Evaluation of Urinary Biomarkers for the Prognosis of Drug-associated Chronic Tubulointerstitial Nephritis

Yingjin Shi, MD, Tao Su, MD, Lei Qu, MB, Caili Wang, MD, Xiaomei Li, MD and Li Yang, MD

Abstract: *Background:* The efficacy of urinary biomarkers for predicting adverse clinical outcomes in drug-induced chronic tubulointerstitial nephritis (D-CTIN) has not been well described. *Methods:* A total of 54 patients with D-CTIN were prospectively followed-up in this study. The urinary excretion of α1-microglobulin and transforming growth factor-β1 and the activity of urinary *N*-acetyl-β-D-glucosaminidase (NAG) and matrix metalloproteinases (MMPs) 2 and 9 at baseline were measured. Changes in the estimated glomerular filtration rate (GFR) over a period of 11 to 54 months (median, 38 months) of follow-up were recorded. The efficacy of urinary biomarkers for differentiating patients with various outcomes was tested. Ten patients with IgA nephropathy and 20 healthy volunteers were enrolled as controls. *Results:* The areas under the receiver operating characteristic curve for urinary NAG, MMP-9, MMP-2 and α1-microglobulin for predicting deterioration of the estimated GFR were 0.879, 0.867, 0.735 and 0.709, respectively ($P < 0.05$ for all). Partial regression coefficient results demonstrated that urinary NAG ($P = 0.02$), MMP-2 ($P = 0.046$) and MMP-9 ($P = 0.041$) were inversely correlated with the rate of GFR decline. *Conclusions:* Urinary NAG, MMP-2 and MMP-9 may be considered as possible candidates for forecasting the progression rate of D-CTIN.

Key Indexing Terms: Tubulointerstitial nephritis; Prognosis; Urinary biomarkers; Matrix metalloproteinase; *N*-acetyl-β-D-glucosaminidase. **[Am J Med Sci 2013;346(4):283–288.]**

Chronic tubulointerstitial nephritis (CTIN), characterized histologically by tubular atrophy and interstitial fibrosis, is a common cause of chronic kidney disease (CKD).[1] Various drugs, including nonsteroidal anti-inflammatory drugs, antibiotics, chemotherapeutic medicine and herbs, are still the predominant causes of CTIN (drug-induced CTIN [D-CTIN]). Clinically, D-CTIN often has an insidious onset with a relatively long disease course. Different rates of progression of renal dysfunction have been found in patients with D-CTIN, even after the withdrawal of the causative drugs.[2] It is very important to continuously monitor and evaluate the active lesions, which allows for prompt intervention and suspension of renal function deterioration. Noninvasive urinary biomarkers, which can be used to evaluate and monitor the pathological process, may allow for prediction of the progression of kidney disease. However, at present, most investigation of biomarkers related to progression of CKD is aimed at glomerular disease.

From the Renal Division (YS, TS, LQ, XL, LY), Department of Medicine, First Hospital, Institute of Nephrology, Peking University, Key Laboratory of Renal Disease, Ministry of Health of China, Beijing, China; and Renal Division (YS, CW), the First Affiliated Hospital of Baotou Medical College, Baotou, China.

Submitted May 8, 2012; accepted in revised form August 30, 2012.

The first 2 authors contributed equally to this project.

This work was supported by the National Natural Science Foundation of China (Grant 81070549 to L.Y.).

The authors have no financial or other conflicts of interest to disclose.

Correspondence: Li Yang, MD, Renal Division, Department of Medicine, First Hospital, Institute of Nephrology, Peking University, Beijing 100034, China (E-mail: li.yang@bjmu.edu.cn).

