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ORIGINAL ARTICLE

Risk factors for non-alcoholic fatty liver disease-associated hepatic fibrosis in type 2 diabetes patients

Asieh Mansour[1] · Mohammad Reza Mohajeri-Tehrani[2] · Majid Samadi[3] · Hadis Gerami[4] · Mostafa Qorbani[5,6] · Nick Bellissimo[7] · Hossein Poustchi[8] · Azita Hekmatdoost[1]

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Abstract

Aims In patients with type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) and liver fibrosis is frequent and presumably associated with increased cardiovascular disease risk and mortality. The objective was to investigate risk factors associated with hepatic fibrosis in patients with type 2 diabetes and NAFLD to provide a basis for the prevention and treatment.

Methods Liver stiffness measurements (LSM) expressed in kilopascals (kPa) and controlled attenuation parameter (CAP) expressed in dB/m were diagnosed by transient elastography. Hepatic steatosis and significant fibrosis were defined as having a CAP score ≥ 260 dB/m and an LSM score ≥ 8 kPa, respectively. Associations between fibrosis categories with anthropometric and metabolic variables were determined; then, variables with statistical significance in the univariate analysis were included in multivariate model.

Results A total of 108 participant with type 2 diabetes and NAFLD (mean age: 44.69 ± 5.57 years; mean duration of diabetes 4.68 ± 4.24 years) were recruited. In these patients, body mass index, obesity, fat mass, waist circumferences, resting energy expenditure, CAP score, fasting insulin, C-peptide, HbA1C, hs-CRP as well as liver enzymes and systolic blood pressure and diastolic blood pressure were positively associated with fibrosis (all $p < 0.05$). Using multivariate logistic regression, serum aspartate aminotransferase (OR 1.12; 95% CI 1.06–1.19), waist circumferences (odds ratio [OR] 1.15; 95% CI 1.05–1.25) and C-peptide (OR 3.81; 95% CI 1.5–9.7) remained as independently associated with liver fibrosis.

Conclusion For participants with type 2 diabetes with coexisting NAFLD, stratification by independent risk factors for fibrosis could have important prognostic value.

Keywords Hepatic fibrosis · Type 2 diabetes · NAFLD · Risk factors · Liver enzymes

Managed by Massimo Federici.

✉ Azita Hekmatdoost
a_hekmat2000@yahoo.com

[1] Department of Clinical Nutrition and Dietetics, Faculty of Nutrition and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, 46, West Arghavan St., Farahzadi Blvd., Shahrak Gharb, Tehran, Iran

[2] Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

[3] Radiology Department, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

[4] Obesity and Eating Habits Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

[5] Non-communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran

[6] Chronic Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran

[7] School of Nutrition, Faculty of Community Services, Ryerson University, Toronto, Canada

[8] Liver and Pancreatobiliary Diseases Research Center, Digestive Diseases Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Introduction

Concurrent type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD) are the most important causes of morbidity and mortality due to higher risk of developing progressive liver damage, and higher incidence of cardiovascular complications [1, 2]. Several meta-analyses have shown that NAFLD is significantly associated with an increased risk of incident diabetes [3–5]. Likewise, recent evidence suggests that patients with T2DM are at the higher risk of developing liver fibrosis and its prevalence is currently increasing (17–37%) compared to general population (2.8–4%) [6]. Moreover, a 6-year-follow-up study showed that liver fibrosis increased the risk of T2DM by fourfold [7] suggesting the direct role of fibrosis in the pathogenesis of T2DM [8]. An increasing number of papers have been published on the association between metabolic syndrome, obesity and liver chronic disease with hepatic fibrosis [9–12]. Despite the recognized association between T2DM and liver fibrosis [13], few studies are available on predictive factors associated with liver fibrosis in T2DM patients [14, 15]. Liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT)) were among possible relevant predictors of liver disease [16]. However, it has emerged that NAFLD patients with advanced fibrosis may have normal ranges of serum liver enzymes levels [17]. Thus, the risk stratification of liver fibrosis to identify patients at risk and the prevention of progression to liver cirrhosis are important challenges in T2DM patients with NAFLD.

