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ORIGINAL ARTICLE

Severity of fatty liver on ultrasound correlates with metabolic and cardiovascular risk

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Abstract Non-alcoholic fatty liver disease is associated with an increased risk of metabolic and cardiovascular diseases. Whether the severity of fatty liver on ultrasound correlates with metabolic or cardiovascular risk remains unclear. A total of 1000 people receiving health examinations were enrolled, and 126 were excluded due to the presence of HBsAg, anti-HCV, known hepatic disorders or alcohol use (>140 g/wk). Significant fatty liver consisted of moderate and severe fatty liver on ultrasound. The definition of central obesity was modified to a waist circumference of >90 cm in men and >80 cm in women. Framingham risk score was used to estimate the risk of cardiovascular disease. A total of 874 subjects (485 women and 388 men with a mean age of 52.07 ± 11.68 years) were included in the final analysis. By using logistic regression analyses stratified by gender, the odds ratio for the prevalence of diabetes mellitus, metabolic syndrome and risk of cardiovascular disease increased with increasing fatty liver status in both genders ($p \leq 0.001$). The difference was not only present between individuals with fatty liver vs. non-fatty liver but also between the mild fatty liver and significant fatty liver groups ($p < 0.05$). In conclusion, the severity of fatty liver on ultrasound could be useful for the risk stratification of metabolic syndrome, diabetes mellitus and cardiovascular disease in clinical practice.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as radiological or pathological confirmation of hepatic steatosis with the clinical exclusion of excessive alcohol intake and other known chronic liver diseases. It is an emerging disease worldwide and has become the leading cause of chronic liver disease in Western countries. In Taiwan, the prevalence is also increasing and the results of a number of studies have been published (Table 1) [1—11]. This rise is in parallel with the obesity and diabetes mellitus pandemics, which are accompanied by the Westernization of food and lifestyle [12—14]. The hepatic histology varies widely in NAFLD, from simple steatosis to non-alcoholic steatohepatitis or fibrosis with the potential to develop end-stage liver disease, including cirrhosis and liver cancer [15—18]. NAFLD has also been recognized as the hepatic manifestation of metabolic syndrome [19,20], which is a surrogate of cardiovascular risk [21]. Recent studies have indicated that patients with NAFLD have increased risks of metabolic syndrome, diabetes mellitus and cardiovascular disease (CVD) [22—24]. Of particular note is that NAFLD patients have higher all-cause mortality than healthy controls [25]. Further studies on different aspects of NAFLD are therefore urgently needed in order to understand more about this global health threat.

In patients with NAFLD, the leading causes of fatality are liver complications and CVD [26]. Although liver biopsy remains the gold standard for the diagnosis and predicting prognosis of liver complications in NAFLD, there are several disadvantages, such as possible bleeding complications and the small sample size compared with the whole liver or interobserver variability [27,28]. Readily available laboratory tests, aspartate aminotransferase-to-platelet ratio index (APRI) and aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio are simple non-invasive indices that have been applied to predict significant fibrosis in patients with NAFLD [29,30]. Non-invasive methods to predict the risk of metabolic or CVD in patients with NAFLD, however, are rare. A recent study demonstrated that the severity of fatty liver on ultrasound was associated with the risk of metabolic syndrome [31]. Whether the severity of fatty liver could be extrapolated to stratify the risk of liver complications or CVD in NAFLD patients remains unknown and deserves additional studies. For this reason, we have investigated the association between fatty liver severity and metabolic risks and hepatic complications in a large cohort of people receiving health examinations.

Table 1 Researches of non-alcoholic fatty liver disease in Taiwan.

