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ORIGINAL ARTICLE

The Prevalence of Advanced Liver Fibrosis Among Patients With Type 2 Diabetes Mellitus: A Single-Centre Experience in Penang, Malaysia

Xe Hui Lee,[1] Lisa Mohamed Nor,[2] Choon Seong Ang,[3] Toh Peng Yeow,[1] Shueh Lin Lim[1]

[1]*Endocrinology Unit, Department of Medicine, Penang General Hospital, Penang, Malaysia*
[2]*Clinical Research Center, Putrajaya Hospital, Wilayah Persekutuan Putrajaya, Malaysia*
[3]*Clinical Research Center, Penang General Hospital, Penang, Malaysia*

Abstract

Objectives. Type 2 diabetes mellitus (T2DM) is an important risk factor for Non-alcoholic fatty liver disease (NAFLD). It worsens the course of NAFLD. We investigated the prevalence of advanced liver fibrosis among patients with T2DM. Our secondary objectives were to describe patient demographics, to explore associated clinical factors, and to compare FIB-4 Index and liver stiffness measurement (LSM).

Methodology. This was a cross-sectional study on 258 patients with T2DM duration of at least 10 years. Transient elastography (FibroScan®) was performed on all subjects. Advanced liver fibrosis was diagnosed based on LSM results. The FIB-4 index formula was used.

Results. The prevalence of advanced liver fibrosis was 22.1%. Associated factors were body mass index (BMI), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), triglyceride (TG) and high-density lipoprotein (HDL) cholesterol. Independent factors were BMI and GGT (p=0.003 and p<0.001). FIB-4 index has 30.0% sensitivity, 85.0% specificity, 38.7% positive predictive value, and 79.4% negative predictive value in detecting advanced liver fibrosis by LSM criteria.

Conclusion. Our study confirmed the high prevalence of advanced liver fibrosis among patients with long-standing T2DM. This study suggests the benefit of advanced liver fibrosis screening in patients with a minimum of 10 years of T2DM, especially those with high BMI and GGT.

Key words: type 2 diabetes mellitus, non-alcoholic fatty liver disease, advanced liver fibrosis, transient elastography, FIB-4 index

INTRODUCTION

NAFLD and T2DM regularly co-exist and act synergistically to drive adverse outcomes. The presence of both NAFLD and T2DM increases the likelihood of the development of complications of diabetes as well as augments the risk of more severe NAFLD, including cirrhosis, hepatocellular carcinoma, and death. The mainstay of NAFLD management is currently to reduce modifiable metabolic risk factors. Achieving good glycaemic control and optimizing weight loss are pivotal to restricting disease progression.[1]

NAFLD is the most common chronic liver disease, affecting 15-40% of the population worldwide.[2] Around 20-30% of patients with NAFLD have non-alcoholic steatohepatitis (NASH), the active form of NAFLD which can cause liver fibrosis. This may eventually progress to cirrhosis and hepatocellular carcinoma in 10-20% of patients.[3-5] In the United States, NASH has already emerged as the second leading aetiology of chronic liver disease among new liver transplant registrants,[6] and is also the second leading cause of hepatocellular carcinoma.[7]

The prevalence of NAFLD and advanced liver fibrosis is high among patients with T2DM. In 2018, a Malaysian study of 571 patients with T2DM by Lee-Lee Lai found the prevalence of transient elastography-diagnosed NAFLD and advanced liver fibrosis to be 72.4% and 21.0% respectively,[8] whereas Kwok found the respective prevalence to be 72.8% and 17.7% in Hong Kong in 2016.[9]

Major guidelines have different recommendations with regards to screening for NAFLD among patients with T2DM. The European Association for the Study of the Liver (EASL) guidelines recommend screening patients with T2DM for NAFLD regardless of serum liver enzyme

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Corresponding author: Xe Hui Lee, MD
Consultant Endocrinologist
Penang General Hospital
1, Jalan Residensi, 10990, Penang, Malaysia
Tel. No.: 04-2225704
Fax No.: 04-2225706
E-mail: xehui@yahoo.com
ORCiD: https://orcid.org/0000-0002-9976-7505

level in view of their high risk for disease progression.[10] On the other hand, the American Association for the Study of Liver Diseases (AASLD) guideline is not in favour of routine screening for NAFLD in patients with T2DM, citing uncertainties surrounding diagnostic tests and treatment options, and the lack of knowledge related to the long-term benefits and cost-effectiveness of screening.[11] Liver biopsy is the gold standard for the assessment of liver fibrosis, but it has its limitations, which include life-threatening complications. Alternative methods of non-invasive laboratory and radiologic testing for the assessment of liver fibrosis in NAFLD have evolved during the past decade, and these methods may be able to overcome the limitations of liver biopsy.[12,13]

An ultrasound-based technique, transient elastography (FibroScan®) is one of the most extensively used and well-validated non-invasive methods for the assessment of liver fibrosis.[14-18] recent meta-analysis showed that transient elastography had a high sensitivity of 94% and specificity of 95% when used to identify fibrosis in patients with NAFLD.[14] However, up to 20% of transient elastography examinations yielded unreliable results, especially among patients with high BMI.[19,20] The use of the XL probe can increase the success rate of examination in obese patients, but proper training is required.[21] To improve test reliability, a minimum of 10 valid readings, with at least a 60% success rate and an interquartile range of ≤30% of the median value, are taken with the results expressed in kilopascals (kPa).[15,22]

We are aware that it is impossible to perform FibroScan® routinely on all patients with long-standing T2DM. Only three tertiary public hospitals in the country offer FibroScan® for free. If done in a private hospital, the charges can amount to 800 Malaysian Ringgit. Hence, various scoring systems of fibrosis have been explored.

