Gender- and disease-specific urinary thioredoxin in chronic kidney disease patients with or without type 2 diabetic nephropathy

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Gender- and disease-specific urinary thioredoxin in chronic kidney disease patients with or without type 2 diabetic nephropathy

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Running Title: Gender-specific urinary thioredoxin
Abstract

Aim

The role of urinary (U-) thioredoxin (Trx), a class of small redox proteins, in physiological and pathological conditions, in addition to its gender specificity, has been insufficiently determined in chronic kidney disease (CKD) patients, especially in diabetes mellitus (DM) nephropathy.

Methods

U-Trx was measured cross-sectionally in 110 CKD outpatients with estimated glomerular filtration rate (eGFR) of >15 mL/min/1.73 m$^2$, namely, in 57 type 2 DM patients (male (M): n=41, female (F): n=16) and 53 non-DM patients (M: n=33, F: n=20), as well as 30 healthy controls (M: n=11, F: n=19). Comparisons were made among controls, DM and non-DM, and between M and F, and clinical parameters were compared in each group. In addition, a comparison between average U-Trx level and the changes of renal function during a one-year period was performed.

Results

U-Trx was significantly higher in females than in males in controls (p<0.05) and in non-DM patients (p<0.05). Multiple regression analysis revealed that urinary protein (UP)/creatinine (Cr) ratio, female sex and HbA1c were independent factors affecting U-Trx among all subjects (adjusted $R^2$=0.468). In DM patients, U-Trx was negatively correlated with eGFR, especially in males, and positively correlated with UP/Cr and NAG in both sexes (all p<0.01), as well as with systolic blood pressure in all (p<0.05). Average U-Trx was positively correlated with the rate of annual eGFR decline of male (p<0.01) but not female DM patients.

Conclusion

U-Trx might have a gender-specific physiological and pathological role and be a potent marker of renal damage in DM nephropathy.
**Key words:** chronic kidney disease, diabetic nephropathy, gender, oxidative stress, thioredoxin

**Introduction**

Thioredoxin (Trx) is a 12-kDa protein with redox-active dithiol in the active site of -Cys-Gly-Pro-Cys- and is ubiquitous in the human body. It is a defensive protein induced by various stresses and has anti-oxidative, anti-apoptotic and anti-inflammatory effects; it may also play crucial roles in the pathophysiological mechanisms behind metabolic disorders, cancer and inflammation [1]. In the kidney, Trx is retained in medullary thick ascending limb (mTAL) and secreted from proximal tubuli into urine during renal ischemia/reperfusion, against which several in vitro studies have shown its protective activity [2]. Recently, *methodology for measuring human U-TRX has been confirmed by Kasuno K and our members and* found markedly high levels of U-Trx not only in ischemic or inflammatory renal diseases, such as tubulointerstitial nephritis and microscopic polyarteritis nodosa, but also especially in AKI [3], which suggested U-Trx as a potent renal biomarker of oxidative injury. However, *the subjects of this study CKD patients were limited to those with estimated glomerular filtration rate (eGFR) of more than 60 mL/min/1.73 m² and did not include cases of diabetes mellitus (DM), provoking progressive renal injury possibly due to high oxidative stress, nor addressed the influence of gender in physiological and pathological conditions.*

In the present study, to investigate the potency of U-Trx measurement as a marker of CKD progression, for the first time, we performed cross-sectional and longitudinal observation of U-Trx levels in CKD patients with eGFR >15 mL/min/1.73 m², including those with DM. In addition, this observation was performed separately in males and females to analyze the influence of gender.
Methods

Subjects

A total of 110 adult CKD outpatients from stages 1 to 4 (eGFR >15 mL/min/1.73 m²) treated at the authors’ hospital between August 2009 and September 2010 were subjects in this study. Fifty-seven had type 2 DM and 53 did not (non-DM). Patients with the following diseases/conditions were excluded: infectious disease, asthma, malignancy, uncontrollable heart failure, cardiovascular and cerebrovascular disease with an onset within 6 months, hepatitis and HBV or HCV carrier, rheumatoid arthritis and collagen disease, arteriosclerosis obliterans higher than Fontaine III, immunosuppressive drug administration within the past year, and general anesthesia or shock within the past month. Among the non-DM patients, 20 cases were diagnosed as follows: 10 IgA nephropathy, 5 non-IgA mesangioproliferative glomerulonephritis (GN), 2 membranous nephropathy, 2 focal glomerular sclerosis, 1 hypertensive nephrosclerosis and 1 IgM nephropathy. The other cases without renal biopsy were clinically diagnosed as follows: 14 hypertensive nephrosclerosis, 3 chronic glomerulonephritis, 1 gouty nephropathy, 2 polycystic kidney disease, 1 renal stone, 1 obesity nephropathy, 1 renal artery stenosis and 10 CKD high-risk patients (4 hypertension, 3 hyperlipidemia and 3 obesity). For blood pressure or lipid control, the following medications were administered: calcium channel blocker, beta-blocker, angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, diuretics and statin.

Thirty control subjects were obtained from among healthy volunteers and participants in a medical check-up of the whole body at Kitano Hospital who were without specific diseases with eGFR >90 mL/min/1.73 m². Written informed consent to take part in the study was obtained from all participants. This study was carried out in compliance with the Helsinki Declaration and approved by the ethics committee at Kitano Hospital (approval No. 08-09-006).
Cross-sectional analysis of urinary and serum Trx in control and CKD patients

We collected serum and urinary samples at the outpatient clinic of the authors’ hospital, and measured urinary (U-) and serum (S-) Trx in controls and CKD patients by ELISA, as described below, and for U-Trx, disease specificity (DM or non-DM) and gender specificity were examined. These data were compared with other clinical parameters: S-Cr, eGFR, U-Cr ratio of U-protein (UP), U-β2-microglobulin (U-β2MG), U-N-acetyl-β-D-glucosidase (U-NAG), U-8-hydroxydeoxyguanosine (U-8-OHdG), hemoglobin (Hb)A1c, total cholesterol, systolic blood pressure (sBP), smoking and medication. Moreover, we performed multiple regression analysis to identify factors independently affecting U-Trx in control and CKD patients.

