Research Article

Plasma Xanthine Oxidoreductase Activity Associated with Glycemic Control in Patients with Pre-Dialysis Chronic Kidney Disease

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Abstract

Introduction: Xanthine oxidoreductase (XOR) activity plays an important role as a pivotal source of reactive oxygen species, which is associated with cardiovascular disease (CVD) events. Patients with CKD have increased risk of CVD events. In the present study, factors associated with plasma XOR activity in pre-dialysis CKD patients were investigated. *Methods:* In this cross-sectional study, plasma XOR activity in 118 pre-dialysis CKD patients (age 68 [57–75] years; 64 males, 26 with diabetes mellitus [DM]) was determined using a newly established highly sensitive assay based on ($^{13}C_2,^{15}N_2$) xanthine and liquid chromatography/triple quadrupole mass spectrometry. *Results:* Plasma glucose, hemoglobin A1c, and estimated glomerular filtration (eGFR) were significantly and positively correlated with plasma logarithmically transformed XOR (ln-XOR) activity. In multiple regression analyses, eGFR and hemoglobin A1c or plasma glucose were significantly, independently, and positively associated with plasma ln-XOR activity after adjusting for several confounders. Plasma XOR activity was significantly higher in CKD patients with ($n = 26$) than in those without ($n = 92$) DM (62.7 [32.3–122] vs. 25.7 [13.4–45.8] pmol/h/mL, $p < 0.001$). A total of 38 patients were taking uric acid-lowering drugs. Multiple regression analysis of CKD patients not administered uric acid-lowering drugs ($n = 80$) showed no significant association between eGFR and plasma ln-XOR activity. In contrast, association between glycemic control and plasma ln-XOR activity was significant even in CKD patients without uric acid-lowering drug treatment. *Conclusions:* These results indicate the importance of glycemic control in CKD patients in regard to decreased XOR, possibly leading to a decrease in CVD events.

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Introduction

CKD is strongly associated with cardiovascular disease (CVD), while a lower estimated glomerular filtration rate (eGFR) has been recognized as a strong and independent risk factor of CVD [1, 2]. Although the high rates of mor-

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bidity and mortality related to CVD in patients with CKD are serious issues, they cannot be explained by traditional CVD risk factors alone, such as hypertension, diabetes mellitus (DM), smoking habit, or hypercholesterolemia [3] as oxidative stress is also involved in CVD development in those patients [3–5]. Xanthine oxidoreductase (XOR) is a rate-limiting enzyme that catalyzes formation of uric acid by oxidative hydroxylation of hypoxanthine and xanthine in purine metabolism [6], and its activation can increase reactive oxygen species, leading to oxidative stress-induced injury in various tissues [7–9]. Plasma XOR activity has been reported to be a predictor of CVD events in patients with pre-dialysis CKD as well as end-stage kidney disease [10]. Thus, determination of plasma XOR activity is clinically important for patients with CKD.

To date, XOR activity assays have been based on determining formation of uric acid from xanthine as a substrate using an ultraviolet detector [11, 12]. However, that traditional measurement method is not considered suitable for measuring uric acid-rich specimens such as human plasma from CKD patients because subtraction of the basal level of uric acid originally contained in the sample is inevitable [13]. In addition, XOR activity is extremely low in humans as compared with that in rodents, making accurate measurement difficult [14].

We recently developed a novel, accurate, and highly sensitive assay to measure XOR activity in human plasma, which utilizes stable isotope-labeled ($^{13}C_2, ^{15}N_2$) xanthine as a substrate and liquid chromatography/mass spectrometry (LC/MS), composed of a Nano Space SI-2 LC system and TSQ-quantum triple quadrupole mass spectrometer (TQMS) [15]. Findings obtained with this novel assay have shown that plasma XOR activity is associated with severity and clinical outcome in patients with chronic heart failure [16], as well as left ventricular ejection fraction in patients with heart disease [17]. In our previous study, plasma glucose was demonstrated to be significantly associated with plasma XOR activity in hemodialysis patients [18]. However, knowledge regarding plasma XOR activity as a biomarker in pre-dialysis CKD patients is limited [19, 20], while the association of plasma XOR activity with various CKD-associated parameters has not been investigated. We report here results of a cross-sectional single-center investigation of 118 pre-dialysis CKD patients, in whom plasma XOR activity was measured using our newly developed method, as well as findings showing a potential relationship among CKD-associated parameters, including hemoglobin A1c, and plasma XOR activity.