Author (year)	Study design	Number of patients	Findings	Reference
Chiang (2010)	Case series	724 without CVD	NAFLD is independently associated with increased CVD risk, especially among elderly subjects and those with increased CRP level	[1]
Kuo (2010)	Case series	54,325	There was an independent association between gout and the risk of NAFLD	[2]
Chen (2010)	Case series	295	NAFLD was also associated with moderate to high risk of CAD	[3]
Hsieh (2009)	Case series	2539	Metabolic syndrome and BMI were related to abnormal liver function test results	[4]
Wang (2009)	Case series	170 NAFLD patients	Serum ALT levels are positively associated with the risk of carotid atherosclerosis in patients with NAFLD	[5]
Tsai (2008)	Case series	876	Metabolic syndrome and some of its components are independent risk factors for NAFLD	[6]
Chu (2007)	Case series	144	TNF-alpha may participate in the pathogenesis of NAFLD	[7]
Huang (2007)	Case series	111 obese patients	Presence of metabolic syndrome, high blood pressure, and high fasting glucose was independently related to increased risk of NASH	[8]
Hsiao (2007)	Case series	16,486	The presence of severe fatty liver in ultrasound correlated significantly with the prevalence and degree of hypertension, abnormal glucose and triglyceride metabolism	[9]
Chen (2007)	Case series	3260 adults	NAFLD appears to be the commonest cause of elevated ALT in Taiwan. The development of NAFLD is closely associated with many metabolic disorders	[10]
Chen (2006)	Case series	3245 adults	NAFLD is closely associated with elevated ALT, obesity, diabetes mellitus, hypercholesterolemia, hypertriglyceridemia, and hyperuricemia	[11]

Abbreviations: ALT = alanine aminotransferase, BMI = body mass index, CAD = coronary artery disease, CRP = C-reactive protein, CVD = cardiovascular disease, NAFLD = non-alcoholic fatty liver disease, NASH = non-alcoholic steatohepatitis, TNF = tumor necrotic factor.

Methods

Patients and methods

A total of 1000 people were enrolled from the health examination center of the Buddhist Tzu Chi General Hospital between October 2007 and December 2008. Demographic, anthropometric, clinical and laboratory data were obtained from each individual. A well-trained study nurse questioned the patients on their drug and alcohol history. Among these, 126 patients were excluded due to the presence of hepatitis-B-virus antigen (HBsAg), hepatitis-C antibody (anti-HCV), known hepatic disorders or alcohol use (>140 g/wk). Framingham risk score, APRI and AST/ALT ratio were adopted to predict the risk of CVD and the severity of hepatic fibrosis.

Clinical features and biochemical examinations

We collected information on age, sex, past history of hypertension and diabetes mellitus, history of drug or alcohol use, body mass index (BMI), waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), ALT, AST, cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose, glycated hemoglobin (HbA_{1c}), hemoglobin, white blood cell and platelet count. BMI was calculated as weight in kilograms divided by height in square meters. Metabolic syndrome was determined by using the National Cholesterol Education Program, Adult Treatment Panel III (2002 panel) guidelines. It was defined as the presence of three or more of the following criteria: central obesity, high blood pressure (≥130/85 mmHg or antihypertensive treatment), decreased HDL-C (<40/50 mg/dl in men/women), increased fasting glucose ≥110 mg/dl or glucose-lowing drug or previously diagnosed diabetes mellitus and increased triglyceride ≥150 mg/dl under fasting conditions or fibrate or nicotinic acid therapy [32]. Central obesity was modified to waist circumference >90 cm in men and >80 cm in women, according to the Department of Health in Taiwan. An automated Sysmex XE-2100 hematology analyzer (Sysmex, Kobe, Japan) assessed the hemogram, and biochemical data were measured using an autoanalyzer (ROCHE ANALYTICS; Roche Professional Diagnostics, Penzberg, Germany).

Ultrasound examination of the liver

All sonograms were obtained from the same machine (LOGIQ-5, GE, Medical System LTD, Seoul, Korea) with a 4 MHz electronic probe. The technical parameters, including gain adjustment and use of tissue harmonics, were optimized on a case-by-case basis. The severity of fatty liver was recorded as non-fatty liver, mild, moderate or severe fatty liver according to the findings of bright liver, hepatorenal echo contrast, the blurring of vessels and deep attenuation of ultrasound signal [33]. Significant fatty liver consisted of moderate and severe fatty liver on ultrasound.

Framingham risk score and aspartate aminotransferase-to-platelet ratio index

Using the information on age, gender, LDL-C, blood pressure, the presence of diabetes and smoking, we were able obtain the estimated and comparative coronary heart disease risk over a period of 10 years based on the Framingham experience in people between 30 and 74 years at baseline [34]. The relative risk was calculated by dividing the estimated risk by the comparative risk, which is defined as the average risk of coronary heart disease over a period of 10 years in age and gender-matched subjects. APRI was calculated using the following formula [35]:

$$\text{AST (IU/mL)/upper normal limit} \times 100/\text{platelet count } (10^9/\text{L}).$$

Study design

On the basis of the severity of fatty liver on ultrasound, patients who were enrolled were divided to three groups as follows:

- significant fatty liver group including subjects with moderate or severe fatty liver;
- mild fatty liver group; and
- control (non-fatty liver) group.