Longitudinal studies of the relationship between average U-Trx level and renal function in CKD patients

We analyzed the relationship between the rate of eGFR decline (%/year) calculated as follows: 
\[
\frac{[\text{eGFR value of one year later (mL/min/1.73 m}^2]\ - \text{initial eGFR value (mL/min/1.73 m}^2)]}{\text{initial eGFR value (mL/min/1.73 m}^2)} \times 100,
\]
and average U-Trx level measured at least 2 times with a 5-7-month interval during 1 year. These longitudinal studies were performed in each sex separately.

Measurement of serum and urinary Trx

Periodic urinary samples were centrifuged for 10 minutes immediately after collection, and the supernatants of the samples as well as the serum samples were cryopreserved at -30 °C in a deep freezer. S- and U-Trx were quantified using a commercial ELISA kit for human Trx (Redox...
Bioscience Inc., Kyoto). In this ELISA study, because the values of U-Trx were preliminarily proved to be stable at physiological pHs (pH 5-8), and not to be influenced by the temperature (freeze/thaw) and a presence of cellular components in urine [3], this ELISA system is suitable for use with clinical urine samples.

Examinations of clinical and laboratory parameters

Estimated GFR was calculated as follows: 194 × Cr^{-1.094} × Age^{-0.287} × 0.739 (if female) [4]. The following kits and methods were used: competitive ELISA kits (New 8-OHdG check ELISA, Fukuroi, Shizuoka) [5] for U-8-OHdG, latex agglutination for HbA1c and U-β2MG, Pyrogallol Red-molybdenum method for UP and N-acetylhexosamine oxidase-peroxidase method for U-NAG. All urinary parameters were divided by U-Cr concentration (mg/dL) and then used.

Statistical analysis

All data except urinary markers are expressed as the mean ± standard deviation (SD). Urinary markers such as UP, U-β2MG, U-NAG, U-8-OHdG and U-Trx were logarithmically transformed before analysis because of their skewed distributions and are reported as the geometric mean (95% confidence interval [CI]). A P value less than 0.05 was considered statistically significant. The difference between each group was analyzed by t-test or one-way ANOVA. The relationship between U-Trx and other clinical parameters was analyzed by Spearman’s rank correlation coefficient or chi-square test and multiple regression analysis. As variables increased, medication was excluded in the definitive presentation of results in multiple regression analysis. All statistical calculations were performed with SPSS Statistics version 19.
Results

Average levels of clinical parameters and S- and U-Trx in CKD patients and controls

As shown in Table 1, DM and non-DM patients showed significantly higher age, S-Cr (lower eGFR), sBP, UP, U-NAG and HbA1c than controls. For UP, U-NAG and HbA1c, those of DM patients were even significantly higher than those of non-DM. U-8-OHdG in both DM and non-DM patients was significantly lower than that of controls. In terms of medication, diuretics and statin were significantly often administered in DM patients. The average levels of S- and U-Trx showed no significant difference among controls, DM and non-DM patients.

The difference of U-Trx between the sexes and among age groups in controls and CKD patients

We investigated the difference of U-Trx between the sexes in each group. As shown in Figure 1, female U-Trx showed significantly higher levels than that of males, in controls and non-DM patients (control, M: n=11, geometric mean [95%CI] 3.3 [1.1-7.7] ng/gCr, F: n=19, 7.5 [1.7-25.7] ng/gCr, p<0.01; non-DM patients, M: n=33, 1.1 [0-4.8] ng/gCr, F: n=20, 7.2 [1.5-25.8] ng/gCr, p<0.00). Evaluation of U-Trx performed separately in each sex revealed that male U-Trx in DM patients was significantly higher than in non-DM patients. Evaluation of the influence of menopause on U-Trx levels revealed significantly higher levels in the female control group under the age of 50 than in the group over 50 (p<0.01). On the other hand, U-Trx levels in the male control group did not show any significant difference between under and over the age of 50 (data not shown).

Multiple regression analysis to identify the factors independently affecting U-Trx

We performed multiple regression analysis to identify factors independently affecting U-Trx, in
all subjects (n=140) including normal controls (n=30), DM (n=57) and non-DM patients (n=53).

The investigated factors were age, sex, eGFR, S-Trx, UP/Cr ratio, U-β2MG/Cr ratio, U-NAG/Cr ratio, U-8-OHdG/Cr ratio, HbA1c, total cholesterol, sBP and smoking. As variables increased, medication was excluded in the definitive presentation of results. S-Cr, CKD(+/-) and DM(+/-) were also excluded because of multicollinearity to eGFR for S-Cr and CKD(+/-) and to HbA1c for DM(+/-) in this analysis. As a result, UP/Cr ratio, female sex and HbA1c were observed as independent risk factors of U-Trx (adjusted R²=0.468) in all subjects.

The relationship between U-Trx and clinical parameters in DM and non-DM patients

Figure 2 shows the clinical parameters that had significant relationships with U-Trx in DM and non-DM patients. As shown in Figure 2, in DM patients (Figure 2-a,c,e,g), U-Trx showed a negative correlation with eGFR in all subjects and in males (all: n=57, r_s=-0.43, p<0.01; M: n=41, p<0.01, r_s=-0.47; F: n=16, r_s=-0.38, p=0.14) (Figure 2-a) and positive correlations with UP in all subjects and in each sex (all: r_s=0.59, p<0.00; M: r_s=0.68, p<0.00; F: r_s=0.68, p<0.01) (Figure 2-c), with U-NAG in all subjects and in each sex (all: r_s=0.55, p<0.00; M: r_s=0.49, p<0.01; F: r_s=0.75, p<0.01) (Figure 2-e) and with sBP in all subjects (all: r_s=0.28, p<0.05; M: r_s=0.18, p=0.26; F: r_s=0.32, p=0.23) (Figure 2-g). On the other hand, in non-DM patients (Figure 2-b,d,f,h), U-Trx showed a positive correlation only with UP in males (n=33, r_s=0.38, p<0.05, Figure 2-d). In order to clarify the influence of gender on these clinical parameters for U-Trx, we performed a comparison of their average levels separately in control, DM and non-DM groups. There was no significant difference in the level of each parameter between the two sexes in each group (Table 2).

The relationship between average U-Trx level and the change of renal function of DM and
The longitudinal study included 31 DM patients (23 male, 8 female) and 36 non-DM patients (24 male, 12 female) for one year. The averages of clinical parameters affecting U-Trx in the cross-sectional analysis were not significantly different between males and females in each of DM and non-DM patients; however, the average U-Trx level of females was significantly higher than that of males in non-DM patients (p=0.009, Table 3). U-NAG levels of DM patients were significantly higher than those of non-DM in both sexes (both p<0.05, Table 3). The relationship between average U-Trx level and the rate of eGFR decline was significantly positive only in male DM patients (r_s=0.607, p<0.01, Table 4). Although this significant relationship was not observed in females in both DM and non-DM, it was noteworthy that all of the correlation coefficients had negative values (Table 4).