In 2009, Shah AG et al., concluded that the FIB-4 index [(Age x AST) / (Platelet x √(ALT)] is superior to 7 other non-invasive markers of fibrosis in patients with NAFLD, namely NAFLD Fibrosis score, Goteburg University Cirrhosis Index, AST:ALT ratio, AST:Platelet ratio index, AST:Platelet ratio, BMI, AST: ALT, diabetes (BARD) score and cirrhosis discriminant score. Their study used a nationwide database of 541 adults with NAFLD; jack knife-validated areas under receiver operating characteristic curves (AUROC) of FIB-4 and 7 other markers were compared. All patients in this dataset had a liver biopsy in the 12 months prior to enrolment.[16]

Data on the prevalence of NAFLD among patients with T2DM in the region of Southeast Asia is lacking. Availability of this data will help to assess the benefits and cost-effectiveness of NAFLD screening among patients with T2DM in this region and worldwide.

Our primary objective was to investigate the prevalence of advanced liver fibrosis by transient elastography among patients with at least 10 years of T2DM. We also aimed to describe the demographic and clinical profiles of the patients with advanced liver fibrosis, explore the factors associated with advanced liver fibrosis among patients with T2DM, and compare FIB-4 Index and LSM on FibroScan®.

METHODOLOGY

Subjects

Patients seen at the diabetes specialist clinic of the endocrinology unit in Penang General Hospital, Malaysia who were at least 35 years old and had long-standing T2DM for at least 10 years were enrolled after they provided written informed consent. Excluded were those with significant alcohol intake (greater than 21 units per week for males and greater than 14 units per week for females); established history of other forms of liver diseases including hepatitis B (positive serum hepatitis B surface antigen), hepatitis C (positive anti-hepatitis C antibody), autoimmune hepatitis (positive autoimmune serology with consistent biopsy result), drug-induced liver disease (history of amiodarone or tamoxifen use), and biliary duct obstruction; history of gastrointestinal bypass or use of drugs known to cause hepatic steatosis (i.e., amiodarone, valproate, tamoxifen, methotrexate, steroids); established history of liver cirrhosis; active substance abuse; history of platelet disorders; congestive cardiac failure who may have secondary liver congestion; presence of a pacemaker (according to FibroScan® manufacturer advice) and those who were pregnant.

Materials and methods

This was a cross-sectional prevalence study which took place from July 2019 to January 2020.

Before each diabetes specialist clinic consult, the subjects were screened and selected based on the study inclusion and exclusion criteria. On the actual visit, anthropometric measurements and vital signs (weight, height, waist circumference, and blood pressure) were taken at the registration counter by a designated nurse. During their consultation with the attending doctors, eligible patients were asked if they were keen to participate. Patients who agreed were sent to the study procedure room to meet the primary investigator after their consultation. The primary investigator would then give verbal and written explanations based on the patient information sheet. Informed consent was obtained and appointment dates for blood sampling and FibroScan® were given.

A total of 321 patients were recruited. Data collection was done based on the Data Collection Sheet. Venous blood samples were obtained at the Penang General Hospital outpatient clinic after a 10-hour overnight fast. Blood was sent for complete blood count, renal profile, liver function tests, fasting blood sugar, fasting lipid profile, glycosylated haemoglobin, aspartate aminotransferase and gamma-glutamyl transpeptidase. This step was omitted if latest

available results were performed not more than 4 months prior to recruitment.

Transient elastography

After blood extraction, the patients proceeded to the gastroenterology clinic for transient elastography using Fibroscan® 502 keyboard (Echosen™, Paris, France). This was a non-invasive imaging done to assess the severity of liver fibrosis. Transient elastography was performed by a single operator with either the M or the XL probe. If a patient failed to obtain a valid result with the M probe due to central obesity, the elastography was repeated using the XL probe.

Adequate pressure of the probe on the skin surface, good layering on TM mode, and a straight imaginary line on A mode were ensured for each measurement. An examination was considered successful if at least 10 valid measurements were obtained, and reliable if the interquartile range (IQR) / median of the LSM was at most 30%.[17] A patient was considered to have advanced fibrosis if the LSM was at least 9.6 kPa using the M probe or at least 9.3 kPa using the XL probe. Cirrhosis is considered if the LSM was at least 11.5 kPa using the M probe or at least 11.0 kPa using the XL probe.[20,21]

The FibroScan® available in our institution could only measure LSM. The machine is unable to measure controlled attenuation parameters (CAP). Hence, we were able to investigate the prevalence of advanced liver fibrosis but not hepatic steatosis.

