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ORIGINAL ARTICLE

Fractional excretion of tumor necrosis factor receptor 1 and 2 in patients with type 2 diabetes and normal renal function

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ABSTRACT

Aims/Introduction: Increased concentrations of serum tumor necrosis factor (TNF) receptors (TNFRs; TNFR1 and TNFR2) are positively associated with the urinary albumin-to-creatinine ratio (ACR), and negatively associated with the estimated glomerular filtration rate (eGFR) in patients with type 2 diabetes. However, the mechanism underlying this increase and the relationship between TNFRs in serum, and urine and kidney measures (ACR and eGFR) are unclear.

Materials and Methods: This was a cross-sectional study that included 499 patients with type 2 diabetes and eGFR ≥60 mL/min/1.73 m^2. The concentrations of TNFRs in serum and urine, and their respective fractional excretion, were measured.

Results: Serum and urinary TNFR levels were positively associated with the ACR, and negatively associated with the eGFR. The fractional excretion of TNFRs did not differ between patients with an eGFR ≥90 and those with an eGFR 60–89 mL/min/1.73 m^2, and also did not correlate with eGFR. After adjustment for relevant covariates, the serum TNFRs were associated with a lower eGFR (60–89 mL/min/1.73 m^2) and an increased ACR (≥30 mg/gCr), but urinary TNFRs were associated with an increased ACR (≥30 mg/gCr) alone, in the multivariate logistic model.

Conclusions: The pattern of fractional excretion TNFRs showed that an increase in serum TNFRs might result from their increased systemic production, including in the kidney, rather than being a simple reflection of GFR decline. Kidney measures appear to be strongly associated with serum TNFRs rather than urinary TNFRs in patients with type 2 diabetes and normal renal function.

INTRODUCTION

Patients with type 2 diabetes and microalbuminuria or macroalbuminuria die more often from cardiovascular diseases than from progression to macroalbuminuria or end-stage renal disease, respectively[1]. Chronic inflammation plays a critical role in the pathophysiology of diabetic kidney disease, and has been considered to be one of the non-traditional mechanisms that contributes to renal impairment in patients with diabetes[2–4]. A growing body of evidence indicates that concentrations of circulating tumor necrosis factor (TNF) receptors (TNFRs; i.e., TNFR1 and 2) are positively correlated with the urinary albumin-to-creatinine ratio (ACR), and negatively correlated with estimated glomerular filtration rate (eGFR) in patients with a wide variety of kidney diseases[5,6]. These biomarkers also predict not only future GFR decline, but also all-cause mortality in patients with diabetes and other kidney diseases[7–9]. However, we do not know the main source of TNFRs in patients with diabetes or whether urinary TNFRs are also related to kidney measures, such as ACR and eGFR.

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The purpose of the present study was to evaluate whether increased concentrations of serum TNFRs simply reflect impaired renal handling of these proteins, and also to determine which biomarkers – serum or urinary TNFRs – are closely associated with kidney measures. We therefore measured serum and urinary TNFR levels, and calculated the fractional excretion (FE) of TNFRs in patients with type 2 diabetes and an eGFR ≥ 60 mL/min/1.73 m^2.

METHODS

Patients, clinical and laboratory measurements

Ethical approval was obtained from the institutional review board of Kure Medical Center and Chugoku Cancer Center, Hiroshima, Japan. The study was carried out in accordance with the guidelines in the Declaration of Helsinki. We recruited Japanese patients with diabetes from Kure Medical Center and Chugoku Cancer Center, as previously described[5]. This is an ongoing study on the natural course of kidney disease in patients with diabetes. In brief, 738 Japanese patients with diabetes agreed to participate in an observational follow-up study. Of those, 499 participants were included in this cross-sectional study, after excluding patients with eGFR <60 mL/min/1.73 m^2 (stage 3–5 chronic kidney disease; n = 140), type 1 diabetes (n = 80) and secondary diabetes (n = 19). We included patients with normal renal function (eGFR ≥60 mL/min/1.73 m^2), because FE strongly depended on their renal function. Of these, two were missing information on smoking status, and seven on diabetic retinopathy.