Discussion

A huge number of studies have shown that oxidative stress plays an important role in the pathogenesis of diabetic nephropathy by a variety of mechanisms [6, 7]. It is also known that there are several defensive mechanisms in renoparenchymal cells against oxidant stress. Among them, Trx is one of the major intracellular antioxidants [8], and is known to have its function inhibited by Trx-interacting protein (Txnip) [9]. Hamada et al. have shown the highly upregulated mRNA expression of Txnip in renal tissue of streptozotocin-induced DM nephropathy in mice with high excretion of oxidative stress markers, such as 8-OHdG, in urine [10]. In humans, the expression of Trx mRNA has also been proved to be higher in biopsy tissue of DN patients than in a normal control [11]. However, a method for detecting tissue mRNA is not available for clinical use.

As for S-Trx, Kakisaka et al. reported its elevation in DM patients with higher non-esterified fatty
acid levels [12]. However, they did not evaluate the significance of renal organ involvement. Kato et al. showed significantly high S-Trx in DN patients under hemodialysis [13]. Considering the molecular size of Trx to be about 12 kDa, in renal failure patients, elevations of both S- and U-Trx are possible due to a decrease of glomerular filtration and damaged tubular reabsorption. Recently, methodology for measuring human U-Trx has been confirmed by Kasuno K and our members [3], and we used this method for detecting U-Trx of CKD subjects in this study. The positive correlation between U-NAG, a known marker of local renal injury, and U-Trx indicated that high U-Trx might be the local (renal) origin of CKD. Considering this complex condition, in the present study, we enrolled patients with eGFR >15 ml/min. S-Trx was not significantly different among DM, non-DM patients and controls, and did not correlate with U-Trx, renal function, HbA1c and the presence of DM. On the other hand, multiple regression analysis in all subjects including normal controls and CKD patients showed that UP and HbA1c independently affected U-Trx. In addition, significant relationships between U-Trx and proteinuria, renal dysfunction and markers of tubular damage such as U-NAG were selectively observed in DM patients, but not in non-DM ones. Although we did not perform comparisons with the morphological changes or fibrogenic markers, these findings suggest that U-Trx is a potent marker of renal damage in DM that is highly accessible in daily clinical use across a relatively wide range of renal functions.

In this study, for the first time, we found that female U-Trx was significantly higher than that in males in normal controls. In addition, female sex was a factor significantly affecting U-Trx in multiple regression analysis in all subjects. There have been several reports about the relationship between female sex and higher Trx excretion in relation to an estrogen-mediated mechanism [14] [15], although these have been limited to the serum but not the urinary level of Trx [16]. In the present study, the result of significantly higher U-Trx in female controls under the age of 50,
possibly before menopause, but not in males, might indicate the influence of gender on the level of U-Trx even in normal conditions. In the present study, we investigated the gender difference of U-Trx in CKD, especially in DM. In these patients, the longitudinal study revealed a positive correlation between average U-Trx and the rate of eGFR decline in males. On the other hand, female patients did not show any positive correlation between them, but instead showed a negative correlation coefficient, although U-Trx levels were not significantly different between the two sexes. Without any additional data such as estrogen levels, the possibility for further analysis is limited; however, the existence of some gender-specific role of U-Trx might be reasonable, especially in DM.

U-8-OHdG has been shown to be a marker of oxidative stress and has sometimes been reported to be identified in CKD patients [17], especially in DM nephropathy [18]. In the present study, however, U-8-OHdG was significantly lower in CKD patients than in controls, in both DM and non-DM conditions. Although the precise reason for this was not sufficiently clear, the negative correlation of U-8-OHdG with the use of ARB and ACEI in CKD patients (data not shown) might indicate the influence of an antioxidant effect of these drugs in CKD patients [19]. On the other hand, in the present study, U-8-OHdG did not show any significant relationship with U-Trx. These results suggest that U-Trx may not be a direct oxidative stress marker, but reflects the reaction against various stresses including oxidation in CKD. Kasuno suggested that U-Trx has a closer relationship with the cellular redox response to oxidative stress than do other urinary biomarkers [3].

There are several limitations in this study. First, the number of samples was limited in longitudinal observation, especially for females. The results of chi-square test for medication, as shown in Table 1, might provide a limited conclusion because the sample was small. Further study
with a larger sample size might give us more convincing results on the utility of U-Trx as one of the important markers of the disease state, especially in DM. Second, the age of the control subjects was significantly young, which might have influenced the effects of estrogen level on Trx secretion. However, even though the average age of the patients with DM was as old as menopause, a significantly high value of U-Trx in females was noted. Third, the UP level of female CKD patients in longitudinal study tended to be low compared with that of males (not significant). In conclusion, although analysis of more patients is needed, U-Trx might be a potent marker for renal damage in DM nephropathy. The different effect on the change of renal function between the two sexes suggests the gender-specific physiological and pathological significance of U-Trx.

Acknowledgement

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Disclosure

The authors have declared no conflicts of interest.

References


2. Kasuno K, Nakamura H, Ono T, Muso E, Yodoi J. Protective roles of thioredoxin, a


Legends to the figures

Figure 1: Urinary thioredoxin levels in each sex in control, DM and non-DM patients

Female U-Trx levels were significantly higher than those of male subjects in control and non-DM patients. In DM patients, female U-Trx levels tended to be higher than in males. **p<0.01. DM, diabetes mellitus; U-Trx, urinary thioredoxin

Figure 2: Correlation between urinary thioredoxin and clinical parameters such as eGFR (a,b), UP (c,d), U-NAG (e,f) and sBP (g,h) in DM (whole, n=57; ● male, n=41; ○ female, n=16) (a,c,e,g) and non-DM patients (whole, n=53; ● male, n=33; ○ female, n=20) (b,d,f,h)

In all DM patients, U-Trx showed a negative correlation with eGFR (a), and positive correlations with UP (c), U-NAG (e) and sBP (g). In non-DM patients, U-Trx level did not show any significant correlation with almost all parameters in both sexes, except for UP level (d). **p<0.01, *p<0.05. DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; sBP, systolic blood pressure; U-NAG, urinary N-acetyl-β-D-glucosaminidase; UP, urinary protein
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Figure 1: Urinary thioredoxin levels in each sex in control, DM and non-DM patients

Female U-Trx levels were significantly higher than those of male subjects in control and non-DM patients. In DM patients, female U-Trx levels tended to be higher than in males. **p<0.01. DM, diabetes mellitus; U-Trx, urinary thioredoxin

78x66mm (300 x 300 DPI)
Figure 2: Correlation between urinary thioredoxin and clinical parameters such as eGFR (a,b), UP (c,d), U-NAG (e,f) and sBP (g,h) in DM (whole, n=57; ● male, n=41; ○ female, n=16) (a,c,e,g) and non-DM patients (whole, n=53; ● male, n=33; ○ female, n=20) (b,d,f,h).

