ORIGINAL RESEARCH

Urinary TGF-β1 was not independently associated with renal function in diabetes mellitus

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Background: Several clinical studies have shown increased level of urinary TGF-β1 in diabetic nephropathy patients and its correlation with urine albumin-to-creatinine ratio (UACR), but other studies showed different results. Because of this contradiction, this study aims to analyze the correlation between urinary TGF-β1 concentration and UACR, and also estimated glomerular filtration rate (eGFR) in type 2 diabetes mellitus (DM) patients by controlling some confounding factors.

Methods: This was a cross-sectional study, and the samples were obtained using consecutive sampling technique. The study was performed on 99 subjects (62 DM normoalbuminuria patients, 27 DM albuminuria patients, and 10 non-DM patients as controls) at Pasar Minggu Community Health Center. Urinary TGF-β1 concentration was measured by ELISA, and UACR was measured using immunoturbidimetry and an enzymatic colorimetric method. The eGFR value was calculated based on serum creatinine using Chronic Kidney Disease Epidemiology Collaboration equation. The results were then subjected to be analyzed statistically.

Results: There was an increase of urinary TGF-β1 in albuminuria (326.49 ± 48.98) as compared to normoalbuminuria (290.54 ± 30.71) and non-DM subjects (229.83 ± 31.90), but the values did not differ statistically ($P=0.790$). In addition, no correlation was observed between urinary TGF-β1 and UACR ($r=-0.084$, $P=0.410$) and eGFR ($r=0.155$, $P=0.125$), but a correlation was found with SBP ($r=-0.224$, $P=0.026$). Linear regression analysis showed that urinary TGF-β1 and HbA1c could predict UACR, but only HbA1c could be considered as a significant predictor of UACR.

Conclusion: There is an increase of urinary TGF-β1 concentrations in albuminuria patients clinically, but not statistically. The concentration of TGF-β1 was not correlated with UACR and eGFR, but correlated with SBP. Since TGF-β1 could be interfered by many factors, including hypertension and its medication, urinary TGF-β1 might not be independently associated with renal function in diabetes.

Keywords: albuminuria, diabetes mellitus, diabetic nephropathy, TGF-β1, UACR

Introduction

Diabetic nephropathy is a common microvascular complication of DM and a severe health problem due to increased morbidity and mortality. The most common cause of chronic renal disease and ESRD in the US is diabetes, which accounts for roughly 50% of all cases, followed by hypertension (25%), with other causes including glomerulonephritis and polycystic kidney disease.[1] In Malaysia, South Korea, and Mexico, ESRD is caused by diabetic nephropathy in more than 50% of the cases.[1] It is predicted that the prevalence will increase by more than two million cases in 2030.[1] The risk of diabetic nephropathy causing ESRD is equivalent to an increase of early mortality in

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DM patients.[1] eGFR and UACR are referred to as biomarkers to determine chronic kidney diseases.[2] However, there are situations where serum creatinine based-eGFR may not be accurate due to the clinical conditions that affect serum creatinine concentration.[3] Inaccuracy also happens in measuring urine albumin since the calculation of excretion rate requires a timed urine specimen. An error could be due to incomplete bladder emptying, incomplete collection, and spills, which lead to the variation in albumin excretion.[3] Also, studies have shown that the progression of diabetic nephropathy is even reported in diabetic patients without any significant changes in urinary albumin levels; for instance, microalbuminuria could reverse to normoalbuminuria in advanced diabetic nephropathy patients.[4,5] The excretion level of albumin also varied due to some clinical characteristics of patients, such as obesity, exercise, diet, smoking, infection, and inflammation.[6-10] These observations indicate that the progression of diabetic nephropathy does not necessarily affect urinary albumin, yet it only reflects an initial reversible phase of kidney damage.[11] Thus, these markers are relatively imprecise to determine the early stage of diabetic nephropathy in DM patients, and a more sensitive diagnostic test of early-stage kidney damage in DM patients is highly needed.[12]

