Serum uric acid as a predictor for development of diabetic nephropathy in type 1 diabetes – an inception cohort study

Short running title: Uric acid and development of diabetic nephropathy

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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.

Research Design and Methods: Prospective observational follow-up study 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 three years after onset of diabetes before any patient developed microalbuminuria.

Results: During a median follow-up of 18.1 years (range 1.0-21.8 years), 23 of 263 patients developed persistent macroalbuminuria (urinary albumin excretion rate >300 mg/24h in at least two out of three consecutive samples). In patients with uric acid levels in the highest quartile (above 249 µmol/l), the cumulative incidence of persistent macroalbuminuria was 22.3 % (95% CI: 10.3-34.3) as compared with 9.5 % (95% CI: 3.8-15.2) in patients with uric acid in the three lower quartiles (log rank test, p=0.006). In a Cox proportional hazard 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 µmol/l increase in uric acid level, p=0.04). Adjustment for confounders did not change the estimate significantly.

Conclusions: Uric acid level early after onset of type 1 diabetes is independently associated with the risk for later development of diabetic nephropathy.
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 clarified (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 clarified. The aim 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 September 1st 1979 and August 31st 1984, were included in an inception cohort (n=277), previously described in detail (4). Diagnosis in all patients included measurement of fasting C-peptide. Of these, 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 three years after onset of diabetes before any patient developed microalbuminuria.

Patients were treated according to set principles and guidelines as previously described (4;5). No specific intervention was carried out. HemoglobinA\textsubscript{1c} was measured from venous blood samples, with a normal range of 4.1-6.4% (4). Each patient had their 24 hour urinary albumin excretion rate (UAER) measured at least once a year. UAER was quantitated using automated immunotopical nephelometric analysis until 1984 (6), from 1984 to 1997 by enzyme immunoassay (7) (sensitivity: 1.1 mg/l, coefficient of variation: 8 %). From 1997, DAKO Turbidimetric method was used to measure UAER with a coefficient of variation of 5 %. A very close correlation between radial immunodiffusion and enzyme immunoassay (r=0.99), and between enzyme immunoassay and the turbidimetric method (r=0.99) was documented, and absence of any systematically error between methods was verified by Bland-Altman plots, before any change in methods was imposed. Persistent microalbuminuria and macroalbuminuria were defined as UAER between 30 and 300 mg/24h and >300 mg/24h in at least two out of three consecutive samples, respectively, with at least 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 coefficient of variation of 1.3 % and 1.5 % in samples from the lowest and the highest quartiles of the uric acid levels, respectively. Measurements were from samples taken three years after onset of diabetes in all subjects, i.e. after initial glycemic stabilization and prior to development of persistent microalbuminuria. At time of sampling, only two patients received antihypertensive treatment (both treated with diuretics, one of them additionally...
with an ACE inhibitor). Normal range of uric acid was 200-450 µmol/l in men and 150-350 µmol/l in women.

Arterial blood pressure was measured at least once a year with a standard mercury sphygmomanometer, and was performed with the patient in the sitting position after approximately 10 minutes rest.

Smoking history was elicited via questionnaire, and patients were classified as smokers if they were smoking more than 1 cigarette per day. Retinopathy was graded as absent, non proliferative or proliferative (5). All patients gave informed consent for the participation in the study.

**Statistical analysis:** Median with interquartile ranges (IQR) is used for variables with skewed distribution, 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 $\chi^2$ test was used for comparison between groups of non continuous 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. As both gender and age can affect both the outcome (development of nephropathy) and the independent variable (uric acid), gender and age were entered as fixed variables in the models. These models allow for adjustment for gender differences, which is practical as the different reference intervals between genders can be disregarded when only evaluating risk without firm cut-off 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).

**RESULTS**

The 263 patients were followed for a median (range) of 18.1 (1.0-21.8) years. Seventy two 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 % (95 % CI: 7.3-17.9). Clinical characteristics of the diabetic patients at baseline, defined as three 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 mean (SD) level of uric acid three 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 towards an overall difference between groups, $p=0.063$ (see Table 1). Looking at the differences between each groups separately, the mean level of serum uric
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Acid was significantly higher in patients who eventually progressed to persistent macroalbuminuria (239.1 [61] \(\mu\)mol/l) vs. patients remaining normoalbuminuric (209.4 [57.8] \(\mu\)mol/l) or who later progressed to microalbuminuria only (210.7 [55.9] \(\mu\)mol/l), p<0.05 for both comparisons. Importantly, no difference in serum creatinine was apparent between groups (see Table 1).

**Development of microalbuminuria:** When comparing patients progressing to microalbuminuria as a combined group irrespective of later progression to macroalbuminuria or not vs. patients remaining normoalbuminuric, no difference in the mean (SD) level of uric acid three years after onset of diabetes was found, 219.8 (58.7) \(\mu\)mol/l in patients later progressing to persistent micro- or macroalbuminuria vs. 209.4 (56.8) \(\mu\)mol/l in patients with persistent normoalbuminuria, p=0.194.

