Urinary Biomarkers for Screening for Renal Scarring in Children with Febrile Urinary Tract Infection: Pilot Study

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Abbreviations and Acronyms

^{99m}Tc-DMSA = ^{99m}technetium dimercapto-succinic acid

ACE = angiotensin-converting enzyme

AGT = angiotensinogen

BMG = beta2-microglobulin

Cr = creatinine

CRP = C-reactive protein

fUTI = febrile urinary tract infection

L-FABP = liver-type fatty acid binding protein

NAG = N-acetyl-β-D-glucosaminidase

NGAL = neutrophil gelatinase associated lipocalin

RAS = renin-angiotensin system

RN = reflux nephropathy

VUR = vesicoureteral reflux

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Purpose: Recurrent febrile urinary tract infections during infancy cause renal scarring, which is characterized by progressive focal interstitial fibrosis and may lead to renal failure. Renal scarring can be diagnosed through scintigraphy, although it seems impractical to perform renal scintigraphy for all infants with febrile urinary tract infections. Therefore, it is important to search for a biomarker to identify the presence of renal scarring. We hypothesized that urinary biomarkers of nephropathy may increase in infants with renal scarring following febrile urinary tract infections.

Materials and Methods: A total of 49 infants who underwent renal scintigraphy for febrile urinary tract infections were enrolled in the study. Several measurements were performed using urine samples, including total proteins, beta2-microglobulins, N-acetyl-β-D-glucosaminidase, neutrophil gelatinase associated lipocalin, liver-type fatty acid binding protein and angiotensinogen. Values were corrected by creatinine and compared between patients with and without renal scarring.

Results: Among urinary biomarkers only angiotensinogen in patients with scarring (median 14.6 μg/gm creatinine) demonstrated significantly higher levels than in patients without scarring (3.6 μg/gm creatinine, $p < 0.001$).

Conclusions: Urinary angiotensinogen may be useful for diagnosing the presence of renal scarring.

Key Words: angiotensinogen, cicatrix, radionuclide imaging, urinary tract infections, vesico-ureteral reflux

Approximately 5% of infants with fever of unknown origin suffer from urinary tract infection.[1] Although reflux nephropathy is related to vesicoureteral reflux, and approximately 8.5% of childhood chronic kidney disease is associated with reflux, its progression to end-stage renal disease is slower than other diseases.[2] The recent concept of reflux nephropathy also includes congenital renal dysplasia, which is often complicated by vesicoureteral reflux and renal parenchymal scarring/atrophy associated with febrile urinary tract infections. Risk factors for renal scar formation in children after febrile urinary tract infections include age at presentation, gender, recurrent infection, peak fever, treatment delay and presence of vesicoureteral reflux.[1,3,4] In 12% to 21% of patients reflux nephropathy, which causes noticeable renal dysfunction at puberty,

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is accompanied by proteinuria and hypertension, and ultimately leads to end-stage renal disease.[1,5]

To reduce the rates of progression from reflux nephropathy to end-stage renal disease, patients with recurrent fUTIs need to be diagnosed early and maintained with careful observation for surgical treatment or continuous antibiotic prophylaxis.[6] While ^{99m}Tc-DMSA renal scintigraphy and magnetic resonance imaging are used to diagnose renal scarring, the former is the most commonly used method.[7] However, its indications are limited, since it is unavailable at many local hospitals and involves radiation exposure. Although it has been postulated that bilateral renal scarring, proteinuria, hypertension and low glomerular filtration rate are prognostic indicators in patients with RN,[2,8] they are less useful in infants. Therefore, noninvasive biomarkers for the diagnosis of renal scarring are desirable.

Reflux nephropathy has been characterized by tubular proteinuria consisting of NAG and BMG, although their significance in RN is still controversial.[9] Recently NGAL and L-FABP have attracted attention as urinary biomarkers for the early diagnosis of acute renal impairment.[10,11] It is also reported that urinary AGT, which mainly derives from the AGT produced in the proximal tubular cells, reflects intrarenal RAS activity and could be a potential biomarker in patients with overt proteinuria.[12–14] Hypothesizing that biomarkers of nephropathy in urine might increase in infants with renal scarring after fUTI, we aimed to identify biomarkers useful for the diagnosis of renal scarring.

