Elevation of Urinary Adipsin in Preeclampsia
Correlation With Urine Protein Concentration and the Potential Use for a Rapid Diagnostic Test

Tao Wang,* Rong Zhou,* Linbo Gao, Yanyun Wang, Changping Song, Yunhui Gong, Jin Jia, Wei Xiong, Li Dai, Lin Zhang, Huaizhong Hu

Abstract—Early diagnosis and treatment of preeclampsia are essential for prevention of seizure development and fetus maturation. Although various methods have been developed for predicting or monitoring the onset of preeclampsia, a simple assay that can be used as a home or point of care test remains unavailable. We attempted to find a urinary protein that could be used as a biomarker for developing such a test. Urinary samples were collected from 124 preeclampsia and 135 healthy pregnant women for screening using a protein array technology and quantification by ELISA. A urinary protein, adipsin, was found significantly increased, and the adipsin creatinine ratio was closely correlated with the urinary 24-hour protein in patients with preeclampsia. When combined with the increased diastolic blood pressure (≥90 mm Hg), the sensitivity was 90.3% and the specificity reached 100.0% for preeclampsia diagnosis. We then developed a laminar flow immunoassay for rapid diagnosis, and the sensitivity and specificity were 89.04% and 100%, respectively, when combined with increased diastolic blood pressure. Because of the easiness of sample collection, assay conduction, and result interpretation, this urine test can be potentially used as a home test for monitoring preeclampsia onset for high-risk pregnant women and as a rapid test for a preliminary diagnosis for emergency patients at hospitals. (Hypertension. 2014;64:846-851.) ● Online Data Supplement

Key Words: preeclampsia ■ protein creatinine ratio ■ urinary adipsin ■ urinary protein ■ urinary rapid test

Preeclampsia, a devastating pregnancy-specific syndrome complicating 2% to 8% of pregnancies, is responsible for ≥60,000 maternal deaths worldwide every year. It is characterized by new-onset hypertension, proteinuria, and edema and usually develops after 20 weeks of gestation.1,2 Early diagnosis and treatment of preeclampsia is essential for prevention of seizure development and maintaining the fetus in the uterus to mature. Many attempts have been made to meet these purposes. It has been found that altered concentration of soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PIGF) can predict the occurrence of preeclampsia weeks before the appearance of the clinical symptoms.3-6 Uterine artery Doppler studies that assess the pulsatility index reveal increased uterine vascular resistance well before the preeclampsia clinical signs arise.7 These predictive tests are valuable for identifying high-risk pregnant women but are incapable of conveniently monitoring the exact occurrence of the disease. In this study, we assumed that in preeclampsia a urinary protein that closely correlated with the 24-hour urine protein could be identified with advanced proteomics technologies and could be developed as a simple rapid assay for point-of-care use and for home monitoring as well.

In 1843, John Lever of Guy’s Hospital in London discovered the presence of albumin by boiling the urine from pregnant women with puerperal convulsions.8 Proteinuria in preeclampsia since then has been studied extensively. The glomerular injury that results in proteinuria is characterized as endotheliosis, manifested as glomerular endothelial swelling with loss of endothelia fenestrae and occlusion of the capillary lumens.9,10 Proteinuria is measured currently by a 24-hour urine protein determination or a dipstick examination on a random urine sample. The 24-hour urine protein quantification is no doubt the most accurate and reliable approach but is time consuming and inconvenient. Dipstick is semiquantitative, easy to use, quick, and inexpensive. However, many studies have reported a poor correlation between the dipstick examination and the 24-hour urine assay with high false-positive or false-negative rates.11,12

We aimed to find a urine protein in preeclampsia that closely correlated with the 24-hour urine protein and was at a
concentration range allowing the development of a rapid laminar flow immunoassay. This rapid test should be sensitive and specific for the preeclampsia diagnosis and can be easily used by both patients and healthcare providers. Through a series of screening assays and confirmative evaluation, we identified, for this first time, that urinary adipsin (also called complement factor D) was a protein that met these requirements.

