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Liver fibrosis is independently associated with diabetic peripheral neuropathy in type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: Non-alcoholic fatty liver disease and type 2 diabetes mellitus are closely related, and often occur simultaneously in patients. Type 2 diabetes increases the risk of diabetic peripheral neuropathy, resulting in intolerable pain and extremity amputation that reduces the quality of life. However, the role of non-alcoholic fatty liver disease in the pathogenesis of diabetic peripheral neuropathy remains unclear. Thus, we evaluated the correlation of liver fibrosis and steatosis, which are representative histological morphologies of non-alcoholic fatty liver disease, with diabetic peripheral neuropathy in type 2 diabetes patients.

Materials and Methods: Five hundred twenty individuals with type 2 diabetes were recruited. All the patients were detected nerve conduction study for diabetic peripheral neuropathy and fibro touch for liver steatosis and fibrosis. Correlation of DPN with liver steatosis and fibrosis were analysed with binary logistic analysis.

Results: Among the 520 patients, the prevalence of liver steatosis, fibrosis and diabetic peripheral neuropathy was 63.0% (n = 328), 18.1% (n = 94) and 52.1% (n = 271), respectively. The prevalence of diabetic peripheral neuropathy was significantly elevated in patients with liver steatosis (55.7 vs 44.9%, P = 0.03) and fibrosis (61.5 vs 50%, P = 0.04), and it increased as liver stiffness measurement increased. Additionally, both hepatic steatosis (odds ratio 1.48, 95% confidence interval 1.04–2.11, P = 0.03) and fibrosis (odds ratio 1.60, 95% confidence interval 1.02–2.51, P = 0.04) were correlated with diabetic peripheral neuropathy. After adjusting for age, sex, weight, height, body mass index, waist hip ratio, duration of type 2 diabetes, blood glucose, homeostatic model assessment of insulin resistance, blood pressure, serum lipid, liver enzyme, urea, uric acid, creatinine and inflammatory factors, liver fibrosis remained associated with diabetic peripheral neuropathy (odds ratio 2.24, 95% confidence interval 1.11–4.53, P = 0.02).

Conclusions: The prevalence of diabetic peripheral neuropathy was elevated in patients with liver steatosis and fibrosis. Liver fibrosis was also independently associated with an increased risk of diabetic peripheral neuropathy.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, with a global prevalence of 22–28% in the general population[1]. It refers to the excessive accumulation of triglyceride in the liver in the absence of competing liver disease etiologies, such as substantial alcohol consumption, chronic viral hepatitis and use of medications that induce steatosis. With the progression of steatosis, lobular inflammation and pericellular fibrosis would occur, which is known as non-alcoholic steatohepatitis, leading to cirrhosis and hepatocellular cancer, and eventually to death[2]. Except for liver-related complications, increasing epidemiological studies have claimed a correlation between other metabolic disorders and NAFLD.

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For example, the incidence of cardiovascular disease (CVD) mortality in the NAFLD population is much higher than that of liver-related mortality[2]. Additionally, a meta-analysis with a sample size of 8,515,431 from 22 countries showed CVD-specific and liver-specific mortalities of 4.79/1,000 person-years and 0.77/1,000 person-years, respectively, in NAFLD patients.

Given that CVD is one of the most common chronic complications of type 2 diabetes mellitus, and the prevalence of NAFLD in type 2 diabetes mellitus patients is almost threefold that in the general population[3,4], tremendous epidemiological studies on the relationship between NAFLD and chronic vascular complications of diabetes have emerged[5]. Currently, NAFLD has been comprehensively reported to correlate with an increase in the incidence of CVD and chronic kidney disease in individuals with type 2 diabetes mellitus[5,6]. However, just a few studies have evaluated the relationship between NAFLD and diabetic peripheral neuropathy (DPN) in patients with type 2 diabetes mellitus and have produced conflicting results[7-12].

Furthermore, DPN is a significant microvascular complication of type 2 diabetes mellitus that contributes to pain, numbness, ulceration and amputation of the distal extremities, leading to compromised quality of life. The prevalence of DPN in the adult type 2 diabetes mellitus population varied significantly from 20 to 60% due to diverse detection methods and ethnicities. In China, the prevalence reached up to 61.8%[13]. Considering the high prevalence and high disability tendency, the etiology and potential treatment of DPN are worth exploring. Currently, the most effective treatments are glucose control and pain management. Nevertheless, the 7.8-year intensive therapy achieving glycemic control compared with conventional therapy showed a decrease in the incidence of autonomic neuropathy, but not a decrease in the incidence of DPN in type 2 diabetes mellitus patients[14]. Thus, an early diagnosis of DPN is also of necessity.

