

Albuminuria Is Associated with Steatosis Burden in Patients with Type 2 Diabetes Mellitus and Nonalcoholic Fatty Liver Disease

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Background: This study aimed to investigate the association between hepatic steatosis burden and albuminuria in Korean patients with type 2 diabetes mellitus (T2DM) and nonalcoholic fatty liver disease (NAFLD).

Methods: We recruited 100 patients with both T2DM and NAFLD, but without chronic kidney disease. Albuminuria was defined as a spot urinary albumin-to-creatinine ratio (ACR) ≥ 30 mg/g. Transient elastography was performed, and the steatosis burden was quantified by controlled attenuation parameter (CAP) with significant steatosis defined as CAP > 302 dB/m.

Results: The prevalence of significant steatosis and albuminuria was 56.0% and 21.0%, respectively. Subjects with significant steatosis were significantly younger and had a significantly shorter duration of T2DM, greater waist circumference, and higher body mass index, total cholesterol, triglyceride, and low density lipoprotein cholesterol levels, than subjects without severe NAFLD (all $P < 0.05$). Albuminuria was higher in patients with significant steatosis than in patients without significant steatosis (32.1% vs. 6.8%, $P = 0.002$). Urinary ACR showed a correlation with CAP ($r = 0.331$, $P = 0.001$), and multiple linear regression analysis revealed a significant association between a high degree of albuminuria and high CAP value ($r = 0.321$, $P = 0.001$). Additionally, multivariate logistic regression analysis demonstrated the independent association between urinary ACR and significant steatosis after adjustment for confounding factors including age, body mass index, duration of T2DM, low density lipoprotein level, and renin-angiotensin system blocker use (odds ratio, 1.88; 95% confidence interval, 1.31 to 2.71; $P = 0.001$).

Conclusion: T2DM patients with NAFLD had a higher prevalence of albuminuria, which correlated with their steatosis burden.

Keywords: Albuminuria; Diabetes mellitus, type 2; Non-alcoholic fatty liver disease

INTRODUCTION

Due to the increasing prevalence of obesity and type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD) has emerged as one of the most common chronic liver diseases, with an estimated worldwide prevalence of 25.2% [1]. The prevalence of NAFLD in patients with T2DM is much greater than in the general population and is reported to be 55.5% [2]. As NAFLD shows a 3% rise in the annual incidence rate, the prevalence of NAFLD is expected to rise as individuals with

obesity and T2DM age [3]. NAFLD is defined by the presence of fat accumulation in hepatocytes, after the exclusion of excessive alcohol use, hepatitis virus infection, iron overloading, autoimmune disease, or medications, which can cause chronic liver disease. NAFLD can advance to non-alcoholic steatohepatitis (NASH) that can progress to cirrhosis, end-stage liver disease, and finally, hepatocellular carcinoma (HCC) [4]. Moreover, NAFLD, *per se*, accounts for the most predominant cause of HCC, even without cirrhotic progression [5]. The risk of HCC rises at least two-fold in populations with T2DM and

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obesity [6].

Albuminuria is an independent risk factor for cardiovascular and renal diseases [7,8]. Additionally, it is a marker of insulin resistance and systemic inflammation. Consequently, an association between albuminuria and other metabolic diseases has been reported [9,10]. Although NAFLD and albuminuria are independently associated with the aggravation of coexisting metabolic diseases, the interactive influence of NAFLD and albuminuria is not fully determined, especially in subjects with normal kidney function. Therefore, we aimed to investigate the association between the degree of steatosis and albuminuria, independent of other metabolic factors. Furthermore, we investigated whether albuminuria is associated with the hepatic burden in NAFLD.

METHODS

Study population

In this cross-sectional study, a total of 100 Korean subjects, aged 18 years or above, with T2DM were recruited at Keimyung University Dongsan Hospital, a tertiary university hospital in Korea. T2DM was defined according to the American Diabetes Association guidelines [11]. This study complied with the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol received ethical approval from the Institutional Review Board at the Keimyung University School of Medicine (2017-09-045). Informed consent was obtained from the patients prior to each examination.

