JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Synergistic association of the copper/zinc ratio under inflammatory conditions with diabetic kidney disease in patients with type 2 diabetes: The Asahi Diabetes Complications Study

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Keywords
Copper/zinc ratio, Soluble tumor necrosis factor-α receptor 1, Diabetic kidney disease

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J Diabetes Investig 2022; 13: 299–307

doi: 10.1111/jdi.13659

ABSTRACT

Aims/Introduction: We aimed to study the relationships among the copper (Cu)/zinc (Zn) ratio, inflammatory biomarkers, and the prevalence of diabetic kidney disease (DKD) in patients with type 2 diabetes.

Materials and Methods: A cross-sectional study was performed on 651 patients with type 2 diabetes. DKD was defined as a urinary albumin-to-creatinine ratio of ≥30 mg/g creatinine and/or an estimated glomerular filtration rate using cystatin C of < 60 mL/min/1.73 m^2. Areas under the curves (AUCs), cutoff values, and thresholds for detecting DKD were determined for the Cu/Zn ratio, soluble tumor necrosis factor-α receptor 1 (sTNFαR1), and high-sensitivity C-reactive protein (hsCRP). Patients were categorized by each cutoff value of sTNFαR1 and the Cu/Zn ratio. Odds ratios (ORs) and biological interactions for the prevalence of DKD were determined.

Results: DKD was identified in 220 patients. AUC/optimal cutoff values were 0.777/1300 pg/mL for sTNFαR1, 0.603/1.1648 for the Cu/Zn ratio, and 0.582/305 ng/mL for hsCRP. The ORs for DKD were higher, but not significantly, in the sTNFαR1 < 1300 and Cu/Zn ≥ 1.1648 group, significantly higher in the sTNFαR1 ≥ 1300 and Cu/Zn < 1.1648 group ($P < 0.0001$), and further synergistically elevated in the sTNFαR1 ≥ 1300 and Cu/Zn ≥ 1.1648 group ($P < 0.0001$) compared with the sTNFαR1 < 1300 and Cu/Zn < 1.1648 group after multivariable adjustment. Levels of sTNFαR1 were significantly higher in the sTNFαR1 ≥ 1300 and Cu/Zn ≥ 1.1648 group than in the sTNFαR1 ≥ 1300 and Cu/Zn < 1.1648 group ($P = 0.0006$).

Conclusions: Under an inflammatory initiation signal of elevated serum sTNFαR1 levels, an increase in the Cu/Zn ratio may further exacerbate inflammation and is synergistically associated with a high prevalence of DKD in patients with type 2 diabetes.

INTRODUCTION

Inflammation plays a major role in progressive nephropathy in diabetes. Inflammatory biomarkers, including soluble tumor necrosis factor-α receptor 1 (sTNFαR1), are associated with a decline in the estimated glomerular filtration rate (eGFR) and

Received 24 May 2021; revised 5 August 2021; accepted 1 September 2021

an increase in the urinary albumin excretion rate in patients with type 1 diabetes[1–3]. In a previous study, circulating sTNFαR1 levels were independently associated with the incidence of end-stage renal disease in patients with type 1 diabetes[4]. In patients with type 2 diabetes, elevated sTNFαR1 levels were also associated with an eGFR <60 mL/min/1.73 m^2[25]. Therefore, measurement of sTNFαR1 levels provides useful information regarding the risk of diabetic kidney disease (DKD).

Zinc (Zn) and its transporters are involved in the synthesis and secretion of insulin and the signaling pathways of insulin action[6]. Zinc deficiency increases inflammatory factors and reactive oxygen species, and leads to immune dysfunction and aggravated inflammation[7]. Zinc deficiency also aggravates fibrosis by increasing inflammation in the kidney[8]. Zinc is extensively distributed in many organs and its levels are relatively high in the kidneys. Therefore, Zn deprivation exacerbates kidney damage[8].

Zinc and copper (Cu) compete at the absorption stage. Excess Cu under inflammatory conditions triggers oxidative stress[9]. Oxidative stress can explain the underlying mechanism of Cu renal toxicity because cellular membrane lipids are altered by lipoperoxidation and cause renal dysfunction[10]. According to a recent meta-analysis, higher serum Cu levels were observed in patients with diabetes than in healthy subjects, and similar results were obtained when stratified by the presence or absence of diabetic complications[11].

Many chronic diseases, such as diabetes and diabetic complications, are associated with abnormal metabolism of Cu and Zn[12]. Increased serum Cu levels and decreased serum Zn levels have been observed in patients with type 1 diabetes[13,14]. Zn deficiency and Cu excess are associated with increased risks of type 2 diabetes and cardiovascular disease[9]. The Cu/Zn ratio is increased in patients with diabetes compared with healthy controls. Cu and Zn levels are negatively correlated, and the Cu/Zn ratio and glycated hemoglobin (HbA1c) levels are positively correlated in patients with diabetes[15]. The Cu/Zn ratio in older Japanese inpatients is increased in the following order: chronic renal failure > diabetes mellitus > controls[16]. The Cu/Zn ratio can be a better indicator of various diseases compared with Zn or Cu levels alone[17–19]. The Cu/Zn ratio also appears to represent the severity of Zn deficiency[19].

