Prevalences of diabetic retinopathy and nephropathy are lower in Korean type 2 diabetic patients with non-alcoholic fatty liver disease

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ABSTRACT

Aims/Introduction: The associations between non-alcoholic fatty liver disease (NAFLD) and chronic vascular complications of type 2 diabetes remain uncertain. We assessed the relationships between NAFLD and chronic vascular complications in patients with type 2 diabetes.

Materials and Methods: Patients with type 2 diabetes ($n = 929$) attending a diabetes clinic of a university hospital were studied retrospectively. Patients who had any clinical evidence of cirrhosis or other causes of chronic liver disease were excluded. Prevalences of chronic microvascular and macrovascular complications were assessed. NAFLD was ascertained by ultrasonography.

Results: The prevalence of NAFLD in patients with type 2 diabetes was 63.3%. The prevalences of diabetic retinopathy and nephropathy were significantly lower in patients with NAFLD than those without NAFLD (33.0 vs 70.2%, $P < 0.001$; 29.3 vs 37.1%, $P = 0.007$, respectively), whereas no difference was found in the prevalence of diabetic neuropathy. The prevalence of diabetic macrovascular complications was lower in type 2 diabetic patients with NAFLD than those without NAFLD (9.2 vs 14.7%, $P = 0.008$). After adjustment for confounding factors, such as age, sex, glycated hemoglobin, fasting serum C-peptide, diabetic duration, body mass index and hypertension, NAFLD remained significantly associated with a lower odds ratio (OR) of diabetic retinopathy (OR 0.440, 95% confidence interval 0.255–0.757, $P = 0.003$) and nephropathy, (OR 0.541, 95% confidence interval 0.358–0.817, $P = 0.003$). In contrast, NAFLD was not significantly associated with macrovascular complications after adjustment for confounding factors.

Conclusions: These results suggest that NAFLD is inversely associated with prevalences of diabetic retinopathy and nephropathy in Korean patients with type 2 diabetes.

INTRODUCTION

Compared with non-diabetic individuals, people with type 2 diabetes are at increased risk of developing non-alcoholic fatty liver disease (NAFLD), and have a higher risk of developing fibrosis and cirrhosis[1–3]. NAFLD affects 30% of the general adult population, and 60–80% of diabetic and obese patients[4,5]. There is now growing evidence that NAFLD, especially in patients with type 2 diabetes, could be linked to an increased risk of developing cardiovascular disease (CVD) independently of other known risk factors[6–10]. In contrast, there is currently very little information on the association between NAFLD and chronic microvascular complications, such as retinopathy, nephropathy and neuropathy, in people with type 2 diabetes. To date, only a few studies have reported that NAFLD is independently associated with diabetic retinopathy or nephropathy[11–13]. We have assessed whether

NAFLD, as diagnosed by ultrasonography, is associated with an increased risk of macrovascular (cerebrovascular disease, coronary artery disease and peripheral arterial disease) and microvascular complications (retinopathy, nephropathy and peripheral polyneuropathy) in a clinical cohort of Korean patients with type 2 diabetes.

MATERIALS AND METHODS

Participants

Data for Korean patients with type 2 diabetes who visited the diabetes clinic at Soonchunhyang University Bucheon Hospital during 2001–2008 were analyzed retrospectively. All patients ($n = 1,410$) who had undergone thorough evaluation for diabetic complications, with regular follow up for more than 2 years, were enrolled. The following patients were excluded: (i) patients ($n = 378$) for whom liver ultrasonography was not carried out, or not available; and (ii) patients ($n = 103$) with any clinical evidence of cirrhosis or other causes of chronic liver disease (i.e., alcohol intake >20 g/day, viral hepatitis, autoimmune hepatitis, hemochromatosis and use of hepatotoxic medications). The remaining 929 patients with type 2 diabetes were included in the final analysis. The present study was approved by the institutional review board of Soonchunhyang University Bucheon Hospital.