As mentioned previously, NAFLD directly or indirectly results in many liver-irrelated disorders. Given the high clustering of NAFLD and type 2 diabetes mellitus, the high prevalence of DPN in type 2 diabetes mellitus patients, and the ambiguous connection between NAFLD and DPN, we evaluated the relationship between NAFLD and DPN in individuals with type 2 diabetes mellitus for better management of DPN.

MATERIALS AND METHODS

Study population

Patients aged >18 years and diagnosed with type 2 diabetes mellitus according to the World Health Organization criteria from the Wujing community, Shanghai, China, were enrolled in the present study using the cluster sampling method. All enrolled patients were admitted to one center for all clinical and laboratory assessments. Patients with excessive alcohol consumption (daily alcohol intake >20 g in women and >30 g in men), viral hepatitis B and C, autoimmune hepatitis, steatosis-inducing drug utilization and hepatocellular carcinoma history were excluded from the study. In total, 520 adult type 2 diabetes mellitus patients were included. Written informed consent was obtained from all participants, and the study was approved by the Ethical Committee of Huashan Hospital.

Demographic and laboratory evaluation

All participants were gathered from the same community center. Age, sex, bodyweight, height, waist circumference, hip circumference and blood pressure were recorded by one doctor. Additionally, fasting serum samples were collected for measurement of blood glucose, glycated hemoglobin, C-peptide, insulin, lipid profile, liver and kidney function, and inflammatory factors.

Evaluation of DPN

Nerve conduction study (NCS) is the most reliable method, except for biopsy, of studying DPN in clinical trials. Hence, NCSs (NDI-097; Shanghai Haishen Medical Electronic Instrument Co., Shanghai, China) were carried out on the motor ulnar, median and peroneal nerves, and the sensory ulnar, median and sural nerves in one community center with temperatures maintained at 22–26°C in the present study. Distal motor latency, motor nerve conduction velocity, motor compound muscle action potential, sensory nerve conduction velocity and sensory nerve action potential amplitude were evaluated. Distal motor latency prolonging, motor nerve conduction velocity slowing or compound muscle action potential descending of the motor nerves was defined as motor nerve dysfunction. Meanwhile, sensory nerve conduction velocity slowing or sensory nerve action potential amplitude descending of the sensory nerves was defined as sensory nerve dysfunction.

At least two nerve dysfunctions, with at least one belonging to the lower extremity nerves, were deemed to have DPN. The cut-off value of each parameter shown in Table S1 was evaluated by Huashan Hospital, Shanghai, China, because the variation in different laboratories and ethnicities was large.

The detailed protocol was carried out, as described in a previous study[15]. Surface electrodes were used in the present study. The recording electrodes were fixed to the skin of the patients using adhesive tape; the skin was prepared by disinfecting the surface. The stimulation and recording sites are listed in Table S2. The length of each nerve was measured using a flexible measuring tape. Finally, a ground electrode was placed between the stimulating and recording electrodes for safety.

Evaluation of liver steatosis and fibrosis

FibroTouch (FT5000), which was as valid as FibroScan, was used to evaluate liver steatosis and fibrosis in the present study[16,17]. The liver stiffness measurement (LSM) value in FibroTouch showed a high coincidence rate with hepatic fibrosis staging according to the liver biopsy, which is the gold standard for diagnosis of cirrhosis and staging of fibrosis or steatosis[18,19]. Transient elastography was carried out by a certified physician who was blinded to the patients' clinical data, and manipulated according to the operations manual. Briefly,

patients were placed in a standard supine position, with their right hands beneath their heads to broaden the intercostal space. Then, the probe was applied to the skin of the seventh to ninth intercostal spaces in a vertical position where the coupling agent was smeared. The controlled attenuation parameter (CAP) was expressed as dB/m, and liver LSM was expressed in kPa.

The CAP and LSM were considered reliable only if 10 successful measurements were obtained, with an interquartile range/median of <30% and a success rate of ≥60%. The cut-off points of CAP and LSM were set at 240 dB/m and 9.5 kPa, respectively, according to Chinese thresholds[19,20]. Therefore, individuals with CAP ≥240 dB/m and LSM ≥9.5 kPa were assigned to steatosis and fibrosis, respectively.