Materials and Methods

Subjects

The research protocol was approved by the Institutional Committee for the Protection of Human Subjects (the Institutional Review Board of West China Second University Hospital, Sichuan University), and all patients provided informed consent. Between September 2010 and October 2012, 259 pregnant women and 65 healthy nonpregnant women at reproductive age were enrolled in this study. Among the pregnant individuals, 124 were diagnosed as preeclampsia (106 primary preeclampsia and 18 superimposed preeclampsia), and 135 were non-preeclampsia pregnant women (Table 1). Preeclampsia was diagnosed as previously described.\textsuperscript{4,13} Severe preeclampsia was confirmed as systolic blood pressure (BP) $\geq 160$ mm Hg, or diastolic BP $\geq 110$ mm Hg on 2 occasions $\geq$ 6 hours apart while the patient is on bed rest, or a proteinuria of 2 g or higher in a 24-hour urine specimen. Primary preeclampsia was diagnosed as new hypertension and quantified proteinuria at or after 20 weeks of pregnancy and resolved by 12 weeks postpartum. Superimposed preeclampsia was confirmed as a development of features of preeclampsia in context of pre-existing hypertension or pre-existing proteinuria or both.\textsuperscript{4,13} Non-preeclampsia pregnant women were recruited according to the following criteria: (1) normotensive pregnant woman and (2) negative result of random urine sample with a urine dipstick test.

Sample Collection

Five to 10 mL of a midstream urine sample was each collected by using a sterile container. Serial urine samples were collected from pregnant women enrolled in the follow-up study. Samples were centrifuged at 1600 rpm and 4°C for 10 minutes. The plasma was then aliquoted, and stored at $-80^\circ$C until use.

Determination of Urine 24-Hour Protein, Urinary Protein, and Creatinine

The volume of a 24-hour urine collection was determined, and the protein concentration was measured with an ADVIA2400 automatic biochemical analyzer (Siemens Ltd, Munich, Germany). Urinary protein and creatinine (Cr) in a spot urine sample were also determined by ELISA using commercial kits purchased from RayBiotech, Inc (Norcross, GA), R&D Systems (Minneapolis, MN), and Bender MedSystems (Vienna, Austria). The experiments were conducted by following the manufacturer’s suggested procedures as described previously.\textsuperscript{14}

Screening Assay Using Antibody Array

Two preeclampsia urine samples and 2 non-preeclampsia pregnant women urine samples were used for a screening assay by using an antibody array (C Series 4000, RayBiotech Inc, Norcross, GA). This array could simultaneously detect 274 human soluble proteins. The experiments were conducted by following the manufacturer’s suggested procedures as described previously.\textsuperscript{14}

Detection of Urinary Adipsin with Strip Test

Strip tests for urinary adipsin were prepared using colloidal gold laminar flow technology. This was a qualitative rapid test for revealing adipsin in urine samples. One milliliter of a urine sample was diluted in a test tube containing 2 mL of sterile PBS. After pipetting up and down 10x, 2 drops of the buffer sample were added onto the sample well of the test card. One or 2 test lines could be observed in the control and test window within 10 to 15 minutes. Presence of the orange-purple test line and control line was determined as positive for adipsin. Absence of the test line and presence of the control line were determined as negative for adipsin.

