Biomarkers of tubulointerstitial damage and function in type 1 diabetes

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ABSTRACT

Objective To evaluate biomarkers of renal tubulointerstitial damage and function in type 1 diabetes with and without diabetic kidney disease.

Research design and methods Cross-sectional case-control study of Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Study participants. Cases (N=43) had incident persistent estimated glomerular filtration rate (eGFR) <60 mL/ min/1.73 m^2 with urinary albumin excretion $\geq$300 mg/24 hour. Controls (N=43) had persistent eGFR $\geq$90 mL/ min/1.73 m^2 and urinary albumin excretion <30 mg/24 hour. Urinary and plasma biomarkers reflecting tubular injury, inflammation, fibrosis, secretion, and synthetic function were measured from stored specimens collected at the first study visit with reduced eGFR (for case participants) or the corresponding study year (for control participants).

Results Mean (SD) age was 51 (9) and 50 (8) years for case and control participants, and mean (SD) duration of diabetes was 30 (6) and 30 (5) years, respectively. Mean (SD) eGFR was 39 (14) and 103 (9) mL/min/1.73 m^2 for case and control participants, and mean (SD) albumin excretion rate was 1978 (2914) and 10 (7) mg/day, respectively. Comparing cases with controls, significant differences were observed in each measured biomarker, including urine epidermal growth factor (mean 5.3 vs 21.2 µg/g creatinine for case vs control participants, respectively), urine monocyte chemoattractant protein-1 (596 vs 123 ng/g creatinine), urine galectin-3 (168 vs 52 µg/g creatinine), plasma soluble tubular necrosis factor receptor-1 (3695 vs 1022 pg/mL), plasma galectin-3 (21.3 vs 11.0 ng/mL), urinary clearances of hippurate (70 vs 167 mL/min) and cinnamoylglycine (77 vs 317 mL/min), and plasma arginine-citrulline ratio (5.6 vs 7.7 µg/µg), each P<0.001.

Conclusions Marked abnormalities in biomarkers of kidney tubular injury, inflammation, fibrosis, secretion, and synthetic function accompany reduced eGFR and albuminuria in type 1 diabetes.

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INTRODUCTION

Elevated urine albumin excretion (albuminuria) and reduced estimated glomerular filtration rate (eGFR) have long been viewed as

Significance of this study

What is already known about this subject?
Kidney disease is a common complication of types 1 and 2 diabetes. Diabetic kidney disease is usually assessed using estimated glomerular filtration rate (eGFR) and urine albumin excretion, which predominantly reflect damage to the kidney glomerulus.

What are the new findings?
Compared with people with type 1 diabetes and normal eGFR and urine albumin excretion, those with abnormal eGFR and urine albumin excretion had significant concomitant abnormalities in urine and plasma biomarkers that reflect damage and impaired function of the tubulointerstitital compartment of the kidney. The results suggest that tubular injury, inflammation, and fibrosis as well as impairments in kidney tubule secretion and synthesis of small molecules are prominent features of diabetic kidney disease.

How might these results change the focus of research or clinical practice?
This study suggests that diverse aspects of tubulointerstitial damage and function should be evaluated in future studies of diabetic kidney disease and provides new information on specific biomarkers that might be useful for this work. Ultimately, such studies may better define the pathophysiology of diabetic kidney disease, help develop new biomarkers to add to eGFR and urine albumin excretion in clinical care, and guide the development of new treatments for diabetic kidney disease.

the cardinal manifestations of diabetic kidney disease (DKD). These DKD manifestations are common in type 1 diabetes, can progress to end-stage renal disease, and are strongly associated with cardiovascular diseases and other adverse health outcomes.[1] However, kidney functions beyond glomerular filtration and conservation of circulating proteins are also critical for maintaining homeostasis and health. In particular, renal tubular cells secrete small molecules and synthesize growth

factors, hormones, and amino acids. Renal tubulointerstitial damage—not glomerular disease—is most strongly associated with progression of chronic kidney disease.[2,3] Further understanding of tubular damage, secretion, and synthesis may lead to better understanding of kidney injury in DKD, the relationship of kidney disease to other diabetes complications, and new therapeutic targets.

