Urinary prostaglandin D synthase as a biomarker in lupus nephritis: a longitudinal study

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Abstract

Objective

Urinary prostaglandin D synthase (uPGDS) has been identified as a biomarker in lupus nephritis (LN) mice model as well as in humans. We studied the effect of therapy for LN on its levels in a longitudinal study and its ability to differentiate between active systemic lupus erythematosus (SLE) patients with and without nephritis.

Methods

Twenty-eight SLE patients with active LN, 6 patients with inactive disease, 12 patients with active non-renal disease and 19 healthy individuals were enrolled. Urine and serum samples were collected at baseline from all patients and at a 3-monthly follow-up from 25 patients in active nephritis group. uPGDS was measured by ELISA and normalised to urinary creatinine excretion.

Results

In the cross-sectional study, median uPGDS was higher in patients with active nephritis (618.5 ng/mg) as compared to healthy controls (141.7ng/mg; p<0.001), active non-renal (130.1ng/mg; p=0.008) and inactive disease (56.2 ng/mg; p=0.002) patients and had modest correlation with urinary protein / creatinine ratio (r=0.39; p=0.014).
In the longitudinal study, median uPDGS reduced from 618.5 ng/mg at baseline (n=28) to 91.9 at 6 months (n=25), 73.3 at 9 months (n=20) and 81.7 ng/mg at 12 months (n=13). uPGDS remained persistently elevated in a patient who developed CKD and showed an increase 2 months before the clinical relapse in another patient with relapse of LN.

Conclusion

Given that uPGDS levels fall after treatment of LN, uPGDS may be used to monitor the efficacy of therapy. It can also differentiate patients with active nephritis and active non renal lupus.

Key words

systemic lupus erythematosus, nephritis, urinary, biomarker, prostaglandin D synthase

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Introduction

Lupus nephritis (LN) has a major impact on the management and outcome of systemic lupus erythematosus (SLE) (1). LN is conventionally diagnosed by the presence of proteinuria, active urinary sediment and/or increased serum creatinine. The conventional methods of evaluation have limitations, for example, proteinuria can mean both disease activity and/or damage. Immunological parameters may be altered in non-renal flare. Thus, at present, renal biopsy is the gold standard for the assessment of lupus nephriti, however, because it is an invasive procedure, it cannot be done repeatedly. Therefore, there is an ongoing quest to look for new urinary biomarkers.

Urinary proteomics has identified a number of potential urinary biomarkers for LN including prostaglandin D synthase (PGDS) in a lupus nephritis mice model (2). Then, urinary PGDS was found to be differentially expressed in patients with active LN as compared to inactive LN using proteomics (3). PGDS is a small acidic glycoprotein molecule which is involved in the arachidonic acid metabolism pathway and converts PGH_2 to PGD_2. PGD_2 acts as a vasodilator, bronchoconstrictor and modulates inflammatory cell infiltration (4, 5).

Urinary PGDS levels are higher in children with active LN as compared to juvenile arthritis, inactive LN and healthy controls (6). In a longitudinal analysis, a worsening of LN was associated with an increase in urinary PGDS levels (6). No data is available on the effect of therapy on urinary levels of PGDS. We evaluated urinary PGDS levels in a cross-sectional study to assess its ability to differentiate active LN from active non-renal lupus. In addition, we studied the effect of treatment on its levels to explore its usefulness in monitoring response to treatment.

Patients and methods

SLE patients satisfying the 1982 ACR criteria were enrolled in the study (7). Patients with active nephritis were defined as those having SLEDAI>4 and evidence of active renal disease (proteinuria >500 mg/day and/or active urinary sediment, increased serum creatinine attributable to SLE), active non-renal disease (SLEDAI>4 but no evidence of renal disease as stated above), or inactive disease (SLEDAI<4) (8). Renal SLEDAI (rSLEDAI) was calculated using 4 parameters related to the kidney in SLEDAI and ranged from 0–16. All urine samples were collected in the morning, spun to remove debris and cells and stored at -80°C in aliquots.