Transient elastography reports were interpreted based on the following scoring card used by Echosens™, Paris, France (Figure 1).

Results were then grouped into

- F0: Normal
- F1: Mild fibrosis
- F2: Significant fibrosis
- F3: Severe fibrosis
- F4: Cirrhosis

Patients with a fibrosis score of F1 were offered two annual transient elastography surveillance. Patients with a fibrosis score of F2 were offered yearly assessment. Those who scored F3 and above were referred to a gastroenterologist for further assessment and surveillance of cirrhosis and hepatocellular carcinoma.

FIB-4 index

As transient elastography is not readily available in many parts of the world, scoring systems of liver fibrosis are important to assess the risk of fibrosis and the indication for this scan. We have chosen the FIB-4 Index as a scoring system option to calculate the risk of liver fibrosis in our subjects. This study compared FIB-4 Index to LSM on FibroScan®.

FIB-4 Index was calculated using the following calculator.[25]

$$\text{FIB-4} = \frac{\text{Age (years)} \times \text{AST Level (U/L)}}{\text{Platelet Count } (10^9/L) \times \sqrt{\text{ALT (U/L)}}}$$

For a fixed specificity of 90% (FIB-4 equal to 1.93), the sensitivity in identifying advanced fibrosis was only 50% (95% CI, 46-55%). A FIB-4 greater than or equal to 2.67 had an 80% positive predictive value and a FIB-4 index less than or equal to 1.30 had a 90% negative predictive value. Using the threshold values of 1.30 and 2.67 for the absence

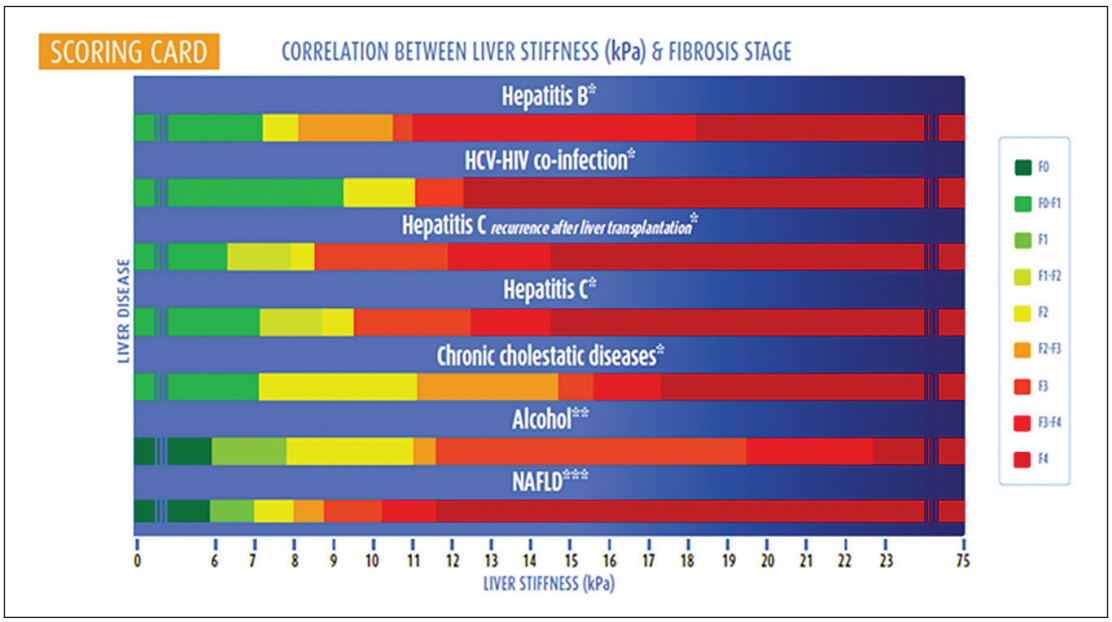

Figure 1. Scoring card for correlation between liver stiffness (kPa) and fibrosis stage (© Echosens™, Paris, France).[18,20,23,24]

and presence of advanced fibrosis, respectively, the FIB-4 index showed 89% accuracy. The FIB-4 index is to be used with caution in patients less than 35 or greater than 65 years old, as the score has been shown to be less reliable in these patients.[16]

Sample size calculation

Through literature search conducted on PubMed, we found six studies looking into the prevalence of liver fibrosis among diabetic patients using FibroScan®. The two studies that we selected were from Malaysia and Hong Kong (Table 1). These studies presumably had a more similar demographic profile with our patients and used the same cut-off values of FibroScan®. Other studies used different FibroScan® cut-offs and studied a different population group.[26-29] Hence, these studies were not considered in our sample size calculation.