In all DM patients, U-Trx showed a negative correlation with eGFR (a), and positive correlations with UP (c), U-NAG (e) and sBP (g). In non-DM patients, U-Trx level did not show any significant correlation with almost all parameters in both sexes, except for UP level (d). **p<0.01, *p<0.05. DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; sBP, systolic blood pressure; U-NAG, urinary N-acetyl-β-D-glucosaminidase; UP, urinary protein.
Table 1  Average levels of clinical parameters and urinary and serum Trx in CKD patients and controls

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<th>Parameter</th>
<th>Controls (n=30)</th>
<th>DM patients (n=57)</th>
<th>Non-DM patients (n=53)</th>
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<tr>
<td>Age (year), mean±SD (range)</td>
<td>38±12 (22-67)</td>
<td>63±13 (34-85) **</td>
<td>58±15 (26-86) **</td>
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<td>Male/Female (n)</td>
<td>11 / 19</td>
<td>41 / 16</td>
<td>33 / 20</td>
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<td>serum Cr (mg/dL), mean±SD (range)</td>
<td>0.66±0.13 (0.5-0.99)</td>
<td>1.27±0.77 (0.41-3.39)**</td>
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<td>eGFR (mL/min/1.73 m²), mean±SD (range)</td>
<td>92.9±10.4 (76-113)</td>
<td>59.4±31.8 (15-116)**</td>
<td>63.2±26.0 (18-129)**</td>
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<td>sBP (mmHg), mean±SD (range)</td>
<td>111±12 (91-135)</td>
<td>137±24 (104-220)**</td>
<td>130±17 (91-201)**</td>
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<td>UP/Cr (mg/gCr)† (95% CI)</td>
<td>3 (0-18)</td>
<td>117 (5-2498)**</td>
<td>80 (5-1087)**</td>
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<td>U-β2MG/Cr (µg/gCr)‡ (95% CI)</td>
<td>83 (50-138)</td>
<td>156 (14-1636)</td>
<td>183 (31-1055)*</td>
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<td>U-NAG/Cr (IU/gCr)†† (95% CI)</td>
<td>3.6 (1.9-6.4)</td>
<td>12.9 (6.8-23.7)**</td>
<td>7.6 (3.9-14.0)**</td>
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<td>U-8-OHdG/Cr (ng/gCr)‡ (95% CI)</td>
<td>14.3 (7.5-26.7)</td>
<td>5.5 (1.5-15.6)**</td>
<td>8.1 (3.2-18.5)**</td>
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<td>Hba1c (%), mean±SD (range)</td>
<td>4.7±0.3 (4.1-5.4)</td>
<td>6.5±1.0 (5.0-9.6)**</td>
<td>4.9±0.4 (4.0-5.9)*</td>
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<td>total cholesterol (mg/dL), mean±SD (range)</td>
<td>183±29 (143-233)</td>
<td>198±44 (135-329)</td>
<td>203±32 (125-289)</td>
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<td>Medication</td>
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<td>CCB (n, %)</td>
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<td>ARB (n, %)</td>
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<td>ACEI (n, %)</td>
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<td>diuretics (n, %)</td>
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<td>1 (2%)</td>
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<td>beta-blocker (n, %)</td>
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<td>4 (8%)</td>
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<tr>
<td>statin (n, %)</td>
<td>0</td>
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<td>12 (23%)</td>
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<td>serum Trx (ng/mL), mean±SD (range)</td>
<td>22.3±14.6 (0.8-55.5)</td>
<td>22.6±8.8 (3.9-48.7)</td>
<td>22.9±9.6 (6.9-52.6)</td>
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<td>U-Trx/Cr ratio (ng/gCr)† (95% CI)</td>
<td>5.6 (1.3-17.8)</td>
<td>3.9 (0.1-20.5)</td>
<td>2.5 (0.0-11.5)</td>
</tr>
</tbody>
</table>

* p<0.05 vs. control, ** p<0.01 vs. control, † p<0.05 vs. non-DM, †† p<0.01 vs. non-DM, ‡ geometric means

Trx, Thioridoxin; CKD, chronic kidney disease; DM, diabetes mellitus; Cr, creatinine; SD, standard deviation; eGFR, estimated glomerular filtration rate; sBP, systolic blood pressure; HbA1c, hemoglobin A1c; U-NAG, urinary N-acetyl-β-D-glucosaminidase; UP, urinary protein; CI, confidence interval; U-β2MG, urinary beta 2-microglobulin; U-8-OHdG, urinary 8-hydroxy-2'-deoxyguanosine; CCB, calcium channel blocker; ARB, angiotensin II receptor blocker; ACEI, angiotensin-converting enzyme inhibitor;
### Table 2: Average levels of clinical parameters affecting urinary Trx by sex separately in CKD patients and controls

