


ORIGINAL ARTICLE

Prevalence of clinically relevant liver fibrosis due to nonalcoholic fatty liver disease in Indian individuals with type 2 diabetes

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Key words

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Abstract

Background and Aim: Type 2 diabetes (T2D) is associated with higher prevalence and worse outcomes of nonalcoholic fatty liver disease (NAFLD). However, data regarding the prevalence of clinically relevant liver fibrosis (CRLF) in Indian individuals with T2D are scarce. We investigated the prevalence of, and factors associated with, CRLF in Indians with T2D.

Methods: We conducted a prospective study of 601 consecutive adults with T2D. Steatosis was diagnosed using ultrasonography. Liver stiffness measurement (LSM) by transient elastography of ≥ 8.0 kPa was taken as cutoff suggesting CRLF. Individuals with LSM > 13.0 kPa underwent dynamic magnetic resonance imaging (MRI) of liver for detecting changes consistent with cirrhosis.

Results: The prevalence of steatosis was 84.2%. Higher body mass index (BMI, $P = 0.022$), alanine aminotransferase (ALT; $P = 0.001$), and lower high-density lipoprotein (HDL; $P = 0.002$) were independent factors associated with steatosis. The prevalence of CRLF was 28.2%. Higher BMI ($P = 0.001$), aspartate aminotransferase (AST; $P < 0.0001$), gamma-glutamyl transpeptidase (GGT; $P < 0.0001$), and concomitant hypertension ($P = 0.03$) were independent factors associated with CRLF. Elevated ALT and AST (≥ 40 units/L) levels were present in 70.6 and 51.6% individuals with CRLF, respectively. Thirty-one (7.2%) individuals had LSM > 13.0 kPa. Among them, 25 individuals underwent dynamic MRI of liver, which revealed features consistent with cirrhosis in 18 patients.

Conclusion: CRLF, an established risk factor for cirrhosis and overall mortality, affects at least one out of four (25%) Indians with T2D. These results support screening of all patients with T2D and NAFLD for liver fibrosis.

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Introduction

Type 2 diabetes (T2D) and nonalcoholic fatty liver disease (NAFLD) often exist together. This is a high-risk population, as the presence of T2D promotes the progression of NAFLD to more severe histology forms.¹ Approximately 50 million individuals in India have coexisting T2D and NAFLD. Among them, an estimated 12.4 million people have NAFLD-related advanced liver fibrosis.² Advanced liver fibrosis is an imminent risk factor for liver-related and overall mortality.³ Yet there is hardly any consensus regarding screening of T2D patients for clinically relevant liver fibrosis (CRLF) or compensated cirrhosis. This is partly because of lack of data regarding true burden of liver fibrosis and cirrhosis in Indian population with T2D.

NAFLD is also strongly associated with the components of metabolic syndrome (obesity, hypertension, hyperglycemia, and dyslipidemia).⁴ The prevalence of metabolic syndrome is rapidly increasing in Indian population probably due to increasing urbanization and rapid transition from undernutrition to overnutrition.^{5–7} There are a few NAFLD prevalence studies from India that have used ultrasonography (USG) as a diagnostic tool for evaluating NAFLD.^{8–11} Unfortunately, USG does not give any information regarding liver fibrosis, which is the main determinant of liver-related and overall mortality.

Transient elastography (TE) is an ultrasound-based technique for fibrosis risk assessment. TE generates two parameters: controlled attenuation parameter (CAP), which gives estimation of liver steatosis, and liver stiffness measurement (LSM), which gives estimation of liver fibrosis.¹²

There is scarcity of data regarding the prevalence of CRLF and compensated cirrhosis due to NAFLD in Indian people with T2D. Therefore, the aim of our study was to investigate the prevalence of, and factors associated with, CRLF and cirrhosis in a large cohort of Indian individuals with T2D.