Measurements

Fasting and postprandial 2-h glucose and C-peptide levels, glycated hemoglobin (HbA1c), total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and high-sensitivity C-reactive protein (hs-CRP) were measured. HbA1c was measured by ion-exchange high-performance liquid chromatography (Bio-Rad, Hercules, CA, USA). The reported values are the mean of the nearest three determinations around the time of evaluation for complications. Serum C-peptide level was determined by immunoradiometric assay (Immunotech, Prague, Czech Republic). Delta C-peptide was defined as postprandial serum C-peptide minus fasting C-peptide level. Homeostasis model assessment insulin resistance index (HOMA-IR) was calculated as follows:

$$HOMA\text{-}IR = \text{fasting plasma glucose (mmol/L)} \times \text{fasting serum insulin (uIU/mL)}/22.5.$$

Liver ultrasonography was carried out by experienced radiologists. The diagnosis of hepatic steatosis was made on the basis of characteristic sonographic features; that is, diffuse hyperechogenicity of the liver relative to kidneys, ultrasound beam attenuation and poor visualization of intrahepatic structures.

Assessment of Diabetic Complications

Information regarding duration of diabetes, familial history of diabetes mellitus, and previous history of hypertension and macrovascular complications (cerebrovascular disease, coronary artery disease and peripheral arterial disease) was obtained from the patients' medical records.

Diabetic retinopathy was evaluated by experienced ophthalmologists. If required, fluorescein angiography was carried out. Diabetic nephropathy included microalbuminuria, overt albuminuria and azotemia. Albuminuria was determined by radioimmunoassay (Immunotech) using spot urine or time-collected urine. Microalbuminuria was defined as an albumin excretion rate of 20–200 µg/min, an albumin-to-creatinine ratio in spot urine of 30–300 mg/g or a 24-h urine protein of 30–300 mg/day. Diabetic neuropathy was diagnosed by nerve conduction velocity testing or the current perception threshold test. Diabetic neuropathy was also established by the presence of typical symptoms and compatible findings on neurological examinations or a history of treatment for neuropathy.

Coronary artery disease was diagnosed based on a patient's hospital medical record of myocardial infarction, angina pectoris, coronary artery bypass surgery, percutaneous coronary angioplasty or electrocardiographic changes typical of ischemia (the presence of Q/Qs pattern, significant ST segment depression, or deep T wave inversion). Cerebrovascular disease was defined by the presence of either transient ischemic attack or strokes. Peripheral arterial disease was defined by the presence of ischemic foot ulcers, gangrene, a history of vascular surgery, significant stenosis on angiography or abnormal ankle–brachial index (≤ 0.9).

Statistical Analysis

Statistical analyses were carried out using SPSS for Windows 14.0 software (SPSS Inc., Chicago, IL, USA). Results are expressed as the mean ± standard deviation. Variables with a skewed distribution, such as blood concentrations of glucose, triglycerides and HbA1c, were log-transformed before analysis. Unpaired Student's t-tests were used to compare between-group differences. The χ^2-test was used to compare frequencies. Multivariate logistic regression analyses were carried out to estimate the odds ratios (ORs) for diabetic vascular complications after adjusting for other clinical and biochemical variables. A P-value of <0.05 was deemed to show statistical significance.

RESULTS

Overall, the 929 participants of the study had a mean age of 57.7 years, mean body mass index (BMI) of 24.8 kg/m^2, mean HbA1c of 8.4% and mean duration of diabetes of 6.2 years. The prevalence of NAFLD in the 929 participants of the study was 63.3% (588/929).