Data on clinical characteristics, including age, sex, weight, height, duration of diabetes, smoking habits, history of cardiovascular disease (CVD), presence of diabetic retinopathy, and use of medication for hypertension, diabetes and dyslipidemia, were collected from medical histories. CVD was defined as angina after percutaneous coronary intervention, myocardial infarction, hemorrhagic stroke or ischemic stroke. Body mass index was calculated by dividing the weight (kg) by height squared (m^2). Glycated hemoglobin A1c (HbA1c) levels were measured by high-performance liquid chromatography. Serum lipids and uric acid were measured by biochemical autoanalyzer. Non-high-density lipoprotein cholesterol (non-HDL-C) levels were calculated by subtracting the HDL-C level from a total cholesterol level. The precision of Japanese GFR equations based on serum cystatin C was significantly better in GFR 90–119 mL/min/1.73 m^2 and total participants compared with that of Japanese GFR equations based on serum creatinine[10]. Therefore, in the present study, renal function was estimated using the following equation that was especially designed for a Japanese population: eGFR (mL/min/1.73 m^2) = (104 × [serum cystatin C]$^{-1.019}$ × 0.996^{Age} [×0.929 for women]) – 8[11]. Urinary albumin and Cr were analyzed by a nephelometry assay (N-assay TIA Micro Alb; Nittobo Medical Co., Ltd., Fukushima, Japan) and an enzymatic method, respectively. The ACR was used as an index of urinary albumin excretion, and expressed as milligrams of albumin per gram of Cr (mg/gCr). Blood and spot urine samples were obtained and stored at −80°C before measurements were taken.

Measurements of serum and urinary TNFRs and FE of TNFRs

Concentrations of serum and urinary TNFRs were detected by enzyme-linked immunosorbent assays (cat. nos. DRT 100, DRT 200; R&D Systems, Minneapolis, MN, USA), as described previously[12]. The FE of TNFRs was calculated using the following equation: FE of TNFRs (%) = 100 × (urinary TNFRs [pg/mL] × serum Cr [mg/dL] / serum TNFRs [pg/mL] × urinary Cr [mg/dL]).

Statistical analysis

Continuous variables are expressed as the mean ± standard deviation or median (25–75th percentile) depending on their distribution. Asymmetric distributed parameters were logarithmically transformed before analysis. Patients were stratified into two groups according to their eGFR (60–89 or ≥90 mL/min/1.73 m^2) or ACR (<30 [normoalbuminuria], 30–299 [microalbuminuria] or ≥300 [macroalbuminuria] mg/gCr). The Mann–Whitney U-test or Kruskal–Wallis test was used for comparisons of continuous variables. Dichotomous variables were assessed using χ^2-tests and they are expressed as percentages. Correlation among the two kidney measures (ACR and eGFR) and the serum and urinary TNFRs were assessed using Spearman's correlation. Univariate logistic regression analysis was carried out to examine the association of baseline variables with lower eGFR or higher ACR. Next, the multivariate model was reduced by minimizing the Akaike's information criterion, and the independent effect of serum and urinary TNFRs on two kidney measures was examined in the presence of relevant clinical covariates. Multivariate linear regression analysis was carried out to determine the contribution of the clinical factors to FE TNFR. Statistical significance was defined as a P-value <0.05. All data were analyzed using SAS 9.4 software (SAS Institute, Cary, NC, USA).

RESULTS

Patients characteristics

The baseline clinical and demographic characteristics of the study patients are presented in Table 1. Overall, the 499 patients with type 2 diabetes were included in a cross-sectional study. The mean age was 64 ± 13 years, and men were predominant (61%; 305 men). The mean body mass index was 25.0 ± 4.5 kg/m^2, duration of diabetes was 15 ± 11 years and HbA1c was 7.4% ± 1.2%. The study patients were stratified into two groups according to eGFR. No difference was found in the duration of diabetes, prior CVD, body mass index, systolic blood pressure, uric acid, HDL-C, non-HDL-C or HbA1c levels between the two groups (i.e., eGFR ≥90 [G1] and eGFR 60–89 [G2] mL/min/1.73 m^2). Patients in the G2 group were older, were more likely to be women, had a history of diabetic retinopathy, higher ACR, lower diastolic blood pressure and lower hemoglobin level, and were mostly non-smokers.

