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Short communication

Podocyte number predicts long-term urinary albumin excretion in Pima Indians with Type II diabetes and microalbuminuria

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Abstract

Aims/hypothesis. The predictive value of glomerular structure on progression of renal disease was examined in patients with Type II (non-insulin-dependent) diabetes and microalbuminuria (urinary albumin-to-creatinine ratio = 30–299 mg/g).
Methods. Kidney biopsy specimens were obtained from 16 diabetic Pima Indians (6 men, 10 women). Progression of renal disease was assessed by measuring urinary albumin excretion 4 years after the biopsy ($UAE_{4\ years}$) and by computing the change in urinary albumin excretion during the study (Δ UAE).
Results. At baseline, the duration of diabetes averaged 13.3 years (range = 4.0–23.8 years) and the mean glomerular filtration rate was 159 ml · min^{-1} · 1.73m^{-2} (range = 98 – 239 ml · min^{-1} · 1.73m^{-2}). Median urinary albumin excretion was 67 mg/g (range = 25–136 mg/g) and it increased to 625 mg/g (range = 9–13471 mg/g) after 4 years; 10 subjects (63 %; 4 men, 6 women) developed macroalbuminuria (urinary albumin-to-creatinine ratio ≥ 300 mg/g). Neither mean arterial pressure nor HbA_{1c} changed substantially during follow-up. Among the glomerular morphologic characteristics, the number of visceral epithelial cells, or podocytes, per glomerulus was the strongest predictor of renal disease progression ($UAE_{4\ years}$, $r = -0.49$, $p = 0.05$; ΔUAE, $r = -0.57$, $p = 0.02$), with fewer cells predicting more rapid progression. Glomerular basement membrane thickness did not predict progression ($UAE_{4\ years}$, $r = 0.11$, $p = 0.67$; ΔUAE, $r = 0.09$, $p = 0.73$) and mesangial volume fraction had only a modest effect ($UAE_{4\ years}$, $r = 0.42$, $p = 0.11$; ΔUAE, $r = 0.48$, $p = 0.06$).
Conclusion/interpretation. Whether lower epithelial cell number per glomerulus among those that progressed was due to cellular destruction, a reduced complement of epithelial cells, or both is uncertain. Nevertheless, these findings suggest that podocytes play an important part in the development and progression of diabetic renal disease. [Diabetologia (1999) 42: 1341–1344]

Key words Type II diabetes mellitus, diabetic nephropathies, epithelial cell, glomerulus, Indians, North American.

The visceral epithelial cell, or podocyte, forms the filtration slit structure that prevents the escape of plasma proteins from the glomerular circulation. Considerable evidence in experimental animals indicates that disruption of the podocyte contributes to the development of glomerular sclerosis [1]. By implication, the progressive rise in urinary protein excretion that characterizes diabetic nephropathy points to a putative role for the podocyte in the pathogenesis of diabetic renal disease. Consistent with this hypothesis is a cross-sectional study in diabetic Pima Indians which found that subjects with clinical nephropathy had fewer podocytes per glomerulus than those without nephropathy [2]. Since podocytes are thought to be incapable of replication, this observation suggests

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Abbreviations: GFR, glomerular filtration rate; GBM thickness, glomerular basement membrane thickness; RPF, renal plasma flow; UAE, urinary albumin excretion

that podocyte loss, or perhaps a low podocyte number per glomerulus, contributes to the development and progression of diabetic glomerulosclerosis [2].

In this longitudinal study, we examined the predictive value of glomerular structure in Pima Indians with Type II (non-insulin-dependent) diabetes and microalbuminuria on urinary albumin excretion 4 years later and on the change in urinary albumin excretion during the study.

Subjects and methods

The protocol was approved by the review boards of the National Institute of Diabetes and Digestive and Kidney Diseases, Stanford University and the Cleveland Clinic Foundation and by the Tribal Council of the Gila River Indian Community. Each subject gave informed consent.