Statistical analysis

Continuous variables are presented as the mean ± standard deviation when normally distributed, and as the median (interquartile range) when skewedly distributed. In contrast, categorical variables are presented as percentages. Comparisons between two groups were carried out using Student's *t*-test for continuous variables, and the χ^2-test for categorical variables. The risk of liver fibrosis and steatosis in the presence of DPN was estimated using binary logistic analysis. Missing data were eliminated. All statistical analyses were carried out using SPSS for Windows (version 21.0; IBM Corp., Armonk, NY, USA).

RESULTS

Clinical characteristics of the study population

A total of 520 patients with type 2 diabetes mellitus were enrolled in the present study. All basic clinical characteristics, including age, sex, body mass index (BMI), duration of type 2 diabetes mellitus, glycemic control, β-cell function, blood lipid, blood pressure, inflammatory indicators, liver function and kidney function indicators, are presented in column 2 of Table 1.

Table 1 | Basic clinical characteristics of whole population

Characteristics	Overall (*n* = 520)
Sex (male), *n* (%)	227 (43.6%)
Age (years)	64.82 ± 6.51
Weight (kg)	64.66 ± 10.09
Height (m)	1.62 ± 0.08
BMI (kg/m^2)	24.47 ± 3.03
WHR	0.91 ± 0.06
Duration of T2DM (years)	8.21 ± 5.59
HbA1c (%)	7.01 ± 1.20
FBG (mmol/L)	7.39 ± 2.16
Fasting insulin (pmol/L)	16.60 (8.89–26.57)
Fasting C-peptide (pg/mL)	155.41 (66.79–247.13)
HOMA-IR	0.74 (0.41–1.24)
Systolic pressure (mmHg)	130 ± 9
Diastolic pressure (mmHg)	80 ± 6
LDL (mmol/L)	2.36 ± 1.00
HDL (mmol/L)	1.01 ± 0.40
TC (mmol/L)	4.30 ± 1.29
TG (mmol/L)	1.60 (1.05–2.36)
ALT (U/L)	9 (7–12)
AST (U/L)	16 (11.25–20)
GGT (U/L)	19.5 (13–28)
Creatinine (μmol/L)	65.76 ± 21.50
Urea (mmol/L)	5.34 ± 1.55
Uric acid (μmol/L)	284.30 ± 90.62
hsCRP (ng/mL)	3.25 (1.43–6.76)
TNF-α (pg/mL)	33.85 (18.93–98.01)
IL-6 (pg/mL)	10.63 (6.39–18.94)
LSM	5.95 (4.70–8.20)
CAP	259.71 ± 41.63

Cohort size, *n* = 520. Continuous variables are expressed as the mean ± standard deviation for normal distributed variables, and the median (interquartile range) for skewed distributed data or as absolute and relative frequencies for categorical variables. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, fat attenuation parameter; FBG, fasting blood glucose; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; LSM, liver stiffness measurement; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α; WHR, waist-to-hip ratio.

Prevalence and clinical characteristics of patients with liver steatosis and fibrosis

Among the 520 patients with type 2 diabetes mellitus, 63.0% (*n* = 328) and 18.1% (*n* = 94) had liver steatosis and fibrosis, respectively (Figure 1). Patients with liver steatosis (CAP ≥240 dB/m) were heavier than those with CAP <240 dB/m and were prone to abdominal obesity. Fasting insulin, C peptide, homeostatic model assessment of insulin resistance and blood pressure levels were also much higher in patients with liver steatosis. The liver enzyme levels of patients with liver steatosis were elevated. In addition, serum uric acid levels were significantly increased. Neither creatinine nor urea levels were found to be altered. Furthermore, serum high-sensitivity C-reactive protein levels were elevated in patients with liver steatosis (columns 2–4, Table 2).

Similarly, patients with liver fibrosis (LSM ≥9.5 kPa) were prone to abdominal obesity, and their fasting insulin, C-peptide, homeostatic model assessment of insulin resistance and blood pressure levels were also higher. Liver enzyme levels in patients with liver fibrosis were elevated. Additionally, their serum uric acid levels increased, but without statistical significance. Neither creatinine nor urea levels were found to be altered. Serum high-sensitivity C-reactive protein levels were also elevated in patients with liver fibrosis (columns 5–7, Table 2).

