Original Article

Prevalence and Risk Factors of Non-alcoholic Fatty Liver Disease in Type 2 Diabetes Mellitus Patients with Normal Serum Aminotransferase Levels

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Objective: Non-alcoholic fatty liver disease [NAFLD] is more common and more severe in patients with type 2 diabetes mellitus [T2DM], however the prevalence and severity of NAFLD in T2DM patients with normal serum aminotransferase [ALT] is unclear. This study aimed to evaluate the prevalence and risk factors of NAFLD and liver fibrosis in T2DM patients with normal serum aminotransferase.

Materials and Methods: T2DM patients with persistently normal serum alanine aminotransferase (ALT, defined by ALT ≤40 IU/L for ≥2 occasions during ≥6 months) were evaluated by controlled attenuation parameter and transient elastography [CAP-TE] between January 2017 and September 2017. Exclusion criteria were T1DM, significant alcohol drinking, chronic viral hepatitis, and the use of medications that may affect NAFLD. The cut-offs for steatosis were CAP 215 dB/m for S1 and CAP 252 dB/m for S2, whereas for fibrosis were TE 7.0 kPa for significant fibrosis and TE 10.0 kPa for advanced fibrosis (NAFLD defined by ≥S1).

Results: One hundred and eighty patients were included; 65.6% were female with median age of 59.5 (range 27 to 80) years. Median body mass index [BMI] was 26.2 (range 16.8 to 42.2) kg/m^2 and 55.6% were obese (BMI ≥25 kg/m^2). The median duration of T2DM was 8 years (range 0.25 to 40) years and 37.8% had microvascular complications. Prevalence of NAFLD was 82.8% (64.4% were ≥S2). Prevalence of NAFLD with significant fibrosis and advanced fibrosis were 24.4% and 11.1%, respectively. By multivariate analysis, independent predictors for steatosis were female,obesity, and triglyceride level and independent predictor for significant and advanced fibrosis was obesity.

Conclusion: NAFLD and fibrosis is relatively common among T2DM patients with normal ALT. Obesity is a predictor for steatosis and fibrosis in this population.

Keywords: Advanced fibrosis, Aminotransferase, Controlled attenuation parameter, Diabetes mellitus, Liver stiffness, NAFLD, Non-alcoholic fatty liver disease, Significant fibrosis, Transient elastography

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Non-alcoholic fatty liver disease [NAFLD] is a chronic liver disease characterized histologically by hepatic steatosis without identifiable cause such as alcohol, viral hepatitis, drugs or toxins. The histopathology of NAFLD can be classified into 2 subgroups; non-alcoholic fatty liver [NAFL] or simple steatosis and non-alcoholic steatohepatitis [NASH]. NAFL is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning degeneration, whereas NASH is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis[(1)].

Currently, NAFLD is the most common form

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of chronic liver disease with an increasing trend worldwide. Risk factors of NAFLD include obesity, type 2 diabetes mellitus [T2DM], dyslipidemia and metabolic syndrome. Prevalence of NAFLD varies among populations (tends to be more common in Western than in Asian populations) and the method used for diagnosis.Two Japanese studies reported an incidence rate of 31 and 86 cases of suspected NAFLD per 1,000 person-years respectively[2,3]. The reported prevalence of NAFLD when defined by ultrasound ranged between 17 to 46% depending on the population studied. The estimated worldwide prevalence of NAFLD ranges from 6.3 to 33% with a median of 20% in the general population, based on various assessment methods[4]. On the other hand, NASH accounts for about one-third of patients with NAFLD, thus the estimated prevalence of NASH is lower, ranging from 3 to 5%. Both excessive body mass index [BMI] and visceral obesity are well recognized risk factors for NAFLD.

Notably, NAFLD appears to be more common and more severe in patients with T2DM. In an Indian study, the prevalence of NAFLD in patients with T2DM was 87% by ultrasonography with further histologic confirmation[5]. In addition, several other factors (e.g. age, gender, diet and ethnicity), also affected the prevalence of NAFLD with variable extents. Patients with NAFLD have an increased risk of overall mortality, mainly from cardiovascular disease, while patients with NASH also have an increased risk of liver-related mortality[6]. Serum ALT levels are unnecessary to be elevated for the diagnosis of NAFLD and it does not correlate with the degree of inflammation and fibrosis among patients with NAFLD[1]. Therefore, a proportion of patients with NAFLD can associate with progressive liver disease and hepatocellular carcinoma even with normal serum ALT levels.