Materials and Methods

Patients

CKD was defined according to criteria proposed by the Kidney Disease: Improving Global Outcomes Organization [21]. Patients diagnosed with CKD were regularly followed up by nephrologists at the departments of nephrology, metabolism, endocrinology, and molecular medicine at Osaka City University Hospital, of whom 118 examined from March to June 2016 were enrolled. The etiological factors included hypertensive nephrosclerosis ($n = 29$), diabetic nephropathy ($n = 26$), IgA nephropathy ($n = 15$), membranous nephropathy ($n = 15$), anti-neutrophil cytoplasmic antibody-associated glomerulonephritis ($n = 5$), autosomal dominant polycystic kidney disease ($n = 4$), minimal change nephrotic syndrome ($n = 3$), tubulointerstitial nephritis ($n = 3$), membranoproliferative glomerulonephritis ($n = 2$), focal and segmental glomerulosclerosis ($n = 2$), lupus nephritis ($n = 1$), and IgA vasculitis ($n = 1$), as well as unknown causes ($n = 12$). Those with hepatitis B, hepatitis C, malignancy, or a clinically overt infection were excluded. Thirty-eight of the 118 CKD patients enrolled were undergoing treatment with uric acid-lowering drugs or an XOR inhibitor, that is, allopurinol or febuxostat.

Blood Sampling

Spot blood and urine samples were collected from all patients in the morning after overnight fasting. The urine samples were kept on ice for 1 h and then centrifuged at 630 *g* for 10 min, as previously described [22]. All laboratory measurements were performed using routine assays with automated methods [23]. eGFR was calculated using the new Japanese coefficient for the abbreviated modification of diet in renal disease study equation, with a correction factor of 0.739 used for females [24]. Serum calcium was corrected based on serum albumin and determined as corrected calcium, as previously described [23]. Serum intact parathyroid hormone (PTH) was measured using a second-generation Elecsys PTH IRMA assay (Roche Diagnostics, Mannheim, Germany), as previously reported [23]. Fibroblastic growth factor 23 (FGF-23), a bone-derived hormone, has a physiological role in regulation of phosphate, 1,25-dihydroxyvitamin D, and PTH [25–28]. Elevated FGF-23 levels have been shown to be associated with CVD events in patients with CKD [29, 30]. It is conceivable that FGF-23 is a potential biomarker that may be useful to improve current clinical practice related to management of CKD mineral and bone disorder (CKD-MBD) [31–33]. Serum full-length FGF-23 was determined using a CL-JACK System (Kyowa Medex Co. Ltd., Tokyo, Japan), a fully automated random access chemiluminescence immunoanalyzer, as previously reported [22]. Plasma samples were kept in a freezer at −80°C until determination of XOR activity.

Determination of Plasma XOR Activity

Plasma XOR activity was determined using freshly thawed samples that had been stored at −80°C until the time of the assay with our recently established assay using stable isotope-labeled ($^{13}C_2, ^{15}N_2$) xanthine with LC/MS (Nano Space SI-2 LC system, Shiseido, Tokyo, Japan, and TQMS, Thermo Fisher Scientific GmbH, Bremen, Germany), as previously described [14, 15]. In brief, 100-μL aliquots of plasma were purified on a Sephadex G25 column and then mixed with Tris buffer (pH 8.5) containing ($^{13}C_2, ^{15}N_2$) xanthine as the substrate and NAD$^+$, with ($^{13}C_3, ^{15}N_3$) uric acid used as an internal standard. The mixtures

were incubated at 37°C for 90 min, then methanol (500 μL) was added and centrifugation was performed at 2,000 g for 15 min at 4°C. Next, the supernatants were transferred to new tubes and dried using a centrifugal evaporator. The residues were reconstituted in 150 μL of distilled water, filtered through an ultrafiltration membrane, and measured using LC/TQMS. Calibration standard samples were measured for ($^{13}C_2,^{15}N_2$) uric acid, and the amounts of ($^{13}C_2,^{15}N_2$) uric acid produced were calculated based on the calibration curve. XOR activity is expressed as pmol/mL/h of ($^{13}C_2,^{15}N_2$) uric acid produced. The intra- and inter-assay coefficients of variation were 6.5 and 9.1%, respectively [15].