The clinical and biochemical characteristics of patients stratified by their NAFLD status are shown in Table 1. Patients with NAFLD were younger, tended to be male, had higher BMI, shorter duration of diabetes, higher prevalence of hypertension, higher total cholesterol, lower HDL cholesterol and higher triglyceride levels than those without NAFLD. Furthermore, fasting and postprandial C-peptide levels, insulin levels, and HOMA-IR values were higher in patients with NAFLD than in

Table 1 | Baseline characteristics of the type 2 diabetic patients according to non-alcoholic fatty liver disease status

	Without NAFLD (n = 341, 36.7%)	With NAFLD (n = 588, 63.3%)	P-value
Age (years)	59.3 ± 11.9	56.7 ± 11.7	0.001
Sex, male (%)	48.7	55.1	0.034
Duration of DM (years)	7.2 ± 7.1	5.6 ± 6.3	0.001
BMI (kg/m^2)	23.3 ± 3.4	25.8 ± 3.5	<0.001
HbA1c (%)	8.3 ± 2.21	8.4 ± 1.1	0.782
SBP (mmHg)	130.5 ± 19.6	132.0 ± 18.9	0.250
DBP (mmHg)	76.8 ± 12.7	78.6 ± 12.1	0.040
Hypertension (%)	60.1	69.1	0.004
Total cholesterol (mg/dL)	190.8 ± 44.1	202.3 ± 45.3	<0.001
LDL cholesterol (mg/dL)	113.6 ± 42.4	123.2 ± 48.6	0.092
HDL cholesterol (mg/dL)	46.4 ± 11.8	43.3 ± 10.4	0.001
Triglycerides (mg/dL)	157.9 ± 104.3	209.9 ± 140.8	<0.001
AST (U/l)	25.1 ± 43.7	29.2 ± 37.5	0.141
ALT (U/l)	30.1 ± 80.9	37.9 ± 68.8	0.119
GGT (U/l)	49.2 ± 52.5	67.5 ± 138.6	0.102
hs-CRP (mg/dL)	0.84 ± 1.84	0.60 ± 1.63	0.129
HOMA-IR	3.3 ± 2.8	5.0 ± 4.8	<0.001
Fasting C-peptide (ng/mL)	2.3 ± 1.4	2.9 ± 1.5	<0.001
Postprandial C-peptide (ng/mL)	5.7 ± 3.3	7.3 ± 4.0	<0.001
Fasting insulin (uIU/mL)	8.6 ± 7.4	12.4 ± 10.4	<0.001
Postprandial insulin (uIU/mL)	30.5 ± 25.9	46.3 ± 40.9	<0.001
Delta C-peptide (ng/mL)	2.6 ± 2.0	4.9 ± 4.5	<0.001
Insulin user (%)	34.1	28.7	0.007
Oral hypoglycemic agents (%)	55.4	58.0	0.532
Lipid lowering agents (%)	56.9	55.5	0.420
ACEI or ARB (%)	44.3	35.3	0.120

Data are shown as mean ± standard deviation. Delta C-peptide = postprandial C-peptide − Fasting C-peptide. ACEI, angiotensin converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin II receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; GGT, gamma-glutamyl transferase; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance index; hs-CRP, high sensitivity C-reactive protein; LDL, low density lipoprotein; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure.

those without NAFLD. The proportion of insulin users was lower in patients with NAFLD. No significant difference between groups was found in glycemic control, serum AST, ALT, GGT and hs-CRP concentrations.

The prevalences of diabetic vascular complications in patients stratified by their NAFLD status are shown in Table 2. The prevalences of diabetic retinopathy and nephropathy were

Table 2 | Prevalence of diabetic vascular complications according to non-alcoholic fatty liver disease status

	Without NAFLD (%)	With NAFLD (%)	P-value
Retinopathy			
NPDR	41.9	21.7	<0.001
PDR	28.3	11.3	
Neuropathy	50.6	47.2	0.181
Nephropathy	37.1	29.3	0.007
Macrovascular complications†	14.7	9.2	0.008

†Macrovascular complications included coronary artery disease, cerebrovascular disease, and peripheral arterial disease. NAFLD, non-alcoholic fatty liver disease; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

significantly lower in type 2 diabetic patients with NAFLD than in those without NAFLD (33.0 vs 70.2%, $P < 0.001$; 29.3 vs 37.1%, $P = 0.007$, respectively), whereas no difference was found in the prevalence of diabetic neuropathy. The prevalence of diabetic macrovascular complications was lower in patients with NAFLD than those without NAFLD (9.2 vs 14.7%, $P = 0.008$).