Methods

Study population

Between September 2017 and March 2018, we reviewed a total of 13,807 medical records of patients with T2DM from the diabetes clinics in the Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences (Tehran, Iran), where patients with diabetes undergoing annual or biannual comprehensive health examinations include treatment goals and acute and/or chronic diabetes-related complications. From a total of 790 subjects who invited to participate, 248 subjects underwent abdominal ultrasonography for possible diagnosis of fatty liver, 119 patients had hepatic steatosis of grade 2 higher, and were examined by transient elastography. Finally, a total of 108 individuals met the inclusion criteria and were included in this analysis (Fig. 1). The investigation was performed in accordance with the Helsinki Declaration and was approved by the Institutional Review (approval number: 1396-028). Written informed consent was taken from all study participants.

Inclusion criteria for the study were patients diagnosed with T2DM based on 1999 World Health Organization (WHO) criteria (fasting blood glucose ≥ 7.0 mmol/L, a 2-h post-load glucose concentration of ≥ 11.1 mmol/L, or individuals with a previous diagnosis (current therapy with hypoglycemic agents)), no current therapy with insulin, controlled attenuation parameter (CAP) score ≥ 260, 30–53 years of age, no known acute or chronic disease except for obesity and hypertension, no pregnancy or lactation, no history of current or past excessive alcohol drinking as defined by an average daily consumption of alcohol > 20 g, negative tests for the presence of viral hepatitis (hepatitis B surface antigen or serum hepatitis C antibody), absence of history and clinical, biochemical, and ultrasound findings consistent with cirrhosis and other chronic liver diseases, no current supplementation with vitamin E and other antioxidants, no history of thyroid dysfunction, cardiovascular disease, renal dysfunction, cancer, and mental disease.

Ultrasonography assessment

Hepatic ultrasonic examination for the diagnosis of fatty liver and gallbladder disease was performed after 8-h overnight fasting by an experienced radiologist who was trained extensively in liver ultrasonography, using a commercially available ultrasound scanner (Siemens Accuson S2000 ultrasound system Germany) equipped with a 2.0–5.0 MHz convex probe. The severity of fatty liver was categorized into four grades: the absence of fatty liver, grade 1 (mild), grade 2 (moderate), or grade 3 (severe) fatty liver based on the findings of bright liver, stronger echoes in the liver than in the kidney, the blurring of blood vessels structure, and deep attenuation of ultrasound signal [18].

Transient elastography

Transient elastography was used to assess severity of steatosis and fibrosis in patients if they were diagnosed with grade 2 or higher by ultrasonography when gallbladder diseases were also ruled out. Liver stiffness measurements (LSM) expressed in kilopascals (kPa), and CAP expressed in dB/m were performed by experienced investigator on patients using the FibroScan® 502 instrument (EchoSense, Paris, France, 5 MHz) after at least 3 h of fasting with either an M probe or XL probe. Examinations with ten successful shots were performed for each patient and the median value was recorded. In the present study, the cutoff for significant steatosis was set as equal or greater than 260 dB/m [19]. Based on these severity of

