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ORIGINAL ARTICLE

Expression of lipocalin-type prostaglandin D synthase in preeclampsia patients: a novel marker for preeclampsia

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Aim: To test the hypothesis that lipocalin-type prostaglandin D synthase (L-PGDS), a marker of vascular endothelial cell disorders, may be a diagnostic for preeclampsia.
Methods: Plasma and urine were collected from 36 preeclamptic patients and 94 normal pregnant women. L-PGDS concentrations were determined by sandwich ELISA assay. Receiver operating characteristic (ROC) curve validated the cut-off point of the assay.
Results: The plasma and urinary L-PGDS concentrations were significantly higher in the preeclamptic patients than the normal pregnant women. Urinary L-PGDS concentrations of the normal pregnant women were higher in the third trimester compared to earlier pregnancy, while plasma concentrations remained unchanged. Urinary L-PGDS levels were significantly higher in early onset of preeclampsia (onset < 32 weeks gestation) compared with late onset and in the severe compared to mild preeclampsia. ROC curve showed the cut-off point of 58.85 μg/dl (sensitivity 76.5%, specificity 75.6%, positive predictive value [PPV] 46.4%, negative predictive value [NPV] 92.1% and area under the curve [AUC] 0.82) in the plasma and 2.195 μg/dl (sensitivity 84.6%, specificity 58.7%, PPV 33.8%, NPV 93.8% and AUC 0.76) in urine.
Conclusions: Our results indicate that plasma and urinary concentrations of L-PGDS may be a potential diagnostic for preeclampsia.

Introduction

Preeclampsia is among the common complications of pregnancy, resulting in maternal and perinatal morbidities and mortalities worldwide.[1–3] The pathophysiology of preeclampsia is considered to be a systemic disorder of vascular endothelial cell involving multiple etiologies that lead to renal dysfunction, HELLP (Hemolysis, Elevated Liver enzymes, Low Platelets) syndrome, eclampsia and other critical medical abnormalities.[2,3] The precise etiology of preeclampsia is still unknown, but inadequate trophoblast invasion and incomplete remodeling of the uterine spiral arteries are considered to be key factors.[3,4] No definitive therapy other than the interruption of pregnancy has been established, and symptomatic treatment remains the predominant form of managing this syndrome.[2,3,5] Therefore, early diagnosis and prediction of preeclampsia as well as precise measurements of severity are essential for appropriate care. Unfortunately, a reliable biomarker has yet to be identified.[5–8] A novel

biomarker of vascular epithelium disorders is lipocalin-type prostaglandin D synthase (L-PGDS). Accordingly we measured urine and blood L-PGDS concentrations in preeclamptic patients and normal pregnancy women and determined whether L-PGDS is a potential biomarker of preeclampsia or not.

Materials and methods

Subjects and samples

Pregnant and postpartum women, including preeclampsia patients, were recruited for this study conducted in the Center for Maternal Fetal and Neonatal Medicine, Saitama Medical Center. Blood and urine samples were collected from the patients. Preeclampsia is diagnosed by newly recognized hypertension with proteinuria after 20 weeks of gestation, and taking up to 12 postpartum weeks to resolve.[2,3] Furthermore, it is classified with severe type (blood pressure ≥ 160/110 mmHg and/or proteinuria ≥ 2 g/day) and mild type (blood pressure ≥ 140/90 mmHg and proteinuria ≥ 300 mg/day). According to the Japan Society of Obstetrics and Gynecology, the onset was distinguished between early and late onset as 32 weeks of gestation.[9–11] For the preeclampsia cases, samples were collected at the time of the onset except following serial samples including postpartum. Postpartum samples were collected 3 or 4 days after the delivery. Blood samples were drawn from a peripheral vein into heparinized tubes, and centrifuged at 1,500 × *g* for 15 minutes at room temperature. Urine samples were collected in sterile containers. The plasma and urine samples were stored in -20°C until use.

Written informed consent was obtained from all patients who participated in this study. The study was approved by the ethics committee of Saitama Medical University (#226).