<table>
<thead>
<tr>
<th>Each groups</th>
<th>Male</th>
<th>Female</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls (n=30)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²), mean±SD(range)</td>
<td>92.3±10.8 (79-113)</td>
<td>93.2±10.5 (76-112)</td>
<td>n.s</td>
</tr>
<tr>
<td>sBP (mmHg), mean±SD(range)</td>
<td>111±10 (105-135)</td>
<td>107±11 (91-127)</td>
<td>n.s</td>
</tr>
<tr>
<td>UP/Cr (mg/gCr)‡ (95% CI)</td>
<td>2 (0-12)</td>
<td>3 (0-23)</td>
<td>n.s</td>
</tr>
<tr>
<td>U-NAG/Cr (IU/gCr)‡ (95% CI)</td>
<td>2.8 (1.7-4.5)</td>
<td>4.1 (2.1-7.5)</td>
<td>n.s</td>
</tr>
<tr>
<td><strong>DM patients (n=57)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²), mean±SD(range)</td>
<td>62.8±30.9 (15-115)**</td>
<td>50.8±33.5 (15-116)††</td>
<td>n.s</td>
</tr>
<tr>
<td>sBP (mmHg), mean±SD(range)</td>
<td>132±19 (104-194)*</td>
<td>151±30 (119-220)††</td>
<td>n.s</td>
</tr>
<tr>
<td>UP/Cr (mg/gCr)‡ (95% CI)</td>
<td>135 (5-2605)**</td>
<td>81 (2-2378)†‡</td>
<td>n.s</td>
</tr>
<tr>
<td>U-NAG/Cr (IU/gCr)‡ (95% CI)</td>
<td>12.5 (6.8-22.4)**</td>
<td>14.1 (7.0-27.4)††</td>
<td>n.s</td>
</tr>
<tr>
<td><strong>Non-DM patients (n=53)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²), mean±SD(range)</td>
<td>58.4±26.9 (18-109)**</td>
<td>71.1±22.8 (42-129)††</td>
<td>n.s</td>
</tr>
<tr>
<td>sBP (mmHg), mean±SD(range)</td>
<td>132±18 (91-201)†</td>
<td>127±16 (102-152)††</td>
<td>n.s</td>
</tr>
<tr>
<td>UP/Cr (mg/gCr)‡ (95% CI)</td>
<td>120 (10-1306)**</td>
<td>41 (1-732)†</td>
<td>n.s</td>
</tr>
<tr>
<td>U-NAG/Cr (IU/gCr)‡ (95% CI)</td>
<td>8.5 (5.1-13.7)**</td>
<td>6.2 (2.6-13.6)</td>
<td>n.s</td>
</tr>
</tbody>
</table>

* p<0.05 vs. male control, ** p<0.01 vs. male control, † p<0.05 vs. female control, †† p<0.01 vs. female control, ‡ geometric means.

Trx, Thioredoxin; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; SD, standard deviation; sBP, systolic blood pressure; UP, urinary protein; Cr, creatinine; CI, confidence interval; U-NAG, urinary N-acetyl-β-D-glucosaminidase; DM, diabetes mellitus
Table 3 Average levels of clinical parameters affecting urinary Trx by sex separately in DM and non-DM patients in longitudinal study

<table>
<thead>
<tr>
<th>Each groups</th>
<th>Male</th>
<th>Female</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM patients (n=31)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=23)</td>
<td>(n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²), mean±SD</td>
<td>45.9±28.8</td>
<td>38.1±18.2</td>
<td>0.849</td>
</tr>
<tr>
<td>sBP (mmHg), mean±SD</td>
<td>139±19</td>
<td>141±26</td>
<td>0.758</td>
</tr>
<tr>
<td>UP/Cr (mg/gCr)² (95% CI)</td>
<td>564 (46-6760)</td>
<td>105 (4-2206)</td>
<td>0.371</td>
</tr>
<tr>
<td>U-NAG/Cr (IU/gCr)² (95% CI)</td>
<td>15.8 (5.9-27.7)</td>
<td>14.0 (5.6-32.8)</td>
<td>0.962</td>
</tr>
<tr>
<td>average U-Trx/Cr (ng/gCr)² (95% CI)</td>
<td>5.9 (0.9-24.2)</td>
<td>6.9 (1.1-29.1)</td>
<td>0.995</td>
</tr>
<tr>
<td>Non-DM patients (n=36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=24)</td>
<td>(n=12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²), mean±SD</td>
<td>49.4±21.4</td>
<td>62.8±16.9</td>
<td>0.370</td>
</tr>
<tr>
<td>sBP (mmHg), mean±SD</td>
<td>135±17</td>
<td>128±11</td>
<td>0.189</td>
</tr>
<tr>
<td>UP/Cr (mg/gCr)² (95% CI)</td>
<td>219 (26-1761)</td>
<td>83 (3-1618)</td>
<td>0.702</td>
</tr>
<tr>
<td>U-NAG/Cr (IU/gCr)² (95% CI)</td>
<td>9.2 (5.8-14.3)*</td>
<td>6.2 (2.2-15.2)</td>
<td>0.355</td>
</tr>
<tr>
<td>average U-Trx/Cr (ng/gCr)² (95% CI)</td>
<td>1.1 (0-4.4)**</td>
<td>7.0 (1.6-23.9)</td>
<td><strong>0.009</strong></td>
</tr>
</tbody>
</table>

*p<0.05 vs. male DM, **p<0.01 vs. male DM, †p<0.05 vs. female DM, ††p<0.01 vs. female DM, ‡geometric means.
Trx, Thioredoxin; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; SD, standard deviation;
sBP, systolic blood pressure; UP, urinary protein; Cr, creatinine; CI, confidence interval; U-NAG, urinary N-acetyl-β-D-glucosaminidase
Table 4 The relationship between average U-Trx levels and the change ratio of eGFR levels of CKD patients by sex separately in the longitudinal study

<table>
<thead>
<tr>
<th>Each groups</th>
<th>average U-Trx/Cr vs. the rate of eGFR decline</th>
<th>r&lt;sup&gt;s&lt;/sup&gt;</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM patients</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Whole(n=31)</td>
<td>0.350</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td>Male(n=23)</td>
<td><strong>0.607</strong></td>
<td><strong>0.002</strong></td>
<td></td>
</tr>
<tr>
<td>Female(n=8)</td>
<td>-0.587</td>
<td>0.126</td>
<td></td>
</tr>
<tr>
<td>Non-DM patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole(n=36)</td>
<td>-0.053</td>
<td>0.757</td>
<td></td>
</tr>
<tr>
<td>Male(n=24)</td>
<td>0.297</td>
<td>0.159</td>
<td></td>
</tr>
<tr>
<td>Female(n=12)</td>
<td>-0.315</td>
<td>0.318</td>
<td></td>
</tr>
</tbody>
</table>

*<sup>r</sup>*, Spearman’s rank correlation coefficient;  
Trx, Thioredoxin; CKD, chronic kidney disease;  
eGFR, estimated glomerular filtration rate;  
DM, diabetes mellitus
Gender- and disease-specific urinary thioredoxin in chronic kidney disease patients with or without type 2 diabetic nephropathy

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Running Title: Gender-specific urinary thioredoxin
Abstract

Aim

The role of urinary (U-) thioredoxin (Trx), a class of small redox proteins, in physiological and pathological conditions, in addition to its gender specificity, has been insufficiently determined in chronic kidney disease (CKD) patients, especially in diabetes mellitus (DM) nephropathy.

