Association of Triglycerides to High-Density Lipoprotein Cholesterol Ratio with Incident Cardiovascular Disease but not End-Stage Kidney Disease among Patients with Biopsy-Proven Diabetic Nephropathy

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Abstract

Increased triglycerides (TG) and decreased high-density lipoprotein cholesterol (HDL-C) are dyslipidemias characteristic of diabetes. Here, we aimed to examine associations of TG/HDL-C ratio with cardiovascular disease (CVD) and kidney dysfunction among patients with diabetic nephropathy. This retrospective observational study consists of patients with biopsy-proven diabetic nephropathy at Nara Medical University Hospital. Exposure of interest was TG/HDL-C ratio measured at kidney biopsy. Outcome variables were kidney histological findings, incident CVD and end-stage kidney disease (ESKD). Multivariable logistic regression models and Cox proportional hazard models were used to examined these associations. A total of 353 subjects were divided into quartiles based on TG/HDL-C ratio: Quartile 1 (reference), <1.96; Quartile 2, 1.96−3.10; Quartile 3, 3.11−4.55; and Quartile 4, ≥4.56. TG/HDL-C ratio was not a predictor of any histological findings in fully adjusted models. During median follow-up periods of 6.2 and 7.3 years, 152 and 90 subjects developed CVD and ESKD, respectively. Higher TG/HDL-C ratio was independently associated with higher incidences of CVD even after adjustments for potential confounders (hazard ratio [95% confidence interval] for Quartile 3 vs. reference; 1.73 [1.08−2.79] and Quartile 4 vs. reference; 1.86 [1.10−3.17]). Although there was a weak association between TG/HDL-C ratio and ESKD in the univariable model, the association was not significant in fully adjusted models. In conclusion, among patients with biopsy-proven diabetic

nephropathy, higher TG/HDL-C ratio was independently associated with higher incidences of CVD but not with kidney outcomes, suggesting different impact of TG/HDL-C ratio on cardiorenal outcomes.

Keywords

cardiovascular disease, diabetic nephropathy, end-stage kidney disease, kidney biopsy, triglycerides to high-density lipoprotein cholesterol ratio

Running title

TG/HDL-C ratio and cardiorenal outcomes

Introduction

Dyslipidemias, including higher levels of triglycerides (TG) and higher low-density lipoprotein cholesterol (LDL-C) and lower levels of high-density lipoprotein cholesterol (HDL-C), are traditional risk factors for cardiovascular disease (CVD)[1-4]. In recent times, a novel indicator of dyslipidemia, TG/HDL-C ratio, is being used in clinical practice. Higher TG/HDL-C ratio, which is often observed in patients with diabetes and/or chronic kidney disease (CKD)[5,6], is considered to reflect insulin resistance and atherogenic small dense LDL-C levels[7,8]. Therefore, apart from increased LDL-C levels, increased TG and decreased HDL-C levels are considered as a residual atherogenic risk in these patients. Higher TG/HDL-C ratio is shown to be associated with a higher incidence of CVD in the general population[9,10]. In these studies, TG/HDL-C ratio could be used as a more reliable predictor of CVD than traditional lipid markers, such as LDL-C and HDL-C[9,11]. In terms of kidney outcomes, TG/HDL-C ratio could be used as a predictor of incident CKD[12] or of a 2-year change in estimated glomerular filtration rate (eGFR)[13]; however, there is a lack of evidence regarding hard kidney endpoints, such as end-stage kidney disease (ESKD).

Diabetes is one of the leading causes of CVD and kidney dysfunction. On the other hand, subjects with diabetes also have a higher prevalence of obesity, hypertension, and other metabolic disturbances[14,15]. Although glucose intolerance would be the main cause of subsequent CVD and kidney dysfunction, it is also possible for other metabolic disturbances,

such as increased TG and/or decreased HDL-C levels, to play an important role in the development of such clinical outcomes. Since the histological changes in diabetes, including glomerular and tubulointerstitial lesions, are known to be useful predictors of subsequent kidney dysfunction[16,17], the association between TG/HDL-C ratio and pathological changes is also intriguing.

In this study, we aimed to examine the association of TG/HDL-C ratio with histological findings, incident CVD and ESKD among patients with biopsy-proven diabetic nephropathy.

Methods

Study design and population

This was a single-center, retrospective observational study. Patients who underwent kidney biopsy between June 1981 and December 2014 at Nara Medical University Hospital and who diagnosed with diabetic nephropathy with class IIa or more severe glomerular lesions using light microscopy, as defined by the guidelines of the Research Committee of the Renal Pathology Society[18], were included. As glomerular basement membrane thickening was not confirmed by electron microscopy, patients with diabetic nephropathy with class I glomerular lesions were not included in the analyses. Patients with missing clinical data or diabetic nephropathy complicated by other kidney diseases (e.g., diabetic nephropathy

complicated with Immunoglobulin A nephropathy) were excluded. The observational period ended either at the end of October 2018 or when a patient was lost to follow-up.

The study protocol was approved by the Nara Medical University Ethics Committee (No. 2005-18) and was registered in the University Hospital Medical Information Network (UMIN) clinical trial registry (UMIN000031121). The opt-in or opt-out approach was used to obtain the consent of this study.

Exposure of interest and outcomes

The exposure of interest was TG/HDL-C ratio calculated at the time of kidney biopsy. The outcome variables were kidney histological findings, incident CVD and ESKD after kidney biopsy.