We aimed to study the relationships among the Cu/Zn ratio, sTNFαR1 levels, and the prevalence of DKD in patients with type 2 diabetes. We also included a routinely measured inflammatory biomarker, high-sensitivity C-reactive protein (hsCRP) for comparison in our analysis.

METHODS

Recruitment of participants

This study was conducted using data from the Asahi Diabetes Complications Study (Asahi Study). The Asahi Study is an ongoing prospective, observational study on the incidence and progression of diabetic complications in patients with diabetes who visited our outpatient clinic that specializes in diabetes care. The patients were registered from November 2014 to December 2017 after obtaining informed consent. The exclusion criteria were as follows: patients who were unable to visit our clinic regularly; those who were on dialysis; pregnant women; and those who were judged to be ineligible to participate in this study (e.g., uncooperative patients and those who had difficulty in communicating). A total of 858 patients were eligible for this study of whom 806 had type 2 diabetes. Of these 806 patients, 652 had measurement of serum Zn and Cu levels and inflammatory markers, including sTNFαR1 and hsCRP. Of these 652 patients, one patient who suffered from an acute urinary tract infection at baseline was excluded. Therefore, at baseline, 651 patients (529 men and 122 women) were analyzed.

The research protocol was reviewed and approved by the Ethics Committee of the Institute of Medical Science, Asahi Life Foundation (No. 07501). The protocol complied with the Japanese government's Ethical Guidelines for Medical and Health Research Involving Human Subjects, which conform to the provisions of the Declaration of Helsinki. All participants gave written informed consent.

Measurement of the serum Cu/Zn ratio

At baseline, blood samples for measuring Cu and Zn levels were collected from participants of the Asahi Study who visited the outpatient clinic by 10:00 AM after overnight fasting. The serum Zn and Cu levels were determined using a colorimetric method[20,21]. The serum Cu/Zn ratio was then calculated.

Measurement of inflammatory biomarkers

Serum sTNFαR1 and hsCRP levels were measured. Levels of sTNFαR1 are more stable than TNF-α levels and remain elevated for extended periods of time. Additionally, sTNFαR1 levels are a powerful indicator of activation of the TNF-α system[22,23]. These inflammatory biomarkers were measured in the morning after overnight fasting. Levels of sTNFαR1 were assayed using an enzyme-linked immunosorbent assay (Human TNF RI/TNFRSF1A Quantikine ELISA Kit; R&D Systems, Minneapolis, USA). Levels of hsCRP were assayed by immunoturbidimetry.

Assessment of DKD

The urinary albumin-to-creatinine ratio (ACR) was measured by a turbidimetric immunoassay. Cystatin C levels, which are a biomarker of renal function, were measured using the colloidal gold agglutination method[24]. Cystatin C is an alternative filtration biomarker that has more linear risk associations than creatinine[25–27]. Therefore, cystatin C is a better biomarker than creatinine for risk classification and can be used reliably in clinical settings[27]. The use of cystatin C-based eGFR enhances the associations between eGFR categories and the risks of death and end-stage renal disease across diverse populations compared with using the creatinine-based eGFR[28]. The eGFR using

cystatin C (eGFRcys) was calculated using the formula advocated by the Japanese Society of Nephrology[29]. DKD was defined as an ACR of ≥ 30 mg/g creatinine and/or an eGFRcys of < 60 mL/min/1.73 m^2. Combining the eGFRcys and ACR significantly improves the risk classification[27].

Clinical and biochemical data collection

The height and weight of each patient were measured, and the body mass index (BMI) was calculated. Blood pressure (BP) was examined once in a seated position by a trained medical technician using an electronic sphygmomanometer (BP-10; OMRON, Kyoto, Japan). The duration of diabetes was obtained from the medical charts by attending physicians. Smoking status and alcohol intake were obtained from interview sheets completed by the study participants. Patients with an alcohol intake of ≥ 40 g/day among men and ≥20 g/day among women were defined as drinkers[30]. After fasting overnight, blood samples were taken in the morning. The HbA1c levels were measured using an analyzer (HLC-723G8, HLC-723G11; TOSOH, Tokyo, Japan) by a high-performance liquid chromatography method. Serum high-density lipoprotein cholesterol (HDLC) levels and serum low-density lipoprotein cholesterol (LDLC) levels were determined using a direct method. Serum triglyceride (TG) levels were determined using an enzymatic method.

Statistical analysis

Baseline clinical characteristics in patients with and without DKD were compared using the Student's *t*-test, Wilcoxon rank-sum test, and χ^2 test. The TG levels, ACR, hsCRP levels, and sTNFαR1 levels were converted to natural logarithms owing to their skewed distribution.