All patients had NAFLD, as diagnosed using abdominal ultrasound or computed tomography and confirmed by a radiologist. Patients were excluded if they fulfilled any one of the following criteria: (1) diagnosis of type 1 diabetes mellitus, gestational diabetes mellitus, or any other form of diabetes other than T2DM; (2) history of addiction to alcohol, heavy alcohol consumption (≥ 210 g/week for men or ≥ 140 g/week for women); (3) other causes of liver disease (e.g., active viral or autoimmune hepatitis), liver cirrhosis, or HCC; (4) estimated glomerular filtration rate (eGFR) < 45 mL/min/1.73 m²; (5) medications associated with fatty liver disease (e.g., amiodarone, methotrexate, tamoxifen, or valproate); and (6) pregnant or nursing women.

Measurement of steatosis burden and albuminuria

All patients underwent physical examination and clinical laboratory tests after an overnight (over 8 hours) fasting period for

baseline parameters. Liver fat content was assessed via the controlled attenuation parameter (CAP), and liver fibrotic burden was assessed via the liver stiffness measurement (LSM). Both CAP and LSM measure ultrasonic attenuations at 3.5 MHz, using signals acquired by transient liver elastography (FibroScan; Echosens, Paris, France) with XL probe. To achieve an accurate CAP value, we calculated only ultrasonic attenuation when the matched LSM was valid while attempting to collect at least 10 valid LSMs. A success rate of $\geq 60\%$ and a ratio for the interquartile range (IQR) to the median value of LSM of $\leq 30\%$ were considered reliable and used for the final analysis [12]. The final CAP value was the median of each individual CAP value using the same valid measurements [13,14]. The steatosis grade (S) was determined using the following CAP-cutoff values that have been reported earlier: 236 dB/m for $S \geq 1$, 270 dB/m for $S \geq 2$, and 302 dB/m for $S \geq 3$. We characterized significant steatosis when CAP was over 302 dB/m [15-17].

Urinary albumin and creatinine levels were measured in a fasting, morning spot urine sample obtained from each participant. Urinary albumin levels were expressed as the urinary albumin-to-creatinine ratio (ACR) to minimize the influence of variations in kidney function. The urine albumin level was measured by an immunoturbidimetric method using an AU680 automated chemistry analyzer (Beckman Coulter Inc., Brea, CA, USA). The urine creatinine level was also measured using the AU680 analyzer (Beckman Coulter Inc.) by the kinetic Jaffe method. Albuminuria was defined as urinary ACR ≥ 30 mg/g.

Clinical parameters and biochemical analysis

Body weight, waist circumference, blood pressure, glycemic parameters (i.e., fasting plasma glucose and glycosylated hemoglobin [HbA1c]), lipids (i.e., total cholesterol, high density lipoprotein cholesterol [HDL-C], low density lipoprotein cholesterol [LDL-C], and triglycerides), and liver enzymes (i.e., aspartate aminotransferase [AST], alanine transaminase [ALT], and gamma-glutamyl transferase [γ -GT]) were measured. An eGFR was calculated using the Modification of Diet in Renal Disease equation [18]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: fasting glucose (mg/dL) \times fasting insulin (μ IU/L)/405. Subjects were considered as obese when their body mass index (BMI) was ≥ 25 kg/m². Central obesity was defined on the basis of the waist circumference criteria from the Korean Society for the Study of Obesity (≥ 90 cm for men and ≥ 85 cm for women)

[19]. Regular exercise was defined as more than 20 minutes per session and at least three times per week. Hypertension was defined as systolic pressure over 140 mm Hg and diastolic pressure over 90 mm Hg, or if the subject was currently prescribed antihypertensive medications. Cardiovascular disease was defined as any prior history of acute coronary syndromes, cerebrovascular accidents, or peripheral arterial diseases. The presence of diabetic neuropathy and diabetic retinopathy was defined by the patient's medical record diagnosed by a neurologist/ophthalmologist, or if the patient was currently prescribed medication for neuropathy/retinopathy.