Data acquisition and definition

The medical records were manually searched to identify all the patients who underwent a kidney biopsy and were diagnosed with diabetic nephropathy as well as to obtain the following data: date of biopsy, demographic data, comorbidities, medication use, laboratory data, and clinical outcomes including renal histological findings. A pathological evaluation was performed independently by at least two pathologists, and differences were resolved through consensus.

TG/HDL-C ratio was calculated by dividing TG level (mg/dL) by HDL-C level (mg/dL). We evaluated the diabetic glomerular, tubule-interstitial, and vascular lesions according to the previous reports[18,19]. Glomerular lesions were categorized as follows: Classes IIa and IIb, mild and severe mesangial expansion, respectively; Class III, nodular sclerosis with <50% global glomerulosclerosis; and Class IV, >50% global glomerulosclerosis. The severity of interstitial fibrosis and tubular atrophy (IFTA) was scored as follows: 0, absent; 1, <25% IFTA; 2, 25%−50% IFTA; and 3, >50% IFTA. The severity of intimal thickening in large vessels was scored as follows: 0, no intimal thickening; 1, intimal thickening less than thickness of media; and 2, intimal thickening greater than thickness of media. The severity of arteriolar hyalinosis was graded as follows: 0, no hyalinosis; 1, one or more partial hyalinosis; 2, approximately 50% hyalinosis; and 3, more than 50% hyalinosis or penetrating hyalinosis[19]. For analyses, these histological findings were reclassified into dichotomous variables (i.e., glomerular lesions: Class ≥IIb or IIa, IFTA: score ≥2 or ≤1, arteriolar hyalinosis: score ≥1 or 0, and intimal thickening: score ≥1 or 0), as described previously[20]. Incident CVD was defined as the first event of fatal or non-fatal acute myocardial infarction, fatal or non-fatal stroke, hospitalization due to worsening of congestive heart failure, coronary revascularization, fatal arrhythmia, major amputation, or cardiac sudden death. ESKD was defined as the requirement for permanent kidney replacement therapy.

Baseline demographics and blood sample results were obtained at the time of biopsy. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or the current use of antihypertensive agents. Blood pressures were measured twice on patients' upper-arm with the auscultatory method using a mercury or hybrid sphygmomanometer at two minutes intervals after they were seated for a few minutes[21]. The average values were used as baseline blood pressures. A history of CVD included that of ischemic heart disease, stroke, hospitalization due to the worsening of congestive heart failure, or major amputation. Renin-angiotensin system (RAS) blockers included angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers. Fasting blood samples were collected. LDL-C was calculated from total cholesterol, HDL-C and TG levels using the Friedewald formula[22]. If need, serum creatinine levels that were measured using the Jaffe method were converted to levels corresponding to the enzymatic method by subtracting 0.207 mg/dL[23]. The eGFR was calculated based on the baseline creatinine level using the equation developed for Japanese populations[24]. Hemoglobin A1c (HbA1c) levels measured in Japan Diabetic Society values were converted to National Glycohemoglobin Standardization Program values[25]. Proteinuria was evaluated using the 24-h urine protein level (or the spot urine protein creatinine ratio if the 24-h urine protein level was not available).

Statistical analysis

Based on TG/HDL-C ratios, the patients were divided into quartiles ranging from Quartile 1 to 4, and Quartile 1, which was the group with the lowest TG/HDL-C ratios, was treated as the reference. The baseline data were expressed as medians with interquartile ranges or numbers with percentages as appropriate. The trends among the groups were tested using the Cochran-Armitage and Jonckheere-Terpstra tests. A multivariable logistic regression model was used to examine the association between TG/HDL-C ratio and kidney histological findings. The data were adjusted for age and sex in model 1. In model 2, the data were adjusted for variables included in model 1 as well as for body mass index (BMI), history of diabetic retinopathy, RAS blockers, statins, systolic blood pressure, eGFR, fasting serum glucose, and proteinuria. Multivariable Cox proportional hazard models were used to examine the association between TG/HDL-C ratio and incident CVD or ESKD. For the analyses of incident CVD, the data were adjusted for age and sex in model 1. In model 2, the data were adjusted for variables included in model 1 as well as for BMI, history of CVD, RAS blockers, and statins. In model 3, the data were adjusted for variables included in model 2 as well as for systolic blood pressure, eGFR, fasting serum glucose, and proteinuria. In model 4, the data were adjusted for variables included in model 3 as well as for IFTA, glomerular lesions, hyalinosis, and intimal thickening. For the analyses of incident ESKD, the data were adjusted for age and sex in model 1. In model 2, the data were adjusted for

variables of model 1 plus BMI, history of diabetic retinopathy, RAS blockers, and statins. In model 3, the data were adjusted for variables of model 2 plus systolic blood pressure, eGFR, fasting serum glucose, and proteinuria. In model 4, the data were adjusted for variables of model 3 plus IFTA, glomerular lesions, hyalinosis, and intimal thickening. Restricted cubic spline (RCS) analysis of each outcome was performed using the same variables as those in model 4. In RCS analysis, TG/HDL-C ratio was used as a continuous variable, and the upper limit value in Quartile 1 was used as the reference TG/HDL-C ratio. In addition, outliers, defined as TG/HDL-C ratio <1 or >8, were excluded from RCS analysis. P value <0.05 was considered to be statistically significant. All analyses were performed using R, version 4.0.5 (R Foundation, Vienna, Austria).

