Urinary α_1-Microglobulin as a Marker of Nephropathy in Type 2 Diabetic Asian Subjects in Singapore

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OBJECTIVE — This study examines urinary α_1-microglobulin as a marker of early nephropathy in type 2 diabetic Chinese, Malays, and Asian Indians in Singapore.

RESEARCH DESIGN AND METHODS — A cross-sectional study was performed on 590 consecutive type 2 diabetic patients (296 males, 294 females) who were on routine follow-up at a primary care clinic. Information was obtained from interviews, case notes, and blood and urine samples. Because the distribution of urinary α_1-microglobulin levels was highly skewed, these levels were log-transformed, and geometric means were calculated. There was correction for variability in urine flow by dividing by urine creatinine levels, given as mg/mmol urine creatinine, and adjustment for confounding variables.

RESULTS — Urinary α_1-microglobulin was higher in men than in women and was directly related to age, but no ethnic differences were apparent. It was directly related to duration of diabetes, with adjusted geometric means of 1.19 and 1.43 mg/mmol urine creatinine for a duration of <10 and ≥ 10 years, respectively ($P = 0.07$). Urinary α_1-microglobulin was highest in patients on insulin, followed by those on oral medication and then those on diet alone (adjusted geometric means: 1.47, 1.36, and 0.86 mg/mmol urine creatinine, respectively; $P = 0.01$). Levels were also higher in patients with poor glucose control, as measured by HbA_{1c}, fasting plasma glucose, and 2-h postprandial plasma glucose ($P < 0.01$ for each). Urinary α_1-microglobulin was directly related to albuminuria, with adjusted geometric means for normoalbuminuria, microalbuminuria, and macroalbuminuria of 1.06, 1.47, and 4.72 mg/mmol urine creatinine, respectively ($P < 0.01$). However, of patients with normoalbuminuria, 33.6% had raised urinary α_1-microglobulin. Likewise, of patients with normal urinary α_1-microglobulin, 27.6% had albuminuria.

CONCLUSIONS — Urinary α_1-microglobulin was related to duration, severity, and control of diabetes. Urinary α_1-microglobulin and albumin were directly related, but in some patients, one was present in the absence of the other. Hence, in addition to albuminuria (which measures glomerular dysfunction), urinary α_1-microglobulin (which measures proximal tubular dysfunction) is useful for the early detection of nephropathy in diabetic subjects.

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α_1-Microglobulin is a glycoprotein with a molecular weight of 26,000–31,000 Da (1), and it exists in blood as a free or unbound form and in complexes with IgA and albumin (2). Because of its low molecular weight, the unbound form is filtered freely through the renal glomerular basement membrane and reabsorbed by the proximal tubular cells (3). Hence, any proximal tubular cell dysfunction results in increased quantities of α_1-microglobulin in the urine.

Because of this property, α_1-microglobulin has been studied as a marker for renal tubular dysfunction in various disorders (4), including heavy metal poisoning (5–7), hypertension (8), multiple myeloma, and monoclonal gammopathy (9). It has also been used to monitor renal function in postoperative patients (10) and in patients on immunosuppressive therapy (11). It has the advantage over β_2-microglobulin, another tubular marker, in that it is stable at a low pH (4).

Relatively few studies have been conducted on urinary α_1-microglobulin in diabetes, and it is not routinely used to investigate and measure renal impairment in diabetic subjects. Urinary α_1-microglobulin has been found to be higher, when compared with normal control subjects, in both type 1 (12) and type 2 diabetic subjects (13) and present even without clinical nephropathy (14,15). In type 2 diabetic subjects, α_1-microglobulin excretion was directly correlated with albuminuria (16) and HbA_{1c} levels (17) and decreased with improved glycemic control (17,18). These studies, however, were conducted on Caucasian populations, and the number of subjects was small (<100).

The risk of developing diabetic nephropathy varies in different populations and it may be genetically determined (19). In the U.K., a higher rate of renal disease in diabetic subjects was found in Asian Indians than in white Caucasians (20). In diabetic subjects, renal failure is the leading cause of death among Chinese in Hong Kong, whereas in the West, the leading cause of death is cardiovascular disease (21).

