Tubulointerstitial Fibrosis of Living Donor Kidneys Associates with Urinary Monocyte Chemoattractant Protein 1

Xiangling Wang^a John C. Lieske^{a,b} Mariam P. Alexander^b
Muthuvel Jayachandran^c Aleksandar Denic^a Jerry Mathew^a
Lilach O. Lerman^a Walter K. Kremers^d Joseph J. Larson^d Andrew D. Rule^{a,d}

^aDivision of Nephrology and Hypertension, Departments of ^bLaboratory Medicine and Pathology, ^cSurgery, Physiology and Biomedical Engineering, and ^dHealth Sciences Research, Mayo Clinic, Rochester, Minn., USA

Key Words

Monocyte chemoattractant protein 1 · Collagen type IV · Tubulointerstitial fibrosis · Implantation biopsies · Living kidney donors

Abstract

Background: Non-invasive biomarkers that detect occult pathology in patients with normal glomerular filtration rate (GFR) and normal urine albumin excretion may help identify patients at risk for chronic kidney diseases. **Methods:** Two promising biomarkers of interstitial fibrosis, urinary monocyte chemoattractant protein 1 (MCP-1) and collagen IV, were assayed among 634 living kidney donors from 2005 to 2011, who had both a frozen pre-donation spot urine sample and a core needle biopsy of their donated kidney at transplantation ('time zero biopsy'). The association of urine MCP-1 and collagen IV with kidney function (GFR and urine albumin excretion), kidney volume on computed tomographic imaging and histological findings was assessed. **Results:** The mean $\pm$ SD age was 45 ± 12 years, 24-hour urine albumin was 4 ± 7 mg and measured GFR (mGFR) was 102 ± 18 ml/min/1.73 m². The median (25th–75th percentile) urine level of MCP-1 was 146 (54–258) pg/ml and of collagen IV was 2.0 (1.0–3.5) μ g/l. Higher urine MCP-1 associated with higher 24-hour urine albumin excretion; higher urine collagen IV asso-

ciated with male gender. On kidney biopsy, any interstitial fibrosis was present in 22% and fibrosis >5% in 4% of donors. The mean MCP-1/Cr ratio was 1.49 pg/mg for 0% fibrosis, 1.80 pg/mg for 1–5% fibrosis, 2.33 pg/mg for 6–10% fibrosis and 4.33 pg/mg for >10% fibrosis. After adjustment for age, sex, mGFR and 24-hour urine albumin, higher urine MCP-1 but not collagen IV associated with interstitial fibrosis and tubular atrophy. **Conclusion:** Urine MCP-1 may detect early tubulointerstitial fibrosis in adults with normal kidney function.

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Introduction

Renal tubulointerstitial fibrosis is a final common manifestation of chronic kidney disease (CKD) regardless of the etiology [1]. The degree and extent of tubulointerstitial fibrosis is a predictor of future renal function [2–4]. While moderate-severe interstitial fibrosis associates with CKD, mild interstitial fibrosis can be evident in patients with normal kidney function [5, 6]. Early detection of renal fibrosis may be important for the timely initiation of intervention to prevent progression of kidney disease. Renal biopsy can be used to clinically assess renal fibrosis [1, 7], but is invasive and associated with a bleed-

ing risk that limits application in patients without a strong clinical indication. Thus, noninvasive markers that detect renal fibrosis in patients with otherwise normal kidney function are needed.

Studies have shown that inflammation contributes to the early pathogenesis of renal fibrosis [3, 8, 9]. Indeed, inflammatory chemokines, particularly monocyte chemoattractant protein 1 (MCP-1), detect interstitial inflammation and fibrosis in lupus nephritis [10]. Urine MCP-1 may have potential as a biomarker for diagnosis, prognosis and response to therapy in a variety of renal diseases including diabetic nephropathy and autosomal dominant polycystic kidney disease (ADPKD) [11–20]. Interestingly, urine MCP-1 can help detect early diabetic nephropathy lesions on biopsy prior to the proteinuric manifestations of the disease [21]. Type IV collagen is a major component of basement membranes [22] involved in the progression of renal fibrosis [1]. Studies have suggested urinary type IV collagen may be a predictor of CKD and its progression in diabetes [23–25]. However, the association of MCP-1 and collagen IV levels with sub-clinical kidney remodeling remains unknown.