Prevalence and clinical characteristics of patients with DPN

Among the patients with type 2 diabetes mellitus, 52.1% (*n* = 271) were diagnosed with DPN according to NCS

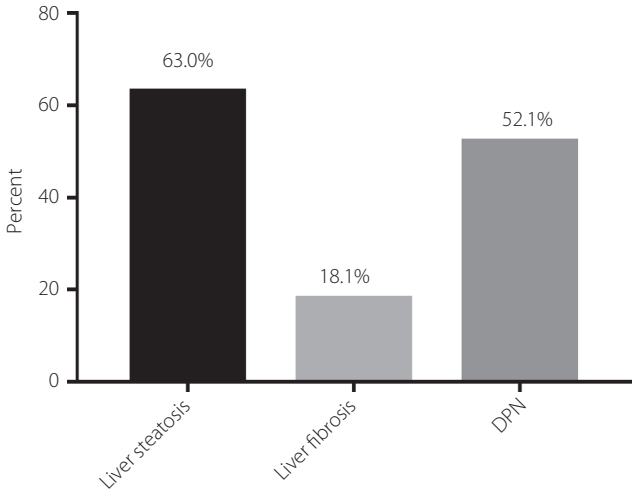

Figure 1 | Prevalence of non-alcoholic fatty liver disease and diabetic peripheral neuropathy (DPN) in type 2 diabetes mellitus patients. Cohort number: 520 type 2 diabetes mellitus patients; liver steatosis: controlled attenuation parameter ≥240 dB/m; liver fibrosis: liver stiffness measurement ≥9.5Kpa. Dysfunction of at least two nerves, among which at least one belonged to lower extremities nerves, was deemed as DPN.

(Figure 1). Patients with DPN were older, heavier and taller. The number of diabetic courses was longer, and both fasting plasma glucose and glycated hemoglobin levels were higher. Additionally, both systolic and diastolic blood pressures were higher. Liver enzymes, kidney function, lipid patterns and inflammatory indicators were not significantly different between patients with and without DPN. The LSM of DPN patients also tended to be higher (interquartile range) (5.80 [4.70–7.70] vs 6.25 [4.80–8.58], $P = 0.09$; columns 8–10, Table 2).

Prevalence of DPN stratified by LSM and CAP

The prevalence of DPN was significantly elevated in patients with liver steatosis (55.7 vs 44.9%, $P = 0.03$, Figure 2c) and liver fibrosis (61.5 vs 50%, $P = 0.04$, Figure 2a). After grouping by quartile values, the prevalence of DPN was elevated as LSM increased (Figure 2b), but a positive relationship did not exist as the CAP increased (Figure 2d).

Associations of hepatic steatosis and fibrosis with the presence of DPN in patients with type 2 diabetes mellitus

The univariate logistic regression analysis showed that both hepatic steatosis (odds ratio 1.48, $P = 0.03$) and fibrosis (odds ratio 1.60, $P = 0.04$) were correlated with DPN. After adjusting for age, sex, weight, height, BMI, waist hip ratio, blood glucose, homeostatic model assessment of insulin resistance, blood pressure, inflammatory indicators and all other risk factors, only fibrosis remained associated with the presence of DPN (odds ratio 2.24, $P = 0.02$; Table 3).

DISCUSSION

In this large cohort of type 2 diabetes mellitus patients in the community sampled by cluster sampling, we found that liver fibrosis diagnosed by FibroTouch was associated with DPN diagnosed by NCS independently of BMI, plasma glucose, lipid profile, insulin resistance, blood pressure, serum liver enzymes, inflammatory factors and other DPN risk factors. The risk of DPN in patients with LSM ≥9.5 kPa was more than twice of that in patients with LSM <9.5 kPa. Meanwhile, liver steatosis was also correlated with DPN, but was not statistically significant after adjusting for BMI and other risk factors. In addition, we found a high prevalence of liver steatosis (63.0%), fibrosis (18.1%) and DPN (52.1%) in the type 2 diabetes mellitus population, which is consistent with previous epidemiological studies[21,22].