The three groups were compared in terms of the presence of diabetes, metabolic syndrome, the risk of CVD assessed by Framingham risk score, the presence of hepatic inflammation or fibrosis determined by elevated ALT levels (men >31 IU/L and women >20 IU/L) and APRI value, respectively [36].

Ethical considerations

The study was performed in accordance with the principles of the 1975 Declaration of Helsinki and approved by the Buddhist Tzu-Chi General Hospital Ethical Committee (98-IRB-018-X). Written informed consent was obtained from each person who took part.

Statistical analysis

Mean and standard deviation were calculated for continuous variables. Percentage was used for categorical variables. In univariate analysis, we compared all the variables between patients with and without fatty liver and patients with mild and significant fatty liver. Chi-squared and Student *t* tests were used to compare the distributions of frequency and the means among different groups, respectively. Logistic regression analysis was used to estimate the odds ratio of having diabetes, metabolic syndrome, relative cardiovascular risk, elevated ALT levels and fibrosis severity in three different ways:

- first, we defined non-fatty liver, mild fatty liver and significant fatty liver as an ordinal variable and determined the odds ratios (ORs) of each increment of the severity of fatty liver;

- second, the ORs were derived from comparing patients with and without fatty liver;
- third, the ORs were derived from comparing patients with mild and significant fatty liver.

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). A p value of <0.05 was considered statistically significant.

Results

Demographic data and comparison between patient and control groups

A total of 874 subjects (485 women and 388 men with a mean age of 52.07 $\pm$ 11.68 years) were included in the final analysis. The prevalence of diabetes and metabolic syndrome were 8.4% and 20.6%, respectively.

Based on the finding of fatty liver on ultrasound, the patients were divided to two groups: fatty liver (52.9%, 462/874); and non-fatty liver (47.1%, 412/874). The individuals with fatty liver had a higher mean age compared to those without (54.00 $\pm$ 10.69 vs. 50.34 $\pm$ 12.51, p <0.001). The percentage of men was higher in the fatty liver group compared with the non-fatty liver group (50.9 % vs. 37.1%, $p < 0.001$). Furthermore, the prevalence of diabetes, central obesity or the components of metabolic syndrome were significantly higher in fatty liver than the non-fatty liver group. In addition, higher BMI, waist circumference, relative risk of CVD, SBP, DBP, triglyceride, LDL-C, AST, ALT and APRI were noted in the fatty liver group, along with lower HDL-C and AST/ALT ratios compared with the control (non-fatty liver) group.

According to the severity of fatty liver found during ultrasound, the fatty liver group was divided into two groups: mild fatty liver (MFL, 63.9%) and significant fatty liver (SFL, 36.1%). Compared to the MFL group, the percentage of men, individuals with diabetes, central obesity, items of metabolic syndrome or history of smoking was significantly higher in the SFL group. In addition, higher BMI, waist circumference, relative risk of CVD, high blood pressure, triglyceride, AST, ALT and APRI readings, and lower HDL-C and AST/ALT ratio were noted in the SFL group. There was no difference in age, platelet count, LDL-C level and the presence of LDL-C >130 mg/dl between the MFL and SFL groups (Table 2).

Prevalence of metabolic diseases

In terms of diabetes, if defining the severity of fatty liver as an ordinal variable, the OR for every increment in severity was 3.3 in women (95% confidence interval (CI) 1.9−5.8) and 2.9 in men (95% CI 1.9−4.6). Furthermore, the risk was significantly higher in patients with fatty liver compared to non-fatty liver as well as in those with SFL compared to MFL. For the metabolic syndrome, the OR for every increment in fatty liver severity was 3.3 in women (95% CI 2.3−4.6) and 3.7 in men (95% CI 2.6−5.3). The risk was significantly higher in patients with fatty liver compared to without, as well as being significantly higher in those with SFL compared to MFL ($p < 0.01$) (Table 3).