The central event in CKD progression is the formation and continuous accumulation of fibrotic tissue.[3] Transforming growth factor (TGF)-β1 is a well-established principal driver of fibrosis via promotion of fibroblast proliferation, extracellular membrane (ECM) synthesis and inhibition of collagenases in multiple organs,[4,5] whereas matrix metalloproteinases (MMPs) 2 and 9 are reportedly the most important proteolytic enzymes involved in remodeling of the ECM.[6] Urinary secretion of TGF-β1 and MMPs is reportedly elevated in glomerular diseases, which may reflect the activity of ECM deposition in the kidney. However, there is very little information about the significance of these urinary markers in tubulointerstitial diseases. In this prospective study, 51 patients with D-CTIN were enrolled and followed-up for 11 to 54 months. The urinary secretion of TGF-β1, MMP-2 and MMP-9 and markers reflecting tubular injury such as *N*-acetyl-β-D-glucosaminidase (NAG) and α1-microglobulin (α1-MG) were examined. This multiple-biomarker panel was chosen because these biomarkers may reflect the formation of interstitial fibrosis, active tubular structural injury and degree of tubular dysfunction and thus indicate both chronicity and the presence of active lesions in the tubulointerstitial compartment. The relationship between these urinary biomarkers and renal insufficiency and histological changes, and their significance in predicting renal function deterioration, was further analyzed. Ten renal function-matched and proteinuria-matched patients with IgA nephropathy (IgAN) were enrolled as controls to compare possible differences in the urinary biomarker patterns between glomerular and tubulointerstitial diseases.

METHODS

Patients With D-CTIN

This study was approved by the Committee on Research Ethics of the Peking University First Hospital. Fifty-one patients (12 men and 39 women, aged 53.06 ± 10.49 years), clinically diagnosed with D-CTIN, were enrolled in this study from October 2005 to July 2006. The diagnosis of D-CTIN was made when patients met the following criteria: (1) chronic kidney dysfunction with an estimated glomerular filtration rate (eGFR) of $<$60 mL/min/1.73 m^2 related to exposure to a culprit drug and (2) obvious tubular dysfunction, including small-molecular weight proteinuria, renal glycosuria, and renal tubular acidosis or Fanconi syndrome. Eleven patients were histologically confirmed to have CTIN.

The exclusion criteria were as follows: (1) CTIN caused by glomerular diseases, autoimmune diseases, connective tissue diseases, tumors or infections; (2) cases accompanied by infectious diseases, obstructive nephropathy, cystic nephropathy, renal artery stenosis, diabetes mellitus, liver cirrhosis, severe heart diseases, lung interstitial fibrosis, chronic obstructive pulmonary disease or malignant tumors; (3) a $>$10-year history of hypertension; or (4) proteinuria of $>$2 g per 24 hours or prominent hematuria (red blood cells $>$20 per high power field).

Copyright © by the Southern Society for Clinical Investigation. Unauthorized reproduction of this article is prohibited.

Control Subjects

Ten patients with IgAN were randomly chosen as the disease control group from those patients with IgAN who were diagnosed during the same period and met the following enrollment criteria: (1) chronic renal dysfunction with an eGFR comparable with that of the D-CTIN group (15–60 mL/min/1.73 m^2), (2) proteinuria of <2 g per 24 hours, (3) red blood cells of <20 per high power field and (4) the absence of acute or chronic infectious diseases.

Twenty healthy volunteers were enrolled in the normal control group; all were age-matched and sex-matched with patients in the D-CTIN group. Serum creatinine measurement, urinalysis, 24-hour urine collection and B-ultrasound examinations were performed to exclude the presence of kidney diseases.

Urine and Plasma Specimens

Blood and early-morning midstream urine samples were collected from all patients at the time of enrollment in the study. The blood and urine samples were centrifuged at 2500 rpm for 10 minutes within 1 hour of collection. The supernatant aliquots were frozen and stored at -80°C until they were tested simultaneously.

Creatinine Measurement and Change in the eGFR

Serum and urinary creatinine was measured by picric acid colorimetric assay. The eGFR was calculated using the CKD-Epidemiology Collaboration formula.[7] The rate of GFR decline was determined by calculation of the slope of the regression line of the eGFR at each time point of follow-up.

Pathological Studies

All kidney biopsy sections were processed for light microscopy examination (hematoxylin and eosin, periodic acid-Schiff and Masson's trichrome stains). Parameters for chronic lesions, including tubular atrophy and interstitial fibrosis, were assessed by a semiquantitative scoring system according to the proportion of lesion areas taking up the total sections (area): $<25\%$, score 1; 25% to 50%, score 2; 50% to 75%, score 3; and $>75\%$, score 4 (modified on the base of Banff 97 classification).[8]

Urinary Albumin, α1-MG and NAG Measurement

Urinary albumin, α1-MG and NAG levels were measured by immune transmission turbidity and a spectrophotometric method. To compensate for differences in urine flow rate,[9] urinary excretion of biomarkers was normalized to urinary creatinine. The results were expressed as micrograms per milligram creatinine for α1-MG and units per milligram creatinine for NAG. The interassay and intra-assay coefficient variations were 5% to 10% for α1-MG and 5.1% to 7.1% for NAG.