Table 1. Clinic Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Healthy Nonpregnant Women (n=65)</th>
<th>Non-PE Pregnant Women (n=135)</th>
<th>PE (n=124)</th>
<th>Mild PE (n=18)</th>
<th>Severe PE (n=106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>23.88±3.34 (13–33)</td>
<td>30.67±4.23 (20–41)</td>
<td>31.73±5.83 (19–45)</td>
<td>32.17±6.02 (24–41)</td>
<td>31.65±5.82 (19–45)</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m$^2$</td>
<td>21.04±2.81 (14.45–25.80)</td>
<td>23.34±5.48 (16.02–36.36)</td>
<td>22.50±3.20 (16.87–27.89)</td>
<td>22.74±3.53 (16.02–36.36)</td>
<td></td>
</tr>
<tr>
<td>Primagravida</td>
<td>...</td>
<td>83</td>
<td>79</td>
<td>18</td>
<td>61</td>
</tr>
<tr>
<td>Current smoking</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Diabetes mellitus</td>
<td>19</td>
<td>29</td>
<td>9</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery, d</td>
<td>272.9±8.49 (271.4–274.3)</td>
<td>246.2±23.2 (177–289)*</td>
<td>256.78±16.39 (234–286)</td>
<td>244.86±23.45 (177–289)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>114.8±8.68 (98–139)</td>
<td>157.05±13.09 (127–172)</td>
<td>158.82±16.87 (127–202)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>72.33±8.00 (53–89)</td>
<td>102.03±8.48 (90–130)*</td>
<td>97.1±5.35 (90–108)</td>
<td>102.86±8.65 (90–130)</td>
<td></td>
</tr>
<tr>
<td>24-hour urinary protein, g</td>
<td>...</td>
<td>2.94±2.37 (0.16–10.17)</td>
<td>0.62±0.33 (0.24–1.35)</td>
<td>3.48±2.31 (0.16–10.17)</td>
<td></td>
</tr>
</tbody>
</table>

BMI indicates body mass index; and PE, preeclampsia.

* $P<0.05$, PE vs non-PE pregnant women.
Statistical Analysis
The levels of adipsin, Acrp30, and FLRG in urine and plasma, adipsin/Cr in urine, and sFlt-1 and PIGF in plasma were expressed as mean value±SD. The statistical significance of adipsin/Cr in patients with preeclampsia and controls was assessed by Student t test using a computer software Prism 5 from GraphPad Software (San Diego, CA). P≤0.05 was considered significant. Correlation analysis of urinary adipsin/Cr and 24-hour urine protein was assessed using Prism 5 as well.

Sensitivity and specificity of the urinary adipsin tests were calculated as follows: sensitivity=number of true positive specimens/[number of true positive specimens+number of false-negative specimens]; specificity=number of true negative specimens/[number of true negative specimens+number of false-positive specimens (FP)].

Results
Screening of Urine Samples
A protein antibody array was used to reveal the urinary protein spectrum in 2 preeclampsia and 2 non-preeclampsia pregnant women. This array was a semiquantitative assay assessed by the density of each analyte spot. Stronger signals that indicate the presence of higher concentration of proteins were seen in the preeclampsia samples (Figure S1 in the online-only Data Supplement). From the analytes that showed much stronger signals in preeclampsia than those of the controls, Acrp30, adipsin, and FLRG were selected for quantitative analyses (Tables S1 and S2).

Adipsin Level in Plasma and Urine
After the assessment of Acrp30, adipsin, and FLRG with a small panel of urine samples, we found that adipsin correlated best with the 24-hour urine protein. Adipsin was then selected for an expanded evaluation. The mean value of adipsin in preeclampsia, non-preeclampsia pregnant women, and healthy nonpregnant women urine samples was 539.93±1134.55 ng/mL, 14.48±35.88 ng/mL, and 5.14±4.95 ng/mL, respectively, and the adipsin/Cr value (Figure 1B) was 693.23±1400.21 mg/g, 15.66±33.39 mg/g (P<0.01, preeclampsia versus non-preeclampsia pregnant women), and 4.46±3.57 mg/g (P<0.01, preeclampsia versus healthy nonpregnant women), respectively.