Kidney biopsy specimens were obtained from 17 diabetic Pima Indians with microalbuminuria (urinary albumin-to-creatinine ratio = 30–299 mg/g) who were participating in a study of the course of renal disease in Type II diabetes [2, 3]. Of these subjects 16 were examined again 4.1 years later (range = 3.7–4.4 years) and are included in this study. Baseline measures of glomerular haemodynamic function, urinary albumin excretion, HbA_{1c} and blood pressure were obtained from a renal clearance study in each subject within 6 months of the kidney biopsy (13 of 16 studies were done within 30 days of the biopsy). Glomerular filtration rate (GFR) and renal plasma flow (RPF) were calculated as the average urinary clearances of iothalamate and para-aminohippurate, respectively. Albumin excretion was expressed as the ratio of urinary albumin to urinary creatinine (mg/g) from a single spot urine specimen. We measured HbA_{1c} by high-performance liquid chromatography. Blood pressure was measured to the nearest 2 mmHg with a mercury sphygmomanometer while the subject rested in a seated position. Diastolic blood pressure was measured at the fifth Korotkoff sound. Mean arterial pressure was calculated as the diastolic pressure plus one-third of the pulse pressure. Hypertension was defined by the criteria of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC VI) as a systolic pressure of 140 mmHg or more, a diastolic pressure of 90 mmHg or more or treatment with antihypertensive medicine [4].

Morphometric studies. Kidney biopsy specimens were obtained under ultrasound guidance using a 14 gauge needle. Biopsy specimens were processed for morphometric examination by light and electron microscopy as described previously [2]. Briefly, the volumes of individual glomeruli in each subject were calculated from the areas of their mid-sections using the maximum planar area method. The volume fraction of cortical interstitium was determined by point counting in five to seven sections spaced 80 μ apart in each core. Glomerular basement membrane (GBM) thickness was measured in each glomerulus using the orthogonal intercept method. Volume densities of glomerular components were determined by the point counting technique. Absolute values for the volumes and areas of the components of glomerular structure were then calculated by multiplying the appropriate densities by values for the glomerular tuft volume. The average number of podocytes per glomerulus was determined using a previously described method [5].

Statistical analysis. Changes in mean arterial pressure, HbA_{1c} and urinary albumin excretion over time were assessed, depending on their distributions, by paired *t* tests or Wilcoxon signed-rank tests. Pearson correlations were used to examine the relation between baseline variables and urinary albumin excretion 4 years later ($UAE_{4\ years}$) or the change in urinary albumin excretion during the study (ΔUAE). Values for urinary albumin excretion were logarithmically transformed for analysis. The ΔUAE represents the natural logarithm of the ratio of the urinary albumin excretion after 4 years to the urinary albumin excretion at baseline. Adequacy of the linear correlations was assessed by inspection of residual plots. A p value less than 0.05 was considered statistically significant.

Results

Clinical and morphological features of the 16 subjects at baseline, as described previously [2], are shown in Table 1. Duration of diabetes averaged 13.3 years (range = 4.0–23.8 years) and glomerular filtration rate 183 ml/min (range 115–271 ml/min). Of the subjects three had hypertension; none was receiving antihypertensive medicines at the initial examination. Neither mean arterial pressure nor HbA_{1c} changed substantially during follow-up; after 4 years, mean arterial pressure increased, on average, by 5 mmHg ($p = 0.12$) and HbA_{1c} by 0.1 % ($p = 0.46$). The median urinary albumin-to-creatinine ratio was 67 mg/g (range = 25–136 mg/g) at baseline and increased to 625 mg/g (range = 9–13471 mg/g) ($p < 0.0001$); ten subjects (63 %; 4 men, 6 women) progressed to macroalbuminuria (urinary albumin-to-creatinine ratio ≥ 300 mg/g).

Epithelial cell number correlated with age ($r = –0.63$, $p = 0.01$), duration of diabetes ($r = –0.49$, $p = 0.05$), and GFR ($r = 0.60$, $p = 0.01$). On average, a 10-year increment in age was associated with 97 fewer (95 % confidence interval, –165 to –28) epithelial cells, a 10-year increment in duration of diabetes with 118 fewer (95 % confidence interval, –238 to 1) epithelial cells and a 50 ml · min^{-1} · $1.73m^{-2}$ increment in GFR with 103 more (95 % confidence interval, 25 to 182) epithelial cells per glomerular tuft.