Risk of cardiovascular disease

The model of the Framingham risk score could not be used to predict cardiovascular risk in people aged 30 to 74 years at baseline, so 770 subjects who belonged to this age group were included in the analyses. Increased risk of CVD was defined as a relative risk >1. The OR for every increment of fatty liver severity was 2.3 in women (95% CI 1.4−3.5) and 2.7 in men (95% CI 1.7−4.1) (Table 3). The CVD risk also increased in patients with fatty liver compared to non-fatty liver as well as in those with SFL compared to MFL ($p < 0.01$) (Table 3).

Risk of elevated ALT levels and fibrosis

The elevated ALT levels were defined by the updated cut-off values (men >31 U/mL and women >20 U/mL). Using logistic regression analyses, the OR for every increment in fatty liver severity was 1.4 in both men and women (95% CI 1.0−2.0). A significant difference was only present between the fatty liver and non-fatty liver group, however, and was not seen between MFL and SFL groups (Table 3).

APRI ($\geqq$1 for cirrhosis or $\geqq$0.5 for significant fibrosis) and AST/ALT ratio ($\geqq$0.8) had been used as clinical markers of hepatic fibrosis in patients of NAFLD. The fatty liver group had significantly higher APRI than the control group. In addition, the SFL group had a higher APRI than the MFL group (Table 2). Using stratified analysis, by gender and age adjustment the likelihood of having APRI $\geqq$0.5 or 1 was 1.8 (95% CI 1.1−2.9) and 5.5 (95% CI 1.3−23.6), respectively, for every increment in fatty liver severity in women. The difference was not present when comparing women with fatty liver and those without or when comparing the MFL and SFL groups. There was no obvious association between the severity of fatty liver and APRI levels in men (Table 3). In those with an AST/ALT ratio $\geqq$0.8, the OR for every increment in fatty liver severity was 0.3 in both men and women (95% CI 0.2−0.4). A significant difference was present when comparing patients with fatty liver and non-fatty liver as well as those with MFL and SFL (Table 3).

Discussion

In this study, we demonstrated that the severity of fatty liver as determined using ultrasound could be useful for the risk stratification of CVDs in clinical practice. Furthermore, we consistently confirmed that patients with NAFLD had a higher risk of developing inherent metabolic diseases, such as metabolic syndrome and diabetes. In addition to this, the prevalence of metabolic diseases and the risk of CVD trended to increase with the severity of fatty liver on ultrasound. Unsurprisingly, those with a fatty liver on ultrasound had a higher probability of hepatic inflammation.

NAFLD has been reported to be associated with diabetes and metabolic syndrome. Our results consistently confirmed that patients with fatty liver had an increased likelihood of metabolic syndrome and diabetes than those without. Moreover, patients with SFL on ultrasound had a higher likelihood of having diabetes and metabolic syndrome compared to those with MFL. This semiquantitative dose-response relationship could provide solid evidence to

Table 2 Comparison of characteristics between patients with and without fatty liver and with different severities of fatty liver.