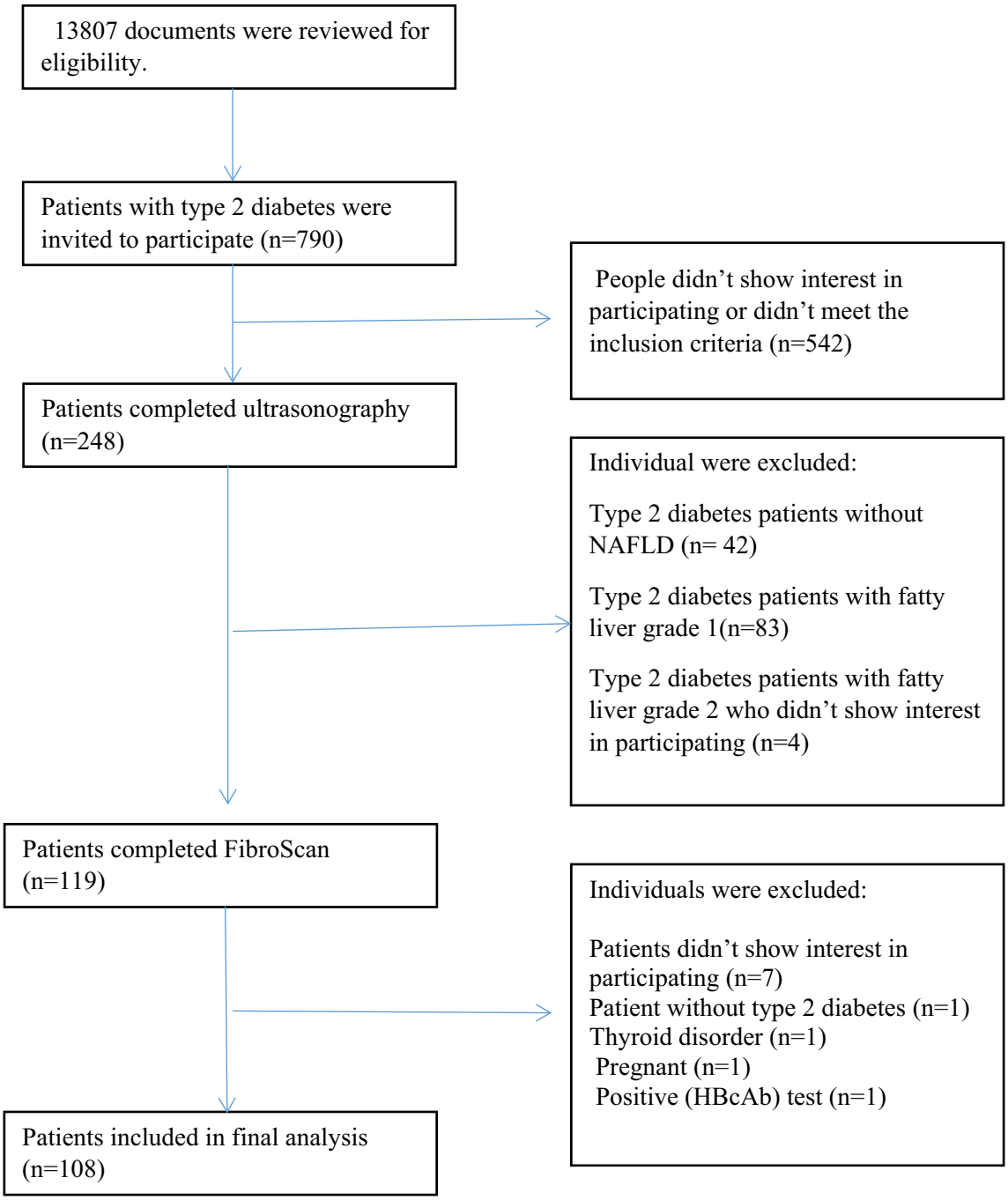

Fig. 1 Recruitment flow of the study participants

fibrosis as detected by transient elastography, the patients were divided into two groups: patients with a CAP score ≥ 260 dB/m but an LSM score < 8 kPa were considered to have only advanced steatosis but no significant fibrosis; patients who had a CAP score ≥ 260 dB/m and an LSM score ≥ 8 kPa were considered to have advanced steatosis and progressed to liver fibrosis.

Clinical and laboratory data

A standard questionnaire was administered to obtain the information on demographic, characteristics, lifestyles, history of diseases and medication usage with face-to-face interviews by trained investigators. Participants' height was obtained without shoes to the nearest 0.1 cm. Weight to

the nearest 0.1 kg and body composition were measured in light clothes while barefoot using Tanita scale (BC 418 MA Segmental Body Composition Analyzer, Tanita, Japan). Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Subjects with a BMI ≥ 30 were classified as obese. Waist circumference (WC) was measured at the midpoint between the lower costal margin and the iliac crest. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained on the left arm, while subjects were in a seated position with a standard calibrated mercury sphygmomanometer and an appropriately sized cuff, after at least 10-min rest. Patients were considered hypertensive based on systolic blood pressure ≥ 130 mm Hg and/or diastolic blood pressure ≥ 85 mm Hg or use of antihypertensive drugs [20]. Regular cigarette smoking in the past 6 months was defined as current smoker. Venous blood was drawn in the morning after an overnight fast. Serum liver enzymes (ALT, AST, and GGT), lipids, creatinine and high-sensitive C-reactive protein (hs-CRP) measured by using ELISA kit (Roche, Germany). LDL cholesterol was calculated using the Friedewald equation. Hemoglobin A1c (HbA1c) levels were measured by a high-performance liquid chromatography analyzer (Tosoh, Tokyo, Japan). Fasting blood glucose concentrations were measured using the glucose oxidase method on an autoanalyzer (Cobas c 311, Roche Dignostics, Risch-Rotkreuz, Switzerland). Serum insulin, C-peptide and thyroid-stimulating hormone (TSH) levels were measured by using ELISA kit (Monobind Inc. Lake Forest, California, USA). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) score was calculated using the formula: HOMA-IR = [Fasting insulin (μU/mL) × Fasting glucose (mmol/L)/22.5].