Table 1 | Characteristics of the study patients by estimated glomerular filtration rate level

Characteristic	G1 eGFR ≥90 (mL/min/1.73 m^2) (n = 251)	G2 eGFR 60–89 (mL/min/1.73 m^2) (n = 248)	P
eGFR (mL/min/1.73 m^2)	106 (98–119)	76 (68–84)	by design
Age (years)	60 ± 14	67 ± 10	<0.0001
Male sex (%)	79.3	42.7	<0.0001
BMI (kg/m^2)	25.2 ± 4.5	24.9 ± 4.5	0.53
Duration of diabetes (year)	14 ± 11	16 ± 11	0.09
ACR (mg/g·Cr)	18 (7–62)	22 (10–101)	0.005
Diabetic retinopathy (%)	27.2	38.4	0.008
HbA1c (%)	7.4 ± 1.2	7.3 ± 1.1	0.14
Insulin treatment (%)	28.3	29.8	0.70
GLP-1RA treatment (%)	5.2	4.8	0.86
Sys BP (mmHg)	137 ± 16	140 ± 17	0.07
Dia BP (mmHg)	81 ± 11	77 ± 11	0.001
Hypertension treatment (%)	46.2	60.9	0.001
RASi treatment (%)	39.0	51.6	0.005
HDL-C (mg/dL)	52 ± 14	53 ± 13	0.43
Non-HDL-C (mg/dL)	132 ± 33	131 ± 33	0.76
Statin treatment (%)	53.8	51.6	0.63
Current smoking (%)	23.6	11.7	<0.0001
Prior CVD (%)	11.2	12.9	0.55
Hemoglobin (g/dL)	14.4 ± 1.6	13.3 ± 1.5	<0.0001
Uric acid (mg/dL)	5.1 ± 1.3	5.3 ± 1.3	0.20
Serum TNFR1 (pg/mL)	1,356 (1,081–1,602)	1,614 (1,290–2,015)	<0.0001
Serum TNFR2 (pg/mL)	2,886 (2,305–3,469)	3,395 (2,806–4,256)	<0.0001
Urinary TNFR1 (ng/gCr)	2,541 (1,688–3,742)	3,008 (2,011–4,474)	0.0008
Urinary TNFR2 (ng/gCr)	4,539 (3,296–6,687)	5,834 (4,070–7,920)	<0.0001
FE TNFR1 (%)	1.30 (0.97–1.93)	1.41 (0.95–2.11)	0.27
FE TNFR2 (%)	1.15 (0.82–1.59)	1.25 (0.90–1.76)	0.09

Data are the mean ± standard deviation, median (quartiles) or percentage. ACR, urinary albumin-to-creatinine ratio; BMI, body mass index; CVD, cardiovascular disease; Dia BP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FE, fractional excretion; GLP-1RA, glucagon-like peptide-1 receptor agonists; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; RASi, renin–angiotensin system inhibitor; Sys BP, systolic blood pressure; TNFR, tumor necrosis factor receptor.

Compared with patients in the G1 group, patients in the G2 group were prescribed antihypertensive drugs more frequently. In contrast, the use of hypoglycemic and antidyslipidemic drugs did not differ between both the groups. Very few patients (n = 10) received treatment with sodium–glucose cotransport protein 2 inhibitor. The serum and urinary TNFR levels in the G2 group were significantly higher than those in the G1 group. The baseline characteristics of the study patients, who were stratified into the two groups according to ACR, are shown online in Table S1.

Correlation between TNFR levels and kidney measures: Spearman's rank correlation

As shown in Table 2, the concentrations of TNFRs in serum and urine significantly and positively correlated with ACR, whereas the concentrations of those significantly and negatively correlated with eGFR. The concentration of TNFRs in serum correlated closely with two renal measures than that of TNFRs in urine (ACR [serum TNFR1, r = 0.37; serum TNFR2, r = 0.30; urinary TNFR1, r = 0.32; urinary TNFR2, r = 0.31], eGFR [serum TNFR1, r = –0.39; serum TNFR2, r = –0.39; urinary TNFR1, r = –0.19; urinary TNFR2, r = –0.24]). The concentrations of TNFRs in serum and urine were weakly correlated with each other (serum TNFR1 vs urinary TNFR1, r = 0.28; serum TNFR1 vs urinary TNFR2, r = 0.22; serum TNFR2 vs urinary TNFR1, r = 0.28; serum TNFR2 vs urinary TNFR2, r = 0.28). Notably, the strong correlation was observed between the two serum or urinary TNFRs (serum TNFR1 and serum TNFR2, r = 0.87; urinary TNFR1 and urinary TNFR2, r = 0.91).