Statistical analysis

Data are presented as mean \pm standard deviation for paramet-

ric variables and as median (IQR) for non-parametric variables or percent for categorical variables. We analyzed differences in the patient characteristics between the two groups using paired *t*-tests for parametric continuous variables and chi-square tests for categorical variables. The urinary ACR, total cholesterol, triglyceride, HDL-C, LDL-C, AST, ALT, γ -GT, insulin, and HOMA-IR values were not normally distributed; analyses, therefore, were performed using the Mann-Whitney *U* test. The association between NAFLD and albuminuria was evaluated using the chi-square test after transforming the variables into tertiles. Correlations between urinary ACR, CAP, and other parameters were analyzed using Spearman's correlation coefficients. Backward multiple linear regression analysis was performed on CAP to analyze the relationship between CAP

Table 1. Baseline characteristics of study population

Characteristic	Total (n=100)	Patients without significant steatosis (n=44)	Patients with significant steatosis (n=56)	P value
Age, yr	53.0 \pm 15.5	56.8 \pm 14.5	49.9 \pm 15.7	0.026
Male sex	53 (53.0)	23 (52.3)	30 (53.6)	0.897
Duration of T2DM, yr	7.1 \pm 7.3	8.9 \pm 8.7	5.7 \pm 5.9	0.036
Body mass index, kg/m ²	26.5 \pm 4.3	24.6 \pm 3.4	28.1 \pm 4.2	<0.001
Obesity	59 (59.0)	16 (36.4)	43 (76.8)	<0.001
Waist circumference, cm	90.7 \pm 10.5	87.4 \pm 9.7	93.3 \pm 10.4	0.005
Central obesity	60 (60.0)	21 (47.7)	39 (69.6)	0.026
Current smoking	23 (23.0)	15 (34.1)	8 (14.8)	0.064
Regular exercise	33 (33.0)	14 (28.0)	19 (38.0)	0.288
SBP, mm Hg	126.7 \pm 12.5	125.1 \pm 13.0	128.0 \pm 12.0	0.229
DBP, mm Hg	72.8 \pm 11.7	70.1 \pm 14.0	74.9 \pm 9.0	0.056
FPG, mg/dL	136.5 \pm 50.1	142.8 \pm 58.8	131.5 \pm 42.0	0.267
HbA1c, %	7.9 \pm 2.0	8.0 \pm 2.2	7.9 \pm 2.0	0.986
PPPG, mg/dL	186.2 \pm 65.2	184.0 \pm 61.5	188.0 \pm 68.6	0.757
BUN, mg/dL	14.9 \pm 5.3	15.9 \pm 6.5	14.2 \pm 3.9	0.097
Creatinine, mg/dL	0.8 \pm 0.2	0.8 \pm 0.2	0.8 \pm 0.2	0.371
eGFR, mL/min/1.73 m ²	98.5 \pm 25.1	93.4 \pm 22.6	102.5 \pm 26.4	0.069
Albumin, mg/dL	4.6 \pm 0.4	4.5 \pm 0.4	4.6 \pm 0.4	0.602
Total cholesterol, mg/dL	150.0 (126.0–181.0)	142.5 (119.5–170.3)	159.0 (136.0–195.0)	0.019
Triglyceride, mg/dL	124.0 (101–177.7)	117.2 (90.4–157.6)	141.0 (111.0–185.0)	0.034
HDL-C, mg/dL	47.1 (36.4–53.4)	48.5 (35.3–58.0)	46.1 (40.1–52.1)	0.726
LDL-C, mg/dL	74.0 (54.5–94.0)	61.5 (47.0–81.0)	80.7 (63.3–100.1)	0.006
AST, IU/L	25.0 (19.0–37.0)	25.0 (19.0–40.8)	24.5 (20.0–36.3)	0.622
ALT, IU/L	29.0 (17.0–51.0)	28.5 (16.3–51.8)	29.5 (19.5–50.5)	0.391

(Continued to the next page)

and demographic and laboratory parameters. The relative risk factors for severe NAFLD were obtained using multiple logistic regression, and the risk was reported as odds ratios (ORs) with 95% confidence intervals (CIs). All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). For all tests, $P < 0.05$ was considered as statistically significant.