Statistical analysis

Statistical analysis was performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive values are expressed as mean ± standard deviation (SD) for continuous variables or percentages for categorical variables. Differences between groups were tested for significance by the independent samples *t* test for variables with normal distribution and Mann–Whitney test for variables without normal distribution (for continuous variables) or Chi-squared test (for categorical variables). Correlation analysis between liver fibrosis with different metabolic and anthropometric indices was assessed using Pearson for continues variable with normal distribution and also using Spearman's for continuous variables that were not normally distributed. The normality of variables was tested by Kolmogorov–Smirnov. The association of anthropometric and metabolic variables with liver fibrosis was investigated through univariate analyses. Then, variables with statistical significance in the univariate analysis were included in multivariate model. Statistical significance was set at $p < 0.05$.

Result

The baseline study population characteristics are summarized in Table 1. A total of 108 participants including 75 men and 33 women were recruited, and the participants' mean age was 44.69 ± 5.57 years and the mean duration of diabetes was 4.68 ± 4.24 years.

LSM measurements in the patients with elevated CAP

Table 1 presents the demographics and clinical characteristics of the participants with CAP ≥ 260 dB/m stratified by liver fibrosis (LSM < 8 kPa vs. LSM ≥ 8 kPa). There were no significant differences in age between two groups ($p < 0.05$). Compared to the subjects without fibrosis, those with significant fibrosis had higher BMI, fat mass, higher values of waist circumferences, REE, CAPscore, insulin, C-peptide, and HbA1C value as well as higher levels of liver enzymes (AST, ALT, and GGT) and also higher hs-CRP levels, systolic and diastolic blood pressure. Moreover, data indicate that patients with significant fibrosis were more likely to be obese (76.92% vs. 47.56%; $p = 0.009$) compared to those without significant fibrosis.

Correlation between liver fibrosis and different variables

Liver fibrosis was significantly correlated with BMI ($r = 0.365$, $p = <0.001$), waist ($r = 0.413$, $p < 0.001$), fat mass ($r = 0.289$, $p = 0.003$), systolic blood pressure ($r = 0.256$, $p = 0.008$), CAP score ($r = 0.301$, $p = 0.002$), fasting insulin ($r = 0.22$, $p = 0.02$), C-peptide ($r = 0.509$, $p = <0.001$), FBS ($r = 0.201$, $p = 0.03$), HDL ($r = -0.215$, $p = 0.02$), AST ($r = 0.518$, $p < 0.001$), GGT ($r = 0.242$, $p = 0.01$), and HOMA-IR score ($r = 0.279$, $p = 0.003$) (Table 2).

Logistic regression analyses of factors affecting LSM

In multivariable logistic regression analysis, among anthropometric variables, only waist circumferences were independently associated with hepatic fibrosis (odds ratio [OR] 1.15; 95% CI 1.05–1.25), and among the laboratory parameters, C-peptide (OR 3.81; 95% CI 1.5–9.7), AST (OR 1.12; 95% CI 1.06–1.19) were also independently associated with liver fibrosis in patients with type 2 diabetes and NAFLD, as shown in Table 3.