Association between TNFR levels and eGFR using univariate and multivariate logistic analysis

Univariate logistic regression analyses showed that many clinical characteristics were associated with a lower eGFR, as shown in Table 3. Multivariate logistic regression analysis was carried

Table 2 | Spearman's correlation coefficients among urinary and serum tumor necrosis factor receptor, urinary albumin-to-creatinine ratio and estimated glomerular filtration rate

Characteristic	eGFR	Serum TNFR1	Serum TNFR2	Urinary TNFR1	Urinary TNFR2
ACR	–0.15**	0.37*	0.30*	0.32*	0.31*
eGFR	–	–0.39*	–0.39*	–0.19*	–0.24*
Serum TNFR1	–	–	0.87*	0.28*	0.22*
Serum TNFR2	–	–	–	0.28*	0.28*
Urinary TNFR1	–	–	–	–	0.91*

*$P < 0.0001$, **$P < 0.0005$. Tumor necrosis factor receptor (TNFR), urinary albumin-to-creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) were handled after common logarithmic transformation.

Table 3 | Univariate and multivariate logistic regression analysis of the factors influencing lower estimated glomerular filtration rate in study patients according to clinical covariates and tumor necrosis factor receptor biomarkers

	Univariate model: OR (95% CI)		Multivariate model: OR (95% CI)			
(One unit of increase)		*P*	Clinical factors only	*P*	Clinical factors and each TNFR†	*P*
Age	1.05 (1.03–1.06)	<0.0001	1.08 (1.05, 1.10)	<0.0001	1.08 (1.06–1.11)	<0.0001
Sex (male)	0.20 (0.13–0.29)	<0.0001	0.07 (0.04, 0.12)	<0.0001	0.004 (0.002–0.012)	<0.0001
Duration of diabetes	1.01 (0.997–1.03)	0.10				
BMI	0.99 (0.95–1.03)	0.58				
Diabetic retinopathy	1.67 (1.14–2.44)	0.008				
Current smoking	0.43 (0.27–0.70)	0.0007				
Sys BP	1.01 (0.999–1.02)	0.07				
Dia BP	0.97 (0.96–0.99)	0.001				
Hypertension treatment	1.81 (1.27–2.59)	0.001				
RASi treatment	1.67 (1.17–2.38)	<0.005				
Uric acid	1.11 (0.97–1.28)	0.12	1.84 (1.50, 2.25)	<0.0001	2.15 (1.68–2.76)	<0.0001
HDL-C	1.00 (0.99–1.02)	0.66				
Non-HDL-C	0.999 (0.99–1.00)	0.75				
Statin treatment	0.92 (0.65–1.30)	0.63				
Hemoglobin	0.64 (0.56–0.73)	<0.0001	0.78 (0.68, 0.91)	0.001	0.91 (0.77–1.08)	0.27
HbA1c	0.87 (0.74–1.01)	0.07				
Insulin treatment	1.08 (0.73–1.59)	0.70				
GLP-1RA treatment	0.93 (0.42–2.08)	0.86				
Prior CVD	1.18 (0.69–2.03)	0.55				
ACR (1 SD = 0.70)	1.30 (1.08–1.55)	0.005	1.35 (1.08, 1.69)	0.007	0.80 (0.60–1.06)	0.13
Serum TNFR1 (1 SD = 0.126)	1.95 (1.59–2.39)	<0.0001			7.81 (4.95–12.30)	<0.0001
Serum TNFR2 (1 SD = 0.127)	1.90 (1.56–2.32)	<0.0001			5.48 (3.68–8.17)	<0.0001
Urinary TNFR1 (1 SD = 0.270)	1.35 (1.13–1.62)	0.001			1.30 (1.01–1.66)	0.04
Urinary TNFR2 (1 SD = 0.237)	1.43 (1.19–1.72)	0.0002			1.23 (0.96–1.57)	0.10