	Non-fatty liver (n = 412)	Fatty liver (n = 462)	p value	Mild fatty liver (n = 295)	Significant fatty liver (n = 167)	p value
Gender						
Women	259 (62.0)	227 (49.1)		160 (54.2)	67 (40.1)	
Men	153 (37.1)	235 (50.9)	<0.001	135 (45.8)	100 (59.9)	0.004
Age (years)						
Mean ± SD	50.34 ± 12.51	54.00 ± 10.69	<0.001	54.16 ± 10.49	53.71 ± 11.05	0.666
Smoking						
No	389 (94.4)	428 (92.6)		279 (94.6)	149 (89.2)	
Yes	23 (5.6)	34 (7.4)	0.288	16 (5.4)	18 (10.8)	0.034
Body mass index[a]						
Mean ± SD	21.74 ± 2.62	24.95 ± 3.56	<0.001	24.08 ± 3.30	26.48 ± 3.49	<0.001
Waist conference (cm)						
Women, mean ± SD	74.48 ± 6.99	81.50 ± 9.08	<0.001	79.42 ± 8.54	86.47 ± 8.43	<0.001
Men, mean ± SD	77.93 ± 7.37	87.23 ± 9.00	<0.001	85.27 ± 8.21	89.88 ± 9.27	<0.001
Central obesity[b]						
No	360 (87.4)	277 (60.0)		204 (69.2)	73 (43.7)	
Yes	52 (12.6)	185 (40.0)	<0.001	91 (30.9)	94 (56.3)	<0.001
Diabetes mellitus[c]						
No	400 (97.1)	401 (86.8)		273 (92.5)	128 (76.7)	
Yes	12 (2.9)	61 (13.2)	<0.001	22 (7.5)	39 (23.4)	<0.001
Increased fasting sugar level[d]						
No	383 (93.0)	366 (79.2)		254 (86.1)	112 (67.1)	
Yes	29 (7.0)	96 (20.8)	<0.001	41 (13.9)	55 (32.9)	<0.001
Systolic blood pressure (mm Hg)						
Mean ± SD	118.95 ± 16.23	124.64 ± 16.52	<0.001	122.70 ± 16.84	128.09 ± 15.38	<0.001
Diastolic blood pressure (mm Hg)						
Mean ± SD	73.31 ± 11.20	76.90 ± 12.41	<0.001	75.57 ± 12.44	79.27 ± 12.03	0.002
High blood pressure[e]						
No	296 (71.8)	258 (55.8)	<0.001	186 (63.1)	72 (43.1)	<0.001
Yes	116 (28.2)	204 (44.2)		109 (37.0)	95 (56.9)	
Triglyceride (mg/dl)						
Mean ± SD	102.73 ± 52.75	155.97 ± 107.23	<0.001	140.45 ± 72.45	138.38 ± 146.47	<0.001
Triglyceride >150 mg/dl						
No	352 (85.4)	276 (59.7)	<0.001	193 (65.4)	83 (49.7)	0.001
Yes	60 (14.6)	186 (40.3)		102 (34.6)	84 (50.3)	
HDL-C (mg/dl)						
Mean ± SD	58.13 ± 15.49	49.15 ± 13.01	<0.001	51.36 ± 13.34	45.24 ± 11.44	<0.001
Decreased HDL-C[f]						
No	327 (79.4)	277 (60.0)		193 (65.4)	84 (50.3)	
Yes	85 (20.6)	185 (40.0)	<0.001	102 (34.6)	83 (49.7)	0.001
LDL-C (mg/dl)						
Mean ± SD	118.34 ± 31.43	130.82 ± 32.05	<0.001	130.21 ± 31.42	131.90 ± 33.19	0.586
LDL-C >130 mg/dl						
No	272 (66.0)	245 (53.0)		162 (54.9)	83 (49.7)	
Yes	140 (34.0)	217 (47.0)	<0.001	133 (45.1)	84 (50.3)	0.281
Relative risk of CVD[g]	0.52 ± 0.31	0.70 ± 0.39	<0.001	0.62 ± 0.35	0.82 ± 0.42	<0.001

(*continued on next page*)

Table 2 (*continued*)

	Non-fatty liver (n = 412)	Fatty liver (n = 462)	p value	Mild fatty liver (n = 295)	Significant fatty liver (n = 167)	p value
ALT (IU/L)						
Mean ± SD	20.83 ± 14.30	32.04 ± 24.58	<0.001	28.11 ± 22.13	38.98 ± 27.10	<0.001
AST (IU/L)						
Mean ± SD	22.38 ± 8.31	25.87 ± 12.18	<0.001	24.37 ± 10.08	28.51 ± 14.88	<0.001
Platelet (10^9/L)						
Mean ± SD	244.63 ± 58.14	248.96 ± 57.84	0.271	250.72 ± 59.72	245.84 ± 54.39	0.385
AST/ALT ratio						
Mean ± SD	1.19 ± 0.36	0.95 ± 0.33	<0.001	1.02 ± 0.35	0.82 ± 0.24	<0.001
APRI[h]						
Mean ± SD	0.28 ± 0.14	0.31 ± 0.18	0.018	0.29 ± 0.15	0.34 ± 0.22	0.005

Abbreviations: ALT = alanine aminotransferase, APRI = aspartate aminotransferase-to-platelet ratio index, AST = aspartate aminotransferase, CHD = coronary hear disease, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, SD = standard deviation.

[a] body mass index = weight (kg)/height (m^2).

[b] waist circumference >90 cm in men and >80 cm in women.

[c] fasting glucose ≥126 mg/dl or patient on glucose-lowing drug or previously diagnosed with diabetes mellitus.

[d] increased fasting glucose ≥110 mg/dl or patient on glucose-lowing drug or previously diagnosed with diabetes mellitus.

[e] systolic blood pressure >130 or diastolic blood pressure >85 mmHg or receiving antihypertensive treatment.

