Effects of Angiogenic Factors, Antagonists, and Podocyte Injury on Development of Proteinuria in Preeclampsia

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Abstract

Proteinuria is universal to all patients with preeclampsia. We examined the urinary podocytes in women with preeclampsia ($n = 14$), gestational hypertension ($n = 14$), and normal pregnancy. Maternal serum and urinary concentrations of vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and the antiangiogenic factor soluble fms-like tyrosine kinase 1 (sFlt-1) were detected. These concentrations were used to evaluate the urinary excretion of podocytes and the alteration of angiogenic factors and to assess their relationships to proteinuria in preeclampsia. Our studies suggest that the urinary podocyte number and angiogenic factors are correlated with random urine albumin/creatinine ratio and blood pressure. Receiver–operating characteristic (ROC) curves of serum and urinary PlGF and the PlGF/sFlt-1 ratio as well as the presence of podocyturia confirmed their usefulness in distinguishing preeclamptic and normotensive pregnant women. In addition, combinations of serum or urinary PlGF or podocyturia tests in parallel or in series provided the best clue for identifying patients with preeclampsia. We considered that the dysregulation of angiogenic factors and its subsequent podocyte injury may contribute to the mechanism of proteinuria development in preeclampsia.

Keywords

angiogenic factors, preeclampsia, proteinuria, podocyte

Introduction

Preeclampsia is a pregnancy-specific disorder affecting at least 5% of all pregnancies worldwide and is thus becoming a major health concern.¹ Among the maternal pathologic hallmarks, which include hypertension, proteinuria, and edema, the most distinctive hallmark of preeclampsia is proteinuria, a reflection of renal injury. However, the causes of preeclampsia are still obscure.

It has been extensively accepted that the proteins lining the podocyte slit diaphragms play a critical role in the maintenance of glomerular permeability.² Recently, the downregulation of podocyte foot process proteins has garnered particular attention in the development of proteinuria in preeclampsia.^{2,3} Additionally, urinary podocyte excretion is related to the podocyte injury observed in various renal diseases and is associated with disease activity as well as severity.⁴⁻⁷ Podocalyxin, a podocyte-specific sialoprotein, has been used for urinary podocyte diagnostics.⁸ Several studies have also revealed that podocyturia is present in preeclampsia.⁹⁻¹¹ The factors that cause podocyte injury in preeclampsia are poorly understood.

Numerous researchers have focused on angiogenic factors secreted by the ischemic placenta, which is partly responsible for the maternal syndrome in pregnancy.¹²⁻¹⁵ These factors include vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and their antagonist, soluble fms-like tyrosine kinase 1 (sFlt-1). We propose that the imbalance of angiogenic factors and their antagonist, sFlt-1, may contribute to podocyte injury and subsequent proteinuria. This study was designed to evaluate the urinary excretion of podocytes in preeclampsia and the alterations of VEGF, PlGF, and sFlt-1. In addition, we aimed

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to assess the relationships of these factors to proteinuria as well as their diagnostic sensitivity and specificity.

Participants and Methods

Participants

From March 2009 to March 2010, women with preeclampsia ($n = 14$), gestational hypertension ($n = 14$), and gestational age-matched normotensive pregnant women ($n = 13$) were recruited from the Department of Gynecology and Obstetrics, The Fifth People's Hospital of Shanghai, Fudan University, China. Preeclampsia was defined as a systolic blood pressure ≥ 140 mm Hg or a diastolic blood pressure ≥ 90 mm Hg after 20 weeks of gestation accompanied by proteinuria $>1+$ by dipstick in a random urine analysis or ≥ 300 mg/24 h with no evidence of urinary tract infection. Gestational hypertension was defined as a systolic blood pressure ≥ 140 mm Hg or a diastolic blood pressure ≥ 90 mm Hg after 20 weeks of gestation in the absence of significant proteinuria ($\leq 1+$ by dipstick or <300 mg/24 h).¹⁶ These patients enrolled in this study were all newly admitted inpatients and all samples were collected before any medication. Women with diabetes mellitus, thyroid, liver, or chronic renal disease, or preexisting chronic hypertension were excluded from the study. The study was approved by the local ethics committee before the experiment was started and followed the ethical standards of the Helsinki Declaration. All participants provided written informed consent to participate in the study.