After adjustment for confounding factors, such as age, sex, HbA1c, C-peptide, diabetic duration, BMI and hypertension, NAFLD remained significantly associated with lower OR of diabetic retinopathy (OR 0.440, 95% confidence interval 0.255–0.759, $P = 0.003$) and nephropathy (OR 0.541, 95% confidence interval 0.358–0.817, $P = 0.003$; Table 3). In contrast, after adjustment for confounding factors, NAFLD was not significantly associated with macrovascular complications (Table 3).

Table 3 | Odds ratios for vascular complications in type 2 diabetic patients with non-alcoholic fatty liver disease

	NAFLD OR (95% CI)	P-value
Retinopathy		
Model 1	0.402 (0.256–0.561)	<0.001
Model 2	0.430 (0.258–0.719)	0.001
Model 3	0.440 (0.255–0.759)	0.003
Nephropathy		
Model 1	0.668 (0.475–0.942)	0.021
Model 2	0.664 (0.455–0.969)	0.034
Model 3	0.541 (0.358–0.817)	0.003
Macrovascular complications*		
Model 1	0.920 (0.577–1.468)	0.728
Model 2	1.049 (0.644–1.710)	0.847
Model 3	0.833 (0.490–1.146)	0.499

Model 1: Adjusted for sex, age. Model 2: Adjusted for sex, age, fasting C-peptide, glycated hemoglobin, diabetes duration. Model 3: Adjusted for the factors in Model 2 + body mass index, hypertension. *Macrovascular complications included coronary artery disease, cerebrovascular disease, and peripheral arterial disease. NAFLD, non-alcoholic fatty liver disease.

DISCUSSION

The present study assessed the relationship of NAFLD with chronic microvascular and macrovascular complications in a cohort of patients with type 2 diabetes. Our major finding was that NAFLD, as diagnosed by characteristic sonographic features, is inversely associated with prevalences of diabetic retinopathy and nephropathy in Korean patients with type 2 diabetes, in contrast to the results of previous studies[11–13]. After adjustment for confounding factors, NAFLD remained significantly associated with lower OR of diabetic retinopathy and nephropathy. In contrast, NAFLD was not associated with diabetic neuropathy or macrovascular complications after adjustment for confounding factors.

The association between NAFLD and microvascular complications (retinopathy, nephropathy and neuropathy) in type 2 diabetes has not been studied thoroughly. Three previous studies, two from Italy[11,12] and one from Romania[13], investigated the association between NAFLD and microvascular complications in type 2 diabetes. Targher et al.[11,12] showed that NAFLD is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in patients with type 2 diabetes. Casoinic et al.[13] showed that NAFLD is positively correlated with microalbuminuria, a marker of early-stage nephropathy in patients with type 2 diabetes. In contrast to these previous studies, the present results showed that NAFLD was inversely associated with prevalences of diabetic retinopathy and nephropathy in Korean patients with type 2 diabetes. This difference in results with previous studies might be related to differences in characteristics of the participants. The present study participants had a lower proportion of men, and lower body mass index, and were younger patients with poorer glycemic control compared with those of the Italian study. Additionally, the patients in the present study underwent a comprehensive assessment for diabetic neuropathy, which has not been assessed in previous studies. In addition, the ethnic differences for pathophysiological characteristics of type 2 diabetic patients could be responsible for the discrepancies between the present results and those of previous studies. In the study by Targher et al.[11], HbA1c was higher in type 2 diabetes with NAFLD than in those without NAFLD. Thus, insulin resistance associated with NAFLD might have led to poorer glycemic control and a higher prevalence of diabetic complications. In contrast, no significant difference in glycemic control between the two groups was found in the present study. Insulin secretory capacity in Asians, including Koreans, has been proposed to be lower compared with Western subjects[14–17]. Higher serum C-peptide and insulin levels in the present study participants with NAFLD might reflect relatively preserved β-cell function, which could have beneficial effects on glycemic control, thus decreasing the occurrence of diabetic complications.

