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Assessment of the diagnostic value of a urinary adipsin rapid strip test for pre-eclampsia: A prospective multicenter study

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Abstract

Aim: The purpose of the present study was to evaluate the clinical value of the rapid strip test of urinary adipsin for the quick diagnosis of pre-eclampsia.

Methods: In a multicenter diagnostic test study, we studied the diagnostic accuracy of the rapid strip test of urinary adipsin in women presenting with pre-eclampsia. A total of 204 pre-eclampsia patients and 254 healthy pregnant women were recruited for this study, respectively. The rapid strip test of urinary adipsin was used to detect the adipsin in the urine of each patient.

Results: The diagnostic value of the rapid strip test of urinary adipsin for pre-eclampsia was demonstrated by its high sensitivity and specificity (95.10% and 97.64%, respectively). The diagnostic accuracy was 96.51%. The consistency analysis showed that the kappa value was 0.93 compared with the gold standard diagnosis of pre-eclampsia.

Conclusion: The rapid strip test of urinary adipsin is a non-invasive test for the diagnosis of pre-eclampsia with high sensitivity and specificity. It could help the quick diagnosis of pre-eclampsia in clinical practice greatly.

Key words: diagnosis, hypertension, pre-eclampsia, urinary adipsin.

Introduction

Pre-eclampsia is a major cause of maternal and perinatal morbidity and mortality that complicates 2–8% of all pregnancies. It is clinically characterized by hypertension and proteinuria.[1,2] Pre-eclampsia has been considered as a multisystem endothelial disease that leads to glomerular endotheliosis, and in severe cases it can lead to renal impairment and failure. Serious perinatal morbidity occurs in the form of preterm delivery and fetal growth restriction. Antenatal care involves a screening program with measurements of

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blood pressure and proteinuria increasing in frequency towards term to detect the hypertensive disorders of pregnancy.[3]

Proteinuria arises when there is damage to the glomerular filtration barrier. Twenty-four-hour urine protein excretion has long been regarded as the gold standard for the diagnosis of pre-eclampsia. However, this test has disadvantages, such as inconvenience for patients, inaccuracy due to incomplete collection, and delay of diagnosis and management, which make its wide use difficult for clinicians.[4] Quick and accurate diagnosis of pre-eclampsia allows for gestational age-specific obstetric management to improve the outcome and minimize complication. On the contrary, a false positive diagnosis may lead to unnecessary interventions. Therefore, a timely correct diagnosis of pre-eclampsia is critically important for obstetricians to prescribe the right treatment.

We previously recommended increased urinary adipsin as an excellent biomarker for the quick detection of pre-eclampsia. Adipsin is a serine protease essential for the activation of the complement alternative pathway and is synthesized mainly by adipocytes and macrophages. It is filtered through the glomerulus and catabolized in the proximal renal tubules. In our previous study, we described how urinary concentrations of adipsin were significantly higher in women with pre-eclampsia and the adipsin-to-creatinine ratio was closely correlated with the urinary 24-h protein in patients with pre-eclampsia. Furthermore, we developed a rapid laminar flow test examining a random urine sample to reveal potential pre-eclampsia in 10–15 min.[5] Because of its convenience, previously described high accuracy, and possibly a wide usage after the confirmation of what has been claimed, we organized a prospective multicenter study that involved nine medical centers in China to evaluate the usefulness of the adipsin rapid strip test for diagnosing pre-eclampsia.

Methods

Subjects

This prospective multicenter observational study was undertaken between March 2014 and September 2014 in nine consultant maternity units in China. 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). Written informed consent was obtained, and baseline demographic and pregnancy-specific information was entered into the study database.

A sample size of 153 achieves 90% power to detect a difference (P1–P0) of −0.015 using a two-sided binomial test. The target significance level is 0.0500. These results assume that the population proportion under the null hypothesis is 0.9999. According to the calculation, 204 pre-eclampsia patients and 254 healthy pregnant women were recruited for this study, respectively (Table 1).