The discriminatory power of the Cu/Zn ratio, sTNFαR1 levels, and hsCRP levels for detecting DKD was determined using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve by univariable logistic regression models. The cutoff values of these biomarkers for detecting the prevalence of DKD were determined using Youden's index by ROC curve analyses.

Additionally, to estimate the thresholds of the Cu/Zn ratio, sTNFαR1 levels, and hsCRP levels for detecting DKD, multivariable logistic regression analyses were performed. The Cu/Zn ratio, sTNFαR1 levels, and hsCRP levels were classified into quintiles, with the lowest quintile as the reference. The covariates were age, sex, diabetes duration, BMI, systolic BP, levels of HbA1c, LDLC, HDLC, and natural logarithm (ln)-transformed TG, current smoking, use of insulin, use of renin–angiotensin–aldosterone system (RAAS) inhibitors, and use of statins.

Furthermore, multivariable logistic regression analyses were used to determine the ORs for the prevalence of DKD related to the patients' categories classified by each cutoff value of sTNFαR1 levels and the Cu/Zn ratio. Model 1 included the dichotomized category of sTNFαR1 levels as an explanatory variable. Model 2 included the dichotomized category of the Cu/Zn ratio as an explanatory variable. Model 3 included both dichotomized categories of sTNFαR1 levels and the Cu/Zn ratio simultaneously to adjust each other. Model 4 included four categories based on each cutoff value of sTNFαR1 levels and the Cu/Zn ratio. Covariates were included in these models using three sets of variables. The first set was age and sex. The second set was age, sex, diabetes duration, BMI, systolic BP, HbA1c levels, LDLC levels, HDLC levels, TG levels (ln-transformed), and current smoking. The third set was the use of insulin, use of an RAAS inhibitor, and use of a statin in addition to the second set of variables. Additionally, the Wilcoxon rank-sum test was used to compare sTNFαR1 levels in the patients' categories.

Subsequently, the biological interaction was calculated using a logistic regression model. The relative excess risk due to interaction (RERI), the attributable proportion due to interaction (AP), and the synergy index (S), which are three measures of biological interaction, were determined as reported by Andersson *et al.*[31]. Specifically, $i = 1$ if the first risk factor is present and $i = 0$ otherwise, $j = 1$ if the second risk factor is present and $j = 0$ otherwise, and RRij is the relative risk in category i, j. Therefore, the relative risks for four categories were RR_{11}, RR_{10}, RR_{01}, and RR_{00} (RR_{00} was the reference category). Three measures of the biological interaction were defined as follows: $RERI = RR_{11} - RR_{10} - RR_{01} + 1$, $AP = RERI/RR_{11}$, and $S = [RR_{11} - 1]/[(RR_{10} - 1) + (RR_{01} - 1)]$. If both RERI and AP are equal to 0 and S is equal to 1, there is no biological interaction. Confidence intervals (CIs) for the three measures of biological interaction were calculated using the delta method[32] referred by Hosmer and Lemeshow[33].

The data were analyzed using SAS Version 9.4 software (SAS Institute, Cary, NC, USA). Two-sided *P*-values < 0.05 were considered statistically significant.

RESULTS

Comparison of clinical features and biomarkers between the DKD and non-DKD groups

Diabetic kidney disease was identified in 220 patients. The clinical features of all patients, patients with DKD, and those without DKD are shown in Table 1. The DKD group was older ($P < 0.0001$), had a longer duration of diabetes ($P = 0.0001$), had a higher BMI ($P = 0.004$), systolic BP ($P = 0.0002$), Cu/Zn ratio ($P < 0.0001$), and ACR ($P < 0.0001$), and had higher levels of HbA1c ($P = 0.001$), TG ($P = 0.004$), creatinine ($P < 0.0001$), cystatin C ($P < 0.0001$), hsCRP ($P = 0.0003$), sTNFαR1 ($P < 0.0001$), and Cu ($P = 0.007$) than the non-DKD group. The DKD group had lower HDLC levels ($P = 0.020$), eGFRcys ($P < 0.0001$), and Zn levels ($P = 0.0005$) than the non-DKD group. The DKD group comprised higher proportions of users of insulin ($P < 0.0001$), antihypertensive agents (RAAS inhibitors, calcium channel blockers, α-, β-, and αβ-blockers, and diuretics; all $P < 0.0001$), and lipid-lowering agents (statins, $P = 0.020$) than the non-DKD group. The DKD group also had a lower proportion of users of oral

anti-diabetes drugs and/or glucagon-like peptide-1 receptor agonists than the non-DKD group ($P = 0.001$).

