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

Tomohito Gohda1*, Nozomu Kamei2, Mitsunobu Kubota4, Kanako Tanaka4, Yoshinori Yamashita3, Hiroko Sakuma1, Chiai Kishida1, Eri Adachi1, Takeo Koshida1, Makio Murakoshi1, Shinji Hagiwara5, Kazuhiko Funabiki5, Seiji Ueda1, Yusuke Suzuki1

1Department of Nephrology, Faculty of Medicine, Juntendo University, Tokyo, Japan, 2Department of Endocrinology and Metabolism, Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital, Hiroshima, Japan, 3Institute for Clinical Research, National Hospital Organization, Kure Medical Center and Chugoku Cancer Center, Hiroshima, Japan, 4Department of Endocrinology and Diabetology, National Hospital Organization, Kure Medical Center and Chugoku Cancer Center, Hiroshima, Japan, and 5Department of Kidney and Hypertension, Juntendo Tokyo Koto Geriatric Medical Center, Tokyo, Japan

Keywords
Biomarker, Diabetic kidney disease, Tumor necrosis factor receptor

*Correspondence
Tomohito Gohda
Tel.: +81-3-5802-1065
Fax: +81-3-3813-1183
E-mail address: goda@juntendo.ac.jp

J Diabetes Investig 2021; 12: 382–389
doi: 10.1111/jdi.13351

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 < 90 mL/min/1.73 m². 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 m², and also did not correlate with eGFR. After adjustment for relevant covariates, the serum TNFRs were associated with a lower eGFR (≥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, respectively1. 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 diabetes2–4. 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 diseases5–6. These biomarkers also predict not only future GFR decline, but also all-cause mortality in patients with diabetes and other kidney diseases7–9. 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.
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 m².

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. 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 m² (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 m²), 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 (m²). 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 the present study, renal function was estimated using the following equation: FE of TNFRs (%) = 100 × (serum TNFRs [pg/mL] × serum Cr [mg/dL]) / serum TNFRs [pg/mL] × urinary Cr [mg/dL]).

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/m², duration of diabetes was 15 ± 11 years and HbA1c was 7.4% ± 1.2%. The study patients were stratified into two groups according to their eGFR (60–89 or ≥90 mL/min/1.73 m²) 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 χ²-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).

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 m²) 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 χ²-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/m², 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 m²). 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.

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. The FE of TNFRs was calculated using the following equation: FE of TNFRs (%) = 100 × (serum TNFRs [pg/mL] × serum Cr [mg/dL]) / serum TNFRs [pg/mL] × urinary Cr [mg/dL]).
Table 1 | Characteristics of the study patients by estimated glomerular filtration rate level

| Characteristic                          | G1 eGFR ≥90 (mL/min/1.73 m²) (n = 251) | G2 eGFR 60–89 (mL/min/1.73 m²) (n = 248) | P       |
|----------------------------------------|----------------------------------------|----------------------------------------|---------|
| eGFR (mL/min/1.73 m²)                  | 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/m²)                            | 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 (%)               | 272                                    | 384                                    | 0.008   |
| Uric acid (mg/dL)                      | 7.4 ± 1.2                              | 7.3 ± 1.1                              | 0.14    |
| Serum TNFR1 (pg/mL)                   | 283                                    | 298                                    | 0.70    |
| Serum TNFR2 (pg/mL)                   | 4.5                                    | 4.8                                    | 0.86    |
| Hemoglobin (g/dL)                      | 137 ± 16                               | 140 ± 17                               | 0.07    |
| Hypertension treatment (%)             | 81 ± 11                                | 77 ± 11                                | 0.0001  |
| Sys BP (mmHg)                          | 462                                    | 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 (%)                          | 1.12                                   | 1.29                                   | 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 antidyshlipidemic drugs did not differ between both the groups. Very few patients (n = 10) received treatment with sodium–glucose cotransport 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...
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 m²) 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

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*         |
| Urinary TNFR2  | -     | -          | -          | -             | -             |