Results

Baseline characteristics of patients with diabetic nephropathy

Among 408 subjects diagnosed as diabetic nephropathy with no other histological complications, 12 and 51 were excluded due to missing data for proteinuria and TG/HDL-C ratio, respectively; the remaining 353 subjects were included in the analysis. The subjects were divided into the quartiles, and the range of TG/HDL-C ratios in each group was as follows: Quartile 1, <1.96; Quartile 2, 1.96−3.10; Quartile 3, 3.11−4.55; Quartile 4, ≥4.56. The baseline characteristics of the subjects are presented in Table 1. Median age was 59

years, and 63.5% were male. Median eGFR was 58.3 mL/min/1.73 m^2. Among 353 subjects, 154 had diabetic retinopathy, and 126 had a history of CVD. Higher BMI; TG, LDL-C, and T-cholesterol levels; and lower HDL-C levels were associated with higher TG/HDL-C ratio. Higher proportion of hypertension and statins were associated with incremental increase in TG/HDL-C ratio. 32 patients underwent TG lowering therapy (such as including fibrates, omega-3 fatty acids, and niacin), while its proportion was not different among the quartiles of TG/HDL-C ratio.

Association of TG/HDL-C ratio with kidney histological findings

Among 353 subjects, 245 had Class IIb or more severe glomerular lesions, 135 had 25% or more severe IFTA, 305 had arteriolar hyalinosis (score 1 or more), and 255 had intimal thickening (score 1 or more). In the univariable analysis, higher TG/HDL-C ratio was associated with the presence of IFTA (25% or more), with odds ratio (OR) [95% confidence interval (CI)] of 2.08 [1.13−3.84] (Quartile 1 vs. Quartile 4); however, there were no significant associations of higher TG/HDL-C ratio with glomerular lesions, arteriolar hyalinosis, or intimal thickening. After the adjustment for potential confounders in model 2, higher TG/HDL-C ratio was not associated with any kidney histological findings (Table 2).

Association of TG/HDL-C ratio with incident CVD after kidney biopsy

Among 353 subjects, 152 (coronary re-vascularizations, 48; strokes, 37; hospitalizations for heart failure, 27; acute myocardial infarctions, 26; fatal arrhythmias, 4; major amputations, 5; and cardiac sudden deaths, 5; Supplementary Table 1) developed incident CVD during a median follow-up period of 6.2 years (5.1/100 person-years). In the Kaplan-Meier method, there was a significant difference in the cumulative incidence between the groups (log-rank *P* = 0.047; Supplementary Fig. 1). In the univariable Cox proportional hazard model, higher categories of TG/HDL-C ratio were associated with a higher incidence of CVD. Compared to Quartile 1 (reference), hazard ratio (HR) [95% CI] for Quartile 3 was 1.59 [1.03−2.46], and HR for Quartile 4 was 1.33 [0.84−2.10]. Even after the adjustment for potential confounders in the fully adjusted model (model 4), higher categories of TG/HDL-C ratio were still independently associated with a higher incidence of CVD (HR for Quartile 3 vs. reference: 1.73 [1.08−2.79] and HR for Quartile 4 vs. reference: 1.86 [1.10−3.17]) (Table 3). The RCS curve showed that HR for incident CVD increased monotonously as TG/HDL-C ratio increased up to 5, following which it reached a plateau (Fig. 1). In sensitivity analysis, this association was significant even after the adjustment for potential confounders in the model 4 plus TG lowering therapy at the time of kidney biopsy (HR for Quartile 3 vs. reference: 1.94 [1.12−3.34] and HR for Quartile 4 vs. reference: 2.38 [1.31−4.31]).

Association of TG/HDL-C ratio with incident ESKD after kidney biopsy

Among 353 subjects, 90 developed ESKD during a median follow-up period of 7.3 years (2.6 /100 person-years). In the Kaplan-Meier method, there was no significant difference in the cumulative incidence between the groups (log-rank $P = 0.076$; Supplementary Fig. 1). In the univariable analysis, the Cox proportional hazard model showed that higher category of TG/HDL-C ratio was incrementally associated with a higher incidence of ESKD. However, even in the Quartile 4, HR [95% CI] for ESKD (vs. reference) was not statistically significant (1.73 [0.99−3.02]). This weak association remained after adjusting for potential confounders apart from the histological findings. After the adjustment for histological findings including glomerular changes, IFTA, and vascular changes in the fully adjusted model (model 4), there was no association between TG/HDL-C ratio and incidence of ESKD (Table 4). The RCS curve revealed monotonous increase in HR for ESKD as TG/HDL-C ratio increased in model 3, but HR was not statistically significant (Fig. 1).

Discussion

This study suggested that TG/HDL-C ratio was a robust predictor for CVD but not for kidney histological findings and renal outcomes in patients with diabetic nephropathy. When considering long-term outcomes, higher TG/HDL-C ratio was significantly associated with a higher incidence of CVD, even after adjusting for potential confounders. The RCS analysis of cardiovascular outcomes confirmed an increase in HR for incident CVD along with an

increase in TG/HDL-C ratio. On the other hand, weak associations of TG/HDL-C ratio with renal histological findings and ESKD were detected; however, these associations were not significant.

The effect of lipid disturbance on histological changes was intriguing; however, we were not able to uncover any possible associations between TG/HDL-C ratio and histological findings. The effect of TG/HDL-C ratio might be small, and glucose toxicity itself might be a dominant factor affecting histological changes[26].