One of the most important negative prognostic predictor for liver-related morbidity among patients with NAFLD is the presence of liver fibrosis[7]. The gold standard to detect and to quantify liver fibrosis is histology by liver biopsy. However, non-invasive methods particularly the measurement of transient elastography, are increasingly utilized as a first-line alternative method, mainly due to its non-invasive nature with reasonable accuracy and cost. Unfortunately, the available data regarding the prevalence of NAFLD and NASH in T2DM patients with normal ALT is quite limited, particularly the data from Asian population.

This study aims to evaluate the prevalence and risk factors of NAFLD in Thai T2DM patients with persistently normal serum ALT levels.

Materials and Methods

Study population

This cross-sectional analytical study was conducted at the Department of Gastroenterology, Rajavithi Hospital, a tertiary care hospital and referral center in Bangkok, Thailand. The protocol of this research was reviewed and approved by the Ethics Committee of Rajavithi Hospital. Written informed consents were obtained from all patients prior to enrollment.

Patients were consecutively recruited from Diabetic Mellitus Outpatient Clinic between January 2017 and September 2017. Inclusion criteria were T2DM patients, aged between 18 to 80 years old, with the AST and ALT levels $\leq$40 IU/L at least 2 occasions during the past 6 months before the day of enrollment. Exclusion criteria were pre-existing chronic liver disease (e.g. hepatitis B and C, autoimmune hepatitis, hemochromatosis, Wilson disease, cirrhosis of any etiologies), hepatobiliary malignancy, metastatic liver disease, excessive alcohol drinking (>21 drinks/week in male and >14 drinks/week in female)[1], T1DM, human immunodeficiency virus [HIV], history of methotrexate use, history of drugs affecting hepatic steatosis (e.g. vitamin E, thiazolidine dione, steroids and hormonal therapy) during the previous 6 months, pregnancy or lactating women, chronic kidney disease stage 4 to 5, and congestive heart failure with New York Heart Classification >grade II.

We calculated the sample size based on the prevalence data from previous study[8], in which the prevalence of NAFLD in T2DM patients with normal serum aminotransferase levels was 50%. The calculated sample size was 171.

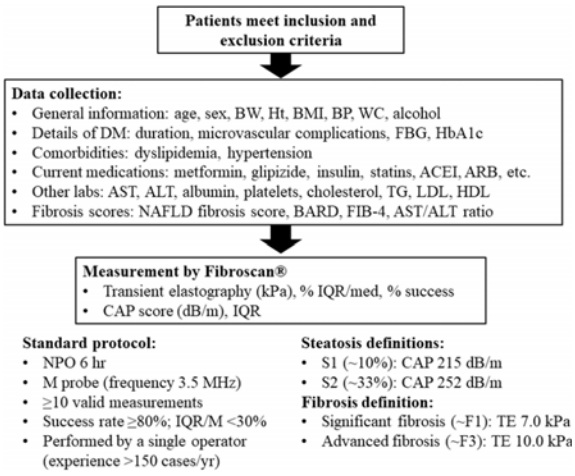
Study design

The study flow chart was summarized in Figure 1. Patients who fulfilled inclusion and exclusion criteria were enrolled and informed consent was provided. Baseline clinical information (e.g. age, sex, body weight, height, BMI, waist circumference, blood pressure, underlying diseases, duration of T2DM, complications of T2DM, and current medications) and laboratory parameters (e.g. fasting blood glucose, HbAIC, AST, ALT, albumin, platelet count, lipid profiles) were collected. Available parameters and scoring system for predicting liver fibrosis in NAFLD including NAFLD fibrosis score[9], BARD score[10], Fibrosis-4 (FIB-4) score[11], and AST/ALT ratio[12,13],

were also calculated in order to analyze the prognostic score which could correlate to fibrosis in NAFLD.

After data collection, allpatients were measured transient elastography [TE] and controlled attenuation parameter [CAP] by Fibroscan (Echosen®, Paris) by an experienced (>150 cases per year) and certified nurse (TT) under a standardized protocol (Figure 1). The interpretation of TE-CAP results was based on the definition recommended by previous studies and the EASL-ALEH clinical practice guidelines[14,15]. NAFLD was defined by CAP score ≥215 dB/m (≥S1 grade steatosis). NAFLD with significant fibrosis was defined by CAP score ≥215 dB/m and TE ≥7.0 kPa and advanced fibrosis was defined as TE ≥10.0 kPa.