Methods

U-Trx was measured cross-sectionally in 110 CKD outpatients with estimated glomerular filtration rate (eGFR) of $\geq 15$ mL/min/1.73 m$^2$, namely, in 57 type 2 DM patients (male (M): n=41, female (F): n=16) and 53 non-DM patients (M: n=33, F: n=20), as well as 30 healthy controls (M: n=11, F: n=19). Comparisons were made among controls, DM and non-DM, and between M and F, and clinical parameters were compared in each group. In addition, a comparison between average U-Trx level and the changes of renal function during a one-year period was performed.

Results

U-Trx was significantly higher in females than in males in controls (p<0.05) and in non-DM patients (p<0.05). Multiple regression analysis revealed that urinary protein (UP)/creatinine (Cr) ratio, female sex and HbA1c were independent factors affecting U-Trx among all subjects (adjusted $R^2=0.468$). In DM patients, U-Trx was negatively correlated with eGFR, especially in males, and positively correlated with UP/Cr and NAG in both sexes (all p<0.01), as well as with systolic blood pressure in all (p<0.05). Average U-Trx was positively correlated with the rate of annual eGFR decline of male (p<0.01) but not female DM patients.

Conclusion

U-Trx might have a gender-specific physiological and pathological role and be a potent marker of renal damage in DM nephropathy.
Key words: chronic kidney disease, diabetic nephropathy, gender, oxidative stress, thioredoxin

Introduction

Thioredoxin (Trx) is a 12-kDa protein with redox-active dithiol in the active site of -Cys-Gly-Pro-Cys- and is ubiquitous in the human body. It is a defensive protein induced by various stresses and has anti-oxidative, anti-apoptotic and anti-inflammatory effects; it may also play crucial roles in the pathophysiological mechanisms behind metabolic disorders, cancer and inflammation [1]. In the kidney, Trx is retained in medullary thick ascending limb (mTAL) and secreted from proximal tubuli into urine during renal ischemia/reperfusion, against which several in vitro studies have shown its protective activity [2]. Recently, methodology for measuring human U-TRX has been confirmed by Kasuno K and our members and found markedly high levels of U-Trx not only in ischemic or inflammatory renal diseases, such as tubulointerstitial nephritis and microscopic polyarteritis nodosa, but also especially in AKI [3], which suggested U-Trx as a potent renal biomarker of oxidative injury. However, the subjects of this study CKD patients were limited to those with estimated glomerular filtration rate (eGFR) of more than 60 mL/min/1.73 m² and did not include cases of diabetes mellitus (DM), provoking progressive renal injury possibly due to high oxidative stress, nor addressed the influence of gender in physiological and pathological conditions.

In the present study, to investigate the potency of U-Trx measurement as a marker of CKD progression, for the first time, we performed cross-sectional and longitudinal observation of U-Trx levels in CKD patients with eGFR >15 mL/min/1.73 m², including those with DM. In addition, this observation was performed separately in males and females to analyze the influence of gender.
Methods

Subjects

A total of 110 adult CKD outpatients from stages 1 to 4 (eGFR >15 mL/min/1.73 m²) treated at
the authors’ hospital between August 2009 and September 2010 were subjects in this study.
 Fifty-seven had type 2 DM and 53 did not (non-DM). Patients with the following
diseases/conditions were excluded: infectious disease, asthma, malignancy, uncontrollable heart
failure, cardiovascular and cerebrovascular disease with an onset within 6 months, hepatitis and
HBV or HCV carrier, rheumatoid arthritis and collagen disease, arteriosclerosis obliterans higher
than Fontaine III, immunosuppressive drug administration within the past year, and general
anesthesia or shock within the past month. Among the non-DM patients, 20 cases were diagnosed
as follows: 10 IgA nephropathy, 5 non-IgA mesangiproliferative glomerulonephritis (GN), 2
membranous nephropathy, 2 focal glomerular sclerosis, 1 hypertensive nephrosclerosis and 1 IgM
nephropathy. The other cases without renal biopsy were clinically diagnosed as follows: 14
hypertensive nephrosclerosis, 3 chronic glomerulonephritis, 1 gouty nephropathy, 2 polycystic
kidney disease, 1 renal stone, 1 obesity nephropathy, 1 renal artery stenosis and 10 CKD high-risk
patients (4 hypertension, 3 hyperlipidemia and 3 obesity). For blood pressure or lipid control, the
following medications were administered: calcium channel blocker, beta-blocker,
angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, diuretics and statin.

Thirty control subjects were obtained from among healthy volunteers and participants in a
medical check-up of the whole body at Kitano Hospital who were without specific diseases with
eGFR >90 mL/min/1.73 m². Written informed consent to take part in the study was obtained
from all participants. This study was carried out in compliance with the Helsinki Declaration and
approved by the ethics committee at Kitano Hospital (approval No. 08-09-006).
Cross-sectional analysis of urinary and serum Trx in control and CKD patients

We collected serum and urinary samples at the outpatient clinic of the authors’ hospital, and measured urinary (U-) and serum (S-) Trx in controls and CKD patients by ELISA, as described below, and for U-Trx, disease specificity (DM or non-DM) and gender specificity were examined. These data were compared with other clinical parameters: S-Cr, eGFR, U-Cr ratio of U-protein (UP), U-β2-microglobulin (U-β2MG), U-N-acetyl-β-D-glucosidase (U-NAG), U-8-hydroxydeoxyguanosine (U-8-OHdG), hemoglobin (Hb)A1c, total cholesterol, systolic blood pressure (sBP), smoking and medication. Moreover, we performed multiple regression analysis to identify factors independently affecting U-Trx in control and CKD patients.

