Urine Periostin as a Biomarker of Renal Injury in Chronic Allograft Nephropathy

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ABSTRACT

Background. Chronic allograft nephropathy (CAN) represents the main cause of renal allograft failure after transplantation. Noninvasive CAN testing is required. Periostin promotes the expression of a mesenchymal phenotype in renal tubules and is a promising urine biomarker for progressive renal injury. Information regarding periostin expression in the setting of CAN remains scarce.

Methods. Subjects were recruited from our outpatient transplantation clinic. Random urine samples were collected from CAN patients (n = 24) and renal transplant patients with normal renal function (transplant controls, n = 18). Control samples were collected from healthy volunteers (n = 18) who had normal renal function. Urine periostin was measured by enzyme-linked immunosorbent assay.

Results. The median urine periostin in CAN patients was significantly higher than in transplant and healthy controls (1.74 vs 0.00 vs 0.14 ng/mg creatinine, respectively; $P < .001$). Urine periostin enzyme-linked immunosorbent assay at a cutoff value of 0.152 ng/mg creatinine demonstrated the sensitivity, specificity, and accuracy for distinguishing CAN patients from transplant patients with normal renal function (91.7%, 77.8%, and 85.7%, respectively). In addition, urine periostin levels correlated directly with urine protein creatinine ratio ($R = 0.566$, $P < .001$) and serum creatinine ($R = 0.522$; $P < .001$), whereas inverse significant correlations were evidenced with estimated glomerular filtration rate ($R = -0.431$; $P < .001$).

Conclusion. The appearance of urine periostin in CAN patients but not in healthy and transplant controls underscores its value as a potential biomarker for chronic progressive renal injury in transplant recipients.

CHRONIC ALLOGRAFT NEPHROPATHY (CAN) is the slow, progressive deterioration in renal dysfunction characterized clinically by an increase in serum creatinine, increasing proteinuria, and progressive hypertension, and histologically by tubular atrophy, interstitial fibrosis, and fibrous neointimal thickening of arterial walls is an almost universal finding in renal transplant recipients [1]. Accurately assessing and monitoring renal function is critically important in CAN patients. Blood urea nitrogen, serum creatinine, formulae to estimate glomerular filtration rate (GFR), and albuminuria are measures currently used to assess the presence and progress of kidney injury [2]. However, these measures are imprecise, are not direct measures of renal tissue injury, and are relatively insensitive to small changes in renal function. Ideally, novel CAN biomarkers should reflect mechanisms and activity of continuing renal injury and predict disease progression and response to treatment.

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The interest of pathway involved in development of fibrosis and tissue remodeling in transplanted kidneys is epithelial–mesenchymal transition (EMT) [3]. This describes the process of phenotypic change that cells of a variety of origins—including mesenchymal cells, resident fibroblasts, and epithelial cells—undergo, leading to fibrosis. Periostin, a member of the matricellular protein family, acts as an adhesion molecule during bone formation, supports osteoblastic cell line attachment, and is involved in cell survival and differentiation [4–7]. Evidence in kidney tissues suggests that periostin reflects the adoption of a mesenchymal phenotype by distal renal tubular cells in response to diverse renal injuries across species [8]. Its renal histopathologic expression patterns and coordinated effect on the induction of a mesenchymal phenotype suggest that periostin may be a biomarker that also participates in the pathogenesis of CAN. Information regarding periostin expression in progressive kidney injury after kidney transplant remains scarce. This study demonstrates urinary and tissue periostin expression in CAN patients.

METHODS

The study protocol was approved by the human subjects institutional review board of the Royal Thai Army Medical Department. Informed consent was obtained from all patients who participated in the study. Random blood and urine samples were collected from CAN patients (n = 24) and renal transplant patients with normal renal function (transplant controls; n = 18). A group of 18 normal age-matched individuals with normal renal function were used as healthy control subjects and stored at −80°C with protease inhibitors until assayed. Individuals with evidence of acute rejection, acute infection, or acute intercurrent illnesses were excluded. Medical history, systolic and diastolic blood pressure, body weight, body mass index, routine laboratory data including blood urea nitrogen, serum creatinine, urine protein creatinine ratio, and estimated GFR were recorded.