ELISA assay

A sandwich ELISA assay with two mouse monoclonal antibodies (mAbs), 1B7 and -7F5, against human L-PGDS was used to determine L-PGDS as previously described.[12,13]

Statistical analysis

Graph Pad Prism 5.0 (Graph Pad Software Inc., San Diego, CA, USA) was used for statistical analysis and drawing graphs. Student's *t*-test or the Mann-Whitney *U* test were used to analyze two-group comparison according to their variances, and the Kruskal-Wallis test followed by Dunn's *post hoc* test were used for analysis of groups of three or more.

Receiver Operating Characteristic (ROC) curves were plotted to evaluate the plasma and urinary L-PGDS levels to probe the sensitivity and specificity of preeclampsia diagnosis and area under the curve (AUC). Positive (PPV) and negative predictive values (NPV) were also calculated. The optimal cut-off point was determined by applying the Youden's index approach.[14] For all statistical analysis, a value of $P < 0.05$ was considered to indicate a statistically significant difference.

Table 1. Clinical profiles of subjects

	NP *n* = 94	PE *n* = 36
Age (year)	32.4 ± 0.5	34.3 ± 0.8
Gestational age at delivery (weeks)	38.2 ± 0.2	34.6 ± 0.7*
Birth weight (g)	2,947.2 ± 55.2	2,089.9 ± 158.5*

All data are expressed as means ± standard errors.
* $P < 0.0001$ compared with normal pregnancy by Mann-Whitney *U* test.
NP, normal pregnancy; PE, preeclampsia.

Results

One hundred thirty women, including 36 preeclamptic patients, were recruited for this study. Seventeen women from the normal pregnant women provided serial samples throughout their pregnancies. Seventeen preeclamptic patients provided samples both during pregnancy and postpartum. Twenty-five of the preeclampsia patients had severe type and 11 had mild type. The clinical profiles are shown in Table 1.

The plasma L-PGDS levels in the normal pregnant women remained essentially constant while urinary L-PGDS levels rose in the third trimester (Table 2). The plasma L-PGDS levels in the preeclamptic patients were significantly higher than those in the normal pregnant women at all measurement time points, however, the urinary L-PGDS levels in the preeclamptic patients were significantly higher than those in the normal pregnant women only in second trimester (Table 2).

Both early and late onset preeclamptic patients had significantly higher L-PGDS levels than the normal pregnant women. Significant change between the two preeclampsia groups was only found in the urinary sample (Figure 1). Both mild and severe types were associated with significantly higher L-PGDS levels than normal pregnancy, but significant difference between the two types of preeclampsia was only found in the urinary samples (Figure 2).

The optimal L-PGDS value for diagnosing preeclampsia based on the Youden's index method were 58.85 μg/dl (sensitivity 76.5%, specificity 75.6%, PPV 46.4% and NPV 92.1%) with the plasma samples and 2.195 mg/gCr (sensitivity 84.6%, specificity 58.7%, PPV 33.8% and

Table 2. **L-PGDS levels**

		First trimester	Second trimester	Third trimester	Postpartum
Plasma (μg/dl)	NP	52.65 ± 1.88 (*n* = 31)	51.68 ± 1.39 (*n* = 46)	47.99 ± 1.78 (*n* = 46)	46.69 ± 1.43 (*n* = 20)
	PE	—	79.55 ± 4.68 (*n* = 4)**	64.00 ± 2.57 (*n* = 30)**	57.30 ± 3.78 (*n* = 19)*
Urine (mg/gCr)	NP	1.54 ± 0.25 (*n* = 26)	1.79 ± 0.23 (*n* = 42)	3.67 ± 0.38 (*n* = 36)***	1.16 ± 0.23 (*n* = 17)
	PE	—	6.61 ± 2.66 (*n* = 3)*	5.99 ± 1.25 (*n* = 22)	3.45 ± 1.50 (*n* = 11)

All data are expressed as means ± standard errors with *n* representing the number of samples in the tables. * $P < 0.02$ compared with the same period of NP by Mann-Whitney *U* test, ** $P < 0.0001$ compared with the same period of NP normal pregnancy by Student *t* test, *** $P < 0.0001$ compared with other periods of NP normal pregnancy by Kruskal-Wallis with Dunn's *post hoc* test.
NP, normal pregnancy; PE, preeclampsia.