The primary outcome was the prevalence of NAFLD (defined by CAP ≥215 dB/m) in T2DM patients with normal serum ALT. The secondary outcomes were risk factors of NAFLD, prevalence and risk factors of significant fibrosis and prevalence and risk factors of advanced fibrosis in T2DM patients with normal serum ALT.



ACEI = angiotensin-converting-enzyme inhibitor; ARB = angiotensin II receptor blocker; AST = aspartate aminotransferase; ALT = alanine aminotransferase; BMI = body mass index; BP = blood pressure; BW = body weight; CAP = controlled attenuation parameter; DM = diabetes mellitus; FBG = fasting blood glucose; HDL = high-density lipoprotein; Ht = height; IQR = interquartile range; IQR/M = interquartile range/median; LDL = low-density lipoprotein; TE = transient elastography; TG = triglyceride; WC = waist circumference

Figure 1. Study flow chart and operational definitions.

Statistical analysis

All statistical analyses were performed using SPSS for Windows version 22.0 (SPSS Inc., Chicago, Illinois, USA). Demographics data and baseline characteristics, including fibrosis scores and TE-CAP data were summarized using descriptive statistics such as mean ± SD or median (range). Categorical variables were compared using the Chi-square test or Fisher exact test. Continuous variables were compared using the independent t-test or the Mann-Whitney U test. Binary logistic regression analysis was used to analyze the independent predictors for NAFLD, NAFLD with significant fibrosis and advanced fibrosis and were presented by odds ratio [OR]. All statistical examinations were 2-tailed with *p*-value <0.05 was defined as statistically significant.

Results

Patient baseline characteristics

A total of 211T2DM patients with normal ALT levels were identified from the hospital records and were all invited to participate in the study. Of these, 31 patients (14.7%) were excluded (10 denied participation, 14 had history of significant alcohol drinking, 4 had positive viral hepatitis serology, and 3 were unable to achieve valid TE-CAP measurement by M-probe). Finally, a total of 180 T2DM patients with persistently normal ALT levelswere enrolled including 118 (65.6%) women and 62 (34.4%) men. The median age was 59.5 (range 27 to 80) years. Mean body weight was 67.8±14.8 kg, mean height was 158.7±8.0 cm, and mean BMI was 26.8±4.8 kg/m^2. Based on the Asian BMI criteria, 78.3% were overweight (BMI ≥23 kg/m^2), 55.6% were obese (BMI ≥25 kg/m^2) and 22.8% were severely obese (BMI ≥30 kg/m^2). Mean waist circumference was 90.1±11.2 cm, and the prevalence of abdominal obesity (female ≥80 cm or male ≥90 cm) was 64.4%. The median duration of T2DM diagnosis was 8 years (range 0.25 to 40 years). Sixty-eight patients (37.8%) had microvascular complications. Concomitant diseases were present in the majority of patients.

Mean fasting plasma glucose was 142±38 mg/dL, and mean HbA_1C was 8.0±6.5%. Mean AST was 20±6 IU/L, ALT was 21±8 IU/L, and 62.8% of patients were considered to have healthy ALT levels according to the AASLD practice guidelines (female ALT ≤19 IU/L, male ALT ≤30 IU/L). Concurrent medications included metformin (88.9%), sulfonylure as (49.4%), statins (88.9%), angiotensin converting enzyme inhibitors (31.7%), angiotensin II receptor blockers (21.7%), insulin (30%), and antiplatelets, mostly aspirin and/or