Materials and methods

Study population. This cross-sectional prospective study was conducted between 1 September 2019 and 31 August 2020 in the Department of Endocrinology and Diabetes, and in

Wellness Clinic of Medanta, The Medicity Hospital, a tertiary care facility in New Delhi NCR, India. We enrolled 601 consecutive patients with T2D, aged 18 years and above, who underwent prespecified set of investigations including fasting plasma glucose, glycated hemoglobin (HbA1c), lipid profile, complete blood counts, liver function tests, kidney function tests, thyrotropin (TSH), abdominal USG and body composition by dual-energy X-ray absorptiometry (DEXA). In addition to these investigations, each individual underwent TE by FibroScan. Exclusion criteria were positive hepatitis B surface antigen or antibody against hepatitis C virus, elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) more than five times the upper limit of normal values, excessive alcohol consumption (>30 g per day for men and >20 g per day for women), congestive heart failure with hepatomegaly, pregnancy, drugs causing fatty liver such as tamoxifen, and amiodarone, end-stage renal disease, active malignancy, and unreliable or invalid TE measurements. The study was approved by the institutional ethics review board (MICR-1184/2020, Gurugram, Haryana, India) and was approved by the ethics committee. Informed consent was obtained from all participants. The study protocol was conducted according to the Helsinki Declaration.

Clinical assessment. Clinical assessment, anthropometric, and demographic data were collected at the day of TE examination. Information on medical history, and current drugs was collected. All investigations were performed on the same day in the fasting state from the center laboratory of the institute. Abdominal ultrasound was used to grade hepatic steatosis from grade 0 to grade 3. Body weight, body mass index (BMI), body fat percentage, and total fat mass (kg) were measured by DEXA using the Hologic Horizon DXA System (USA) with Discovery software, version 12.3 (Bellingham, WA, USA). Normal weight, overweight, and obesity were defined as BMI < 23 kg/m², BMI between ≥23 and <25 kg/m², and BMI > 25 kg/m², respectively, in accordance with World Health Organization (WHO) Asia Pacific guidelines.¹³ Patient was considered to have hypertension if the blood pressure was ≥140/90 mmHg, if the patient was taking antihypertensive drugs or if there was positive medical

history. Dyslipidemia was defined as positive medical history, use of lipid-lowering drugs, or if the serum low-density lipoprotein (LDL) cholesterol level was >100 mg/dL. Hypothyroidism was defined as positive medical history, use of thyroxine, or if the serum TSH level was >10 mIU/L. Established CVD was defined as a positive history of angioplasty and/or coronary artery bypass graft surgery.

Transient elastography. TE was performed with a FibroScan device (EchoSens, Paris, France), in fasting conditions for more than 4 h, with the patient in a supine position, right arm in maximum abduction, by intercostal approach, in the right liver lobe. In each patient, we aimed for 10 valid LSM, with a success rate of measurements >60%. The examination was performed using the M probe (standard probe—transducer frequency 3.5 MHz) or the XL probe (transducer frequency 2.5 MHz). M and XL probes were used according to the European recommendation on M and XL probe selection. A median value of 10 valid LSM was calculated, and the results were expressed in kilopascals (kPa). Reliable measurements were defined as the median value of 10 valid LSM with an interquartile range interval/median ratio (IQR/M) < 30%. LSM \geq 8.0 kPa was taken as cut-off suggesting CRLF.¹⁴

Dynamic liver magnetic resonance imaging. A subset of individuals with LSM >13.0 kPa underwent dynamic magnetic resonance imaging (MRI) of liver for further verification of cirrhosis, portal hypertension, and to look for any space occupying lesions. MRI was performed at the same hospital.

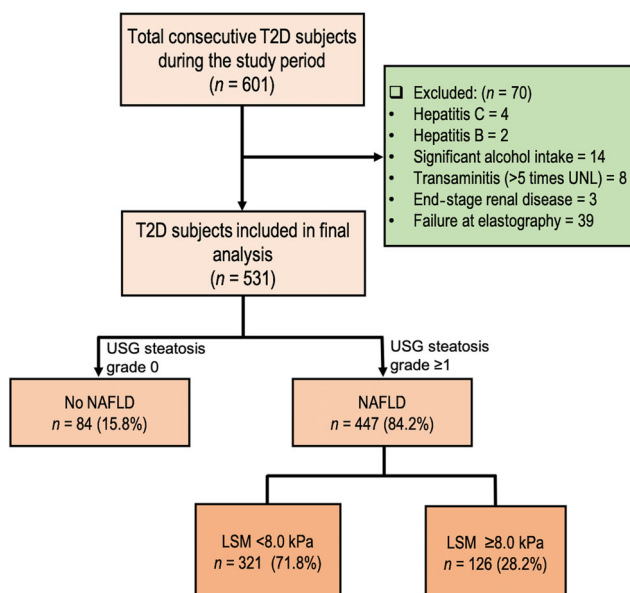


Figure 1 Derivation of the study cohort. LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; T2D, type 2 diabetes; USG, ultrasonography.