Immunofluorescence for the Quantification of Urinary Podocytes

The quantification of urinary podocytes was performed as previously reported.^{17,18} Fresh first void morning urine (50–100 mL) was collected for 3 consecutive days within 1 week before labor, and the number of urinary podocytes was counted daily. No special drugs that impact on the urinary samples were used during the course for more than 1 week before delivery. Each urine specimen was centrifuged at 1000 g for 5 minutes within 30 minutes after voiding. One-half milliliter of phosphate-buffered saline (PBS), pH 7.2, was used to wash and resuspend the sediment. The sample was then centrifuged for 5 minutes at 1000g onto poly-L-lysine-coated glass slides using cytospin (TXT3; Shanghai Lu Xiangyi Centrifuge Instrument Co, Ltd, Shanghai, China). The slides were air-dried and fixed in acetone for 5 minutes at 4°C. The urinary sediment was incubated with antihuman podocalyxin monoclonal antibody (R&D Systems Inc, Minneapolis, Minnesota) at 10 μ g/mL for 60 minutes at 37°C. Then the slides were incubated with Cy2-labeled anti-mouse immunoglobulin G (IgG; United Chemicon Inc, Rosemont, Illinois) at a dilution of 1:100 for 30 minutes at 37°C. Cell nuclei were counterstained with Hoechst 33342 (Sigma-Aldrich Corp, Carlsbad, California) for 5 minutes at a concentration of 5 μ g/mL. Immunofluorescence microscopy was used to examine the slides. Cells staining positive for both podocalyxin and nuclei were defined as entire podocytes. The number of urinary podocytes was

expressed as cells/mL and was reported as the average number of podocytes from 3 samples for each individual. Two of the authors were engaged in the blinded quantitative analysis. The random urine albumin/creatinine ratio (ACR), which strongly correlates with the 24-hour urine protein excretion, was also estimated.⁴

Enzyme-Linked Immunosorbent Assays

Maternal fasting blood samples and fresh first void morning urine samples were collected before labor for this assay. The serum was centrifuged within 30 minutes of collection at 3000g for 10 minutes, and the urinary samples were centrifuged at 2000g for 10 minutes, immediately thereafter, the serum and the clear supernatants were frozen at -80°C and stored for less than 12 months. The samples were thawed only once for this study. Serum and urinary VEGF (4A Biotech, Beijing), PlGF (Rapidbio Inc Shanghai LangKa Trade Co, Ltd, Shanghai, China), and sFlt-1 (Rapidbio Inc) concentrations were measured with commercially available enzyme-linked immunosorbent assays (ELISAs) performed in duplicate according to the manufacturers' instructions. The minimal detectable doses of VEGF, PlGF, and sFlt-1 were 7, 2.5, and 20 pg/mL, respectively. The interassay and intra-assay coefficients of variation were all less than 10%. The correlation coefficient of the standard curves was $>.99$.

Statistical Analysis

The SPSS 13.0 was used for all statistical analyses. All normally distributed parameters were each expressed as the mean $\pm$ SD, and the skewed distributed parameters were each expressed as the median (interquartile range; [IQR]). One-way analyses of variance (ANOVAs) between groups and Student-Newman-Keuls (SNK-q) were used to compare normally distributed data. The Mann-Whitney *U* test was used for the comparison of nonnormally distributed parameters. Pearson and Spearman correlations were used for parametric data and nonparametric data, respectively. Receiver–operating characteristic (ROC) curves were plotted, and the area under the curve (AUC) was analyzed. All *P* values were 2-tailed, and $P < .05$ was defined as statistically significant.

Results

Clinical Profiles of the Participants

The demographical and clinical data of the patients are presented in Table 1. Compared with the normal pregnancy group, blood pressure was significantly higher in women with preeclampsia and gestational hypertension. The gestational age was shorter, and the infant birth weights were lower in women with preeclampsia. Additionally, blood urea nitrogen (BUN), serum creatinine, uric acid, ACR, and proteinuria were significantly higher, while the estimated glomerular filtration rate (eGFR) and the creatinine clearance were lower in women with preeclampsia than in other 2 groups. However, it should be noted that despite this difference, the BUN and the serum creatinine values were still within the normal range in all 3 groups. The uric acid was also slightly higher,

Table 1. Clinical Profiles and Laboratory Data.

Characteristic	Normal Pregnancy (n = 13)	Preeclampsia (n = 14)	Gestational Hypertension (n = 14)
Age, years	26.00 ± 1.63	29.64 ± 4.80	27.21 ± 5.41
Body mass index, kg/m	26.90 ± 2.36	27.71 ± 3.78	29.17 ± 2.45
Blood pressure, mm Hg			
Systolic	117.31 ± 9.04	150.71 ± 19.30 ^d	146.21 ± 9.36 ^d
Diastolic	74.23 ± 5.72	98.21 ± 8.68 ^d	96.29 ± 7.78 ^d
Gestational age at delivery, week	39.67 ± 2.57	36.77 ± 2.94 ^d	39.50 ± 1.30
Birth weight of infants, g	3578.08 ± 297.84	2655.83 ± 651.21 ^d	3394.29 ± 367.68
Bood urea nitrogen, mmol/L	2.92 ± 7.26	5.03 ± 2.33 ^c	2.83 ± 1.03
Creatinine, μmol/L	44.38 ± 9.10	54.79 ± 12.58 ^c	44.08 ± 7.34
Uric acid, μmol/L	230.77 ± 65.29	391.57 ± 134.78 ^d	298.15 ± 87.52 ^b
eGFR(MDRD), mL/min/1.73m ²	197.32 ± 50.69	117.94 ± 36.31 ^d	158.10 ± 32.67 ^b
Creatinine clearance (mL/min)	191.65 ± 42.16	155.31 ± 42.58 ^b	206.78 ± 43.96
ACR, mg/g, median (IQR)	15.95 (7.37, 32.75)	257.97 (93.11, 729.64) ^d	9.88 (2.63, 24.43)
Proteinuria (qualification)	Negative	2+ ~ 4+	Negative / 1+
Proteinuria, g/24 h	No data	2.391(0.815, 7.331)	0.175(0.163, 0.254) ^e

Abbreviations: eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; ACR, urine albumin/creatinine ratio; IQR, interquartile range.