Although this cross-sectional study could not elucidate the potential mechanisms for the inverse association between NAFLD and microvascular complications of diabetes, there are several possible explanations. First, as aforementioned, patients with NAFLD in the present study might have preserved β-cell function, which leads to more stable glycemic control and a decrease in microvascular complications. Second, we could not exclude the possibility that the shorter duration of diabetes and relatively younger age of patients with NAFLD compared with those without NAFLD might have contributed to a lower prevalence of diabetic complications, even though we adjusted for those factors in logistic regression analysis. In addition, patients with NAFLD, who had higher BMI and insulin resistance, might have encouraged more intensive lifestyle modification, such as diet control and exercise. Therefore, they could have achieved similar glycemic control within a shorter period of time compared with those without NAFLD, which might be related to a lower prevalence of diabetic complications.

The present results showed that serum fasting, postprandial and delta C-peptide levels were higher in patients with NAFLD than in those without NAFLD. This finding suggested that patients with NAFLD in the present study could have preserved β-cell function. Unfortunately, we do not have data for a more accurate index of β-cell function estimated by dynamic tests, such as oral or intravenous glucose tolerance tests or hyperglycemic clamp. However, postprandial 2-h serum C-peptide levels are known to be useful indicators of residual β-cell function in type 2 diabetes patients[18,19], and are closely associated with microvascular complications. Lower serum C-peptide level was found to be associated with higher prevalences of retinopathy and nephropathy in patients with type 2 diabetes[20–22]. In a previous study of patients with type 1 diabetes, residual β-cell function was reported to be an independent protective factor against the development of microvascular, but not macrovascular, complications[23]. C-peptide levels could directly impact the development and/or progression of microvascular complications, as has been shown by others[18,19].

Targher et al.[11] suggested that the possible molecular mediators linking NAFLD with retinopathy and chronic kidney disease (CKD) could include the increased release of some pathogenic mediators from the liver, such as advanced glycation end-products, reactive oxygen species, CRP, interleukin (IL)-6 and tumor necrosis factor (TNF)-α. Several studies have shown that these potential mediators of vascular and/or renal injury are higher in obese and/or diabetic patients with NAFLD than in those without NAFLD[24–27]. However, these studies were mostly based on non-alcoholic steatohepatitis (NASH), and there has been little evidence that inflammatory mediators are increased in patients with simple steatosis. The majority of our study participants with NAFLD might have simple steatosis rather than NASH.

Previous data in some ethnic populations suggested that the presence of NAFLD might increase coronary heart disease risk independent of components of the metabolic syndrome[11,28,29]. Recent data suggest that the presence of NAFLD in type 2 diabetes might also be linked to increased CVD risk independently

of components of the metabolic syndrome[6–8,30,31]. The present our study, NAFLD seemed to be associated with a lower prevalence of CVD in unadjusted analysis. However, after adjustment for confounding factors, NAFLD was not associated with macrovascular complications. Reasons for the discrepancy with previous studies are unclear, but might be related to the lower prevalence of macrovascular complications in our cohort. The present study participants had lower mean BMI, lower prevalence of obesity and lower insulin resistance index compared with the Western populations.

The present study had several limitations. First, this was a cross-sectional analysis and could not determine causal relationships. Prospective studies are required to confirm the associations between NAFLD and chronic vascular complications of type 2 diabetes. Second, the present study population was a cohort of patients cared for at a single center. Thus, our results might not be generalizable to all patients with type 2 diabetes. However, the majority of our subjects were typical patients with type 2 diabetes commonly encountered in outpatient diabetes clinics. This single-center study also conferred a high degree of consistency regarding laboratory data, ultrasonographic finding and the evaluation for diabetic complications. Third, the relatively short diabetic duration was a limitation in evaluating the occurrence of chronic diabetic vascular complications.

Despite these limitations, the present results suggest that NAFLD is inversely associated with prevalences of diabetic retinopathy and nephropathy in Korean patients with type 2 diabetes. Furthermore, large-scale prospective studies are required to elucidate causal associations between NAFLD and the chronic vascular complications of type 2 diabetes.

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