Table 1. Two studies selected as references for sample size calculation[8,9]

Authors	Year	Population	Prevalence of liver fibrosis (%)	Sample size calculated
Lee et al.	2018	571 patients with T2DM in Malaysia	21.0	255
Kwok et al.	2016	1918 patients with T2DM in Hong Kong	17.7	224

We computed the sample size with 80% certainty (power) and alpha of 0.05. The calculation is based on the formula for sample size without finite population correction.[30]

$n = Z^2 P(1-P) / d^2$ where,

n = sample size

Z = Z statistic for a level of confidence = 1.96 for 95% level of confidence

P = Expected prevalence = 0.21

d = Precision = 0.05

Based on the study by Lee et al., we needed to include at least 255 patients. Accounting for an expected 20% drop-out rate, we planned to recruit a minimum of 319 patients.

Statistical analysis

The data analysis was done using SPSS version 22. Descriptive data were expressed as mean ± standard deviation (SD) unless otherwise stated. For demographic comparisons between patients with and without advanced liver fibrosis, the Chi-square or Fisher exact test was used for categorical variables, and independent *t*-test or Mann-Whitney U test was used for differences between continuous variables. Pearson correlation coefficient was used to evaluate correlations between LSM and FIB-4 Index.

Ethical Statement

The study was listed in the Malaysian National Medical Research Register (NMRR) (reference number NMRR-19-654-46941). The study protocol was approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia.

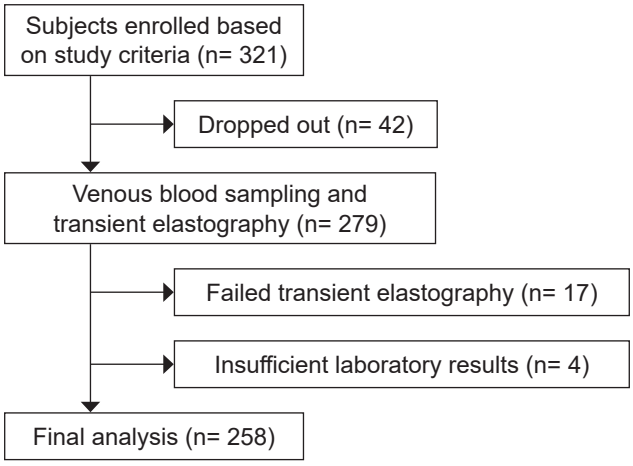

Figure 2. Study participant flow.

RESULTS

A total of 321 patients were recruited. Forty-two (13%) patients dropped out after recruitment for various reasons, 279 patients underwent venous blood sampling and transient elastography, 17 (6%) patients were unable to obtain valid results on transient elastography and 4 had insufficient results to proceed with further data analysis. Data from 258 patients were used for our final analysis (Figure 2).

Overview of the study population

The mean age of the 258 patients included in the analysis was 61.64 ± 10.35 years old. More than half were female (n=135, 52.3%). In the cohort, 38.0% were Chinese (n=98), 30.2% were Malays (n=78) and 28.7% were Indians (n=74). The more common associated co-morbidities were hypertension (n=188, 72.9%), ischemic heart disease (IHD) (n=67, 26.0%) and chronic kidney disease stage 3 and above (n=69, 26.7%).

The anthropometric indices showed that 21.3% of the subjects (n=55) were obese with a mean BMI of 27.04 ± 4.11 kg/m^2. The majority had central obesity (n=226, 87.6%) with a mean waist circumference of 95.69 ± 9.84 cm (Table 2).

Laboratory assessment

The median fasting blood sugar was 8.0 mmol/L and mean glycated hemoglobin was 8.3%. Median triglyceride (1.4 mmol/L), LDL cholesterol (2.1 mmol/L) and HDL cholesterol (1.2 mmol/L) levels were normal. Six patients had incalculable LDL cholesterol values because their triglyceride levels were more than 4.5 mmol/L (Table 2).

Prevalence of advanced liver fibrosis and associated factors

The prevalence of advanced fibrosis based on transient elastography was 22.1% (57 out of the 258 patients) (Table 3). Using simple logistic regression, the factors associated

Table 2. Baseline demographic and clinical profiles of the 258 subjects

Variables	n (%)	Mean ± SD
Age, in years		61.64 ± 10.35
Gender		
Male	123 (47.7)	
Female	135 (52.3)	
Ethnicity		
Chinese	98 (38.0)	
Malay	78 (30.2)	
Indian	74 (28.7)	
Others	8 (3.1)	
Hypertension	188 (72.9)	
Blood pressure control		SBP 134.68 ± 19.28 DBP 71.95 ± 9.29
Ischemic heart disease	67 (26.0)	
eGFR		
≥60 ml/min/1.73m^2	189 (73.3)	
<60 ml/min/1.73m^2	69 (26.7)	
BMI (kg/m^{-2}) †		27.04 ± 4.11
<30 kg/m^{-2}	203 (78.7)	
≥30 kg/m^{-2}	55 (21.3)	
Central obesity	226 (87.6)	
WC (cm)		95.69 ± 9.84
Male		97.05 ± 9.39
Female		94.46 ± 10.10
FBS (mmol/L) †		8.0 (5.15)
HbA1c (%)		8.3 ± 1.6 (67 ± 18)
Platelet count (10^9/L) †		269.50 (79.25)
Albumin (g/L)		37.97 ± 3.31
ALT (U/L) †		21.0 (16.0)
AST (U/L) †		20.0 (11.0)
GGT (U/L) †		29.0 (26.0)
TC (mmol/L) †		4.0 (1.2)
TG (mmol/L) †		1.40 (0.8)
LDL (mmol/L) †$		2.10 (1.0)
HDL (mmol/L) †		1.20 (0.4)