We hypothesized that urine MCP-1 and collagen IV levels may detect early tubulointerstitial fibrosis in adults with normal kidney function. We studied a sample of well-characterized living kidney donors carefully screened for CKD risk factors and studied for kidney function. We tested the association of urine MCP-1 and collagen IV levels with kidney function, kidney volume on imaging and structural findings on kidney biopsy.

Methods

Study Population

This study was approved by the Institutional Review Board at the Mayo Clinic, Rochester Minnesota. Our study population consisted of living kidney donors evaluated at the Mayo Clinic (Rochester, Minn., USA) from 2005 to 2011 who had a pre-donation spot urine sample collected along with a core needle biopsy of their donated kidney during surgery. Each kidney donor underwent a renal CT angiography (CTA) scan and a standardized battery of tests as part of his or her pre-donation evaluation [26]. The kidney function tests included glomerular filtration rate (GFR) measured by iothalamate clearance and 24-hour urine albumin. Hypertension was defined by treatment with any antihypertensive therapy, systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg.

Kidney Volume Measurement

As described previously [27], kidney donors underwent a renal CTA scan as a part of pre-donation examination and the CT images from the angiogram phase were downloaded into a local workstation for processing. The kidney cortical and medullary vol-

umes were estimated using image analysis software (ITK-SNAP version 2.2, University of Pennsylvania, Philadelphia, Pa., USA; www.itksnap.org) to semi-automatically segment the cortex and medulla from transverse images obtained during the angiogram (arterial) phase. The ratio of cortex volume to medulla volume was determined.

Kidney Histology

As described previously [5, 28], implantation renal biopsies were a routine part of the transplantation surgery intended to provide baseline histology for the recipient. An 18-gauge needle core biopsy of the cortex was taken with a biopsy gun (1.7 cm specimen slot) at the time of implantation (intraoperative) after revascularization of the kidney in the recipient. The tissue specimen was fixed in formalin and embedded in paraffin. Nephron size on biopsy was subsequently characterized by mean glomerular (non-sclerotic) volume, cortex volume per glomerulus (inverse of glomerular density) and mean profile tubular area as previously described [28]. Nephrosclerosis on biopsy was also characterized by glomerulosclerosis, ischemic glomeruli, interstitial fibrosis and tubular atrophy and arteriosclerosis (intimal thickening or arteriolar hyalinosis). The number of non-sclerotic glomeruli, globally sclerotic glomeruli and ischemic glomeruli (identified by periglomerular fibrosis, capsule thickening and wrinkled tuft) were counted to determine percentage of globally sclerotic glomeruli and percentage of globally sclerotic and ischemic glomeruli. As previously described [6], a renal pathologist characterized the proportion of cortical area occupied by fibrosis (0, 1–5, 6–10, and >10%) and number of distinct tubular atrophy/fibrosis foci on Mason's trichrome stained slides. Fibrosis was defined by interstitial expansion with increased collagen staining by trichrome. In this population, minimal or no inflammation was present in the fibrotic regions and any fibrosis was always focal rather than diffuse. Tubular atrophy/fibrosis foci were defined by the distinct regions within otherwise normal appearing parenchyma of thickened tubular basement membranes with surrounding fibrosis (regardless of size). The percentage of luminal stenosis was determined from the artery (if any) most orthogonal to its axis by the ratio of the intimal area to the intimal plus luminal area [6]. These measures of nephrosclerosis correlate with decreased kidney cortical to medullary volume ratio, increased kidney surface roughness and renal cysts [6]. Donors with a suboptimal biopsy section defined by compression artifact, <2 mm² of cortex or <2 glomeruli were excluded.