Regarding long-term outcomes, previous cohort studies have suggested that higher TG/HDL-C ratio is associated with a higher incidence of CVD in the general population[10] and in patients with CKD without diabetes[9]; however, it was unknown whether the association was true among patients with diabetes. Our analyses yielded the similar results, and TG/HDL-C ratio was proven to be a universal predictor of CVD. A possible mechanism underlying the association between higher TG/HDL-C ratio and cardiovascular outcomes is the involvement of insulin resistance and small dense LDL. Higher TG/HDL-C ratio has been considered to reflect higher insulin resistance and higher small dense LDL levels[7,8]. Insulin resistance increases the release of free fatty acids from adipose tissue, stimulates the production of TG-rich very low-density lipoprotein (VLDL), and suppresses TG-rich VLDL catabolism by the reduced activity of lipoprotein lipase (LPL)[27,28]. Moreover, an increase in TG-rich VLDL levels induces LDL and HDL to become smaller and denser, resulting in an

increase in small dense LDL and low HDL-C concentrations[29]. Small dense LDL has a low binding affinity for LDL receptors. It penetrates the vessel wall and becomes oxidized and atherogenic[30-32].

On the other hand, our study did not reveal any association between TG/HDL-C and incident ESKD. Although the information regarding kidney outcomes is limited, two reports have indicated an association between TG/HDL-C and kidney dysfunction[12,13]. One cohort study[12], including diabetic patients without retinopathy or CKD, demonstrated that higher TG/HDL-C ratio was associated with a higher incidence of microvascular composite outcomes (retinopathy and/or CKD). The other study[13] also confirmed that higher TG/HDL-C ratio was associated with a faster decline in eGFR among the general population who participated in nationwide health checkups, and the association between TG/HDL-C ratio and kidney outcomes was reconfirmed using other alternative outcomes (incident CKD, decline in eGFR by ≥5 mL/min/1.73 m^2/year, progression of CKD G-stage accompanied by a ≥25% decline in eGFR from baseline, or >30% decline in eGFR from baseline during the 2-year follow-up period). Although, these results seemed to be inconsistent with our results, the outcomes used in the two previous studies were soft outcomes such as incident CKD and decline in eGFR, but not a hard outcome, such as incident ESKD, which was used in this study. Another possible explanation for the discrepancy in results related to kidney outcomes is that the number of participants in this study was not sufficient to observe significant

differences. Indeed, TG/HDL-C ratio was weakly associated with both kidney histological changes and ESKD development. Interestingly, the association between TG/HDL-C ratio and incidence of ESKD was completely diminished after the adjustment for kidney histological changes (Figure 1B and 1C), suggesting that TG/HDL-C ratio has a significant effect on diabetic histological changes in the kidney. Nevertheless, we confirmed that the effect of TG/HDL ratio on kidney histology and renal outcomes was smaller than that on CVD. Previous studies showed that hypercholesterolemia and hyperlipoproteinemia induced by apolipoprotein E and LDL receptor double knockout mice advanced atheroma in aorta and its branches (macrovasclular disease) but not in intrarenal vascular (microvascular disease) [33,34]. In other words, dyslipidemia including high TG / low HDL-C may less affect in kidney disease (mainly associated with microvascular disease) than in macrovascular disease such as CVD. This might be the reason why our study showed the different impact of TG/HDL-C ratio on cardiorenal outcomes.

This study has several strengths. First, all patients were diagnosed histologically with diabetic nephropathy, implying that this study did not include patients with diabetes accompanied by other kidney diseases, such as hypertensive nephrosclerosis. Second, the follow-up periods were long enough to monitor the patients for the development of incident CVD and ESKD. Third, to the best of our knowledge, this is the first study to simultaneously

examine the association of TG/HDL-C ratio with CVD and that with ESKD among patients with biopsy-proven diabetic nephropathy.

Our study has several limitations. First, this was an observational study and there might be other unknown confounders. Second, TG/HDL-C ratio was used as an exposure under the assumption that it is a surrogate marker for small dense LDL-C; however, we did not directly measure small dense LDL-C levels. We did not confirm the type of dyslipidemia pathology that actually reflects TG/HDL-C ratio. Third, although the value of TG and HDL-C levels might be affected by sex, the inclusion criteria for biopsy-proven diabetic nephropathy made it difficult to include enough participants to perform an analysis stratified by sex. Fourth, TG/HDL-C ratio was assessed only at the baseline. It could change during the follow-up period among some patients. Moreover, some patients could receive TG-lowering drugs after kidney biopsy. As is described in the previous study[35], CKD is also associated with increased incidence of new onset of high TG/HDL-C ratio, which could accelerate worsening kidney outcome. This might affect the association between TG/HDL-C ratio and kidney outcome. On the other hand, only 8 patients newly underwent TG lowering therapy after kidney biopsy, and it was considered that our results would not change regardless of the therapy.

Perspective of Asia

Many intervention studies about dyslipidemia have undergone previously. However, most of enrolled patients were Europeans or Americans, and there was not enough evidence about the affect of dyslipidemia on CVD and kidney outcomes in Asians. REDUCE-IT trial showed that icosapent ethyl reduced cardiovascular risk[36]. The subgroup analyses revealed that the effect was not different among ethnic groups, including Asians. FIELD study, conducted in Australia, showed that fibrate decrease the incidence of CVD but not ESKD in people with CKD[37]. Therefore, our study suggested that dyslipidemia might affect CVD more than kidney outcomes in Asians as well as other ethnic groups.