Table 1. Demographic and laboratory data of the 180 patients

Variables	Mean ± SD or n (%)	Median (range)
Age (years)	58.5±12.0	59.5 (27 to 80)
Sex		
Female	118 (65.6)	
Male	62 (34.4)	
Body weight(kg)	67.8±14.8	66 (23.3 to 108)
Height(cm)	158.7±8.0	159 (135 to 178)
BMI (kg/m^2)	26.8±4.8	26.2 (16.8 to 42.2)
≥23	141 (78.3)	
≥25	100 (55.6)	
≥30	41 (22.8)	
Waist (cm)	90.1±11.2	89 (66 to 127)
Female ≥80, male ≥90	116 (64.4)	
Systolic blood pressure (mmHg)	132.4±17.9	132 (94 to 191)
Diastolic blood pressure (mmHg)	73.6±11.6	74 (20 to 98)
Alcohol	4 (2.2)	
Hypertension	129 (71.7)	
Dyslipidemia	163 (90.6)	
DM (years)	9.7±8.2	8 (0.25 to 40)
≥10 years	82 (45.6)	
Microvascular complications	68 (37.8)	
Labs		
Fasting plasma glucose (mg/dL)	142.0±38.2	137 (65 to 374)
HbA_1C (%)	7.55±1.8	7.1 (4.2 to 14.8)
AST (IU/L)	20.4±5.7	20 (10 to 40)
ALT (IU/L)	20.7±7.5	20 (6 to 39)
Healthy ALT (female ≤19, male ≤30)	113 (62.8)	
Albumin (g/dL)	4.4±0.4	4.5 (2.9 to 5.2)
Platelets (per mm^3)	288,973±81,054	283,500 (113,000 to 669,000)
Cholesterol (mg/dL)	178.7±41.8	170 (94 to 414)
LDL cholesterol (mg/dL)	108.9±32.7	103 (48 to 214)
Triglyceride (mg/dL)	157.0±101.3	137 (45 to 823)
HDL cholesterol (mg/dL)	53.2±15.0	50 (10 to 96)
Medications		
Metformin	160 (88.9)	
Sulfonylurea	89 (49.4)	
Statin	160 (88.9)	
ACEI	57 (31.7)	
ARB	39 (21.7)	
Insulin	54 (30)	
Antiplatelets	51 (28.3)	

ACEI = angiotensin-converting-enzyme inhibitor; ARB = angiotensin II receptor blocker; AST = aspartate aminotransferase; ALT = alanine aminotransferase; BMI = body mass index; DM = diabetes mellitus; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SD = standard deviation

clopidogrel (28.3%).The demographic, clinical and laboratory characteristics of the patient are summarized in Table 1 and Table 2.

Four scoring systems including NAFLD fibrosis score, BARD score, FIB-4 score, and AST/ALT ratio, were calculated in order to predict liver fibrosis, and were summarized in Table 2. Advance fibrosis was predicted in 2.8%, 89.4%, 0.6%, and 79.4% according to these scores, respectively.

The measurements of TE-CAP were summarized in Table 2 and Figure 2. Prevalence of significant (TE ≥7 kPa) and advanced fibrosis (TE ≥10

Table 2. Fibrosis scoring systems and measurement of transient elastography

Variables	Mean±SD or n (%)	Median (range)
NAFLD fibrosis score	-1.5±1.36	-1.34 (-6.8 to 1.99)
No fibrosis	86 (47.8)	
Intermediate	89 (49.4)	
Fibrosis	5 (2.8)	
BARD		
BARD 1	19 (10.6)	
BARD 2	18 (10)	
BARD 3	93 (51.7)	
BARD 4	50 (27.8)	
FIB-4	1.02±0.47	0.93 (0.26 to 2.68)
No fibrosis	134 (74.4)	
Intermediate	44 (24.4)	
Fibrosis	1 (0.6)	
AST/ALT ratio	1.07±0.35	1 (0.56 to 2.71)
AST/ALT ratio ≥0.8	143 (79.4)	
AST/ALT ratio ≥1.0	93 (51.7)	
Transient elastography (kPa)	6.2±2.7	5.3 (2.9 to 18.8)
Significant fibrosis	51 (28.3)	
Advanced fibrosis	20 (11.1)	
Significant fibrosis with CAP ≥215	44 (24.4)	
Advanced fibrosis with CAP ≥215	17 (9.4)	
IQR/median (%)	12.6±5.5	12 (2 to 32)
CAP (dB/m)	269.3±62	274 (112 to 400)
≥S1 steatosis	149 (82.8)	
≥S2 steatosis	116 (64.4)	
≥S3 steatosis	58 (32.2)	
IQR	32.7±21	27 (4 to 189)

AST = aspartate aminotransferase; ALT = alanine aminotransferase; CAP = controlled attenuation parameter; IQR = interquartile range

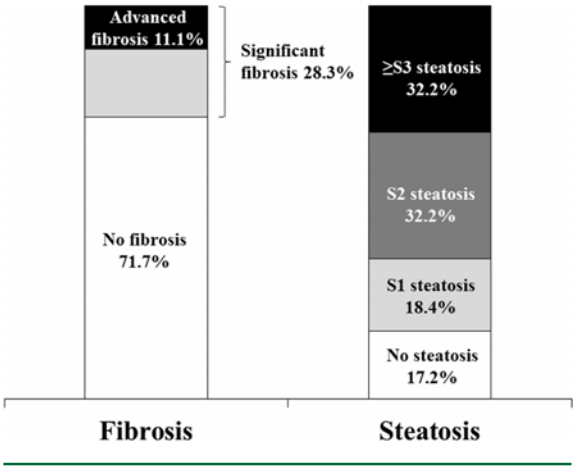


Figure 2. Prevalence of liver fibrosis and steatosis according to the measurement of transient elastography (TE) and controlled attenuation parameter (CAP).