It is therefore important to study nephropathy in Asian diabetic subjects, who are at high risk of developing it. Singapore, a Southeast Asian country, is composed of Chinese (76.7%), Malays (13.9%), Asian Indians (7.9%), and other ethnic groups (1.5%). In this study, we examined urinary α_1-microglobulin as a

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

marker of nephropathy in type 2 diabetic subjects attending a primary care clinic in Singapore.

RESEARCH DESIGN AND METHODS

Study population

This cross-sectional study was carried out at Toa Payoh Polyclinic, a government primary care clinic. It involved 604 consecutive patients, attending by appointment for routine follow-up management of type 2 diabetes. Individuals were classified as having type 2 diabetes when their case records satisfied all of the following criteria: *1*) either documented diagnosis of diabetes according to World Health Organization criteria (22) or doctor-diagnosed diabetes as shown by referral letters from general practitioners or hospital specialists; *2*) no record of any episode of ketoacidosis, to exclude type 1 diabetes (23), and *3*) first-line treatment of dietary restriction alone or oral hypoglycemic agents, to exclude type 1 diabetes. Patients who were on insulin because of secondary drug failure were included. Patients with renal failure due to causes other than diabetes were not included.

Of the 604 patients recruited, 14 patients with serum creatinine levels above the laboratory cutoff of ≥141 μmol/l were excluded from further analysis. Hence, 590 patients were included in the study. Informed consent was obtained before inclusion in the study, and there were no procedures other than those usually done during these visits.

Demographic and clinical information

Demographic data, method and date of diagnosis of diabetes, presence of hypertension, and current treatment were obtained from interview and case notes. Hypertension was defined as either documented diagnosis in case notes according to World Health Organization criteria (24) or doctor-diagnosed hypertension as evidenced from referral letters by general practitioners or hospital specialists.

Laboratory measurements

Blood and urine (second morning sample) samples were taken. For standardization, all appointments were in the morning. Blood measurements were performed in the clinic laboratory, and urine examinations were performed in our departmental laboratory.

Using the blood samples, plasma glucose measurements (fasting and 2-h postprandial) were performed on the day of attendance using the glucose oxidase method. Within 3 months, after storing at −20°C, HbA_{1c} was measured by high-performance liquid chromatography, and serum creatinine was measured by Jaffe's method (25).

The urine samples were stored at −20°C and were subsequently thawed and centrifuged. Creatinine was measured by Jaffe's method (5), and albumin was measured by enzyme-linked immunosorbent assay using commercially available polyclonal antibodies. α_1-Microglobulin was measured using a commercial test kit (Imzyme α_1-m; Fujirebio, Inc.) based on the latex immunoassay technique. The detection limit for α_1-microglobulin was 0.05 mg/l, with within-run and between-run coefficients of variation of 5 and 10%, respectively. Reproducibility was 95% within batches and 90% between batches. Buffering was not necessary because the substances measured were not pH sensitive.

Analysis

All statistical analyses were performed using the SPSS for Windows statistical package (26).

Because these were spot urine samples, urinary proteins were adjusted for variability in urine flow by dividing by urine creatinine levels and were expressed as mg/mmol urine creatinine. Because the frequency distribution of urinary α_1-microglobulin levels was markedly skewed to the right, they were log-transformed, which produced a near-normal distribution, for parametric analysis. Hence, geometric means were presented rather than arithmetic means.

For α_1-microglobulin, the normal cutoff of 1.70 mg/mmol urine creatinine (15 mg/g urine creatinine) was used (4). Albuminuria was grouped into three subgroups: normoalbuminuria (<2 mg/mmol urine creatinine), microalbuminuria (2–20 mg/mmol urine creatinine), and macroalbuminuria (>20 mg/mmol urine creatinine) (27).

Means were compared using Student's *t* tests and ANOVA, and adjusted means were obtained using ANCOVA. Bonferroni's test was used for post hoc comparison of differences in means. Multiple linear regression was used to obtain standardized β values to determine the predictors of α_1-microglobulin excretion.