Table 1 Demographic, anthropometric, and biochemical characteristics of patients with CAP score ≥ 260 dB/m, according LSM categories

Characteristics	Total ($n=108$)	Liver steatosis but no significant liver fibrosis ($n=82$)	Significant liver fibrosis ($n=26$)	p value
Age (years)[a]	44.69 ± 5.57	44.13 ± 5.71	46.46 ± 4.75	0.06
Gender male *n* (%)[c]	75 (69.4)	54 (65.85)	21 (80.77)	0.15
Smoking, current *n* (%)[c]	24 (22.2)	18 (21.95)	6 (23.08)	0.9
Diabetes duration (years)[a]	4.68 ± 4.24	4.64 ± 4.21	4.82 ± 4.43	0.86
BMI (kg/m^2)[a]	30.72 ± 3.82	30.03 ± 3.35	32.89 ± 4.44	< 0.001
Obesity, *n* (%)[c]	59 (54.63)	39 (47.56)	20 (76.92)	0.009
Fat mass (kg)[a]	27.23 ± 8.52	25.92 ± 7.99	31.33 ± 8.95	0.004
FFM (kg)[a]	62.2 ± 10.87	61.07 ± 10.18	65.7 ± 12.36	0.05
Waist circumference (cm)[a]	108.07 ± 9.43	105.83 ± 8.23	114.94 ± 9.69	< 0.001
REE (kcal)[a]	1828.86 ± 354.40	1791.729 ± 353.56	2009.6 ± 308.98	0.02
CAP score (dB/m)[a]	317.11 ± 36.70	310.49 ± 35.60	338 ± 32.53	< 0.001
Fasting blood sugar (mg/dL)[a]	152.46 ± 51.95	150.69 ± 53.43	158.04 ± 47.53	0.53
Fasting insulin (uIU/mL)[b]	11.53 ± 4.81	11.03 ± 3.89	13.14 ± 6.81	< 0.001
C-peptide (ng/mL)[a]	1.86 ± 0.77	1.69 ± 0.63	2.4 ± 0.91	< 0.001
HOMA-IR score[a]	4.27 ± 2.23	4.05 ± 1.99	4.96 ± 2.8	0.07
HbA1c (%)[a]	8.33 ± 3.95	7.90 ± 1.75	9.68 ± 7.38	0.04
AST (U/L)[b]	26.19 ± 10.70	23.17 ± 8.36	35.73 ± 11.80	< 0.001
ALT (U/L)[b]	22.55 ± 10.66	20.36 ± 8.46	29.42 ± 13.79	0.001
GGT (U/L)[b]	42.16 ± 32.31	35.59 ± 16.46	63 ± 54.75	0.001
AST/ALT[b]	1.26 ± 0.5	1.21 ± 0.34	1.41 ± 0.81	0.39
hs-CRP (mg/L)[b]	3.79 ± 5.99	3.09 ± 2.87	6.07 ± 11.10	0.04
Total cholesterol (mg/dL)[a]	166.29 ± 36.95	165.21 ± 37.81	169.73 ± 34.59	0.59
HDL-cholesterol (mg/dL)[a]	35.08 ± 8.97	35.76 ± 9.40	32.96 ± 7.17	0.16
LDL cholesterol (mg/dL)[a]	89.93 ± 30.7	88.33 ± 31.85	94.92 ± 26.77	0.34
Triglycerides (mg/dL)[b]	212.51 ± 193.96	214.71 ± 217.04	205.54 ± 91.23	0.35
Cr (mg/dL)[a]	0.93 ± 0.18	0.91 ± 0.17	0.98 ± 0.2	0.07
TSH (uIU/mL)[b]	2.1 ± 1.37	1.98 ± 1.31	2.50 ± 1.5	0.06
Systolic blood pressure (mmHg)[a]	124.21 ± 15.72	121.76 ± 13.82	131.92 ± 18.92	0.003
Diastolic blood pressure (mmHg)[a]	85.18 ± 11	83.78 ± 10.90	89.61 ± 10.29	0.01
Hypertension, *n* (%)[c]	66 (61.11)	46 (56.1)	20 (76.92)	0.05
Physical activity (METs h/day)[a]	31.48 ± 4.70	31.69 ± 4.76	30.79 ± 4.54	0.4
Use of oral anti-diabetic drugs (%)[c]				
Metformin (%)	94 (87)	71 (86.6)		0.8
DPP4 inhibitors (%)	30 (27.8)	23 (28)	7 (26.9)	0.9
Sulfonylureas (%)	37 (34.3)	24 (29.3)	13 (50)	0.05
Use of lipid-regulating drugs (%)				
Statin (%)[c]	40 (37)	33 (40.3)	7 (26.9)	0.22
Use of antihypertensive drugs (%)[c]				
Angiotensin II receptor blockers (%)	16 (14.8)	12 (14.6)	4 (15.4)	0.92
Beta blockers (%)	10 (9.3)	7 (8.5)	3 (11.5)	0.64