^a Negative, the 24-hour urine protein excretion in women with normal pregnancy was presumed to 150 mg (it should be <150 mg in normal pregnancy). It was used for the following correlation analysis: ^b $P < .05$, ^c $P < .01$, ^d $P < .001$ compared to normal pregnancy; ^e $P < .001$ compared to the preeclampsia group.

Figure 1. Urinary podocytes examined by immunofluorescence using antihuman podocalyxin antibody. The nuclei were stained by Hoechst 33342 ($\times 200$). The cells directed by arrows are intact podocytes in urine.

and the eGFR was lower in women with gestational hypertension than in those with normal pregnancies. No significant differences in other demographical data were observed.

Levels of Urinary Podocyte

Positive immunofluorescent staining using antihuman podocalyxin antibodies and nuclear counterstaining were used to identify intact podocytes (Figure 1). The number of urinary podocytes in women with preeclampsia (median: 3.10 cells/mL, $P < .001$) and gestational hypertension (1.41 cells/mL, $P < .05$)

Figure 2. Comparison of podocyturia levels among normal pregnancies and patients. The results, expressed as the number of urinary podocyte in 1 mL urine, demonstrate that the number of urinary podocytes in preeclampsia significantly increased. The data are shown as medians (interquartile ranges). $*P < .05$, $***P < .001$ vs normal pregnancy group; $\#P < .05$ vs gestational hypertension group.

was significantly higher than that in normal pregnancy (0.52 cells/mL). Furthermore, this number was significantly higher in women with preeclampsia than in women in the gestational hypertension group ($P < .05$; Figure 2).

Serum and Urinary VEGF, PlGF, and sFlt-1 Concentration Detection as well as Serum and Urinary PlGF/sFlt-1 Ratio

As shown in Figure 3 (A, B, and C), serum VEGF concentrations in women with preeclampsia (44.05 ± 25.01 pg/mL)

Figure 3. The concentration of serum and urinary VEGF (A), PlGF (B), and sFlt-1 (C) as well as the PlGF/sFlt-1 ratio (D) in women with normal pregnancies and in patients with hypertension or preeclampsia. The data are shown as the means $\pm$ SEs $^*P < .05$, $^{**}P < .01$, $^{***}P \leq .001$. VEGF indicates vascular endothelial growth factor; PlGF, placental growth factor; sFlt-1, antiangiogenic factor soluble fms-like tyrosine kinase 1.

were higher than in women with normal pregnancies (25.84 ± 14.22 pg/mL, $P < .05$) or gestational hypertension (34.75 ± 15.67 pg/mL, $P > .05$). Urinary VEGF concentrations did not differ significantly among the 3 groups. Serum and urinary PlGF concentrations in women with preeclampsia (serum: 52.59 ± 7.46 ; urine: 12.39 ± 5.30 pg/mL) were significantly lower than in women with gestational hypertension (serum: 60.39 ± 4.74 , $P = .001$; urine: 26.25 ± 8.25 pg/mL, $P < .05$) or normal pregnancies (serum: 65.40 ± 3.76 , $P < .001$; urine: 31.81 ± 26.65 pg/mL, $P < .01$). Serum PlGF concentrations in women with gestational hypertension were also significantly lower than in women with normal pregnancies ($P < .05$), and urinary PlGF concentrations did not differ significantly between women with gestational hypertension and those with normal pregnancies. Serum and urinary sFlt-1 concentrations in women with preeclampsia (serum: 538.33 ± 53.28 , $P < .05$; urine: 812.09 ± 75.78 pg/mL, $P < .01$) and gestational hypertension (serum: 546.11 ± 66.80 , $P < .05$;

urine: 813.34 ± 68.03 pg/mL, $P < .01$) were significantly higher than in women with normal pregnancies (serum: 466.82 ± 111.57 ; urine: 679.35 ± 188.88 pg/mL). We then analyzed the serum and urinary PlGF/sFlt-1 ratios (Figure 3D). The serum and urinary PlGF/sFlt-1 ratios in women with preeclampsia (serum: $P < .001$; urine: $P < .01$) and gestational hypertension (serum: $P < .001$; urine: $P < .05$) were significantly lower than in women with normal pregnancies, and the ratios were slightly lower in women with preeclampsia compared to those with gestational hypertension, although the difference was not statistically significant.