Measurement of Urine MCP-1 and Collagen IV

At the time of their pre-transplant evaluation, a spot urine sample from the donors was collected, centrifuged for 10 min at 2,000 rpm at 4°C and the supernatant was frozen at –70°C. Frozen aliquots for this study were subjected to one freeze-thaw cycle prior to this study. Urine MCP-1 was measured using the Quantikine Human CCL2/MCP1 ELISA kit (R&D Systems, Minneapolis, Minn., USA) as previously described [29]. Urine collagen IV was measured using the type IV urinary collagen ELISA kit (Agratus Medical, Daiichi Fine Chemical Co., Ltd., Japan). Urine creatinine was measured using the enzymatic creatinine kit (Roche Diagnostics, Indianapolis, Ind., USA). Duplicate measurements were performed for each urine specimen and those with a difference >20% underwent repeat analysis. MCP-1 and collagen IV concentrations were analyzed with and without normalization to urine creatinine. Both urine MCP-1 with and without normalization to urine cre-

Table 1. Characteristics of living kidney donors (n = 634 unless noted otherwise)

Characteristics	Value
Demographic	
Mean age, years	44.9±11.5
Male	272 (43)
White	560 (88)
Kidney function and risk factors	
mGFR*, ml/min/1.73 m ²	102.2±17.6
24-Hour urine albumin**, mg	4.3±7.3; 2.0 (0.0–6.0)
Hypertension	94 (15)
BMI, kg/m ²	28±5
Biopsy measures[#]	
Mean (non-sclerotic) glomerular volume, mm ³	0.003±0.001
Cortex per glomerulus, mm ³	0.07±0.05
Mean profile tubular area (including lumen), mm ²	4,547.9±1,467.2
Percentage of globally sclerotic glomeruli	
0	366 (62)
1–5	90 (15)
6–10	64 (11)
11–15	33 (6)
>15	41 (7)
Percentage of globally sclerotic/ischemic glomeruli	
0	332 (57)
1–5	89 (15)
6–10	72 (12)
11–15	37 (6)
>15	54 (9)
Percentage of luminal stenosis	
<50	406 (76)
50–75	110 (21)
>75	15 (3)
Percentage of cortical fibrosis	
0	463 (79)
1–5	104 (18)
6–10	16 (3)
>10	5 (1)
Number of fibrosis and atrophic tubules foci	
0	463 (79)
1	83 (14)
2+	42 (7)
CT scan measures^E	
Total cortex volume (left + right) ^{EE} , ml	202±39
Total medulla volume (left + right), ml	85±21
Cortex/medulla volume	2.5±0.6

Data are presented as the mean ± SD, median (25th–75th percentile) or n (%). BMI = Body mass index.

* n = 620; ** n = 602; [#] n = 594; ^E n = 602; ^{EE} cortical volume was directly segmented in 587 and estimated from total kidney volume in 15 donors.

atinine have been presented in prior studies [11, 13–17, 30]. Normalization to urine creatinine addresses variation due to urine concentration, but urine creatinine also reflects muscle mass.

Statistical Analysis

All data are presented as the mean ± SD, median (25th–75th percentile) or n (%). MCP-1, MCP-1/Cr, collagen IV and collagen IV/Cr concentrations were natural log transformed for regression analysis. Linear regression models were used to determine the association of MCP-1, MCP-1/Cr, collagen IV and collagen IV/Cr with age, sex, kidney function, kidney volumes and kidney pathology (nephron size and nephrosclerosis measures). Regression slopes are described either per change of the original unit, per doubling or per SD of the original unit, according to which of these provided the best interpretation. Multivariate models with adjustment for age, sex, measured GFR (mGFR) and 24-hour urine albumin were performed.

Results

Study Population

Frozen urine was available for urinary MCP-1 and collagen IV measurement in 634 living kidney donors assessed between 2005 and 2011. Clinical, biopsy and CT characteristics of the 634 donors are shown in table 1. The median (25th–75th percentile) urine level of MCP-1 was 146 (54–258) pg/ml and of collagen IV was 2.0 (1.0–3.5) µg/l.