Statistical analysis. Categorical variables are shown as number and percentages, and continuous variables as means with standard deviation (parametric data) or medians with interquartile range (25th and 75th percentiles, nonparametric data). The chi-square test was used for comparing proportions expressed as percentages. Linear and logistic regression were used for univariate and multivariate analyses of factors that may influence LSM and

Table 1 Baseline characteristics of all subjects

Characteristics	All subjects (n = 531)
Age, years	53.1 (10.8)
Males, n (%)	361 (68.0)
Females, n (%)	170 (32.0)
Weight, kg	75.8 (13.5)
BMI (kg/m ²)	27.5 (4.1)
Body fat percentage	34.7 (7.1)
Total fat mass, kg	26.4 (8.1)
Diabetes duration, years	7.0 (3–12)
Obesity (BMI \geq 25 (kg/m ²), n (%))	412 (75.9)
Hypertension (n, %)	288 (54.2)
Dyslipidemia (n, %)	347 (65.3)
Established CVD (n, %)	47 (8.9)
Hypothyroidism, (n, %)	67 (12.6)
Fasting plasma glucose, mg/dL	153 (67)
Hemoglobin A1c (%)	7.4 (1.8)
Urea, mg/dL	29.0 (13.0)
Creatinine, mg/dL	0.85 (0.44)
Uric acid, mg/dL	5.3 (1.4)
Na, mmol/L	139.7 (2.2)
K, mmol/L	4.6 (0.4)
Total bilirubin, mg/dL	0.6 (0.3)
Albumin, g/dL	4.4 (0.4)
Aspartate aminotransferase, IU/L	30 (23–40)
Alanine aminotransferase, IU/L	32 (22–50)
Gamma-glutamyl transpeptidase, IU/L	30 (21–44)
Alkaline phosphatase, IU/L	89 (73–108)
Hemoglobin, g/dL	13.5 (12.4–14.7)
Total leucocyte count $\times 10^3/\text{mm}^3$	7.31 (6.11–8.68)
Platelets $\times 10^3/\text{mm}^3$	206 (154–265)
Total cholesterol, mg/dL	164 (138–197)
Triglycerides, mg/dL	139 (103–191)
HDL, mg/dL	38 (32–45)
LDL, mg/dL	105 (80–131)
Thyrotropin (TSH), mIU/mL	2.4 (1.5–3.5)
Steatosis by ultrasonography	
Grade 0, n (%)	84 (15.8)
Grade 1, n (%)	266 (50.1)
Grade 2, n (%)	158 (29.8)
Grade 3, n (%)	23 (4.3)
Transient elastography	
Liver stiffness measurement (LSM), kPa,	6.0 (4.6–8.2)
Clinically relevant fibrosis, LSM \geq 8.0 kPa, n (%)	147 (27.7)
LSM-based cirrhosis, LSM >13.0 kPa, n (%)	31 (7.2%)

Data are presented as mean (SD), median (25th–75th percentile), or percentage.

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

CAP values. Furthermore, 95% confidence intervals (CIs) were calculated for each predictive test, and a two-tailed *P*-value < 0.05 was considered significant for all statistical tests. All the statistical analyses were performed using SPSS V.22.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics. A total of 531 subjects with T2D were analyzed after inclusion and exclusion criteria were applied. Seventy subjects were excluded due to various reasons as shown in Figure 1. The mean age of our cohort was 53.1 (10.8) years and 68% were males. The median duration of T2D diagnosis was 7.0^{3–12} years and mean HbA1c was 7.4 (1.8). The mean BMI of the cohort was 27.5 (4.1) kg/m², and the median LSM was 6.0 (4.6–8.2) kPa. The demographics, biochemical results, and comorbidities of all study participants with T2D are summarized in Table 1.

Overall, 447 (84.2%) participants had hepatic steatosis. Among them, 50.1, 29.8, and 4.3% individuals had ultrasound-based grade 1, grade 2, and grade 3 steatosis, respectively (Table 1). One hundred twenty-six participants (28.2%) had LSM ≥ 8.0 kPa, suggesting CRLF. Grading of CAP-based steatosis and LSM-based fibrosis is given in Table S1.