RESULTS

Baseline characteristics of the study population

Baseline characteristics according to the status of NAFLD are shown in Table 1. The prevalence of significant steatosis and albuminuria was 56.0% and 21.0%, respectively. Subjects with significant steatosis were significantly younger and had a significantly shorter duration of T2DM. They had greater waist

circumferences, higher BMI, total cholesterol, triglycerides, and LDL-C, than subjects without severe NAFLD (all $P < 0.05$). Furthermore, the proportion of central obesity and obesity was significantly higher in subjects with significant steatosis, whereas the proportion of hypertension and cardiovascular diseases was similar between the two groups. Liver function tests, including ALT, AST, γ -GT levels, were mostly within the normal range for both groups, with no significant difference between the values. The prevalence of albuminuria was higher in patients with significant steatosis than in subjects without significant steatosis (32.1% vs. 6.8%, $P = 0.002$). Ninety-seven patients were prescribed metformin, and 35 patients were using angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARB), and the proportion of anti-diabetic medications and ACE inhibitors/ARB was similar between the severe and non-severe NAFLD groups. In addition,

Table 1. Continued

Characteristic	Total (n=100)	Patients without significant steatosis (n=44)	Patients with significant steatosis (n=56)	P value
γ -GT, IU/L	35.0 (21.0–55.0)	35.0 (17.3–52.8)	33.0 (23.0–61.0)	0.428
HOMA-IR	2.1 (1.2–4.0)	1.8 (1.3–3.8)	2.2 (1.4–4.3)	0.242
CAP, dB/m	302.3 \pm 47.2	260.3 \pm 29.9	335.3 \pm 28.2	<0.001
Liver stiffness measurement, kPa	6.1 \pm 2.6	5.7 \pm 2.7	6.4 \pm 2.5	0.194
Urinary ACR, mg/g creatinine	5.0 (1.7–17.2)	2.8 (1.1–8.0)	8.8 (2.4–50.8)	0.002
Albuminuria	21 (21.0)	3 (6.8)	18 (32.1)	0.002
Comorbidities				
Hypertension	36 (35.0)	18 (40.9)	18 (32.1)	0.365
Cardiovascular disease	10 (10.0)	6 (13.6)	4 (7.1)	0.283
Diabetic retinopathy	6 (6.0)	4 (9.1)	2 (3.0)	0.249
Diabetic neuropathy	16 (16.0)	9 (20.5)	7 (12.5)	0.281
Medications				
Metformin	97 (97.0)	43 (97.7)	54 (96.4)	0.706
Sulfonylurea	30 (30.0)	14 (31.8)	16 (28.6)	0.725
Thiazolidinedione	7 (7.0)	1 (2.3)	6 (10.7)	0.101
Insulin	24 (24.0)	9 (20.5)	15 (26.8)	0.462
Lipid lowering agents	70 (70.0)	29 (65.9)	41 (73.2)	0.429
ACE inhibitor/ARB	35 (35.0)	18 (40.9)	17 (30.4)	0.272

Values are presented as mean \pm standard deviation, number (%), or median (interquartile range).

T2DM, type 2 diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; PPPG, postprandial plasma glucose; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; AST, aspartate transaminase; ALT, alanine transferase; γ -GT, gamma glutamyltransferase; HOMA-IR, homeostatic model assessment of insulin resistance; CAP, controlled attenuation parameter; ACR, albumin-to-creatinine ratio; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

the fibrotic burden, LSM was comparable between the two groups.

Correlation between CAP and urinary ACR

After stratifying into CAP and urinary ACR tertiles (<2.39, 2.39 to 11.41, >11.41 mg/g), individuals with a higher CAP had a higher urinary ACR than those with a lower CAP (Fig. 1A). When stratified by severity of NAFLD (S0, S1, S2, and S3 stages assigned to 12, 14, 18, and 56 subjects, respectively), the proportion of the highest urinary ACR tertile and significant albuminuria gradually increased and was the highest in subjects with S3 stage NAFLD (Fig. 1B and C). To assess the association between CAP and other metabolic components, we performed a linear correlation analysis. As shown in Table 2, BMI, waist circumference, ALT, γ -GT, and urinary ACR positively correlated with CAP, displaying statistical significance, whereas age and duration of T2DM showed a negative correlation with CAP. Furthermore, to determine which factors were significantly associated with CAP, we performed multiple linear analyses (Table 3). After adjusting for age and sex in Model 1, BMI, waist circumference, and urinary ACR were independently associated with CAP. In Model 2, adjusting further for the geodemographic parameters, including duration of T2DM, diabetic retinopathy, and ACE inhibitors/ARB use, led to a significant association of triglyceride level, BMI, waist circumference, and urinary ACR with CAP. On further adjusting Model 2 for laboratory findings (HbA1c, ALT, γ -GT, and triglyceride)

