

Serum Uric Acid as a Predictor for Development of Diabetic Nephropathy in Type 1 Diabetes

An Inception Cohort Study

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OBJECTIVE—Experimental and clinical studies have suggested that uric acid may contribute to the development of hypertension and kidney disease. Whether uric acid has a causal role in the development of diabetic nephropathy is not known. The objective of the present study is to evaluate uric acid as a predictor of persistent micro- and macroalbuminuria.

RESEARCH DESIGN AND METHODS—This prospective observational follow-up study consisted of an inception cohort of 277 patients followed from onset of type 1 diabetes. Of these, 270 patients had blood samples taken at baseline. In seven cases, uric acid could not be determined; therefore, 263 patients (156 men) were available for analysis. Uric acid was measured 3 years after onset of diabetes and before any patient developed microalbuminuria.

RESULTS—During a median follow-up of 18.1 years (range 1.0–21.8), 23 of 263 patients developed persistent macroalbuminuria (urinary albumin excretion rate >300 mg/24 h in at least two of three consecutive samples). In patients with uric acid levels in the highest quartile (>249 $\mu\text{mol/l}$), the cumulative incidence of persistent macroalbuminuria was 22.3% (95% CI 10.3–34.3) compared with 9.5% (3.8–15.2) in patients with uric acid in the three lower quartiles (log-rank test, $P = 0.006$). In a Cox proportional hazards model with sex and age as fixed covariates, uric acid was associated with subsequent development of persistent macroalbuminuria (hazard ratio 2.37 [95% CI 1.04–5.37] per 100 $\mu\text{mol/l}$ increase in uric acid level; $P = 0.04$). Adjustment for confounders did not change the estimate significantly.

CONCLUSIONS—Uric acid level soon after onset of type 1 diabetes is independently associated with risk for later development of diabetic nephropathy. *Diabetes* 58:1668–1671, 2009

Diabetes is the leading cause of end-stage renal disease (ESRD) in the Western world, and the number of patients diagnosed each year with ESRD due to diabetes is rising (1). The complex pathogenesis for the development of diabetic nephropathy is not fully understood (2). One factor that has been associated with cardiovascular and renal disease is serum uric acid. Recently, experimental and clinical studies have suggested that uric acid may contribute to the development of hypertension, the metabolic syndrome, and kidney disease (3). The role of uric acid in the development of diabetic nephropathy is not understood. The objective of the present study is therefore to evaluate uric acid as a predictor of persistent micro- and macroalbuminuria in an inception cohort of type 1 diabetic patients followed from onset of diabetes.

RESEARCH DESIGN AND METHODS

All newly diagnosed type 1 diabetic patients, consecutively admitted to the Steno Diabetes Center between 1 September 1979 and 31 August 1984, were included in an inception cohort ($n = 277$), described previously in detail (4). Diagnosis in all patients included measurement of fasting C-peptide. A total of 270 patients had blood samples taken at baseline. In seven cases, uric acid was not determined; therefore, 263 patients (156 men) were available for analysis. Uric acid was measured 3 years after onset of diabetes and before any patient developed microalbuminuria.

Patients were treated according to set principles and guidelines as described previously (4,5). No specific intervention was carried out. A1C was measured from venous blood samples, with a normal range of 4.1–6.4% (4). Each patient had a 24-h urinary albumin excretion rate (UAER) measured at least once per year. UAER was quantitated by using automated immunotopical nephelometric analysis until 1984 (6) and by using enzyme immunoassay from 1984 to 1997 (7) (sensitivity 1.1 mg/l; coefficient of variation [CV] 8%). From 1997, the DAKO Turbidimetric method was used to measure UAER with a CV of 5%. A very close correlation between radial immunodiffusion and enzyme immunoassay ($r = 0.99$) as well as between enzyme immunoassay and the turbidimetric method ($r = 0.99$) was documented, and absence of any systematic error between methods was verified by Bland-Altman plots before any change in methods was imposed. Persistent microalbuminuria and macroalbuminuria were defined as UAER of 30–300 mg/24 h and >300 mg/24 h, respectively, in at least two of three consecutive samples, with $\geq 30\%$ increase in UAER above the baseline level (4).