Research design and methods

We performed a cross-sectional case-control study nested within the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study. The goal of the study was to identify candidate biomarkers of tubulointerstitial damage and function that are markedly abnormal in DKD in order to prioritize promising biomarkers for additional longitudinal studies.

We studied all participants with incident persistent eGFR <60 mL/min/1.73 m^2 (on two consecutive study visits), urinary albumin excretion rate (AER) ≥300 mg/24 hour, and available biosamples (N=43). Controls (N=43) were randomly selected from DCCT/EDIC Study participants who maintained persistent eGFR ≥90 mL/min/1.73 m^2 and AER <30 mg/24 hour through the study visit on which the corresponding case participant developed incident eGFR <60 mL/min/1.73 m^2. Control participants were matched to cases on duration of diabetes and DCCT cohort (primary vs secondary intervention).

Using stored urine and plasma specimens collected at the time of incident persistent eGFR <60 mL/min/1.73 m^2 (for cases) or at the same study year (for controls), we measured biomarkers reflecting tubular injury, inflammation, fibrosis, secretion, and synthetic function. These included urine epidermal growth factor (EGF), urine monocyte chemoattractant protein-1 (MCP-1), plasma soluble tubular necrosis factor receptor-1 (sTNFR-1), urine and plasma galectin-3, the urinary clearances of hippurate and cinnamoylglycine, and plasma arginine-citrulline ratio. These biomarkers were chosen to represent diverse aspects of tubulointerstitial damage and function that are not captured well by eGFR and albuminuria, prioritizing those most likely to be feasible and useful based on published literature.[4-9]

Aliquots of plasma and urine were collected concurrently using standardized procedures and stored at <−70°C. For 66 participants (with samples collected prior to August 2012), urine aliquots were taken from 4-hour collections. For 20 participants (with samples collected after August 2012), urine was taken from a random morning sample, based on a change in the EDIC Study protocol. Urine EGF, MCP-1, and sTNFR-1 were measured using ELISA assays from R&D Systems. Urine and plasma galectin-3 were measured using ELISA assays from BGMedicine. Plasma and urine hippurate and cinnamoylglycine and plasma arginine and citrulline were measured by mass spectrometry.[8,10]

Urinary EGF, MCP-1, and sTNFR-1 were standardized to urine creatinine concentration. The urinary clearances of hippurate and cinnamoylglycine were calculated within the subset of 66 participants with timed urine collections as urinary excretion rate divided by plasma concentration. Differences in biomarkers were tested using the paired T-test, based on the case-control design.

RESULTS

Mean age was 50.5 years and mean duration of diabetes was 30.1 years at the time of biospecimen collection. Case and control participants were similar with regard to characteristics used for matching (DCCT cohort and duration of diabetes) as well as age and gender (table 1). Mean (SD) eGFR was 39 (14) and 103 (9) mL/min/1.73 m^2 for case and control participants, and mean (SD) albumin excretion rate was 1978 (2914) and 10 (7) mg/day, respectively. Blood pressure and hemoglobin A1c (HbA1c) (particularly time-weighted HbA1c) were higher comparing case with control participants.

The distributions of each measured biomarker differed significantly comparing cases with controls (table 2). The urinary excretion of EGF was markedly lower in case versus control participants, while the urinary excretion of MCP-1 and galectin-3 were markedly higher. Plasma sTNFR-1 and galectin-3 concentrations were markedly higher in case versus control participants, while plasma arginine-citrulline ratio (a marker of tubular synthetic function) was lower. The urinary clearances of hippurate and cinnamoylglycine were significantly lower in case versus control participants. For urinary biomarkers, results were similar among subsets of participants with timed compared with random urine samples.