In conclusion, TG/HDL-C ratio was robustly associated with the incidence of CVD but not with kidney pathology or the incidence of ESKD in biopsy-proven diabetic nephropathy. The results on the association between TG/HDL-C ratio and kidney outcomes might be inconclusive, and further studies are warranted for kidney outcomes in patients with diabetes.

Authors' Contributions

Research idea and study design: TU, MN, ME, and KT; data acquisition: MN, ME, KM, and KS; and supervision or mentorship: ME, MN, HTam, HTas, RF, FF, TK, KM, MM, KS, and KT. All authors provided intellectual content of critical importance to the work described and approved the final version of the manuscript.

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Conflict of Interest Statement

Nothing to disclose.

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Point of view

・Clinical relevance

TG/HDL-C ratio was associated with the incidence of CVD but not with kidney pathology or the incidence of ESKD in biopsy-proven diabetic nephropathy.

・Future direction

Further studies are warranted for the association between TG/HDL-C ratio and kidney outcomes in patients with diabetes.

・Consideration for the Asian population

Although the difference of effects of dyslipidemia therapy among ethnic groups is unclear, dyslipidemia might affect CVD more than kidney outcomes in Asians as well as other ethnic groups.

Table 1: Baseline characteristics according to the quartile of TG/HDL-C ratio

		TG/HDL-C ratio				
		Quartile 1 <1.96 N=89	Quartile 2 1.96 to 3.10 N=88	Quartile 3 3.11 to 4.55 N=88	Quartile 4 >4.55 N=88	*P* for trend
Age, years		61.0 (49.0–66.0)	59.0 (54.0–65.3)	60.5 (52.0–67.0)	56.5 (46.5–63.3)	0.08
Sex, male, n (%)		53 (59.6)	56 (63.6)	52 (59.1)	63 (71.6)	0.17
BMI, kg/m^2		22.7 (20.1–25.1)	23.3 (21.6–25.7)	23.7 (22.4–26.5)	24.5 (22.5–26.8)	<0.001
SBP, mmHg		132 (120–142)	130 (120–140)	139 (124–151)	133 (120–153)	0.13
DBP, mmHg		74 (64–80)	73 (66–82)	79 (69–82)	74 (68–83)	0.26
Hypertension, n (%)		57 (64.0)	61 (69.3)	71 (80.7)	66 (75.0)	0.04
Diabetic retinopathy, n (%)[a]		35 (40.7)	40 (47.1)	35 (39.8)	44 (51.2)	0.32
History of CVD, n (%)		33 (37.1)	23 (26.1)	42 (47.7)	28 (31.8)	0.8
RAS blockers, n (%)		29 (32.6)	27 (30.7)	28 (31.8)	34 (38.6)	0.39
Statins, n (%)		7 (7.9)	17 (19.4)	15 (17.0)	18 (20.5)	0.04
TG lowering therapy, n (%)[b]		9 (12.3)	6 (7.8)	9 (12.9)	8 (10.3)	0.94
Hemoglobin, g/dL		12.9 (11.7–13.8)	13.5 (11.8–14.7)	13.4 (11.4–14.7)	13.2 (12.0–15.2)	0.1
Serum albumin, g/dL		4.0 (3.4–4.3)	4.0 (3.3–4.3)	4.1 (3.7–4.4)	4.0 (3.3–4.4)	0.23
Serum creatinine, mg/dL		0.80 (0.60–1.20)	0.90 (0.70–1.30)	0.99 (0.80–1.20)	1.09 (0.70–1.50)	0.01
eGFR, mL/min/1.73 m^2		65.2 (42.1–90.3)	56.9 (41.3–79.5)	57.8 (39.2–74.8)	52.5 (36.6–82.3)	0.07
Fasting serum glucose, mg/dL		137 (117–175)	123 (108–188)	131 (106–179)	144 (109–202)	0.77
HbA1c, %[c]		7.5 (6.5–8.7)	7.8 (6.7–9.1)	7.6 (6.5–9.1)	7.7 (6.6–8.6)	0.9
TG, mg/dL		86 (72–98)	120 (101–142)	159 (136–180)	239 (195–329)	<0.001
T-cholesterol, mg/dL		188 (161–215)	210 (172–234)	212 (184–242)	222 (198–254)	<0.001
HDL-C, mg/dL		57 (48–68)	47 (41–57)	43 (36–48)	37 (31–41)	<0.001
LDL-C, mg/dL[d]		113 (87–133)	134 (106–159)	137 (109–161)	136 (114–167)	<0.001
Proteinuria, g/day or g/gCr		0.36 (0.10–2.20)	0.60 (0.20–2.55)	0.40 (0.13–1.65)	0.73 (0.20–4.21)	0.06
Glomerular lesion, n (%)	IIa	27 (30.3)	22 (25.0)	33 (37.5)	26 (29.5)	0.74
	IIb	36 (40.4)	34 (38.6)	30 (34.1)	26 (29.5)	
	III	21 (23.6)	23 (26.1)	22 (25.0)	30 (34.1)	
	IV	5 (5.6)	9 (10.2)	4 (3.4)	6 (6.8)	
IFTA, n (%)	0	7 (7.9)	3 (3.4)	7 (8.0)	10 (11.4)	0.28
	1	54 (60.7)	51 (58.0)	51 (58.0)	35 (39.8)	
	2	11 (12.4)	14 (15.9)	14 (15.9)	21 (23.9)	
	3	17 (19.1)	20 (22.7)	16 (18.2)	22 (25.0)	
Hyalinosis, n (%)	0	12 (13.5)	11 (12.5)	15 (17.0)	10 (11.4)	0.76
	1	19 (21.3)	13 (14.8)	17 (19.3)	13 (14.8)	
	2	8 (9.0)	9 (10.2)	11 (12.5)	11 (12.5)	

