Urinary Transforming Growth Factor-β1 and α1-Microglobulin in Children and Adolescents With Type 1 Diabetes

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OBJECTIVE — Transforming growth factor (TGF)-β1 is an important mediator in the pathogenesis of diabetic nephropathy. Urinary TGF-β1 reflects TGF-β1 production in the kidney, and α1-microglobulin tubular dysfunction. These 2 markers were studied in the early phases of type 1 diabetes.

RESEARCH DESIGN AND METHODS — There were 113 type 1 diabetic children and adolescents (mean ± SD: age 14.1 ± 2.9 years, and diabetes duration 7.4 ± 2.9 years, HbA1c 9.3 ± 1.5%) and 39 healthy subjects (age 13.8 ± 2.8 years) who participated in the study. Of the diabetic patients, 105 were normoalbuminuric (2–3 consecutive overnight urinary albumin excretion rates [AERs] < 20 µg/min) and 8 had microalbuminuria (at least 2 AERs 20–200 µg/min). Overnight urinary TGF-β1 and α1-microglobulin levels were measured and the results expressed as the ratio to urinary creatinine concentration.

RESULTS — Data are medians (range). Diabetic patients had higher urinary TGF-β1 levels than those of control subjects: 0.9 ng/mg (0.05–122.3) vs. 0.3 ng/mg (0.05–2.2) creatinine, respectively (P = 0.003). Urinary TGF-β1 levels correlated with urinary glucose (r = 0.2, P = 0.03) and α1-microglobulin (r = 0.2, P = 0.02) levels, but not with HbA1c, AER, age, or duration of diabetes. In 43 patients with urinary TGF-β1 above the control levels, urinary TGF-β1 levels correlated with urinary glucose (r = 0.6, P < 0.001) and α1-microglobulin (r = 0.6, P < 0.001) levels. Diabetic patients had higher urinary α1-microglobulin levels than those of control subjects: 4.8 µg/mg (0.6–48.8) vs. 2.7 µg/mg (0.8–11.6) creatinine, respectively (P < 0.001). α1-Microglobulin levels correlated with AER (r = 0.2, P = 0.02), HbA1c (r = 0.3, P = 0.001), urinary glucose (r = 0.5, P < 0.001), and urinary TGF-β1 levels.

CONCLUSIONS — An early rise in urinary TGF-β1 levels was observed in young type 1 diabetic patients. Urinary TGF-β1 is associated with 2 interrelated tubular markers, α1-microglobulin and urinary glucose.

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Since the discovery of the central role of transforming growth factor (TGF)-β in fibrotic kidney disease (1), urinary TGF-β activity has been studied in experimental models of kidney disease together with renal TGF-β mRNA and protein expression, and indexes of fibrosis (2,3). A simultaneous increase in urinary TGF-β and glomerular TGF-β mRNA and protein was observed in a rabbit model of an antibody-mediated crescentic nephritis (4). In people with various glomerular diseases, urinary TGF-β correlated with the grade of interstitial fibrosis (5). In patients with membranous glomerulonephritis, urinary TGF-β1 at baseline was lower in those patients who later reached remission than in patients whose disease had a more progressive course (6). In type 2 diabetic patients, urinary TGF-β1 is elevated (7) and associated with histologically proven severe mesangial expansion (8). These studies show the usefulness of urinary TGF-β measurement in kidney disease during clinical follow-up. Urinary TGF-β1 levels in children and adolescents with a relatively short duration of type 1 diabetes are not known. We, therefore, studied the urinary TGF-β1 excretion in children and adolescents with type 1 diabetes and compared the data with 2 conventional markers of glomerular and tubular injury, urinary albumin, and α1-microglobulin (9,10), and with parameters of glycemic control.