Data are presented as mean ± SD or number (%). Hypertension was defined as systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or use of antihypertensive agent. Obesity was defined as BMI ≥ 30 kg/m^2

[a]Value for independent sample *t* test

[b]Value for Mann–Whitney test

[c]Value for Chi-squared test

BMI body mass index, *FFM* fat free mass, *REE* resting energy expenditure, *CAP* controlled attenuation parameter score, *HOMA-IR* homeostasis model assessment insulin resistance, *HbA1c* hemoglobinA1c, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *hs-CRP* high-sensitivity C-reactive protein, *Cr* creatinine, *TSH* thyroid-stimulating hormone, *METs* metabolic equivalents

Table 2 Correlation coefficient of fibrosis (kPa) with anthropometric and clinical characteristics in type 2 diabetes patients with NAFLD

Characteristics	Fibroscore	*p* value
BMI (kg/m^2)[a]	0.365	< 0.001
Obesity, *n* (%)[a]	0.252	0.008
Fat mass (kg)[a]	0.289	0.003
FFM (kg)[a]	0.143	0.143
Waist (cm)[a]	0.413	< 0.001
Systolic blood pressure (mmHg)[a]	0.256	0.008
Diastolic blood pressure (mmHg)[a]	0.125	0.19
Hypertension, *n* (%)[a]	0.183	0.05
REE (kcal)[a]	0.179	0.09
CAP score (dB/m)[a]	0.301	0.002
Fasting insulin (uIU/mL)[b]	0.22	0.02
C-peptide (ng/mL)[a]	0.509	< 0.001
FBS (mg/dL)[a]	0.201	0.03
HOMA-IR score[a]	0.173	0.07
HBA1c[a]	0.127	0.19
Cr (mg/dL)[a]	0.093	0.33
TG (mg/dL)[b]	0.175	0.07
Cholesterol (mg/dL)[a]	0.111	0.25
HDL (mg/dL)[a]	− 0.215	0.02
LDL (mg/dL)[a]	0.103	0.29
AST (U/L)[b]	0.518	< 0.001
ALT (U/L)[b]	0.331	0.290
GGT (U/L)[b]	0.242	0.01
AST/ALT[b]	0.062	0.52
hs-CRP (mg/L)[b]	0.150	0.12
TSH[b]	0.121	0.21
Physical activity (METs h/day)[a]	− 0.137	0.15
HOMA-IR score[a]	0.279	0.003
Diabetes duration[a]	0.08	0.4

Hypertension was defined as systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or use of antihypertensive agent. Obesity was defined as BMI ≥ 30 kg/m^2

[a]Value for Pearson

[b]Value for Spearman

BMI body mass index, *FEM* fat free mass, *TBW* total body water, *REE* resting energy expenditure, *CAP* controlled attenuation parameter Score, *HOMA-IR* homeostasis model assessment insulin resistance, *HbA1c* hemoglobinA1c, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *hs-CRP* hs-CRP (mg/L): high-sensitivity C-reactive protein, *Cr* Creatinine, *TSH* thyroid-stimulating hormone, *METs* metabolic equivalents

Discussion

To our knowledge, this is the first study that evaluated the predictive risk factors of hepatic fibrosis in patients with T2DM and NAFLD. Our results have shown that BMI, fat mass, waist circumferences, obesity, REE, systolic blood pressure and diastolic blood pressure, fasting insulin, C-peptide, HbA1C as well as liver enzymes and CAP score were positively associated with significant fibrosis; however, after adjusting for confounding variables, association between hepatic fibrosis with only AST, C-peptide, and waist circumference remained significant.