$ Missing values: LDL, 6. n=252
† Presented as median (IQR)
SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; WC, waist circumference; FBS, fasting blood sugar; HbA1c, glycated haemoglobin; TC, total cholesterol; LDL, low-density lipoprotein cholesterol

with advanced liver fibrosis were BMI, ALT, AST, GGT, TG, and HDL (Table 4).

Figure 3 shows the proportion of patients with advanced liver fibrosis based on different BMI cut-offs. For BMI greater than or equal to 23.0, 25.1% of patients who are overweight have advanced liver fibrosis. If the BMI cut-off is set at 27.5 kg/m^2 based on Malaysian obesity guidelines, 27.4% of patients have advanced liver fibrosis. However, when the BMI cut-off is set at 30 kg/m^2 based on WHO guidelines, the percentage of patients with advanced liver fibrosis increased to 38.2%.

By multiple logistic regression analysis, independent factors associated with advanced fibrosis were BMI and GGT (p=0.003 and p<0.001 respectively). Patients who were obese by WHO definition are 3.14 times more likely to develop advanced liver fibrosis (95% CI, 1.49 - 6.61). Patients who have elevated GGT are 8.39 times more likely to develop advanced liver fibrosis (95% CI, 4.20 - 16.78). This model predicted 81.7% of cases correctly with 68.2% sensitivity and 83.0% specificity. The model did not show any multicollinearity or interaction. Hosmer-Lemeshow test was not significant (p=0.967).

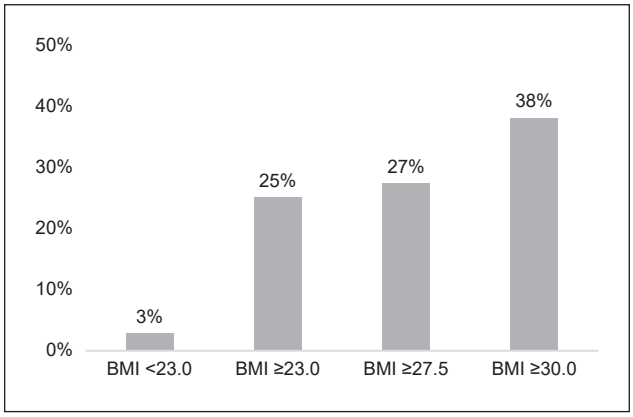

Figure 3. The proportion of patients with advanced liver fibrosis based on different BMI cut-offs.

FIB-4 index for the diagnosis of advanced liver fibrosis

The FIB-4 index should be used with caution among patients less than 35 or greater than 65 years old, as the score has been shown to be less reliable in these patients in the study by Shah et al.[20] We only included patients who are 35 years old and above. Including only the patients between 35 to 65 years old with a FIB-4 index cut-off of 1.31, the sensitivity, specificity, positive predictive value, and negative predictive value in detecting advanced liver fibrosis by LSM criteria were 30.0%, 85.0%, 38.7% and 79.4% respectively.

Serum ALT level for the diagnosis of advanced liver fibrosis

For the diagnosis of advanced liver fibrosis according to the WHO criteria, ALT cut-off was set at 30 U/L for males and 19 U/L for females. In our cohort, the combined sensitivity, specificity, positive predictive value, and negative predictive value of serum ALT were 64.9%, 63.7%, 33.6% and 86.5%, respectively.

DISCUSSION

In our cohort who had T2DM for at least 10 years, 22.1% had increased LSM suggestive of advanced liver fibrosis.

To date, there are two similar studies done in Southeast Asia. In 2016, a study published in Hong Kong recorded a prevalence of advanced liver fibrosis of 17.7%,[9] while another study done in Malaysia in 2018 recorded a prevalence of 21.0%.[8] The study cohort in Hong Kong had better glycaemic control (HbA1c 7.4%; FBS 7.4 mmol/L) compared to our cohort (HbA1c 8.3%; FBS 8.0 mmol/L). Their population had a mean waist circumference of 92.9cm, whereas ours had a mean waist circumference of 95.7cm. The BMI, cholesterol and blood pressure control were similar.

