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Original Investigation

Associations of Urinary Levels of Kidney Injury Molecule 1 (KIM-1) and Neutrophil Gelatinase-Associated Lipocalin (NGAL) With Kidney Function Decline in the Multi-Ethnic Study of Atherosclerosis (MESA)

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Background: Whether elevations in levels of urinary biomarkers of tubular injury (urine neutrophil gelatinase-associated lipocalin [NGAL] and kidney injury molecule 1 [KIM-1]) are associated with future risk of kidney disease has not been investigated.

Study Design: 1:1 nested case-control study.

Setting & Participants: 686 participants in the Multi-Ethnic Study of Atherosclerosis (MESA).

Predictor: NGAL and KIM-1 were measured at baseline, expressed as log-transformed continuous variables, and categorized into deciles.

Outcomes: Kidney function was estimated by cystatin C level using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation. Incident CKD stage 3 was defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 and an eGFR decrease >1 mL/min/1.73 m^2 per year, and rapid kidney function decrease was defined as decrease ≥ 3 mL/min/1.73 m^2 per year.

Measurements: Cases were defined as persons with eGFR >60 mL/min/1.73 m^2 who subsequently developed incident CKD stage 3 and/or had rapid kidney function decrease by the MESA year-5 visit. Controls were matched for age, sex, race, diabetes, and baseline eGFR. We adjusted for age, hypertension, and presence of albuminuria (albumin-creatinine ratio ≥ 30 mg/g).

Results: Of 343 cases, 145 had incident CKD stage 3, 141 had rapid kidney function decrease, and 57 had both. Mean eGFR for controls was 81 $\pm$ 10 mL/min/1.73 m^2 at baseline and 80 $\pm$ 10 mL/min/1.73 m^2 at follow-up compared with 82 $\pm$ 13 and 58 $\pm$ 10 mL/min/1.73 m^2 for cases. Each doubling of KIM-1 level (in picograms per milliliter) was associated with an OR of 1.15 (95% CI, 1.02-1.29) for incident CKD stage 3 and/or rapid kidney function decrease. Compared with the lowest 90%, the highest decile of KIM-1 level was associated with an OR of 2.02 (95% CI, 1.15-3.56) for the outcome; these associations were independent of albuminuria. NGAL levels (in nanograms per milliliter) were not associated with incident CKD stage 3 and/or rapid kidney function decrease (OR, 1.04; 95% CI, 0.99-1.10). Results were similar when KIM-1 and NGAL levels were standardized for urine creatinine.

Limitations: The case-control design limits the ability to account for persons who died or were not available for follow-up.

Conclusions: Urinary KIM-1 level is associated with future risk of kidney disease independent of albuminuria. Urinary biomarkers of tubular injury are a promising tool for identifying persons at risk of CKD.
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INDEX WORDS: Kidney injury molecule 1 (KIM-1); neutrophil gelatinase-associated lipocalin (NGAL); kidney function decline.

Chronic kidney disease (CKD), defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2, is highly prevalent[1] and is associated with increased risk of death, cardiovascular disease, and progression to chronic kidney failure.[2] Identification of persons at risk of developing CKD is paramount in designing prevention strategies. Recent work has focused on understanding risk factors for the development of CKD.[3] However, clinically available markers detect CKD only when the disease is estab-

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lished, there is extensive kidney damage, and the window for primary prevention has closed. Albuminuria (defined as a urinary albumin-creatinine ratio [ACR] ≥30 mg/g) has been advocated as an early marker of kidney disease. However, albuminuria primarily reflects glomerular damage,[4] and national data suggest that most nondiabetic CKD in the United States may be nonalbuminuric.[1] In persons with preserved GFR, novel markers of kidney injury potentially could identify persons at risk of the development of CKD to allow investigation of targeted prevention strategies.

Urinary biomarkers of kidney injury, such as urinary kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL), have emerged as predictors of acute kidney injury before decreases in eGFR are detectable.[5-8] High levels of urinary KIM-1 and NGAL are associated with the extent of acute kidney injury, length of hospitalization, outcomes after cardiac surgery, and death.[9-11] Whether these urinary markers of acute injury are associated with a longer term kidney function decrease in the ambulatory setting is not known. Urinary KIM-1 and NGAL are particularly promising markers to identify persons at risk of CKD because they are expressed by tubular epithelial cells in response to injury and tubulointerstitial damage is a common pathway in the progression of most forms of kidney disease.[12,13]

We designed this nested case-control study to evaluate the association of urinary KIM-1 and NGAL levels with kidney function decrease and incident CKD stage 3 in the Multi-Ethnic Study of Atherosclerosis (MESA). We hypothesized that higher levels of these biomarkers would predict rapid kidney function decrease and the development of CKD.