The diagnosis of pre-eclampsia was based on the development of blood pressure higher than 140/90 mmHg on two separate occasions 6 h apart in association with proteinuria ≥(+) by dipstick testing or proteinuria ≥300 mg per 24 h. Superimposed pre-eclampsia was confirmed as a development of features of pre-eclampsia in the context of pre-existing hypertension or pre-existing proteinuria or both.[6] Non-pre-eclamptic pregnant women were recruited according to the following criteria: (i) normotensive pregnant woman; and (ii) negative result of random urine sample with a urine dipstick test. Women with a history of renal disease or positive urine occult blood test were excluded from the study.

Sample collection and detection of urinary adipsin with the strip test

The study was designed in a single-blinded manner to evaluate the diagnostic value of the urinary adipsin rapid strip test for the diagnosis of pre-eclampsia. Urine

Table 1 Clinical characteristics of the two groups

	Pre-eclampsia group (n = 204)	Non-pre-eclampsia group (n = 254)	P-value
Age (years)	30.96 ± 5.5	30.4 ± 4.3	0.201
Gravidity			
1	79 (38.7%)	111 (43.7%)	
2	53 (26.0%)	62 (24.4%)	0.552
≥3	72 (35.3%)	81 (31.9%)	
Gestational age (weeks)	33.99 ± 4.2	37.4 ± 3.8	<0.001
Systolic blood pressure (mmHg)	154.7 ± 16.0	114.9 ± 9.8	<0.001
Diastolic blood pressure (mmHg)	97.2 ± 12.0	71.8 ± 8.4	<0.001

was collected from pregnant women who attended an antenatal ward over a 6-month interval. A sample of 5–10 mL midstream urine was collected using a sterile container. Full urinalysis was recorded using the Arkray Ax4030 automated urinalysis device (Arkray Inc. Japan). A 24-h urine sample was collected using a clean container for detection of total protein; 24-h urine protein was detected by using a urine protein quantization kit (Beijing Leadman Biochemical Co.).

Rapid 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 phosphate buffered saline. After pipetting up and down 10 times, two drops of the buffered sample were added onto the sample well of the test card. One or two test lines could be observed in the control and test window within 10–15 min at room temperature. The presence of both the orange-purple test line and the control line was determined as positive for adipsin. The absence of the test line and the presence of the control line were determined as negative for adipsin.

Statistical analysis

Descriptive statistics are reported for quantitative traits as means and SD and for categorical traits as percentages. The number of subjects with positive or negative results of the urinary adipsin strip tests was counted. Sensitivity, specificity, and diagnostic accuracy of urinary adipsin rapid strip tests were calculated as follows: sensitivity = number of true-positive specimens (TP)/ [TP + number of false-negative specimens (FN)]; specificity = number of true-negative specimens (TN)/ [TN + number of false-positive specimens (FP)]; diagnostic accuracy = (TP + TN)/(TP + FP + TN + FN). All statistical tests were carried out at the two-sided 0.05 significance level.

Results

A total of 474 pregnant women were recruited for this study and 16 patients were rejected because of incomplete information or positive urine occult blood test. The remaining 458 pregnant women met the study requirement. Of the recruited subjects, there were 204 pre-eclampsia and 254 non-pre-eclampsia controls. No significant difference was observed between the test and the control groups in terms of the maternal age and gravidity; however, gestational age ($P < 0.01$), and systolic and diastolic blood pressure ($P < 0.01$) at the time of sample collection were significantly different between the pre-eclampsia individuals and the controls (Table 1).