Urine TGF-β1 Measurement

Urinary TGF-β1 concentrations at the protein level were determined using the human TGF-β1 ELISA Kit (R&D Systems, Minneapolis, MN). The assays were performed according to the manufacturer's instructions. To activate latent TGF-β1, 0.5 mL of urinary supernatant was incubated with 0.1 mL of 1 N HCl. After mixing and incubating for 10 minutes at room temperature, the acidified samples were neutralized by adding 0.1 mL of 1.2 N NaOH/0.5 M HEPES, and the sample was assayed immediately. The enzymatic reaction was detected at 450 nm with a reference wavelength of 570 nm in an automatic microplate reader (X-680; Bio-Rad, Hercules, CA). The detection limit was 4.6 pg/mL. All tests were performed in duplicate. Urine TGF-β1 levels were standardized by the amount of creatinine in the urine and expressed as picograms per milligram creatinine.

Urinary MMP-2 and MMP-9 Activity Measurement

The activity of urinary MMP-2 and MMP-9 was tested using gelatin zymography.[10,11] The amount of urine loaded on the gel was 5 to 15 μL depending on the creatinine content. Briefly, 10% running gels containing 1 mg/mL gelatin were overlaid with a 4% stacking gel. Urine samples were loaded with equal amounts of Tris-glycine-sodium dodecyl sulfate sample buffer, and electrophoresis was performed at a constant voltage of 100 V. Gels were incubated for renaturation at room temperature in a buffer containing 50 mM Tris, pH 7.5; 5 mM $CaCl_2$; 1 μM $ZnCl_2$; and 2.5% Triton X-100 for 30 minutes. After exchange of developing buffer (containing 50 mM Tris, pH 7.5; 5 mM $CaCl_2$; 11 μM $ZnCl_2$; 0.02% NaN_3; and 1% Triton X-100), gels were further incubated for 36 hours at 37°C. Gels were then stained with Coomassie blue to visualize white negative bands indicating the presence of protease activity. A mixture of MMP-9 and MMP-2 (Chemicon International, Temecula, CA) served as a positive control and molecular standard. Zymographic bands were scanned, and the optical density was determined using the Bio-Rad Gel Doc system.

Treatment and Follow-up of Patients With D-CTIN

Follow-up visits were conducted regularly for all patients with D-CTIN at the Nephrology Department of Peking University First Hospital. Each patient's clinical condition and course were documented. Routine treatments included diet adjustment, blood pressure control, anemia correction and other systematic support treatments, and these were performed to maintain a blood pressure of $<140/90$ mm Hg and a hemoglobin level of >110 g/L. All enrolled patients were evaluated every 3 months, and the serum creatinine level was assessed at each visit.

Statistical Analysis

Statistical analysis was performed using SPSS software, version 11.0. Data are presented as mean $\pm$ standard deviation or median and range for continuous variables and as proportions for categorical variables. Differences in urinary biomarker levels among groups were tested with the Mann-Whitney *U* test and the nonparametric Kruskal-Wallis test. Frequencies were examined using the χ^2 test for categorical variables. Correlations were assessed according to Pearson for parametric data and Spearman for nonparametric data; patients with IgAN and healthy subjects were not included in the correlation analysis. Urinary biomarkers and other factors for ΔeGFR were analyzed by a multiple linear regression model. A 2-sided P value of <0.05 indicated statistical significance. To distinguish between patients in whom the decline in the eGFR was <2 and >2 mL/min/1.73 m^2 per year, receiver operating characteristic curve analyses of the urinary biomarker levels were carried out to determine the area under the curve (AUC) and to calculate the sensitivity and specificity using the most discriminate thresholds.

RESULTS

Demographic and Clinicopathologic Data of the Participants

As shown in Table 1, the healthy volunteers were age-matched and sex-matched to the patients with D-CTIN. The patients with IgAN were younger than those in the other

Copyright © by the Southern Society for Clinical Investigation. Unauthorized reproduction of this article is prohibited.

Download English Version:

https://daneshyari.com/en/article/2863602

Download Persian Version:

https://daneshyari.com/article/2863602

Daneshyari.com