Correlation of uric acid and urinary albumin excretion rate in patients with type 2 diabetes mellitus in Taiwan

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Background. Uric acid is detrimental to the kidneys in animal models. However, its role in human diabetic nephropathy has not been extensively studied. This study evaluated the association between serum uric acid and urinary albumin-to-creatinine ratio (ACR) among patients with type 2 diabetes mellitus in Taiwan.

Methods. A total of 343 patients (144 men and 199 women), aged 62.8 ± 10.8 years and not using uric acid–lowering agents, diuretics, or alcohol, were recruited. Serum uric acid and urinary ACR were determined. Normoalbuminuria, microalbuminuria, and macroalbuminuria were defined as ACR <30.0, 30.0 to 299.9, and ≥300.0 mg/gCr, respectively.

Results. The respective uric acid levels for normoalbuminuria (N = 166), microalbuminuria (N = 130), and macroalbuminuria (N = 47) were 5.2 ± 1.6 mg/dL, 5.6 ± 1.9 mg/dL, and 6.7 ± 2.1 mg/dL (P < 0.001). The mean ± SD (minimum-maximum) values of uric acid for the first to the fourth quartile were 3.4 ± 0.6 (1.7–4.2), 4.9 ± 0.4 (4.3–5.4), 6.0 ± 0.3 (5.5–6.5), and 8.1 ± 1.2 (6.6–12.2), respectively. Prevalence of abnormal albuminuria (microalbuminuria plus macroalbuminuria) for the respective quartiles were 38.4%, 51.2%, 50.6%, and 66.3% (P trend <0.01). In men, uric acid correlated positively with triglycerides, natural logarithmic [ln (ACR)] (γ = 0.168, P < 0.05). In women, uric acid correlated positively with triglycerides, ln (ACR) (γ = 0.277, P < 0.01) and body mass index (borderline significant P < 0.1), but negatively with calculated creatinine clearance. The standardized regression coefficient for ln (ACR) and the odds ratio for abnormal albuminuria for every 1 mg/dL increment of uric acid after adjusting for calculated creatinine clearance and other confounders were 0.138 (P < 0.05) and 1.183 (1.025–1.364), respectively. The results after excluding 127 cases with a history of hypertension were similar.

Conclusion. Serum uric acid is an independent correlate of urinary ACR in Taiwanese patients with type 2 diabetes mellitus.

Uric acid is a product of purine metabolism in humans and approximately two thirds of it is excreted by the kidneys [1]. Gastrointestinal excretion is also observed, especially in patients with renal insufficiency [2]. Elevated uric acid levels can result from increased generation or decreased elimination. Increased generation, in turn, can be caused by ingesting a purine-rich diet or alcohol, by certain genetic disorders (such as the Lesch-Nyhan syndrome), and by increased turnover of cells (such as in myeloproliferative diseases or tumor lysis syndrome) [1, 3]. On the other hand, decreased renal excretion can be a consequence of decreased glomerular filtration rate (GFR), increased tubular reabsorption induced by volume depletion when using diuretics, or inhibition of renal tubular secretion induced by inhibition of the anion-exchange transport system by lactate or keto acids [1, 3].

Hyperuricemia is associated with an increased risk of hypertension and cardiovascular events [1, 3, 4]. A recent follow-up study carried out in 6403 Japanese subjects with normal renal function at baseline showed that a serum uric acid level of >8.0 mg/dL was associated with a 2.9-fold higher risk of developing renal insufficiency within 2 years in men and a 10.0-fold higher risk in women, when compared to a level of <5.0 mg/dL [5]. Similarly, high uric acid level was associated with increased risk of progression of renal disease in another study carried out in type 2 diabetic patients in Italy [4]. These studies suggested that increased uric acid levels could be injurious to the kidneys and were predictive of the progression of renal disease.

A recently developed animal model, which induces mild hyperuricemia without urate crystal deposition and acute renal failure by feeding rats with oxonic acid (an inhibitor of uricase, which degrades uric acid to allantoin), provides most of the information and answers to renal injuries induced by chronic hyperuricemia not directly related to the urate crystal deposition observed in humans. Hypertension can be induced in these rats made mild hyperuricemia after feeding oxonic acid for 3 weeks,
probably through mechanisms involving an increase in juxtaglomerular renin and a decrease in macula densa neuronal nitric oxide synthase (NOS) [6]. Lowering uric acid levels by allopurinol (a xanthine oxidase inhibitor) or benziodarone (a uricosuric agent) could prevent the development of hypertension in these animals [6].