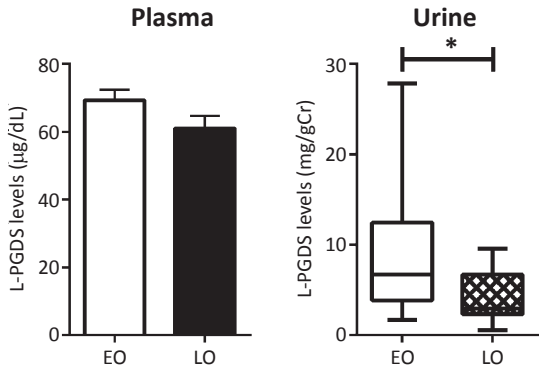

Figure 1. **L-PGDS levels classified by gestational age at onset of preeclampsia.**
n = 20 in EO and 14 in LO for plasma samples, and 9 in EO and 17 in LO for urinary samples.
As the data were normally distributed in plasma but not for urinary samples, histograms were used for plasma and box and whisker plots were used for urine. There was significantly higher urinary L-PGDS level in the early onset group compared to those in the late onset when the cut-off set at 32 weeks. * $P < 0.05$ by Mann-Whitney *U* test.
EO, early onset; LO, late onset.

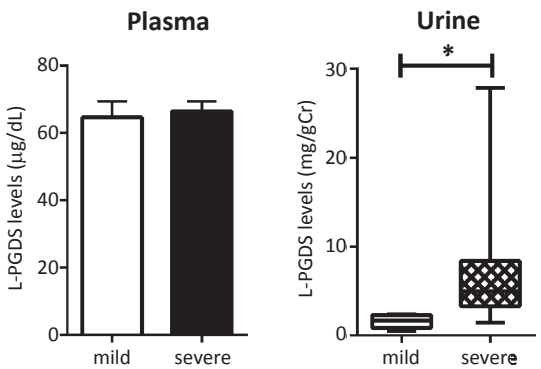

Figure 2. **L-PGDS levels classified by severity of preeclampsia.**
n = 10 in mild and 24 in severe for plasma samples, and 5 in mild and 21 in severe for urinary samples.
As the data were normally distributed for plasma but not for urinary samples, histograms were used for plasma and box and whisker plots were used for urine. Urinary L-PGDS levels from the severe types were significantly higher than those from mild types. * $P < 0.002$ by Mann-Whitney *U* test.

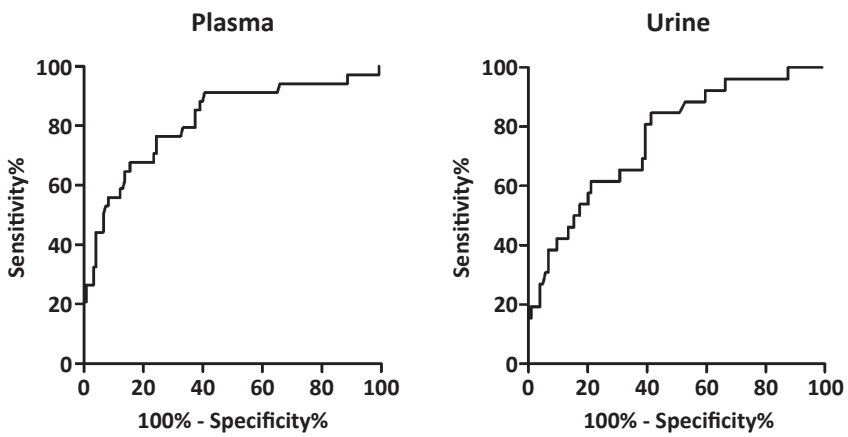

Figure 3. **ROC curve of the plasma and urinary.**

NPV 93.8%) with the urine samples (Figure 3). The AUC were 0.82 and 0.76 in plasma and urinary, respectively.