Of the 204 pre-eclampsia cases, 194 were detected as positive and 10 as negative by the adipsin rapid strip test. Meanwhile, of the 254 controls, 248 individuals showed negative results and six individuals presented positive results for the adipsin strip test. The sensitivity and the specificity were 95.1% and 97.7%, respectively, as summarized in Table 2. We also calculated the likelihood ratio for a positive and negative result. The likelihood ratio for a positive result was 40.26, and the likelihood ratio for a negative result was 0.05. The diagnostic accuracy of urinary adipsin in women with pre-eclampsia was 96.51%. Compared with the gold standard for the diagnosis of pre-eclampsia, the consistency analysis showed that the kappa value was 0.93 ($P < 0.05$). We also subdivided all of our study participants into the gestational diabetes mellitus (GDM) group and the non-GDM group and calculated the indexes respectively. We did not find a significant difference between the results of the two groups. There were also no differences between the results of the non-GDM group and the original group (see the supplementary material). These data indicate a very strong agreement between the adipsin strip test and the gold standard for the diagnosis of pre-eclampsia.

Discussion

The histological hallmark of pre-eclampsia in the kidney is glomerular endotheliosis. It is characterized by the swelling of endothelial cells, enlarged glomerular volume with hypertrophy, and a loss of glomerular endothelial fenestrate.[7,8] Kidney injuries in pre-eclampsia patients are most commonly defined by the total excretion of urinary protein in 24 h. However, the validity of standard urinary protein measurements for the diagnosis of pre-eclampsia has been questioned, partly because

Table 2 Diagnostic value of urinary adipsin rapid strip test

Urinary adipsin rapid strip test	Sensitivity (%)	Specificity (%)	LR+	LR–	Diagnostic accuracy (%)
	95.1	97.6	40.3	0.05	96.5

LR+, positive likelihood ratio; LR–, negative likelihood ratio.

the extent of proteinuria in pre-eclampsia does not correlate well with disease severity.[9,10] The test itself is without problems, however the collection is time-consuming, inconvenient, and subject to errors, such as incomplete collection or contamination leading to inaccuracies. Delays may occur in the management plan while results are awaited, and verification of diagnosis of pre-eclampsia may not be possible if patients deliver before the urine collection is complete. The laboratory assay methods used also vary widely, and the incidence of significant proteinuria has been shown to vary depending on the assay used.[10,11] For this reason, a more rapid and accurate diagnostic test that is capable of predicting kidney injury of pre-eclampsia patients would be valuable. The adipsin rapid strip test is potentially a useful test for the preliminary diagnosis and for self-assessment by high-risk pregnant women.

This prospective multicenter study was therefore conducted to assess the usefulness of the urinary adipsin rapid strip test for the diagnosis of pre-eclampsia. We proved that the rapid strip test is accurate for the diagnosis of pre-eclampsia in symptomatic women across a wide gestational age range with an overall sensitivity of 95.10% and a specificity of 97.64%. The diagnostic accuracy was as high as 96.51%. Further analysis showed that GDM was not an influence factor of the results. Our results suggest that the rapid strip test is a useful adjunct to the timely diagnosis of pre-eclampsia. It is easy to conduct, non-invasive, and yields results within 10–15 minutes.

This study included the use of multiple centers encompassing a wide demographic profile in China and a pragmatic approach to enrollment with minimal exclusion criteria, enabling generalizability. The main research question was chosen to be clinically relevant. Final diagnoses were adjudicated following review of the database records with strict criteria.

In summary, the rapid strip test of urinary adipsin is a highly promising method for the quick detection of pre-eclampsia. We consider that the addition of the urinary adipsin measurement to the current clinical assessment of pre-eclampsia could improve risk stratification, achieve a quick diagnosis, and enable individualized management of pregnant women with the disease. This will potentially reduce the associated maternal morbidity and unnecessary health service usage as an earlier and effective intervention can be administered.

Disclosure

None declared.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Table S1: Comparison of diagnostic value of urinary adipsin rapid strip test between GDM group and non-GDM group.
Table S2: Comparison of diagnostic value of urinary adipsin rapid strip test between original group and non-GDM group.