In addition, mild hyperuricemia also induces glomerular hypertension [7] and renal afferent arteriolar thickening [8], at the same time accelerates the progression of renal disease [9]. A more recent study using computer image analysis to quantify the glomerular size in a blinded manner has effectively demonstrated that glomerular hypertrophy with a 30% increase in size was observed in rats made mildly hyperuricemic [10]. More important, glomerular hypertrophy can be prevented by simultaneous treatment with allopurinol or benziodarone [10].

Albuminuria and glomerulosclerosis also develop after prolonged hyperuricemia for 6 months [10]. Treatment of hypertension with hydrochlorothiazide cannot prevent and treatment with enalapril can only partially prevent glomerular hypertrophy, despite of a comparable success in blood pressure control. This suggests that the hyperuricemia-induced glomerular hypertrophy is independent of blood pressure control and that activation of the renin-angiotensin system may only partially explain the induction of glomerular hypertension by uric acid [10].

Based on these detrimental effects of uric acid on the kidneys observed in the animal model, it is reasonable to hypothesize that elevated uric acid may contribute to the development of increased urinary albumin excretion in diabetic patients, independent of hypertension. To the best of our knowledge, this issue has not been examined previously. Therefore, the purpose of this study was to evaluate the association between uric acid and urinary albumin excretion rate in type 2 diabetic patients in Taiwan.

METHODS

Study subjects

The study was approved by the Department of Health, Taiwan, and the subjects voluntarily participated in the study. A total of 343 (144 men and 199 women) type 2 diabetic patients, aged 62.8 ± 10.8 years, were recruited consecutively from an outpatient diabetes clinic at the National Taiwan University Hospital. The patients did not show a history of diabetic ketoacidosis at the onset of diabetes and were being treated with either oral antidiabetic drugs or insulin at the time of recruitment. For those under insulin treatment, none received such treatment within 1 year of diagnosis of diabetes mellitus.

Patients using uric acid–lowering agents, diuretics, or alcoholic beverages were excluded because the confounding effect of these substances might not be completely controlled by adjustments in the multivariate analyses if the timing, the dosage, the duration, or the reasons of their use were diverse. Patients with acute illness, malignancy, fever, or urinary tract infection were likewise excluded.

Measurement of urine and blood biospecimens

The subjects were instructed not to participate in any vigorous physical activity 1 day before examination. Urine specimens and blood samples were collected in the early morning after fasting for at least 12 hours. First void and mid-stream urine was collected, followed by venous blood sampling.

Urinary albumin concentration was measured quantitatively by means of a particle-enhanced turbidimetric immunoassay (Biolatex®) (Logrono, Spain). Urinary creatinine concentration was measured after 10× dilution on an automatic biochemistry analyzer (Cobas Mira S) (Roche Diagnostica, Basel, Switzerland) with reagents obtained from Randox Laboratories Ltd. (Antrim, UK). Urinary albumin-to-creatinine ratio (ACR) was calculated by dividing the urinary albumin concentration in micrograms by the urinary creatinine concentration in milligrams. ACR <30.0 μg/mg was defined as normoalbuminuria, 30.0 to 299.9 μg/mg as microalbuminuria, and ≥300.0 μg/mg as macroalbuminuria and ≥300.0 μg/mg as macroalbuminuria and microalbuminuria plus macroalbuminuria [11]. Abnormal albuminuria was defined as an ACR ≥30.0 μg/mg (i.e., microalbuminuria plus macroalbuminuria).

Venous blood samples were collected in the morning after the subjects fasted overnight for more than 12 hours. Fasting plasma glucose and serum total cholesterol, triglycerides, uric acid, and creatinine were measured by an automatic biochemistry analyzer (Cobas Mira S) (Roche Diagnostica) with reagents obtained from Randox Laboratories Ltd. (Antrim, UK). Creatinine clearance (mL/min) was calculated from the Cockcroft-Gault formula as [(140 − age in years) × body weight in kilograms]/(72 × serum creatinine in mg/dL) [12]. For women, the calculated values were multiplied by 0.85 [12].