Prevalence of NAFLD and associated risk factors.

The prevalence of NAFLD was 84.2%. Relative to T2D subjects without NAFLD (non-NAFLD), BMI (28.0 *vs* 25.1 kg/m², *P* < 0.0001), body fat (27.1 *vs* 22.1 kg, *P* < 0.0001), AST (34.8 *vs* 29.2 U/L, *P* = 0.003), ALT (41.3 *vs* 32.0 U/L, *P* = 0.002), GGT (44.9 *vs* 30.0 U/L, *P* = 0.004), and triglycerides (168 *vs* 145 mg/dL, *P* = 0.048) were significantly higher in NAFLD subjects (Table 2). No significant difference in age, duration of diabetes, and platelet levels were observed. Higher proportion of subjects with NAFLD were obese (BMI ≥ 25 kg/m²) (80.5 *vs* 52.4%, *P* < 0.0001).

Risk factors associated with NAFLD included higher BMI, body fat percentage, AST, ALT, GGT, TG, and lower high-density lipoprotein (HDL) levels. Obesity was also associated with NAFLD from univariate logistic regression. Multivariate analyses revealed that only higher BMI (odds ratio [OR], 1.168, confidence interval [CI], 1.02–1.33, *P* = 0.022), ALT (OR, 1.031, 1.01–1.04, *P* = 0.001), and lower HDL (OR, 0.969, 0.95–1.00, *P* = 0.002) were independent factors associated with steatosis (Table 3).

Prevalence of CRLF and associated risk factors.

CRLF was detected in 126 (28.2%) participants. Relative to T2D subject without CRLF (LSM < 8.0 kPa), higher BMI (29.4 *vs* 27.4 kg/m², *P* < 0.0001), AST (44 *vs* 31 U/L, *P* < 0.0001), ALT (53 *vs* 37 U/L, *P* < 0.0001), GGT (71 *vs* 35 U/L, *P* < 0.0001), and ALP (99 *vs* 91 U/L, *P* = 0.018) were significantly higher in subjects with CRLF (LSM ≥ 8.0 kPa). No significant difference in age, duration of diabetes, triglycerides, and platelet levels were observed (Table 4). Hypertension was also associated with CRLF from univariate logistic regression. Multivariate analysis revealed that only higher BMI (OR, 1.084, CI, 1.03–1.14, *P* = 0.002), AST (OR, 1.031, 1.01–1.06, *P* = 0.016),

and GGT (OR, 1.02, 1.01–1.02, *P* < 0.0001) and hypertension (OR, 1.729, 1.10–2.72, *P* = 0.018) were independent factors associated with CRLF (Table 5).

Prevalence of transaminitis in individuals with T2D and CRLF.

Eighty-nine (70.6%) individuals with steatosis and CRLF had elevated ALT (≥ 40 U/L). Sixty-five (51.6%) individuals with steatosis and CRLF had elevated AST (≥ 40 U/L). Thirty-seven (29.4%) and 61 (48.4%) individuals with steatosis and CRLF had normal ALT and AST, respectively (Table S2).