DISCUSSION

We observed marked abnormalities in biomarkers of tubulointerstitial damage and function comparing participants with type 1 diabetes, incident eGFR <60 mL/min/1.73 m^2, and AER ≥300 mg/24 hour to those without evidence of kidney disease. Differences in all eight candidate biomarkers were large and statistically significant, despite the modest sample size. The eight biomarkers represent diverse aspects of tubulointerstitial damage and function, including tubular injury, inflammation, fibrosis, secretion, and synthetic function. Our results demonstrate that DKD in type 1 diabetes is characterized by a broad range of abnormalities beyond reduced eGFR and albuminuria, and suggest that diverse facets of tubulointerstitial damage and function should be evaluated in future studies.

Tubular damage, inflammation, and tubulointerstitial fibrosis are acknowledged mechanisms of DKD.[2,6,11-13] Therefore, the observation that markers of these processes are abnormal among patients with established DKD is not surprising. However, the large differences we observed reinforce the concept that tubulointerstitial damage is a critical feature of DKD. Moreover, markers of tubular injury, inflammation, fibrosis, secretion, and

Table 1 Clinical characteristics of included DCCT/EDIC Study participants with and without kidney disease

	Kidney disease status*	
	Controls (N=43)	Cases (N=43)
Clinical characteristics		
Demographic data		
Age (years)	50 (8)	51 (9)
Duration of diabetes (years)	30 (5)	30 (6)
Female gender	20 (47)	17 (40)
DCCT intensive therapy	21 (49)	14 (33)
DCCT primary cohort	22 (51)	22 (51)
Physical examination data		
Body mass index (kg/m^2)	29.3 (5.2)	30.4 (6.0)
Systolic BP (mmHg)	122 (14)	137 (21)
Diastolic BP (mmHg)	75 (9)	76 (13)
Laboratory data		
eGFR (mL/min/1.73 m^2)	103 (9)	39 (14)
Albumin excretion rate (mg/day)	10 (7)	1978 (2914)
Current HbA1c (%)	8.0 (1.0)	8.6 (1.7)
DCCT/EDIC time-weighted HbA1c (%)	7.9 (0.6)	9.2 (1.1)
EDIC time-weighted HbA1c (%)	7.9 (0.7)	9.1 (1.3)
LDL cholesterol (mg/dL)	95 (29)	105 (39)
HDL cholesterol (mg/dL)	60 (19)	52 (18)
Triglycerides (mg/dL)	77 (35)	131 (73)

Cell contents are mean (SD) or N (%).
*Cases were defined by incident persistent eGFR <60 mL/min/1.73 m^2 with urinary AER ≥300 mg/24 hour; control subjects were randomly selected from the pool of DCCT/EDIC Study participants who maintained persistent eGFR ≥90 mL/min/1.73 m^2 and AER <30 mg/24 hour through the study visit on which the corresponding case participant developed incident eGFR <60 mL/min/1.73 m^2 and were additionally matched to cases on duration of diabetes and DCCT cohort.
AER, albumin excretion rate; BP, blood pressure; DCCT/EDIC, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; LDL. low-density lipoprotein.

Table 2 Biomarkers of tubulointerstitial damage and function among included DCCT/EDIC Study participants with and without kidney disease

	Kidney disease status*		
	Controls (N=43)	Cases (N=43)	P value for difference
Urine biomarkers†			
EGF (µg/g)	21.2 (8.7)	5.3 (2.8)	<0.0001
MCP-1 (ng/g)	123 (100)	596 (860)	<0.0001
Galectin-3 (µg/g)	52 (35)	168 (145)	<0.0001
Plasma biomarkers			
sTNFR-1 (pg/mL)	1022 (256)	3695 (1289)	<0.0001
Galectin-3 (ng/mL)	11.0 (5.3)	21.3 (6.6)	<0.0001
Arginine-citrulline ratio (µg/µg)	7.7 (2.8)	5.6 (1.9)	0.0004
Urinary clearance‡			
Hippurate (mL/min)	167 (72)	70 (79)	<0.0001
Cinnamoylglycine (mL/min)	317 (150)	77 (71)	<0.0001