Uric acid was measured from samples that had been stored in freezers at -20°C by colorimetric slide test (Vitros 5.1 FS; Ortho Clinical Diagnostics), with a CV of 1.3 and 1.5%, respectively, in samples from the lowest and highest quartiles of the uric acid levels. Measurements were from samples taken 3 years after onset of diabetes in all subjects, i.e., after initial glycemic stabilization, and prior to development of persistent microalbuminuria. At the time of sampling, only two patients received antihypertensive treatment (both treated with diuretics; one additionally treated with an ACE inhibitor). The normal range of uric acid was 200–450 $\mu\text{mol/l}$ in men and 150–350 $\mu\text{mol/l}$ in women.

Arterial blood pressure was measured at least once per year with a standard mercury sphygmomanometer and was performed with the patient in a seated position after ~ 10 min rest. Smoking history was determined via

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TABLE 1

Characteristics of 263 type 1 diabetic patients followed from onset of diabetes divided into patients with persistent normoalbuminuria, patients who later progressed to microalbuminuria, and patients who later progressed to microalbuminuria and further to macroalbuminuria

	Persistent normoalbuminuria	Microalbuminuria	Macroalbuminuria	<i>P</i>
<i>n</i>	191	49	23	
Male sex (<i>n</i> %)	107/56	33/67	16/70	0.21
Height (cm)	172 ± 13	168 ± 16	171 ± 11	0.10
Weight (kg)	60.6 ± 13.3	58.5 ± 17.0	63.1 ± 13.4	0.41
Age (years)	28 ± 13	29 ± 18	28 ± 13	0.88
Systolic blood pressure (mmHg)	122 ± 16	129 ± 20	128 ± 15	0.009
Diastolic blood pressure (mmHg)	76 ± 10	80 ± 13	81 ± 10	0.016
UAER (mg/24 h)	8 (5–13)	11 (7–17)	11 (8–16)	0.004
A1C (%)	9.7 ± 2.2	10.2 ± 1.8	10.1 ± 2.0	0.36
Serum cholesterol (mmol/l)	5.4 ± 1.5	5.5 ± 1.4	5.9 ± 1.5	0.359
Serum creatinine (μmol/l)	80 ± 15	77 ± 16	79 ± 13	0.443
Serum uric acid (μmol/l)	209.4 ± 57.8	210.7 ± 55.9	239.1 ± 61.0	0.063

Data are mean ± SD or median (interquartile range) unless otherwise indicated. Data are from 3 years after onset of diabetes. *P* values are overall comparison between the three groups.

questionnaire, and patients were classified as smokers if they were smoking more than one cigarette per day. Retinopathy was graded as absent, nonproliferative, or proliferative (5). All patients provided informed consent for the participation in the study.

Statistical analysis. Variables with skewed distribution are median (interquartile range); all other values are given as means ± SD. For non-normally distributed variables, comparisons between groups were performed using the Mann-Whitney *U* test, whereas one-way ANOVA or unpaired Student's *t* tests were used for normally distributed variables. A χ^2 test was used for comparison between groups of noncontinuous variables. To evaluate uric acid as a causal determinant of development of persistent micro- or macroalbuminuria in an explanatory model, a Cox proportional hazards regression model was used, including baseline levels of variables that either previously had been shown to be associated with the level of uric acid or were correlated with uric acid in the present study, correcting for different lengths of follow-up. Uric acid was entered in the model as a continuous variable. Because both sex and age can affect the outcome (development of nephropathy) and the independent variable (uric acid), sex and age were entered as fixed variables in the models. These models allow for adjustment for sex differences, which is practical because the different reference intervals between sexes can be disregarded when only evaluating risk without firm cutoff values.

The cumulative incidence of persistent micro- and macroalbuminuria was calculated based on the entire follow-up period with a life-table method. Groups were compared using the log-rank test. Statistical significance was assumed for *P* < 0.05. All statistical calculations were performed with SPSS for Windows, version 15.0 (SPSS, Chicago, IL).