Optimal cutoff values of the Cu/Zn ratio, sTNFαR1 levels, and hsCRP levels for detecting DKD

The discriminatory abilities of the Cu/Zn ratio, sTNFαR1 levels, and hsCRP levels for detecting DKD were determined using ROC curves (Figure 1). The AUC (95% CI) and optimal cutoff values were 0.777 (0.736–0.817) and 1300 pg/mL for sTNFαR1 levels, 0.603 (0.558–0.648) and 1.1648 for the Cu/Zn ratio, and 0.582 (0.536–0.628) and 305 ng/mL for hsCRP levels, respectively. The sensitivity (%), specificity (%), the positive predictive value (%), and the negative predictive value were 60.0, 84.0, 65.7, and 80.4% for sTNFαR1 levels, 60.5, 57.1, 41.8, and 73.9% for the Cu/Zn ratio, and 76.4, 37.4, 38.4, and 75.6% for hsCRP levels, respectively. The AUC of sTNFαR1 levels was significantly larger than that of hsCRP levels or that of the Cu/Zn ratio (both $P < 0.0001$). The AUC of hsCRP levels was the smallest among these three biomarkers.

Evaluation of the thresholds of sTNFαR1 levels, the Cu/Zn ratio, and hsCRP levels for detecting DKD

Odds ratios (ORs) and 95% CIs by quintiles of sTNFαR1 levels, the Cu/Zn ratio, and hsCRP levels were calculated using multivariable logistic regression analyses (Figure 2). Covariates are defined in the Methods section. The ORs of the quintiles of sTNFαR1 levels were significantly elevated from the fourth quintile (1230–1400 pg/mL) (OR, 2.07; 95% CI, 1.08–3.96;

Table 1 | Baseline clinical characteristics of all participants and participants with and without DKD

	All (n = 651)	Non-DKD (n = 431)	DKD (n = 220)	P-value
Male sex	529 (81.3)	350 (81.2)	179 (81.4)	0.96
Age (years)	65.1 ± 9.7	63.5 ± 9.2	68.3 ± 10.0	<0.0001
Diabetes duration (years)	17.1 ± 9.5	16.1 ± 9.2	19.2 ± 9.8	0.0001
BMI (kg/m^2)	24.5 ± 3.6	24.2 ± 3.5	25.1 ± 3.8	0.004
Systolic BP (mmHg)	130.4 ± 12.5	129.1 ± 12.5	132.9 ± 12.0	0.0002
Diastolic BP (mmHg)	75.9 ± 8.8	76.3 ± 8.5	75.1 ± 9.3	0.095
HbA1c (%) (mmol/mol)	7.0 ± 0.8 (53 ± 9)	7.0 ± 0.8 (52 ± 8)	7.2 ± 1.0 (55 ± 11)	0.001
LDL-C (mmol/L)	2.69 ± 0.58	2.71 ± 0.59	2.64 ± 0.56	0.13
HDL-C (mmol/L)	1.48 ± 0.39	1.51 ± 0.40	1.43 ± 0.38	0.020
TG (mmol/L)*	1.17 ± 0.68	1.12 ± 0.66	1.27 ± 0.72	0.004
Creatinine (μmol/L)	73.5 ± 20.7	68.4 ± 13.0	83.6 ± 28.0	<0.0001
Cystatin C (mg/L)	0.96 ± 0.24	0.88 ± 0.13	1.12 ± 0.32	<0.0001
eGFRcys (mL/min/1.73 m^2)	79.0 ± 19.4	85.1 ± 15.4	67.1 ± 20.9	<0.0001
Urine albumin-to-creatinine ratio (mg/g creatinine)*	16.1 ± 40.3	8.0 ± 6.1	63.0 ± 175.2	<0.0001
hsCRP (ng/mL)*	524.8 ± 845.3	468.4 ± 706.2	655.9 ± 1141.4	0.0003
sTNFαR1 (pg/mL)*	1190.3 ± 343.8	1079.5 ± 209.5	1441.6 ± 490.5	<0.0001
Cu (μg/dL)	98.2 ± 15.7	97.0 ± 15.6	100.5 ± 15.5	0.007
Zn (μg/dL)	84.3 ± 11.5	85.4 ± 11.3	82.1 ± 11.6	0.0005
Cu/Zn	1.186 ± 0.253	1.155 ± 0.242	1.247 ± 0.265	<0.0001
Current smoker	126 (19.4)	90 (20.9)	36 (16.4)	0.17
Alcohol intake†	82 (12.6)	53 (12.3)	29 (13.2)	0.75
Use of antidiabetes agents				
Oral antidiabetes drugs and/or GLP-1RA	426 (65.4)	301 (69.8)	125 (56.8)	0.001
Insulin	173 (26.6)	92 (21.4)	81 (36.8)	<0.0001
Use of antihypertensive agents				
RAAS inhibitors	328 (50.4)	185 (42.9)	143 (65.0)	<0.0001
Calcium-channel blockers	270 (41.5)	134 (31.1)	136 (61.8)	<0.0001
α-, β-, αβ-blockers	66 (10.1)	26 (6.0)	40 (18.2)	<0.0001
Diuretics	37 (5.7)	13 (3.0)	24 (10.9)	<0.0001
Use of lipid-lowering agents				
Statins	358 (55.0)	223 (51.7)	135 (61.4)	0.020
Fibrates	30 (4.6)	17 (3.9)	13 (5.9)	0.26