kPa) was 28.3% and 11.1%, respectively. Prevalence of NAFLD with ≥S1 steatosis (CAP ≥215 dB/m) was 82.8%, and with ≥S2 steatosis (CAP ≥252 dB/m) was 64.4%. Prevalence of NAFLD with significant fibrosis was 24.4%, and advanced fibrosis was 9.4%.

Predictors for liver steatosis

In the univariate analysis for ≥S1 steatosis, significant predictors were female, body weight, BMI ≥25 kg/m^2, waist circumference, triglyceride, and AST/ALT <1 (Table 3). In the multivariate analysis, significant predictors were female (OR 3.42, 95% CI 1.43 to 8.19, $p = 0.006$], BMI ≥25 kg/m^2 (OR 3.81, 95% CI 1.53 to 9.47), $p = 0.004$), and triglyceride (OR 1.01, 95% CI 1 to 1.02, $p = 0.022$) (Table 3).

In the univariate analysis for ≥S2 steatosis, significant predictors were body weight, BMI ≥25 kg/m^2, waist circumference, HbA1C, triglyceride, and AST/ALT <1. In the multivariate analysis, significant

Table 3. Logistic regression analysis of ≥S1 steatosis

Univariate analysis of ≥S1 steatosis Variables	≥S1 steatosis (n = 149)	No (n = 31)	Odds ratio (95% CI)	*p*-value
Age (years)	59.2±11.2	55.2±14.8	1.03 (1.00 to 1.06)	0.094
Female	104 (69.8)	14 (45.2)	2.81 (1.18 to 6.70)	0.009*
Body weight (kg)	69.6±14.1	58.7±15	1.07 (1.03 to 1.10)	<0.001*
BMI (kg/m^2)	27.6±4.7	23.3±3.6	1.33 (1.16 to 1.51)	<0.001*
≥25	91 (61.1)	9 (29)	3.84 (1.55 to 10.08)	0.001*
Waist (cm)	91.4±11.1	83.0±9.4	1.08 (1.03 to 1.14)	0.001*
Female ≥80, Male ≥90	105 (70.5)	11 (35.5)	4.34 (1.79 to 10.83)	<0.001*
LDL cholesterol (mg/dL)	108.9±30.5	108.8±42.5	1.00 (0.99 to 1.01)	0.998
Triglyceride (mg/dL)	166.5±105.9	111.8±59.0	1.01 (1.00 to 1.02)	0.003*
HDL cholesterol (mg/dL)	52.3±14.0	57.7±18.6	0.98 (0.95 to 1.00)	0.071
AST/ALT ≥1	71 (47.7)	22 (71)	0.37 (0.14 to 0.91)	0.018*
Multivariate analysis for ≥S1 steatosis Variables			**Adjusted odds ratio (95% CI)**	***p*-value**
Female			3.42 (1.43 to 8.19)	0.006*
BMI ≥25 kg/m^2			3.81 (1.53 to 9.47)	0.004*
Triglyceride			1.01 (1.00 to 1.02)	0.022*
AST/ALT ≥1			0.50 (0.19 to 1.29)	0.150

AST = aspartate aminotransferase; ALT = alanine aminotransferase; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein

predictors were BMI ≥25 kg/m^2 (OR 2.72, 95% CI 1.36 to 5.42, $p = 0.004$), and triglyceride (OR 1.01, 95% CI 1 to 1.02, $p = 0.001$).

Predictors for significant fibrosis

In the univariate analysis of significant fibrosis, significant predictors were body weight, BMI ≥25 kg/m^2, waist circumference, dyslipidemia, triglyceride, and HDL.In the multivariate analysis, the only significant predictor for significant fibrosis was BMI ≥25 kg/m^2 (OR 3.13, 95% CI 1.39 to 7.03, $p = 0.006$).

In the univariate analysis of NAFLD with significant fibrosis, significant predictors were body weight, BMI ≥25 kg/m^2, waist circumference, microvascular complications, triglyceride, HDL, and AST/ALT <1 (Table 4). In the multivariate analysis for NAFLD with significant fibrosis, the only significant predictor was BMI ≥25 kg/m^2 (OR 4.02, 95% CI 1.68 to 9.6, $p = 0.002$) (Table 4).