Several studies have suggested that intrinsic renal cells can produce inflammatory cytokines and growth factors, such as TGF-β1, during the progression of diabetic nephropathy.[13,14] As a fibrogenic cytokine, TGF-β1 is considered as a key mediator in diabetic nephropathy.[15] Recent evidence suggests that TGF-β1 is involved in the pathogenesis of diabetic nephropathy because of its prosclerotic properties.[16] TGF-β1 is a multifunctional cytokine circulating in a biologically inactive form in human plasma.[17] Among its many actions, regulation of cell proliferation and ECM production appear prominent. Excessive production of TGF-β1 is thought to occur in cases with fibrosis of the kidney, liver, skin, and other organs.[18] The expression of TGF-β1 is known to be increased in patients with type 2 DM, especially those with high HbA1c. Hyperglycemia in DM patients can activate ROS, PKC, polyol pathway, production of AGEs, and hexosamine pathway, which will transcribe TGF-β1.[13,19,20] The presence of TGF-β1 causes renal cell hypertrophy and accumulation of ECM, which are involved in the pathophysiology of kidney damage.[12,20]

Several clinical studies have shown increased level of urinary TGF-β1 in diabetic nephropathy patients and its correlation with UACR.[18,20,21] However, other studies showed different results suggesting no correlation between urinary TGF-β1 and UACR in type 2 DM.[22] Because of this contradic-

tion, the present study aims to analyze the levels of urinary TGF-β1 in type 2 DM patients with normoalbuminuria and albuminuria, and to evaluate its correlation with the UACR, and also eGFR. The results of this research are expected to confirm whether TGF-β1 can be used as a marker for diabetic nephropathy.

Materials and methods
Study design and subjects
This was a cross-sectional study undertaken in April 2017 and was approved by Ethics Committee, Faculty of Medicine, Universitas Indonesia, Dr Cipto Mangunkusumo Hospital (151/UN2.F1/ETIK/II/2017). The study population included patients attending Pasar Minggu Community Health Center. A total of 99 subjects were divided into three groups: 27 patients with type 2 DM with albuminuria, 62 patients with type 2 DM with normoalbuminuria, and 10 non-DM controls.

Sample collection and assessment
Patients were enrolled if they met the following inclusion criteria: age ≥25 years old, in fasting state for at least 8 hours before sample collection, not severely anemic (Hb ≥10.7 g/dL), not having hematuria, and willing to sign written informed consent. Demographic and baseline data, including body weight, height, and blood pressure, were recorded. After enrollment, patients were asked to go home and return on the following day for study. They were instructed to fast for 8 hours before returning on the following day.

On the day of study, patients were asked to collect their first urine in the morning in a 30 mL plastic container. The urine obtained was divided into two equal parts: one was used for TGF-β1 measurement and the other was sent to a commercial laboratory (Prodia) to determine UACR. UACR was measured using immunoturbidimetry and an enzymatic colorimetric method. Urinary TGF-β1 level was measured using ELISA kit according to the procedure mentioned in the package insert (Catalog No. EH0287; Fine Test, Wuhan, China). Absorbance was measured at 450 nm. Blood was obtained by finger prick for HbA1c determination. HbA1c was determined using HbA1c analyzer (Alere Afinion AS100; Abbott, Chicago, IL, USA).

Statistical analysis
Descriptive statistics were used for descriptive data. Comparison between groups was done using a chi-squared test for categorical variables. Kruskal–Wallis and Mann–Whitney statistics were used to analyze nonparametric data, and

ANOVA was used for parametric data. Correlation between two variables was analyzed using Spearman or Pearson test. A *P*-value of <0.05 was considered statistically significant. All analyses were performed using SPSS version 20 (IBM Corp., Armonk, NY, USA).

Results

The characteristic data included gender, age, body mass index, height, weight, exercise routine, smoking habit, duration of DM, oral antidiabetic use, blood pressure, hypertension, urinary albumin, urinary creatinine, HbA1c, UACR, and eGFR, and are presented in Table 1. There were significant differences in age, HbA1c, albuminuria, and UACR, but not in other characteristics. Regarding the sample groups, there were significant differences in the average age between albuminuria and non-DM group (*P*<0.001), but not between albuminuria and normoalbuminuria type 2 DM groups. Similar studies also showed significant differences in age between DM patients and non-DM subjects.[14] Increased age increases the risk of DM as indicated by the increasing proportion of patients with DM as the age increased. Increase in proportion of DM with increasing age is also due to the

increased interference with glucose tolerance.[15] HbA1c also showed a significant difference (*P*<0.001). The average HbA1c levels in non-DM subjects, normoalbuminuria subjects, and albuminuria subjects were 5.53%, 8.28%, and 9.52%, respectively. Based on the average HbA1c levels, it is clear that normoalbuminuria and albuminuria groups have uncontrolled HbA1c levels. It suggests that high levels of HbA1c will increase the risk of diabetic nephropathy.[16,17]

Most of the subjects in this study had normoalbuminuria. The mean UACR differed greatly between normoalbuminuria and albuminuria groups (8.19 and 189.89 µg/mg, respectively). On the other hand, the TGF-β1 concentration was not significantly different (*P*=0.790) between the three groups. However, the albuminuria group showed the highest TGF-β1 concentration (326.49 pg/mg creatinine), followed by normoalbuminuria group (290.54 pg/mg creatinine), which had a higher average concentration compared to the non-DM group (229.83 pg/mg creatinine).

