Increased Urinary Excretions of Immunoglobulin G, Ceruloplasmin, and Transferrin Predict Development of Microalbuminuria in Patients With Type 2 Diabetes

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Microalbuminuria is generally considered as the best available noninvasive predictor of diabetic nephropathy. However, several studies have shown that increases in certain urinary proteins (immunoglobin G [IgG] [1–3], transferrin [Tf] [4–7], and ceruloplasmin [CRL] [3,8]) were found in normoalbuminuric diabetic patients. Recently, we reported that in normoalbuminuric diabetic patients, there was a strong linear correlation among these urinary proteins (9). To assess whether increased urinary excretions of IgG, CRL, and Tf can predict development of microalbuminuria, we conducted a 5-year follow-up study of type 2 diabetic patients with normoalbuminuria at baseline.

RESEARCH DESIGN AND METHODS

— In 1998 and 1999, we recruited 140 type 2 diabetic patients with normoalbuminuria from outpatients. Normoalbuminuria was defined as a urinary albumin-to-creatinine ratio <30 mg/gCr in all of three serial spot urine samples. No subjects had a history of disease other than diabetes, hypertension, or dyslipidemia.

Since urinary IgG, CRL, and Tf can be easily affected by meal protein (10,11), timed overnight urine samples were collected on three different days within 2 or 3 months for analyses of these urinary proteins and *N*-acetylglucosaminidase (NAG) (3,8–13). Geometric means of the results expressed as protein-to-creatinine (Cr) ratios including albumin (U-Alb/Cr, U-IgG/Cr, U-CRL/Cr, U-Tf/Cr, and U-NAG/Cr, respectively) were calculated. Increases in these parameters at baseline were defined as exceeding the upper limits of healthy control subjects (Table 1). Means of HbA_{1c} (A1C) and serum lipids were calculated from data in three blood samples taken in the morning after the urine collections.

These patients were followed at our outpatients' clinic once a month. The development of microalbuminuria was defined as two consecutive U-Alb–to–Cr ratios of spot urines ≥30 mg/gCr. During 5-year follow-up, 3 patients died and 20 patients discontinued their visits to our clinic. Finally, 117 patients (83.6%) were available for analysis of the development of microalbuminuria.

All participants in this study gave their consent after being fully informed of the study protocol.

RESULTS

— Of 117 patients, 17 (14.5%) progressed to microalbuminuria. Baseline A1C, mean A1C during the follow-up, and rates of retinopathy and use of insulin at baseline were significantly higher in progressors than in nonprogressors. Blood pressure levels during the follow-up were slightly higher in progressors than in nonprogressors (not statistically significant). Other parameters exhibited no difference between progressors and nonprogressors (Table 1) (Mann-Whitney *U* test or Pearson's χ^2 test).

At baseline, U-IgG/Cr, U-CRL/Cr, and U-Tf/Cr of progressors were significantly higher than those of nonprogressors, but there was no difference in U-NAG/Cr. The rate of progression to microalbuminuria was significantly higher in patients with increased U-IgG/Cr (8 of 17, 47.1%) than in patients without increased U-IgG/Cr (9 of 100, 9%) ($P = 0.0003$; odds ratio [OR] 8.99 [95%CI 3.16–25.6]). Similar results were also obtained for U-CRL/Cr and U-Tf/Cr but not for U-NAG/Cr (Table 1).

In a multiple logistic regression analysis, the model included the clinical parameters at baseline and during the follow-up (Table 1) as variables. Increased U-IgG/Cr was selected as a representative of urinary proteins for multicollinearity among U-IgG/Cr, U-CRL/Cr, and U-Tf/Cr. In the model, use of insulin and retinopathy at baseline, high diastolic blood pressure during the follow-up, and increased U-IgG/Cr at baseline were independent variables for development of microalbuminuria ($P = 0.049$, 0.039, 0.031, and 0.0098, respectively; OR 7.03 [95% CI 1.01–48.84], 7.57 [1.10–51.96], 3.12 [1.10–8.80 per 5 mmHg], and 10.87 [1.78–66.5], respectively).

CONCLUSIONS

— Increased urinary excretions of IgG, CRL, and Tf had equal predictive value for development of microalbuminuria in normoalbuminuric type 2 diabetic patients. Kazumi et al. reported that increased urinary Tf excretion could predict development of microalbuminuria in normoalbuminuric type 2 diabetic patients (14). We confirmed their result and found that increased urinary

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Abbreviations: Cr, creatinine; CRL, ceruloplasmin; IgG, immunoglobin G; NAG, *N*-acetylglucosaminidase; Tf, transferrin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical characteristics of 117 normoalbuminuric type 2 diabetic patients*