Table 2. Correlation between CAP and other parameters

Parameter	r	P value
Age, yr	-0.321	0.001
BMI, kg/m ²	0.449	<0.001
Waist circumference, cm	0.339	0.001
Duration of T2DM, yr	-0.266	0.007
Presence of diabetic retinopathy	-0.081	0.421
ACE inhibitor/ARB use	0.018	0.858
FPG, mg/dL	-0.101	0.316
HbA1c, %	-0.065	0.521
PPPG, mg/dL	-0.035	0.730
AST, IU/L ^a	0.192	0.056
ALT, IU/L ^a	0.319	0.001
γ -GT, IU/L ^a	0.281	0.005
Triglyceride, mg/dL ^a	0.281	0.005
LDL-C, mg/dL ^a	0.101	0.322
HOMA-IR ^a	0.113	0.301
eGFR, mL/min/1.73 m ²	0.166	0.099
Urinary ACR, mg/g creatinine ^a	0.331	0.001

CAP, controlled attenuation parameter; BMI, body mass index; T2DM, type 2 diabetes mellitus; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; PPPG, postprandial plasma glucose; AST, aspartate transaminase; ALT, alanine transferase; γ -GT, gamma glutamyltransferase; LDL-C, low density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; eGFR, estimated glomerular filtration rate; ACR, albumin-to-creatinine ratio.

^aLog transformed.

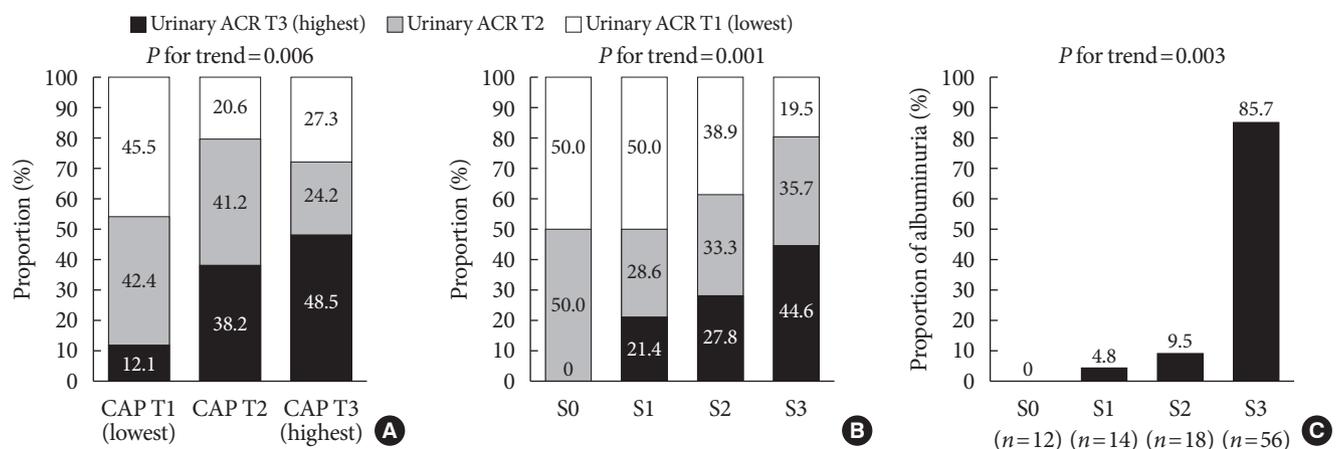


Fig. 1. Association between albuminuria and steatosis burden. (A) Association of urinary albumin-to-creatinine ratio (ACR) with controlled attenuation parameter (CAP) by tertiles. T1 for the lowest tertile, T2 for middle tertile, and T3 for the highest tertile group. (B) Association of urinary ACR tertiles according to the steatosis grade. T1 for the lowest tertile, T2 for middle tertile, and T3 for the highest tertile group. (C) Proportion of albuminuria (urinary ACR \geq 30 mg/g) according to the steatosis grade. The steatosis grade (S) was determined using the following CAP-cutoff values: 236 dB/m for S \geq 1, 270 dB/m for S \geq 2, and 302 dB/m for S \geq 3.