Table 3 Multivariate analysis for factors associated with liver fibrosis (LSM ≥ 8.0 kPa) in type 2 diabetes patients with NAFLD (sample size *n* = 108)

	OR	95% CI	*p* value
C-peptide (ng/mL)	3.81	1.5–9.7	0.005
Waist circumference (cm)	1.15	1.05–1.25	0.001
AST (U/L)	1.12	1.06–1.19	< 0.001

AST aspartate aminotransferase, *OR* odds ratio, *CI* confidence interval

Previous studies have reported that insulin resistance may play an important role in fibrosis progression [13, 21]. It has been shown that pancreatic islet hypertrophy is more frequent in patients with cirrhosis; it was supposed that an adaptive response of pancreatic beta cells to increased insulin resistance lead to augmented insulin accumulated in blood circulation [22]. A study by Paradis et al. [23] has highlighted an association between exposure to insulin and glucose with liver fibrosis progression in non-alcoholic steatohepatitis (NASH) patients through the overexpression of connective tissue growth factor (CTGF). Moreover, hyperinsulinemia stimulates hepatocytes to secret extra matrix, which results in progression of hepatic fibrogenesis [13]. Leighton et al. reported that high C-peptide levels were associated with hepatic fibrosis. Both insulin and connecting 31-amino acid peptide (C-peptide) are produced and released in equal amounts from the pancreatic beta cell secretory granules; C-peptide can therefore be used to assess endogenous insulin secretion [24]. The serum levels of C-peptide and insulin are determined, in principle, by the rate and mechanism of their elimination [25]. The degradation rate of C-peptide is threefold lower than insulin [26], and unlike insulin, C-peptide is not extracted by the liver and has a peripheral clearance at a constant rate [27]. In the present study, C-peptide levels were significantly correlated with fibrosis, whereas insulin concentrations were not. To take into account that the C-peptide levels area more reliable indicator of insulin secretion rather than insulin itself [27], our observation suggests that insulin secretion is one of the key metabolic pathways for liver damage in T2M patients independently of its serum levels. These observations indicate that insulin hypersecretion, and not impaired degradation, accounts for the increase in NAFLD-related fibrosis in T2DM. Although decreased hepatic insulin extraction in patients with NAFLD and T2DM has long been recognized [28–30], in a large cohort of NAFLD and biopsy confirmed NASH patients, Bril et al. [30] have determined that reduced hepatic whole body insulin clearance may be associated with

excess deposition of fat in the liver, and it is not a reliable parameter for fibrosis estimation in patients with NAFLD. This implies that hyperinsulinemia and impaired insulin clearance observed in cirrhosis may not be related to the presence of hepatic fibrosis itself [24], and it is the consequence of increased pancreatic beta cells responds to blood glucose, by contrast hepatic insulin extraction does not seem to play a direct role in the progression of fibrosis [31]. Previous studies demonstrated that hepatic fat accumulation effect on the ability of insulin to suppress endogenous glucose production [32, 33], thereby increase the requirement of insulin [34]. Weather-evolved hepatic fibrosis level may influence increase in requirements of insulin in diabetic patients via an effect on suppression of endogenous glucose production by insulin are still remained to be illustrated.

We observed that increased insulin secretion (higher C-peptide), but not insulin resistance (higher HOMA-IR) would be injurious specifically to the liver cells and promote fibrosis development. This finding is in line with previous study that had shown that glucose effectiveness (ability of glucose to enhance its own disposal and suppress hepatic glucose production independent of insulin) decreases as the metabolic score increases in obese subjects [35].

Our data demonstrated that abdominal obesity is a stronger predictor of liver fibrosis rather than generalized obesity among men and women aged 30–53 years who have T2DM and NAFLD. These results concur with previous studies in children and adolescents [36, 37] and adult [38], suggesting that once NAFLD is present, waist circumference is one of the major determinant of liver injury severity, occurring independent of the degree of liver steatosis, insulin resistance, and age [38]. However, one previous study demonstrated the association between waist circumference and severity of steatosis, while it was not correlated with hepatic fibrosis, demonstrating visceral fat not to be directly associated with liver damage. Although the exact mechanisms by which central obesity exerts its damaging effects on liver remain controversial, a number of plausible explanations have been proposed. The waist circumferences is often used as a surrogate marker of visceral fat, and visceral fat compared with subcutaneous fat is considered an independent predictor of hepatic steatosis and is associated with liver fibrosis [37, 39]. Under condition of increased amount of visceral adipose tissue, release of toxic-free fatty acid increases in the blood, resulting in hepatic lipid deposition [36]. Moreover, visceral fat could lead to increased inflammatory cytokines and adipokines production including interleukin 6 (IL-6), which play a pivotal role in liver damage progression [38].