Table 2 Characteristics of T2D individuals with and without NAFLD

Characteristics	Non-NAFLD (<i>n</i> = 84)	NAFLD (<i>n</i> = 447)	<i>P</i> value
Age, years	53.0 (12.3)	53.2 (10.5)	0.908
Males, <i>n</i> (%)	55 (65.5)	306 (68.5)	0.591
Females, <i>n</i> (%)	29 (34.5)	141 (31.5)	
Weight, kg	67.8 (11.9)	77.3 (13.3)	<0.0001*
BMI (kg/m ²)	25.1 (3.9)	28.0 (3.9)	<0.0001*
Body fat percentage	32.5 (7.4)	35.1 (6.9)	0.002*
Total fat mass, kg	22.1 (7.0)	27.1 (8.1)	<0.0001*
Diabetes duration, years	8.6 (6.8)	8.4 (6.5)	0.778
Obesity (BMI ≥ 25 kg/m ²), <i>n</i> (%)	44 (52.4)	355 (80.5)	<0.0001*
Hypertension (<i>n</i> , %)	47 (56)	241 (54)	0.731
Dyslipidemia (<i>n</i> , %)	54 (64.3)	293 (65.5)	0.823
Established CVD (<i>n</i> , %)	7 (8.3)	40 (8.9)	0.855
Hypothyroidism (<i>n</i> , %)	9 (10.7)	58 (13.0)	0.567
Fasting plasma glucose, mg/dL	158 (80)	151 (64)	0.377
Hemoglobin A1c (%)	7.2 (1.8)	7.4 (1.8)	0.367
Total bilirubin, mg/dL	0.58 (0.36)	0.59 (0.3)	0.788
Albumin, g/dL	4.36 (0.4)	4.35 (0.4)	0.564
Aspartate aminotransferase, IU/L	29.2 (11.9)	34.8 (16.2)	0.003*
Alanine aminotransferase, IU/L	32.0 (20.4)	41.3 (25.9)	0.002*
Gamma-glutamyl transpeptidase, IU/L	30.0 (27.4)	44.9 (45.5)	0.004*
Alkaline phosphatase, IU/L	94.4 (31.0)	93.5 (28.9)	0.684
Hemoglobin, g/dL	13.9 (1.7)	13.7 (1.8)	0.215
Total leucocyte count × 10 ³ /mm ³	7.424 (1.99)	7.597 (2.03)	0.474
Platelets × 10 ³ /mm ³	225 (77)	218 (79)	0.434
Total cholesterol, mg/dL	173 (46)	168 (46)	0.325
Triglycerides, mg/dL	145 (76)	168 (102)	0.048*
HDL, mg/dL	46 (19)	39 (11)	<0.0001*
LDL, mg/dL	110 (41)	106 (39)	0.613
Thyrotropin (TSH), mIU/mL	3.2 (3.5)	2.9 (2.7)	0.300
LSM, kPa	6.9 (5.4)	7.2 (4.2)	0.600

*Statistically significant.

Data are presented as mean (SD) or percentage as indicated.

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein, LSM, liver stiffness measurement.

Table 3 Factors associated with hepatic steatosis in univariate and multivariate analyses

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
Age	1.001	0.98–1.02	0.908			
Gender	1.144	0.7–1.87	0.591			
BMI	1.268	1.17–1.37	<0.0001	1.168	1.02–1.33	0.022*
Body fat percentage	1.061	1.02–1.1	0.002			
Obesity (BMI \geq 25 (kg/m ²))	3.75	2.30–6.12	<0.0001			
Diabetes duration, years	0.963	0.93–1.08	0.628			
Hypertension	0.921	0.58–1.47	0.731			
Dyslipidemia	1.057	0.65–1.72	0.823			
Established CVD	1.081	0.47–2.5	0.856			
Hypothyroidism	1.243	0.59–2.62	0.568			
Hemoglobin A1c	1.064	0.93–1.22	0.361			
Albumin	1.88	0.98–3.02	0.392			
AST	1.034	1.01–1.06	0.002			
ALT	1.031	1.01–1.04	<0.0001	1.031	1.01–1.04	0.001*
GGT	1.019	1.01–1.03	0.004			
Platelets	0.999	0.99–1.00	0.433			
Triglycerides	1.003	1–1.01	0.048			
HDL	0.969	0.95–0.98	<0.0001	0.969	0.95–1.00	0.002*
LDL	0.998	0.99–1	0.612			
Thyrotropin (TSH)	0.964	0.9–1.03	0.308			

*Statistically significant.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CVD, cardiovascular disease; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Prevalence of cirrhosis and associated risk factors. Thirty-one (7.2%) patients with NAFLD were found to have LSM $>$ 13.0 kPa. Of these, 22 (71%) were males, and mean age of 56.9 (8.7) years. The baseline characteristics of all NAFLD patients with LSM $>$ 13.0 kPa are given in Table S3. Multivariate analysis revealed that higher AST (OR, 1.04, 1.02–1.06, $P <$ 0.0001) and GGT (OR, 1.01, 1.01–1.02, $P <$ 0.0001) were independent factors associated with cirrhosis (Table S4). Out of 31 patients with LSM $>$ 13.0 kPa, 25 patients underwent dynamic MRI of liver and 18 patients were diagnosed as having features consistent with cirrhosis. Among them, ultrasound showed changes of cirrhosis in only two patients.

Discussion

Our study demonstrated a remarkably high prevalence of NAFLD among type 2 diabetes mellitus (T2DM) patients, which was 84.2% using USG. The prevalence of CRLF (LSM \geq 8.0 kPa) was 28.2%. Furthermore, among 31 subjects with LSM \geq 13.0 kPa, 25 underwent dynamic MRI of liver, and 18 were found to have definitive cirrhosis.