[f] HDL-C <40/50 mg/dl in men/women.

[g] relative risk is derived from dividing the estimated risk of CVD by the comparative risk that is defined as the average risk of CVD.

[h] APRI is derived from the formula of AST (U/L)/upper normal limit × 100/platelet count (10^9/L).

support the association of NAFLD with metabolic syndrome and diabetes.

Ultrasound, being cheap, readily available and without the risk of radiation exposure, is the most common modality by which NAFLD is diagnosed in clinical practice [37,38]. Our results further confirmed that it could be used as a tool to stratify the risk of metabolic syndrome and diabetes in individuals with NAFLD.

A case-control study found that patients with NAFLD had a higher prevalence of plaques and greater intima-media thickness of the carotid arteries than controls [39]. Another study proved the relationship between the severity of liver histology and carotid atherosclerosis, regardless of classic CVD risk factors [40]. In addition, patients with NAFLD are reported to have an increased risk of CVD and all-cause mortality compared to the general population [41]. The identification of surrogates that could further stratify the risk of CVD in patients with NAFLD is therefore clinically important. For example, serum ALT level has been found to be positively associated with the risk of carotid atherosclerosis in NAFLD patients. In this study, using Framingham risk score we have gathered data showing that the severity of fatty liver on ultrasound correlates with CVD risk. The patients with SFL had the greatest likelihood of CVD compared to healthy controls and those with MFL had a lower risk than patients with SFL. On the basis of these findings, even patients with MFL should be screened and treated for the presence of other CVD risk factors.

Serum ALT has long been recognized as a marker of hepatic inflammation. In patients who have no viral or other known causes of hepatitis, elevated serum ALT level is a clinical indicator of NAFLD, but is only found in a portion of these patients [42]. Our data showed that the likelihood of ALT elevation tended to increase with the severity of fatty liver detected during ultrasound. This suggests that hepatic steatosis could damage hepatocytes through the "second hit" as opposed in the pathogenesis of NAFLD [43]. Our earlier study indicated that NAFLD patients with ALT elevation had an increased risk of carotid atherosclerosis compared to those with normal ALT levels, suggesting that serum ALT levels could serve as a clinical marker that is predictive of CVD risk [44]. Taking these data together, our findings strengthen the association between fatty liver, elevated ALT levels and CVD risk.

APRI is a non-invasive marker that predicts the severity of hepatic fibrosis in patients with chronic hepatitis C virus infection, even in patients using hemodialysis [45,46]. Recent studies have further proved its use in the diagnosis of other causes of hepatitis, such as hepatitis B virus infection and NAFLD [47]. An AST/ALT ratio ≧0.8 was also used to predict the presence of significant fibrosis in patients with NAFLD. By using the AST/ALT ratio, we found that the presence of significant fibrosis was less likely with increasing fatty liver severity and that healthy controls were more likely to have significant fibrosis. This result is quite unreasonable in clinical practice. Further studies are therefore warranted to determine whether the AST/ALT ratio could be used to predict hepatic fibrosis in patients with NAFLD. Where the APRI was >0.5 or 1, the presence of significant fibrosis was increasingly likely with the increasing severity of fatty liver in women but not in men. This significant finding in women was not present when comparing patients with fatty liver and those without as well as comparing the MFL and SFL groups. For this reason, the relationship between the clinical markers of hepatic

Table 3 Odds ratio of liver or non-liver related complication of non-alcoholic fatty liver disease among significant, mild fatty liver and non-fatty liver groups by logistic regression analyses adjusted by age (every 10-year increment).