RESULTS

The 263 patients were followed for a median of 18.1 years (interquartile range 1.0–21.8). Of these, 72 patients progressed to persistent microalbuminuria and 23 progressed further to persistent macroalbuminuria. This resulted in a cumulative incidence of persistent microalbuminuria of 32.2% (95% CI 25.7–38.7) and a cumulative incidence of macroalbuminuria of 12.6% (7.3–17.9). Clinical characteristics of the diabetic patients at baseline, defined as 3 years after onset of diabetes, are summarized in Table 1. No patients had diabetic retinopathy at baseline.

All patients had serum uric acid values within the reference interval. However, within the normal range, a significant difference in the means ± SD level of uric acid 3 years after onset of diabetes and before any patient developed micro- or macroalbuminuria was found when comparing the three groups. In a one-way ANOVA test comparing the mean levels of uric acid in the three groups, there was a trend toward an overall difference among groups; *P* = 0.063 (Table 1). Looking at the differences

between each group separately, the mean level of serum uric acid was significantly higher in patients who eventually progressed to persistent macroalbuminuria (239.1 ± 61 μmol/l) versus patients remaining normoalbuminuric (209.4 ± 57.8 μmol/l) or later progressing to microalbuminuria only (210.7 ± 55.9 μmol/l); *P* < 0.05 for all comparisons. Importantly, no differences in serum creatinine were apparent among groups (Table 1).

Development of microalbuminuria. When comparing patients progressing to microalbuminuria as a combined group irrespective of later or no progression to macroalbuminuria versus patients remaining normoalbuminuric, no difference in the mean ± SD level of uric acid 3 years after onset of diabetes was found (219.8 ± 58.7 μmol/l in patients later progressing to persistent micro- or macroalbuminuria vs. 209.4 ± 56.8 μmol/l in patients with persistent normoalbuminuria; *P* = 0.194).

In a Cox proportional hazards model with sex and age as fixed covariates, uric acid was not independently associated with subsequent development of persistent microalbuminuria (hazard ratio [HR] 1.05 [95% CI 0.66–1.69] per 100 μmol/l increase in uric acid level; *P* = 0.83). Adjustments for baseline level of BMI, glycemic control, UAER, serum creatinine, serum cholesterol, and mean arterial blood pressure did not change the estimate significantly.

Development of macroalbuminuria. In a Cox proportional hazards model with sex and age as fixed covariates, uric acid was independently associated with subsequent development of persistent macroalbuminuria (HR 2.37 [95% CI 1.04–5.37] per 100 μmol/l increase in uric acid level; *P* = 0.04). Adjustment for baseline level of BMI, glycemic control, UAER, serum creatinine, serum cholesterol, and mean arterial blood pressure did not change the estimate significantly (adjusted HR 2.93 [1.25–6.86] per 100 μmol/l increase in uric acid level; *P* = 0.013). In patients with uric acid levels in the highest quartile (>249 μmol/l but within the normal range), the cumulative incidence of persistent macroalbuminuria was 22.3% (10.3–34.3) compared with 9.5% (3.8–15.2) in patients with uric acid in the three lower quartiles (crude log-rank test, *P* = 0.006; after Bonferroni correction, *P* = 0.012) (Fig. 1).

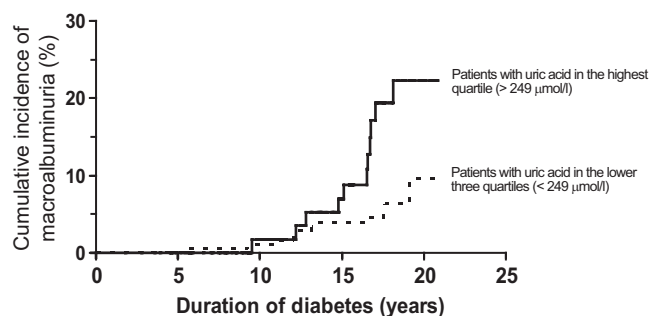


FIG. 1. Cumulative incidence of persistent macroalbuminuria in 263 type 1 diabetic patients with onset of diabetes from 1979 to 1984. Divided by quartiles of uric acid 3 years after onset of type 1 diabetes, comparing patients with uric acid in the highest quartile ($>249 \mu\text{mol/l}$) with patients with uric acid in the three lower quartiles ($<249 \mu\text{mol/l}$); $P = 0.006$.