Relationships Among Clinical Profiles, Podocyturia, and Proangiogenic and Antiangiogenic Factors

The analysis of the correlation among renal function, proteinuria (ACR), podocyturia, and the serum and urinary levels of VEGF, PlGF, sFlt-1, and the PlGF/sFlt-1 ratios are shown

Table 2. Relationships Among Clinical Profiles, Podocyturia, Angiogenic Factors, and Antiangiogenic Factors as Assessed by Pearson or Spearman Correlation Coefficients.^{a,b}

Parameter	r/ P Value (n = 41)							
	Podocyturia	ACR	BUN	SCr	UA	eGFR	SBP	DBP
Podocyturia	1	.631/<.001	.465/.005	.291/.090	.472/.004	-.387/.024	.534/0.001	.559/<.001
Serum VEGF	.520/.002	.506/.001	.193/.246	.273/.098	.432/.007	-.334/.016	.528/0.001	.403/.011
Urinary VEGF	-.148/.404	.169/.305	-.032/.849	-.031/.855	.004/.981	.138/.415	-.098/.552	-.224/.170
Serum PIGF	-.427/.009	-.407/.008	-.360/.023	-.266/.097	-.442/.004	.395/.013	-.543/<.001	-.581/<.001
Urinary PIGF	-.228/.180	-.326/.037	-.248/.122	-.046/.777	-.278/.082	-.027/.871	-.280/.076	-.214/.179
Serum sFlt-1	.319/.058	.079/.624	.015/.926	.057/.727	.223/.166	-.100/.545	.168/.294	.220/.166
Urinary sFlt-1	.308/.068	-.014/0.929	.114/.485	.000/.998	.219/.174	-.016/.923	.385/.013	.327/.037
Serum PIGF/ sFlt-1	-.540/.001	-.392/0.011	-.222/.169	-.166/.305	-.434/.005	.256/.116	-.451/.003	-.498/.001
Urinary PIGF/ sFlt-1	-.269/.113	-.333/.034	-.206/.201	-.113/0.487	-.272/0.089	-.033/.842	-.167/.296	-.051/.750

in Table 2. There was a positive correlation between podocyturia and ACR, BUN, UA, SBP, and DBP as well as a negative correlation between podocyturia and eGFR. We also identified a significantly positive correlation between serum VEGF and podocyturia, ACR, UA, SBP, DBP as well as a negative correlation between serum VEGF and eGFR ($P < .05$). Serum PIGF levels negatively correlated with podocyturia, ACR, BUN, UA, SBP, and DBP but positively correlated with eGFR. Urinary PIGF was inversely associated with ACR. Urinary sFlt-1 positively correlated with blood pressure. In addition, the serum PIGF/sFlt-1 ratio was inversely correlated with podocyturia, ACR, UA, SBP, and DBP. The urinary PIGF/sFlt-1 ratio correlated inversely with ACR.

Furthermore, there was a significant correlation between serum and urinary VEGF, PIGF, sFlt-1, and the PIGF/sFlt-1 ratio (Figure 4A-D). Serum PIGF was positively correlated with urinary PIGF (r/P : .342/.028), and serum sFlt-1 was positively correlated with urinary sFlt-1 (r/P : .624/<.001). The serum PIGF/sFlt-1 ratio was positively correlated with the urinary PIGF/sFlt-1 ratio (r/P : .805/<.001). Urinary PIGF was inversely correlated with urinary sFlt-1 (r/P : -.721/<.001).

Collectively, podocyturia was closely correlated with proteinuria, renal function, and blood pressure. Furthermore, serum angiogenic factors and the serum PIGF/sFlt-1 ratio were also closely correlated with podocyturia, renal function, and blood pressure.

Usefulness of Podocyturia, Angiogenic Factors, and Antiangiogenic Factors for the Identification of Preeclampsia and Normal Pregnancy

To assess the use of podocyturia, angiogenic factors, and antiangiogenic factors for identifying preeclampsia and normotensive pregnancies, the ROC curves were plotted (Figure 5A-E). The AUC of podocyturia, 0.868 (95% CI: 0.751-0.985, $P < .001$), was greater than that of VEGF (serum: 0.675; 95% CI, 0.483-0.866, $P = .079$ and urine: 0.550; 95% CI, 0.354-0.746, $P = .613$) or sFlt-1 (serum: 0.590; 95% CI, 0.414-0.766, $P = .350$ and urine: 0.567; 95% CI, 0.383-0.752, $P = .483$) but was comparable to that of PIGF (serum:

0.872; 95% CI, 0.744-1.000, $P < .001$ and urine: 0.811; 95% CI, 0.681-0.941, $P = .001$) and the PIGF/sFlt-1 ratio (serum: 0.847; 95% CI, 0.728-0.965, $P < .001$ and urine: 0.815; 95% CI, 0.686-0.943, $P = .001$). In summary, podocyturia, serum and urinary PIGF, and the PIGF/sFlt-1 ratio are very useful for distinguishing preeclampsia and normal pregnancy.