Tumor necrosis factor receptor (TNFR) and urinary albumin-to-creatinine ratio (ACR) were handled after common logarithmic transformation. †The effect of each TNFR marker was examined separately while controlling for clinical factors. Odds ratios (OR) for clinical factors are from the multivariate model with serum TNFR1. BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; Dia BP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GLP-1RA, glucagon-like peptide-1 receptor agonists; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; RASi, renin–angiotensin system inhibitor; Sys BP, systolic blood pressure.

out to evaluate the association of eGFR with clinical parameters. Age, sex, hemoglobin and ACR levels remained significant clinical factors of lower eGFR (<90 mL/min/1.73 m^2) in the multivariate analysis. We next hypothesized that each TNFR in serum and urine might be associated with eGFR independently of clinical factors. To test this possibility, we repeated multivariate analysis. In these models, serum TNFRs and urinary TNFR1 remained significant after taking into account all relevant covariates. Finally, to examine the independent effects of TNFRs in serum and urine for lower eGFR, we added TNFR1

in serum and urine simultaneously to clinical model. In this model, serum TNFR1, but not urinary TNFR1, remained significant (serum TNFR1 odds ratio [(OR] 7.89, 95% confidence interval [CI] 6.46–9.63; $P < 0.001$; urinary TNFR1 0.96; 0.72–1.29, $P = 0.80$).

Although urinary TNFR levels did not differ between the male and female patients, the serum TNFR levels in the male patients were significantly higher than those in the female patients (Table S2). Therefore, we carried out a subanalysis stratified by sex (Table S3). By and large, the impact of TNFR levels on eGFR was mostly similar for all the patients, although serum TNFR levels were only associated with eGFR in female patients.

Association between TNFR levels and ACR: Univariate and multivariate logistic analysis

Similar analysis was carried out to evaluate the association of ACR with clinical parameters. As shown in Table 4, findings from the multivariate logistic regression analysis showed that the presence of diabetic retinopathy, prior CVD, HbA1c, systolic blood pressure and uric acid remained significant clinical factors of higher ACR (≥30 mg/gCr). After adjusting for relevant covariates, each TNFR in serum and urine remained significant. The impact of serum and urinary TNFR levels on ACR was almost equivalent. Furthermore, to examine the independent effects of TNFRs in serum and urine for higher ACR, we added TNFR1 in serum and urine simultaneously to the clinical model. High concentrations of both TNFRs in serum and urine were significantly associated with higher ACR, even after adjustment for clinical factors (serum TNFR1 OR,1.61, 95% CI 1.28–2.02; $P < 0.001$; urinary TNFR1 OR 1.57, 95% CI 1.25–1.96, $P < 0.0001$). In addition, the level of each TNFR in the serum and urine was associated with ACR, even after stratification by sex (Table S4).

Table 4 | Univariate and multivariate logistic regression analysis of the factors influencing higher albuminuria (micro- or macro-albuminuria) in study patients according to clinical covariates and tumor necrosis factor receptor biomarkers

	Univariate model: OR (95% CI)		Multivariate model: OR (95% CI)			
(One unit of increase)		*P*	Clinical factors only	*P*	Clinical factors and each TNFR†	*P*
Age	1.01 (0.99–1.02)	0.30				
Sex	1.47 (1.02–2.13)	0.04				
BMI	1.06 (1.02–1.10)	0.006				
Duration of diabetes	1.03 (1.01–1.05)	0.0007				
Diabetic retinopathy	2.18 (1.49–3.20)	<0.0001	2.10 (1.41–3.13)	0.0003	1.87 (1.24–2.82)	0.003
Current smoking	1.34 (0.85–2.14)	0.21				
Sys BP	1.02 (1.01–1.03)	0.0001	1.02 (1.01–1.03)	0.0009	1.02 (1.01–1.03)	0.002
Dia BP	1.01 (0.998–1.03)	0.09				
Hypertension treatment	1.68 (1.17–2.41)	0.005				
RASi treatment	1.97 (1.37–2.82)	0.0003				
Uric acid	1.18 (1.02–1.35)	0.02	1.21 (1.04–1.41)	0.01	1.12 (0.96–1.31)	0.16
Hemoglobin	0.96 (0.86–1.07)	0.47				
HbA1c	1.21 (1.04–1.42)	0.02	1.28 (1.14–3.66)	0.004	1.25 (1.06–1.48)	0.009
Insulin treatment	1.38 (0.93–2.04)	0.11				
GLP-1RA treatment	1.92 (0.85–4.32)	0.12				
HDL-C	0.99 (0.98–1.01)	0.23				
Non-HDL-C	1.00 (0.997–1.01)	0.37				
Statin treatment	1.07 (0.75–1.53)	0.71				
Prior CVD	2.09 (1.21–3.61)	0.008	2.04 (1.14–3.66)	0.02	1.56 (0.85–2.87)	0.16
eGFR (1 SD = 0.10)	0.85 (0.70–1.02)	0.08				
Serum TNFR1 (1 SD = 0.126)	2.02 (1.65–2.47)	<0.0001			1.80 (1.45–2.23)	<0.0001
Serum TNFR2 (1 SD = 0.127)	1.79 (1.47–2.17)	<0.0001			1.62 (1.31–1.99)	<0.0001
Urinary TNFR1 (1 SD = 0.270)	1.82 (1.49–2.23)	<0.0001			1.79 (1.43–2.22)	<0.0001
Urinary TNFR2 (1 SD = 0.237)	1.75 (1.43–2.15)	<0.0001			1.64 (1.32–2.04)	<0.0001