RESEARCH DESIGN AND METHODS

Patients
A total of 186 type 1 diabetic patients were recruited in a consecutive manner from the outpatient clinics of the Hospital for Children and Adolescents, University of Helsinki, and the Pediatric Department, Aurora Hospital, Helsinki, Finland, during the years 1994–1996. The recruitment criteria were a minimum age of 7 years at the Hospital for Children and Adolescents and 10 years at the Aurora Hospital, and a duration of diabetes ≥ 3 years. There were 39 healthy subjects recruited for a single-timed overnight urinary sample for albumin excretion rate (AER), TGF-β1, and α1-microglobulin measurements. The study protocol was approved by the ethical committees of the 2 hospitals. Altogether there were 113 patients (57 female and 56 male; 53 from the Hospital for Children and Adolescents and 60 from the Aurora Hospital). All of the patients had given their informed consent and collected 2 or 3 timed overnight urinary samples at 3-month intervals for definition between normal and microalbuminuria. The remaining 73 patients were excluded.
because either no sample for TGF-β1 measurement or an inadequate number of overnight urine collections for AER measurement was obtained. Glycemic control, duration of diabetes, insulin dose, or serum creatinine levels did not differ between the included and excluded patients. Patients defined as normoalbuminuric had at least 2 AERs <20 µg/min and patients defined as microalbuminuric had at least 2 AERs 20–200 µg/min. Their mean age was 14.1 ± 2.9 years (mean ± SD), duration of diabetes 7.4 ± 2.9 years, a mean HbA1c concentration of 3 consecutive visits of 9.3 ± 1.5%, a mean serum creatinine level of 71 ± 13 µmol/l, and a median AER of 6 (2–196) µg/min. All of the patients had insulin treatment (0.95 ± 0.2 U/kg body wt). Eight microalbuminuric patients were older and had had diabetes for a longer time than patients with normal AER (mean age 16.8 ± 3.1 vs. 13.9 ± 2.8 years, P = 0.009; mean duration of diabetes 10.1 ± 3.0 vs. 7.2 ± 2.8 years, P = 0.01). Two microalbuminuric patients were on ACE inhibitor medication. The mean age of healthy control subjects was 13.8 ± 2.8 years and their median AER was 3 (1–29) µg/min.

At the Hospital for Children and Adolescents, the blood pressure was recorded at 3 consecutive visits at 3-month intervals in a sitting position by an automatic device (Dinamap; Critikon, Tampa, FL). TGF-β1 and α1-microglobulin were analyzed from a single urine sample.

Clinical laboratory tests
The HbA1c concentration was measured by an instant immunological device (DCA-2000; Bayer Diagnostics, Leverkusen, Germany) at the Hospital for Children and Adolescents, and by high-performance liquid chromatography (Diamat analyzer; Bio-Rad, Anaheim, CA) at the Aurora Hospital (normal range 4–6% with an interassay coefficient of variation [CV] of 2–4% at both hospitals). Urinary albumin excretion was measured by immunoturbidimetry at the Hospital for Children and Adolescents (Hitachi 911 Analyzer; Hitachi, Tokyo) and at the Aurora Hospital (Cobas Mira Analyzer; Roche Diagnostics, Basel). Interassay CV was 9.7% at 43 mg/l at the Hospital for Children and Adolescents, and 5.6% in the range of 13–50 mg/l at the Aurora Hospital. The urinary creatinine level was measured by the Jaffe kinetic method (Hitachi 911; Hitachi). Urinary glucose was measured by an automated hexokinase method with a normal value of <0.1 g/l and an interassay CV of 2% (Hitachi 917; Hitachi).

Assay for TGF-β1
The urinary concentration of TGF-β1 (active plus acid-activatable latent) was measured by enzyme-linked immunosorbent assay. In brief, microtiter plates were coated with 0.1 µg/well monoclonal mouse antibody to TGF-β1-3 (Genzyme Diagnostics, Cambridge, MA). To activate latent TGF-β1, samples were incubated with 100 mmol/l HCl at 4°C for 2 h. Activated samples were added to the wells and left overnight. The detection antibody was chicken IgG-antibody to human TGF-β1 (R&D Systems, Minneapolis, MN), followed by sheep antibody to chicken IgG (Serotec, Oxford, U.K.), and alkaline phosphatase-conjugated donkey antibody to sheep IgG (Serotec). Natural, human TGF-β1 (R&D Systems, Abingdon, U.K.) served as a standard. The detection limit was 70 ng/l. All assays were performed as duplicates. The intra-assay and interassay CVs were 5.9 and 8.1%, respectively.