	3	50 (56.2)	55 (62.5)	45 (51.1)	54 (61.4)	
	0	18 (22.0)	18 (21.4)	15 (18.5)	18 (23.4)	
Intimal thickening, n (%) [e]	1	21 (25.6)	27 (32.1)	27 (33.3)	30 (39.0)	0.24
	2	43 (52.4)	39 (46.4)	39 (48.1)	29 (37.7)	

Subjects were divided into quartiles (Quartile 1 to 4) based on TG/HDL-C ratios. Data are shown as median (interquartile range) or number (%).

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, CVD: cardiovascular disease, RAS: renin-angiotensin system, eGFR: estimated glomerular filtration rate, HbA1c: hemoglobin A1c, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, IFTA: interstitial fibrosis and tubular atrophy.

a: 8 patients had missing information regarding diabetic retinopathy.

b: 55 patients had missing information regarding TG lowering therapy.

c: 26 patients had missing information regarding HbA1c level.

d: 6 patients had missing information regarding LDL-C level.

e: 19 patients had missing information regarding intimal thickening.

Table 2: Odds ratios for each pathological finding associated with the quartile of TG/HDL-C ratios

TG/HDL-C ratio	Quartile 1 <1.96	Quartile 2 1.96 to 3.10	Quartile 3 3.11 to 4.55	Quartile 4 >4.55
IFTA (≥2)				
Crude	1.00 (reference)	1.37 (0.74–2.55)	1.13 (0.60–2.11)	2.08 (1.13–3.84)
Model 1	1.00 (reference)	1.39 (0.73–2.64)	1.16 (0.61–2.22)	2.49 (1.30–4.76)
Model 2	1.00 (reference)	1.30 (0.52–3.27)	0.96 (0.37–2.49)	1.84 (0.72–4.69)
Glomerular lesions (≥IIb)				
Crude	1.00 (reference)	1.31 (0.67–2.53)	0.73 (0.39–1.36)	1.04 (0.55–1.98)
Model 1	1.00 (reference)	1.31 (0.67–2.56)	0.72 (0.38–1.35)	1.18 (0.61–2.29)
Model 2	1.00 (reference)	1.12 (0.50–2.48)	0.70 (0.32–1.51)	0.79 (0.34–1.83)
Arteriolar hyalinosis (≥1)				
Crude	1.00 (reference)	1.09 (0.45–2.62)	0.76 (0.33–1.73)	1.22 (0.50–2.98)
Model 1	1.00 (reference)	1.06 (0.43–2.59)	0.73 (0.32–1.70)	1.31 (0.52–3.31)
Model 2	1.00 (reference)	0.83 (0.31–2.22)	0.75 (0.29–1.89)	1.07 (0.37–3.10)
Intimal thickening (≥1)				
Crude	1.00 (reference)	1.03 (0.49–2.16)	1.24 (0.58–2.66)	0.92 (0.44–1.94)
Model 1	1.00 (reference)	1.03 (0.49–2.17)	1.25 (0.57–2.71)	1.03 (0.48–2.22)
Model 2	1.00 (reference)	0.82 (0.36–1.85)	0.86 (0.37–2.00)	0.68 (0.29–1.59)

Model 1: adjusted for age and sex.

Model 2: adjusted for the variables included in Model 1 plus BMI, history of diabetic retinopathy, RAS blockers, statins, systolic blood pressure, eGFR, fasting serum glucose and proteinuria.

TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, IFTA: interstitial fibrosis and tubular atrophy, BMI: body mass index, RAS: renin-angiotensin system, eGFR: estimated glomerular filtration rate.

Table 3: Hazard ratios for incident cardiovascular disease according to the quartile of TG/HDL-C ratio

TG/HDL-C ratio	Quartile 1 <1.96	Quartile 2 1.96 to 3.10	Quartile 3 3.11 to 4.55	Quartile 4 >4.55
Crude	1.00 (reference)	0.90 (0.55–1.47)	1.59 (1.03–2.46)	1.33 (0.84–2.10)
Model 1	1.00 (reference)	0.95 (0.58–1.56)	1.74 (1.12–2.70)	1.61 (1.01–2.56)
Model 2	1.00 (reference)	1.10 (0.66–1.83)	1.59 (1.02–2.47)	1.58 (0.99–2.54)
Model 3	1.00 (reference)	1.17 (0.70–1.95)	1.63 (1.05–2.56)	1.62 (1.01–2.62)
Model 4	1.00 (reference)	1.20 (0.69–2.09)	1.73 (1.08–2.79)	1.86 (1.10–3.17)

Model 1: adjusted for age and sex.
Model 2: adjusted for the variables included in Model 1 plus BMI, history of cardiovascular diseases, RAS blockers and statins.
Model 3: adjusted for the variables included in Model 2 plus systolic blood pressure, eGFR, fasting serum glucose and proteinuria.
Model 4: adjusted for the variables included in Model 3 plus IFTA, glomerular lesions, arteriolar hyalinosis and intimal thickening.
TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, BMI: body mass index, RAS: renin-angiotensin system, eGFR: estimated glomerular filtration rate, IFTA: interstitial fibrosis and tubular atrophy.