Tumor necrosis factor receptor (TNFR) and estimated glomerular filtration rate (eGFR) were handled after common logarithmic transformation. †The effect of each TNFR marker was examined separately while controlling for clinical factors. Odds ratios (OR) for clinical factors are from the multivariate model with serum TNFR1. BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; Dia BP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GLP-1RA, glucagon-like peptide-1 receptor agonists; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; RASi, renin–angiotensin system inhibitor; Sys BP, systolic blood pressure.

FE of TNFRs in patients with type 2 diabetes and normal renal function

To elucidate the source of TNFRs, we measured the FE of TNFRs in patients with type 2 diabetes. In the present study, we included patients with normal renal function (eGFR ≥60 mL/min/1.73 m^2), because FE strongly depended on their renal function. No difference was found in the FE of TNFRs between the two groups (Table 1), and the FE of TNFRs did not correlate with eGFR (TNFR1 $r = -0.05$; TNFR2 $r = -0.07$). Next, to examine the factors clinically relevant to FE TNFR, the patients were stratified into two groups according to the FE TNFR levels. Many clinical factors were associated with FE TNFR levels, as shown in Table S5. However, these clinical factors could explain only a fraction of FE TNFR in multivariate linear regression analysis (Table 5). In contrast, a scatter plot of the relationship between FE TNFR and serum or urinary TNFR levels showed that urinary TNFR levels are strongly associated with FE TNFR levels. However, serum TNFR levels are not strongly associated with FE TNFR levels (Figure 1).

DISCUSSION

The present cross-sectional study showed that two kidney measures (eGFR and ACR) were more strongly associated with serum TNFRs than with urinary TNFRs in patients with type 2 diabetes and normal renal function. Furthermore, after taking into account relevant clinical factors, serum TNFRs remained independently associated with both kidney measures, whereas urinary TNFRs were associated with only the ACR. The serum TNFR levels of G2 were significantly higher than those in G1; however, no difference was found in the FE of TNFRs between the two groups. Furthermore, the FE of TNFRs also did not correlate with the eGFR. Therefore, increased serum TNFR levels are possibly explained not so much by the decreased renal loss, but rather by the elevated production from any tissue, including the kidney.

We previously reported that TNFRs in serum and urine were positively associated with ACR, and negatively associated with eGFR in patients with immunoglobulin A nephropathy[13,14]. As with the present study, serum TNFR levels were strongly associated with eGFR and ACR compared with urinary TNFR levels, although approximately one-quarter of patients had decreased renal function. Idasiak-Piechocka *et al.*[15] showed that age and urinary TNFR1 levels are independently associated with baseline renal function in patients with biopsy-proven glomerulonephritis, and relatively maintained renal function. In contrast to their study, the present study showed that serum TNFRs, except urinary TNFRs, are factors that contribute to eGFR in patients with type 2 diabetes and normal renal function. Compared with the present study, their renal function (estimated Cr clearance: 75 ± 39 mL/min/1.73 m^2), which was estimated by the Cockcroft and Gault formula, seems to be low, although the estimated method of renal function was different between both studies. Serum TNFRs might be sensitive markers for eGFR compared with urinary TNFRs, especially in patients with normal renal function. Indeed, the Joslin group had shown that serum TNFRs, except urinary TNFRs, predict stage 3 CKD in patients with type 1 diabetes and baseline normal renal function[8].