METHODS

The MESA

MESA is a large National Heart, Lung, and Blood Institute–sponsored study designed to understand subclinical cardiovascular disease and its progression in a multiethnic cohort. Details for recruitment and design have been published previously.[14] Briefly, between 2000 and 2002, MESA recruited 6,814 men and women aged 45-84 years who were free of cardiovascular disease and who self-identified as white, black, Hispanic, or Chinese. Persons were recruited from Baltimore City and Baltimore County, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan and the Bronx, NY; and St Paul, MN. Participants returned for 3 visits, in 2002-2004 (examination 2), 2004-2005 (examination 3), and 2005-2007 (examination 4). Repeated measurements of kidney function were obtained at visits 3 and 4. The institutional review boards at all participating centers approved the study, and all participants gave written informed consent.

Measures of Kidney Function

Kidney function was assessed by creatinine and cystatin C level. All assays were performed in frozen serum specimens that were stored at −70°C. Serum creatinine was measured by rate reflectance spectrophotometry using thin-film adaptation of the creatine amidinohydrolase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics Inc, www.orthoclinical.com) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN) and calibrated to the Cleveland Clinic. Cystatin C was measured by means of a particle-enhanced immunonephelometric assay (N Latex Cystatin C; Siemens) with a nephelometer (BNII; Siemens, www.siemens.com) and corrected for assay drift. We used the CKD-EPI (CKD Epidemiology Collaboration) equations to estimate GFR from creatinine ($eGFR_{cr}$) and from cystatin C ($eGFR_{cys} = 76.7 \times [\text{cystatin C}]^{-1.19}$).[15]

Selection of Cases and Controls

We excluded persons with $eGFR_{cr}$ <60 mL/min/1.73 m^2 at the baseline visit, following CKD guideline definitions.[16] Cases were defined as persons with eGFR >60 mL/min/1.73 m^2 (by both creatinine and cystatin C) at baseline who subsequently developed incident CKD stage 3 and/or had rapid kidney function decrease by the MESA year-5 visit (n = 343). Controls were individually matched for age, sex, race, diabetes, and baseline eGFR. Specifically, cases were matched to controls within 10 years of age (45-54, 55-64, 65-74, and 75-84 years) and within 10 mL/min/1.73 m^2 of baseline eGFR as 60-69, 70-79, 80-89, 90-99, 100-109, 110-119, and >120 mL/min/1.73 m^2.

In this study, incident CKD stage 3 was defined as both reaching $eGFR_{cys}$ <60 mL/min/1.73 m^2 and having an $eGFR_{cys}$ decrease >1 mL/min/1.73 m^2 per year. We used this definition to reduce misclassification due to small changes around the CKD threshold. Rapid decrease was defined as $eGFR_{cys}$ decrease ≥3 mL/min/1.73 m^2 per year. This definition has been associated with increased risk of death and cardiovascular outcomes independent of baseline eGFR.[17,18] For both outcomes, we based our definitions on $eGFR_{cys}$ because we have shown that $eGFR_{cys}$ significantly reduces misclassification of CKD status based on creatinine level.[19,20] Controls were individually matched for age, sex, race, diabetes, and baseline eGFR. Total sample size was 343 cases and 343 controls.

Measurement of Urinary KIM-1 and NGAL

Urinary KIM-1 and NGAL were measured from previously frozen stored urine samples. Urinary soluble KIM-1 and NGAL were measured by a microbead-based assay as previously described.[21] The inter- and intra-assay coefficients of variation for KIM-1 and NGAL were <8%. Urine creatinine was measured by the Jaffé assay using Randox Daytone Analyzer (Randox Laboratories Ltd, www.randox.com). The inter- and intra-assay coefficients of variation for creatinine were <3%. KIM-1 and NGAL concentrations also were standardized for urinary creatinine measured concurrently. All laboratory personnel performing measurements were blinded to case-control status, and all statistical analyses were performed at the MESA coordinating center (by R.K.).

Covariates

Information for age and self-reported race/ethnicity was obtained using standardized questionnaires. Blood pressure measurements were obtained using the Dinamap automated blood pressure device (Dinamap Monitor Pro 100; GE Medical Systems, www.gehealthcare.com). Three sequential measurements were obtained and the average of the second and third measurements was recorded. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or current use of antihypertensive medication. Diabetes was defined as either fasting glucose level ≥126 mg/dL or use of oral