Statistical analysis
Both urinary TGF-β1 and α1-microglobulin levels were expressed as the ratio to urinary creatinine levels (ng/mg creatinine and µg/mg creatinine, respectively). The samples with urinary TGF-β1 levels under the detection limit were not divided by urinary creatinine, but a uniform value of 0.05 ng/mg creatinine was used. The Mann-Whitney U test was used for comparisons between groups and the Pearson test for correlations. For the correlation and regression analyses, urinary TGF-β1 and α1-microglobulin levels, urinary glucose concentration, and AER were logarithmically transformed. Multiple linear regression analysis was used to explore relationships among multiple variables. A P value <0.05 was considered statistically significant.

**RESULTS**

**Urinary TGF-β1**
Type 1 diabetic patients had higher urinary TGF-β1 levels than those of control subjects (data are medians [range]): 0.9 ng/ml (0.05–122.3) vs. 0.3 ng/ml (0.05–2.2) creatinine, respectively (P = 0.003). Microalbuminuric patients did not differ from normoalbuminuric patients in TGF-β1 levels (P = 0.4, Fig. 1). The urinary TGF-β1 concentration correlated with the concurrent urinary glucose concentration (r = 0.2, P = 0.03) and the α1-microglobulin concentration (r = 0.2, P = 0.02). However, the
Urinary TGF-β1 in type 1 diabetic youths

Urinary TGF-β1 concentrations showed no correlation with the HbA1c levels in patients with type 1 diabetes (r = −0.03, P = 0.7), nor with age in diabetic patients or control subjects. The mean systolic and diastolic blood pressure of 3 consecutive visits, available in 51 diabetic patients, did not correlate with TGF-β1 levels (r = 0.2, P = 0.1 for diastolic pressure and 0.2 for systolic blood pressure). AER or duration of diabetes did not correlate with urinary TGF-β1 levels.

In the multiple linear regression model containing α1-microglobulin and urinary glucose, neither of these variables showed independent association with urinary TGF-β1 with an adjusted R² of 0.04.

Of the diabetic patients, 43 (38%) had urinary TGF-β1 concentrations exceeding the levels seen in the control subjects (>2.25 ng/mg creatinine). In these 43 patients, urinary TGF-β1 levels correlated with urinary glucose (r = 0.6, P < 0.001, Fig. 2) and α1-microglobulin levels (r = 0.6, P < 0.001), HbA1c (r = 0.3, P = 0.07) and the mean systolic pressure, available in 15 of 43 patients (r = 0.4, P = 0.11) did not reach significance. In the multiple linear regression model containing α1-microglobulin and urinary glucose, α1-microglobulin independently associated with urinary TGF-β1 (P = 0.02) with adjusted R² of 0.35.

Urinary α1-microglobulin
Type 1 diabetic patients had higher urinary α1-microglobulin levels than those of the control subjects: 4.8 μg/mg (0.6-48.8) vs. 2.7 μg/mg (0.8-11.6) creatinine, respectively (P < 0.001). In diabetic patients, urinary α1-microglobulin correlated with urinary AER (r = 0.2, P = 0.02), HbA1c (r = 0.3, P < 0.001), and with urinary glucose (r = 0.5, P < 0.001) in addition to urinary TGF-β1. Multiple linear regression analysis of these 4 variables showed that urinary glucose (P < 0.001) and TGF-β1 (P = 0.046) independently influenced urinary α1-microglobulin levels (adjusted R² 0.28). HbA1c and AER were not significant (P = 0.07 and 0.08, respectively).

Urinary AER
In diabetic patients, urinary AER correlated with age (r = 0.4, P < 0.001), duration of diabetes (r = 0.3, P = 0.001), systolic blood pressure (r = 0.4, P = 0.01), and urinary α1-microglobulin (r = 0.2, P = 0.02). In the linear regression model containing these variables, duration of diabetes was independently associated with AER (P = 0.04).

CONCLUSIONS — We observed an increased urinary TGF-β1 excretion in 38% of the children and adolescents with type 1 diabetes when compared with that of healthy children. In diabetic patients, urinary TGF-β1 correlated weakly with urinary glucose and α1-microglobulin. According to the regression analysis, these 2 factors were interrelated and could explain only little of the variation in urinary TGF-β1. In the subgroup of patients with increased urinary TGF-β1, ~35% of the variation in TGF-β1 excretion could be explained by either of these parameters. An interesting feature was the presence of low urinary TGF-β1 excretion in the majority (62%) of young type 1 diabetic patients, despite high HbA1c and urinary glucose concentrations. Thus, in a substantial group of the diabetic patients, hyperglycemia does not lead to an increased urinary TGF-β1 excretion. This may be due to the presence of protective factors, which are unidentified, but genetic regulation may play a role (11).