One might wonder where the TNFRs come from. The kidneys do not seem to be the main source of TNFRs, considering the strong relationship between kidney measures (eGFR and ACR) and serum TNFRs, as compared with urinary TNFRs. In fact, the pattern of the FE of TNFRs showed that increased serum TNFRs might result from their increased systemic production, including in the kidney, rather than being a simple reflection of GFR decline. Recently, Niewczas *et al.*[16] made a reasonable guess about the sources of TNFRs in humans using three different approaches, as follows. First, they showed that the levels of many serum TNFR superfamily members including TNFRs predict future end-stage renal disease in patients with diabetes, and that patients with a fast decline in renal function also have increased levels of urinary TNFR superfamily members in accordance with serum levels, as compared with patients who have no or slow renal function decline many years before the onset of end-stage renal disease. Therefore, those researchers suggested that elevated serum TNFR levels caused systemic overproduction rather than impaired renal handling. Next, they used serum and kidney biopsy samples obtained from Pima people with type 2 diabetes. Accordingly,

Table 5 | Stepwise multivariate regression analysis of the factors associated with fractional excretion tumor necrosis factor receptor 1 and fractional excretion tumor necrosis factor receptor 2

Variable	Parameter estimate	SE	*t*-value	$P > t$
Fractional excretion tumor necrosis factor receptor 1				
Age	0.005	0.0010	4.56	<0.0001
Male sex	0.084	0.0239	3.51	0.0005
HbA1c	0.028	0.0099	2.89	0.004
Duration of diabetes	0.003	0.0011	2.69	0.007
ACR	0.043	0.0159	2.68	0.008
HDL-C	0.002	0.0008	2.60	0.02
Uric acid	−0.023	0.0091	−2.56	0.006
RASi treatment	0.052	0.0226	2.30	0.02
Multiple R^2: 0.206; Adjusted multiple R^2: 0.193				
Fractional excretion tumor necrosis factor receptor 2				
Age	0.004	0.0009	4.47	<0.0001
Male sex	0.046	0.0021	2.26	0.0005
HbA1c	0.017	0.0087	2.01	0.004
Duration of diabetes	0.003	0.0010	2.69	0.007
ACR	0.032	0.0143	2.25	0.008
HDL-C	0.003	0.0007	3.41	0.01
RASi treatment	0.038	0.0204	1.86	0.02
Multiple R^2: 0.170; Adjusted multiple R^2: 0.159				

Urinary albumin-to-creatinine ratio (ACR) was handled after common logarithmic transformation. HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; RASi, renin–angiotensin system inhibitor; SE, standard error.