Urinary albumin excretion 4 years after biopsy ($UAE_{4\ years}$) correlated with epithelial cell number ($r = –0.49$, $p = 0.05$; Fig. 1) and the duration of diabetes ($r = 0.52$, $p = 0.04$), but not with HbA_{1c} ($r = 0.07$, $p = 0.80$), mean arterial pressure ($r = –0.28$, $p = 0.30$), glomerular filtration rate ($r = –0.37$, $p = 0.15$), GBM thickness ($r = 0.11$, $p = 0.67$) or mesangial volume fraction ($r = 0.42$, $p = 0.11$). Weak correlations were found with age and RPF (Table 1). The change in urinary albumin excretion during the 4-year study (ΔUAE) correlated with epithelial cell number ($r = –0.57$, $p = 0.02$; Fig. 1), age ($r = 0.55$, $p = 0.03$), duration of diabetes ($r = 0.59$, $p = 0.02$) and modestly with GFR ($r = –0.46$, $p = 0.07$) and mesangial volume fraction ($r = 0.48$, $p = 0.06$).

Table 1. Clinical and morphological features of the 16 diabetic Pima Indians (6 men, 10 women) at baseline and the correlation coefficients between these variables and urinary albumin excretion 4 years later ($UAE_{4\ years}$) or the change in urinary albumin excretion during the study (ΔUAE)

	Mean (SEM)	Range	$UAE_{4\ years}$		ΔUAE	
			r	*p*	*r*	*p*
Age (years)	40 (2)	24–55	0.46	0.07	0.55	0.03
Duration of diabetes (years)	13.3 (1.5)	4.0–23.8	0.52	0.04	0.59	0.02
Body mass index (kg/m^2)						
Men	30.1 (3.3)	20.5–43.5	–0.31	0.56	–0.41	0.42
Women	34.5 (1.1)	30.7–41.9	–0.19	0.60	–0.14	0.69
MAP (mm Hg)	94 (2)	80–107	–0.28	0.30	–0.32	0.22
HbA_{1c} (%)	10.5 (0.5)	6.7–13.5	0.07	0.80	–0.09	0.74
Urinary albumin-to-creatinine ratio (mg/g)	67[a]	25–136	–0.20	0.47	–0.44	0.09
GFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	159 (11)	98–239	–0.37	0.15	–0.47	0.07
RPF ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	777 (37)	483–1108	–0.46	0.07	–0.38	0.14
GBM thickness (nm)	503 (20)	384–635	0.11	0.67	0.09	0.73
Vv mesangium (%)	28 (2)	19–43	0.42	0.11	0.48	0.06
Vv interstitium (%)	24 (1)	10–34	–0.02	0.93	–0.05	0.85
Glomerular volume ($\times 10^6\ \mu m^3$)	7.1 (0.5)	4.2–11.6	–0.23	0.40	–0.28	0.30
Foot process width (nm)	653 (32)	463–943	–0.06	0.81	–0.01	0.96
Epithelial cell number	466 (36)	263–778	–0.49	0.05	–0.57	0.02

[a] Median. MAP, mean arterial pressure; Vv mesangium, volume fraction of mesangium; Vv interstitium, volume fraction of the cortical interstitium

Discussion

In a previous study [2], diabetic Pima Indians with clinical nephropathy had fewer epithelial cells per glomerulus than those with less advanced renal disease. Because the study was cross-sectional, however, the temporal relation between epithelial cell number and progression of renal disease could not be determined; the lower epithelial cell number could either precede or be a consequence of the clinical nephropathy. The present study confirms that the lower epithelial cell number precedes the development of more advanced renal disease. Other morphological measures did not have strong relations with subsequent urinary albumin excretion but age and duration of diabetes were associated with progression.