Table 3. Multiple linear regression analysis for CAP

Variable	Model 1		Model 2		Model 3		Model 4	
	STD β	<i>P</i> value						
Duration of T2DM, yr	-0.160	0.135	-	-	-	-	-	-
Diabetic retinopathy	-0.414	0.680	-	-	-	-	-	-
ACE inhibitor/ARB use	0.382	0.704	-	-	-	-	-	-
HbA1c, %	-0.177	0.083	-0.157	0.131	-	-	-	-
ALT, IU/L ^a	0.153	0.119	0.195	0.081	-	-	-	-
γ -GT, IU/L ^a	0.096	0.344	0.060	0.574	-	-	-	-
Triglyceride, mg/dL ^a	0.198	0.062	0.213	0.047	-	-	-	-
BMI, kg/m ²	0.391	0.001	0.380	<0.001	0.364	<0.001	-	-
Waist circumference, cm	0.287	0.003	0.284	0.006	0.300	0.003	-	-
Urinary ACR, mg/g creatinine ^a	0.305	0.001	0.321	0.001	0.299	0.004	0.321	0.001

Model 1: adjusted for age and sex; Model 2: adjusted for Model 1+duration of T2DM, diabetic retinopathy and ACE inhibitor/ARB use; Model 3: adjusted for Model 2+glycated hemoglobin, alanine aminotransferase^a, gamma glutamyl transferase^a and triglyceride^a; Model 4: adjusted for Model 3+body mass index and waist circumference.

CAP, controlled attenuation parameter; STD, standardized coefficient; T2DM, type 2 diabetes mellitus; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; HbA1c, glycosylated hemoglobin; ALT, alanine transferase; γ -GT, gamma glutamyltransferase; BMI, body mass index; ACR, albumin-to-creatinine ratio.

^aLog transformed.

(Model 3), BMI, waist circumference, and urinary ACR remained independently associated factors. Finally, Model 3 adjusted for BMI and waist circumference (Model 4) showed a significant association between urinary ACR and CAP.

Association between significant steatosis and urinary ACR

We demonstrated a relationship between CAP and urinary ACR; likewise, we analyzed the clinical and demographical factors related to significant steatosis. Table 4 shows that age, BMI, waist circumference, duration of T2DM, triglycerides, LDL-C, and urinary ACR demonstrated a significant association with significant steatosis. Similar to the linear association, younger age and shorter duration of T2DM was associated with a higher risk of significant steatosis ([OR, 0.97; 95% CI, 0.94 to 0.99; *P*=0.030 for age], [OR, 0.94; 95% CI, 0.89 to 0.99; *P*=0.034 for the duration of T2DM]), along with higher BMI, waist circumference, triglycerides, LDL-C, and urinary ACR (OR ranges 1.02 to 3.79; all *P*<0.05). To determine the independence of these risk factors, we performed multivariable logistic regression analysis. After adjusting covariate factors including age, duration of T2DM, BMI, use of ACE inhibitors or ARB, LDL-C, and BMI, LDL-C, urinary ACR still remained statistically significant ([OR, 1.34; 95% CI, 1.15 to 1.57; *P*<0.001 for BMI], [OR, 4.33; 95% CI, 1.20 to 15.63; *P*=0.025 for

LDL-C], [OR, 1.88; 95% CI, 1.31 to 2.71; *P*=0.001 for urinary ACR]).

DISCUSSION

This prospective and cross-sectional study demonstrates a significant association between CAP and urinary ACR, and an increased risk of significant steatosis in subjects with albuminuria and T2DM. Urinary ACR showed a significant positive correlation with CAP and independently predicted fatty burden of NAFLD after adjustment for clinical variables, including age, sex, waist circumference, BMI, duration of T2DM, presence of diabetic retinopathy and the use of ACE inhibitor/ARB, along with laboratory test results including HbA1c, ALT, γ -GT, triglycerides, and LDL-C. After further adjustments, we observed an approximately 2-fold rise in the adjusted risk of significant steatosis in patients with albuminuria and T2DM (OR, 1.88; 95% CI, 1.31 to 2.71; *P*=0.001).