Although the relationship between NAFLD and diabetic microvascular complications, including diabetic kidney disease and retinopathy, has been researched comprehensively, just six currently available studies referred to DPN and drew contradictory conclusions. As shown in Table S3, five of the studies were unicentric cross-sectional studies from Italy, Australia, Korea, China and India[8-12], while the other one was a multicenter cross-sectional study from Italy[7]. Among them, Mantovani *et al.*[8] showed positive correlations between the prevalence of NAFLD diagnosed by ultrasonography and DPN assessed by the Michigan Neuropathy Screening Instrument method in adult outpatients with type 1 diabetes, but Vendhan *et al.*[11] reported no difference in the risk of neuropathy (evaluated by vibratory perception threshold) stratified by NAFLD in young outpatients with type 1 diabetes. Distinct ethnicities, ages and neuropathy diagnostic methods might have caused the inconsistency. Contradictions were also found in studies involving type 2 diabetes mellitus patients. Kim *et al.*[7] and Lombardi *et al.*[10] reported that both liver steatosis and fibrosis were not correlated with DPN in outpatients with type 2 diabetes mellitus. Another study from China claimed negative correlations between the prevalence of NAFLD and the duration of diabetes and DPN in inpatients with type 2 diabetes mellitus, which was not comprehensively discussed in that article. From our perspective, subjective bias of the diagnosis by ultrasound, the neuropathy symptoms and strict lifestyle interventions of patients with longer diabetic duration might be the explanations[12]. In contrast, Williams *et al.*[9] discovered a higher vibratory perception threshold associated with liver fibrosis due to NAFLD in inpatients with type 2 diabetes mellitus. In summary, currently, the effects of NAFLD on DPN have been elusive because of divergent diagnostic methods, ethnicity and single-centered origin of the recruited population. Thus, further research is necessary.

The present results are valuable, as the correlation of DPN and liver steatosis and fibrosis secondary to NAFLD in a large cohort using FibroTouch and NCS simultaneously have been evaluated for the first time. FibroTouch is a valid and sensitive

Table 2 | Basic clinical characteristics of population stratified by fat attenuation parameter, liver stiffness measurement and diabetic peripheral neuropathy