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Clinical assessment of the specificity of an adipsin rapid test for the diagnosis of preeclampsia

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Clinical assessment of the specificity of an adipsin rapid test for the diagnosis of preeclampsia

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ABSTRACT

Objective: To evaluate the specificity of the adipsin rapid test in clinical practice for the diagnosis of preeclampsia (PE). *Methods*: A total of 1144 pregnant women were recruited in this study: 44 pregnant women with PE and 1100 healthy pregnancies as controls. Urine samples were collected and used, respectively, for the adipsin rapid test and the urinary dipstick test for protein detection. Sensitivity and specificity were calculated on the basis of the detection results. *Results*: In the 1144 women examined with the adipsin rapid test for PE diagnosis, the sensitivity and specificity were 93.2% and 98.8%, respectively; the total accuracy was 98.6%. For the 1144 women tested with urinary dipstick, the sensitivity and specificity were 93.2% and 40.5%, respectively; and the total accuracy was 42.5%. *Conclusion*: Both the adipsin rapid test and the urinary dipstick test are noninvasive and inexpensive rapid tests for the diagnosis of PE. However, the adipsin rapid test was proven more reliable since it had a higher sensitivity, specificity, and accuracy.

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Adipsin; adipsin rapid test; preeclampsia; specificity; urine dipstick

Introduction

Preeclampsia (PE), affecting at least 5–8% of all pregnancies, is a rapidly progressive disease characterized by high blood pressure and the presence of protein in the urine (1,2). Typically, PE occurs after 20 weeks gestation and up to 6 weeks postpartum. PE and other hypertensive disorders of pregnancy are a leading cause of maternal and infant illness and death, responsible for 76,000 maternal and 500,000 infant deaths each year (3,4). Early diagnosis and management of PE is essential for an optimal clinical outcome (5,6).

In a normal pregnancy, the renal length and volume increases by approximately 1 cm and 30%, respectively, primarily due to the increase in renal vascular and interstitial volume rather than a change in the number of nephrons (7–9). Consequently, protein may appear in urine in healthy pregnancies due to a physiological leakage. This leakage

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Tao Wang and Rong Zhou contributed equally to this work.

could lead to an increased urinary protein level and a positive result for urinary dipstick test in healthy pregnancies. Indeed, the urinary dipstick for detection of protein as a diagnostic test for PE is notoriously poor for the specificity (10–12).

We have previously described that the urinary adipsin concentration quantitatively correlated with the urinary 24 h protein, and it was proven to be a sensitive biomarker for the diagnosis of PE (13). Urinary adipsin has now been developed into a lateral flow immunoassay-based rapid strip test. In this study, we aimed to examine the specificity of this rapid test in a large sample study, and the results are proved to be important for the assessment of the usefulness of the rapid test in the clinical practice.

Materials and methods

Subjects

The research protocol was approved by the Institutional Review Board of West China Second University Hospital, Sichuan University, and all patients provided informed consent. Between November 2013 and October 2014, 1144 pregnant women, 1100 healthy pregnancies, and 44 PE patients were enrolled in this study. All participants were Chinese, predominantly of Han race. The mean age and the age range of PE patients were comparable to those of the healthy pregnancies (Table 1). Healthy pregnancies were recruited according to the following criteria: (1) normotensive pregnant woman; (2) negative result of blood cell with random urine sample under microscopy. PE was confirmed according to the following criteria: (1) any consecutive readings of diastolic blood pressure (DBP) ≥90 mmHg or systolic blood pressure (SBP) ≥140 mmHg at more than one occasion at least 4 h apart; (2) 24 h urinary protein excretion exceeding 300 mg, or a persistent value ≥30 mg/dl (1+ on a dipstick) protein in random urine samples. Clinical symptoms such as abdominal discomfort, headache, blurring of vision may be complicated with the patients. Both primary PE and superimposed PE were included in this study. Primary PE was diagnosed as new hypertension and quantified proteinuria at or after 20 weeks of pregnancy, and resolved by 12 weeks postpartum. Superimposed PE was confirmed

Table 1. Clinical characteristic of the study subjects.