There was no significant correlation between urinary TGF-β1 and UACR (*r*=−0.084, *P*=0.410), and eGFR (*r*=0.155, *P*=0.125) (Table 2). This result showed that in patients with albuminuria, there was an increase in concen-

Table 1 Characteristics of the study subjects

Variables	Non-DM subjects (n=10)	DM subjects		P
		Normoalbuminuria (n=62)	Albuminuria (n=27)	
Gender				0.616
Male	1 (10.0)	13 (21.0)	7 (25.9)	
Female	9 (90.0)	49 (79.0)	20 (74.1)	
Age (years)	48.80±8.64	61.37±6.79	61.11±7.25	<0.001[a,b]
Body weight (kg)	64.35±10.53	62.72±10.44	64.13±11.72	0.911
Body height (cm)	158.10±7.31	154.22±6.82	156.72±9.67	0.302
Body mass index (kg/m²)	25.64±3.06	26.40±4.30	26.15±3.88	0.909
Daily exercise	4 (40.0)	45 (72.6)	21 (77.8)	0.072
Smoking habit	1 (10.0)	1 (1.6)	1 (3.7)	0.174
DM duration (years)	NA	7.08±4.42	8.96±6.06	0.267
Using oral antidiabetic	NA			0.358
Monotherapy		33 (53.2)	11 (40.7)	
Combination		29 (46.8)	16 (59.3)	
Blood pressure				
Systolic (mmHg)	119.00±7.38	121.77±11.94	120.74±9.97	0.842
Diastolic (mmHg)	76.00±5.16	78.23±7.13	76.67±6.79	0.518
Hypertension	0 (0.00)	33 (53.2)	13 (48.1)	0.818
HbA1c (%)	5.53±0.19	8.28±1.68	9.52±2.19	<0.001[a,b,c]
Urinary creatinine (mg/dL)	71.45±7.88	75.56±6.88	77.68±10.28	0.839
Urinary albumin (mg/L)	<3	8.60±1.58	169.52±60.46	<0.001[a,b,c]
UACR (µg/mg)	0.00±0.00	8.19±1.21	189.89 ± 44.51	<0.001[a,b,c]
eGFR (mL/min/1.73)	103.70±4.53	82.24±2.97	86.33±5.72	0.010[a,b]
Urinary TGF-β1 (pg/mg creatinine)	229.83±31.90	290.54±30.71	326.49±48.98	0.790

Notes: Data expressed as n (%) or mean ± SD/SEM. [a]Significant difference between albuminuria and non-DM group. [b]Significant difference between normoalbuminuria and non-DM group. [c]Significant difference between albuminuria and normoalbuminuria group.
Abbreviations: DM, diabetes mellitus; UACR, urine albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; NA, not applicable.

Table 2 Correlation between urinary TGF-β1 and renal function parameters in this study and the previous studies

Author, year	Country	Subject	Renal function parameter	r	P
Shaker et al (2014)[20]	Egypt	53 albuminuria T2DM patients	UACR	0.623	<0.001**
Rivarola et al (1999)[21]	Brazil	13 persistent proteinuria, 6 microalbuminuria, and 4 normoalbuminuria T2DM patients	Proteinuria (g/24 hours)	0.7	<0.05*
Titan et al (2012)[18]	Brazil	56 macroalbuminuria T2DM patients	Proteinuria (g/24 hours)	0.5	<0.001**
			Estimated creatinine clearance	0.12	0.39
Kim et al (2011)[22]	UK	63 T2DM patients	UACR	0.120	0.347
Present study	Indonesia	89 T2DM patients	UACR	0.084	0.410
			eGFR	0.155	0.125

Abbreviations: T2DM, type 2 diabetes mellitus; UACR, urine albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate. *P<0.05; **P<0.001.

tration of urinary TGF-β1 clinically, but not statistically. However, we found a significant correlation between urinary TGF-β1 and SBP (Table 3).

Table 4 shows the linear regression analysis using a backward method. The analysis showed that urinary TGF-β1 and HbA1c could predict UACR, but only HbA1c could be considered as a significant predictor of UACR.

