Increased plasma kidney injury molecule-1 suggests early progressive renal decline in non-proteinuric patients with type 1 diabetes

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Progressively decreasing glomerular filtration rate (GFR), or renal decline, is seen in patients with type 1 diabetes (T1D) and normoalbuminuria or microalbuminuria. Here we examined the associations of kidney injury molecule-1 (KIM-1) in plasma and urine with the risk of renal decline and determine whether those associations are independent of markers of glomerular damage. The study group comprised patients with T1D from the 2nd Joslin Kidney Study of which 259 had normoalbuminuria and 203 had microalbuminuria. Serial measurements over 4 to 10 years of follow-up (median 8 years) of serum creatinine and cystatin C were used jointly to estimate eGFRcr-cys slopes and time of onset of CKD stage 3 or higher. Baseline urinary excretion of IgG_2 and albumin were used as markers of glomerular damage, and urinary excretion of KIM-1 and its plasma concentration were used as markers of proximal tubular damage. All patients had normal renal function at baseline. During follow-up, renal decline (eGFRcr-cys loss 3.3% or more per year) developed in 96 patients and 62 progressed to CKD stage 3. For both outcomes, the risk rose with increasing baseline levels of plasma KIM-1. In multivariable models, elevated baseline plasma KIM-1 was strongly associated with risk of early progressive renal decline, regardless of baseline clinical characteristics, serum TNFR1 or markers of glomerular damage. Thus, damage to proximal tubules may play an independent role in the development of early progressive renal decline in non-proteinuric patients with T1D.

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End-stage renal disease (ESRD) is a major health problem responsible for high morbidity and premature mortality in patients with type 1 diabetes (T1D).[1,2] Progressive renal decline leading to ESRD begins while renal function is normal and usually proceeds inexorably along a linear trajectory.[3] It develops in about 10% of patients while urinary albumin excretion is normal (NA), 30% of those with microalbuminuria (MA) and 50% of those with proteinuria.[3–6] We refer to this decline as *early progressive renal decline*: early, because it starts when renal function is normal and progressive because, once initiated, it continues until ESRD is reached.[3–6]

The disease process underlying early progressive renal decline is unknown. Several systemic factors have been implicated: poor glycemic control, elevated blood pressure, and elevated serum levels of uric acid, TNFR1 and TNFR2.[5–10] Morphological kidney studies of early progressive renal decline are nonexistent with the exception of the RASS clinical trial reported by Mauer *et al.* During the 5 year trial involving healthy T1D participants, significant decline in eGFR occurred in 25%. This decline was not associated with any morphological lesion in glomeruli assessed in baseline biopsies.[11] Whether it was associated with morphological lesions in tubular and interstitial compartments is unknown as this was not assessed.

To gain a view of processes taking place in kidneys, an alternative to an examination of morphology in kidney biopsies is an examination of biomarkers in plasma and urine that are specific for glomerular or tubular damage. For example, urinary albumin excretion has been viewed as a marker of glomerular damage although, in truth, it is also a marker of tubular injury that impairs albumin reabsorption. Similarly, urinary excretion of various IgG classes reflects abnormalities in the glomerular filtration barrier, and we have developed sensitive assays to measure their concentrations in urine.[12] An example of a biomarker specific to proximal tubular cell injury is the urinary concentration of kidney injury molecule-1 (KIM-1). This protein was originally discovered using representational difference analysis in an

Table 1 | Distribution of baseline plasma and urine concentrations of KIM-1 in study subgroups: non-diabetic controls (NDM), T1D patients with normoalbuminuria (NA) or microalbuminuria (MA)

	ND	T1	T2	T3	Total
Panel A: Stratum of baseline plasma KIM-1[a]					
NDM	61.5% [46]	22.4% [17]	15.8% [12]	1.3% [1]	100% [76]
NA	35.1% [91]	28.6% [74]	20.1% [52]	16.2% [42]	100% [259]
MA	20.7% [42]	17.2% [35]	29.6% [60]	32.5% [66]	100% [203]
Panel B: Stratum of baseline urine KIM-1[b]					
NDM	40.3% [31]	23.4% [18]	25.9% [20]	10.4% [8]	100% [77]
NA	32.5% [82]	19.5% [49]	25.4% [64]	22.6% [57]	100% [252]
MA	11.1% [22]	32.8% [65]	26.8% [53]	29.3% [58]	100% [198]

[a]Definition of strata of baseline concentration of KIM-1 plasma: ND, not detectable (<0.2 pg/ml). T1-T3, tertiles of the distribution of detectable values of plasma KIM-1 in all T1D patients. Cut points for tertiles (33rd and 67th percentiles) were 11 and 21 pg/ml. [b]Definition of strata of baseline concentration of KIM-1 in urine: ND, not detectable (<0.2 pg/ml). T1-T3, tertiles of the distribution of detectable values of urinary KIM-1 in all T1D patients. Cut points for tertiles (33rd and 67th percentiles) were 58 and 208 pg per mg of urinary creatinine.