In addition, the sensitivity, specificity, and other evaluation parameters were evaluated. Using different cutoff values, podocyturia, PIGF and the PIGF/sFlt-1 ratio exhibited different sensitivities, specificities, and other evaluation parameters for the diagnosis of preeclampsia (Tables 3-7). The cutoff point with the highest Youden's index was defined as the optimized cutoff value. In this context, serum PIGF <60 pg/mL, urinary PIGF <25 pg/mL, and podocyturia ≥ 1 cells/mL were determined to combine in parallel or in series differently. These combinations may provide clue for future studies about the early diagnosis of preeclampsia (Table 8). The PIGF/sFlt-1 ratio in the serum (<0.12) or in urine (<0.025) had the maximal diagnostic value.

Discussion

Preeclampsia is a hypertensive syndrome of pregnancy with consistent involvement of the kidney and is characterized by proteinuria and, frequently, edema.¹⁹ However, the source of proteinuria is still unclear. It is generally recognized that the injury of endothelial cells is the predominant renal injury in preeclampsia.²⁰ Recent studies have provided novel insights into the role of podocyte injury in proteinuria and the development of preeclampsia.

The excretion of podocytes into the urine in various glomerular diseases is considered to result from the detachment of podocytes from the glomeruli and is closely linked to proteinuria.^{21,22} Podocalyxin, the main podocyte surface antigen, has been used reliably in immunofluorescence for the detection of urinary podocytes.¹⁸ Based on these reports, we studied the podocyturia in our preeclamptic participants. The present results suggest that the number of urinary podocyte in women with preeclampsia is significantly higher than in women with gestational hypertension or normal pregnancies. Furthermore, the number of urinary podocytes is higher in women with

Figure 4. The relationship between the concentrations of serum and urinary PIGF (A), between the concentrations of serum and urinary sFlt-1 (B), and between the serum and urinary PIGF/sFlt-1 ratios (C) as well as the relationship between the concentration of urinary PIGF and urinary sFlt-1 in all participants. VEGF indicates vascular endothelial growth factor; PIGF, placental growth factor; sFlt-1, antiangiogenic factor soluble fms-like tyrosine kinase 1.

gestational hypertension than in women with normal pregnancies, although this may be attributed to the elevated blood pressure. Moreover, podocyuria is positively correlated with ACR, renal function, and blood pressure and is inversely correlated with eGFR.

Although the mechanisms responsible for the emergence of the preeclampsia phenotype in women are poorly understood, it has been largely accepted to be associated with an abnormality in the levels of various angiogenic factors, including VEGF, PIGF, and the antiangiogenic factor sFlt-1, all of which are critical pathogenetic elements of preeclampsia. Podocytes secrete VEGF and neuropilin, a VEGF receptor, are expressed on podocytes, thereby providing evidence that the effect of preeclampsia on podocyte health could even be the primary event.²³ However, the cross talk between podocytes and endothelial cell remains to be investigated.² Sugimoto et al²⁴ reported that the intravenous infusion of sFlt-1 into normal healthy mice caused glomerular endotheliosis and proteinuria through a mechanism that was likely associated with the downregulation of nephrin, a

specific protein of the podocyte slit diaphragm. The low serum and urinary levels of PIGF were associated with an increased risk for subsequent preeclampsia.^{15,25} Several researchers have reported that circulating free VEGF and PIGF are decreased in preeclampsia due to their binding to the antagonistic receptor sFlt-1, present in elevated levels.²⁶⁻²⁸ Other investigations suggest that the elevated levels of serum and plasma VEGF in patients with preeclampsia may also contribute to the extravasations of proteins and the subsequent development of proteinuria.²⁹⁻³² This study indicates that serum VEGF concentrations in women with preeclampsia are significantly higher than in women with normal pregnancies and are slightly higher than in women with gestational hypertension. However, there is no significant difference in the urinary VEGF concentrations. Elevated serum VEGF levels may be involved in the activation and dysfunction of endothelial cells.³³ It is possible that the hyperdynamic circulation that characterizes the latent phase of preeclampsia causes vascular shear stress, which in turn increases the levels of circulating VEGF. Despite the increased

Figure 5. The ROC curve of the number of urinary podocytes (A), the concentrations of serum and urinary VEGF (B), PIGF (C), and sFlt-1 (D) and the PIGF/sFlt-1 ratio (E) for the identification of preeclampsia. The AUC of podocyturia is 0.868 (95% CI: 0.751-0.985, $P < .001$); the AUC of serum and urinary VEGF is 0.675 (95% CI, 0.483-0.866, $P = .079$) and 0.550 (95% CI, 0.354-0.746, $P = .613$) respectively; the AUC of serum and urinary PIGF is 0.872 (95% CI, 0.744-1.000, $P < .001$) and 0.811 (95% CI, 0.681-0.941, $P = .001$), respectively; the AUC of serum and urinary sFlt-1 is 0.590 (95% CI, 0.414-0.766, $P = .350$) and 0.567 (95% CI, 0.383-0.752, $P = .483$), respectively; the AUC of the serum and urinary PIGF/sFlt-1 ratio is 0.847 (95% CI, 0.728-0.965, $P < .001$) and 0.815 (95% CI, 0.686-0.943, $P = .001$), respectively. Null hypothesis: true area = 0.5. VEGF indicates vascular endothelial growth factor; PIGF, placental growth factor; sFlt-1, antiangiogenic factor soluble fms-like tyrosine kinase 1; ROC, receiver-operating characteristics.