Characteristics	Normal pregnancies (n = 1100)	PE patients (n = 44)
Maternal age (years) Mean ± SD (range)	30.4 ± 4.03 (21–43)	31.49 ± 5.09 (22–44)
Pre-pregnancy BMI (kg/m^2) Mean ± SD (range)	21.06 ± 2.51 (13.45–25.68)	29.56 ± 3.88 (21.91–39.12)
Diabetes mellitus	45	11
Gestational age at sample collection (Week^{+Day})[a] Mean ± SD (range)	18.14 ± 55.3 (10^{+2}–39^{+4})	36.03 ± 3.76 (27^{+6}–41^{+1})[b]
Systolic blood pressure (mmHg) Mean ± SD (range)	115.94 ± 9.91 (87–139)	150.11 ± 10.68 (130–170)[†]
Diastolic blood pressure (mmHg) Mean ± SD (range)	60.69 ± 3.61 (51–89)	96.09 ± 8.61 (74–120)[†]
24 h urinary protein (g) Mean ± SD (range)	Not available	2.91 ± 2.92 (0.16–13.29)

[a]Gestational age when PE was diagnosed for patients.
[b]$P < 0.05$, PE versus normal pregnancies.

as development of features of PE in context of pre-existing hypertension or pre-existing proteinuria, or both (14–16).

Sample collection and determination of urine 24 h protein

A 24 h urine sample was collected continuously in 24 h using a clean container. The volume of a 24 h urine collection was determined, and the protein concentration was measured with an ADVIA2400 automatic biochemical analyzer (Siemens Ltd., Munich, Germany).

Sample collection and determination of urine dipstick for protein

A mid-stream urine sample (5–10 ml) was collected using a sterile container. Urinary protein in a spot urine sample was determined with an ADVIA2400 automatic biochemical analyzer (Siemens Ltd.) following the procedures suggested by the manufacturer.

Sample collection and adipsin rapid test

A urine sample of 1 ml was diluted in a test tube containing 3 ml of sterile phosphate buffered saline (PBS). After mixing samples up and down 10 times, 2–3 drops of the buffered sample was added onto the sample well of the test card. One or two test lines could be observed in the control and test window within 10–15 min. The presence of the orange-purple test line and the orange-purple control line was determined as positive. The absence of the test line with the presence of the control line was determined as negative (Figure 1).

Statistical analysis

The number of subjects, patients, or healthy pregnancies, of positive or negative results, was enumerated. Sensitivity, specificity, positive predictive value, negative predictive value, validity, mistake diagnostic rate, and omission diagnostic rate of the adipsin rapid test and the urine dipstick test were calculated as follows: sensitivity = number of true positive specimens (TP)/[TP + number of false negative specimens (FN)]; specificity = number of true negative specimens (TN)/[TN + number of false positive specimens (FP)]; positive predictive value = TP/(TP + FP); negative predictive value = TN/(FN + TN); validity = (TP + TN)/(TP + FP + TN + FN); mistake diagnostic rate = FP/(FP + TN); and omission diagnostic rate = FN/(TP + FN).

Results

A total of 1144 pregnant women were recruited for this study, 1100 healthy individuals and 44 PE patients. Urine samples from the same subject were tested with the adipsin rapid test and the urinary dipstick. Out of the 1100 controls, 1087 cases showed negative results and 13 cases showed positive results for the adipsin rapid test. For the 1100 healthy individuals examined using urine dipstick, 445 cases showed negative results and 655 cases showed positive results. Meanwhile, out of the 44 PE cases, 41 were detected positive and 3 negative by the adipsin rapid test; when the urinary dipstick was used, the results were same as for the adipsin test, 41 cases positive and 3 cases negative.

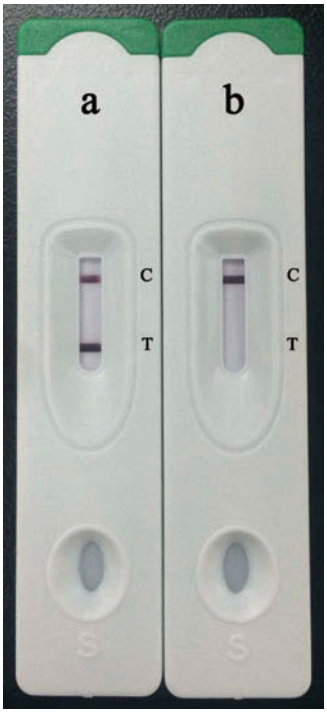

Figure 1. Test results of adipsin rapid test: (a) presence of the orange-purple test line and the orange-purple control line was determined as positive for PE. (b) Absence of the test line and presence of the control line was determined as negative for PE. C, control line; T, test line; and S, sample well.