Characteristics	CAP <240 dB/m	CAP ≥240 dB/m	*P*	LSM <9.5 kPa	LSM ≥9.5 kPa	*P*	Non-DPN	DPN	*P*
Sex (M), *n* (%)	86 (45%)	140 (42.6%)	0.58	198 (46.5%)	28 (29.8%)	0.004*	86 (34.1%)	144 (52.4%)	**<0.001**
Age (years)	65.66 ± 6.58	65.12 ± 5.99	0.34	65.21 ± 6.36	65.80 ± 5.52	0.41	64.62 ± 6.22	65.94 ± 6.14	**0.02**
Weight (kg)	59.28 ± 8.77	67.69 ± 9.39	<0.001*	64.27 ± 9.97	66.57 ± 10.07	0.03*	62.82 ± 9.80	65.72 ± 10.16	**0.001**
Height (m)	1.62 ± 0.08	1.63 ± 0.08	0.20	1.63 ± 0.08	1.60 ± 0.07	0.002*	1.61 ± 0.08	1.63 ± 0.07	**0.008**
BMI (kg/m^2)	22.48 ± 2.39	25.56 ± 2.86	<0.001*	24.02 ± 2.81	26.24 ± 3.58	<0.001*	24.08 ± 2.92	24.75 ± 3.23	**0.01**
WHR	0.89 ± 0.06	0.92 ± 0.05	<0.001*	0.90 ± 0.05	0.93 ± 0.06	<0.001*	0.91 ± 0.05	0.91 ± 0.06	0.18
Duration of T2DM (years)	9.33 ± 6.24	7.67 ± 5.16	<0.001*	8.27 ± 5.65	8.18 ± 5.44	0.89	7.38 ± 5.29	9.10 ± 5.53	**<0.001**
HbA1c (%)	6.87 ± 1.32	7.06 ± 1.06	0.09	6.93 ± 1.16	7.27 ± 1.15	0.01*	6.79 ± 0.95	7.20 ± 1.37	**<0.001**
FBG (mmol/L)	7.20 ± 1.93	7.41 ± 2.14	0.28	7.25 ± 2.00	7.71 ± 2.32	0.06	7.10 ± 1.65	7.62 ± 2.53	**0.007**
Fasting insulin (pmol/L)	13.60 (7.83–22.88)	18.64 (11.00–28.88)	<0.001*	16.17 (8.61–24.83)	21.29 (12.26–34.33)	<0.001*	18.23 (10.24–27.04)	15.53 (8.46–26.46)	0.17
Fasting C-peptide (pg/mL)	135.63 (54.28–204.49)	171.36 (75.21–301.89)	<0.001*	147.90 (57.05–230.00)	225.04 (112.47–430.92)	<0.001*	173.17 (82.17–256.78)	142.02 (57.61–258.52)	0.14
HOMA-IR	0.60 (0.33–1.02)	0.85 (0.48–1.40)	<0.001*	0.71 (0.39–1.16)	1.04 (0.66–1.70)	<0.001*	0.84 (0.41–1.22)	0.72 (0.42–1.26)	0.59
Systolic pressure (mmHg)	128 ± 10	131 ± 7	<0.001*	130 ± 9	133 ± 8	0.001*	129 ± 8	132 ± 9	**<0.001**
Diastolic pressure (mmHg)	79 ± 5	81 ± 5	<0.001*	80 ± 5	82 ± 5	0.001*	80 ± 5	81 ± 5	**<0.001**
LDL (mmol/L)	2.29 ± 1.00	2.39 ± 1.02	0.29	2.37 ± 1.01	2.28 ± 1.01	0.47	2.41 ± 1.06	2.31 ± 0.96	0.25
HDL (mmol/L)	1.04 ± 0.46	0.94 ± 0.35	0.01*	1.00 ± 0.41	0.88 ± 0.34	0.006*	0.97 ± 0.40	0.98 ± 0.38	0.76
TC (mmol/L)	4.09 ± 1.29	4.35 ± 1.36	0.04*	4.25 ± 1.35	4.25 ± 1.33	0.99	4.32 ± 1.37	4.20 ± 1.30	0.36
TG (mmol/L)	1.25 (0.91–2.01)	1.78 (1.19–2.61)	<0.001*	1.56 (1.02–2.29)	1.82 (1.27–2.87)	0.002*	1.69 (1.09–2.44)	1.49 (1.01–2.30)	0.16
ALT (U/L)	8.00 (6.00–10.00)	9.00 (7.00–13.00)	<0.001*	9.00 (7.00–11.00)	11.00 (8.00–15.00)	<0.001*	9.00 (7.00–11.00)	9.00 (7.00–12.00)	0.52
AST (U/L)	15.00 (11.00–18.00)	17.00 (12.00–22.00)	<0.001*	15.00 (11.00–19.00)	17.50 (11.25–24.00)	0.03*	16.00 (11.00–20.00)	15.50 (12.00–20.00)	0.96
GGT (U/L)	17.00 (11.75–22.00)	22.00 (15.00–32.00)	<0.001*	19.00 (12.00–27.00)	23.00 (15.00–38.75)	0.006*	19.00 (13.00–26.50)	20.00 (13.00–29.00)	0.49
Creatinine (μmol/L)	64.63 ± 20.76	64.80 ± 20.85	0.93	65.06 ± 20.45	63.30 ± 22.33	0.47	63.20 ± 21.0	66.57 ± 20.7	0.08
Urea (mmol/L)	5.42 ± 1.64	5.30 ± 1.53	0.43	5.34 ± 1.60	5.38 ± 1.41	0.82	5.39 ± 1.62	5.33 ± 1.51	0.67
Uric acid (μmol/L)	258.93 ± 84.21	296.80 ± 94.23	<0.001*	279.19 ± 91.23	297.76 ± 96.28	0.09	283.8 ± 88.5	282.6 ± 95.5	0.89
hsCRP (ng/mL)	1.95 (0.82–4.41)	4.14 (1.97–7.52)	<0.001*	2.97 (1.29–6.03)	4.56 (2.14–8.26)	0.003*	3.29 (1.59–7.08)	3.20 (0.96–6.04)	0.12
TNF-α (pg/mL)	35.50 (19.96–123.52)	31.86 (18.85–89.96)	0.14	33.74 (19.46–96.17)	33.95 (18.83–111.96)	0.94	35.50 (20.36–116.22)	32.12 (18.82–73.21)	0.21
IL-6 (pg/mL)	10.77 (6.34–20.46)	10.63 (6.46–18.36)	0.72	10.72 (6.22–18.98)	10.51 (6.88–18.53)	0.39	9.42 (5.75–22.31)	10.47 (6.40–17.77)	0.92
LSM	5.30 (4.33–6.90)	6.60 (5.00–9.40)	<0.001*	5.50 (4.50–6.90)	12.30 (10.90–14.40)	<0.001*	5.80 (4.70–7.70)	6.25 (4.80–8.58)	0.09
CAP	217.85 ± 16.94	283.94 ± 32.92	<0.001*	252.24 ± 39.46	292.32 ± 40.50	<0.001*	259.18 ± 43.61	259.79 ± 41.57	0.87

Cohort size, *n* = 520. Continuous variables are expressed as the mean ± standard deviation for normal distributed variables, and the median (interquartile range) for skewed distributed data or as absolute and relative frequencies for categorical variables. *P*-values represent results of comparisons between two groups with the χ^2-test (for categorical variables), the unpaired Student's *t*-test and the Mann–Whitney test (for normally and not normally distributed continuous variables). *Statistically significant *P*-values. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, fat attenuation parameter; FBG, fasting blood glucose; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LDL, low density lipoprotein; LSM, liver stiffness measurement; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α; WHR, waist-to-hip ratio.