Values are n (%) or mean ± standard deviation. BMI, body mass index; BP, blood pressure; Cu, copper; DKD, diabetic kidney disease; eGFRcys, estimated glomerular filtration rate using cystatin C; GLP-1 RA, glucagon-like peptide-1 receptor agonist; HbA1c, glycated hemoglobin; HDLC, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDLC, low-density lipoprotein cholesterol; *RAAS*, renin–angiotensin–aldosterone system; sTNFαR1, soluble tumor necrosis factor-α receptor 1; TG, triglycerides; UA, uric acid; Zn, zinc. *Ln-transformed. †Alcohol intake ≥40 g/day for men and ≥20 g/day for women.

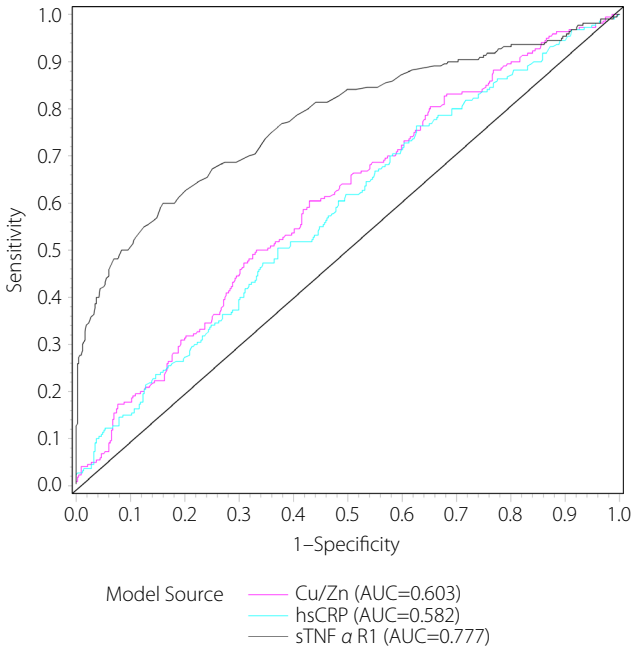

Figure 1 | Receiver operating characteristic curves of sTNFαR1 levels, the Cu/Zn ratio, and hsCRP levels for detecting diabetic kidney disease. sTNFαR1, soluble tumor necrosis factor-α receptor 1; Cu, copper; Zn, zinc; hsCRP, high-sensitivity C-reactive protein.