DISCUSSION

In the present prospective observational study of an inception cohort followed from onset of type 1 diabetes and for a median of 18 years, uric acid was not a predictor of persistent microalbuminuria. In contrast, we demonstrate that the level of uric acid early in the course of type 1 diabetes is significantly and independently associated with later development of persistent macroalbuminuria. A significantly higher proportion of patients developing overt nephropathy among patients with serum uric acid in the highest quartile at baseline was found. These results support the idea that uric acid may be involved in the pathogenesis of microvascular complications in diabetes.

Hyperuricemia may be a marker of or by itself be responsible for microvascular disease through stimulation of the renin angiotensin system and inhibition of endothelial nitric oxide (8). Animal models of induced hyperuricemia have demonstrated an association with renal disease (9–11). In humans, hyperuricemia has been associated with hypertension and, recently, with initiation and progression of nondiabetic renal disease (3,8,12). In those with diabetes, Rosolowsky et al. (13) have reported from a cross-sectional study that serum uric acid in the high-normal range was associated with impaired renal function in patients with type 1 diabetes and normo- or microalbuminuria.

Thus far, no studies have evaluated the impact of uric acid on the development of diabetic kidney disease. In our present study of 263 type 1 diabetic patients followed from onset of diabetes, we were able to demonstrate that the level of uric acid early in the course of type 1 diabetes is significantly associated with later development of diabetic kidney disease, but we could not establish an association with persistent microalbuminuria. However, patients progressing to microalbuminuria may be a more heterogeneous group than previously assumed, which could explain why the level of uric acid was not elevated in the microalbuminuric patients as such. Our data suggest that uric acid may only be elevated in the progressors. Our findings in the present study emphasize the importance of the use of a solid and robust end point when evaluating risk markers for disease.

The patients in our inception cohort are unselected, all type 1 diabetic patients irrespective of age at diagnosis were included, and our population has a higher mean age than other studies of type 1 diabetic patients. Consequently, our results cannot be directly generalized to patients with type 1 diabetes, and earlier onset of disease

but must be validated in such populations. As only two patients received antihypertensive treatment at the time of sampling for measurement of uric acid, the level of uric acid is unlikely to be confounded by use of diuretics in our population. One limitation in the present study is that clinical blood pressure measurements were used. Clearly, a more precise method, such as ambulatory blood pressure measurements over 24 h (14), reduces variability in measurement and makes estimates more precise. Genetic factors influencing the level of uric acid (15) or other unknown factors not measured in our study may have an impact on the relationship between uric acid and the development of diabetic nephropathy. The possibility of sublimation of the frozen and stored samples cannot be ruled out. However, if sublimation occurred, this would affect all samples, and patients with high values would still have high values within the same population, although at a lower level.

Diabetic kidney disease is strongly associated with cardiovascular mortality (16) and may reflect a more generalized vascular process (17). Elevated uric acid not only has been demonstrated to be associated with kidney disease but also has been linked to endothelial dysfunction, development of hypertension, and cardiovascular disease irrespective of renal involvement (3). Elevated uric acid may be a candidate for a common link between micro- and macrovascular disease in diabetic patients.

In conclusion, we found levels of circulating uric acid in the higher end of the normal range to be an independent predictor for development of overt diabetic nephropathy, thus supporting the concept that uric acid may be involved in the pathogenesis of diabetic microvascular complications. Consequently, our study suggests that a long-term treatment trial with allopurinol is warranted.

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R.J.J. has patent applications with the University of Washington and the University of Florida related to lowering uric acid as a means to reduce blood pressure and the metabolic syndrome and to slow diabetic kidney disease. He did not have any role in the analysis of the study. No other potential conflicts of interest relevant to this article were reported.

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