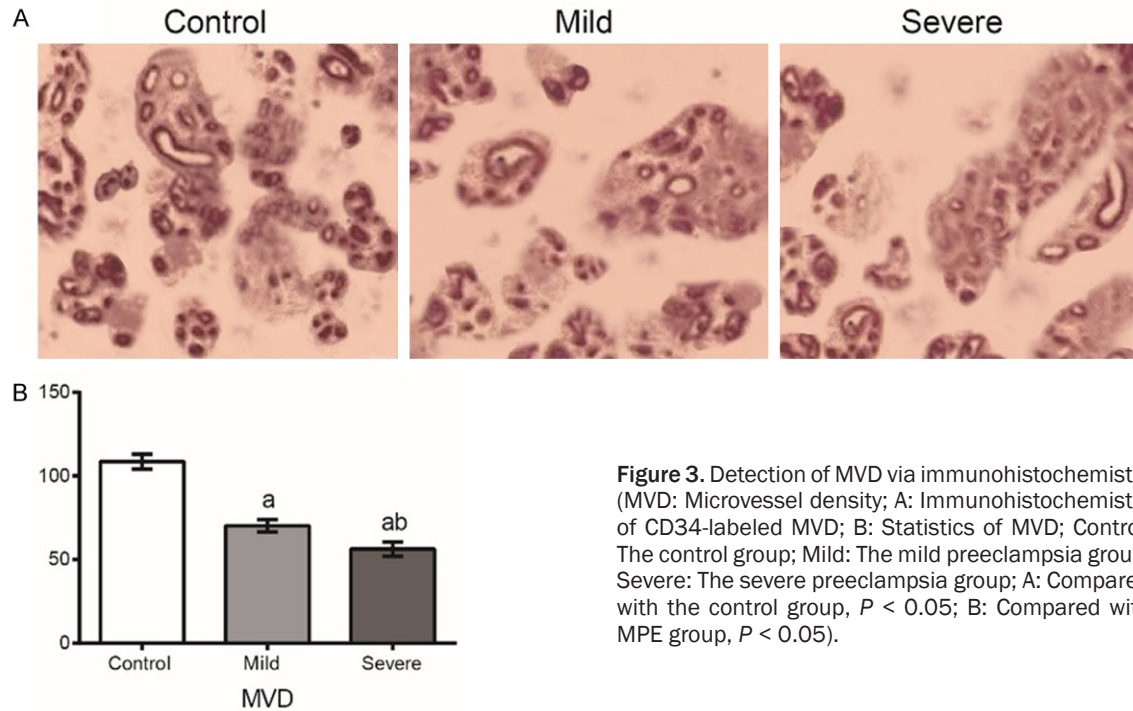
Immunohistochemistry

Tissues were washed by PBS for 3 times, then fixed by 4% paraformaldehyde, and embedded by paraffin and sliced for immunohistochemistry [21]. Firstly, parafin section was dewaxed and hydrated, followed by the addition of 3%

hydrogen peroxide for 15 min for sealing activity of endogenous peroxidase. And then, parafin sections were heated by vapour for 30 min for antigen recovery. After antigen recovery, parafin sections were sealed at PBS greenhouse containing 1% bovine serum albumin (BSA) for 1 h, incubated at greenhouse for 30 min with the addition of primary antibody, and then incubated overnight at 4°C. Rabbit polyclonal anti-VEGF and CD34 (Abcam, Cambridge, MA, USA) were used for primary antibody and incubated at 4°C. POD-conjugated goat anti-rabbit (1:5000) was used for secondary antibody, incubated at room temperature for 30 min and colored by horseradish peroxidase (HRP) (Bio-Rad Laboratories, Hercules, Calif., USA). The colored sections were observed under a light microscope at × 400 magnification, and then the positive cells and microvessel numbers were counted via ImageJ software (V1.49, National Institutes of Health, Bethesda, MD, USA). The microvessel was featured with brown positive cells, positive staining of CD34 staining with tubular structure, and less than 8 blood cells in lumen. Microvessel density (MVD) = total number of microvessels/total number of sections (every 20 sections in each sample were counted).