Statistical Analysis

Statistical analyses were performed using GraphPad Prism for Windows, version 6.0 (GraphPad Software, San Diego, CA), and the JMP software package, version 10 (SAS Institute Inc., Cary, NC). Values are expressed as mean ± SD. Comparisons between CKD patients with and without uric acid-lowering drug administration, and with and without DM were made using unpaired Student's t-test or Mann-Whitney U test for continuous variables, while a χ^2 test was used for categorical variables. Plasma XOR activity was natural logarithm transformed (ln) to normalize the skewed distribution. Correlations between plasma logarithmically transformed XOR (ln-XOR) activity and clinical data were examined by Pearson's analysis. Independent associations between the variables and XOR activity in CKD patients were assessed by multiple regression analysis. Multiple regression analysis conducted previously showed that glycated albumin, a marker of glycemic control, was independently associated with plasma XOR activity in diabetic hemodialysis patients [18]. When plasma glucose was added instead of glycated albumin, those analysis findings showed that it was also independently associated with plasma XOR activity. Thus, hemoglobin A1c (model 1) and plasma glucose (model 2) were separately included as explanatory variables in the present multiple regression analyses. p values <0.05 were considered to indicate statistical significance. Datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Results

Clinical Characteristics of CKD Patients

The clinical characteristics of the 118 CKD patients enrolled in this study are shown in Table 1. The median value [interquartile range] for eGFR, and serum creatinine levels were 31.7 [16.1–45.1] mL/min/1.73 m², and 1.59 [1.19–2.79] mg/dL, respectively, while the mean (±SD) value for uric acid in serum was 6.7 ± 1.5 mg/dL. Plasma XOR activity in all CKD patients was 29.2 [14.2–65.1] pmol/h/mL, lower than the mean (±SD) value previously reported for healthy subjects (89.1 ± 55.1 pmol/h/mL, n = 20) [15], but higher than that shown in patients undergoing hemodialysis (21.4 ± 13.5 pmol/h/mL, n = 163) [18].

Table 1. Clinical characteristics of CKD patients (n = 118)

Clinical variable	
Male/female	64/54
DM/non-DM	26/92
Age, years	68 [57–75]
BMI, kg/m²	21.6 [19.5–25.0]
eGFR, mL/min/1.73 m²	31.7 [16.1–45.1]
Creatinine, mg/dL	1.59 [1.19–2.79]
BUN, mg/dL	24.5 [19.0–38.3]
Total protein, g/dL	6.7±0.66
Albumin, g/dL	3.8 [3.5–4.0]
Alanine transaminase, IU/L	14 [10–20]
Aspartate transaminase, IU/L	20 [16–23]
Uric acid, mg/dL	6.7±1.5
Hemoglobin, g/dL	12.6±1.77
Iron, μg/dL	88.4±29.4
Ferritin, ng/mL	83.6 [34.5–169]
Plasma glucose, mg/dL	99 [87–117]
Hemoglobin A1c, %	5.6 [5.4–6.1]
CRP, mg/L	0.06 [0.02–0.16]
Phosphate, mg/dL	3.6 [3.3–4.3]
Corrected calcium, mg/dL	9.5 [9.2–9.7]
Intact PTH, pg/mL	70.5 [38.6–1 26]
FGF-23, pg/mL	73 [51–113]
Urinary protein, g/gCr	1.12 [0.25–3.22]
XOR, pmol/h/mL	29.2 [14.2–65.1]
Uric acid-lowering drug administration (allopurinol/febuxostat)	38 (13/25)

Data are expressed as mean ± SD. Median values [interquartile range] are shown for variables with skewed distributions. CRP, C-reactive protein; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone; XOR, xanthine oxidoreductase.