Table 4: Hazard ratios for incident end-stage kidney disease according to the quartile of TG/HDL-C ratio

TG/HDL-C ratio	Quartile 1 <1.96	Quartile 2 1.96 to 3.10	Quartile 3 3.11 to 4.55	Quartile 4 >4.55
Crude	1.00 (reference)	0.85 (0.45–1.62)	1.15 (0.63–2.09)	1.73 (0.99–3.02)
Model 1	1.00 (reference)	0.88 (0.46–1.67)	1.17 (0.64–2.13)	1.96 (1.10–3.46)
Model 2	1.00 (reference)	0.68 (0.34–1.34)	1.06 (0.57–1.96)	1.52 (0.84–2.73)
Model 3	1.00 (reference)	0.72 (0.36–1.44)	1.26 (0.67–2.38)	1.50 (0.82–2.74)
Model 4	1.00 (reference)	0.84 (0.42–1.69)	1.26 (0.66–2.42)	1.04 (0.54–2.02)

Model 1: adjusted for age and sex.
Model 2: adjusted for the variables included in Model 1 plus BMI, history of diabetic retinopathy, RAS blockers and statins.
Model 3: adjusted for the variables included in Model 2 plus systolic blood pressure, eGFR, fasting serum glucose and proteinuria.
Model 4: adjusted for the variables included in Model 3 plus IFTA, glomerular lesions, arteriolar hyalinosis and intimal thickening.
TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, BMI: body mass index, RAS: renin-angiotensin system, eGFR: estimated glomerular filtration rate, IFTA: interstitial fibrosis and tubular atrophy.

Figure legend

Fig. 1: The restricted cubic spline analyses of incident cardiovascular disease and end-stage kidney disease.

A. Hazard ratio for cardiovascular disease was adjusted for covariates in model 4.

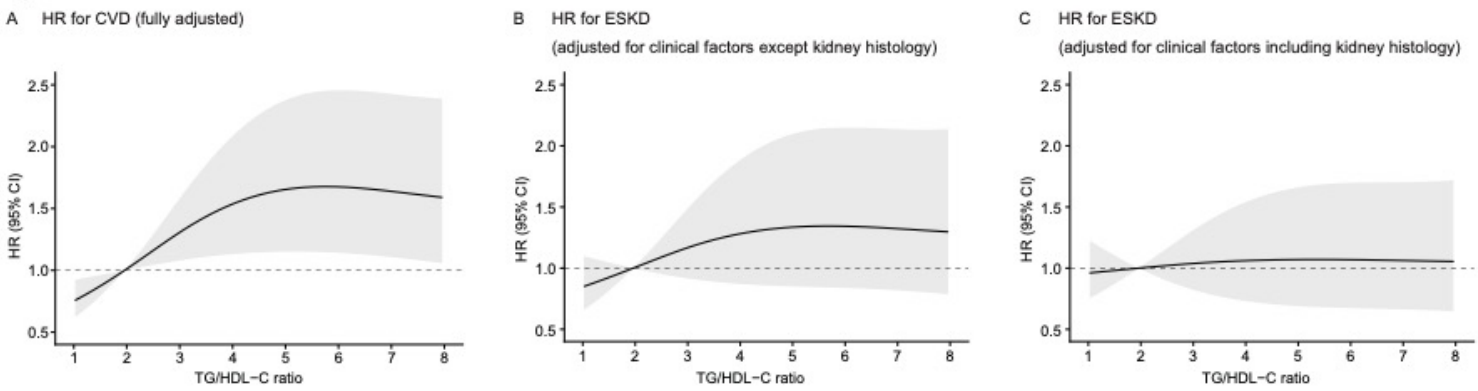
B. Hazard ratio for end-stage kidney disease was adjusted for covariates in model 3.

C. Hazard ratio for end-stage kidney disease was adjusted for covariates in model 4.

Subjects with outliers (TG/HDL-C ratio was less than 1 or more than 8) were excluded, and 291 out of 353 subjects were included in the analysis.

HR: hazard ratio, CI: confidence interval, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol.

Figure1.