There are five large TE-based studies among individuals with coexisting T2D and NAFLD, one each from Hong Kong (2015), Malaysia (2019), Singapore (2020), Croatia (2020), and the United States (2021).^{15–19} These studies revealed high prevalence of NAFLD among individuals with T2D, ranging from 70% in the United States, 72.4% in Malaysia, 72.8% in Hong Kong, 78.7% in Singapore, and 83.6% in Croatia. Our study is the first from India and revealed a high prevalence of USG-based NAFLD (84.2%). Some differences in prevalence of NAFLD among these nations are because of using different CAP cutoffs and may be because of different populations.

We found that higher BMI, ALT, and lower HDL were independent risk factors associated with liver steatosis. Kwok *et al.* demonstrated that female gender, higher BMI, fasting plasma glucose, ALT, and triglycerides were independently associated with increased CAP.¹⁵ We did not find female gender as a risk factor. We acknowledge that our study population had fewer female representation (32%), probably fewer females getting access to tertiary medical facility in our region. Other risk factors for NAFLD were similar to the study by Kwok *et al.* In the study by Lai *et al.*,¹⁶ NAFLD was found to be independently associated with central obesity, triglycerides, and ALT levels. Chen *et al.*¹⁷ showed that higher ALT, obesity, and metabolic syndrome were independently associated with increased CAP. Mikolasevic *et al.*¹⁸ found that higher BMI, longer duration of T2D, and higher triglycerides were independently associated with NAFLD in the multivariate analysis.

The clinical burden from NAFLD-related complications is expected to be considerable because T2D is known to be an accelerating factor for NAFLD progression and associated with increased mortality. NAFLD is largely an asymptomatic condition and only manifests at late stage of liver disease. We, therefore, demonstrated the prevalence of complication of NAFLD as CRLF. In our study, the prevalence of CRLF was 28.2% among people with coexisting T2D and NAFLD, which is much higher than that of the general population^{14,15,20} as well as of T2D population. The prevalence of increased LSM in subjects with T2D were 17.7% (Hong Kong),¹⁵ 21.0% (Malaysia),¹⁶ 13.08% (Singapore)¹⁷; moderate (26.9%) and advanced (12.6%) liver fibrosis (Croatia)¹⁸; and 21% (LSM \geq 7.0 kPa) (United States).¹⁹ In our study, we found much higher prevalence of CRLF. Mikolasevic *et al.* used LSM cutoff of \geq 7.0 kPa for defining moderate liver fibrosis, still the prevalence of increased LSM was lower than that of ours (26.9%

Table 4 Characteristics of T2DM subjects with NAFLD, with or without clinically relevant liver stiffness (LSM \geq 8.0 kPa)

Characteristics	LSM <8.0 kPa <i>n</i> = 321	LSM \geq 8.0 kPa <i>n</i> = 126	<i>P</i> value
Age, years	53.0 (10.7)	53.7 (10.1)	0.526
Males, <i>n</i> (%)	206 (69.4)	90 (67.7)	0.726
Females	91 (30.6)	43 (32.3)	
Weight, kg	76.1 (13.1)	80.1 (13.4)	0.004*
BMI (kg/m ²)	27.4 (3.6)	29.4 (4.4)	<0.0001*
Body fat percentage	34.9 (7.2)	35.6 (6.4)	0.343
Total fat mass, kg	26.6 (8.4)	28.5 (6.9)	0.033*
Diabetes duration, years	8.1 (6.7)	8.3 (6.0)	0.793
Obesity (BMI \geq 25 (kg/m ²))	229 (77.9)	111 (84.1)	0.140
Hypertension (<i>n</i> , %)	153 (51.5)	84 (63.2)	0.025*
Dyslipidemia (<i>n</i> , %)	191 (64.3)	95 (71.4)	0.148
Established CVD (<i>n</i> , %)	24 (8.1)	14 (10.5)	0.409
Hypothyroidism (<i>n</i> , %)	37 (12.5)	23 (17.3)	0.181
Fasting plasma glucose, mg/dL	148 (66)	160 (57)	0.093
Hemoglobin A1c (%)	7.3 (1.9)	7.7 (1.6)	0.041*
Total bilirubin, mg/dL	0.6 (0.3)	0.6 (0.3)	0.374
Albumin, g/dL	4.4 (0.3)	4.4 (0.4)	0.850
Aspartate aminotransferase, IU/L	31 (14)	44 (19)	<0.0001*
Alanine aminotransferase, IU/L	37 (23)	53 (30)	<0.0001*
Gamma-glutamyl transpeptidase, IU/L	35 (26)	71 (69)	<0.0001*
Alkaline phosphatase, IU/L	91 (28)	99 (30)	0.018*
Hemoglobin, g/dL	13.8 (1.8)	13.3 (2.0)	0.016*
Total leucocyte count $\times 10^3$ /mm ³	7.537 (2.08)	7.750 (1.93)	0.320
Platelets $\times 10^3$ /mm ³	220 (80)	211 (78)	0.278
Total cholesterol, mg/dL	172 (46)	158 (42)	0.003*
Triglycerides, mg/dL	167 (107)	171 (89)	0.760
HDL, mg/dL	40 (11)	38 (11)	0.107
LDL, mg/dL	109 (39)	103 (39)	0.092
Thyrotropin (TSH), mIU/mL	3.0 (2.8)	2.6 (2.7)	0.187