Most patients in the active nephritis group underwent renal biopsy. Patients with class II LN were treated with steroids, whereas patients with class V LN were treated with either mycophenolate or monthly cyclophosphamide along with steroids. Patients with class III/ IV LN were treated according to the Euro-lupus protocol (9) followed by maintenance therapy with either azathioprine or mycophenolate (8). Patients in the active nephritis group were followed up at 3-monthly intervals. Response to treatment was assessed by SLEDAI and rSLEDAI, and urine samples were collected.

Urinary PGDS was measured by commercial ELISA kits (Cayman Chemical Company, Ann Arbor, USA). All values were normalised to urinary creatinine excretion. Urine samples from 19 healthy females were used as controls. All inter-group comparisons were made using non-parametric tests. $p<0.05$ was considered significant.

Results

A total of 46 SLE patients (28 with active nephritis, 6 with inactive disease and 12 with active non-renal disease) were included in the study. Median urinary protein to creatinine ratio was 2.67 in the active nephritis group, while it was 0.43 in the inactive disease and 0.22 in the active non-renal group (Table I).

In the cross sectional analysis, normalised median uPGDS level in active nephritis was higher than in inactive disease (p=0.002), active non-renal disease (p=0.008) and healthy control groups (p=0.001) (Table I; Fig. 1) Normalised uPGDS showed modest correlation with protein creatinine ratio (r=0.39; p=0.014) but not with SLEDAI and rSLEDAI (Fig. 2).

Table I. Baseline characteristics of patients in three categories.

	Active nephritis	Active non-renal	Inactive disease
Number	28	12	6
F:M	27:1	11:1	6
Median age (y)	26.5 (13-50)	24 (14-48)	30.5 (14-48)
Median SLEDAI	20 (6-32)	9 (5-18)	3 (0-4)
Median rSLEDAI	8 (4-16)	0 (0)	0 (0)
Median C3 (mg/dl)	50.7 (16.9-156)	91.1 (31.4-139)	115.5 (75.9-146)
Low C3 (n)	14	4	0
Median C4 (mg/dl)	7.95 (5.6-30)	17.3 (5.6-32.2)	26.1 (16.7-44)
Low C4 (n)	21	6	0
Median anti-dsDNA (IU/ml)	200 (24-200)	96.5 (6.25-200)	24.25 (8.43-200)
Median Upr/Ucr ratio	2.67 (0.11-7.5)	0.22 (0.04-1.4)	0.43 (0-0.5)
Median creatinine (mg/dl)	0.9 (0.55-2.5)	0.94 (0.7-1.2)	0.82 (0.64-1.1)
Median uPGDS (ng/ml)	28040 (3000-334300)	20000 (1250-50000)	1901.82 (1808-192640)
Median normalised uPGDS (ng/mg)	618.5 (28.8-3039.1)	130.1 (14.3-790.8)	56.2 (16.6-663.1)

Of the 28 patients with active nephritis, renal histology was Class II in 4, class III in 11, class IV in 6 and class V in 3. Four patients where renal biopsy could not be carried out due to contraindications were treated as proliferative lupus nephritis in accordance with the Euro-lupus protocol. Of these 28 patients, 25 patients were treated at our centre and completed a 6-month follow-up, 20 patients completed a 9-month follow- up and 13 patients completed a 1-year follow-up. Out of these patients, 1 patient did not respond to treatment and received rituximab but developed chronic kidney disease (CKD) at 6 months. The rest of the 24 patients responded to treatment and attained complete remission by 6 months. Of these, 1 patient had a relapse at 11 months of follow-up.

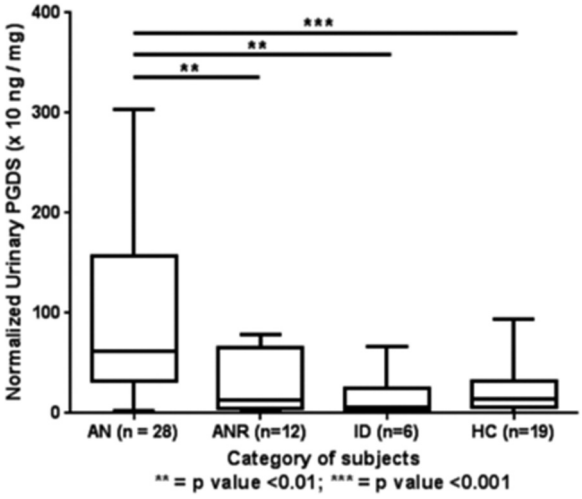

Fig. 1. Urinary PGDS values normalised for urinary creatinine excretion at the baseline in the 3 groups of patients and healthy controls