Correlations of Plasma ln-XOR Activity with Clinical Parameters in Pre-Dialysis CKD Patients

Correlations between various clinical parameters and plasma ln-XOR activity were determined using simple regression analyses. In the present CKD patients, alanine transaminase, aspartate transaminase, eGFR (Fig. 1A), hemoglobin A1c (Fig. 1B), and plasma glucose (Fig. 1C) were each significantly and positively correlated with plasma ln-XOR activity (r = 0.204, p = 0.027; r = 0.217, p = 0.018; r = 0.274, p = 0.003; r = 0.269, p = 0.003; and r = 0.384, p < 0.001, respectively), while serum creatinine, BUN, phosphate, and FGF-23 were significantly and negatively correlated with that activity (r = −0.270, p = 0.003; r = −0.342, p < 0.001; r = −0.224, p = 0.019; and r = −0.222, p = 0.017, respectively) (Table 2). eGFR was significantly and positively correlated with plasma ln-XOR activity in the pre-dialysis CKD patients
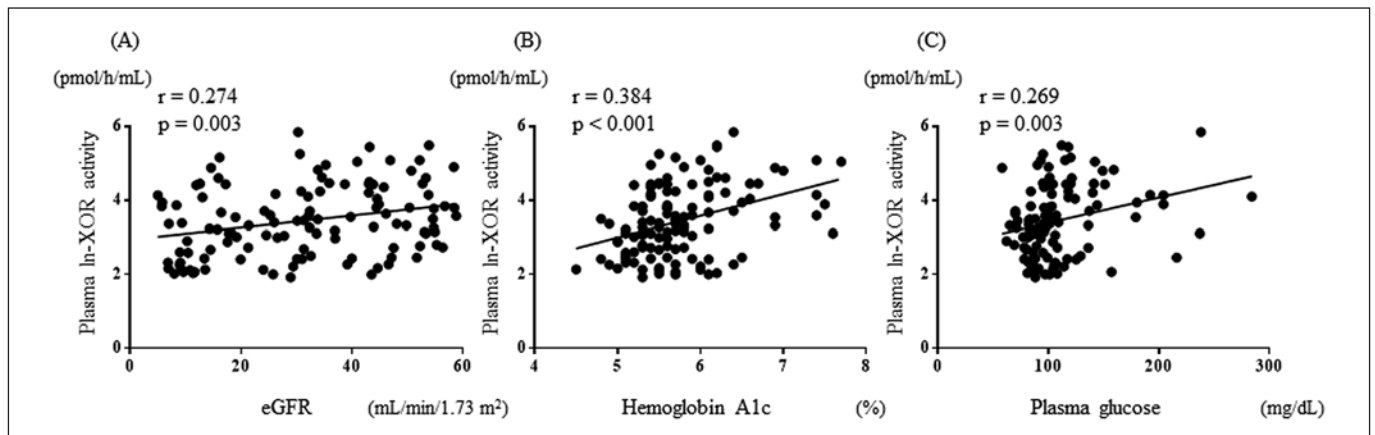
Fig. 1. Correlations between plasma ln-XOR activity and clinical parameters in pre-dialysis CKD patients. eGFR (**A**), hemoglobin A1c (**B**), and plasma glucose were significantly (**C**) positively correlated with plasma ln-XOR activity. eGFR, estimated glomerular filtration rate; XOR, xanthine oxidoreductase; ln-XOR, logarithmically transformed XOR.

receiving uric acid-lowering drugs (n = 38, r = 0.376 p = 0.020) as well as in those not receiving those drugs (n = 80, r = 0.224 p = 0.046) (online suppl. Fig. 1; see www.karger.com/doi/10.1159/000516610 for all online suppl. material).

Association of Various CKD Parameters with Plasma ln-XOR Activity

Next, the associations of various CKD clinical parameters with plasma ln-XOR activity were examined. In multiple regression analysis results, the variables age, gender, eGFR, uric acid, aspartate transaminase, FGF-23, and presence of diabetes were included as explanatory variables. The results showed that presence of diabetes was significantly and positively associated with plasma ln-XOR activity, independent of the other confounders. Thus, for multiple regression analysis, hemoglobin A1c was included as an explanatory variate in model 1 and plasma glucose was included in model 2 (Table 3).

In the present CKD patients, eGFR (β = 0.223, p = 0.028) and hemoglobin A1c (β = 0.332, p < 0.001) were significantly and independently associated with plasma ln-XOR activity (R^2 = 0.261, p < 0.001) (Table 3, model 1), as were eGFR (β = 0.281, p = 0.007) and plasma glucose (β = 0.250, p = 0.007) (R^2 = 0.222, p < 0.001) (Table 3, model 2). In contrast, age, gender, BMI, serum uric acid, aspartate transaminase, and the FGF-23 level did not have a significant association with plasma ln-XOR activity in the present CKD patients.