Association of Urine MCP-1 with Kidney Function, Volume and Biopsy Findings

As shown in table 2, in unadjusted analysis, both urine MCP-1 concentration and urine MCP-1/Cr ratio associated with higher urine albumin excretion, larger medullary volume and more tubulointerstitial fibrosis (either percentage of cortical fibrosis or number of tubular atrophy and interstitial fibrosis foci). Online supplemental figure 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000446851) shows MCP-1 concentration and MCP-1/Cr ratio increase with higher percentage of cortical fibrosis and with more tubular atrophy and interstitial fibrosis foci. The mean MCP-1/Cr ratio was 1.49 pg/mg for 0% fibrosis, 1.80 pg/mg for 1–5% fibrosis, 2.33 pg/mg for 6–10% fibrosis and 4.33 pg/mg for >10% fibrosis. After adjustment for age, sex, mGFR and urine albumin excretion, both higher urine MCP-1 concentration and higher urine MCP-1/Cr ratio still associated with more tubulointerstitial fibrosis. The association of urine MCP-1 with urine albumin per doubling ($\beta = 0.17$, $p < 0.0001$) and the association of urine MCP-1/Cr with urine albumin per doubling ($\beta = 0.08$, $p < 0.0001$) remained statistically significant after adjusting for age, sex and mGFR.

Table 2. Association of urine MCP-1 with kidney function, volume, and biopsy findings

Characteristics	ln MCP-1		ln MCP-1/Cr		ln MCP-1		ln MCP-1/Cr	
	unadjusted β value	p value	unadjusted β value	p value	adjusted β value*	p value	adjusted β value*	p value
Age, 10 years	−0.02	0.69	0.10	<0.0001				
Male	0.51	<0.0001	−0.007	0.91				
Kidney function and risk factors								
mGFR, SD	0.002	0.97	−0.01	0.64				
24-Hour urine albumin, doubling	0.16	<0.0001	0.07	0.0001				
Hypertension	0.03	0.82	0.23	0.005	−0.02	0.90	0.09	0.32
Kidney volume, SD								
Cortical volume	0.17	0.001	0.01	0.69	0.05	0.48	0.04	0.30
Medullary volume	0.14	0.01	0.08	0.01	0.06	0.29	0.06	0.09
Cortex/medulla	0.06	0.44	−0.08	0.09	0.02	0.83	−0.02	0.66
Nephron size, SD								
Mean (non-sclerotic) glomerular volume	0.12	0.02	0.06	0.06	0.05	0.39	0.05	0.10
Cortex per glomerulus	0.13	0.01	0.05	0.12	0.08	0.14	0.03	0.33
Mean profile tubular area	0.09	0.08	0.007	0.83	0.03	0.59	−0.007	0.83
Nephrosclerosis								
Percentage of globally sclerotic glomeruli	0.64	0.30	0.90	0.02	0.87	0.17	0.60	0.12
Percentage of globally sclerotic/ischemic glomeruli	0.81	0.15	0.95	0.005	0.95	0.11	0.63	0.08
Percentage of luminal stenosis	0.10	0.68	0.18	0.22	0.11	0.66	−0.02	0.88
Percentage of cortical fibrosis group	0.24	0.01	0.23	<0.0001	0.28	0.004	0.21	0.0005
Number of fibrosis and atrophic tubules foci	0.25	0.005	0.19	0.0004	0.28	0.002	0.16	0.005

* Adjusted for age, sex, 24-hour urine albumin and mGFR.

Association of Urine Collagen IV with Kidney Function, Volume and Biopsy Findings

As shown in online supplemental table 1, in unadjusted analysis, urine collagen IV concentration associated with younger age, male gender, higher urine albumin excretion, larger cortical volume, larger medullary volume, larger non sclerotic glomerular volume, larger cortex per glomerulus and larger mean profile tubular area. These associations were no longer evident (or were reversed) for urine collagen IV/Cr ratio. After adjustment for age, sex, mGFR and urine albumin excretion, higher urine collagen IV concentration associated with larger cortex per glomerulus and more globally sclerotic/ischemic glomeruli, while higher urine collagen IV/Cr associated with lower cortex/medulla ratio.