*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

| (One unit of increase) | Univariate model: OR (95% CI) | Multivariate model: OR (95% CI) |
|------------------------|-------------------------------|---------------------------------|
|                        | P                             | Clinical factors only           | Clinical factors and each TNFR† | P                |
| Age                    | 1.05 (1.03–1.06)              | <0.0001                         | 1.08 (1.05, 1.10)               | <0.0001          |
| Sex (male)             | 0.20 (0.13–0.29)              | <0.0001                         | 0.07 (0.04, 0.12)               | <0.0001          |
| Duration of diabetes   | 1.01 (0.997–1.03)             | 0.10                            | 1.84 (1.50, 2.25)              | <0.0001          |
| BMI                    | 0.99 (0.95–1.03)              | 0.58                            | 2.15 (1.68–2.76)               | <0.0001          |
| Diabetic retinopathy   | 1.67 (1.14–2.44)              | 0.008                           | 0.78 (0.68, 0.91)              | 0.001            |
| Current smoking        | 0.43 (0.27–0.70)              | 0.0007                          | 0.91 (0.77–1.08)               | 0.27             |
| Sys BP                 | 1.01 (0.99–1.02)              | 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          |
| HDL-C                  | 1.00 (0.99–1.02)              | 0.66                            | 2.15 (1.68–2.76)               | <0.0001          |
| 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            |
| HbA1c                  | 0.87 (0.74–1.01)              | 0.07                            | 0.91 (0.77–1.08)               | 0.27             |
| 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            |
| Serum TNFR1 (1 SD = 0.126) | 1.95 (1.59–2.39)             | <0.0001                         | 0.80 (0.60–1.06)               | 0.13             |
| Serum TNFR2 (1 SD = 0.127) | 1.90 (1.56–2.32)             | <0.0001                         | 7.81 (4.95–12.30)              | <0.0001          |
| Urinary TNFR1 (1 SD = 0.270) | 1.35 (1.13–1.62)            | 0.001                           | 5.48 (3.68–8.17)               | <0.0001          |
| Urinary TNFR2 (1 SD = 0.237) | 1.43 (1.19–1.72)            | 0.0002                          | 1.30 (1.01–1.66)               | 0.04             |
|                        |                               |                                | 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.
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 (\( \geq 30 \text{mg/gCr} \)). 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**

| Clinical factors only | Clinical factors and each TNFR† |
|-----------------------|--------------------------------|
| **(One unit of increase)** | **Univariate model: OR (95% CI)** | **Multivariate model: OR (95% CI)** |
| Age | 1.01 (0.99–1.02) | 2.10 (1.41–3.13) |
| Sex | 1.47 (1.02–2.13) | 0.003 |
| BMI | 1.06 (1.02–1.10) | 1.87 (1.24–2.82) |
| Duration of diabetes | 1.03 (1.01–1.05) | 0.0007 |
| Diabetic retinopathy | 2.18 (1.49–3.20) | 0.0001 |
| Current smoking | 1.34 (0.85–2.14) | 0.21 |
| Sys BP | 1.02 (1.01–1.03) | 0.0009 |
| Dia BP | 1.01 (0.99–1.03) | 1.02 (1.01–1.03) |
| Hypertension treatment | 1.68 (1.17–2.41) | 0.09 |
| RASi treatment | 1.97 (1.37–2.82) | 0.0003 |
| Uric acid | 1.18 (1.02–1.35) | 1.21 (1.04–1.41) |
| Hemoglobin | 0.96 (0.86–1.07) | 0.04 |
| Hba1c | 1.21 (1.04–1.42) | 0.02 |
| Insulin treatment | 1.38 (0.93–2.04) | 0.02 |
| GLP-1RA treatment | 1.92 (0.85–4.32) | 0.12 |
| HDL-C | 0.99 (0.98–1.01) | 0.12 |
| Non-HDL-C | 1.00 (0.99–1.01) | 0.23 |
| Statin treatment | 1.07 (0.75–1.53) | 0.37 |
| Prior CVD | 2.09 (1.21–3.61) | 0.37 |
| eGFR (1 SD = 0.10) | 0.85 (0.70–1.02) | 0.07 |
| Serum TNFR1 (1 SD = 0.126) | 2.02 (1.65–2.47) | 0.02 |
| Serum TNFR2 (1 SD = 0.127) | 1.79 (1.47–2.17) | <0.0001 |
| Urinary TNFR1 (1 SD = 0.270) | 1.82 (1.49–2.23) | <0.0001 |
| Urinary TNFR2 (1 SD = 0.237) | 1.75 (1.43–2.15) | <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 m²), 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. 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. showed that age and urinary TNFR 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 m²), 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.