Table 3. Different Cutoff Points for Podocyturia and the Evaluated Index for Preeclampsia Diagnosis.

Cutoff Points, Cells/mL	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %	Agreement Rate, %	Youden Index, <i>r</i>	Positive LR	Negative LR
0.6	100	53.85	70.00	100	77.78	0.54	2.17	0
0.8	100	61.54	73.68	100	81.48	0.62	2.60	0
1.0	100	76.92	82.35	100	88.89	0.77	4.33	0
2.0	85.71	84.62	85.71	84.62	85.19	0.70	5.57	0.17
3.0	57.14	100	100	68.42	77.78	0.57	—	0.43

Abbreviation: LR, likelihood ratio.

serum levels of total VEGF, the levels of free-flowing VEGF may be decreased in preeclampsia compared to normal pregnancy.³¹ Thus, the decrease in the freely circulating levels of VEGF, rather than the increased total VEGF levels, may be involved in the pathogenesis of preeclampsia.³⁴ Our detection of serum and urinary PIGF and sFlt-1 concentrations as well as their alterations among the 3 groups is consistent with previous work,^{14,15,25,27,35,36} although the levels of these detection were lower than those reported in some literature.^{14,15} The discrepancy may be produced by some factors as follows: the gestational age and the severity of preeclampsia; the time of

preeclampsia onset; the ethnic difference; the situation of placenta and fetus from different patients which involve the secretion of VEGF, PIGF, and sFlt-1. In addition, the experimental conditions such as the length of storage time of the samples and the sensitivity of detective reagents, and so on may also contribute to the discrepancy. The elevation of sFlt-1 levels, which is well documented in preeclampsia, may disrupt the slit diaphragm proteins, thereby causing proteinuria.³ Gestational hypertension may be an intermediate stage that has some features of both normal pregnancy and preeclampsia. Furthermore, the correlation analysis may support the idea that the

Table 4. Different Cutoff Points for Serum PIGF and the Evaluated Index for Preeclampsia Diagnosis.

Cutoff Points, Cells/mL	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %	Agreement Rate, %	Youden Index, <i>r</i>	Positive LR	Negative LR
55	57.14	100	100	68.42	77.78	0.57	—	0.43
57	71.43	100	100	76.47	85.19	0.71	—	0.29
60	85.71	92.31	92.31	85.71	88.89	0.78	11.14	0.15
63	92.86	76.92	81.25	90.91	85.19	0.70	4.02	0.09
65	92.86	53.85	68.42	87.50	74.07	0.47	2.01	0.13
70	100	15.38	56.00	100	59.29	0.15	1.18	0

Abbreviations: LR, likelihood ratio; PIGF, placental growth factor.

Table 5. Different Cutoff Points for Urinary PIGF and the Evaluated Index for Preeclampsia Diagnosis.

Cutoff Points, pg/mL	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %	Agreement Rate, %	Youden Index, <i>r</i>	Positive LR	Negative LR
8	21.43	84.62	60.00	50.00	51.85	0.06	1.39	0.93
10	35.71	76.92	62.50	52.63	51.85	0.13	1.55	0.84
12	42.86	69.23	60.00	52.94	51.85	0.12	1.39	0.83
14	62.29	53.85	60.00	58.33	51.85	0.18	1.39	0.66
16	85.71	46.15	63.16	75.00	51.85	0.32	1.59	0.31
20	92.86	46.15	65.00	85.71	51.85	0.39	1.72	0.15
25	100	46.15	66.67	100	51.85	0.46	1.86	0
30	100	38.46	63.64	100	51.85	0.38	1.63	0

Abbreviations: LR, likelihood ratio; PIGF, placental growth factor.

Table 6. Different Cutoff Points for the Serum PIGF/sFlt-1 Ratio and the Evaluated Index for Preeclampsia Diagnosis.

Cutoff Points, pg/mL	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %	Agreement Rate, %	Youden Index, <i>r</i>	Positive LR	Negative LR
0.10	57.14	100	100	68.42	77.78	0.57	—	0.43
0.11	71.43	92.31	90.91	75.00	81.48	0.64	9.29	0.31
0.12	92.86	76.92	81.25	90.91	85.19	0.70	4.02	0.09
0.13	100	38.46	62.64	100	70.37	0.38	1.63	0

Abbreviations: LR, likelihood ratio; PIGF, placental growth factor; sFlt-1, antiangiogenic factor soluble fms-like tyrosine kinase 1.

Table 7. Different Cutoff Points for the Urinary PIGF/sFlt-1 Ratio and the Evaluated Index for Preeclampsia Diagnosis.