Table 2. Correlations between clinical parameters and plasma ln-XOR activity in all patients (simple regression analyses; n = 118)

Clinical variable	Correlation with plasma ln-XOR activity	
	r	p value
Age, years	0.166	0.072
BMI, kg/m²	0.111	0.229
eGFR, mL/min/1.73 m²	0.274	0.003*
Creatinine, mg/dL	−0.270	0.003*
BUN, mg/dL	−0.342	<0.001*
Total protein, g/dL	−0.096	0.303
Albumin, g/dL	−0.092	0.321
Alanine transaminase, IU/L	0.204	0.027*
Aspartate transaminase, IU/L	0.217	0.018*
Uric acid, mg/dL	0.032	0.733
Hemoglobin, g/dL	0.066	0.477
Iron, µg/dL	−0.001	0.942
Ferritin, ng/mL	−0.092	0.330
Plasma glucose, mg/dL	0.269	0.003*
Hemoglobin A1c, %	0.384	<0.001*
CRP, mg/L	0.098	0.303
Phosphate, mg/dL	−0.224	0.019*
Corrected calcium, mg/dL	0.103	0.281
Intact PTH, pg/mL	−0.068	0.481
FGF-23, pg/mL	−0.222	0.017*
Urinary protein, g/gCr	−0.059	0.526

BMI, body mass index; BUN, blood urea nitrogen; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone; XOR, xanthine oxidoreductase; ln-XOR, logarithmically transformed XOR. Data include simple correlation coefficient (r) and the level of significance (p). *p < 0.05.

Clinical Parameters Associated with Plasma ln-XOR Activity in Pre-Dialysis CKD Patients without DM

Plasma XOR activity levels were significantly higher in CKD patients with ($n = 26$) than in those without ($n = 92$) DM (62.7 [32.3–122] vs. 25.7 [13.4–45.8] pmol/h/mL, $p < 0.001$) (Fig. 2A). While the presence of diabetes can be related to increased plasma XOR activity, the number of CKD patients with DM was relatively small in the present study. Therefore, we performed multiple regression analyses to elucidate parameters associated with plasma ln-XOR activity in those without DM, which showed that eGFR (β = 0.255, $p = 0.028$) and hemoglobin A1c (β = 0.251, $p = 0.023$) were significantly and independently associated with plasma ln-XOR activity in CKD patients without DM ($R^2 = 0.285$, $p < 0.001$) (Table 4, model 1). Furthermore, eGFR (β = 0.337, $p = 0.004$) and plasma glucose (β = 0.245, $p = 0.019$) were significantly and independently associated with plasma ln-XOR activity in pre-dialysis CKD patients without DM ($R^2 = 0.288$, $p < 0.001$), while uric acid was also significantly and independently associated with plasma ln-XOR activity in those patients (β = 0.227, $p = 0.032$) (Table 4, model 2).

Plasma ln-XOR Activity in Pre-Dialysis CKD Patients with and without Uric Acid-Lowering Drug Administration

Uric acid-lowering drugs such as allopurinol or febuxostat are known to be XOR inhibitors and are being used by 38 of the present CKD patients. eGFR was not significantly different between patients with and those without such administration ($n = 80$) (30.1 [8.89–44.7] vs. 32.4 [16.2–46.1] mL/min/1.73 m^2, $p = 0.338$), whereas the plasma XOR activity level was significantly lower in patients with than that in patients without such administration (11.7 [8.48–28.0] vs. 40.6 [22.6–83.9] pmol/h/mL, $p < 0.001$) (Fig. 2B). Since uric acid-lowering drugs are known to inhibit XOR activity, we performed multiple regression analysis to further identify parameters independently associated with plasma ln-XOR activity in pre-dialysis CKD patients who were not taking uric acid-lowering drugs (Table 5). Age (β = 0.221, $p = 0.014$), aspartate transaminase (β = 0.327, $p < 0.001$), and hemoglobin A1c (β = 0.368, $p < 0.001$) were significantly and independently associated with plasma ln-XOR activity ($R^2 = 0.475$, $p < 0.001$), while eGFR did not have an association in that model (Table 5, model 1). When plasma glucose was included instead of hemoglobin A1c (model 2), age (β = 0.192, $p = 0.045$), male gender (β = −0.268, $p = 0.007$), aspartate transaminase (β = 0.318, $p = 0.001$), and plasma glucose