To find out whether the difference of the urinary adipsin concentration was originated from a difference in the blood, plasma samples were examined (Figure 1A). The adipsin concentrations were 6221.05±2527.65 ng/mL, 5183.55±1988.51 ng/mL, and 6685.56±1003.20 ng/mL, respectively, in preeclampsia, non-preeclampsia pregnant women, and healthy nonpregnant women (P<0.05, preeclampsia versus non-preeclampsia pregnant women; P>0.05, preeclampsia versus healthy nonpregnant women; P<0.05, non-preeclampsia pregnant women versus healthy nonpregnant women). Based on the results, because the adipsin concentration was only slightly higher in patients with preeclampsia, the increase of adipsin in urine of patients with preeclampsia does not seem to be originated from a difference of the adipsin plasma concentration.

Urinary adipsin concentration was evaluated in association with the pregnancy outcome (Figure S2). Pregnant women with high adipsin concentration tended to deliver the baby prematurely (P<0.01), and the new born babies tended to have a lower body weight (P<0.01) and length (P<0.01) at birth.

Serial urine samples were collected from 15 preeclampsia and 6 non-preeclampsia pregnant women before and after the delivery. Adipsin/Cr level decreased in patients with preeclampsia from 506.63±970.01 mg/g before delivery to 7.09±11.79 mg/g at 1 month after delivery, whereas the ratio in non-preeclampsia pregnant women also slightly decreased from 8.85±5.86 mg/g to 4.94±3.65 mg/g during the same period (Figure S3). This indicates that with the resolving of preeclampsia disease activity, adipsin excretion could decrease to the baseline level.

Correlation Between Urinary Adipsin/Cr and sFlt-1/PIGF
Urine adipsin/Cr was analyzed for correlation with a well established preeclampsia biomarker, sFlt-1/PIGF ratio. Compared with the controls, patients with preeclampsia had a much higher adipsin/Cr in urine, a higher sFlt-1, and a lower PIGF in plasma (Figure S4). Moreover, a linear correlations was observed between PIGF or sFlt-1/PIGF and urinary adipsin/Cr (r²=0.08, P<0.05; r²=0.18, P<0.01, n=64; Figure S5).

Diagnostic Value of Urinary Adipsin for Preeclampsia
Among the 124 patients with preeclampsia, 96 patients were diagnosed based on a 24-hour urine protein quantification and
28 patients based on a dipstick test. As presented in Figure 2, a correlation was noted between the urine adipsin/Cr and the 24-hour urine protein in patients with preeclampsia ($r^2=0.21$, $P<0.01$). This correlation suggests that measurement of urinary adipsin could be used as a biomarker for the diagnosis of preeclampsia (Table 2).

Because the diagnosis of preeclampsia is based on the increase of both BP and urine proteins, the assessment of the diagnostic value of adipsin is combined with the BP elevation. When diastolic BP ≥90 mm Hg and adipsin/Cr ≥15 mg/g was used as the cutoff, the sensitivity of the test was 90.3% and the specificity reached 100% because of the adoption of the dual parameter (Figure 3; Table 2). Moreover, if the adipsin/Cr is combined with sFlt-1/PIGF, the sensitivity can be further increased (Table S3), indicating that the urinary and blood biomarkers are complementary for preeclampsia diagnosis.

**Adipsin Strip Test for Preeclampsia Diagnosis**

Because the urinary adipsin seemed to be a good biomarker for preeclampsia diagnosis, and its urinary level was at nanogram level, a colloid gold-based laminar flow immunoassay, that usually can detect biomarkers at ≥2 ng/mL, could be developed as a point-of-care rapid test.

However, a reliable rapid urine test depends on the diagnostic value of adipsin in a random urine sample, and the adipsin concentration in a random urine sample has to be representative for the adipsin/Cr to maintain a high sensitivity and specificity. A strong correlation between adipsin absolute concentration (ng/mL) and adipsin/Cr (mg/g) was observed in both patients with preeclampsia ($r^2=0.5215$, $P<0.01$) and non-preeclampsia pregnant women ($r^2=0.7132$, $P<0.01$). Based on the analyses, a regression equation can be generated as $\log_2 y = 0.925 \log_2 x - 0.065$, where $x$ indicates the urinary adipsin and $y$ is the urinary adipsin/Cr. The cutoff point value for adipsin/Cr, 15 mg/g, then corresponds to 12 ng/mL of the absolute value of adipsin. When combined with diastolic BP ≥ 90 mm Hg, the sensitivity was 88.7% for the diagnosis of preeclampsia, close to that of adipsin/Cr (90.3%), indicating that a useful rapid test based solely on adipsin concentration in a random urine sample can be developed.