effort to identify mRNAs and their encoded proteins that are up-regulated after acute ischemic kidney injury.[13]

KIM-1, also known as Hepatitis A Virus Cellular Receptor 1 (HAVCR1) and T cell Ig mucin 1 (TIM1), is a transmembrane glycoprotein specifically over-expressed in damaged proximal tubules. The ectodomain of KIM-1 (approximately 90 kDa) is cleaved by matrix metalloproteinases and released into the urine.[13–16] Since its discovery, KIM-1 has emerged as a sensitive and specific urinary biomarker of kidney injury in both rodent models and humans.[17–21] After injury to proximal tubules, excess KIM-1 protein may be released not only into the urine but also into the circulation.[18] An elevated circulating concentration of KIM-1, independent of albuminuria, predicts the risk of ESRD in patients with proteinuria and T1D.[18]

In this study of non-proteinuric patients with T1D and normal renal function, we sought to test the hypothesis that plasma and urinary KIM-1, markers of proximal tubule damage, are elevated prior to any detectable change in glomerular permeability or albuminuria. Thus proximal tubule injury may represent an early feature and potential causative factor in the development of early progressive renal decline in T1D.

RESULTS

Distribution of markers of tubular and glomerular damage in the study group

The study group comprised non-proteinuric T1D patients whose renal function was normal (eGFRcr-cys > 60 ml/min) when enrolled into the 2nd Joslin Kidney Study (as described previously[5,7–10,19]) and who were followed for 4–10 years. The present study includes 259 patients with normoalbuminuria (NA), 203 with microalbuminuria (MA), and a comparison group of 77 healthy individuals without diabetes (NDM). Four markers were examined at baseline: two markers of tubular damage (urinary and plasma concentrations of KIM-1) and two markers of glomerular damage (urinary concentrations of albumin and IgG2).

Distributions of baseline plasma and urinary concentrations of KIM-1 in the three study sub-groups (NDM, NA and MA) are compared in Table 1. Plasma concentrations of KIM-1 below the detection limit (0.2 pg/ml) were designated not detectable (ND). Detectable plasma concentrations of KIM-1 in T1D patients with NA or MA were combined into one distribution and divided into tertiles. This created four strata of baseline plasma concentrations of KIM-1 (ND, T1-T3). Plasma KIM-1 was not detectable in 61.5% of NDM, 35.1% of NA and 20.7% of MA (Table 1, Panel A). The frequency of plasma KIM-1 concentrations in the lowest tertile was similar in all three study sub-groups, while the frequency of elevated concentrations (upper two tertiles) rose from 17.1% of NDM to 36.3% of NA and to 62.1% of MA. The distributions of corresponding strata of urinary KIM-1 in the three study sub-groups (Table 1, Panel B) is similar to the pattern in Panel A with the notable exception of a higher frequency of elevated (upper two tertiles) concentrations of urinary KIM-1 (36.3%) in NDM as compared to plasma KIM-1 (17.1%).

Characteristics of the study group according to strata of plasma KIM-1

To identify variables associated with higher baseline levels of plasma KIM-1, we examined other measured markers and relevant clinical variables in the NA and MA groups according to the four plasma KIM-1 strata. Progressively higher plasma KIM-1 concentrations were accompanied by progressively higher concentrations of KIM-1, albumin and IgG2 (Table 2). Data regarding NAG in urine and TNFR1 in serum, as previously reported by us,[7,8,19] were used in the present study. Their levels increased with plasma levels of KIM-1 (Table 2). Regarding clinical characteristics, while age, HbA1c, and percent treated with ACE-I or ARBs increased, duration of diabetes, systolic and diastolic blood pressures and eGFRcr-cys did not differ across the four strata.

Both the duration of follow-up and the number of serum creatinine and cystatin C measurements available to estimate eGFRcr-cys and determine trajectories of change during follow-up were similar across strata. Three aspects of the trajectories of eGFRcr-cys were used to represent the association between plasma KIM-1 and early progressive renal decline. The slope of eGFRcr-cys decline became steeper, from 1.4 to 3.2%/year (medians) between the lowest and highest stratum of plasma KIM-1. Consequently, the proportion of "decliners" (patients with eGFRcr-cys loss $\geqslant$ 3.3% per year, as defined previously[5]) increased from 5% to 47% between the same strata, and the incidence rate of progression to impaired renal function (CKD stage $\geqslant$ 3) increased from 2 to 54/1000 person-years. Each of these associations was highly significant (test for trend $P < 0.001$).

The associations between concentrations of assayed markers and patient characteristics at baseline were examined with Spearman rank correlation (r_s) (Table 3). Tubular and glomerular markers were significantly correlated with each other but the coefficients were weak to moderate (r_s varied