The diagnostic values of the adipsin rapid test and the urinary dipstick test were calculated and are summarized in Table 2. Both the adipsin rapid test and the urinary dipstick were shown to have a high sensitivity at 93.2%, while adipsin rapid test had a much better specificity (98.8%) than that of the urine dipstick (40.5%). The total accuracy was confirmed to be 98.6% for the adipsin rapid test and 42.5% for the urinary dipstick.

Discussion

Early diagnosis and management of PE is very important for an optimal clinical outcome. In recent years, various methods have been developed for monitoring the onset of PE, but unfortunately, a simple assay that can be used as a home or point-of-care test remains unavailable (14,16,17). We previously described urinary adipsin as a highly sensitive

Table 2. Diagnostic value of adipsin rapid test and urine dipstick for protein.

	Adipsin rapid test	Urine dipstick for protein
Sensitivity	93.2%	93.2%
Specificity	98.8%	40.5%
Positive predictive value	75.9%	5.9%
Negative predictive value	99.7%	99.3%
Validity	98.6%	42.5%
Mistake diagnostic rate	1.2%	59.5
Omission diagnostic rate	6.8%	6.8%

biomarker for the diagnosis of PE, because its concentration quantitatively correlated with the urinary 24 h protein. A significant difference of the adipsin quantity in urine between PE patients and healthy pregnancies was revealed by examination with an enzyme-linked immunosorbent assay (ELISA) (13).

In the current study, the adipsin rapid test was proved to yield results in 10–15 min after applying a diluted urine sample onto the test card. Meanwhile, high sensitivity and specificity were revealed to be 93.2% and 98.8%, respectively. These results were improved compared with what we previously described at 89.04% and 80.9%, respectively. This is better than expected, as the specificity was probably a result of the increased sample size of the healthy pregnancies. Furthermore, the adipsin rapid test had a much better specificity compared with the urinary dipstick. Urine dipstick is semi-quantitative, easy to use, quick, and inexpensive. However, many studies have reported a poor correlation between the dipstick examination and the 24 h urine assay with high false positive or false negative rates (18–20). The current study yielded similar results showing that it had a poor specificity for the diagnosis of PE.

In conclusion, both the adipsin rapid test and the urinary dipstick are noninvasive and inexpensive rapid tests for the diagnosis of PE. However, the adipsin rapid test has a higher specificity. Because the rapid test is easy to use and interpret, it is suitable for home tests and as a point-of-care test at clinics.

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Online Supplement

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

by

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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

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

*, $P < 0.05$ 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)

Groups	Concentration (ng/ml)	
	Acrp30	adipsin
PE (n=33)	292.08±168.08*	806.75±1507.75*
Non-PE pregnant women (n=45)	99.26±88.82	12.25±13.46
Healthy non-pregnant women (n=11)	4.98±3.41	2.33±1.48

*, $P < 0.05$ 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*

Parameters	sFlts-1/PIGF	adipsin/Cr	sFlts-1/PIGF + adipsin/Cr†	sFlts-1/PIGF + adipsin/Cr + DBP‡
Sensitivity (%)	76.6	93.8	96.9	96.9
Specificity (%)	98.2	82.5	80.7	100

*, 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 adipsin/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 adipsin/Cr was higher than the cutoff value, it was determined as positive.