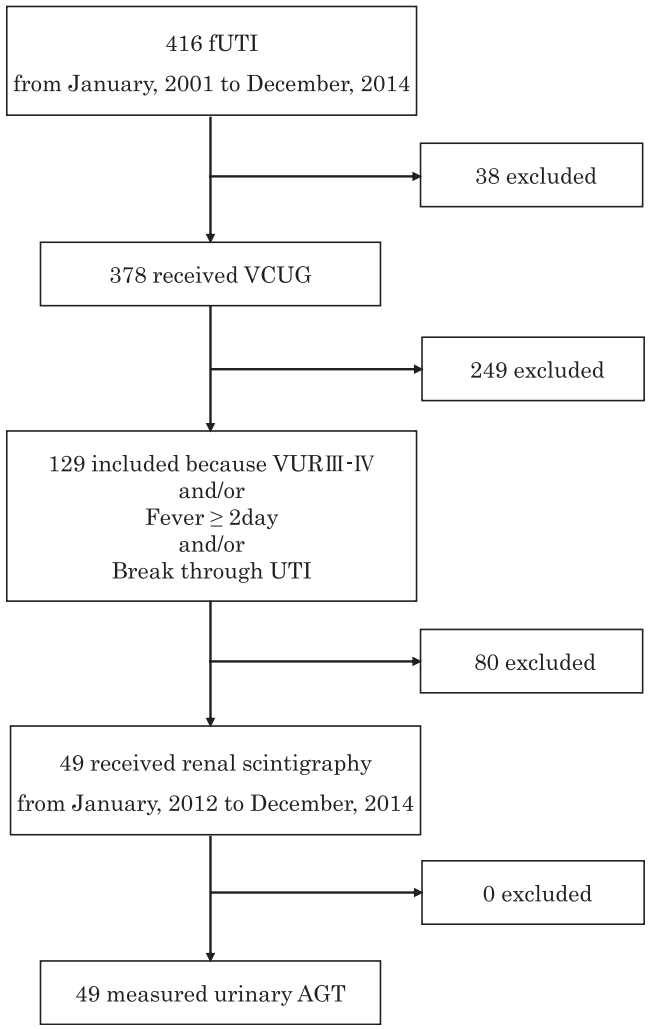

Figure 1. Flow chart depicts study enrollment and inclusion/exclusion criteria. *VCUG,* voiding cystourethrography.

METHODS

Subjects

We prospectively studied 49 patients in whom urinary AGT was measured from among 416 patients who were diagnosed with fUTIs at our institution between January 2001 and December 2014 (fig. 1). Parents provided written informed consent.

Risk factors for renal scarring that indicated ^{99m}Tc-DMSA scintigraphy included grade III or higher VUR, urinary tract infection that was febrile for 2 days or longer and recurrent fUTIs. fUTI was diagnosed in patients who had fever and single bacteria of 10^3 cfu/ml or more in urine specimens obtained by catheterization. Patients with other diseases such as viral illnesses due to respiratory syncytial virus or influenza virus were excluded. ^{99m}Tc-DMSA scintigraphy was performed during the chronic phase, ie 4 months or longer after onset of fUTI, to eliminate the findings from acute inflammatory changes similar to those of scarring.[15] In all patients the results of ^{99m}Tc-DMSA scintigraphy were evaluated by a single radiologist who was blinded to patient characteristics (age, gender, name of disease and presence or absence of VUR).

Sampling and Measurement

All urine samples were collected on the same day as ^{99m}Tc-DMSA scintigraphy, and urine specimens were centrifuged at 1,500 rotations per minute for 5 minutes. The supernatants were harvested and stored at -80C until measurements. At urine sampling blood samples were also collected from 24 subjects and were centrifuged at 3,000 rotations per minute for 5 minutes. The supernatants were stored at -80C for analysis of serum AGT.

The stored blood and urine samples were thawed on the day when several measurements were performed by enzyme linked immunosorbent assay, including serum AGT, urinary AGT, urinary NGAL and urinary L-FABP. Meanwhile, serum levels of creatinine and urinary levels of total protein, NAG, BMG and creatinine were measured on the same day of sampling by enzymatic method, pyrogallol red method, PNP-GlcNAc substrate method, radioimmunoassay and enzymatic method, respectively. Serum AGT and urinary AGT were measured with the human total angiotensinogen assay kit (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) using 10,000-fold