Statistical analysis

Data analysis was made by SPSS 19.0software (SPSS Inc, Chicago, IL, USA). Measurement data expressed as mean ± standard deviation (SD) and tested by Gaussian distribution. Comparisons between two groups were made by *t*-test, and comparisons among multiple groups by One-Way analysis of variance (ANOVA) (after

Figure 3. Detection of MVD via immunohistochemistry (MVD: Microvessel density; A: Immunohistochemistry of CD34-labeled MVD; B: Statistics of MVD; Control: The control group; Mild: The mild preeclampsia group; Severe: The severe preeclampsia group; A: Compared with the control group, *P* < 0.05; B: Compared with MPE group, *P* < 0.05).

homogeneity test of variances). Comparisons between any two means and any two groups among multiple groups were made by least significant difference (LSD)-t test, and correlations were assessed via Pearson correlation analysis. Comparisons of categorical data between two groups were tested by I^2-test, and correlations were evaluated by Spearman correlation analysis (0.8 < r < 1.0 were regarded as closely correlated) [10]. *P* < 0.05 was considered as statistically significant.

Result

Baseline characteristics

Table 1 showed the baseline characteristics. Significant differences were found in comparisons of delivery time, age of pregnancy, history of abortion, cesarean or not, IUGR, placental abruption, IUFD, fetal death, ROM before delivery, and PPH among the control, MPE and SPE groups (all *P* < 0.05).

Comparisons of blood pressure (BP) and birth weight between the healthy and PE patients

Statistical results of BP showed that SBP and DBP in the control (SBP: 110.7 ± 8.9 mmHg; DBP: 74.6 ± 5.2 mmHg), the MPE (SBP: 146.6 ± 7.7 mmHg; DBP: 97.5 ± 6.5 mmHg) and the

SPE (SBP: 177.8 ± 9.4 mmHg; DBP: 114.3 ± 13.1 mmHg) groups were increased in turn, and comparisons among these three groups indicated statistically significant differences (all *P* < 0.05) (**Figure 1A** and **1B**). **Figure 1C** presented that birth weight in the control, MPE and SPE groups (3316.7 ± 328.7 g; 3129.8 ± 381.7 g; 2810.7 ± 345.9 g) were decreased in turn, and comparisons of those suggesting statistically significant differences (all *P* < 0.05). These results showed that the severity of PE was accompanied by increased BP and decreased birth weight.

Comparisons of PIGF expression between the healthy and PE patients

The results of PIGF detection showed that PIGF expressions in the control, MPE and SPE groups were decreased in turn (**Figure 2**) (all *P* < 0.05). Compared to the control group (382.6 ± 56.7 pg/ml), both SPE (294.8 ± 36.8 pg/ml) and MPE (227.3 ± 41.7 pg/ml) groups had decreased PIGF expression (all *P* < 0.05). The result showed that the expression of PIGF was significantly decreased with the severity of PE.

Comparisons of MVD between the healthy and PE patients

The result of immunohistochemistry showed that MVD in the control, MPE and SPE groups

Table 2. Correlation coefficient of PIGF in placenta of PE patients and symptoms of PE patients

	PLGF	P-Value
IUGR	-0.352	0.001
Placental abruption	-0.316	0.002
IUFD	-0.238	0.023
Fetal death	-0.308	0.003
ROM before delivery	-0.287	0.006
PPH	-0.331	0.001
SBP	-0.776	< 0.001
DBP	-0.767	< 0.001
Birth weight	0.635	< 0.001
MVD	0.753	< 0.001

PIGF: placental growth factor; PE: preeclampsia; IUGR: intrauterine growth retardation; IUFD: intrauterine fetal death; ROM: rupture of fetal membranes; PPH: postpartum hemorrhage; SBP: systolic pressure; DBP: diastolic pressure; MVD: microvessel density.

were decreased in turn (**Figure 3A**) (all $P < 0.05$). Statistical results revealed that both MPE (70.2 ± 3.7) and SPE (56.3 ± 4.2) groups had lower CD34-labeled MVD when compared with that in the control group (108.6 ± 4.5) (all $P < 0.05$). The result revealed that the MVD was significantly decreased with the severity of PE.