There have been several attempts made to elucidate the association between NAFLD and albuminuria. Although previous studies consistently indicated a strong association between NAFLD and chronic kidney disease (CKD) [20,21], the results from investigations regarding NAFLD and albuminuria are conflicting; especially in populations with T2DM. A meta-

Table 4. Logistic regression analysis for significant steatosis

Parameter	Odds ratio (95% CI)	P value
Univariate logistic regression analysis		
Age, yr	0.97 (0.94–0.99)	0.030
Male sex	1.05 (0.48–2.32)	0.897
BMI, kg/m ²	1.29 (1.13–1.48)	<0.001
Waist circumference, cm	1.02 (1.02–1.11)	0.008
Duration of T2DM, yr	0.94 (0.89–0.99)	0.034
ACE inhibitors or ARB use	0.27 (0.28–1.44)	0.273
HbA1c, %	1.00 (0.83–1.21)	0.986
HOMA-IR ^a	1.32 (0.85–2.05)	0.223
AST, IU/L ^a	1.22 (0.52–2.86)	0.643
ALT, IU/L ^a	1.36 (0.74–2.51)	0.328
γ-GT, IU/L ^a	1.33 (0.76–2.32)	0.317
Triglyceride, mg/dL ^a	2.43 (1.04–5.68)	0.040
LDL-C, mg/dL ^a	3.79 (1.36–10.55)	0.011
Urinary ACR, mg/g creatinine ^a	1.54 (1.17–2.03)	0.002
Multivariable logistic regression analysis		
Age, yr	1.02 (0.98–1.06)	0.443
BMI, kg/m ²	1.34 (1.15–1.57)	<0.001
Duration of T2DM, yr	0.97 (0.89–1.05)	0.475
ACE inhibitors or ARB use	0.38 (0.12–1.14)	0.085
LDL-C, mg/dL ^a	4.33 (1.20–15.63)	0.025
Urinary ACR, mg/g creatinine ^a	1.88 (1.31–2.71)	0.001

CI, confidence interval; BMI, body mass index; T2DM, type 2 diabetes mellitus; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; AST, aspartate transaminase; ALT, alanine transferase; γ-GT, gamma glutamyltransferase; LDL-C, low density lipoprotein cholesterol; ACR, albumin-to-creatinine ratio.

^aLog transformed.

analysis study showed a 2.25-fold elevated risk of albuminuria among patients with NAFLD without diabetes; however, they could not find any significant association between albuminuria and NAFLD among subjects with diabetes [22]. A Chinese cross-sectional study of 1,763 patients with T2DM and NAFLD (ascertained using transient elastography), showed only a significant association between fibrotic burden and albuminuria, but not between steatosis burden and albuminuria, in subjects with T2DM [23]. Compared to the Chinese study, our study population was relatively younger (mean age 53.0±15.5 years vs. 60.7±11.5 years in the Chinese study), and had a shorter

duration of T2DM (7.1±7.3 years vs. 10.8±8.5 years in the Chinese study). Moreover, we excluded patients with liver cirrhosis, splenomegaly, or chronic liver diseases other than NAFLD, based on imaging studies, along with subjects with CKD to clarify the association between the degree of steatosis and albuminuria. These factors could account for the discrepancies between the two studies. In concordance with our results, studies have confirmed the association between NAFLD and albuminuria in prediabetic patients and those diagnosed recently or with a shorter duration of T2DM [24,25]. As albuminuria is regarded as an early clinical manifestation of diabetic kidney disease (DKD), and fatty burden accumulation precedes fibrotic change, the association between the degree of steatosis and albuminuria might emerge in the early course of T2DM. This finding is clinically relevant to identify high-risk patients of NAFLD for further screening and evaluation.

With respect to risk factors for NAFLD in populations with T2DM, traditional metabolic risk factors such as BMI, waist circumference, and dyslipidemia were independent variables associated with CAP and the aggravation of NAFLD in our study. In addition to these traditional markers, albuminuria showed at least a comparable potential to predict NAFLD severity. In other words, patients with T2DM and additional metabolic diseases are at a higher risk of developing advanced NAFLD and may benefit from a liver assessment. Moreover, it is important to note that among patients with T2DM, urinary ACR test may be useful for screening NAFLD as well as DKD. Among current valid liver assessments, transient elastography has the advantage of providing quantitative measurements, with validations done in different populations, showing high reproducibility [17,26]. In contrast to the scoring system, confounding factors such as age and liver enzymes do not influence the results of transient elastography [27–29].