In a Cox proportional hazard model with sex and age as fixed covariates, uric acid was not independently associated with subsequent development of persistent microalbuminuria (hazard ratio 1.05 [95% CI: 0.66-1.69] per 100 \(\mu\)mol/l increase in uric acid level, p=0.83). Adjustments for level of 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 hazard model with sex and age as fixed covariates, uric acid was independently associated with subsequent development of persistent macroalbuminuria (hazard ratio 2.37 [95% CI: 1.04-5.37] per 100 \(\mu\)mol/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 hazard ratio 2.93 [95% CI: 1.25-6.86] per 100 \(\mu\)mol/l increase in uric acid level, p=0.013). In patients with uric acid levels in the highest quartile (above 249 \(\mu\)mol/l, but within the normal range), the cumulative incidence of persistent macroalbuminuria was 22.3 % (95% CI: 10.3-34.3) as compared with 9.5 % (95% CI: 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), Figure 1.

**CONCLUSIONS**

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 (RAS) 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
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recently with initiation and progression of non-diabetic renal disease (3;8;12). In diabetes, Rosolowsky et al 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 (13).

So 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 diabetic kidney disease, but we could not establish an association with persistent microalbuminuria. However, patients progressing to microalbuminuria may be a more heterogenous group than previously assumed, which could explain why the level 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 endpoint when evaluating risk markers for disease.

The patients in our inception cohort are unselected, as 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 can not 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 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 hours (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 can not be ruled out. However, if sublimation occurred, this would affect all samples, and patients with high values would still have high values with in the same population although at a lower level.

Diabetic kidney disease is strongly associated with cardiovascular mortality (16), may reflect a more generalized vascular process (17). Elevated uric acid has not only been demonstrated to be associated with kidney disease, but also 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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Conflict of interest statements for authors: Dr Johnson does have some patent applications with the University of Washington and the University of Florida related to lowering uric acid as a means to reduce blood pressure, metabolic syndrome and slow diabetic kidney disease. He did not have any role in the analysis of the study. None of the other authors have any conflicts of interest to declare. The corresponding author - Peter Hovind - has full access to all the data in the study and has final responsibility for the decision to submit for publication.

Ethical approval: The local ethical committee, Copenhagen, Denmark, approved the experimental design, and all patients gave written informed consent.

Figure Legend

Figure 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 three years after onset of type 1 diabetes, comparing patients with uric acid in the highest quartile (>249 µmol/l) with patients with uric acid in the three lower quartiles (>249 µmol/l), p=0.006.
REFERENCES


Table 1. Characteristics of 263 type 1 diabetic patients followed from onset of diabetes – divided into persistent normoalbuminuric, patients who later progressed into microalbuminuria, and patients who later progressed to microalbuminuria and further to macroalbuminuria (progressors)

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Persistent normoalbuminuric</th>
<th>Group 2 Microalbuminuria (no progression)</th>
<th>Group 3 Macroalbuminuria (progressors)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (%) male</td>
<td>107 (56)</td>
<td>33 (67)</td>
<td>16 (70)</td>
<td>0.21</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172 ± 13</td>
<td>168 ± 16</td>
<td>171 ± 11</td>
<td>0.10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.6 ± 13.3</td>
<td>58.5 ± 17.0</td>
<td>63.1 ± 13.4</td>
<td>0.41</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>28 ± 13</td>
<td>29 ± 18</td>
<td>28 ± 13</td>
<td>0.88</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>122 ± 16</td>
<td>129 ± 20</td>
<td>128 ± 15</td>
<td>0.009</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>76 ± 10</td>
<td>80 ± 13</td>
<td>81 ± 10</td>
<td>0.016</td>
</tr>
<tr>
<td>Urinary albumin excretion rate (mg/24 hours)*</td>
<td>8 (5-13)</td>
<td>11 (7-17)</td>
<td>11 (8-16)</td>
<td>0.004</td>
</tr>
<tr>
<td>Glycosylated haemoglobin A1c (%)</td>
<td>9.7 ± 2.2</td>
<td>10.2 ± 1.8</td>
<td>10.1 ± 2.0</td>
<td>0.36</td>
</tr>
<tr>
<td>Serum Cholesterol (mM)</td>
<td>5.4 ± 1.5</td>
<td>5.5 ± 1.4</td>
<td>5.9 ± 1.5</td>
<td>0.359</td>
</tr>
<tr>
<td>Serum Creatinine (µM)</td>
<td>80 ± 15</td>
<td>77 ± 16</td>
<td>79 ± 13</td>
<td>0.443</td>
</tr>
<tr>
<td>Serum Uric acid (µmol/l)</td>
<td>209.4 ± 57.8</td>
<td>210.7 ± 55.9</td>
<td>239.1 ± 61.0</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Data are mean ± SD unless otherwise stated, *) Median (interquartile range).
Data are from three years after onset of diabetes. P-values are overall comparison between the three groups.
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Patients with uric acid in the highest quartile (> 249 µmol/l)

Patients with uric acid in the lower three quartiles (< 249 µmol/l)