Cutoff Points	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %	Agreement Rate, %	Youden Index, <i>r</i>	Positive LR	Negative LR
0.010	21.43	84.62	60.00	50.00	51.85	0.06	1.39	0.93
0.015	50.00	69.23	63.64	56.25	59.26	0.19	1.63	0.72
0.020	78.57	46.15	61.11	66.67	62.96	0.25	1.46	0.46
0.025	92.86	46.15	65.00	85.71	70.37	0.39	1.72	0.15
0.050	100	38.46	63.64	100	70.37	0.38	1.63	0

Abbreviations: LR, likelihood ratio PIGF, placental growth factor; sFlt-1, antiangiogenic factor soluble fms-like tyrosine kinase 1.

imbalance of serum and urinary VEGF, PIGF, and sFlt-1 induces podocyte injury and leads to proteinuria in preeclampsia. As kidney biopsies are rarely performed during preeclampsia, the development of noninvasive screening and diagnostic strategies is essential for the early detection of this disease. The ROC curves show that podocyuria, serum and urinary PIGF levels, and the PIGF/sFlt-1 ratio were superior to VEGF and

sFlt-1 levels alone for identifying preeclampsia. Furthermore, the analysis of sensitivity and specificity found that higher sFlt-1 concentrations and lower PIGF levels increase the predictive value of podocyuria. The combination of podocyuria and/or PIGF may be helpful for aiding in the identification of preeclampsia. The PIGF/sFlt-1 ratio may also have utility as an indicator of preeclampsia.

Table 8. Different Sensitivities and Specificities Using Podocyturia and Serum and Urinary PIGF Alone, in Parallel or in Series.

	Test Method	Sensitivity, %	Specificity, %
1	Serum PIGF <60 pg/mL	85.71	92.31
2	Urinary PIGF <25 pg/mL	100	46.15
3	Podocyturia $\geq$ 1 cells/mL	100	76.92
1 and 2	In parallel	100	42.60
1 and 2	In series	85.71	95.86
2 and 3	In parallel	100	35.50
2 and 3	In series	100	87.57
1 and 3	In parallel	100	71.00
1 and 3	In series	85.71	98.22

Abbreviations: PIGF, placental growth factor.

Of course, the limitation should also be addressed. As the sample size was relatively small in this study, there may be little numerical discrepancy. Nevertheless, the overall trend of the results were consistent with literature,^{14,15,25,26,36} which proved that the analyses from the small sample size was still worthy of credible so that the generated conclusion was also meaningful.

In conclusion, deregulation of pro- and antiangiogenic factors and subsequent podocyte injury may represent a possible mechanism for the development of proteinuria in preeclampsia. Podocyturia, PIGF, and PIGF/sFlt-1 ratios may provide clue for discovering early diagnostic markers for preeclampsia. Further prospective studies of larger cohorts are needed to explore this in detail.

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Authors' Note

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References

- Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet*. 2005; 365(9461):785-799.
- Collino F, Bussolati B, Gerbaudo E, et al. Preeclamptic sera induce nephrin shedding from podocytes through endothelin-1 release by endothelial glomerular cells. *Am J Physiol Renal Physiol*. 2008;294(5):F1185-F1194.
- Garovic VD, Wagner SJ, Petrovic LM, et al. Glomerular expression of nephrin and synaptopodin, but not podocin, is decreased in kidney sections from women with preeclampsia. *Nephrol Dial Transplant*. 2007;22(4):1136-1143.
- Achenbach J, Mengel M, Tossidou I, et al. Parietal epithelia cells in the urine as a marker of disease activity in glomerular diseases. *Nephrol Dial Transplant*. 2008;23(10):3138-3145.
- Petermann A, Floege J. Podocyte damage resulting in podocyturia: a potential diagnostic marker to assess glomerular disease activity. *Nephron Clin Pract*. 2007;106(2):c61-c66.
- Yu D, Petermann A, Kunter U, Rong S, Shankland SJ, Floege J. Urinary podocyte loss is a more specific marker of ongoing glomerular damage than proteinuria. *J Am Soc Nephrol*. 2005; 16(6):1733-1741.
- Hara M, Yanagihara T, Kihara I, Higashi K, Fujimoto K, Kajita T. Apical cell membranes are shed into urine from injured podocytes: a novel phenomenon of podocyte injury. *J Am Soc Nephrol*. 2005;16(2):408-416.
- Skoerme A, Konieczny A, Schiffer M. Glomerular epithelial cells in the urine: what has to be done to make them worthwhile? *Am J Physiol Renal Physiol*. 2009;296(2):F230-F241.
- Garovic VD, Wagner SJ, Turner ST, et al. Urinary podocyte excretion as a marker for preeclampsia. *Am J Obstet Gynecol*. 2007;196(4):320.e1-320.e7.
- Aita K, Etoh M, Hamada H, et al. Acute and transient podocyte loss and proteinuria in preeclampsia. *Nephron Clin Pract*. 2009; 112(2):c65-c70.
- Karumanchi SA, Lindheimer MD. Preeclampsia and the kidney: footprints in the urine. *Am J Obstet Gynecol*. 2007;196(4):287-288.
- Roberts L, LaMarca BB, Fournier L, Bain J, Cockrell K, Granger JP. Enhanced endothelin synthesis by endothelial cells exposed to sera from pregnant rats with decreased uterine perfusion. *Hypertension*. 2006;47(3):615-618.
- Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med*. 2006;355(10):992-1005.
- Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*. 2004; 350(7):672-683.
- Levine RJ, Thadhani R, Qian C, et al. Urinary placental growth factor and risk of preeclampsia. *JAMA*. 2005;293(1):77-85.
- . Report of the National High Blood Pressure Education Program Working Group on high blood pressure in pregnancy. *Am J Obstet Gynecol*. 2000;183(1):S1-S22.
- Nakamura T, Ushiyama C, Hirokawa K, et al. Effect of cerivastatin on proteinuria and urinary podocytes in patients with chronic glomerulonephritis. *Nephrol Dial Transplant*. 2002;17(5):798-802.
- Hara M, Yanagihara T, Kihara I. Cumulative excretion of urinary podocytes reflects disease progression in IgA nephropathy and Schonlein-Henoch purpura nephritis. *Clin J Am Soc Nephrol*. 2007;2(2):231-238.
- Shah DM. Role of the renin-angiotensin system in the pathogenesis of preeclampsia. *Am J Physiol Renal Physiol*. 2005;288(4): F614-625.