	Fatty liver			OR (95% CI)	*p* value	Age-adjusted OR (95% CI)	*p* value
	Non	Mild	Significant				
Diabetes mellitus							
Women							
No	253 (97.7)	152 (95.6)	52 (77.6)	3.8 (2.2-6.4)[a]	<0.001	3.3 (1.9-5.8)[a]	<0.001
Yes	6 (2.3)	7 (4.4)	15 (22.4)	4.5 (1.8-11.4)[b]	0.001	3.7 (1.4-9.4)[b]	0.006
				6.3 (2.4-16.2)[c]	<0.001	5.8 (2.2-15.2)[c]	<0.001
Men							
No	147 (96.1)	121 (89.0)	76 (76.0)	2.7 (1.8-4.2)[a]	<0.001	2.9 (1.9-4.6)[a]	<0.001
Yes	6 (3.9)	15 (11.0)	24 (24.0)	4.9 (2.0-11.8)[b]	<0.001	4.8 (2.0-11.8)[b]	0.001
				2.5 (1.3-5.2)[c]	0.009	2.9 (1.4-6.1)[c]	0.004
Metabolic syndrome							
Women							
No	239 (92.3)	123 (77.4)	32 (47.8)	3.6 (2.6-5.0)[a]	<0.001	3.3 (2.3-4.6)[a]	<0.001
Yes	20 (7.7)	36 (22.6)	35 (52.2)	5.5 (3.2-9.4)[b]	<0.001	4.7 (2.7-8.1)[b]	<0.001
				3.7 (2.0-6.9)[c]	<0.001	3.6 (1.9-6.6)[c]	<0.001
Men							
No	145 (94.7)	100 (73.5)	55 (55.0)	3.4 (2.4-4.8)[a]	<0.001	3.7 (2.6-5.3)[a]	<0.001
Yes	8 (5.2)	36 (26.5)	45 (45.0)	9.5 (4.4-20.3)[b]	<0.001	9.6 (4.4-20.6)[b]	<0.001
				2.3 (1.3-3.9)[c]	0.003	2.6 (1.5-4.6)[c]	0.001
ALT elevation[d]							
Women							
No	225 (86.8)	129 (81.1)	51 (76.1)	1.5 (1.1-2.0)[a]	0.021	1.4 (1.0-2.0)[a]	0.031
Yes	34 (13.1)	30 (18.9)	16 (23.9)	1.7 (1.0-2.7)[b]	0.034	1.6 (1.0-2.7)[b]	0.048
				1.3 (0.7-2.7)[c]	0.394	1.3 (0.7-2.7)[c]	0.407
Men							
No	135 (88.2)	113 (83.1)	79 (79.0)	1.4 (1.0-2.0)[a]	0.048	1.4 (1.0-2.0)[a]	0.040
Yes	18 (11.8)	23 (16.9)	21 (21.0)	1.7 (1.0-3.1)[b]	0.072	1.8 (1.0-3.3)[b]	0.048
				1.3 (0.7-2.5)[c]	0.426	1.2 (0.6-2.3)[c]	0.620
RR ≧1.0[e]							
Women							
No	198 (94.3)	130 (92.2)	46 (75.4)	2.3 (1.5-3.6)[a]	<0.001	2.3 (1.4-3.5)[a]	<0.001
Yes	12 (5.7)	11 (7.8)	15 (24.6)	2.4 (1.2-5.0)[b]	0.014	2.3 (1.1-4.7)[b]	0.027
				3.9 (1.7-9.0)[c]	0.002	3.8 (1.6-8.9)[c]	0.002
Men							
No	129 (94.9)	113 (89.0)	69 (72.6)	2.7 (1.8-4.2)[a]	<0.001	2.7 (1.7-4.1)[a]	<0.001
Yes	7 (5.2)	14 (11.0)	26 (27.4)	4.1 (1.8-9.3)[b]	0.001	4.4 (1.9-10.2)[b]	0.001
				3.0 (1.5-6.2)[c]	0.002	2.7 (1.3-5.5)[c]	0.009
APRI[f] ≧0.5							
Women							
No	250 (96.5)	149 (93.7)	58 (86.6)	2.1 (1.3-3.4)[a]	0.004	1.8 (1.1-2.9)[a]	0.026
Yes	9 (3.5)	10 (6.3)	9 (13.4)	2.5 (1.1-5.8)[b]	0.024	2.0 (0.9-4.7)[b]	0.094
				2.3 (0.9-6.0)[c]	0.084	2.0 (0.8-5.4)[c]	0.163
Men							
No	147 (96.1)	128 (94.1)	90 (90.0)	1.7 (1.0-2.8)[a]	0.058	1.7 (1.0-2.9)[a]	0.056
Yes	6 (3.9)	8 (5.9)	10 (10.0)	2.0 (0.8-5.2)[b]	0.145	2.0 (0.8-5.1)[b]	0.165
				1.8 (0.7-4.7)[c]	0.244	1.8 (0.7-4.9)[c]	0.217
APRI ≧1.0							
Women							
No	258 (99.6)	159 (100.0)	63 (94.0)	6.3 (1.5-26.6)[a]	0.012	5.5 (1.3-23.6)[a]	0.022
Yes	1 (0.4)	0 (0.0)	4 (6.0)	4.6 (0.5-41.9)[c]	0.171	3.7 (0.4-33.5)[c]	0.248
Men							
No	151 (98.7)	135 (99.3)	99 (99.0)	0.8 (0.2-3.0)[a]	0.774	0.8 (0.2-3.1)[a]	0.788
Yes	2 (1.3)	1 (0.7)	1 (1.0)	0.6 (0.1-4.6)[b]	0.663	0.6 (0.1-4.5)[b]	0.645
				1.4 (0.1-22.1)[c]	0.827	1.3 (0.1-21.0)[c]	0.871