Table 3. Characteristics of patients with and without advanced fibrosis by Fibroscan®

Variables	Patients with advanced fibrosis, n=57		Patients without advanced fibrosis, n=201		*p*[b]
	Mean ± SD	n (%)	Mean ± SD	n (%)	
Age, in years	61.1 ± 8.6		61.8 ± 10.8		0.657
Gender					0.340
Female		33 (57.9)		102 (50.7)	
Male		24 (42.1)		99 (49.3)	
Ethnicity					0.651[c]
Chinese		19 (33.3)		79 (39.3)	
Malay		21 (36.8)		57 (28.3)	
Indians		16 (28.1)		58 (28.9)	
Others		1 (1.8)		7 (3.5)	
Systolic blood pressure	137.1 ±17.8		134.0 ± 19.7		0.283
Diastolic blood pressure	72.5 ± 8.7		71.8 ± 9.5		0.599
Blood pressure control					0.780
Controlled		34 (59.6)		124 (61.7)	
Uncontrolled		23 (40.4)		77 (38.3)	
Ischemic heart disease (Yes)		10 (17.5)		57 (28.4)	0.100
Serum creatinine, in mmol/L	78.0 (28.5) †		81.0 (34.0) †		0.822[d]
eGFR					0.673
≥60 ml/min/1.73m^2		43 (75.4)		146 (72.6)	
<60 ml/min/1.73m^2		14 (24.6)		55 (27.4)	
Insulin usage (Yes)		45 (78.9)		147 (73.1)	0.375
BMI, in kgm^{-2}					0.001
<30 kg/m^2		36 (63.2)		167 (83.1)	
≥30 kg/m^2		21 (36.8)		34 (16.9)	
WC, in cm	99.7 ± 10.1		94.6 ± 9.5		<0.001
Central Obesity[#]					0.162
No		4 (7.0)		28 (13.9)	
Yes		53 (93.0)		173 (86.1)	
FBS, in mmol/L					0.207
≤7.0		18 (31.6)		82 (40.8)	
>7.0		39 (68.4)		119 (59.2)	
HbA1c, in %					0.106
≤6.5		3 (5.3)		26 (12.9)	
>6.5		54 (94.7)		175 (87.1)	
Platelet Count, in x 10^9/L					0.124[c]
<150		2 (3.5)		1 (0.5)	
≥150		55 (96.5)		200 (99.5)	
Albumin in g/L					0.750
<35		8 (14.0)		25 (12.4)	
35-52		49 (86.0)		176 (87.6)	
ALT, in U/L[*]					<0.001
Normal		20 (35.1)		128 (63.7)	
Abnormal		37 (64.9)		73 (36.3)	
AST, in U/L					<0.001
<32		37 (64.9)		179 (89.1)	
≥32		20 (35.1)		22 (12.9)	
GGT, in U/L					<0.001
<40		16 (28.1)		156 (77.6)	
≥40		41 (71.9)		45 (22.4)	
TC, in mmol/L					0.273
<5.2		51 (89.5)		168 (83.6)	
≥5.2		6 (10.5)		33 (16.4)	
TG, in mmol/L					0.009
≤1.7		30 (52.6)		143 (71.1)	
>1.7		27 (47.4)		58 (28.9)	
LDL in mmol/L[$]					0.375
≤2.6		45 (83.3)		154 (77.8)	
>2.6		9 (16.7)		44 (22.2)	
HDL in mmol/L[&]					0.012
Normal		26 (45.6)		129 (64.2)	
Abnormal		31 (54.4)		72 (35.8)	

[a] The diagnosis of advanced fibrosis was based on LSM ≥9.6 kPa using the M probe or ≥9.3 kPa using the XL probe
[b] All variables were analysed using chi square tests (if categorical) or Student t-tests (if continuous) unless stated otherwise
[c] The variable was analysed using Fisher exact test
[d] The variable was analysed using Mann-Whitney U test as it is non-parametric
† Median (Interquartile range)
[#] Waist circumference: Male ≥90 cm; Female ≥80 cm
[*] ALT: normal: <30 U/L Male; <19 U/L Female. Abnormal: ≥30 U/L Male; ≥19 U/L Female
[$] Missing values: LDL, 6. n=252
[&] HDL: normal: >1.0 mmol/L Male; >1.2 mmol/L Female. Abnormal ≤1.0 mmol/L Male; ≤1.2 mmol/L Female

Table 4. Variables associated with the presence of advanced fibrosis by Fibroscan® using simple logistic regression and multiple logistic regression