Table 1—*Profile of the 590 patients*

Variable	% (*n*)
Age-group	
<50 years	14.7 (87)
50–69 years	65.6 (387)
≥70 years	19.7 (116)
Sex	
M	50.2 (296)
F	49.8 (294)
Ethnic group	
Chinese	80.7 (476)
Malay	6.9 (41)
Indian	11.4 (67)
Others	1.0 (6)
With hypertension	44.4 (262)
Duration of diabetes	
<10 years	65.9 (389)
≥10 years	31.4 (201)
Treatment of diabetes	
Diet only	11.4 (67)
Oral medications	84.9 (501)
Oral and insulin	1.2 (7)
Insulin only	2.5 (15)
HbA_{1c} (%)*	
<7	41.1 (239)
7 to <9	31.6 (184)
≥9	27.3 (159)
Fasting plasma glucose (mmol/l)*	
<6.7	17.4 (102)
6.7 to <7.8	21.9 (128)
≥7.8	60.7 (355)
2-h postprandial glucose (mmol/l)*	
<10.0	47.3 (202)
10 to <11.1	11.7 (50)
≥11.1	41.0 (175)
Albuminuria (mg/mmol urine creatinine)	
Normo- (<2)	63.9 (375)
Micro- (2–20)	30.8 (181)
Macro- (>20)	5.3 (31)

*Information not available for all subjects.

RESULTS

Descriptive profile

There were similar numbers of men (n = 296) and women (n = 294). The mean age was 60.5 years (SD 10.3, median 61.0), with a range of 28–87 years. Median duration of diabetes was 6 years (SD 6.1, mean 7.5, range <1–33) (Table 1).

Table 2—*Urinary α_1-microglobulin by age, sex, ethnic group, and hypertension status*

Variable	*n*	Geometric mean of α_1-microglobulin (mg/mmol urine creatinine)	*P**
Age-group			
<50 years	87	0.85	
50–69 years	387	1.30	<0.01†
≥70 years	116	1.58	
Sex			
M	296	1.41	0.03
F	294	1.14	
Ethnic group			
Chinese	476	1.31	
Malay	41	1.06	0.50
Indian	67	1.18	
Others	6	0.83	
Hypertension			
No	328	1.20	0.19
Yes	262	1.36	

*Two-sided by Student's *t* test or ANOVA; †statistically significant between <50 years and 50–69 years ($P < 0.01$) and between <50 years and ≥70 years ($P < 0.01$) by Bonferroni test.

Urinary α_1-microglobulin in the subgroups

α_1-Microglobulin excretion increased with age, with geometric means of 0.85, 1.30, and 1.58 mg/mmol urine creatinine in age-groups <50, 50–69, and ≥70 years, respectively. It was higher in men (1.41 mg/mmol urine creatinine) than in women (1.14 mg/mmol urine creatinine). There were no differences in α_1-microglobulin by ethnic group. It was higher in hypertensive subjects than in nonhypertensive subjects, although the difference was not statistically significant (Table 2).

Urinary α_1-microglobulin and progression of diabetes

Patients with longer duration of diabetes had higher urinary α_1-microglobulin levels. Although subjects with diabetes for ≥10 years were older (median age 63 years) than subjects with diabetes <10 years (median age 60 years), the difference remained significant after controlling for age together with other variables, with adjusted means of 1.43 mg/mmol urine creatinine (95% CI 1.22–1.67) for duration ≥10 years and 1.19 mg/mmol urine creatinine (1.06–1.33) for duration <10 years (Table 3).

With regard to mode of treatment, adjusted mean α_1-microglobulin levels were highest in patients on insulin (1.47 mg/mmol urine creatinine, 95% CI 0.90–2.30) than in those on oral medication (1.36 mg/mmol urine creatinine, 1.20–1.46) and lowest in those on dietary control alone (0.86 mg/mmol urine creatinine, 0.66–1.08).