Other risk factors

The patients’ age, gender, body mass index, duration of diabetes, history of hypertension (all of the patients with a history of hypertension were treated with antihypertensive agents), systolic blood pressure, diastolic blood pressure, insulin therapy, and smoking habit were recorded or measured. These were then treated as potential confounders in regression analyses. Patients who smoked one or more cigarettes per day were defined as smokers. Duration of diabetes was defined as the time period in years between the time being recruited into the study and the time diabetes was diagnosed. Blood pressure was measured on the right arm after 20 minutes rest in a sitting position with a mercury sphygmomanometer by the auscultatory method.
Body height (in centimeters) was measured by having the subjects stand with their heels, buttocks, and heads against a wall. A flat object was placed on top of the subjects’ head, and their height was marked on a tape measure affixed to the wall. Body weight was measured in kilograms with a standard portable scale. Body height and body weight were measured with the patient wearing light clothes and without socks and shoes. Body mass index was calculated as body weight in kilograms divided by the square of the body height in meters.

## Statistical analyses

Data were expressed as mean ± SD or percentage. A $P < 0.05$ was considered statistically significant, while $0.05 < P < 0.1$ was borderline significant.

Because the distribution of ACR was highly skewed, the natural logarithm of ACR (ln (ACR)) was used for statistical analyses. One-way analysis of variance (ANOVA) followed by multiple comparison test with the least significant difference was used to compare the differences of uric acid levels among the three subgroups of ACR (i.e., normoalbuminuria, microalbuminuria, and macroalbuminuria). The linear test for trend was used to determine if the increasing trend in the prevalence of abnormal albuminuria associated with increasing quartiles of uric acid levels was significant.

Pearson correlation coefficients between uric acid and continuous covariates and ln (ACR) were calculated for different genders. Student $t$ test was used to compare the differences in uric acid levels between diabetic men and women, patients with or without a history of hypertension, patients using and those not using insulin, and smokers and nonsmokers.

Linear regression models were created using ln (ACR) as a dependent variable and uric acid as an independent variable. Four models were created: (1) without adjustment; (2) adjusted for age and gender; (3) adjusted for age, gender, and variables associated with uric acid with $P < 0.1$ in univariate analyses (i.e., body mass index, triglycerides, and calculated creatinine clearance); and (4) adjusted for all covariates (i.e., age, gender, body mass index, duration of diabetes, smoking, insulin use, history of hypertension, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, total cholesterol, triglycerides, and calculated creatinine clearance).

Similarly, logistic regression models were created to estimate the odds ratios for abnormal albuminuria. Because the development of renal histopathology induced by hyperuricemia in the animal model was prevented by factors not identical with the use of different antihypertensive agents [8, 10], it was difficult to simultaneously overcome the potential confounding effects of a variety of antihypertensive agents used in patients having a history of hypertension, especially in those who concomitantly used several antihypertensive agents. Therefore, the above linear and logistic regression models were further analyzed separately in patients with and those without a history of hypertension.

## RESULTS

Table 1 shows the baseline characteristics of the study subjects. While comparing uric acid levels between men and women, patients with and those without a history of hypertension, patients using or not using insulin, and smokers and nonsmokers, a significant difference was observed only in the comparison between men and women (5.9 ± 1.9 vs. 5.3 ± 1.8) ($P = 0.005$). The mean ± SD (minimum-maximum) of uric acid for the first to the fourth quartile was 3.4 ± 0.6 (1.7–4.2), 4.9 ± 0.4 (4.3–5.4), 6.0 ± 0.3 (5.5–6.5), and 8.1 ± 1.2 (6.6–12.2), respectively. Prevalence of abnormal albuminuria for the quartiles of uric acid levels was 38.4%, 51.2%, 50.6%, and 66.3%, respectively ($P$ trend <0.01).

Figure 1 shows the percentile distributions of uric acid among patients with normoalbuminuria ($N = 166$) (●), with microalbuminuria ($N = 130$) (■), and with macroalbuminuria ($N = 47$) (▲).
multiple comparison tests by the least significant difference showed that the differences of uric acid levels between any two subgroups were all significant ($P < 0.05$).

Table 2 shows the Pearson correlation coefficients between uric acid and the continuous covariates and ln (ACR) by gender. Uric acid was correlated significantly with triglycerides and ln (ACR) in males; with triglycerides, ln (ACR), calculated creatinine clearance (in a negative pattern), and body mass index (borderline significant) in females; and with triglycerides, ln (ACR) calculated creatinine clearance (in a negative pattern), and body mass index in all patients.