Variable	Simple logistic regression				Multiple logistic regression		
	Crude OR	95% CI	X^2 stat (df)	*p*	Adjusted OR	95% CI	*p*
Age, in years	0.994	0.97 - 1.02	0.198 (1)	0.656			
Gender			0.913 (1)	0.341			
Male	1.000	ref					
Female	1.335	0.74 - 2.42					
Ethnicity			1.934 (3)	0.597			
Chinese	1.000	ref					
Malay	1.532	0.76 – 3.11		0.238			
Indian	1.147	0.54 – 2.42		0.719			
Others	0.594	0.07 – 5.12		0.636			
BP Control			0.078 (1)	0.780			
Controlled	1.000	ref					
Uncontrolled	1.089	0.60 – 1.99					
IHD			2.873 (1)	0.104			
No	1.000	ref					
Yes	0.538	0.25 – 1.14					
eGFR			0.180 (1)	0.673			
Stage <3	1.000	ref					
Stage ≥3	0.864	0.44 – 1.70					
Insulin usage	1.378	0.68 – 2.80	0.813 (1)	0.376			
BMI, in kgm^{-2}			9.608 (1)	0.002	3.136	1.49 - 6.61	0.003
<30 kg/m^2	1.000	ref					
≥30 kg/m^2	2.865	1.49 – 5.50					
WC, in cm	1.055	1.02 – 1.09	12.194 (1)	0.001			
Central Obesity[#]			2.185 (1)	0.171			
No	1.000	ref					
Yes	2.145	0.72 – 6.39					
FBS, in mmol/L			1.621 (1)	0.209			
≤7.0	1.000	ref					
>7.0	1.493	0.80 – 2.79					
HbA1c, in %			3.041 (1)	0.118			
≤6.5	1.000	ref					
>6.5	2.674	0.78 – 9.18					
Platelet Count, in x 10^9/L			2.761 (1)	0.108			
<150	7.273	0.65 – 81.70					
≥150	1.000	ref					
Albumin in g/L			0.100 (1)	0.750			
<35	1.149	0.49 – 2.71					
35-52	1.000	ref					
ALT, in U/L[*]			14.774 (1)	<0.001			
Normal	1.000	ref					
Abnormal	3.244	1.75 – 6.00					
AST, in U/L			16.533 (1)	<0.001			
<32	1.000	ref					
≥32	4.398	2.18 – 8.87					
GGT, in U/L			47.000 (1)	<0.001	8.394	4.20 -16.78	<0.001
<40	1.000	ref					
≥40	8.883	4.56 – 17.29					
TC, in mmol/L			1.288 (1)	0.277			
<5.2	1.000	ref					
≥5.2	0.599	0.24 – 1.51					
TG, in mmol/L			6.633 (1)	0.010			
≤1.7	1.000	ref					
>1.7	2.219	1.21 – 4.06					
LDL in mmol/L[$]			0.822 (1)	0.376			
≤2.6	1.000	ref					
>2.6	0.700	0.32 – 1.54					
HDL in mmol/L[&]			6.275 (1)	0.013			
Normal	1.000	ref					
Abnormal	2.136	1.18 – 3.88					

Multiple logistic regression Forward LR method was used to identify significant variables
Multicollinearity and interaction were checked and not found.
Hosmer-Lemeshow test was not significant (p=0.967).
The Pseudo R2 was 0.286 and the model predicted 81.7% of cases correctly.
Missing values: LDL, 6.
[#] Waist circumference: Male ≥90 cm; Female ≥80 cm
* ALT: normal: <30 U/L Male; <19 U/L Female. Abnormal: ≥30 U/L Male; ≥19 U/L Female
[$] Missing values: LDL, 6. n=252
[&] HDL: normal: >1.0 mmol/L Male; >1.2 mmol/L Female. Abnormal ≤1.0 mmol/L Male; ≤1.2 mmol/L Female

According to the Malaysian National Health and Morbidity Survey in 2019, 19.7% of our adult population was obese.[31] Our cohort reflected that, with 20.9% of the patients being obese. In our study model, BMI and GGT were identified as two independent factors associated with advanced liver fibrosis. Reducing the high prevalence of obesity will reduce the prevalence of NAFLD and advanced liver fibrosis.

Based on the WHO expert consultation published in 2004, the recommended BMI cut-off for obesity was 30 kg/m^2. For many Asian populations, additional trigger points for public health action were identified with a BMI of at least 23 kg/m^2 representing increased risk and a BMI of at least 27.5 kg/m^2 representing high risk.[32] With this in mind, we categorised our cohort based on different BMI cut-offs. According to Figure 2, at different BMI cut-offs less than 23.0 kg/m^2, greater than or equal to 23.0 kg/m^2, greater than or equal to 27.5 kg/m^2 and greater than or equal to 30.0 kg/m^2, the prevalence of advanced liver fibrosis was 2.9%, 25.1%, 27.4% and 38.2% respectively. There was a marked increase of 10.8% prevalence between the cut-offs of 27.5 kg/m^2 and 30 kg/m^2. Therefore, identifying obesity at a lower cut-off of 27.5 kg/m^2 in our local population instead of 30 kg/m^2 will allow earlier public health and clinical intervention to reduce the prevalence of NAFLD and advanced liver fibrosis.

Currently, treatment approaches for patients with T2DM and NAFLD include weight loss with lifestyle modification, medications such as GLP-1 RA or SGLT2-inhibitors, bariatric surgery, optimising control of cardiovascular risks factors (i.e., T2DM, hypertension and dyslipidaemia) and liver-directed therapies such as pioglitazone. Pioglitazone has shown to improve liver histology in patients with and without T2D with biopsy-proven NASH.[33] These treatment modalities are needed to prevent or slow the progression of NAFLD to advanced fibrosis.