Another factor associated with liver fibrosis was AST, which are found in the circulation in response to hepatocyte injury or death [40], and has also been linked to diabetes [41, 42]. Our study confirmed that the increases in serum AST level, not the ratio of AST to ALT, are associated with significant liver fibrosis. According to a study by Williams and Hoofnagle [43], AST/ALT greater than 1 was a good indicator of the presence of cirrhosis in patients with NAFLD. Moreover, Park et al. concluded that the ratio ≥ 1 appears to reflect the fibrosis grades [44]. However, in our study, the increase in AST/ALT was only moderate in the fibrotic group compared with the non-fibrotic group. Thus, it did not allow us to estimate its relation with fibrosis progression. Our finding supports the study by Teshale et al. [45] that AST/ALT ratio was not a reliable indicator for the detection of fibrosis. Serum levels of aminotransferases are typically different due to the differences in the rate of their release into the circulation and the rate of removal from the circulation [44]. ALT is found in the cytoplasm, whereas AST is found in mitochondria and cytoplasm. Clearance of these enzymes from circulation may occur at different rates and through different mechanisms. The half-life in the circulation of ALT is approximately 47 h, approximately 17 h for total AST and, on average, the mitochondrial AST has a much longer circulating half-life (about 87 h) [46]. The major organ responsible for AST clearance is the liver, which removes AST by sinusoidal cells [46]. The AST levels exceed the ALT levels which could be due to increasing degree of fibrosis leading to mitochondrial structural damage that reflects leakage of mitochondrial AST into plasma [44], and/or the reduction in AST clearance by sinusoidal cells [43]. In the chronic hepatocellular injury, serum ALT is more commonly increased than AST; however, as more severe hepatocellular damages occur, leading to ALT activity typically declined, and AST will be higher than ALT after the development of cirrhosis (advanced stage of fibrosis) [47]. However, normal values of liver function enzymes do not rule out the possibility of a significant stage of fibrosis [15, 17]. Although the significant correlation was noted between AST and liver fibrosis, this association was present despite mean values remained within normal range indicating that liver function tests may not be a useful marker in assessment of fibrosis risk in T2DM patients who may have NAFLD [15].

This study has several strengths. This is the first study to show the relationship between anthropometric or metabolic parameters and liver fibrosis in patients with T2DM and NAFLD using one of noninvasive tests. In addition, the population consisted of T2DM patient newly diagnosed with NAFLD and without use of any drugs for treatment of NAFLD. Nevertheless, some important limitations of this study should be mentioned. First, the key limitation of our study is the observational nature, which precludes interpretations of causality observed in our study. Second, the number of cases in our study was not large, leading to a small number of subjects in the group with LSM ≥ 8. Third, the influence of travel distance from screening site was the main reason of potential participant refusal to participate in the

study that may lead to selection bias. Moreover, it warrants consideration that our analysis was limited to participants without insulin injection, whereas it has been suggested that insulin therapy is associated with hepatocellular cancer risk in patients with T2DM [48]. In addition, Because of restriction of inclusion criteria to patients middle aged and who without micro- and macro-diabetic complications, the generalizability of the results of this study to other diabetic population is questionable.

Conclusion

In conclusion, our results indicate that T2DM patients with elevated visceral fat, serum AST and/or high C-peptide levels are at risk for developing significant liver fibrosis. This association exists even when hepatic enzymes are within normal limits. Thus, hepatic enzyme assessments may not be a suitable screening method for diagnosis of hepatic fibrosis in these patients. It is suggested that T2DM patients with abdominal obesity and elevated serum C-peptide, and AST should be considered for assessment of hepatic fibrosis by transient elastography and/or other easily accessible metric such as ZJU index [49] or liver biopsy depending on local practice and guidelines.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures were in accordance with the ethical standards of the institutional research committee (Ethics Committee of the National Institute for Medical Research Development and National Nutrition and Food Technology Research Institute) and with the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

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