As shown by previous studies, α1-microglobulin correlated with HbA1c and concurrent urinary glucose (12,13). In the present study TGF-β1 excretion and urinary glucose were independent determinants for urinary α1-microglobulin in the multiple regression model. Thus, an increase in TGF-β1 was associated with a marker of tubular dysfunction. This is a new finding suggesting a link between TGF-β1 excretion and tubular damage. Urinary excretion of α1-microglobulin can be decreased if metabolic control improves (13). In contrast to urinary TGF-β1, which is of renal origin (7), α1-microglobulin is derived from plasma and reabsorbed in proximal tubulus. Therefore, the observed correlation between α1-microglobulin and urinary glucose may reflect the temporary effect of glycosuria on the function of proximal tubulus.

Experimental hyperglycemia can increase TGF-β1 production either directly (14–17) or through the formation of advanced glycation end products (18) in the kidney cell culture. However, glycemic control did not predict urinary TGF-β1 levels in adult type 1 diabetic patients (19).

In the present study, HbA1c did not associate with urinary TGF-β1, whereas urinary glucose correlated with TGF-β1. This suggests the importance of glucose as a local stimulus for TGF-β1. Besides glomerular expression, TGF-β1 is produced by tubulointerstitial cells (20), which may contribute to urinary TGF-β1 levels. In these cells, the effect of urinary glucose may be an important factor to enhance urinary TGF-β1 (14,17).

On the other hand, as studies of non-diabetic kidney disease indicate, hyperglycemia is not necessary for the induction of TGF-β1 production in the kidney (2,5). Pure cyclic mechanical force causing mesangial stretching and capillary expansion mimicking glomerular hypertension has been shown to stimulate production of extracellular matrix in rat mesangial cells (21), and TGF-β1 is involved in this process (22,23).

Systemic hypertension leading to elevated intraglomerular pressure is rarely
present in children and adolescents with type 1 diabetes. Instead, glomerular hyperfiltration is prevalent in 40–60% of children and adolescents with type 1 diabetes (24,25), which may contribute to renal TGF-β1 production in diabetic children. This assumption is indirectly supported by the study of Berg et al. (26), in which a high filtration fraction predicted the increase in mesangial matrix volume in normoalbuminuric adolescents and young adults with type 1 diabetes. In the present study, the availability of blood pressure recordings from only half of the study population limited the interpretation of the blood pressure results with respect to urinary TGF-β1. We found correlation (r = 0.4) between the mean systolic blood pressure and urinary TGF-β1, although it was not significant (P = 0.11), probably due to the small number of patients. This finding resembles a recent observation of the correlation between circulating TGF-β1 and systemic blood pressure levels (27).

Although urinary TGF-β1 measurement has been suggested as a marker for diabetic nephropathy (8,19), not all studies have shown the association of urinary TGF-β1 with diabetic nephropathy (28). Also, we did not find a difference in urinary TGF-β1 excretion between microalbuminuric and normoalbuminuric patients. Unfortunately, due to the small number of microalbuminuric patients (n = 8) and the fact that 2 of them received ACE inhibitors with a potential decreasing effect on urinary TGF-β1 secretion (2), we could not reach definite conclusions. Interestingly, urinary TGF-β1 did not associate with AER, suggesting that the mechanism behind increased urinary TGF-β1 is different from that of increased AER.

The association between urinary TGF-β1 and diabetic nephropathy may not be a direct one. In a recent study, both mechanical stretching and high glucose increased TGF-β1 secretion in cultured rat mesangial cells, but only these 2 together induced accumulation of collagen mediated by TGF-β1 (29). Thus, the pathological interplay between different factors may be complex, and the presence of TGF-β1 in urine can be seen as a marker for a capacity of an individual to produce TGF-β1, a potentially harmful growth factor in relation to diabetic nephropathy. As a new finding, our data indicate an early rise in urinary TGF-β1 after diagnosis of diabetes associated with α1-microglobulin, a marker of tubular dysfunction.

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References

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