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, Niewczasz et al. 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,
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, 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.

ACKNOWLEDGMENTS
The authors thank Mami Yamashita MD, Kiyomi Yagyu, Mizuho Yoshizaki, Shusaku Maeda MD, Yukie Saito, Naoko Kishida, Mikie Shitaune, Machiko Ikeda, Terumi Shibata, Tomoko Tsaboi MD, Sayaka Oomuro and Yoshiko Kimura for their cooperation with the data collection. The authors thank Enago (www.enago.jp) for the English language review. The current study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant number 17K09711, 20K08617 to TG).

DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. Adler AI, Stevens RJ, Manley SE, et al. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64), Kidney Int 2003; 63: 225–232.
2. Gohda T, Tomino Y. Novel biomarkers for the progression of diabetic nephropathy: soluble TNF receptors. Curr Diab Rep 2013; 13: 560–566.
3. Gohda T. A paradigm shift for the concept of diabetic nephropathy. *Juntendo Med J* 2014; 60: 293–299.

4. Murakoshi M, Gohda T, Suzuki Y. Circulating tumor necrosis factor receptors: a potential biomarker for the progression of diabetic kidney disease. *Int J Mol Sci* 2020; 21: 1957.

5. Gohda T, Nishizaki Y, Murakoshi M, et al. Clinical predictive biomarkers for normoalbuminuric diabetic kidney disease. *Diabetes Res Clin Pract* 2018; 141: 62–68.

6. Kamei N, Yamashita M, Nishizaki Y, et al. Association between circulating tumor necrosis factor-related biomarkers and estimated glomerular filtration rate in type 2 diabetes. *Sci Rep* 2018; 8: 15302.

7. Gohda T, Maruyama S, Kamei N, et al. Circulating TNF receptors 1 and 2 predict mortality in patients with end-stage renal disease undergoing dialysis. *Sci Rep* 2017; 7: 43520.

8. Gohda T, Niewczas MA, Ficociello LH, et al. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. *J Am Soc Nephrol* 2012; 23: 516–524.

9. Niewczas MA, Gohda T, Skupien J, et al. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. *J Am Soc Nephrol* 2012; 23: 507–515.

10. Horio M, Imai E, Yasuda Y, et al. Performance of GFR equations in Japanese subjects. *Clin Exp Nephrol* 2013; 17: 352–358.

11. Horio M, Imai E, Yasuda Y, et al. GFR estimation using standardized serum cystatin C in Japan. *Am J Kidney Dis* 2013; 61: 197–203.

12. Lee JE, Gohda T, Walker WH, et al. Risk of ESRD and all cause mortality in type 2 diabetes according to circulating levels of FGF-23 and TNFR1. *PLoS One* 2013; 8: e58007.

13. Sonoda Y, Gohda T, Suzuki Y, et al. Circulating TNF receptors 1 and 2 are associated with the severity of renal interstitial fibrosis in IgA nephropathy. *PLoS One* 2015; 10: e0122212.

14. Murakoshi M, Gohda T, Sonoda Y, et al. Effect of tonsillectomy with steroid pulse therapy on circulating tumor necrosis factor receptors 1 and 2 in IgA nephropathy. *Clin Exp Nephrol* 2017; 21: 1068–1074.

15. Idasiak-Piechocka I, Oko A, Pawliczak E, et al. Urinary excretion of soluble tumour necrosis factor receptor 1 as a marker of increased risk of progressive kidney function deterioration in patients with primary chronic glomerulonephritis. *Nephrol Dial Transplant* 2010; 25: 3948–3956.

16. Niewczas MA, Pavkov ME, Skupien J, et al. A signature of circulating inflammatory proteins and development of end-stage renal disease in diabetes. *Nat Med* 2019; 25: 805–813.

17. Beckerman P, Qiu C, Park J, et al. Human kidney tubule-specific gene expression based dissection of chronic kidney disease traits. *EBioMedicine* 2017; 24: 267–276.

**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.