Liver biopsy is considered the reference standard but is impractical to apply to a large study population. With its known limitations, the development and application of new imaging modalities and diagnostic scores can reduce the need for liver biopsy. In 2012, FibroScan® with CAP measurement emerged as a novel non-invasive, easy-to-perform tool developed to assess both hepatic steatosis and fibrosis simultaneously with high sensitivity and specificity.[34] Screening for NAFLD using FibroScan® among individuals with T2DM was recommended in the 2017 Asia–Pacific Working Party on Non–Alcoholic Liver Disease guidelines.[35]

Our FibroScan® machine model could only measure LSM to diagnose fibrosis, but not CAP to assess liver fat. Diagnosing NAFLD early in the disease spectrum is important because early intervention especially with lifestyle modification and treatment of associated comorbidities already mentioned will slow NAFLD disease progression.

Fibrosis assessment is also clinically important. Fibrosis is the most powerful (and possibly the only independent) prognostic factor for liver-related outcomes in NAFLD, including hepatocellular carcinoma development and mortality.[36,37] Having any fibrosis, particularly significant fibrosis with a fibrosis score of at least F2, is associated with increased mortality.[38,39] Currently, there are six phase III trials investigating five agents (cenicriviroc, elafibranor, obeticholic acid, resmetirom and aramchol) that could potentially lead to histological resolution of NASH, no worsening of fibrosis, and even improvement of fibrosis score by at least 1 stage.[40-45]

Although a good modality for liver fibrosis screening, FibroScan® is not readily available in many health centres. Even if it is available, it is impossible to apply it universally to the large number of diabetic patients. In 2009, Shah et al., concluded that the FIB-4 index is superior to 7 other non-invasive markers of fibrosis in patients with NAFLD.[16]

In our study, using a cut-off of 1.31, the FIB-4 index showed a sensitivity, specificity, positive predictive value, and negative predictive value in detecting advanced liver fibrosis by LSM criteria of 30.0%, 85.0%, 38.7% and 79.4% respectively. With a high specificity and negative predictive value, the FIB-4 index can be used to exclude advanced liver fibrosis in centres where FibroScan® is not readily available. A prospective follow-up study of this cohort will be good to determine if the changes in FIB-4 scores correspond to changes in fibrosis over time.

Elevated serum ALT is often used as an indicator for further liver assessment in our clinical setting. However, it is important to note that serum ALT is not ideal for NAFLD screening as it may be normal across the spectrum of the disease,[46] and may even be normal or low in advanced liver fibrosis. Our study showed that ALT has low sensitivity and specificity in diagnosing advanced liver fibrosis.

It is important to note that the WHO guideline and Prati et al., defined the upper limit of normal ALT as 30 U/L for men and 19 U/L for women.[47,48] Usually, our local laboratories give a higher cut-off. For example, the laboratory in our study centre gives a single cut-off of 33 U/L. This would have misled many uninformed doctors and missed a large proportion of patients at risk of liver disease.

All available modalities have their strengths and limitationsand it is important to help decide when to use the appropriate test in the evaluation of patients with NAFLD and advanced liver fibrosis. It is important to identify the risk factors associated with advanced liver fibrosis to prioritise those at highest risk.

Our study had adequate sample size and power to examine the intended primary objective. We applied one of the best non-invasive tests for liver fibrosis to date. All FibroScan® examinations were performed by one dedicated

experienced operator to provide accurate and reliable LSM results. Lastly, we had comprehensive anthropometric and blood parameters for assessment.

Despite our best effort, there were nevertheless, several limitations. First, this was a single-centre study done in a tertiary care centre. This may not reflect the true prevalence in our population. Second, diagnosis of hepatitis B, C and autoimmune hepatitis were based on known medical history alone. Third, our FibroScan® machine model was only able to measure LSM to diagnose fibrosis, but not CAP to assess liver fat. Fourth, our centre did not have a dedicated ultrasonographer to perform hepatobiliary ultrasound to correlate with the FibroScan® findings. Lastly, our study did not include liver biopsy to assess the histological correlation with the LSM finding on FibroScan®.

CONCLUSION

Our study has confirmed the high prevalence of advanced liver fibrosis based on transient elastography among patients with long-standing T2DM. This study suggests the benefit of advanced liver fibrosis screening in patients with T2DM greater than 10 years in duration especially those with high BMI and GGT. Transient elastography and FIB-4 index have limitations as do other non-invasive tests for fibrosis. Understanding the caveats associated with the utility of each modality will optimize their use in clinical practice.

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Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
XHL: Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft preparation, writing – review and editing, visualization, supervision, project administration, funding acquisition; **LMN:** Formal analysis, investigation, resources, data curation, writing – review and editing, visualization; **CSA:** Formal analysis, investigation, resources, data curation, writing – review and editing, visualization; **TPY:** Conceptualization, methodology, validation, resources, writing – review and editing, visualization, supervision; **SLL:** Conceptualization, methodology, validation, writing – review and editing, visualization, supervision, project administration.

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