A laminar flow test using colloid gold as the detection signal was then developed. This assay had a detection power of ≥4.0 ng/mL (data not shown). Urine samples were, therefore, diluted by adding 2 volumes of buffer for the assessment of 75 patients with preeclampsia and 89 non-preeclampsia pregnant women. The strip test (Figure 4) alone showed a sensitivity of 89.04% and a specificity of 80.9% for the diagnosis of preeclampsia. When combined with an increased diastolic BP reading, the specificity was again 100%.

### Table 2. Sensitivity and Specificity of Urinary Adipsin/Cr and Elevated Diastolic Blood Pressure for Diagnosis of Preeclampsia

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Adipsin/Cr ≥15.0</th>
<th>Diastolic Blood Pressure ≥90 mm Hg and Adipsin/Cr ≥15.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, %</td>
<td>90.3</td>
<td>90.3</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>77.8</td>
<td>100</td>
</tr>
</tbody>
</table>

Cr indicates creatinine.

**Discussion**

Adipsin is a serine protease essential for the activation of the complement alternative pathway and is synthesized mainly by adipocytes and macrophages.15–17 Its plasma concentration was determined in the present study, and no significant differences were noted among preeclampsia, non-preeclampsia pregnant, and healthy nonpregnant women; however, we found that urinary concentrations of adipsin were significantly higher in women with preeclampsia. It is known that adipsin plasma levels increase 10-fold in end-stage renal failure, suggesting that its metabolism is closely linked to the renal function.18–20 Pascual et al21 studied adipsin metabolism in humans by injecting purified radiolabeled adipsin into 5 healthy individuals and 12 patients with various renal diseases or renal failure. The proportion of adipsin elimination in the urine was increased in patients with tubular dysfunction, indicating that under normal circumstances adipsin is filtered through the glomeruli and reabsorbed by tubular cells.22 Based on this observation and the pathological changes of glomeruli in preeclampsia, the adipsin concentration changes in the urine of patients with preeclampsia can be explained. At the time of full preeclampsia activity, the damaged glomeruli filter an excessive amount of adipsin into the preurine, surpassing the reabsorption capacity of the tubules, and that leads to...
the increased adipsin concentration in urine. After delivery of the child, the preeclampsia activity reduced, and the urine adipsin subsequently reduced to the basal level because of the decreased filtration through the glomeruli.

Adipsin does not seem to be a protein that is involved in the pathogenesis of preeclampsia. Instead, it happens to be a protein that has an output trend similar to the 24-hour urine protein, making it a good biomarker for the diagnosis of preeclampsia. Furthermore, the adipsin concentration in urine is at the nanogram scale, suitable for developing a rapid test using laminar flow immunoassay.

For pregnancies that have a high risk to develop preeclampsia, based on clinical parameters or sFlt-1 and PIGF,3–6 home-based self-monitoring of BP in combination with the adipsin strip test seems to be a feasible approach for early detection of the preeclampsia onset. Because of the easiness of the test conduction and result interpretation, it requires only a brief education for the test conductor. Moreover, the combination of the BP and adipsin rapid test has a high sensitivity of 89% with an even higher specificity (100%). When double positive results, increased BP and a positive adipsin test, are noted, the patient should seek for medical help as early as possible. Nonetheless, a major concern is that the sensitivity of the adipsin rapid test is only 89%, and the remaining 11% of the potential preeclampsia patients will be missed by the home test. This is indeed an issue, and the sensitivity should be improved in the future by including another biomarker that is complementary to adipsin.