20. Strevens H, Wide-Svensson D, Hansen A, et al. Glomerular endotheliosis in normal pregnancy and pre-eclampsia. *BJOG*. 2003;110(9):831-836.
21. Lemley KV, Lafayette RA, Safai M, et al. Podocytopenia and disease severity in IgA nephropathy. *Kidney Int*. 2002;61(4):1475-1485.
22. Nakamura T, Kawagoe Y, Ogawa H, et al. Effect of low-density lipoprotein apheresis on urinary protein and podocyte excretion in patients with nephrotic syndrome due to diabetic nephropathy. *Am J Kidney Dis*. 2005;45(1):48-53.
23. Harper SJ, Xing CY, Whittle C, et al. Expression of neuropilin-1 by human glomerular epithelial cells in vitro and in vivo. *Clin Sci (Lond)*. 2001;101(4):439-446.
24. Sugimoto H, Hamano Y, Charytan D, et al. Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. *J Biol Chem*. 2003;278(15):12605-12608.
25. Thadhani R, Mutter WP, Wolf M, et al. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *J Clin Endocrinol Metab*. 2004;89(2):770-775.
26. Lam C, Lim KH, Karumanchi SA. Circulating angiogenic factors in the pathogenesis and prediction of preeclampsia. *Hypertension*. 2005;46(5):1077-1085.
27. Berkane N, Lefevre G, Hertig A. Angiogenic factors in preeclampsia: so complex, so simple? *Nephrol Dial Transplant*. 2007;22(10): 2753-2756.
28. Hawfield A, Freedman BI. Pre-eclampsia: the pivotal role of the placenta in its pathophysiology and markers for early detection. *Ther Adv Cardiovasc Dis*. 2009;3(1):65-73.
29. Baker PN, Krasnow J, Roberts JM, Yeo KT. Elevated serum levels of vascular endothelial growth factor in patients with preeclampsia. *Obstet Gynecol*. 1995;86(5):815-821.
30. Hunter A, Aitkenhead M, Caldwell C, McCracken G, Wilson D, McClure N. Serum levels of vascular endothelial growth factor in preeclamptic and normotensive pregnancy. *Hypertension*. 2000;36(6):965-969.
31. Bosio PM, Wheeler T, Anthony F, Conroy R, O'Herlihy C, McKenna P. Maternal plasma vascular endothelial growth factor concentrations in normal and hypertensive pregnancies and their relationship to peripheral vascular resistance. *Am J Obstet Gynecol*. 2001;184(2):146-152.
32. Munaut C, Lorquet S, Pequeux C, et al. Hypoxia is responsible for soluble vascular endothelial growth factor receptor-1 (VEGFR-1) but not for soluble endoglin induction in villous trophoblast. *Hum Reprod*. 2008;23(6):1407-1415.
33. Akercan F, Cirpan T, Terek MC, et al. The immunohistochemical evaluation of VEGF in placenta biopsies of pregnancies complicated by preeclampsia. *Arch Gynecol Obstet*. 2008;277(2):109-114.
34. Sheppard SJ, Khalil RA. Risk factors and mediators of the vascular dysfunction associated with hypertension in pregnancy. *Cardiovasc Hematol Disord Drug Targets*. 2010;10(1):33-52.
35. Hladunewich M, Karumanchi SA, Lafayette R. Pathophysiology of the clinical manifestations of preeclampsia. *Clin J Am Soc Nephrol*. 2007;2(3):543-549.
36. Troisi R, Braekke K, Harsem NK, Hyer M, Hoover RN, Staff AC. Blood pressure augmentation and maternal circulating concentrations of angiogenic factors at delivery in preeclamptic and uncomplicated pregnancies. *Am J Obstet Gynecol*. 2008;199(6):653.e1- 653.e10.