Longitudinal studies of the relationship between average U-Trx level and renal function in CKD patients

We analyzed the relationship between the rate of eGFR decline (%/year) calculated as follows: 
\[
\frac{[\text{eGFR value of one year later (mL/min/1.73 m}^2] - \text{initial eGFR value (mL/min/1.73 m}^2)]}{\text{initial eGFR value (mL/min/1.73 m}^2)} \times 100, \text{ and average U-Trx level measured at least 2 times with a 5-7-month interval during 1 year. These longitudinal studies were performed in each sex separately.}
\]

Measurement of serum and urinary Trx

Periodic urinary samples were centrifuged for 10 minutes immediately after collection, and the supernatants of the samples as well as the serum samples were cryopreserved at -30 °C in a deep freezer. S- and U-Trx were quantified using a commercial ELISA kit for human Trx (Redox
Bioscience Inc., Kyoto). In this ELISA study, because the values of U-Trx were preliminarily proved to be stable at physiological pHs (pH 5-8), and not to be influenced by the temperature (freeze/thaw) and a presence of cellular components in urine [3], this ELISA system is suitable for use with clinical urine samples.

Examinations of clinical and laboratory parameters

Estimated GFR was calculated as follows: $194 \times \text{Cr}^{-1.094} \times \text{Age}^{-0.287} \times 0.739$ (if female) [4]. The following kits and methods were used: competitive ELISA kits (New 8-OHdG check ELISA, Fukuroi, Shizuoka) [5] for U-8-OHdG, latex agglutination for HbA1c and U-β2MG, Pyrogallol Red-molybdenum method for UP and N-acylhexosamine oxidase-peroxidase method for U-NAG. All urinary parameters were divided by U-Cr concentration (mg/dL) and then used.

Statistical analysis

All data except urinary markers are expressed as the mean ± standard deviation (SD). Urinary markers such as UP, U-β2MG, U-NAG, U-8-OHdG and U-Trx were logarithmically transformed before analysis because of their skewed distributions and are reported as the geometric mean (95% confidence interval [CI]). A $P$ value less than 0.05 was considered statistically significant. The difference between each group was analyzed by t-test or one-way ANOVA. The relationship between U-Trx and other clinical parameters was analyzed by Spearman’s rank correlation coefficient or chi-square test and multiple regression analysis. As variables increased, medication was excluded in the definitive presentation of results in multiple regression analysis. All statistical calculations were performed with SPSS Statistics version 19.
Results

Average levels of clinical parameters and S- and U-Trx in CKD patients and controls

As shown in Table 1, DM and non-DM patients showed significantly higher age, S-Cr (lower eGFR), sBP, UP, U-NAG and HbA1c than controls. For UP, U-NAG and HbA1c, those of DM patients were even significantly higher than those of non-DM. U-8-OHdG in both DM and non-DM patients was significantly lower than that of controls. In terms of medication, diuretics and statin were significantly often administered in DM patients. The average levels of S- and U-Trx showed no significant difference among controls, DM and non-DM patients.

The difference of U-Trx between the sexes and among age groups in controls and CKD patients

We investigated the difference of U-Trx between the sexes in each group. As shown in Figure 1, female U-Trx showed significantly higher levels than that of males, in controls and non-DM patients (control, M: n=11, geometric mean [95%CI] 3.3 [1.1-7.7] ng/gCr, F: n=19, 7.5 [1.7-25.7] ng/gCr, p<0.01; non-DM patients, M: n=33, 1.1 [0.4.8] ng/gCr, F: n=20, 7.2 [1.5-25.8] ng/gCr, p<0.00). Evaluation of U-Trx performed separately in each sex revealed that male U-Trx in DM patients was significantly higher than in non-DM patients. Evaluation of the influence of menopause on U-Trx levels revealed significantly higher levels in the female control group under the age of 50 than in the group over 50 (p<0.01). On the other hand, U-Trx levels in the male control group did not show any significant difference between under and over the age of 50 (data not shown).

Multiple regression analysis to identify the factors independently affecting U-Trx

We performed multiple regression analysis to identify factors independently affecting U-Trx, in
all subjects (n=140) including normal controls (n=30), DM (n=57) and non-DM patients (n=53).

The investigated factors were age, sex, eGFR, S-Trx, UP/Cr ratio, U-β2MG/Cr ratio, U-NAG/Cr ratio, U-8-OHdG/Cr ratio, HbA1c, total cholesterol, sBP and smoking. As variables increased, medication was excluded in the definitive presentation of results. S-Cr, CKD(+/-) and DM(+/-) were also excluded because of multicollinearity to eGFR for S-Cr and CKD(+/-) and to HbA1c for DM(+/-) in this analysis. As a result, UP/Cr ratio, female sex and HbA1c were observed as independent risk factors of U-Trx (adjusted R²=0.468) in all subjects.

The relationship between U-Trx and clinical parameters in DM and non-DM patients

Figure 2 shows the clinical parameters that had significant relationships with U-Trx in DM and non-DM patients. As shown in Figure 2, in DM patients (Figure 2-a,c,e,g), U-Trx showed a negative correlation with eGFR in all subjects and in males (all: n=57, rₛ=-0.43, p<0.01; M: n=41, p<0.01, rₛ=-0.47; F: n=16, rₛ=-0.38, p=0.14) (Figure 2-a) and positive correlations with UP in all subjects and in each sex (all: rₛ=0.59, p<0.00; M: rₛ=0.68, p<0.00; F: rₛ=0.68, p<0.01) (Figure 2-c), with U-NAG in all subjects and in each sex (all: rₛ=0.55, p<0.00; M: rₛ=0.49, p<0.01; F: rₛ=0.75, p<0.01) (Figure 2-e) and with sBP in all subjects (all: rₛ=0.28, p<0.05; M: rₛ=0.18, p=0.26; F: rₛ=0.32, p=0.23) (Figure 2-g). On the other hand, in non-DM patients (Figure 2-b,d,f,h), U-Trx showed a positive correlation only with UP in males (n=33, rₛ=0.38, p<0.05, Figure 2-d). In order to clarify the influence of gender on these clinical parameters for U-Trx, we performed a comparison of their average levels separately in control, DM and non-DM groups. There was no significant difference in the level of each parameter between the two sexes in each group (Table 2).

The relationship between average U-Trx level and the change of renal function of DM and
non-DM patients in the longitudinal study

The longitudinal study included 31 DM patients (23 male, 8 female) and 36 non-DM patients (24 male, 12 female) for one year. The averages of clinical parameters affecting U-Trx in the cross-sectional analysis were not significantly different between males and females in each of DM and non-DM patients; however, the average U-Trx level of females was significantly higher than that of males in non-DM patients (p=0.009, Table 3). U-NAG levels of DM patients were significantly higher than those of non-DM in both sexes (both p<0.05, Table 3). The relationship between average U-Trx level and the rate of eGFR decline was significantly positive only in male DM patients (r_s=0.607, p<0.01, Table 4). Although this significant relationship was not observed in females in both DM and non-DM, it was noteworthy that all of the correlation coefficients had negative values (Table 4).