Table 3. Results of multivariate analysis to elucidate various CKD parameters associated with plasma ln-XOR activity in all patients ($n = 118$)

	Model 1		Model 2	
	β	p value	β	p value
Age, years	0.103	0.246	0.147	0.101
Gender	0.106	0.237	0.102	0.267
BMI	0.098	0.268	0.097	0.285
eGFR	0.223	0.028	0.281	0.007
Uric acid	0.148	0.102	0.140	0.131
Aspartate transaminase	0.100	0.274	0.067	0.484
FGF-23	−0.080	0.384	−0.136	0.151
Hemoglobin A1c	0.332	<0.001	–	–
Plasma glucose	–	–	0.250	0.007
R^2	0.261 ($p < 0.001$)		0.222 ($p < 0.001$)	

BMI, body mass index; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; XOR, xanthine oxidoreductase; β, standardized correlation coefficient; R^2, multiple coefficients of determination; ln-XOR, logarithmically transformed XOR.

(β = 0.307 $p = 0.002$) were significantly and independently associated with plasma ln-XOR activity ($R^2 = 0.436$, $p < 0.001$).

Discussion/Conclusion

Clinical parameters associated with plasma XOR activity in pre-dialysis CKD patients were investigated, with measurements of plasma XOR activity performed using a newly established highly sensitive assay based on ($^{13}C_2$,$^{15}N_2$) xanthine and LC/TQMS. The presence of diabetes, hemoglobin A1c, plasma glucose, and eGFR were found to be significantly and independently associated with plasma ln-XOR activity after adjusting for other confounders. Notably, these associations between glycemic control and plasma ln-XOR activity were significant even in CKD patients without DM. As for CKD patients not taking uric acid-lowering drugs, the association between eGFR and plasma XOR activity was not significant, whereas the association between glycemic control and plasma XOR activity was significant in that subgroup.

In previous studies of DM and XOR activity, hepatic activity was 1.6-fold greater and the plasma XOR level was 3.7-fold higher in streptozotocin-induced diabetic rodents as compared with the respective control groups [34, 35].
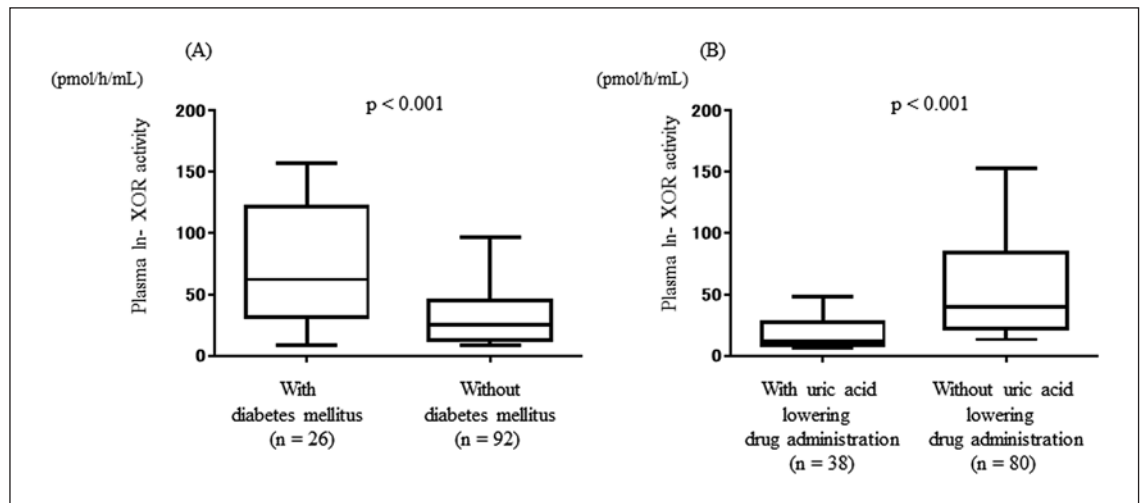
Fig. 2. Plasma XOR activity shown by box plots in patients with and without DM (**A**), and patients with and without administration of uric acid-lowering drugs (**B**). Box plots indicate 25th, 50th, and 75th percentile levels. **A** Plasma XOR activity was significantly higher in pre-dialysis CKD patients with than in those without DM. **B** Plasma XOR activity was significantly higher in pre-dialysis CKD patients without than in those with administration of uric acid-lowering drugs. XOR, xanthine oxidoreductase; DM, diabetes mellitus. *$p < 0.05$.