Immunohistochemical Renal Periostin Expression

Kidney tissues from CAN patients were randomly collected for periostin immunostaining. Four-micron sections of formalin-fixed, paraffin-embedded tissue were deparaffinized and rehydrated. Endogenous peroxidase activity was quenched by incubation in

Table 1. Clinical and Biochemical Parameters in Transplant Controls, Healthy Controls, and Patients With Chronic Allograft Nephropathy (CAN)

	CAN Subjects (n = 24)	Transplant Controls (n = 18)	Healthy Controls (n = 18)
Male (n, %)	18 (75)*,†	7 (38.9)	3 (16.7)
Age (y)	45.33 ± 9.6	48.78 ± 13.13	50.22 ± 4.77
BMI (kg/m^2)	22.20 ± 2.93	24.28 ± 5.18	24.34 ± 3.16
Systolic blood pressure (mm Hg)	125.37 ± 13.50	123.56 ± 14.85	119.17 ± 9.41
Diastolic blood pressure (mm Hg)	71.38 ± 9.41	67.72 ± 8.50	74.39 ± 8.35
Duration of transplant (y)	9.00 ± 7.00	7.73 ± 5.79	-
Duration of dialysis (y)	4.08 ± 3.27	3.92 ± 4.35	-
Type of transplant (n, %)			
Living-related kidney transplant	11 (45.8)	10 (55.6)	
Cadaveric kidney transplant	13 (51.2)	8 (44.4)	-
History of rejection			
Antibody-mediated rejection	7 (29.17)	0 (0)	
Acute cellular rejection	12 (50)*	2 (11.1)	-
Comorbid diseases (n, %)			
Diabetes mellitus	3 (12.5)	1 (5.6)	1 (5.6)
Hypertension	20 (83.3)†	16 (88.9)	0 (0)
Dyslipidemia	22 (91.7)†	16 (88.9)	3 (16.7)
Cerebrovascular disease	1 (4.2)	1 (5.6)	0 (0)
Ischemic heart disease	0 (0)	1 (5.6)	0 (0)
Immunosuppressive agents			
Tacrolimus	19 (79.2)*	8 (44.4)	
Cyclosporine	2 (8.3)*	10 (55.6)	
Sirolimus	3 (12.5)	1 (5.6)	
Mycophenolate mofetil	14 (58.3)	6 (33.3)	
Myfortic	10 (41.7)	9 (50)	
Azathioprine	0 (0)	2 (11.1)	
Prednisolone	24 (100)	18 (100)	-
Serum 25(OH) vitamin D level (ng/mL)	28.05 ± 16.75	26.20 ± 6.38	23.20 ± 4.13
Intact PTH (pg/mL)	93.52 ± 82.92	81.69 ± 41.69	-
Serum creatinine (mg/dL)	2.01 ± 1.18*,†	0.97 ± 0.11	0.74 ± 0.15
Estimated GFR (mL/min/1.73 m^2)	57.44 ± 20.49*,†	68.91 ± 10.21	90.03 ± 12.71
Urine protein/creatinine ratio	1.49 ± 2.67*	0.14 ± 0.09	-

BMI, body mass index; GFR, glomerular filtration rate; PTH, parathyroid hormone.
All data expressed in mean ± SD.
*$P < .05$ versus transplant controls.
†$P < .05$ versus healthy controls.

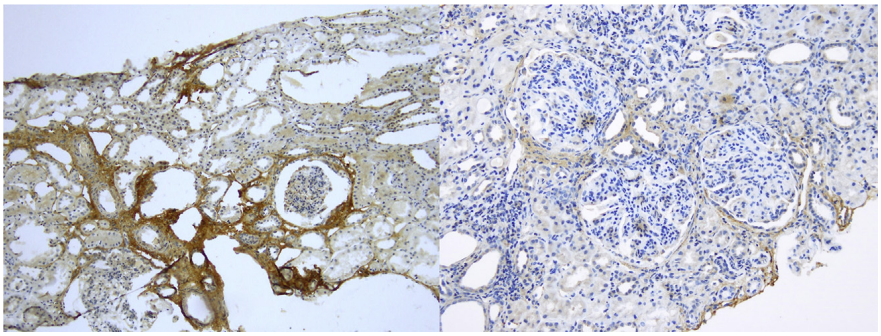


Fig 1. Renal periostin immunostaining in patients with CAN. Representative sections of kidney tissues in 2 patients with CAN displayed cytoplasmic staining for periostin, most prominently in the tubules, interstitium and periglomeruli, frequently in the periadventitial area around arteries and arterioles. (Original magnification, ×400.)