Discussion

We found that urine MCP-1, but not urine collagen IV level, was associated with tubulointerstitial fibrosis in a cohort of healthy living kidney donors. This finding was

robust, as it was observed whether or not urine MCP-1 concentration was indexed to urine creatinine concentration and whether or not we adjusted for age, sex, GFR and urine albumin excretion. Kidney disease models implicate MCP-1 in the development of interstitial fibrosis via recruitment and activation of monocytes/macrophages [31–34]. Blockade of MCP-1/CCR2 signaling ameliorated renal fibrosis in a mouse unilateral ureteral obstruction nephropathy model associated with a decrease in type I collagen deposits and reduced TGF-β expression [35].

Recently, multiplex-based cytokine profiling of urine demonstrated that MCP-1 performed better than a wide range of cytokines as a biomarker of SLE disease activity [30]. Studies implicate MCP-1 in the pathogenesis of tubulointerstitial damage in congenital obstructive nephropathy, where its urinary excretion may represent a powerful prognostic marker [36]. Studies in ADPKD revealed urinary excretion of MCP-1 was increased in the majority of adult patients [16] and was associated with total renal volume, independent of albuminuria [17]. In ANCA-associated vasculitis, urinary MCP-1 was useful to assess renal involvement and monitor the response to

therapy [37]. In addition, studies in patients with type 2 diabetic nephropathy suggest that urinary MCP-1 excretion may be useful to evaluate the degree of renal fibrosis [38]. Furthermore, a recent study of normotensive and normoalbuminuric female patients with type 1 diabetes revealed that elevation of urinary MCP-1 excretion preceded clinical manifestations and was associated with the changes in kidney interstitial volume, but not glomerular lesions [21]. In the current study, we extend these observations to show that urine MCP-1 may constitute a biomarker of mild tubulointerstitial fibrosis among adults with normal kidney function.

Another notable finding was the association of urine MCP-1 with increased 24-hour urine albumin excretion even in this normal range for the healthy donors studied. Population studies have shown that modest increases in urine albumin excretion, well within the normal range of <30 mg, are predictive of higher mortality and ESRD risk [39–41]. It is unclear if the source of the MCP-1 is interstitial versus glomerular, since increased glomerular leak of albumin or decreased tubular reabsorption could both result in increased urinary albumin excretion. Early studies implicated locally produced MCP-1 in the initiation and progression of tubulointerstitial damage [42, 43]. However, there has been an increasing body of evidence that MCP-1 also plays a role in the progression of glomerular lesions [31, 42, 44, 45]. Correlation of urine MCP-1 with urinary albumin or total protein excretion has been documented among patients with various kidney disorders [13, 46].

The other candidate urine biomarker in this study, urine collagen IV, associated with a lower cortex/medulla ratio even after adjustment for age, sex, mGFR and urine albumin. We have recently shown that a lower cortex/medulla ratio can be reflective of underlying tubulointerstitial fibrosis on biopsy [6], but we found no evi-

dence that collagen IV associates with tubulointerstitial fibrosis on biopsy. One may hypothesize that urinary collagen IV may have more utility for detecting tubulointerstitial fibrosis in relatively more advanced kidney disease.

This study has certain limitations. The cohort consisted of healthy kidney donors, and thus per design all the pathology detected was relatively mild. The mild tubulointerstitial fibrosis in this population is unlikely to be of clinical significance without the onset of a separate progressive disease process. Furthermore, kidney needle core biopsies are a limited tissue specimen and this decreases the precision of the structural findings measured and the ability to assess the renal medulla. Nevertheless, our unique data set included a large number of biopsies, urine measures, kidney function measures and quantitative CT parameters in normal adults. Since biopsies are not available in normal individuals, use of biomarkers such as urine MCP-1 may allow the study of mild subclinical kidney pathology.

In conclusion, our study highlights MCP-1 as a useful marker to detect occult tubulointerstitial fibrosis. Urinary MCP-1 might allow further stratification of kidney function beyond the traditional measures of GFR and urinary albumin excretion.

Acknowledgment

This study was supported with funding from the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK090358). The authors thank Michael Linnes for performing the ELISA analysis.

Disclosure Statement

All the authors declared no competing interests.

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