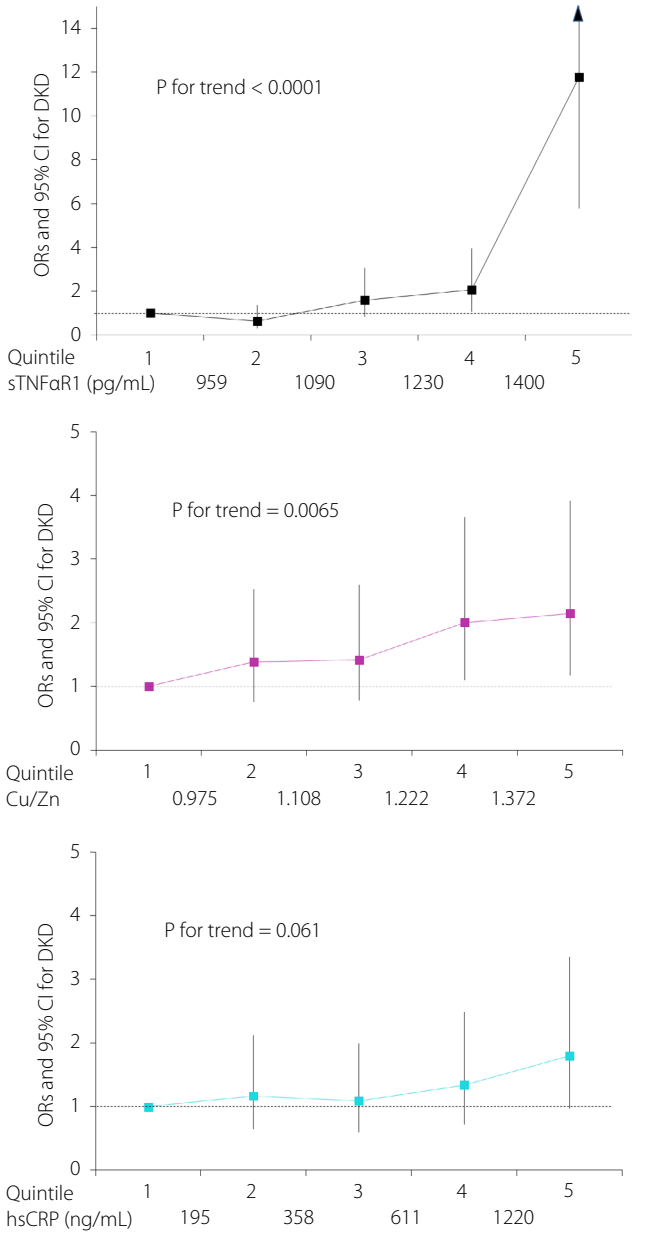

Figure 2 | Evaluation of the thresholds of sTNFαR1 levels, the Cu/Zn ratio, and hsCRP levels for detecting diabetic kidney disease. Data were adjusted for age, sex, diabetes duration, body mass index, systolic blood pressure, glycated hemoglobin levels, low-density lipoprotein cholesterol levels, high-density lipoprotein cholesterol levels, triglyceride levels (ln-transformed), current smoking, use of insulin, use of a renin–angiotensin–aldosterone system inhibitor, and use of a statin.

$P = 0.029$), including the corresponding cutoff value. The ORs of the quintiles of the Cu/Zn ratio were also significantly elevated from the fourth quintile (1.222–1.372) (OR, 2.00; 95% CI, 1.09–3.66; $P = 0.024$) and were slightly higher than the corresponding cutoff value. Therefore, the thresholds were identified as 1230 pg/mL for sTNFαR1 levels and 1.222 for the Cu/Zn ratio. In contrast, ORs of the quintiles of hsCRP levels were not significantly elevated (OR of the fifth quintile, 1.80; 95% CI, 0.97–3.35; $P = 0.063$). Therefore, the threshold for hsCRP levels was unclear. The corresponding cutoff value based on the lowest AUC among these three biomarkers was also useless for discrimination.

ORs for DKD related to the patients' categories classified by each cutoff value of sTNFαR1 levels and the Cu/Zn ratio

Multivariable logistic regression analyses were performed to determine the ORs for the prevalence of DKD in association with the patients' categories classified by each cutoff value of an sTNFαR1 level of 1300 pg/mL and a Cu/Zn ratio of 1.1648 (Table 2). Covariates are described above in the Statistical analysis subsection. In models 1 and 2, the sTNFαR1 ≥ 1300 group and the Cu/Zn ≥ 1.1648 group were significantly associated with DKD after adjusting for age and sex (both $P < 0.0001$), after multivariable adjustment ($P < 0.0001$, $P = 0.0002$, respectively), and after further adjustment ($P < 0.0001$, $P = 0.002$, respectively). In model 3, the sTNFαR1 ≥ 1300 group and the Cu/Zn ≥ 1.1648 group were significantly associated with DKD independent of each other after adjusting for age and sex ($P < 0.0001$, $P = 0.014$, respectively), after multivariable

adjustment ($P < 0.0001$, $P = 0.016$, respectively), and after further adjustment ($P < 0.0001$, $P = 0.046$, respectively). In model 4, patients were divided into four categories on the basis of each cutoff value of sTNFαR1 levels and the Cu/Zn ratio. The ORs for DKD related to these four groups (the sTNFαR1 < 1300 and Cu/Zn < 1.1648 group was the reference) were calculated. The ORs for DKD were elevated, but not significantly, in the sTNFαR1 < 1300 and Cu/Zn ≥ 1.1648 group. The ORs for DKD were significantly elevated in the sTNFαR1 ≥ 1300 and Cu/Zn < 1.1648 group after adjusting for age and sex ($P < 0.0001$), after multivariable adjustment ($P < 0.0001$), and after further adjustment ($P < 0.0001$). The ORs were further significantly elevated in the sTNFαR1 ≥ 1300 and Cu/Zn ≥ 1.1648 group after adjusting for age and sex ($P < 0.0001$), after multivariable adjustment ($P < 0.0001$), and after further adjustment ($P < 0.0001$).

Additionally, sTNFαR1 levels were significantly higher in the sTNFαR1 ≥ 1300 and Cu/Zn ≥ 1.1648 group than in the sTNFαR1 ≥ 1300 and Cu/Zn < 1.1648 group ($P = 0.0006$). Similarly, sTNFαR1 levels tended to be slightly higher in the sTNFαR1 < 1300 and Cu/Zn ≥ 1.1648 group than in the sTNFαR1 < 1300 and Cu/Zn < 1.1648 group ($P = 0.057$).