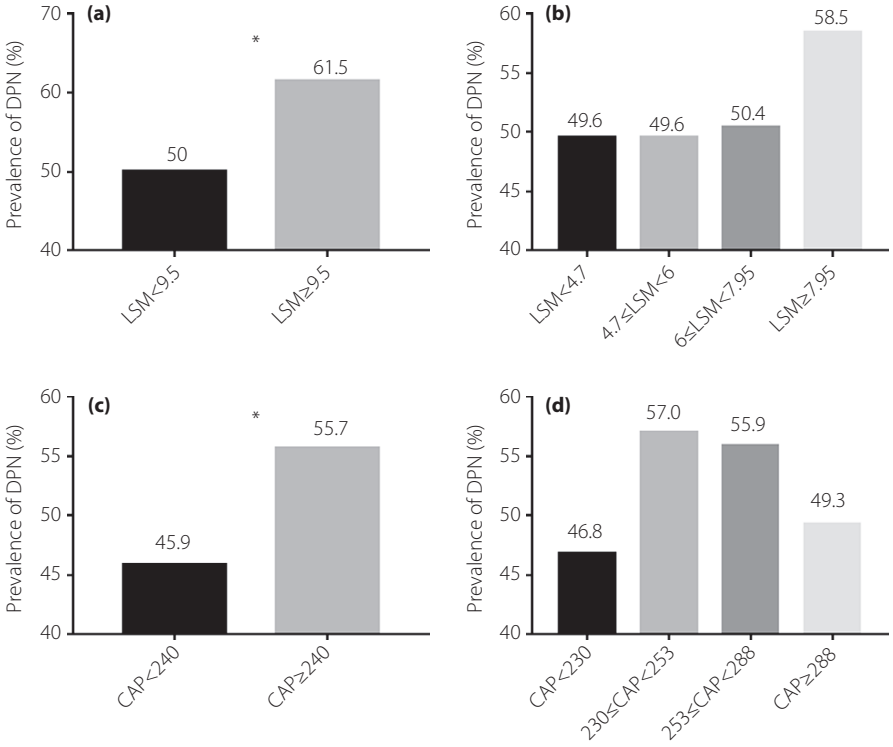

Figure 2 | Prevalence of diabetic peripheral neuropathy (DPN) stratified by liver stiffness measurement (LSM) and controlled attenuation parameter (CAP). Cohort number: 520 type 2 diabetes mellitus patients. Patients were grouped by cut-off values of (a) LSM and (c) CAP. Patients were grouped by quartile values of (b) LSM and (d) CAP. Dysfunction of at least two nerves, among which at least one belonged to lower extremities nerves, was deemed as DPN.

non-invasive approach to assess steatosis and fibrosis, similar to FibroScan[17,23]. According to previous studies, the use of FibroScan in the prediction of DPN was applied in just two studies in which the diagnosis of DPN was mainly dependent on symptoms rather than NCS. In the present study, thorough detection of nerves by NCS provided an objective evaluation of the extent of injury to large fiber nerves. Therefore, the correlation between liver fibrosis and DPN shown by the present results was credible and objective.

Pathways implicated in DPN include the hexosamine pathway, advanced glycation end-products accumulation, excess reactive oxygen species, inflammation and insulin resistance[24]. Patients with liver steatosis and fibrosis in the present study were more likely to have abdominal obesity and to be in an insulin-resistant state. Furthermore, blood pressure and plasma lipid patterns were worse. All the aforementioned factors are widely accepted risk factors for NAFLD and DPN[25]. In line with previous studies, elevated serum uric acid levels were observed in patients with liver steatosis. Uric acid is known to play an important role in metabolic diseases, including NAFLD, type 2 diabetes mellitus and DPN, by triggering inflammation, mitochondrial oxidative stress and insulin resistance[26]. Thus, it is likely that their common etiologies contribute to DPN and NAFLD. In contrast, liver fibrosis per se, which increases the risk of DPN regardless of confounding factors, might also be involved in DPN pathogenesis. Bile acid is an amphipathic steroid molecule synthesized from cholesterol in the liver. Studies over the past two decades have suggested that bile acid might function as a signaling molecule through a variety of receptors to regulate their own synthesis and other metabolic processes, such as glucose, lipid and energy homeostasis, and was implicated in the occurrence of liver fibrosis[27,28]. TGR5, one of the receptors of bile acid, is widely distributed and expressed in neurons[27], which might mediate its role in the pathogenesis of DPN through inflammation. Interacting with TGR5, bile acid would inhibit nuclear factor-κB activation[29,30]. Apart from DPN, liver fibrosis, but not steatosis, was independently associated with albuminuria[31], and regardless of the presence or severity of other histological features, fibrosis stage independently is the most relevant liver biopsy feature associated with overall and liver-related mortality or liver transplantation[32]. Furthermore, inhibiting triglyceride synthesis improves hepatic steatosis, but exacerbates liver damage and fibrosis. In other words, triglyceride synthesis actually helps to protect