Table 4. Results of multivariate analysis to elucidate various CKD parameters associated with plasma ln-XOR activity in pre-dialysis CKD patients without DM ($n = 92$)

	Model 1		Model 2	
	β	p value	β	p value
Age, years	0.072	0.492	0.101	0.311
Gender	0.150	0.162	0.191	0.072
BMI	0.128	0.233	0.110	0.313
eGFR	0.255	0.028	0.337	0.004
Uric acid	0.181	0.096	0.227	0.032
Aspartate transaminase	0.132	0.227	0.104	0.347
FGF-23	−0.073	0.485	−0.116	0.267
Hemoglobin A1c	0.251	0.023	–	–
Plasma glucose	–	–	0.245	0.019
R^2	0.285 ($p < 0.001$)		0.288 ($p < 0.001$)	

eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; XOR, xanthine oxidoreductase; DM, diabetes mellitus; ln-XOR, logarithmically transformed XOR; β, standardized correlation coefficient; R^2, multiple coefficients of determination.

Table 5. Results of multivariate analysis to elucidate various CKD parameters associated with plasma ln-XOR activity in pre-dialysis CKD patients not being administered for uric acid-lowering drugs ($n = 80$)

	Model 1		Model 2	
	β	p value	β	p value
Age, years	0.221	0.014	0.192	0.045
Gender	−0.185	0.056	−0.268	0.007
BMI	0.157	0.077	0.151	0.102
eGFR	0.044	0.673	0.096	0.368
Uric acid	0.065	0.507	0.008	0.937
Aspartate transaminase	0.327	<0.001	0.318	0.001
Hemoglobin A1c	0.368	<0.001	–	–
Plasma glucose	–	–	0.307	0.002
R^2	0.475 ($p < 0.001$)		0.436 ($p < 0.001$)	

eGFR, estimated glomerular filtration rate; XOR, xanthine oxidoreductase; β, standardized correlation coefficient; R^2, multiple coefficients of determination; ln-XOR, logarithmically transformed XOR.

Furthermore, plasma XOR activity was recently found to be significantly and positively associated with hemoglobin A1c in patients with type 1 DM ($n = 71, r = 0.292, p = 0.013$) [36], while BMI, fasting plasma glucose, insulin, and hemoglobin A1c were shown to be significantly and positively correlated with plasma XOR activity in healthy subjects not receiving any medication [37]. In the present study, plasma XOR activity was significantly greater in pre-dialysis CKD patients with than in those without DM. Of importance, other clinical parameters including kidney

function were not significantly different between the groups (online suppl. Table 1), suggesting that impaired glycemic control itself is an important factor related to increased plasma XOR activity. Patients with DM have insulin resistance [38, 39], which is known to impair glycolysis, resulting in activation of the pentose phosphate pathway, followed by promotion of a hepatic de novo purine synthetic pathway [40], thereby triggering increased XOR activity. Although the mechanism remains unknown, redundant fructose in diabetic patients may also increase XOR activity by increasing ATP degradation to AMP, leading to increased nucleotides, which are uric acid precursors [41].

Recently, plasma XOR activity was found to be associated with vascular endothelial dysfunction in patients with type 1 DM [36]. In addition, elevated plasma XOR activity has been reported to be associated with increased CVD events in heart failure patients with preserved ejection function [42] and higher mortality in cardiovascular intensive care patients [43], as well as an independent predictor of CVD events in CKD and hemodialysis patients [10]. Interestingly, Kawachi et al. [44] demonstrated that short-term glycemic control treatment (2 weeks hospitalization) decreased plasma XOR activity in patients with type 2 DM. Together, findings showing an association between glycemic control and plasma XOR activity in patients with type 1 diabetes [36], patients under hemodialysis in our previous study [18], and pre-dialysis CKD patients in the present study suggest the importance of glycemic control in regard to decreased XOR, possibly leading to a decrease in CVD events.