Biological interaction

The biological interaction was calculated to investigate the combined effects of sTNFαR1 levels of ≥ 1300 pg/mL and a Cu/Zn ratio of ≥ 1.1648 on the prevalence of DKD. The values of the estimates (95% CIs) of RERI, AP, and S were 6.205 (0.926–11.485), 0.560 (0.297–0.823), and 2.605 (1.270–5.342), respectively, for DKD after adjusting for age and sex. The values of the estimates (95% CIs) of RERI, AP, and S were 5.318 (0.591–10.044), 0.558 (0.285–0.831), and 2.656 (1.234–5.718) after multivariable adjustment, and 4.328 (0.024–8.632), 0.513 (0.205–0.821), and 2.395 (1.084–5.295) after further adjustment, respectively (footnotes in Table 2). Consequently, in all of the adjusted models, the lower limits of the 95% CIs of RERI and AP were > 0 and that of S was > 1. Therefore, the biological interaction between sTNFαR1 levels ≥ 1300 pg/mL and a Cu/Zn ratio ≥ 1.1648 for DKD was significant.

DISCUSSION

Under an inflammatory initiation signal of elevated serum sTNFαR1 levels, an increase in the Cu/Zn ratio can further exacerbate inflammation and is synergistically associated with a high prevalence of DKD in patients with type 2 diabetes. The inflammatory response under Zn deficiency is aggravated by dysfunction of the T helper type 2 lymphocyte-M2 macrophage pathway[7]. Additionally, Zn deficiency significantly aggravates diabetes-induced renal inflammation, oxidative stress, and fibrosis[34]. An increased intake of Cu in diabetic rats induces a dyslipidemic profile, oxidative damage, and renal dysfunction[10]. These previous research findings support the results of our clinical study. Therefore, the Cu/Zn ratio may play a crucial role in the development of DKD under inflammatory conditions. The serum Cu/Zn ratio may be a clinically important indicator that enables risk stratification for DKD.

There was no definite association between hsCRP levels as an inflammatory biomarker and the prevalence of DKD in our study, which is in line with the results of the Diabetes Control and Complications Trial[1,2]. Levels of hs-CRP reflect nonspecific inflammatory conditions and these cause large fluctuations within or between individuals. This may be one of the reasons why hsCRP levels were not useful for discrimination of DKD.

A previous study showed that the Cu/Zn ratio was a sensitive predictor of all-cause mortality in the older population[35]. Univariable analysis in this previous study showed that CRP levels were also a predictor of all-cause mortality, but multivariable analysis eliminated this significance. These findings are consistent with our study, and are supported by the fact that DKD is an important predictor of all-cause mortality in patients with diabetes. In our study, when hsCRP levels were entered into the models as a continuous variable, they were significantly associated with the prevalence of DKD. However, when hsCRP levels and the Cu/Zn ratio were simultaneously entered into the models, only the Cu/Zn ratio remained significant. Therefore, the Cu/Zn ratio appeared to be a more sensitive biomarker than hsCRP levels for DKD.

A previous study showed that one of the strongest determinants of a renal decline in patients with type 1 diabetes was the baseline serum sTNFαR1 level[3]. This previous study also reported that renal function declined in 10% of patients with normoalbuminuria and in 35% of those with microalbuminuria. Early progressive renal decline preceded the incidence of microalbuminuria and its progression to macroalbuminuria. In our cross-sectional study, normoalbuminuria with an eGFRcys < 60 mL/min/1.73 m^2 was found in 52/220 patients with DKD (almost 24% of the total DKD patients). The AUCs (95% CIs) of serum sTNFαR1 levels and the Cu/Zn ratio for detecting an eGFRcys < 60 mL/min/1.73 m^2 were 0.934 (0.909–0.960) and 0.635 (0.577–0.693), respectively. Those for detecting normoalbuminuria with an eGFRcys < 60 mL/min/1.73 m^2 were 0.866 (0.828–0.903) and 0.616 (0.537–0.694), respectively. Therefore, serum sTNFαR1 levels may be an earlier signal for the beginning of DKD than the serum Cu/Zn ratio. An increased serum Cu/Zn ratio can accelerate the development of DKD under elevated serum sTNFαR1 levels.

The threshold for serum sTNFαR1 levels was 1230 pg/mL and that for the serum Cu/Zn ratio was 1.222 in our study. In the Diabetes Control and Complications Trial, the mean change in albumin excretion rates sharply increased at the median value of 1261 (interquartile range, 1122.5–2910.8) pg/mL, which was the highest tertile of baseline sTNFαR1 levels[1]. This finding supports our results. Another study showed that the potential threshold of the serum Cu/Zn ratio for diagnosing dysgeusia derived from Zn deficiency was 1.1[19]. The threshold of the Cu/Zn ratio for the prevalence of DKD in our study was slightly higher than the threshold for the diagnosis of dysgeusia. This

Table 2 | Odds ratios for the prevalence of DKD related to the patients' categories classified by each cutoff value of sTNFαR1 levels and the Cu/Zn ratio