Urinary α_1-microglobulin and control of diabetes

Urinary α_1-microglobulin levels were significantly higher in patients with poorer diabetic control, as measured by HbA_{1c}, fasting plasma glucose, and 2-h postprandial glucose (Table 3).

Among these indicators of diabetic control, HbA_{1c} was the best predictor of urinary α_1-microglobulin, after adjustment for age, sex, ethnic group, and hypertension status, using multiple linear regression. The regression coefficients were 0.25 for HbA_{1c}, −0.02 for fasting plasma glucose, and 0.11 for 2-h postprandial glucose.

Urinary α_1-microglobulin and albuminuria

Mean α_1-microglobulin levels significantly increased with severity of albuminuria. Adjusted geometric means for normoalbuminuria, microalbuminuria, and macroalbuminuria were 1.08, 1.43, and 4.43 mg/mmol urine creatinine, respectively (Table 4).

Furthermore, with increasing levels of albuminuria, the proportions of subjects with α_1-microglobulin ≥1.70 mg/mmol urine creatinine also increased. Of patients with normoalbuminuria, 33.6% had abnormally raised urinary levels of α_1-microglobulin, whereas with microalbuminuria, it was 53.6%, and with macroalbuminuria, 64.5%.

However, among patients with albuminuria, 44.8% had urinary α_1-microglobulin levels within normal limits. Furthermore, of the 344 patients with normal α_1-microglobulin, 84 (24.4%) had microalbuminuria and 11 (3.2%) had macroalbuminuria. Hence, in patients with normal α_1-microglobulin, 27.6% had albuminuria.

There was no difference between patients with albuminuria and normal α_1-microglobulin and patients with raised α_1-microglobulin and normoalbuminuria with regard to age, ethnic group, hypertension status, duration of diabetes, type of treatment received, and diabetic control. A higher proportion of men than women (60.3 vs. 43.2%) had raised α_1-microglobulin and normoalbuminuria.

CONCLUSIONS — This is the first study on urinary α_1-microglobulin in type 2 diabetic subjects in an Asian population.

Urinary α_1-microglobulin was directly related to duration of diabetes and progression of diabetes (as indicated by type of treatment). Urinary α_1-microglobulin was also directly related to poorer control of diabetes, as measured by HbA_{1c}, fasting plasma glucose, and 2-h postprandial glucose, which is similar to findings in Caucasian populations (17,18). A study in Japan found that the development or progression of early diabetic nephropathy was inhibited by good glucose control (28). Furthermore, of these indicators of diabetic control, HbA_{1c} was the strongest predictor of urinary α_1-microglobulin, which is consistent with the concept that tubular nephropathy is the result of long-standing hyperglycemia rather than transient hyperglycemia, because HbA_{1c} measures glycemic control in the last 3 months, whereas fasting and 2-h postprandial glucose measure glycemic control at one point in time. These findings in Singapore indicate that urinary α_1-microglobulin is a good marker of the degree of renal impairment in diabetic subjects.

Although albumin excretion in the

Table 3—*Urinary α_1-microglobulin by duration, treatment, and control of diabetes*

Variable	*n*	Geometric mean of α_1-microglobulin (mg/mmol urine creatinine) Unadjusted	*P*	Adjusted* (95% CI)	*P*
Duration					
<10 years	389	1.17	0.02	1.19 (1.06–1.33)	0.07
≥10 years	201	1.47		1.43 (1.22–1.67)	
Treatment					
Diet only	67	0.84†		0.86† (0.66–1.08)	
Oral medications only	501	1.32†	0.01	1.36† (1.20–1.46)	0.01
Insulin with and without oral medications	22	1.60		1.47 (0.90–2.30)	
HbA_{1c} (%)					
<7	239	1.04†		1.05† (0.83–1.09)	
7 to <9	184	1.33†	<0.01	1.33† (1.13–1.55)	<0.01
≥9	159	1.71†		1.90† (1.59–2.25)	
Fasting plasma glucose (mmol/l)					
<6.7	102	1.10†		0.87† (0.70–1.08)	
6.7 to <7.8	128	1.16	<0.01	1.14 (1.06–1.38)	<0.01
≥7.8	355	1.43†		1.46† (1.30–1.64)	
2-h postprandial glucose (mmol/l)					
<10.0	202	1.04†		1.01† (0.87–1.18)	
10.0 to <11.1	50	1.46	<0.01	1.41 (1.03–1.92)	<0.01
≥11.1	175	1.59†		1.63† (1.40–1.96)	