Cell contents are mean (SD).
*Cases were defined by incident persistent eGFR <60 mL/min/1.73 m^2 with urinary AER ≥300 mg/24 hour; control subjects were randomly selected from the pool of DCCT/EDIC Study participants who maintained persistent eGFR ≥90 mL/min/1.73 m^2 and AER <30 mg/24 hour through the study visit on which the corresponding case participant developed incident eGFR <60 mL/min/1.73 m^2 and were additionally matched to cases on duration of diabetes and DCCT cohort.
†Urinary biomarkers are expressed per gram of urine creatinine.
‡Urinary clearances are restricted to 66 participants with timed urine collections.
AER, albumin excretion rate; DCCT/EDIC, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications; eGFR, estimated glomerular filtration rate; MCP-1, monocyte chemoattractant protein-1; sTNFR-1, plasma soluble tubular necrosis factor receptor-1.

synthetic function are not currently used in clinical care and may ultimately be useful adjuncts to eGFR and albuminuria. Our data identify promising biomarkers to evaluate in future longitudinal studies that evaluate this possibility.

Recently, in a diverse population of people with chronic kidney disease (most without diabetes), an agnostic analysis starting with transcriptomics of kidney biopsy tissue ultimately identified reduced urinary EGF as a strong predictor of progressive kidney disease.[4] EGF is a growth factor produced by distal tubular cells that promotes tubular cell repair and regeneration. Membrane-bound TNFR-1 is involved in apoptosis, survival, and key aspects

of inflammation and immune response. The soluble form, sTNFR-1, was previously associated with rapid kidney function decline and incident eGFR <60 mL/min/1.73 m^2 in the Joslin study of type 1 diabetes.[5] Urine MCP-1, a more specific marker of renal inflammation, was associated with eGFR loss in type 2 diabetes.[6] Plasma concentration of GDF-15, a member of the TGF-β cytokine superfamily, correlated with GDF-15 expression in renal tubular cells and was associated with progression of kidney disease in populations with established chronic kidney disease (with and without diabetes).[7]

Tubular functions, including secretion and synthetic function, have been less comprehensively studied in DKD. Many proposed uremic toxins are small organic molecules that circulate bound to plasma proteins, such as albumin, and are poorly filtered but actively secreted by proximal tubular cells. Hippurate and cinnamoylglycine are examples of such small molecules, which accumulate with kidney disease in a manner that is not necessarily proportional to reduction in GFR.[8] Renal tubular cells also perform many synthetic functions, such as the production of arginine from citrulline.[9] Additional renal synthetic functions include

production of hydroxylated vitamin D metabolites, gluconeogenesis, ammoniagenesis, and erythropoietin production. Accumulation of uremic solutes and reduced synthesis of important nutrients and hormones may promote insulin resistance, atherosclerosis, and other pathologic processes contributing to adverse health outcomes of chronic kidney disease.[8,14,15] Our results suggest that biomarkers of tubulointerstitial damage and function previously examined in other populations and evaluated in this study may be useful in type 1 diabetes.

The main limitation of our study is the cross-sectional design, from which we cannot determine the sequence of change in renal biomarkers or the utility of tubulointerstitial biomarkers for predicting kidney disease progression or other complications. Compared with control participants, those with DKD had higher systolic blood pressure and a history of worse glycemic control, but we cannot determine the extent to which hyperglycemia and hypertension contributed to tubulointerstitial damage and impaired tubular function. We are unable to discern whether urine biomarkers arise from the tubulointerstitial compartment of the kidney or appear in the urine through filtration. In addition, our candidate biomarker approach necessarily excludes some potentially important biomarkers. Study strengths include the simultaneous evaluation of biomarkers reflecting multiple aspects of tubulointersititial damage and function, the well-characterized cohort with robust control subjects, the use of novel and precise mass spectrometry assays for some analytes, and the strength of the observed associations.

CONCLUSION

In type 1 diabetes, marked abnormalities in biomarkers of kidney tubular injury, inflammation, fibrosis, secretion, and synthetic function accompany reduced eGFR and albuminuria. Longitudinal studies are needed to determine the time course over which these biomarkers of tubulointerstitial function change relative to eGFR and albuminuria and to determine whether these biomarkers are associated with DKD progression and complications. Biomarkers of tubulointerstitial damage and function may be useful for the development of new therapies targeting DKD.

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