Table 3 | Logistic regression analyses-association between liver steatosis or fibrosis with the presence of diabetic peripheral neuropathy in type 2 diabetes mellitus patients

	Liver steatosis		Liver fibrosis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Mode 1	1.48 (1.04–2.11)	0.03*	1.60 (1.02–2.51)	0.04*
Mode 2	1.63 (1.13–2.36)	0.01*	1.95 (1.21–3.12)	0.006*
Mode 3	1.40 (0.92–2.14)	0.12	1.75 (1.07–2.88)	0.03*
Mode 4	1.47 (0.88–2.47)	0.15	2.26 (1.22–4.18)	0.01*
Mode 5	1.59 (0.94–2.69)	0.09	2.41 (1.29–4.50)	0.006*
Mode 6	1.59 (0.91–2.77)	0.10	2.34 (1.23–4.46)	0.01*
Mode 7	1.56 (0.89–2.74)	0.12	2.18 (1.13–4.22)	0.02*
Mode 8	1.78 (0.97–3.25)	0.06	2.24 (1.11–4.53)	0.02*

*Statistically significant *P*-values. Mode 1: liver steatosis or fibrosis per se; mode 2: on the basis of mode 1, adjusting age and sex; mode 3: on the basis of mode 2, adjusting weight, height, body mass index and waist-to-hip ratio; mode 4: on the basis of mode 3, adjusting duration of type 2 diabetes mellitus, glycated hemoglobin , fasting blood glucose, fasting insulin, fasting c peptide and homeostatic model assessment of insulin resistance; mode 5: on the basis of mode 4, adjusting low-density lipoprotein, high-density lipoprotein, triglyceride and total cholesterol; mode 6: on the basis of mode 5, adjusting alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, urea, uric acid and creatinine; mode 7: on the basis of mode 6, adjusting systolic and diastolic blood pressure; mode 8: on the basis of mode 7, adjusting inflammatory factors including high-sensitivity C-reactive protein, tumor necrosis factor-α and interleukin-6.

hepatocytes from lipotoxicity by buffering the accumulation of free fatty acids[33]. However, the mechanism by which only fibrosis is independently associated with DPN remains obscure.

Currently, the most effective treatments for DPN are glucose control and pain management[34]. However, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) randomized trial showed that intensive glucose control did not reduce the risk of advanced measures of microvascular outcomes, and caused higher mortality, although it delayed the onset of neuropathy[35]. Thus, the relationship between liver fibrosis and DPN provides new insights into the etiology and early diagnosis of DPN, and the development of a potential therapy.

However, the present study had several limitations. Due to the cross-sectional design of the study, the causal relationship between NAFLD and DNP cannot be determined, but the preliminary correlation of NAFLD and the presence of DPN in type 2 diabetes mellitus individuals is still significant. Thus, future prospective studies and the underlying mechanism of the correlation between DPN and fibrosis requires further analysis. Furthermore, all recruited patients were from communities in Shanghai, China; therefore, generalization to patients with other ethnicities is inappropriate.

In conclusion, we found that the prevalence of DPN was higher in patients with liver steatosis and fibrosis secondary to NAFLD in the type 2 diabetes mellitus population from communities. Additionally, liver fibrosis is an independent risk factor for the presence of DPN after adjusting for all other confounding indicators.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Cut-off values for parameters of nerve conduction studies.
Table S2 | Sites for stimulation and recording of different nerves.
Table S3 | Summaries of published studies about the correlation between non-alcoholic fatty liver disease and diabetic peripheral neuropathy.