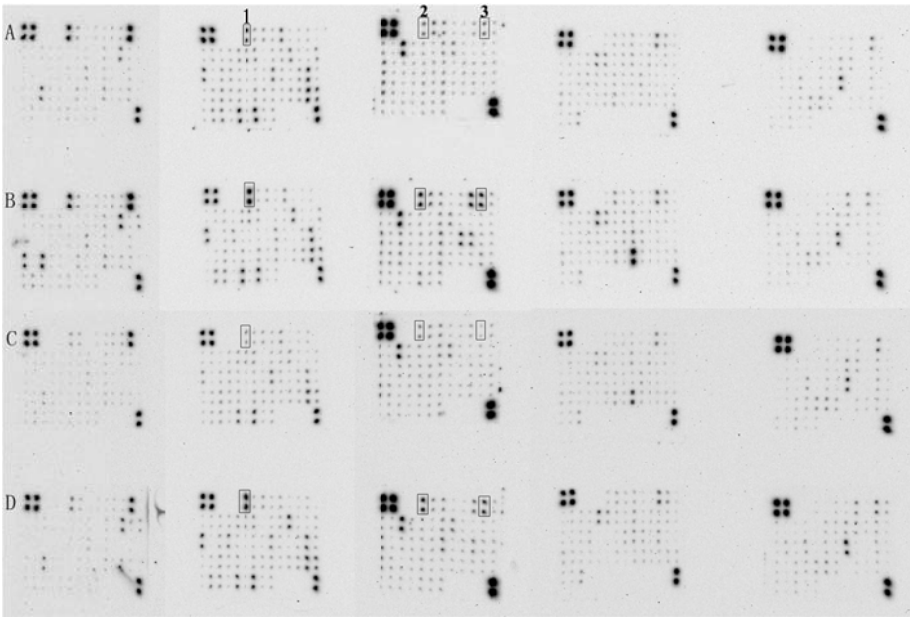

Figure S1. An antibody array was used to screen urine samples of two preeclampsia patients (B, D) and urine samples of two healthy pregnancies (A, C). The signals of Acrp30 (1), Adipsin (2), and FLRG (3) were much stronger in the PE patients than in the healthy pregnancies.

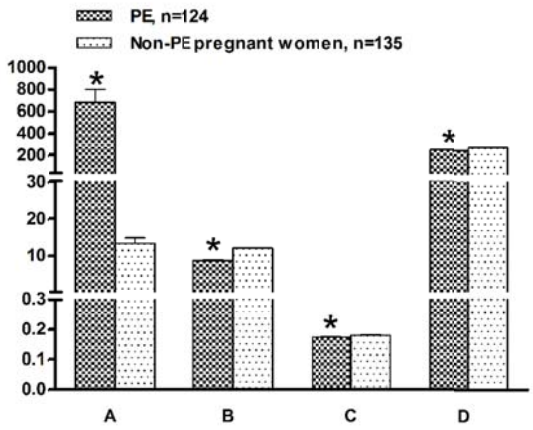

Figure S2. PE patients had a much higher adipsin/Cr value, and these patients tended to deliver premature children with a lower body weight and length at birth. A, adipsin/Cr value (mg/g); B, infant birth weight normalized by pregnancy days (g/day); C, infant birth length normalized by pregnancy days (cm/day); D, delivery day (day); *: $P<0.01$

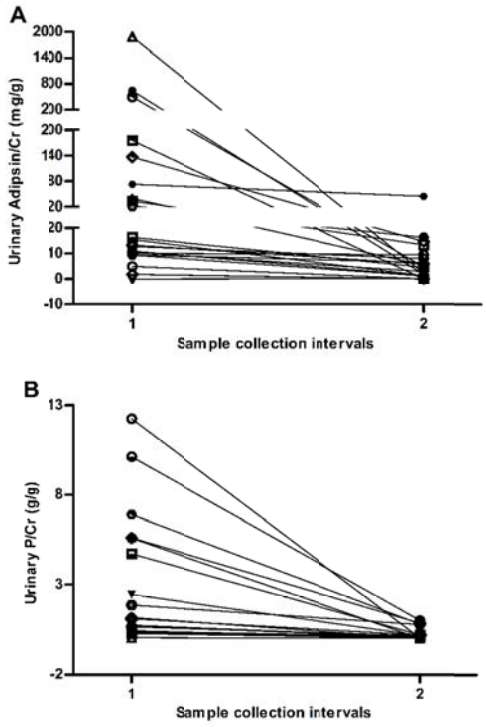

Figure S3. Return of urinary Adipsin/Cr (A) and urinary P/Cr (B) to baseline levels for PE (n=15) and healthy pregnancies (n=6) after the delivery of the child. Urine samples were collected before the delivery or at least 1 month postpartum. 1, before the delivery; 2, 1-16 months postpartum.