*Adjusted for age, sex, ethnic group, and hypertension status by ANCOVA; †statistically significant between these subgroups by the Bonferroni's test.

urine mainly results from glomerular dysfunction, its presence is also contributed by its defective re-absorption by the proximal tubular cells. The presence of α_1-microglobulin in urine, however, is indicative solely of reduced re-absorption capacity of the proximal tubule because it is filtered freely through the glomeruli. Hence, α_1-microglobulin is a better marker of proximal tubular dysfunction.

A direct relation between urinary albumin and α_1-microglobulin was found in our study in Asian subjects, as in Caucasian subjects (16). This finding indicates that, in diabetic nephropathy, both the glomeruli and proximal tubules are involved. However, in our study, the relation of urinary albumin and α_1-microglobulin was not absolute. In particular, one-third of patients with normoalbuminuria had raised α_1-microglobulin, and nearly one-third of patients with normal α_1-microglobulin had albuminuria. Similarly, it has been reported that in cadmium-induced nephropathy, proximal tubular dysfunction indicators including α_1-microglobulin can be increased more than glomerular dysfunction indicators such as albumin and transferrin (29).

This finding in Singapore indicates that in diabetic nephropathy, glomerular dysfunction (with albuminuria) occurs first in some patients and proximal tubular dysfunction (with raised urine α_1-microglobulin) occurs first in other patients. This result could be clarified by a longitudinal study. However, it seems that for diabetic subjects, both urinary albumin and α_1-microglobulin should be measured to identify early renal impairment, because there was no difference between these two groups of patients.

We used one urine sample (second morning sample) for estimating urinary α_1-microglobulin and corrected for urine flow by calculating protein-to-creatinine ratios. This method of urine collection was more convenient for the patients and increased their cooperation. Non-timed spot urine samples were advocated for use in place of the traditional 24-h collection, because there was excellent correlation between the protein content of a 24-h urine sample and the protein-to-creatinine ratio in a single urine sample (30,31). Other authors also concluded that second morning urine samples and the determination of excretion rates are adequate to overcome the problems of reference limits in urine protein determination (32).

In conclusion, we found that urinary α_1-microglobulin was related to the duration, severity, and control of diabetes in this Asian population, indicating that it is a good marker of the severity of renal impairment in type 2 diabetic subjects. Also, although urinary α_1-microglobulin and albumin are related, in early nephropathy, one may be present in the absence of the other. Hence, in addition to urinary albumin (which mainly measures glomerular dysfunction), urinary α_1-microglobulin (which measures proximal

Table 4—*Urinary α_1-microglobulin by degree of albuminuria*

	n	% (*n*) with α_1-microglobulin ≥1.70 mg/mmol urine creatinine	Geometric mean of α_1-microglobulin (mg/mmol urine creatinine) Unadjusted†	Adjusted*† (95% CI)
Normoalbuminuria (<2 mg/mmol urine creatinine)	375	33.6 (126)	1.05	1.08 (0.97–1.21)
Microalbuminuria (2–20 mg/mmol urine creatinine)	181	53.6 (97)	1.51	1.43 (1.23–1.67)
Macroalbuminuria (>20 mg/mmol urine creatinine)	31	64.5 (20)	4.61	4.43 (3.05–6.42)

*Adjusted for age, sex, ethnic group, hypertension status, and HbA_{1c} by ANCOVA; †$P < 0.01$ by trend χ^2 test and $P < 0.01$ between each subgroup pair by the Bonferroni's test.

tubular dysfunction) is useful for the early detection and monitoring of renal disease in diabetic subjects.

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