Correlation between PIGF in placenta of PE patients and PE symptoms

Correlation analysis revealed a negative correlation between PIGF and symptoms of PE patients at peripartum, such as IUGR, placental abruption, IUFD, fetal death, ROM before delivery, and PPH ($r = -0.352$, $P = 0.001$; $r = -0.316$, $P = 0.002$; $r = -0.238$, $P = 0.023$; $r = -0.308$, $P = 0.003$; $r = -0.287$, $P = 0.006$; $r = -0.331$, $P = 0.001$) (**Table 2**). Such negative correlation was also found between PIGF and SBP and DBP ($r = -0.776$, $P < 0.001$; $r = -0.767$, $P < 0.001$). While, PIGF in placenta of PE patients was found positively correlated with birth weight and MVD ($r = 0.635$, $P < 0.001$; $r = 0.753$, $P < 0.001$). These results showed that PIGF in placenta of PE patients was closely correlated with symptoms of PE.

Discussion

Our study provides the demonstration of the relationship between PIGF and symptoms of PE. Our study demonstrated that the expression level of PIGF can be the detection index of PE, which severs as a detection index for diagnosis and treatment of PE.

Higher BP was found in SPE group in our study when compared with the control and MPE groups, respectively. PE is deemed as a common clinical entity in pregnancy, which can result in massive morbidity and mortality of pregnancy, and prediction of PE can provide an opportunity for preventing infaust obstetric and death of neonates [22]. Previous evidence demonstrated that PE is related to a disorder of angiogenic factors which may cause endothelial dysfunction, the implication being that PE is mainly a disease of the vascular endothelium [23]. The placenta, where PIGF mainly expressed in, is a place of action of mediators of all the processes, and plays a key role in the pathogenesis of PE [24]. Besides, vascular growth is also deemed as a critical factor contributes to successful pregnancy during implantation and placentation, and vascular insufficiencies during placentation are thought to result in many kinds of obstetrical complications [9]. In normal pregnancy, PIGF levels would increase with the gestation in maternal circulation and descend toward term [25]. And, our results also found a negative correlation was also found between PIGF in placenta of PE patients and SBP, DBP. Consistent with our result, Troisi *et al.* also clarified that second-to-third-trimester BP were inversely correlated with the PIGF in PE pregnancies [26].

Besides, we also revealed that the expression of PIGF was significantly decreased with the severity of PE. Indeed, study conducted by Teixeira *et al.* also demonstrated that PIGF concentrations were significantly reduced in PE compared with normal controls ($P = 0.0001$) [27]. And PIGF expression was negatively correlated with birth weight. Previous evidence showed that lower PIGF expression might be one important factor influencing the absorption and utilization of nutrient in fetus, resulting in restriction of growth [28]. Study conducted by *Pinheiro* and his colleagues also revealed that birth weight was positively correlated with serum PIGF ($r = 0.52$, $P = 0.0003$) among all PE patients, which was also in accord with our result [29].

Furthermore, PIGF in placenta of PE patients was found positively correlated with MVD. To

the best of knowledge, PlGF can induce the proliferation, migration and activation of vascular endothelial cells, especially the microvascular endothelial cells, and can also act as chemotactic factor of endothelial cell growth factor to regulate the growth of endothelial cells [30]. The same as VEGF, PlGF was also demonstrated can form heterodimers that show only weak biological activity, suggesting that PlGF may negatively regulate VEGF-induced angiogenesis via formation of biologically inactive heterodimers [31]. In addition, PlGF can promote migration of monocytes and endothelial cells, and increase the permeability of endothelial cells, suggesting that PlGF plays a prominent role in inducing angiogenesis and maintaining the normal structure and function of blood vessel [3].

In conclusion, we show that, among the study we conducted, a negative correlation between decreased PlGF expression and increased BP, and a positive correlation between birth weight and MVD were found. These findings suggesting that the expression level of PlGF can be the detection index of PE, which severs as a detection index for diagnosis and treatment of PE. Nevertheless, the specific functions of PlGF expression in symptoms of PE were not revealed in our study, which need further investigation.

Acknowledgements

Disclosure of conflict of interest

None.

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