Whether lower epithelial cell number per unit of glomerular volume among those that progressed reflects inherent phenotypic variability or epithelial cell destruction at this early stage of disease is not known. Perhaps both factors are involved. Data on podocyte number are not available in non-diabetic Pima Indians, but autopsy studies indicate that Pimas have larger glomeruli than Caucasians [6]. Pima Indians with diabetes of recent onset also have larger glomeruli than non-diabetic Caucasians but no more podocytes per glomerulus, suggesting there are ethnic differences in podocyte density [2]. On the other hand, sustained mechanical stress from long-term exposure to glomerular hypertension could cause epithelial cell injury [7] and could explain the lower cell count in those that subsequently progressed, as well as the inverse relations between age or diabetes duration and epithelial cell number. The opportunity for mechanical stress exists, since the GFR was strikingly increased in the study subjects, averaging $159\ ml \cdot min^{-1} \cdot 1.73 m^{-2}$ after 13 years of diabetes. Nevertheless, hyperfiltration is almost certainly not the sole determinant of epithelial cell injury, since GFR was positively associated with epithelial cell number at baseline and higher GFR did not predict progression of renal disease in this small study or in a previous study a larger cohort from the same group of Pima Indians [3].

Podocyte destruction could also be due to thickening of the glomerular basement membrane and expansion of the glomerular mesangium, which together could lead to closure of glomerular capillary loops and cellular obliteration. These factors probably did not contribute greatly to epithelial cell damage at this stage of disease, however, because the magnitude of basement membrane thickening and mesangial expansion in Pima Indians with microalbuminuria is small relative to those with advanced nephropathy and is nearly indistinguishable from subjects with diabetes of recent onset [2]. Moreover, glomerular basement membrane thickness did not predict the level of urinary albumin excretion after 4 years and mesangial volume fraction was only a weak predictor, although these measures strongly predicted higher urinary albumin excretion in Type I (insulin-dependent) diabetes [8, 9]. A study of Italians with Type II diabe-

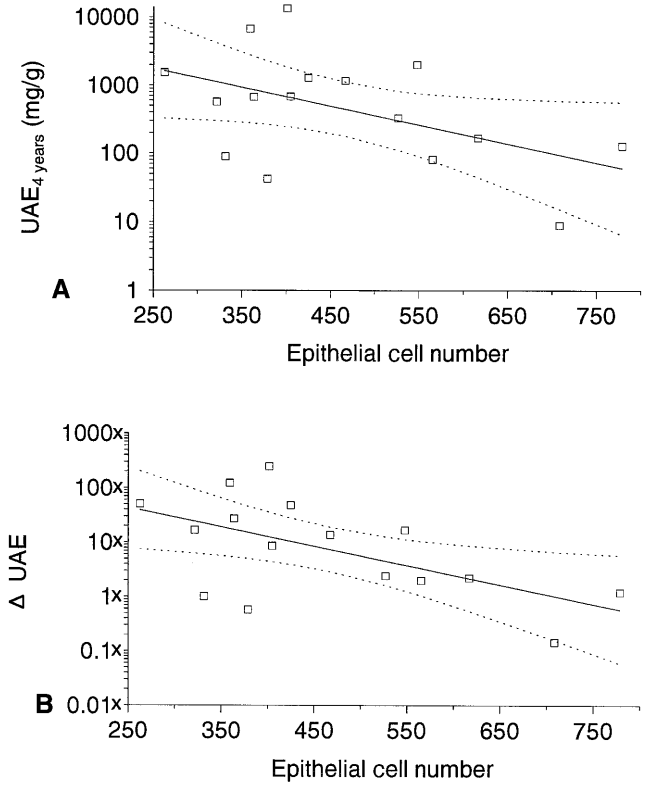

Fig. 1 A–B. Epithelial cell number at baseline and urinary albumin excretion 4 years later ($UAE_{4\ years}$; $r = -0.49$, $p = 0.05$; **A**) or the change in urinary albumin excretion during the study (Δ UAE; $r = -0.57$, $p = 0.02$; **B**) in 16 Pima Indians with Type II diabetes and microalbuminuria at baseline. Regression lines and 95% confidence intervals for the mean urinary albumin excretion after 4 years or change in urinary albumin excretion are shown

tes found that the pattern of underlying glomerular involvement did not reliably predict progression of renal disease [10], but the number of epithelial cells per glomerulus was not determined.

To summarize, a growing body of evidence suggests that podocytes play an important part in the development of glomerular sclerosis [1]. Although our study is small and the effects are therefore measured imprecisely, the results do suggest that the podocyte influences the progression of renal disease in Pima Indians with Type II diabetes and microalbuminuria.

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