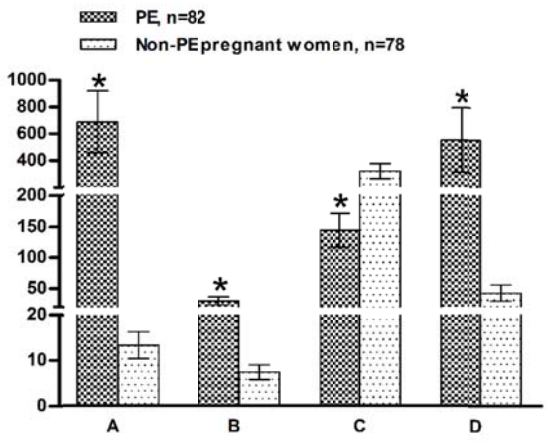

Figure S4. Compared to the controls PE patients had a much higher adipsin/Cr in urine, sFlt-1 in plasma, a lower PIGF in plasma, and a higher sFlt-1/PIGF ratio. A, adipsin/Cr (mg/g); B, sFlt-1 (ng/ml); C, PIGF (pg/ml); D, sFlt-1 and PIGF ratio; *: $P<0.01$

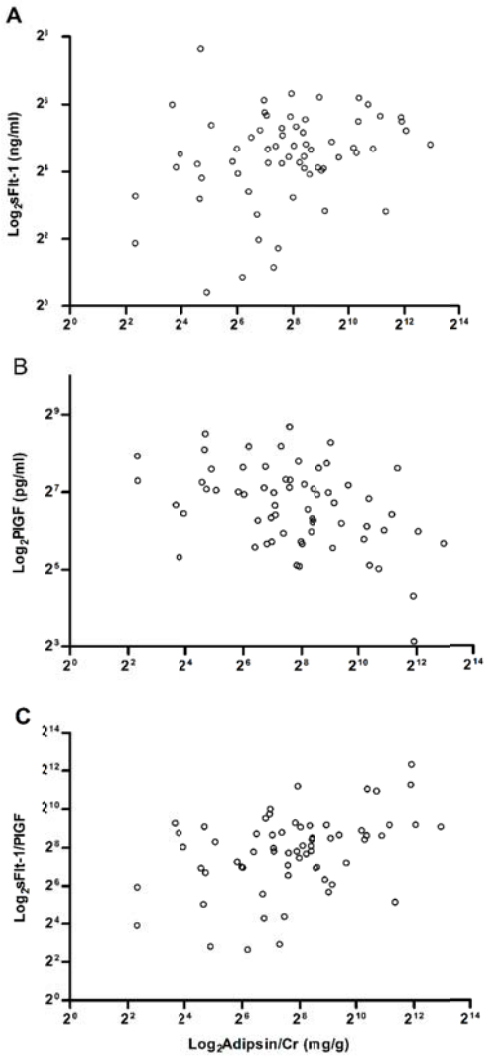

Figure S5. A linear correlations was observed between PlGF, ratio of sFlt-1/PIGF and urinary adipsin/Cr (r^2=0.08, P<0.05; r^2=0.18, P<0.01, n=64), but not between sFlt-1 and urinary adipsin/Cr (r^2=0.005, P>0.05) in PE patients. Correlation between sFlt-1 and urinary adipsin/Cr (A); correlation between PlGF and urinary adipsin/Cr (B); correlation between ratio of sFlt-1/PIGF and urinary adipsin/Cr (C).