Prevalence of DKD (n = 220)	N with/without DKD	Age and sex adjusted		Multivariable adjusted*		Multivariable adjusted†	
		OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Model 1							
sTNFαR1 ≥ 1300 pg/mL	132/69	6.93 (4.71–10.20)	<0.0001	5.87 (3.91–8.81)	<0.0001	5.51 (3.65–8.32)	<0.0001
Model 2							
Cu/Zn ≥ 1.1648	133/185	2.02 (1.43–2.86)	<0.0001	1.99 (1.38–2.88)	0.0002	1.80 (1.23–2.62)	0.002
Model 3							
sTNFαR1 ≥ 1300 pg/mL	132/69	6.49 (4.40–9.57)	<0.0001	5.48 (3.64–8.26)	<0.0001	5.25 (3.47–7.94)	<0.0001
Cu/Zn ≥ 1.1648	133/185	1.62 (1.10–2.37)	0.014	1.63 (1.10–2.42)	0.016	1.51 (1.01–2.26)	0.046
Model 4							
sTNFαR1 ≥ 1300 pg/mL and Cu/Zn ≥ 1.1648	90/32	11.07 (6.55–18.72)‡	<0.0001	9.53 (5.51–16.49)§	<0.0001	8.43 (4.81–14.77)¶	<0.0001
sTNFαR1 ≥ 1300 pg/mL and Cu/Zn < 1.1648	42/37	4.61 (2.65–8.05)	<0.0001	3.95 (2.23–6.98)	<0.0001	3.90 (2.19–6.94)	<0.0001
sTNFαR1 < 1300 pg/mL and Cu/Zn ≥ 1.1648	43/153	1.25 (0.77–2.04)	0.36	1.26 (0.77–2.09)	0.36	1.20 (0.72–2.00)	0.48
sTNFαR1 < 1 300 pg/mL and Cu/Zn < 1.1648	45/209	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	

DKD was defined as a urinary albumin-to-creatinine ratio ≥ 30 mg/g creatinine and/or an eGFRcys < 60 mL/min/1.73 m^2. AP, attributable proportion due to interaction; BMI, body mass index; CI, confidence interval; Cu, copper; DKD, diabetic kidney disease; eGFRcys, estimated glomerular filtration rate using cystatin C; HbA1c, glycated hemoglobin; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; OR, odds ratio; RERI, relative excess risk due to interaction; S, synergy index; sTNFαR1, soluble tumor necrosis factor-α receptor 1; Zn, zinc. *Adjusted for age, sex, diabetes duration, BMI, systolic blood pressure, HbA1c levels, LDLC levels, HDLC levels, triglyceride levels (ln-transformed), and current smoking. †Adjusted for age, sex, diabetes duration, BMI, systolic blood pressure, HbA1c levels, LDLC levels, HDLC levels, triglyceride levels (ln-transformed), current smoking, use of insulin, use of a renin–angiotensin–aldosterone system inhibitor, and use of a statin. ‡Biological interaction: RERI, 6.205 (0.926–11.485); AP, 0.560 (0.297–0.823); S, 2.605 (1.270–5.342). §Biological interaction: RERI, 5.318 (0.591–10.044); AP, 0.558 (0.285–0.831); S, 2.656 (1.234–5.718). ¶Biological interaction: RERI, 4.328 (0.024–8.632); AP, 0.513 (0.205–0.821); S, 2.395 (1.084–5.295).

discrepancy between the studies may be due, at least in part, to a higher Cu/Zn ratio in patients with diabetes compared with those without diabetes.

To the best of our knowledge, this is the first study to evaluate the relationship between biomarkers, including the Cu/Zn ratio and sTNFαR1 levels, and the prevalence of DKD. The strengths of our study include numerous measurements of biomarkers, such as the Cu/Zn ratio and sTNFαR1 levels. However, some limitations should be acknowledged. First, the study design was cross-sectional; therefore, any causality could not be established. Our hypothesis that an increase in the Cu/Zn ratio under inflammatory conditions is synergistically associated with DKD can provide the basis for a prospective cohort study in the future. Second, all participants in the present study were from a single Japanese clinic, and therefore, generalizability is limited.

In conclusion, under an inflammatory initiation signal of elevated serum sTNFαR1 levels, an increase in the Cu/Zn ratio further exacerbates inflammation and is synergistically associated with a high prevalence of DKD in patients with type 2 diabetes. The Cu/Zn ratio may be a clinically important indicator that enables risk stratification for DKD. Maintaining an appropriate Cu/Zn ratio of <1.1648 might be crucial for avoiding the acceleration of DKD in patients with type 2 diabetes.

ACKNOWLEDGMENTS

We express special thanks to all of the Asahi Study participants. We also thank Ms Mineko Okayasu, Ms Keiko Honjo, Ms Yoko Iigaya, Ms Yuki Inoue, and Mr Nobuhiro Tachibana for their excellent secretarial assistance. This study had no funding support.

DISCLOSURE

The authors declare no conflicts of interest.
Approval of the research protocol: Ethics Committee of the Institute of Medical Science, Asahi Life Foundation (approval number: 07501).
Informed consent: Yes.
Approval date of registry and the registration no. of the study/-trial: November 26, 2014, UMIN000015744.
Animal studies: N/A.

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