endogenous enzyme block solution. Staining was performed at 4°C overnight with antibodies to polyclonal periostin (BioVendor, Candler, NC; 1:250) followed by incubation with dextran polymer conjugated with horseradish peroxidase and affinity-isolated immunoglobulin for 30 minutes at room temperature. For controls, nonspecific polyclonal immunoglobulin G was used as primary antibody.

Measurement of Urinary Periostin Level

The 96-well microplates were coated overnight with 1 μg/mL (0.1 μg/well) of antiperiostin antibody (R&D Systems, Minneapolis, MN). Plates were washed three times with 0.05% Tween 20 in phosphate-buffered saline then blocked with reagent diluent for ≥1 hour. A total of 100 μL of all standards and patient samples was added to the 96-well plate and incubated for 2 hours. After a 1-hour incubation with rabbit polyclonal anti-periostin antibody (Abcam, Cambridge, UK; 1:1000), a 20-minute incubation with dextran polymer conjugated with horseradish peroxidase, and a 20-minute incubation with substrate solution, stop solution was added to each well. Periostin absorbance was calculated by measuring at 450 nm, correcting for plate artifact at 570 nm, and using a log-transformed standard curve.

Statistical Analysis

Data were expressed as mean values ± standard deviation unless otherwise specified and analyzed with a *t* test or the Mann–Whitney rank-sum test using SPSS (SPSS, Inc., Chicago, IL). For multiple comparisons, we used analysis of variance, followed by the least significance difference test. Pearson's correlation coefficients and multiple regression analyses were performed to explore the relationship between urine periostin and kidney injury parameters. The performance characteristics of urinary periostin level in diagnosis of CAN were described using the area under a receiver operator characteristic curve. Receiver operator characteristic analysis was used to calculate the area under the curve for urine periostin and to find the best cutoff values for distinguishing transplant controls from CAN. $P = \leq .05$ was considered significant.

RESULTS

General Data

Data are summarized in Table 1. Mean age, body mass index, blood pressure, type of renal transplant, duration of transplant, comorbid diseases, and immunosuppressive agents were similar in both transplant controls and CAN groups. Male was significantly higher in patients with CAN. As expected, serum creatinine and urine protein creatinine ratio were significantly elevated in patients with CAN compared with both transplant controls and healthy controls. Likewise, the estimated GFR was significantly lower in the patients with CAN.

Immunohistochemistry Demonstrated Increments in Renal Periostin in CAN Transplant Tissues

A pair of kidney tissues from CAN patients and transplant controls was randomly collected for periostin immunostaining. Periostin was not detected in cortical control kidneys. In contrast, representative sections of kidneys in the CAN patients showed positive periostin immunostaining in areas of glomerulosclerosis, periglomerular, and interstitial areas around arteries and arterioles (Figure 1). Periostin also showed tubular cytoplasmic staining at sites of interstitial inflammation and fibrosis (Figure 1). These data demonstrated that periostin protein expression was observed mainly in the area of glomeruli, tubules, and interstitial fibrosis after the process of chronic progressive injury after kidney transplant.

Urine Periostin Was Higher in CAN Patients Than in Transplant and Healthy Controls

A standard curve was generated using known concentrations of recombinant periostin resulting in a linearized R^2 of 0.981 (data not shown). Urine periostin was measured