Discussion

The generation of AGEs through nonenzymatic oxidative reaction of amino acids from proteins occurs in renal tissue and plasma in patients with hyperglycemia, and leads to renal complications in patients with DM.[23–25] AGEs also release specific cellular response factors of profibrotic cytokines, such as TGF-β1.[26] Studies have suggested that TGF-β1 could be a hallmark to estimate the progression of diabetic renal disease because it stimulates ECM accumulation and promotes renal cell hypertrophy.[27] The result of this study showed that there was an increase of TGF-β1 in albuminuria patients when compared to normoalbuminuria and non-DM patients, but the values did not differ statistically, and no correlation was observed between UACR and urinary TGF-β1. This result was also supported by a previous study.[22] On the contrary, several studies found positive correlation between UACR and urinary TGF-β1, and also between glucose concentration and HbA1c.[20,21,28–30] The increase of urinary TGF-β1 in albuminuria patients was seen during the early stages of diabetic nephropathy, and the increase was more with the development of diabetic nephropathy.[31] In this study, we did not found any correlation between urinary TGF-β1 and HbA1c, UACR, and eGFR, but there was a correlation with SBP. However, after multivariate analysis, we found that only HbA1c could be considered as a significant predictor of UACR.

The above results can be due to many other biological factors in the body that can contribute to the increase of TGF-β1. Several studies have shown that TGF-β1 levels are increased in various pathological conditions. One of the conditions is

Table 3 Correlation between urinary TGF-β1 and other parameters in all subjects

	Spearman's rho	P
SBP (mmHg)	0.224	0.026*
DBP (mmHg)	0.019	0.855
HbA1c (%)	−0.143	0.157

Note: *P<0.05, significant.

Table 4 Linear regression analysis of variables predicting UACR in all subjects

Dependent variables	β	P
Model 1		
HbA1c (%)	0.298	0.003*
SBP (mmHg)	0.023	0.830
DBP (mmHg)	−0.138	0.202
Urinary TGF-β1 (pg/mg creatinine)	0.151	0.136
Model 2		
HbA1c (%)	0.300	0.003*
DBP (mmHg)	−0.129	0.191
Urinary TGF-β1 (pg/mg creatinine)	0.155	0.114
Model 3		
HbA1c (%)	0.280	0.005*
Urinary TGF-β1 (pg/mg creatinine)	0.155	0.116
Model 4		
HbA1c (%)	0.264	0.008*

Note: *P<0.05, significant. Model 1, no adjustment; Model 2, adjusted for SBP; Model 3, adjusted for SBP and DBP; Model 4, adjusted for SBP, DBP, and urinary TGF-B1

increased blood pressure in the patients, related to the renin–angiotensin system, which can activate TGF-β1.[32–35] In this study, the urinary TGF-β1 concentration was correlated with SBP. It is suggested that the absence of correlation between TGF-β1 and UACR and eGFR was because most of the study subjects received antihypertensive medication, especially angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, which can potentially decrease the level of urinary TGF-β1.[22] It is reported that consumption of losartan can cause a decrease in urinary TGF-β1 levels of DM patients with diabetic nephropathy.[34] Drugs used for cardiovascular disease are also known to reduce TGF-β1 concentration.[32,36,37]

The oxidative stress together with activation of TGF-β1 has the potential to cause various fibrosis-related diseases.[38] The clinical background of subjects revealed a history of cardiovascular diseases, ranging from hyperlipidemia to heart failure, and that some patients were on cardiovascular therapy, such as telmisartan, simvastatin, and isosorbide dinitrate. Additionally, the decreased blood pressure was also suspected to lead to the decrease in urinary TGF-β1 in diabetic nephropathy patients. Although these conditions are commonly observed in the kidneys in a pathological state, TGF-β1 concentration decreases when the blood pressure, especially SBP, decreases.[32,39]

Conclusion

There was an increase of urinary TGF-β1 concentrations in albuminuria patients clinically, but not statistically. The concentration of TGF-β1 was not correlated with UACR and eGFR, but correlated with SBP. Therefore, urinary TGF-β1 concentration might not be an independent marker for the renal function in DM.

Abbreviations

AGEs, advanced glycation end products

DBP, Diastolic Blood Pressure

DM, diabetes mellitus

ECM, extracellular matrix

eGFR, estimated glomerular filtration rate

ESRD, end-stage renal disease

SBP, Systolic Blood Pressure

TGF B1, Transforming Growth Factor B1

UACR, urine albumin-to-creatinine ratio

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Disclosure

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