Predictors for advanced fibrosis

In the univariate analysis of advanced fibrosis, significant predictors were body weight, BMI ≥25 kg/m^2, waist circumference, microvascular complications, HDL, and insulin use. In the multivariate analysis, the only significant predictor for advanced fibrosis was BMI ≥25 kg/m^2 (OR 6.48, 95% CI 1.4 to 29.86, $p = 0.017$) (Table 5).

Discussion

In this prospective cross-sectional study using CAP as a diagnostic method, we demonstrated that the prevalence of NAFLD in T2DM patients with normal ALT levels was high (≥S1 steatosis 82.8% and ≥S2 steatosis 64.4%) similarly to or even higher than of the data from Western countries. Previous study by Portillo-Sanchez et al[8] using liver magnetic resonance spectroscopy [H-MRS] as a diagnostic method reported that prevalence of NAFLD in T2DM patients with normal ALT was 50%. Recent study by Saokaew et al reported that the prevalence of NAFLD in Thailand was 21.3% in general population and 36.3% in patients with DM (OR 2.31). It should be noted that about 85% of patients in this study have ALT <40 IU/L)[16]. Unfortunately, to our knowledge, there was not much available data on the prevalence of NAFLD specifically in T2DM patients with normal ALT levels from Thailand. The high prevalence of NAFLD in this study may be partly explained by the high prevalence of obesity (55.6% according to the Asian BMI criteria),

Table 4. Logistic regression analysis of non-alcoholic fatty liver disease with significant fibrosis

Univariate analysis of NAFLD with significant fibrosis Variables	Significant fibrosis with CAP ≥215 (n = 44)	No (n = 136)	Odds ratio (95%CI)	*p*-value
Age (years)	58.0±11.9	58.6±12.0	1.00 (0.97 to 1.02)	0.765
Female	31 (70.5)	87 (64)	1.34 (0.61 to 3.07)	0.431
Body weight (kg)	74.2±14.3	65.7±14.4	1.04 (1.02 to 1.06)	0.001*
BMI (kg/m^2)	29.5±5.0	26±4.4	1.17 (1.08 to 1.26)	<0.001*
≥25	35 (79.5)	65 (47.8)	4.25 (1.81 to 10.76)	<0.001*
Waist (cm)	96.4±12.9	88±9.8	1.07 (1.04 to 1.11)	<0.001*
Female ≥80, male ≥90	35 (79.5)	81 (59.6)	2.64 (1.13 to 6.72)	0.016*
Microvascular complications	23 (52.3)	45 (33.1)	2.21 (1.05 to 4.68)	0.023*
Triglyceride (mg/dL)	196.6±148.7	144.1±76.6	1.00 (1.00 to 1.01)	0.010*
HDL cholesterol (mg/dL)	48.3±11.3	54.8±15.7	0.97 (0.94 to 0.99)	0.015*
AST/ALT ≥1	16 (36.4)	77 (56.6)	0.44 (0.20 to 0.93)	0.019*
Multivariate analysis of NAFLD with significant fibrosis Variables			Adjusted odds ratio (95% CI)	*p*-value
BMI ≥25 kg/m^2			4.02 (1.68 to 9.60)	0.002*
Microvascular complications			2.03 (0.94 to 4.36)	0.070
Triglyceride			1.00 (1.00 to 1.01)	0.422
HDL cholesterol			0.98 (0.96 to 1.01)	0.199
AST/ALT ≥1			0.55 (0.25 to 1.22)	0.140

AST = aspartate aminotransferase; ALT = alanine aminotransferase; BMI = body mass index; HDL = high-density lipoprotein; NAFLD = non-alcoholic fatty liver disease

long duration of DM (45.6% have had DM for ≥10 years) and multiple features of metabolic syndrome among subjects in this study.

Prevalence of NAFLD with significant fibrosis in this study was 24.4% and advanced fibrosis was 11.1%. Independent predictors for steatosis were female, obesity, and triglyceride level, and the only independent predictor for fibrosis was obesity. Obesity has long been known to be associated with the presence of NAFLD in general population. Thus, it is also known to be associated with steatohepatitis and advanced fibrosis among patients with NAFLD, with or without T2DM[1,7]. However, it should be noted that 15 to 21% of NAFLD patients in Asia-Pacific in some series were found to be non-obese[17]. Similar to patients with NAFLD in general, the findings in this study reconfirm the important role of obesity in the prediction of NAFLD and fibrosis among those patients with T2DM and persistently normal ALT.