	Control subjects	Progressors	Nonprogressors	*P* value or OR (95% CI) (progressors vs. nonprogressors)
n (male/female)	20 (9/11)	17 (6/11)	100 (54/46)	NS
Baseline data				
Age	57.1 ± 3.9	62.7 ± 5.7	60.0 ± 9.3	NS
BMI (kg/m^2)	22.7 ± 2.0	22.8 ± 2.0	22.7 ± 3.1	NS
Known duration (years)		11.8 ± 8.1	10.1 ± 6.4	NS
A1C (%)	5.0 ± 0.21	7.4 ± 0.90	6.9 ± 0.99	0.045
SBP (mmHg)	117.1 ± 13.4	128.9 ± 19.5	127.2 ± 12.2	NS
DBP (mmHg)	73.1 ± 9.5	74.5 ± 13.2	74.2 ± 10.1	NS
Total cholesterol (mmol/l)	5.4 ± 0.73	5.2 ± 0.73	5.0 ± 0.74	NS
Triglycerides (mmol/l)	1.4 ± 0.88	1.5 ± 0.71	1.2 ± 0.78	NS
HDL cholesterol (mmol/l)	1.6 ± 0.48	1.5 ± 0.40	1.5 ± 0.47	NS
Retinopathy (%)		47.1	20.0	0.029
Antihypertensive drug use (%)		23.5	28.0	NS
ACE inhibitor use (%)		11.8	13.0	NS
Insulin use (%)		47.1	17.0	0.0052
Smoking (%)	35.0	29.4	42.0	NS
U-Alb/Cr (mg/gCr)	5.3 (3–14.8)	9.0 (3.7–29)	6.5 (1.1–24)	0.0003
U-IgG/Cr (mg/gCr)	1.9 (1.2–4.6)	3.9 (0.96–7.7)	2.4 (0.83–6.5)	0.0031
High/normal (*n* = 17/100)		8/9	9/91	0.0003
				8.99 (3.16–25.6)
U-CRL/Cr (μg/gCr)	42 (12–90)	71 (38–170)	53 (0.36–84)	0.0094
High/normal (*n* = 24/93)		8/9	16/84	0.034
				4.67 (1.67–13.1)
U-Tf/Cr (μg/gCr)	140 (53–440)	290 (150–970)	140 (29–1300)	0.0001
High/normal (*n* = 15/102)		6/11	9/91	0.0027
				5.52 (1.81–16.8)
U-NAG/Cr (U/gCr)	2.2 (0.16–5.0)	3.7 (0.47–11)	3.8 (0.067–11)	NS
High/normal (*n* = 36/81)		8/9	28/72	NS
5-year mean A1C (%)		7.4 ± 0.60	7.0 ± 0.87	0.0052
5-year mean SBP (mmHg)		130.6 ± 15.3	125.8 ± 11.5	NS
5-year mean DBP (mmHg)		74.6 ± 9.9	71.9 ± 8.6	NS
5-year mean total cholesterol (mmol/l)		5.0 ± 0.44	4.9 ± 0.72	NS
Last U-Alb/Cr (mg/gCr)		57.5 (31.1–154)	10.2 (1.4–37.5)	<0.0001

Data are expressed as means ± SD or median (range), unless otherwise indicated. U-Alb/Cr, U-IgG/Cr, U-CRL/Cr, U-Tf/Cr, and U-NAG/Cr represent ratios of urinary albumin, IgG, CRL, Tf, and NAG to urinary creatinine in overnight urine samples at baseline, respectively. High/normal represents the number of patients whose levels of each parameter did or did not exceed the upper limits of the healthy control subjects. Last U-Alb/Cr represents U-Alb/Cr in spot urine samples at last value during 5-year follow-up. Since some cases of progressors reversed to normoalbuminuria by intensive glycemic and blood pressure control or use of angiotensin II receptor blockers and/or ACE inhibitors after definite microalbuminuria development, last U-Alb/Cr of progressors were expressed at development of microalbuminuria. Comparisons between progressors and nonprogressors are performed using Mann-Whiteney *U* test for continuous data and Pearson's χ^2 test for categorical data. DBP, diastolic blood pressure; NS, not statistically significant ($P \geq 0.05$); SBP, systolic blood pressure.

IgG and CRL can also predict development of microalbuminuria.

Our previous study showed that transiently enhanced glomerular filtration rate in response to acute protein loading did not cause an increase in urinary albumin excretion but parallel increases in urinary excretions of IgG, CRL, and Tf in healthy volunteers (10,11). Our findings suggest that these urinary proteins reflect changes in renal hemodynamics more sensitively than albumin. Parallel increases in these urinary proteins in timed overnight urine samples were found in patients with impaired glucose tolerance (15) and in type 2 diabetic patients with normoalbuminuria (3,9). Furthermore, strict glycemic control reversed increases in these urinary proteins without change in albuminuria (3). Given the finding that hyperglycemia induces an increase in intraglomerular hydraulic pressure (16–18) in early stages of diabetic nephropathy, parallel increases in these urinary proteins likely reflect increased intraglomerular hydraulic pressure, and increased intraglomerular hydraulic pressure does not produce increase in albuminuria. Accordingly, the present results are consistent with the animal studies that increased intraglomerular hydraulic pressure plays a pivotal role in development of diabetic glomerulosclerosis (16–18).

Although A1C levels at baseline and during the follow-up were higher in progressors than in nonprogressors, multiple logistic regression analysis did not indicate that the glycemic control level was an independent predictor of development of microalbuminuria. Several landmark studies of type 2 diabetes showed that poor glycemic control is a risk factor for development of microalbuminuria (19,20). Our negative finding may be explained by the relatively small differences in A1C between progressors and nonprogressors and the relatively lower levels of

A1C compared with those in such studies (19,20). Considering that insulin use and retinopathy at baseline were indicated as independent predictors of development of microalbuminuria in the present study, progressed state of microangiopathy at baseline may be a more powerful predictor than the glycemic control level during 5-year follow-up.

Diastolic blood pressure level during follow-up was shown to be an independent predictor of development of microalbuminuria by multiple logistic regression analysis. This result was consistent with that of U.K. Prospective Diabetes Study (21). The higher reading of diastolic blood pressure correlates with progressors and the lower with nonprogressors.

In conclusion, we found that increases in urinary IgG, CRL, and Tf had equal predictive value for development of microalbuminuria in normoalbuminuric diabetic patients. Increases in these urinary proteins appear to be more sensitive indicators of renal hemodynamic changes, such as enhanced intraglomerular hydraulic pressure, than urinary excretion of albumin.

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