The use of the adipsin test as a point-of-care diagnostic test can provide a quick suggestive answer for the diagnosis of preeclampsia at hospitals. The test will generate a result within 10 to 15 minutes, quicker than any urinary tests that are available nowadays. Furthermore, this rapid test seemed to have a better value than the dipstick test, which has a sensitivity and specificity in the range of 51% to 81% and 47% to 91%, respectively.22–25 Financially, inexpensive materials are used to build the strip test, and the use of such a kit could be more affordable than a dipstick test.

Because this is the first time that adipsin is described as a urinary biomarker based on the kidney injury in preeclampsia, further studies are needed to validate these findings and correlate urinary adipsin to adverse maternal outcomes such as severe hypertension, HELLP syndrome, and eclampsia besides intrauterine growth restriction. Adipsin needs to be investigated in women with underlying renal diseases (lupus, diabetes mellitus, and chronic hypertension) in terms of defining the specificity to preeclampsia.

In summary, the urinary adipsin concentration quantitatively correlates well with the urinary 24-hour protein and seems to be a good biomarker for the diagnosis of preeclampsia. Based on these observations, a laminar flow immunoassay has been developed as a rapid test that seems to be useful as a home test or a point-of-care test.

**Perspectives**

A home urine test can help monitoring the preeclampsia occurrence. The current adipsin strip test is easy to use and can potentially become such a test. Further studies are required to verify whether the test can reveal the disease in combination with the BP measurement before the clinical symptoms arise.

**Sources of Funding**

This study was financially supported by grants from National Natural Science Foundation of China (no. 81170593), Science and Technology Department of Sichuan Province (2013SZ0022), and Program for Changjiang Scholars and Innovative Research Team at Sichuan University (IRT0935).

**Disclosures**

None.

**References**


with soluble fms-like tyrosine kinase 1 to placental growth factor ratio. 


Novelty and Significance

What Is New?

- We discovered urinary adipsin creatinine ratio, for the first time, to be closely correlated with the urinary 24-hour protein in preeclampsia.

What Is Relevant?

- Urinary adipsin could be used for monitoring the onset and a rapid diagnosis of preeclampsia.

Summary

Urinary adipsin/creatinine ratio, when combined with increased diastolic blood pressure, can be used for the diagnosis of preeclampsia with a sensitivity of 90.3% and a specificity of 100.0%. A laminar flow immunoassay was developed, and this rapid urine test can generate results within minutes and retains a high sensitivity and specificity for preeclampsia diagnosis.
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by

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Running title: Urinary Adipsin in Preeclampsia

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The quantitative screening was conducted by ELISA in two rounds. The first round included FLRG, Acrp30 and adipsin (Table S1), and the second round included Acrp30 and adipsin (Table S2). FLRG and Acrp30 were excluded from a further evaluation because of their much smaller difference than adipsin between the PE patients and the non-PE controls.

Concentrations of sFlt-1 and PIGF in plasma samples were determined in duplicate by ELISA using commercial kits purchased from R&D Systems (Minneapolis, MN), and expressed as mean ± standard deviation (SD). The statistical significance of adipsin/Cr in PE patients and controls was assessed by Student t test using a computer software Prism 5 from GraphPad Software (San Diego, CA). P value ≤0.05 was considered significant (Figure S4). Correlation analysis between urinary adipsin/Cr and PIGF, or ratio of sFlt-1/PIGF (Figure S5), and determination of the diagnostic sensitivity and specificity (Table S3) were conducted using Prism 5 as well.
Table S1. FLRG, Acrp30, and Adipsin Concentration in Urine Samples from PE and Healthy Pregnant Controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration (ng/ml)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FLRG</td>
<td>Acrp30</td>
<td>adipsin</td>
</tr>
<tr>
<td>PE (n=12)</td>
<td>15.47±21.40*</td>
<td>23.94±15.69*</td>
<td>740.04±1067.92*</td>
</tr>
<tr>
<td>Non-PE pregnant women (n=17)</td>
<td>0.25±0.81</td>
<td>10.42±9.75†</td>
<td>13.80±18.49†</td>
</tr>
<tr>
<td>Healthy non-pregnant women (n=11)</td>
<td>0.18±0.37</td>
<td>1.06±2.61</td>
<td>5.58±5.50</td>
</tr>
</tbody>
</table>