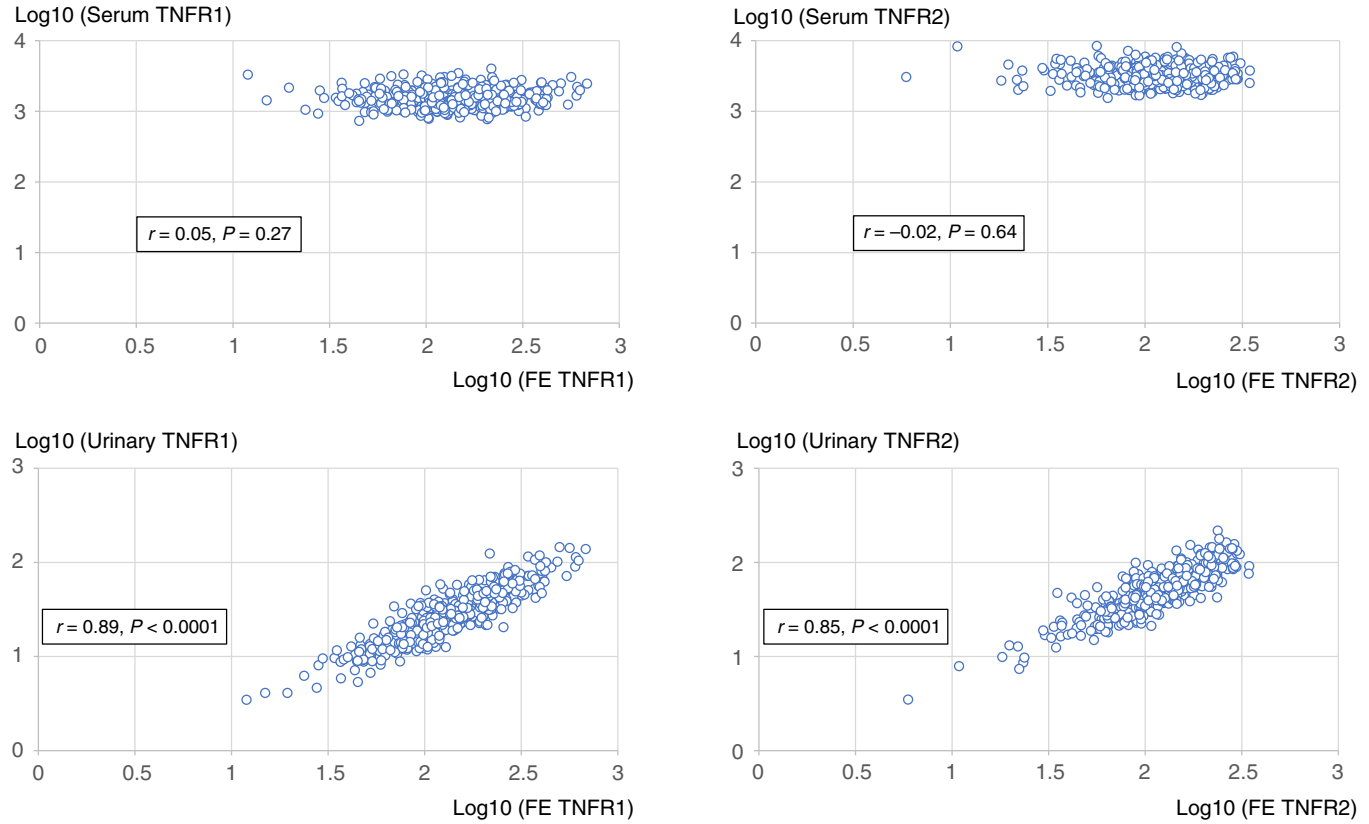

Figure 1 | Scatter plot of the relationship between fractional excretion (FE) tumor necrosis factor receptor (TNFR) and serum or urinary TNFR levels.

no correlation was found between circulating TNFR levels and their gene expression in the tubulointerstitium, although there was only a weak association between circulating TNFRs and glomerular expressions of these genes. Finally, using diabetic kidney specimens from the 1,000 Kidney Genome Project[17], the researchers examined the relationship between the messenger ribonucleic acid (mRNA) levels of renal TNFRs and the histopathological indices of diabetic kidney disease, such as glomerular sclerosis, tubulointerstitial fibrosis and lymphocyte infiltration. The mRNA expression levels of TNFRs in the glomeruli and TNFR1 mRNA in the tubulointerstitium did not relate to the histopathological indices, although TNFR2 mRNA expression in the tubulointerstitium was weakly associated with tubulointerstitial fibrosis and lymphocyte infiltration. Further research is required to elucidate the main sources of TNFRs in patients with diabetes.

In conclusion, the present study suggests that serum TNFR, except urinary TNFR, levels are associated with eGFR after adjustment for clinical covariates in patients with type 2 diabetes and normal renal function. Increased serum TNFR levels are possibly due to elevated production of any tissue, including kidneys. Further studies are required to determine the source of TNFRs in diabetes patients.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Characteristics of the study patients by albuminuria level.
Table S2 | Serum and urinary tumor necrosis factor receptor levels of the study patients by sex.
Table S3 | Multivariate logistic regression analysis of the factors influencing lower estimated glomerular filtration rate in the study patients by sex.
Table S4 | Multivariate logistic regression analysis of the factors influencing higher urinary albumin-to-creatinine ratio (micro- or macro-albuminuria) in the study patients by sex.
Table S5 | Characteristics of the study patient by fractional excretion tumor necrosis factor receptor levels.