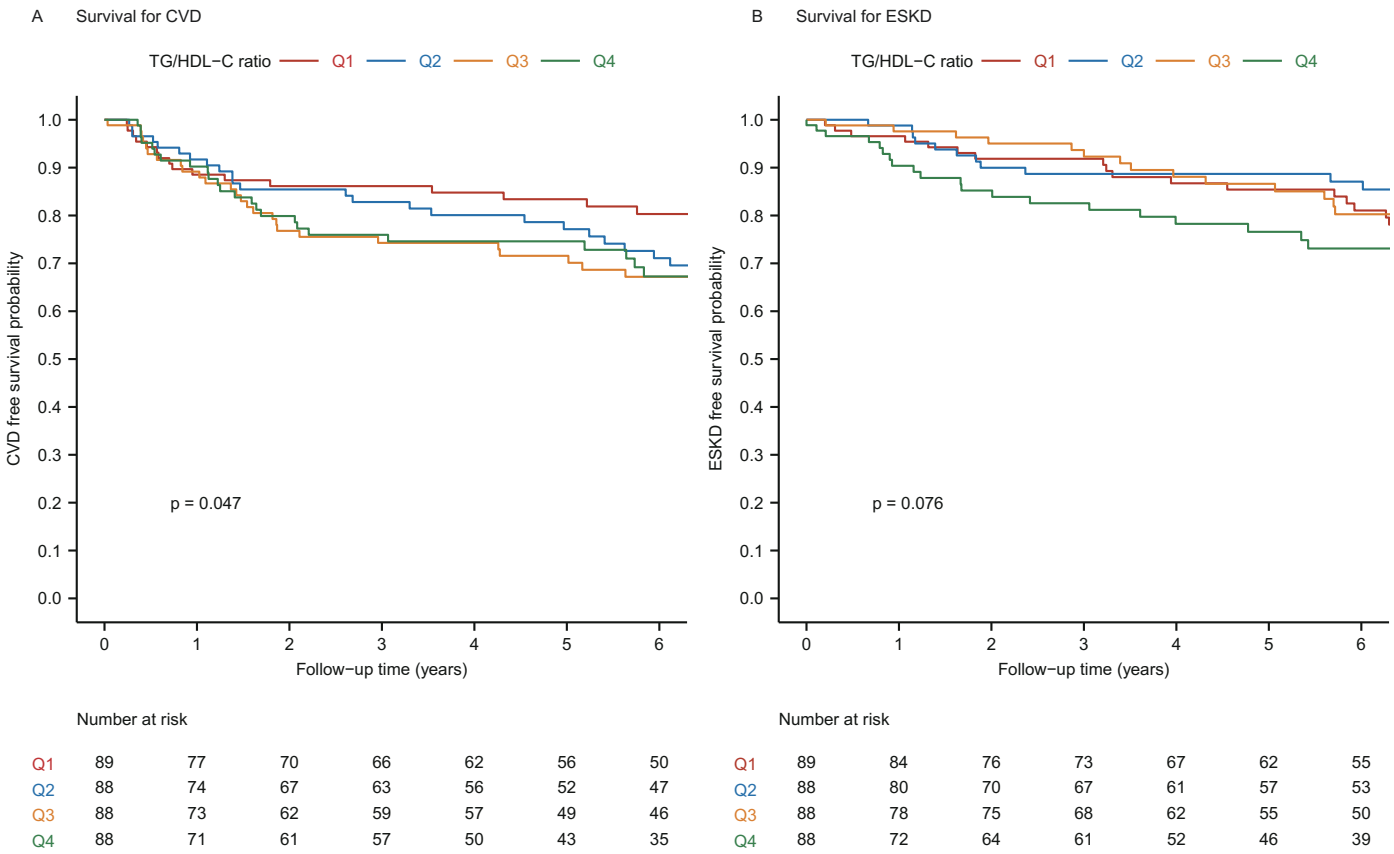

A HR for CVD (fully adjusted)
B HR for ESKD
(adjusted for clinical factors except kidney histology)
C HR for ESKD
(adjusted for clinical factors including kidney histology)
HR (95% CI)
TG/HDL−C ratio
2.5
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8

Supplementary Table 1: Details for incident cardiovascular disease

TG/HDL-C ratio category	Overall	Number of events (%)			
		Quartile 1 <1.96	Quartile 2 1.96 to 3.10	Quartile 3 3.11 to 4.55	Quartile 4 >4.55
All-cause cardiovascular disease	152	34 (22.4)	29 (19.1)	49 (32.2)	40 (26.3)
Acute myocardial infarction	26 (17.1)	3 (8.8)	9 (31.0)	7 (14.3)	7 (17.5)
Stroke	37 (24.3)	9 (26.5)	5 (17.2)	10 (20.4)	13 (32.5)
Hospitalization for heart failure	27 (17.8)	6 (17.6)	3 (10.3)	13 (26.5)	5 (12.5)
Coronary revascularization	48 (31.6)	12 (35.3)	10 (34.5)	16 (32.7)	10 (25.0)
Fatal arrhythmia	4 (2.6)	1 (2.9)	0 (0)	1 (2.0)	2 (5.0)
Major amputation	5 (3.3)	2 (5.9)	1 (3.4)	0 (0)	2 (5.0)
Cardiac sudden death	5 (3.3)	1 (2.9)	1 (3.4)	2 (4.1)	1 (2.5)

TG: triglycerides, HDL-C: high-density lipoprotein cholesterol.

Supplemental Figure1.



Number at risk

Q1	89	77	70	66	62	56	50
Q2	88	74	67	63	56	52	47
Q3	88	73	62	59	57	49	46
Q4	88	71	61	57	50	43	35

Number at risk

Q1	89	84	76	73	67	62	55
Q2	88	80	70	67	61	57	53
Q3	88	78	75	68	62	55	50
Q4	88	72	64	61	52	46	39

A. Kaplan-Meier survival curves for incident cardiovascular disease

B. Kaplan-Meier survival curves for incident end-stage kidney disease

TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, Q1−4: Quartile 1−4, CVD: cardiovascular disease, ESKD: end-stage kidney disease.

Graphic abstract

TG/HDL-C ratio is associated with cardiovascular disease but not kidney outcomes

- retrospective observational study
- 353 patients with biopsy-proven diabetic nephropathy
- Patients were divided into quartiles based on TG/HDL-C ratio (Q1-4): Q1 (reference), <1.96; Q2, 1.96−3.10; Q3, 3.11−4.55; and Q4, ≥4.56.