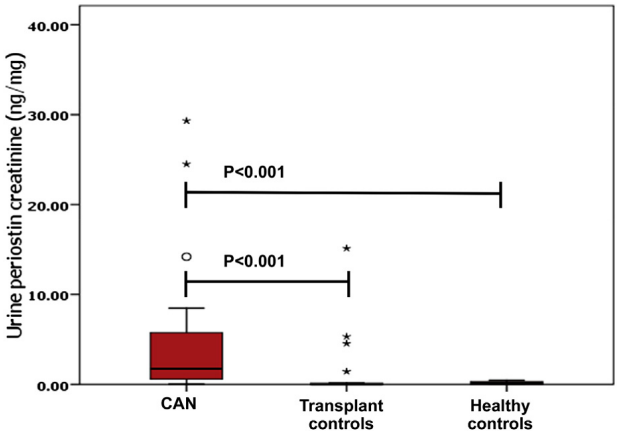
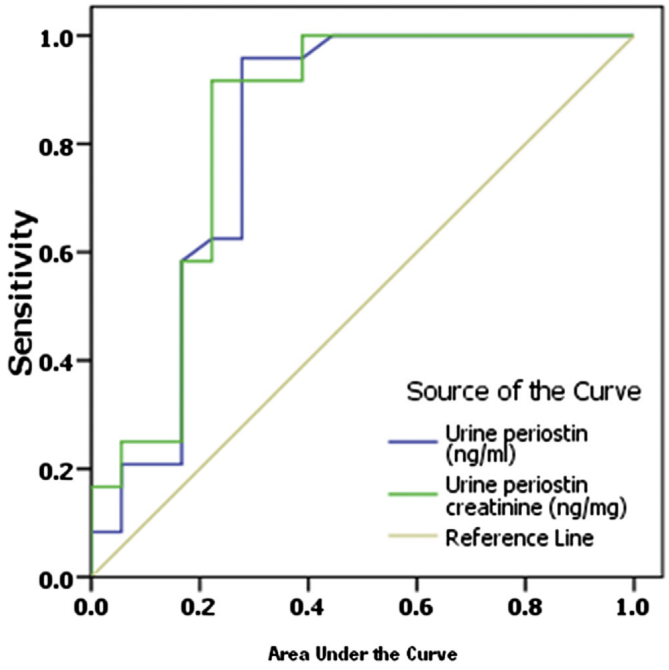


Fig 2. Urine periostin enzyme-linked immunosorbent assay distinguishes CAN from healthy controls and transplant controls.



Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Urine periostin (ng/ml)	.813	.077	.001	.661	.964
Urine periostin creatinine (ng/mg)	.833	.072	.000	.692	.975

The test result variable(s): Urine periostin (ng/ml) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Fig 3. Receiver operating characteristic curves of urinary periostin considering CAN as the status variable. The area under the curve for urine periostin/cr (ng/mg) and urine periostin concentration (ng/mL) were 0.83 (95% confidence interval [CI], 0.69–0.97) and 0.81 (95% CI, 0.66–0.96), respectively, when compared with transplant controls. Both urinary periostin/cr (ng/mg) and periostin (ng/mL) areas were statistically different with respect to that of the diagnostic reference line ($P < .001$).

by enzyme-linked immunosorbent assay in CAN patients (n = 24), transplant controls (n = 18), and healthy controls (n = 18), and corrected for urine creatinine. The median urine periostin in transplant controls (0 ng/mg) and healthy controls (0.14 ng/mg) were significantly less than in patients with CAN (1.74 ng/mg; $P < .001$; Figure 2). These data support the proposition that urine periostin is an indicator of kidney injury after developing progressive allograft nephropathy.

Specificity and Sensitivity of Urine Periostin for Diagnosing CAN

The area under the curve for urine periostin/cr (ng/mg) and urine periostin concentration (ng/mL) were 0.83 (95% confidence interval [CI], 0.69–0.97) and 0.81 (95% CI, 0.66–0.96), respectively, when compared with transplant controls (Figure 3). The best cutoff levels of urine periostin were 0.152 ng/mg (sensitivity 91.7%, specificity 77.8%, accuracy 85.7%) and 0.035 ng/mL (sensitivity 95.8%, specificity 72.2%, accuracy 85.7%) for diagnosing CAN. Thus, the urine periostin measurement demonstrated high sensitivity and specificity for diagnosing CAN.