*Statistically significant.

Data are presented as mean (SD) or percentage as indicated.

BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus.

vs 28.2% at cutoff of \geq 8.0 kPa). This is not surprising as our population has several risk factors predisposing them to NAFLD-related complications. It has been demonstrated that healthy, normal-weight Asian Indians are profoundly insulin resistant and hyperinsulinemic compared with age- and BMI-matched Caucasians. Moreover, Asian Indians have greater amounts of total, visceral, and subcutaneous fat compared with Caucasians matched for BMI.²⁰ Asian Indians also have lower HDL and higher LDL and triglycerides compared with Caucasians. These data demonstrate that altered body composition is associated with insulin resistance, hyperinsulinemia, and dyslipidemia in Asian Indians, and this may explain their heightened risk for diabetes and associated conditions.²¹ In non-obese Asian Indians with T2D, subcutaneous fat and intra-abdominal obesity, including fatty liver, were higher than BMI-matched nondiabetic subjects.²² These observations indicate that our population are particularly at risk for developing metabolic complications including NAFLD-related conditions.

The substantial number of subjects with increased CRLF is concerning, especially since there are usually no associated symptoms. Our study demonstrated higher BMI, AST, GGT, and concomitant hypertension as independent factors associated with increased LSM. In the Hong Kong study,

longer duration of diabetes, higher BMI, increased ALT, and lower HDL cholesterol were associated with increased LSM.¹⁵ In the Malaysia study, higher ALT, and GGT; and lower HDL cholesterol and platelet levels were associated with increased LSM.¹⁶ In the Singapore study, higher AST and CAP** values; and lower platelet count and concomitant hypertension were independent factors associated with increased liver stiffness.¹⁷ In the Croatia study, independent factors associated with advanced fibrosis were female gender, higher BMI, ALT and GGT levels.¹⁸ Overall, the risk factors associated with increased LSM are similar in all the studies. Some differences might be because of different populations and number of subjects included for the analysis. We did not find any association of duration of diabetes with CRLF. Out of the four aforementioned studies, only Hong Kong study had shown association of duration of diabetes with liver fibrosis.¹⁵

Older age had been reported in several studies as an association factor with the development of liver fibrosis,^{23,24} however this was not demonstrated in our study and in other aforementioned similar four studies. One possible explanation could be that the presence of T2D accelerated the progression of NAFLD which lead to a higher prevalence of liver fibrosis independent of age. Indeed

Table 5 Factors associated with clinically relevant liver stiffness (LSM ≥ 8.0 kPa) in univariate and multivariate analyses in patients with NAFLD