Several non-invasive fibrosis scoring systems calculated from multiple simple laboratory parameters, in particular NAFLD fibrosis score, BARD score, FIB-4 score, and AST/ALT ratio, have been widely utilized in clinical practice. They can be used to identify patients with advanced fibrosis among NAFLD with the acceptable sensitivity and high negative predictive values so that liver biopsy could potentially be avoided in a significant proportion of patients. These non-invasive scoring systems have been developed in the Western population, and subsequently also validated in Thai population[18]. Interestingly, fibrosis scores including NAFLD fibrosis score, BARD score, FIB-4 score, and AST/ALT ratio calculated showed no correlation with the degree of liver fibrosis in this study. It should be noted that these scores include AST and/or ALT levels as one parameter in the equation so that the performance of the scores may be reduced in the lower ALT ranges of <40 IU/L as in this study. However, the performance of non-invasive fibrosis scoring systems in this specific group of NAFLD population warrants further evaluations.

Apart from metabolic risk factors, elevation

Table 5. Logistic regression analysis of advanced fibrosis

Univariate analysis of advanced fibrosis Variables	Advanced fibrosis (n = 20)	No advanced fibrosis (n = 160)	Odds ratio (95% CI)	*p*-value
Age (years)	56.4±10.8	58.8±12.1	0.98 (0.95 to 1.02)	0.396
Female	11 (55)	107 (66.9)	0.61 (0.21 to 1.77)	0.292
Body weight (kg)	77.3±13.7	66.6±14.5	1.05 (1.02 to 1.08)	0.003*
BMI(kg/m^2)	30.4±5.9	26.4±4.5	1.17 (1.07 to 1.29)	0.001
≥25	17 (85)	83 (51.9)	5.26 (1.43 to 28.86)	0.005*
Waist(cm)	98.8±13.5	89.0±10.5	1.08 (1.03 to 1.13)	0.001*
Female≥80, Male≥90	15 (75)	101 (63.1)	1.75 (0.57 to 6.46)	0.296
Microvascular complications	12 (60)	56 (35)	2.79 (0.97 to 8.31)	0.030*
Triglyceride(mg/dL)	199.2±154.9	151.7±91.8	1.00 (1.00 to 1.01)	0.068
HDL cholesterol(mg/dL)	44.6±9.2	54.3±15.2	0.95 (0.91 to .99)	0.010*
Insulin	10 (50)	44 (27.5)	2.64 (0.91 to 7.56)	0.038*
NAFLD fibrosis score	-1.2±1.1	-1.5±1.4	1.24 (0.86 to 1.80)	0.250
No fibrosis	7 (35)	79 (49.4)	0.55 (0.18 to 1.59)	0.225
Intermediate	12 (60)	77 (48.1)	1.62 (0.57 to 4.81)	0.317
Fibrosis	1 (5)	4 (2.5)	2.05 (0.04 to 22.10)	0.521
BARD				
BARD 1	1 (5)	18 (11.3)	0.42 (0.01 to 2.96)	0.391
BARD 2-4	19 (95)	142 (88.8)	2.41 (0.34 to 105.54)	0.391
FIB-4	1.0±0.5	1.0±0.5	0.87 (0.31 to 2.41)	0.785
No fibrosis	17 (85)	117 (73.1)	2.08 (0.56 to 11.60)	0.251
Intermediate	3 (15)	41 (25.6)	0.51 (0.09 to 1.91)	0.297
Fibrosis	0	1 (0.6)	0 (0 to 1.00)	0.723
AST/ALT	1.0±0.5	1.1±0.3	0.75 (0.18 to 3.15)	0.693
AST/ALT ≥1.0	7 (35)	86 (53.8)	0.46 (0.15 to 1.33)	0.114
Multivariate analysis for advanced fibrosis Variables			**Adjusted odds ratio (95% CI)**	***p*-value**
BMI ≥25 kg/m^2			6.48 (1.40 to 29.86)	0.017*
Microvascular complications			2.42 (0.81 to 7.26)	0.115
HDL cholesterol			0.96 (0.93 to 1.00)	0.073
Insulin use			1.85 (0.62 to 5.53)	0.273