Table 3 shows the standardized regression coefficients obtained from the linear regression models for estimating ln (ACR) and the odds ratios for abnormal albuminuria by every 1 mg/dL increment of uric acid before and after adjustment for covariates in all of the patients and in 216 patients without a history of hypertension. Uric acid was significantly associated with ln (ACR) in all of the linear regression models except in the model adjusting for age, gender, body mass index, triglycerides, and calculated creatinine clearance in patients without a history of hypertension. The $P$ value for this one was borderline significant.

Similarly, the odds ratios for abnormal albuminuria derived from logistic regression were all significant, with $P$ values < 0.05 except for the same model as in the linear regression having borderline significance. None of these analyses in the 127 patients with a history of hypertension and under treatment for such was statistically significant (data not shown).

**DISCUSSION**

The findings of a close relationship between uric acid and urinary albumin excretion rate in diabetic patients in this study (Tables 2 and 3) (Figs. 1 and 2) are relatively novel. These results supported the observations in rats that mild hyperuricemia can cause renal injury and albuminuria [6–10]. However, the association was not observed in the 127 patients with a history of hypertension and under antihypertensive treatment (data not shown). This finding was interesting but not unexpected, because blockade of the renin-angiotensin system by angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers which are now so commonly used in the diabetic patients can substantially block uric acid–mediated effects [6, 8, 10]. A secondary analysis did show that the correlation coefficient between uric acid and ln (ACR) in 74 (52 women and 22 men) hypertensive patients not using these agents was highly significant ($\gamma = 0.325, P = 0.005$).

A problem of multicollinearity might exist in the model adjusting for all potential confounders, because age and duration of diabetes, and systolic and diastolic blood pressure were highly correlated pairs, with respective correlation coefficients of 0.373 ($P < 0.001$) and 0.483 ($P < 0.001$). However, multicollinearity does not bias the estimated coefficients in the regression models. It merely inflates the variance and may lead to insignificant regression coefficients [13].

It is true that elevated uric acid levels can be a consequence of impaired renal function [14]. However, uric acid per se can also be detrimental to the kidneys as shown in animal and human studies [5–10, 15–17]. Elevated uric acid can induce renin expression from the juxtaglomerular cells and inhibit NOS expression in the macula densa [6]. Uric acid also impairs endothelial function [15] and stimulates the production of cytokines from leukocytes [16] and chemokines from vascular smooth muscle cells [17]. As a result, hyperuricemic rats develop hypertension and show renal injury consisting of afferent arteriopathy, mild tubulointerstitial fibrosis, intraglomerular hypertension, glomerular hypertrophy, and eventually, glomerulosclerosis and albuminuria [6–10]. These histopathologic changes induced by elevated uric acid in animals are also common features of diabetic nephropathy in humans [18].

It is very important to note that most of the reported renal changes in rats can be prevented by maintaining serum uric acid levels in the normal range by allopurinol [7, 10], but only partially prevented by the treatment of hypertension with enalapril [6, 8, 10] or lornarslan [8], and not at all by diuretics [8, 10]. These observations highly suggest a pathogenic role of uric acid in the renal abnormalities independent of blood pressure and imply a possible efficacy to lower urinary albumin excretion rate in diabetic patients by bringing down the uric acid levels. A recent human study did prove that allopurinol treatment could normalize endothelial dysfunction in type 2 diabetic patients with mild hypertension [19].

Theoretically, it is also possible that both elevated uric acid and urinary albumin excretion rate are manifestations of a common underlying pathogenesis of insulin resistance. In humans, uric acid is the final breakdown
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Table 2. Correlation coefficients between uric acid and continuous covariates and ln(ACR)

<table>
<thead>
<tr>
<th></th>
<th>Men (N = 144)</th>
<th>Women (N = 199)</th>
<th>All (N = 343)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−0.057</td>
<td>0.085</td>
<td>0.012</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.128</td>
<td>0.131</td>
<td>0.126</td>
</tr>
<tr>
<td>Diabetic duration</td>
<td>−0.040</td>
<td>−0.006</td>
<td>−0.025</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.095</td>
<td>0.023</td>
<td>0.042</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.077</td>
<td>0.098</td>
<td>0.081</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>0.019</td>
<td>−0.022</td>
<td>−0.030</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.118</td>
<td>0.056</td>
<td>0.047</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.198</td>
<td>0.302</td>
<td>0.240</td>
</tr>
<tr>
<td>Calculated creatinine clearance</td>
<td>−0.114</td>
<td>−0.214</td>
<td>−0.126</td>
</tr>
<tr>
<td>Ln (ACR)</td>
<td>0.168</td>
<td>0.277</td>
<td>0.217</td>
</tr>
</tbody>
</table>

Ln (ACR) is natural logarithmic urinary albumin-to-creatinine ratio.