Correlation of Urine Periostin With Kidney Injury Parameters

Urine periostin level had a positive significant correlation with use of tacrolimus ($r = 0.392$; $P = 0.001$), history of antibody-mediated rejection ($r = 0.476$; $P < .001$), history of acute cellular rejection ($r = 0.419$; $P = .006$), serum creatinine ($r = 0.552$; $P < .001$), and urine protein creatinine ratio ($r = 0.566$; $P = .001$). However, it had an inverse significant correlation with age ($r = -0.409$; $P < .001$), and estimated GFR ($r = -0.431$; $P < .001$; Table 2). After

Table 2. Correlation Coefficients of Urine Periostin and Other Variable Factors

Factors	*r*	*P* Value
Age (y)	−0.409	.001
Male	0.422	.001
Hypertension	0.265	.040
Immunosuppressive agents		
Cyclosporine	−0.560	<.001
Tacrolimus	0.392	.001
Anti-interleukin 2	0.376	.014
History of rejection		
Antibody-mediated rejection	0.476	.001
Acute cellular rejection	0.419	.006
Serum creatinine (mg/dL)	0.522	<.001
Glomerular filtration rate (mL/min/1.73 m^2)	−0.431	.001
Urine protein/urine creatinine ratio	0.566	<.001

multiple regression analyses, only significant correlations were found between percentages of tubulointerstitial fibrosis and increasing urine periostin ($r = 0.535$; $P = .007$).

DISCUSSION

This study describes the renal expression and urine excretion of periostin in CAN patients. Urine periostin/cr demonstrated relative high sensitivity and specificity for diagnosing CAN. These data support the proposition that periostin might be an additional promising tissue and urine biomarker for chronic kidney injury after kidney transplantation.

Periostin is a cell adhesion molecule for pre-osteoblasts, and participates in osteoblast recruitment and spreading [4–7]. Periostin may contribute to renal tissue remodeling in a manner analogous to its functions in other injured tissues [9,10]. In our study, periostin staining was noted predominantly in peri-adventitial areas surrounding tubular atrophy, arteries, and arterioles and in the obsolescent glomeruli and periglomerular area. Consistent with our findings, periostin was not reported in normal kidneys [11] and periostin was expressed predominantly in renal tubules in situ, in cells shed into the lumen, in glomeruli undergoing obsolescence, arterioles, and in the tubulointerstitium in animal model after progressive renal injury [8]. In addition, biopsies from patients with glomerulopathies and renal dysfunction showed enhanced periostin expression in the mesangium, tubular interstitium, and sites of fibrosis. Thus, the data suggest that the de novo expression of periostin during different types of kidney injury and its excretion in kidney tissues are common events during progressive kidney injury.

The main pathway of developed CAN is EMT describing the process of phenotypic change that cells of a variety of origins, including mesenchymal cells, resident fibroblasts, and epithelial cells undergo, leading to fibrosis [12]. Currently, periostin is discussed as a major player in tissue fibrosis, but is also a critical component of mechanically challenged biological structures, including kidney tissues. Moreover, several reports describe periostin overexpression in malignant cells that had undergone EMT and metastasized [13–15]. The de novo periostin expression in the tubulointerstitium areas is most likely from myofibroblasts as described in postischemic myocardium or diseased heart valves [16]. We speculate that periostin in the CAN setting is also involved in tissue remodeling and stimulation of tubulointerstitium undergoing the EMT pathway [17], as is demonstrated in animal models after 5/6 nephrectomy [8] and various tumor cells [17]. Increasing the urinary activity of periostin might be a reasonably specific measure of renal fibrosis with EMT in transplant recipients. In agreement with these previously reported studies conducted on cardiac fibrosis, neoplastic tissues, and chronic kidney disease, this study also demonstrates that urine periostin levels correlated directly with percentages of tubulointerstitial fibrosis in CAN patients and several risk factors for developing renal fibrosis including proteinuria, use of calcineurin inhibitors, and history of transplant rejection.

To our knowledge, this is the first study to test the hypothesis of whether urinary periostin is associated with adverse renal profiles and declining of GFR in patients with established CAN. However, the present data are not sufficient to conclude that urinary periostin level should be used routinely for clinical decision making. Further research should focus on a large population with a prospective cohort study, to clarify the diagnostic and prognostic capabilities of individual and multiple biomarkers.

In conclusion, although urinary periostin seems to be promising diagnostic marker in transplant recipients with chronic progressive renal disease, further investigation is required to combine with 1 or several urinary biomarkers to increase their diagnostic and prognostic precision in patients with CAN.

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