The possible mechanisms for the association between albuminuria and NAFLD might be explained by insulin resistance and systemic inflammation. Albuminuria reflects insulin resistance, even in a healthy population, and the reduced insulin sensitivity promotes hepatic steatosis [30,31]. An increase in systemic inflammatory markers (interleukin 6, tumor necrosis factor α, fibrinogen, monocyte chemoattractant protein-1) is observed in subjects with albuminuria, attributing to NAFLD, and its progression [32–34]. Recently, the concept of crosstalk between the liver and kidney has emerged, involving the renin-angiotensin system. Adipocytes (fat cells) express components of the renin-angiotensin system, impacting the ACE (angioten-

sin II) angiotensin II type 1 receptor axis, which is also expressed in the liver and kidney, leading to organ damage [35]. In addition, the altered activation of nutrient/energy sensors sirtuin-1 and adenosine monophosphate-activated kinase, and impaired antioxidant defense mediated by nuclear factor erythroid 2-related factor-2 (NRF2), support the crosstalk between liver and kidney [36]. Patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene, which is expressed in the liver, adipose tissue, and kidney, is also linked with the crosstalk. *PNPLA3* is a lipid droplet-associated protein that has hydrolase activity toward triglycerides and retinyl esters, leading to hepatic steatosis. Recent studies have also reported the association of *PNPLA3* polymorphism with albuminuria [37,38].

Previous studies reported the increased prevalence of NAFLD with age [39,40]. However, recent studies have also demonstrated an increased presence of NAFLD in young adults with T2DM [41,42]. A Danish cross-sectional study showed that the risk of NAFLD nearly doubled in patients diagnosed with T2DM before the age of 40 [41]. In addition, NAFLD and NASH have shown an increased prevalence in young adults, and, if unchecked, could lead to liver transplantation [43]. In agreement with recent studies, we observed that patients with significant steatosis were younger and more obese than those without significant steatosis. However, after adjusting for age and BMI, this association was dismissed. A selection bias might have affected the results achieved in our study since we actively performed imaging studies in young and recently diagnosed T2DM patients. However, such patients usually do not show any symptoms of NAFLD, and are, therefore, left treated. Screening and early identification of NAFLD should, therefore, not be neglected in this population, and screening should be emphasized as for older patients with T2DM.

We acknowledge that our study has some limitations. Firstly, despite the acceptance of transient elastography for detecting NAFLD, this diagnostic tool does not replace liver biopsy, which is the gold standard for detecting NAFLD. Liver biopsy is regarded as a precise diagnostic tool for evaluating NAFLD and NASH. However, owing to its complications, non-invasive diagnostic devices, including transient elastography, have been widely utilized. Additionally, although we applied XL probe in the current study to enhance its accuracy, CAP could be overestimated in patients with morbid obesity. Secondly, since this was a cross-sectional study, we could not elucidate a causal relationship between the observed findings. Thirdly, since this study was based on a population visiting a single tertiary uni-

versity hospital, our results may not be reflective of the entire Korean population. In addition, selection bias could be present and might have affected the results. Further multi-center, large-scale, longitudinal studies are required to show a clear association between albuminuria and hepatic burden in T2DM.

In conclusion, our results show that patients with T2DM and NAFLD frequently have albuminuria, which is associated with a higher risk of significant steatosis. Additionally, we showed that the level of urinary ACR was associated with an increased hepatic steatosis burden in NAFLD patients. Hepatic complication should be kept in mind in T2DM patients with albuminuria. Further studies with a larger number of patients and longer periods of observation, along with liver biopsy, are needed to elucidate the association more clearly between albuminuria occurrence and NAFLD progression.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: E.H., H.S.K.

Acquisition, analysis, or interpretation of data: E.H., M.K.K., B.K.J., H.S.K.

Drafting the work or revising: E.H., M.K.K., H.S.K.

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