(*continued on next page*)

Table 3 (*continued*)

	Fatty liver			OR (95% CI)	*p* value	Age-adjusted OR (95% CI)	*p* value
	Non	Mild	Significant				
AST/ALT ratio ≧0.8							
Women							
No	12 (4.6)	24 (15.1)	21 (31.3)	0.3 (0.2-0.5)[a]	<0.001	0.3 (0.2-0.5)[a]	<0.001
Yes	247 (95.4)	135 (84.9)	46 (68.7)	0.2 (0.1-0.4)[b]	<0.001	0.2 (1.0-0.4)[b]	<0.001
				0.4 (0.2-0.8)[c]	0.006	0.4 (0.2-0.7)[c]	0.004
Men							
No	21 (13.7)	51 (37.5)	66 (66.0)	0.3 (0.2-0.4)[a]	<0.001	0.3 (0.2-0.4)[a]	<0.001
Yes	132 (86.3)	85 (62.5)	34 (34.0)	0.2 (0.1-0.3)[b]	<0.001	0.2 (0.1-0.2)[b]	<0.001
				0.3 (0.2-0.5)[c]	<0.001	0.3 (0.2-0.6)[c]	<0.001

Abbreviations: ALT = alanine aminotransferase, APRI = aspartate aminotransferase-to-platelet ratio index, AST = aspartate aminotransferase, CI = confidence interval, OR = odds ratio, RR = relative risk.
[a] ordered odds ratio.
[b] fatty liver vs. non fatty liver.
[c] significant fatty liver vs. mild fatty liver.
[d] men >31 IU/L and women >20 IU/L.
[e] sup>relative risk is derived from dividing the estimated risk of coronary heart disease by the comparative risk that is defined as the average risk of coronary heart disease.
[f] APRI is derived from the formula of AST (IU/mL)/upper normal limit × 100/platelet count (10^9/L).

fibrosis and the severity of fatty liver on ultrasound could not be confirmed in this study.

Our study had potential strengths and limitations. This cohort consisted of consecutive individuals presenting for health examination, so selection bias was avoided. Furthermore, patients with viral hepatitis, the habit of drinking (>140 g/wk) or known causes of hepatitis were excluded to avoid confounding effects on the development of hepatic steatosis, inflammation or fibrosis. This study had three limitations. First, the calculated risk of CVD was relative to the average risk of CVD in the population included in the Framingham study. Although we acknowledge that the average CVD risk might be different between Western and Asian countries, we considered that the relative risks of the three groups in this study on the basis of the reference age- and sex-matched population was still representative of the relative possibility of CVD events. Second, although NAFLD was diagnosed by ultrasound examination rather than liver histology, it is thought that ultrasound is commonly used in clinical practice to diagnose NAFLD and that using invasive liver biopsy to assess the risk of metabolic diseases in NAFLD patients may not be cost-effective. Third, the "two hit" hypothesis of NAFLD suggests that the accumulation of triglyceride in hepatocytes is the first hit, followed by the second hit of necroinflammatory processes. Our result demonstrated the association between hepatic steatosis and ALT elevation (necroinflammation). Their causal relationship could not be confirmed, however, because of the cross-sectional study design. In addition, liver biopsy rather than APRI or AST/ALT ratio is the gold standard for assessing the severity of hepatic fibrosis. Further prospective studies with biopsy-proven NAFLD patients are required to find out more about the impact of steatosis on hepatic fibrosis.

In summary, the severity of fatty liver on ultrasound is clinically useful for predicting the likelihood of metabolic diseases, including diabetes mellitus, metabolic syndrome and the risk of CVD.

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