*, P < 0.05 compared with urine sample of non-PE pregnant women
†, P < 0.05 compared with urine sample of healthy non-pregnant women
Table S2.  Acrp30 and Adipsin Concentration in Urine Samples from PE and Healthy Pregnant Controls Concentration (ng/ml)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration (ng/ml)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Acrp30</td>
</tr>
<tr>
<td>PE (n=33)</td>
<td>292.08±168.08*</td>
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<tr>
<td>Non-PE pregnant women (n=45)</td>
<td>99.26±88.82</td>
</tr>
<tr>
<td>Healthy non-pregnant women (n=11)</td>
<td>4.98±3.41</td>
</tr>
</tbody>
</table>

*, P < 0.05 compared with urine sample of non-PE pregnant women and healthy non-pregnant women
Table S3. Urinary Adipsin in Combination with sFlts-1/PIGF Increases the Preeclampsia Diagnostic Sensitivity*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>sFlts-1/PIGF</th>
<th>adipin/Cr</th>
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<th>sFlts-1/PIGF + adipin/Cr + DBP‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>76.6</td>
<td>93.8</td>
<td>96.9</td>
<td>96.9</td>
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<tr>
<td>Specificity (%)</td>
<td>98.2</td>
<td>82.5</td>
<td>80.7</td>
<td>100</td>
</tr>
</tbody>
</table>

*, Patients (n=64) and controls (n=57) that had both urine and plasma samples were included for analysis. Results for sensitivity and specificity were, therefore, not identical to those when all patients and controls were included.
†, When either sFlts-1/PIGF or urinary adipin/Cr was higher than the cutoff value, it was determined as positive.
‡, Based on increased DBP (≥ 90 mmHg), when either sFlts-1/PIGF or urinary adipin/Cr was higher than the cutoff value, it was determined as positive.
**Figure S1.** An antibody array was used to screen urine samples of two preeclampsia patients (B, D) and urine samples of two healthy pregnancies (A, C). The signals of Acrp30 (1), Adipsin (2), and FLRG (3) were much stronger in the PE patients than in the healthy pregnancies.
Figure S2. PE patients had a much higher adipsin/Cr value, and these patients tended to deliver premature children with a lower body weight and length at birth. A, adipsin/Cr value (mg/g); B, infant birth weight normalized by pregnancy days (g/day); C, infant birth length normalized by pregnancy days (cm/day); D, delivery day (day); *: P<0.01
Figure S3. Return of urinary Adipsin/Cr (A) and urinary P/Cr (B) to baseline levels for PE (n=15) and healthy pregnancies (n=6) after the delivery of the child. Urine samples were collected before the delivery or at least 1 month postpartum. 1, before the delivery; 2, 1-16 months postpartum.
Figure S4. Compared to the controls PE patients had a much higher adipsin/Cr in urine, sFlt-1 in plasma, a lower PIGF in plasma, and a higher sFlt-1/PIGF ratio. A, adipsin/Cr (mg/g); B, sFlt-1 (ng/ml); C, PIGF (pg/ml); D, sFlt-1 and PIGF ratio; *: P<0.01
Figure S5. A linear correlations was observed between PIGF, ratio of sFlt-1/PIGF and urinary adipsin/Cr ($r^2=0.08$, $P<0.05$; $r^2=0.18$, $P<0.01$, $n=64$), but not between sFlt-1 and urinary adipsin/Cr ($r^2=0.005$, $P>0.05$) in PE patients. Correlation between sFlt-1 and urinary adipsin/Cr (A); correlation between PIGF and urinary adipisin/Cr (B); correlation between ratio of sFlt-1/PIGF and urinary adipisin/Cr (C).