Discussion

L-PGDS, also known as *β*-trace, is the enzyme that catalyzes the isomerization of prostaglandin H_2 (PGH_2), a common precursor of various prostanoids, to prostaglandin D_2 (PGD_2).[13,15] It is localized in the central nervous system,[16] retina,[17] vascular endothelial cells and male and female genital organs of various mammals.[16,18–20] L-PGDS/*β*-trace is also abundant in various types of biological fluids such as cerebrospinal fluid, ascites, seminal plasma, and amniotic fluid.[21–26] Although the biological role of this enzyme or its prostanoid product remains unclear, several studies have shown L-PGDS to be increased in patients with renal insufficiency,[13,27,28] diabetes mellitus,[29,30] hypertension,[31] angina[32] and osteoarthritis.[33] In addition, Taba *et al*.[34] showed that laminar shear stress loading onto endothelial cells caused increased expression of mRNA for L-PGDS. Thus, L-PGDS was suggested to be a potential indicator of vascular endothelial disorders.

Kristensen et al.[35] showed patients with preeclampsia to have significantly elevated plasma concentrations of *β*-trace, *β*2-microglobulin and cystatin C as compared to normal pregnant women in the third trimester. We demonstrated preeclamptic patients to have significantly higher plasma L-PGDS levels not only in the third trimester but also in the second trimester and postpartum, as compared to normal pregnant women. Consistent with our results, other groups have shown plasma *β*-trace levels to be unchanged until 36 weeks of gestation.[36,37] Thus, measurement of plasma L-PGDS in preeclamptic patients may reveal a difference from normal pregnant women, possibly allowing early diagnosis or prediction of preeclampsia.

We also demonstrated significantly higher urinary L-PGDS levels in preeclamptic patients than in normal pregnant women. To our knowledge, no prior studies have focused on urinary L-PGDS levels in preeclamptic patients. Our data obtained from urinary samples of normal pregnant women showed significantly higher L-PGDS only in the third trimester, whereas plasma L-PGDS levels do not change during pregnancy. The rise in urinary L-PGDS concentration in the third trimester in normal pregnant women could simply be part of the normal increase in urinary protein. The concentrations of urinary L-PGDS in preeclamptic patients are higher than in normal women in both second and third trimesters, raising the possibility that local renal vascular endothelial dysfunction, an important aspect of the pathophysiology of preeclampsia, is responsible. Plasma and urinary L-PGDS may be released from different sources, and the elevation has different pathophysiological meanings. Further experiments are needed to explain the high urinary L-PGDS levels of third-trimester in normal pregnant women.

Classifying this disease by gestational age of onset is still controversial, but abundant evidence shows maternal complications, including maternal and fetal mortality and morbidity, to be higher when the onset is more remote from term (early) as compared to near term (late) onset.[38–40] The Japan Society of Obstetrics and Gynecology defined the borderline for distinguishing between early and late onset as 32 weeks of gestation,[9–11] while other investigators have advocated 34 weeks of gestation as the cut-off.[41–44] For the subclassification system based on onset time and severity of preeclampsia, statistically significant differences were only found in the urinary samples when the borderline was set at 32 gestational weeks (34 weeks data were not shown). For the purposes of this test, these results support a cut-off of 32 weeks rather than 34 weeks to distinguish early from late onset preeclampsia. Urinary L-PGDS is more sensitive than plasma L-PGDS in classifying preeclampsia. Our data suggest that plasma concentrations of L-PGDS might be the best diagnostic tool for preeclampsia but urine concentrations perform better for subclassification of the disease process. Thus, to measure both plasma and urinary L-PGDS levels is important for understanding preeclampsia.

L-PGDS in the plasma and urine were validated by plotting the ROC curves. They had moderate accuracy as assessed by AUC. As the PPV is low and NPV is high in both plasma and urinary L-PGDS levels, measurement of L-PGDS levels may be useful to rule out the diagnosis of preeclampsia. However, there still remains the possibility that increasing the number of preeclamptic patients studied may increase the PPV.

A limitation of this study is that we were able to recruit only a few patients who developed preeclampsia in the second trimester. More experience with preeclampsia in the second trimester may confirm the evidence that L-PGDS rises in preeclamptic patients.

In conclusion, L-PGDS levels in plasma and urine of preeclampsia patients were shown to be significantly higher than those of women with normal pregnancies, perhaps due principally to renal vascular endothelium dysfunction. Plasma and urinary L-PGDS may be released from different sources, and its elevation has different pathophysiological meanings. Thus, L-PGDS levels in plasma and urine may serve as novel biomarkers for early diagnosis or for ruling out the diagnosis of preeclampsia.

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Conflict of interest

The authors have nothing to disclose.

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