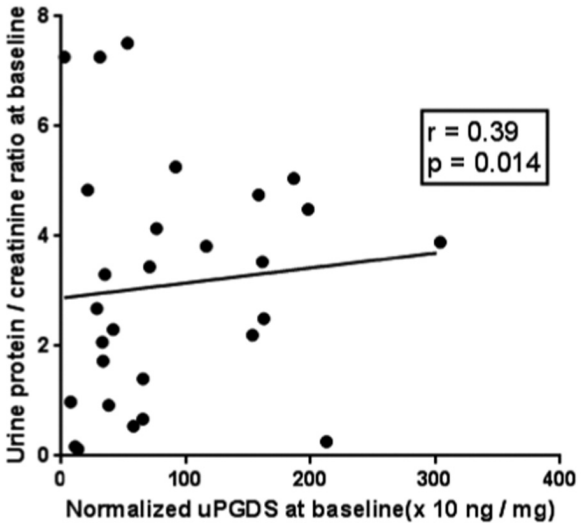

Fig. 2. Relationship of normalised urinary PGDS with baseline urinary protein/creatinine ratio in the active nephritis group (n=28)

With treatment, median rSLEDAI decreased from 8 at baseline to 0 at all subsequent visits. Spot urinary protein to creatinine ratio decreased from 2.67 at baseline to <0.5 at all subsequent visits (Table II). uPGDS values for these patients also showed a significant decrease from the baseline value of 618.5 (28.8–3039.1) ng/mg. (Table II, Fig. 3) A patient who developed CKD at 6 months had a high uPGDS value at baseline (41.89 ng/mg), which further increased at the end of 6 months (390.85 ng/mg) of follow up. The patient who showed relapse at 11 months had an increase in uPGDS value in the 9-month sample though, at that time, there was no urinary abnormality detected (uPGDS: baseline:139.0 ng/mg; 6 months:100.0 ng/mg; 9 months: 479.6 ng/mg).

Discussion

PGDS is a low molecular weight protein of the lipocalin family. Members of lipocalin family *e.g.* neutrophil gelatinase-associated lipocalin (NGAL) have been extensively studied as biomarkers in renal diseases including

Table II. Change in different disease activity parameters and uPGDS in AN group with treatment over 1 year.

	Baseline		6 months		9 months		1 year	
Number	28		25		20		13	
Median SLEDAI	20	(6-32)	2	(0-8)	2	(0-8)	2	(0-2)
Median rSLEDAI	8	(4-16)	0	(0-4)	0	(0-4)	0	(0)
Median C3 (mg/dl)	50.7	(16.9-156)	86.45	(48.7-139)	89.1	(41.3-168)	102	(39.6-166)
Low C3 (n)	14		1		3		1	
Median C4 (mg/dl)	7.95	(5.6-30)	17.2	(5.6-30.9)	18.95	(5.7-23.9)	20.85	(7.0-46)
Low C4 (n)	21		5		4		1	
Median anti-dsDNA (IU/ml)	200	(24-200)	44.8	(6.25-200)	67.45	(7.1-200)	78.15	(27.7-170.8)
Median Upr/Ucr ratio	2.67	(0.11-7.5)	0.28	(0.01-3.37)	0.33	(0.7-2.2)	0.29	(0.09-1.19)
Median creatinine (mg/dl)	0.9	(0.55-2.5)	0.82	(0.5-1.27)	0.8	(0.6-1.3)	0.91	(0.6-1.18)
Median uPGDS (ng/ml)	28040	(3000-334300)	5800	(2400-117960)	3980	(12-28850)	7810	(12-56440)
Median normalised uPGDS (ng/mg)	618.5	(28.8-3039.1)	91.9	(35.7-3908.5)	73.3	(0.1-929.3)	81.7	(0.8-1489.2)

lupus nephritis (10-12). We chose to study PGDS as there were no longitudinal studies in adult patients with LN and because it has been found to be a predictor of nephritis relapse in paediatric lupus nephritis (6). Furthermore, it is synthesised locally as evidenced by the presence of intact form of L-PGDS in cells of loop of Henle and glomeruli and it is processed in lysosomes to produce the truncated form which is excreted in the urine (13).