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
Age, years	1.001	0.99–1.03	0.525			
Gender	0.892	0.57–1.38	0.610			
BMI (kg/m ²)	1.135	1.07–1.2	<0.0001	1.084	1.03–1.140	0.002*
Body fat percentage	1.014	0.98–1.04	0.343			
Diabetes duration, years	1.004	0.97–1.04	0.793			
Obesity (BMI ≥ 25 (kg/m ²))	1.775	1.0–3.16	0.051			
Hypertension	1.650	1.08–2.52	0.020	1.729	1.10–2.72	0.018*
Dyslipidemia	1.184	0.76–1.84	0.451			
Established CVD	1.418	0.71–2.81	0.318			
Hypothyroidism	1.405	0.78–2.52	0.255			
Fasting plasma glucose	1.00	1.00–1.01	0.194			
Hemoglobin A1c	1.121	1.00–1.25	0.043			
Total bilirubin	1.374	0.68–2.77	0.373			
Albumin	0.946	0.54–1.67	0.850			
AST	1.052	1.04–1.07	<0.0001	1.031	1.01–1.06	0.016*
ALT	1.024	1.02–1.03	<0.0001			
GGT	1.023	1.01–1.03	<0.0001	1.02	1.01–1.02	<0.0001*
Platelets	0.999	1.00–1.00	0.282			
Triglycerides	1.00	1.00–1.00	0.760			
HDL	0.984	0.96–1.00	0.108			
LDL	0.94	0.99–1.00	0.063			
Thyrotropin (TSH)	0.957	0.87–1.05	0.337			

*Statistically significant.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CVD, cardiovascular disease; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease.

this was demonstrated by a study reporting similarly the higher probability of liver fibrosis in T2D patients independent of age.¹⁴

Our study also demonstrated that 31 (7.2%) subjects had LSM >13.0 kPa, which is a high risk for cirrhosis. Among them, 25 patients underwent dynamic MRI of liver and 18 patients were diagnosed with definitive cirrhosis. All these patients were asymptomatic and had compensated cirrhosis. Diagnosis of all these patients would have been missed if there was no screening by TE. Among them, USG could detect changes of cirrhosis in only two patients. Higher AST and GGT values were independently associated with cirrhosis in the multivariate analysis.

Nonalcoholic steatofibrosis (NASF) may be an important marker for clinical usage in patients with NAFLD. Using biopsy data, it has been demonstrated that NASH and NASF are similarly and significantly associated with liver-related mortality, but only NASF is associated with overall mortality in patients with NAFLD.²⁵ Although liver biopsy is the gold standard to assess fibrosis and changes of NASH, it is often not performed except in clinical trials, due to risk of complications. What is interesting is that steatosis and fibrosis can be assessed using noninvasive methods, such as USG, CAP, and MRI proton density fat fraction for steatosis; and serological fibrosis scores, TE, and magnetic resonance elastography for fibrosis. Indeed, it has been demonstrated that NASF (steatosis by ultrasound and fibrosis by NAFLD fibrosis score) predicted overall mortality in a large cohort of patients with NAFLD.²⁶ In our study, we have assessed steatosis using USG and fibrosis using TE. Thus, we have assessed steatofibrosis

(NASF) in our cohort, which may better predict liver-related and overall mortality in this cohort. However, it needs further validation whether NASF has an edge over NASH or not.

Our study had several limitations, including its cross-sectional nature and absence of biopsy in patients with advanced fibrosis. However, we used TE, which is a noninvasive technique to evaluate hepatic fibrosis. TE has been endorsed as an alternative to liver biopsy by international guidelines in guiding clinical management of NAFLD.^{27,28} Therefore, TE is a validated modality in assessing NAFLD fibrosis in a real-world setting where liver biopsy is not practical for all subjects. Another limitation of our study is that we assessed individuals with T2D who prefer to get treatment from a tertiary care center. These individuals usually had multiple metabolic comorbidities. Therefore, our study population is a high-risk group, and the results may not apply to community people with T2D. The strength of our study is a large sample size of consecutive Indian patients with T2D. Another strength is that we assessed steatofibrosis with USG and TE, which is a practical way of screening all people with T2D in a clinical setting.

Conclusions

NAFLD and CRLF is highly prevalent in Indian people with T2D. Patients with T2D and higher BMI, AST, and GGT values or comorbidity of hypertension have higher risk for increased liver fibrosis. Our study supports the role of screening for NAFLD-related liver fibrosis in T2D population.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Table S1. CAP and LSM-based grading of steatosis and fibrosis of all patients with T2D (n = 531).

Table S2. Transaminitis in people with T2D and NAFLD, with and without clinically relevant liver fibrosis.

Table S3. Baseline characteristics of T2D patients with NAFLD and cirrhosis (LSM > 13.0 kPa).

Table S4. Factors associated with cirrhosis (LSM > 13.0 kPa) in univariate and multivariate analyses.