AST = aspartate aminotransferase; ALT = alanine aminotransferase; BMI = body mass index; HDL = high-density lipoprotein

of ALT appears to be another important predictor for the presence of NAFLD. Based on study from Thailand, serum ALT >40 IU/L is an independently strong predictor of NAFLD with an adjusted OR of 4.49[16]. However, serum ALT levels may not necessarily correlate with the degree of inflammation and fibrosis in patients with NAFLD, and a proportion of NAFLD patients with normal ALT levels can have significant steatosis, inflammation and/or fibrosis in their livers. These patientsgenerally perceive their livers are healthy. Thus, they have also been overlooked by many physicians because the traditional concept that the levels of ALT less than the upper limits of normal (<40 IU/L) are truly normal. However, studies have used the data from the NHANES databases to assess risk of morbidity and mortality in relationship to abnormal liver tests with one study demonstrating that patients with ALT <40 IU/L, but with ALT >30 U/L for men and >19 U/L for women were associated with significant increase in liver-related mortality (11.2 folds) and DM-related mortality (3.3 folds)[19]. Although the American guidelines proposed a decrease in the upper limits of normal ALT from 40 IU/L to 30 IU/L in men and 19 IU/L in women, this recommendation has not

been widely implemented[20]. Based on our findings, the prevalence of NAFLD in T2DM patients was very high even with serum ALT levels within the normal range, either by the traditional cut-off of the recent US recommendations. Previous studies have shown that liver fibrosis is the important predictor that correlates with liver-related mortality in T2DM patients with NAFLD[6,7]. In addition, almost 30% of T2DM patients with normal serum ALT may have significant liver fibrosis, which put them at increased risk of developing progressive liver disease in the future. Therefore, it is very important to identify these higher risk patients in order to provide early interventions such as intensive life-style modification and pharmacological treatment before the development of advanced fibrosis. Taken together, it should be kept in mind that all T2DM patients are at risk of having NAFLD regardless of their ALT levels. Assessments for liver biochemical test and/or non-invasive fibrosis scores may not be adequate to assess liver steatosis and fibrosis in T2DM patients since they are unable to diagnose NAFLD and may also miss to diagnose about one-third of patients with significant fibrosis. Evaluation with non-invasive imaging techniques (e.g. TE-CAP or magnetic resonance elastography) appear to be helpful as a screening modality, particularly if the patients are obese. Patients with significant liver disease detected by this screening technique should receive more aggressive life-style/pharmacologic interventions and, in selected cases, undergoing further liver evaluations. Nevertheless, this suggestion requires further validations especially in terms of accuracy and cost-effectiveness.

This study has several limitations. First, this was a single-center study with relatively limited number of patients, and we also acknowledge that our patient population was predominantly overweight and obese female. Therefore, larger studies with more number and more heterogeneity of patients are further required to confirm our findings. Another major limitation of our study was that we used TE-CAP for the diagnosis of NAFLD and liver fibrosis. This method is non-invasive, inexpensive, and reasonably accurate so that it is more practical in the real-life practice. However, we acknowledge that the accuracy of TE-CAP is inferior to that of the more invasive or complicated methods such as liver biopsy and magnetic resonance techniques[15].Of note, there have been concerns regarding the accuracy of TE-CAP in severely obese individuals as patients with BMI>30 kg/m^2 may be associated with falsely elevated CAP[21,22]. However, in the present study, patients with BMI >30 kg/m^2 accounted for only 22.8% in which we believe that a possible small effect in a minor proportion of patients would not significantly affect our outcome. Furthermore, previous study had suggested that higher CAP values (>300 dB/m) may cause overestimation of TE measurement causing falsely positive detection of significant fibrosis among patients with severe steatosis[23]. However, majority of our patients have liver steatosis <S3 (67.8%) and again a potential small effect in a minor proportion of patients is not likely to affect our outcome.

Conclusion

NAFLD and fibrosis is relatively common among T2DM patients with normal ALT. Obesity appears to be a good predictor for steatosis and fibrosis in this population. Patients with T2DM with normal ALT levels should be screened for the presence of liver disease, particularly if they are obese.

What is already known on this topic?

NAFLD is more common and more severe in patients with T2DM. Serum ALT levels are not necessary to be elevated for the diagnosis of NAFLD and it also do not correlate well with the degree of inflammation and fibrosis among patients with NAFLD.

What this study adds?

NAFLD is very common (83%) among T2DM patients with normal ALT. Furthermore, the prevalence of NAFLD with significant fibrosis and advanced fibrosis was 24% and 11%, respectively. Obesity is a good predictor for steatosis and fibrosis in this population.

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Potential conflicts of interest

None.

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