Cross-sectional analysis of our data showed that uPGDS was significantly elevated in patients with active nephritis as compared to active non-renal, inactive disease and heathy controls. This means that urinary PGDS is specific for renal involvement in SLE and can differentiate between active SLE with LN and active SLE without LN. However, Suzuki *et al*. had a group with non-renal lupus though it is not clear if they had active disease since the mean extra renal SLEDAI is only 3.5. Similarly, another cross-sectional study in adults which was published while this work was ongoing, found uPGDS to be specific for active LN as compared to non-LN glomerular disease. However, they did not include a group of active non-renal disease (3).

At baseline, uPGDS also showed modest correlation with urinary protein/creatinine ratio but not with other conventional parameters of disease activity. This supports the renal specific nature of uPGDS as urinary protein/creatinine ratio is a composite marker of glomerular filtration rate and disease activity.

The argument that uPGDS could simply be the filtered serum PGDS is unlikely as Somparn *et al*. did not find any correlation between estimated glomerular filtration rate (a marker of glomerular injury/leakage) and levels of urinary PGDS, suggesting that serum PGDS is not the major source of uPGDS. In addition, Wu *et al*. showed that the local production of PGDS in kidneys is the major contributor to uPGDS, in addition to a small contribution from serum (2).

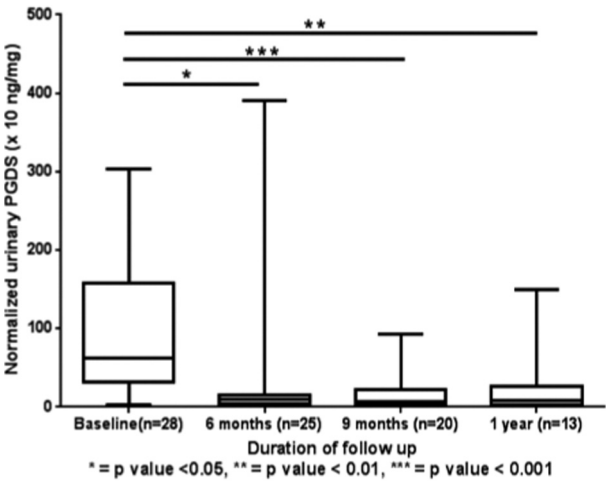

Fig. 3. Urinary PGDS values in the inactive nephritis group at baseline, 6 months, 9 months and 1 year of follow-up

Our results of the longitudinal study in active nephritis group suggest that uPGDS levels correlate with renal disease activity. Its levels decrease as the renal disease activity is reduced with treatment. The only other longitudinal study available on the role of uPGDS in paediatric SLE patients describes its role in predicting relapse but does not mention the effect of treatment on its levels (8). This implies that uPGDS can also be used to monitor response to treatment.

With the limited data of 2 patients, uPGDS may have the potential to predict poor renal response to treatment and relapse. In paediatric LN, uPGDS rose almost 3 months prior to the clinical relapse (6). This could signify that the immune events start earlier than the overt relapse and due to its lower molecular weight (20–30 kDa) as compared to albumin (67 kDa), it may get filtered through the glomerular membrane earlier (2). Persistently elevated levels may suggest poor outcome as was shown in the other patient who developed CKD at the end of 6 months. This may be related to a good correlation of uPGDS levels with tubular atrophy on renal histology as suggested by Brunner *et al*. (14).

The strengths of our study lie in the inclusion of a group of patients with active non-renal disease and longitudinal follow-up on the effect of treatment. Lack of data on prediction of relapse is the major limitation of our study. Other limitations include small sample size, lack of serum PGDS levels and incomplete follow-up of all patients in the active nephritis group.

Thus, in conclusion, uPGDS is a novel potential urinary biomarker of LN that

is specific for renal involvement and is reduced after successful treatment. It needs further evaluation both as renal activity marker and predictor of relapse in a large multicentre study.

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