The correlation between kidney function and plasma XOR activity remains controversial. In a previous study in which plasma XOR activity was measured using a traditional method, Gondouin et al. [10] reported that increased XOR activity in accordance with a decrease in kidney function was seen in 51 pre-dialysis CKD patients as well as 50 other CKD patients undergoing hemodialysis. In contrast, a significant and inverse correlation between plasma ln-XOR activity and kidney dysfunction was shown in the present study. Furthermore, when the correlation was examined separately in patients receiving or not receiving uric acid-lowering drugs, it remained significant in both groups. These significant correlations were consistent with findings presented in 3 other recent studies, in which plasma XOR activity was measured in our novel method. Otaki et al. [16] investigated plasma XOR activity in patients with chronic heart failure and found that in their lower eGFR group (53 ± 25 mL/min/1.73 m^2) to be significantly reduced as compared to the normal eGFR group (68 ± 26 mL/min/1.73 m^2). Ad-

ditionally, Shirakabe et al. [45] demonstrated that the serum creatinine level was independently associated with low plasma XOR activity in patients with acute heart failure, while Terawaki et al. [19] showed a strong correlation between plasma XOR activity and eGFR ($r = 0.751$, $p = 0.002$) in pre-dialysis CKD patients ($n = 13$). We speculate that this discrepancy may have been due to differences in measurement methods used to determine plasma XOR activity. With the traditional method, elevated endogenous uric acid in a CKD patient may influence elevated XOR activity, since the plasma XOR level is determined based on uric acid after addition of xanthine as a substrate to the sample. In contrast, our measurement method is not affected by endogenous uric acid in samples from CKD patients, since only isotope-labeled ($^{13}C_2$,$^{15}N_2$) xanthine is measured. Findings noted in previous studies [16, 19, 45] obtained by use of our novel measurement method support the inverse correlation between plasma XOR activity and kidney dysfunction noted in the present study.

Although XOR plays a pivotal role in uric acid production, no significant association between the serum uric acid level and plasma ln-XOR activity was observed in the present study. Recent studies that used our method have found serum uric acid to be positively correlated with plasma XOR activity in healthy subjects [37, 46, 47]. In contrast, in pre-dialysis CKD patients examined using the same method, serum uric acid was shown to be negatively correlated with plasma ln-XOR activity ($r = -0.562$, $p = 0.043$) [19]. Since uric acid excretion is dependent on kidney function [48, 49], it is possible that its level in serum is affected by decreased urinary uric acid excretion in CKD patients, rather than XOR activity itself.

Regarding CKD-MBD, calcium, phosphate, and intact-PTH were not found to be correlated with plasma ln-XOR activity in patients with hemodialysis [18], though FGF-23, an important factor of CVD events in CKD patients [50], was not evaluated in that study. In the present results, FGF-23 was shown to be significantly and negatively correlated with plasma ln-XOR activity, whereas it did not have a significant association with plasma ln-XOR activity after adjusting for several confounders, suggesting that CKD-MBD status is not associated with plasma ln-XOR activity.

The present study has some limitations. First, the number of patients examined was relatively small mainly because all were enrolled from a single institution. Notably, there were few diabetic CKD patients in whom the association between glycemic control status and plasma XOR activity was to be thoroughly investigated as the present study participants were followed up by nephrologists, not

by diabetologists. Nevertheless, hemoglobin A1c and plasma glucose were significantly and positively associated with plasma XOR activity after adjustments with several confounders in all CKD patients, even when a small number of diabetic patients were included. Additional large-scale studies are needed to investigate whether glycemic control status is associated with plasma ln-XOR activity in diabetic patients with or without CKD. Additionally, this study had a cross-sectional design, and the results did not demonstrate causality of the examined parameters, that is, poor glycemic control, associated with increased plasma XOR activity. Future studies are required to examine whether plasma XOR activity is reduced in pre-dialysis CKD patients by strict glycemic control, while any link between glycemic control and XOR activity should be confirmed using experimental animal models. In addition, in vitro studies may also be necessary to determine the potential effect of plasma glucose on XOR activity.

In conclusion, this is the first study to show a significant positive association of plasma XOR activity with hemoglobin A1c and plasma glucose levels in pre-dialysis CKD patients. The results suggest that glycemic control in CKD patients is important for decreased plasma XOR activity, leading to a decrease in CVD events.

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