*0.05 < P < 0.1; **P < 0.05; ***P < 0.01.

Table 3. Standardized regression coefficients for natural logarithmic albumin-to-creatinine ratio [ln (ACR)] and odds ratios (OR) for abnormal albuminuria by uric acid treated as a continuous variable

<table>
<thead>
<tr>
<th>Models</th>
<th>Standardized β</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (N = 343)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without adjustment</td>
<td>0.217</td>
<td>1.251 (1.107–1.413)</td>
</tr>
<tr>
<td>Adjusted for age and gender</td>
<td>0.225</td>
<td>1.274 (1.124–1.444)</td>
</tr>
<tr>
<td>Adjusted for age, gender, body mass index, triglycerides, and calculated creatinine clearance</td>
<td>0.131</td>
<td>1.156 (1.008–1.325)</td>
</tr>
<tr>
<td>Adjusted for age, gender, body mass index, history of hypertension, insulin use, smoking, diabetic duration, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, total cholesterol, triglycerides, and calculated creatinine clearance</td>
<td>0.138</td>
<td>1.183 (1.025–1.364)</td>
</tr>
<tr>
<td>Patients without hypertension history (N = 216)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without adjustment</td>
<td>0.212</td>
<td>1.278 (1.095–1.492)</td>
</tr>
<tr>
<td>Adjusted for age and gender</td>
<td>0.232</td>
<td>1.319 (1.125–1.548)</td>
</tr>
<tr>
<td>Adjusted for age, gender, body mass index, triglycerides, and calculated creatinine clearance</td>
<td>0.129</td>
<td>1.189 (0.995–1.421)</td>
</tr>
<tr>
<td>Adjusted for age, gender, body mass index, insulin use, smoking, diabetic duration, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, total cholesterol, triglycerides, and calculated creatinine clearance</td>
<td>0.147</td>
<td>1.243 (1.029–1.503)</td>
</tr>
</tbody>
</table>

*P < 0.01; **P < 0.05; ***P < 0.01.

Product of adenosine, which plays an important role in the pathophysiology of insulin resistance [20]. Adenosine can also cause increased renal uric acid retention [20]. Moreover, hyperinsulinemia resulting from insulin resistance can decrease the renal excretion, increase the renal reabsorption, and increase the production of uric acid [21]. On the other hand, microalbuminuria is also an integral component of the metabolic syndrome characterized by insulin resistance [11, 22].

Uric acid can also be a risk factor for hypertension and cardiovascular disease [23–26]. This association has been shown to be stronger in women than in men in some studies [24, 27]. However, when secondary analyses were performed separately in either gender, the results were consistent in both men and women (data not shown). In clinical practice today, we rarely think of measuring uric acid as a risk factor of cardiovascular disease, not to say as a risk factor of abnormal albuminuria. Because hyperuricemia is so common among diabetic patients and since it is relatively easy to lower uric acid levels with medications, this study suggested that not only the importance of uric acid as a risk factor for cardiovascular disease should be reconsidered, its pathogenic role in other clinical manifestations such as renal injury and increased urinary albumin excretion rate is worthy of further investigation.

This study has some limitations. First, it involved only a single collection of urine, which did not completely conform to the requirement of multiple urine collections over 3 to 6 months for the diagnosis of microalbuminuria [11]. Second, the validity to extrapolate the relationship between uric acid and urinary albumin excretion rate to non diabetic subjects requires confirmation. Third, referral bias could not be excluded because of the hospital-based design of the present study.

CONCLUSION

The present study strongly suggests a close link between uric acid and increased urinary albumin excretion rate in type 2 diabetic patients in Taiwan. Because hyperuricemia is very common in type 2 diabetics and the treatment of elevated uric acid is relatively easy, it is of clinical
importance to clarify the pathogenic role of uric acid in renal disease and to evaluate whether the increased urinary albumin excretion rate can be prevented by lowering uric acid levels with medications.

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