Discussion

A huge number of studies have shown that oxidative stress plays an important role in the pathogenesis of diabetic nephropathy by a variety of mechanisms [6, 7]. It is also known that there are several defensive mechanisms in renoparenchymal cells against oxidant stress. Among them, Trx is one of the major intracellular antioxidants [8], and is known to have its function inhibited by Trx-interacting protein (Txnip) [9]. Hamada et al. have shown the highly upregulated mRNA expression of Txnip in renal tissue of streptozotocin-induced DM nephropathy in mice with high excretion of oxidative stress markers, such as 8-OHdG, in urine [10]. In humans, the expression of Trx mRNA has also been proved to be higher in biopsy tissue of DN patients than in a normal control [11]. However, a method for detecting tissue mRNA is not available for clinical use.

As for S-Trx, Kakisaka et al. reported its elevation in DM patients with higher non-esterified fatty
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acid levels [12]. However, they did not evaluate the significance of renal organ involvement. Kato et al. showed significantly high S-Trx in DN patients under hemodialysis [13]. Considering the molecular size of Trx to be about 12 kDa, in renal failure patients, elevations of both S- and U-Trx are possible due to a decrease of glomerular filtration and damaged tubular reabsorption. Recently, methodology for measuring human U-Trx has been confirmed by Kasuno K and our members [3], and we used this method for detecting U-Trx of CKD subjects in this study. The positive correlation between U-NAG, a known marker of local renal injury, and U-Trx indicated that high U-Trx might be the local (renal) origin of CKD. Considering this complex condition, in the present study, we enrolled patients with eGFR >15 ml/min. S-Trx was not significantly different among DM, non-DM patients and controls, and did not correlate with U-Trx, renal function, HbA1c and the presence of DM. On the other hand, multiple regression analysis in all subjects including normal controls and CKD patients showed that UP and HbA1c independently affected U-Trx. In addition, significant relationships between U-Trx and proteinuria, renal dysfunction and markers of tubular damage such as U-NAG were selectively observed in DM patients, but not in non-DM ones. Although we did not perform comparisons with the morphological changes or fibrogenic markers, these findings suggest that U-Trx is a potent marker of renal damage in DM that is highly accessible in daily clinical use across a relatively wide range of renal functions.

In this study, for the first time, we found that female U-Trx was significantly higher than that in males in normal controls. In addition, female sex was a factor significantly affecting U-Trx in multiple regression analysis in all subjects. There have been several reports about the relationship between female sex and higher Trx excretion in relation to an estrogen-mediated mechanism [14] [15], although these have been limited to the serum but not the urinary level of Trx [16]. In the present study, the result of significantly higher U-Trx in female controls under the age of 50,
possibly before menopause, but not in males, might indicate the influence of gender on the level of U-Trx even in normal conditions. In the present study, we investigated the gender difference of U-Trx in CKD, especially in DM. In these patients, the longitudinal study revealed a positive correlation between average U-Trx and the rate of eGFR decline in males. On the other hand, female patients did not show any positive correlation between them, but instead showed a negative correlation coefficient, although U-Trx levels were not significantly different between the two sexes. Without any additional data such as estrogen levels, the possibility for further analysis is limited; however, the existence of some gender-specific role of U-Trx might be reasonable, especially in DM.

U-8-OHdG has been shown to be a marker of oxidative stress and has sometimes been reported to be identified in CKD patients [17], especially in DM nephropathy [18]. In the present study, however, U-8-OHdG was significantly lower in CKD patients than in controls, in both DM and non-DM conditions. Although the precise reason for this was not sufficiently clear, the negative correlation of U-8-OHdG with the use of ARB and ACEI in CKD patients (data not shown) might indicate the influence of an antioxidant effect of these drugs in CKD patients [19]. On the other hand, in the present study, U-8-OHdG did not show any significant relationship with U-Trx. These results suggest that U-Trx may not be a direct oxidative stress marker, but reflects the reaction against various stresses including oxidation in CKD. Kasuno suggested that U-Trx has a closer relationship with the cellular redox response to oxidative stress than do other urinary biomarkers [3].

There are several limitations in this study. First, the number of samples was limited in longitudinal observation, especially for females. The results of chi-square test for medication, as shown in Table 1, might provide a limited conclusion because the sample was small. Further study
with a larger sample size might give us more convincing results on the utility of U-Trx as one of the important markers of the disease state, especially in DM. Second, the age of the control subjects was significantly young, which might have influenced the effects of estrogen level on Trx secretion. However, even though the average age of the patients with DM was as old as menopause, a significantly high value of U-Trx in females was noted. Third, the UP level of female CKD patients in longitudinal study tended to be low compared with that of males (not significant). In conclusion, although analysis of more patients is needed, U-Trx might be a potent marker for renal damage in DM nephropathy. The different effect on the change of renal function between the two sexes suggests the gender-specific physiological and pathological significance of U-Trx.

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Disclosure

The authors have declared no conflicts of interest.

References


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Legends to the figures

**Figure 1: Urinary thioredoxin levels in each sex in control, DM and non-DM patients**

Female U-Trx levels were significantly higher than those of male subjects in control and non-DM patients. In DM patients, female U-Trx levels tended to be higher than in males. **p<0.01.** DM, diabetes mellitus; U-Trx, urinary thioredoxin

**Figure 2: Correlation between urinary thioredoxin and clinical parameters such as eGFR (a,b), UP (c,d), U-NAG (e,f) and sBP (g,h) in DM (whole, n=57; • male, n=41; ○ female, n=16) (a,c,e,g) and non-DM patients (whole, n=53; ◆ male, n=33; ◇ female, n=20) (b,d,f,h)**

In all DM patients, U-Trx showed a negative correlation with eGFR (a), and positive correlations with UP (c), U-NAG (e) and sBP (g). In non-DM patients, U-Trx level did not show any significant correlation with almost all parameters in both sexes, except for UP level (d). **p<0.01, *p<0.05.** DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; sBP, systolic blood pressure; U-NAG, urinary N-acetyl-β-D-glucosaminidase; UP, urinary protein