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ORIGINAL ARTICLE

Association between non-alcoholic fatty liver disease evaluated by transient elastography with extracranial carotid atherosclerosis in a multiethnic Asian community

Eugene Choon-Li Tan,* Mei-Ling Sharon Tai,† Wah-Kheong Chan* and Sanjiv Mahadeva*

*Gastroenterology and Hepatology Unit, Department of Medicine, Faculty of Medicine and †Division of Neurology, Department of Medicine, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia

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atherosclerosis, carotid intima-media thickness, Fibroscan, non-alcoholic fatty liver disease, transient Elastography, ultrasound carotid Doppler.

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Correspondence
Dr Mei-Ling S Tai, Division of Neurology, Department of Medicine, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia.
Email: sharont1990@gmail.com

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Abstract

Background and Aim: There is not much data on the association between non-alcoholic fatty liver disease (NAFLD) and advanced fibrosis assessed using Fibroscan with carotid intima-media thickness (CIMT) in the general population. The objective of this study was to evaluate the association between NAFLD and advanced fibrosis, as diagnosed by Fibroscan, with an increased CIMT in the Malaysian population.

Methods: A cross-sectional study of government officers and their family members attending a health screening at a public healthcare facility was conducted. All subjects underwent clinical evaluation, biochemical testing, anthropometry, ultrasound carotid Doppler, and Fibroscan examination.

Results: Data for 251 subjects were analyzed (mean age 47.1 ± 12.4 years, 74.1% male). Prevalence of NAFLD and advanced fibrosis were 57.4 and 17.5%, respectively. Independent factors associated with NAFLD were waist circumference (odds ratio [OR] = 1.077, 95% confidence interval [CI] 1.038–1.118, $P < 0.001$) and serum alanine aminotransferase (ALT) (OR = 1.039, 95% CI 1.005–1.074, $P = 0.024$). Independent factors associated with advanced fibrosis were male gender (OR = 4.847, 95% CI 1.369–17.155, $P = 0.014$) and serum aspartate aminotransferase (AST) (OR = 1.057, 95% CI 1.003–1.113, $P = 0.036$). Prevalence of increased CIMT was 29.0%. Independent factor associated with increased CIMT was older age (OR = 1.146, 95% CI 1.067–1.231, $P < 0.001$). Of the subjects, 34.5% with NAFLD had increased CIMT compared to 19.1% of the subjects without NAFLD ($P = 0.063$). Advanced fibrosis was not associated with increased CIMT.

Conclusions: Prevalence of NAFLD, advanced liver fibrosis, and increased CIMT were high. NAFLD and advanced liver fibrosis appeared not to be associated with increased CIMT. However, a larger sample size is needed to demonstrate whether there is any association.

Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) is rising all over the world.[1] Non-alcoholic steatohepatitis (NASH), the more severe form of NAFLD, can progress to fibrosis and cirrhosis. NASH is a leading cause of liver transplantation due to hepatic failure and liver cancer.[2] Recent noninvasive modalities, such as liver stiffness measurement (LSM) with transient elastography, have gradually replaced liver biopsy for the estimation of liver fibrosis.[3] Controlled attenuation parameter (CAP), which measures the decrease in amplitude of ultrasound as it transmits through liver tissue, is excellent for the evaluation of significant hepatic steatosis. CAP is estimated using the same radiofrequency data used for the assessment of LSM with Fibroscan, an ultrasound-based vibration-controlled transient elastography device.[4] Carotid intima-media thickness (CIMT) is used to detect subclinical atherosclerosis.[5] Progression of CIMT leads to plaque development, carotid stenosis and, subsequently, a higher risk of stroke.[5] In a previous cross-sectional study, NAFLD evaluated by liver ultrasonography was found to be associated with an increased CIMT.[6] A systematic review reported that 13% of NAFLD patients, diagnosed through simple transabdominal ultrasound scanning, have an increased CIMT.[7] To date, there is only one study on the prevalence of NAFLD in the general population in Malaysia.[8] So far, there has been no report on the relationship of NAFLD, as evaluated by Fibroscan, with CIMT. The primary objective of this study was to evaluate the association between

NAFLD, as diagnosed by Fibroscan, with an increased CIMT in the Malaysian population. The secondary objective was to assess the association between advanced liver fibrosis with increased CIMT. We also evaluated the risk factors associated with NAFLD, advanced fibrosis, and increased CIMT.

Methods

Ethics approval was obtained from University Malaya Medical Centre (MREC ID No.: 201410-677) before the study was conducted. The study was a cross-sectional study on consecutive government officers and their family members who attended a health screening at a public healthcare facility from August 2015 to January 2016. Study participants with significant alcohol intake or those known to have human immunodeficiency virus (HIV) infection, chronic hepatitis B virus infection, chronic hepatitis C virus infection, or other chronic liver diseases were excluded from participating in this study. Pregnant women were also not included in this study.

Demographic, anthropometric, clinical, and laboratory data were obtained using standard protocol. The intake of alcohol was estimated with the quantity–frequency method.[9] Significant alcohol intake was defined as ≥21 units per week for men and ≥14 units per week for women. The subject's height and weight was measured using standardized equipment. Body mass index (BMI) was calculated by dividing weight (in kilogram) by the square of height (in meters). Obesity was defined as BMI ≥25.0 kg per m^2. Waist circumference was measured at the mid-point between the lowest margin of the least palpable rib and the top of the iliac crest, and this was measured in the standing position. Central obesity was defined as waist circumference >90 cm in the men and >80 cm in the women.[10] Blood pressure was measured in a sitting position using standard electronic blood pressure measuring equipment. The subject was defined as having hypertension if he or she was on antihypertensive medication or had systolic blood pressure (SBP) of ≥140 mmHg and diastolic blood pressure (DBP) ≥90 mmHg. The Global Physical Activity Questionnaire, which was developed by the World Health Organization, was used to measure the physical activity in three domains, namely, activity at work, travel to- and-from places, and recreational activities.[11]

Blood was taken from all the subjects after an overnight fast for full blood count, liver profile, fasting lipid profile, hepatitis B and C, and HIV screening. An oral glucose tolerance test was performed for all subjects. A subject was considered to have dyslipidemia if he or she was on lipid-lowering medication or if the subject had the serum total cholesterol (TC) ≥5.2 mmol/L, serum low-density lipoprotein (LDL) ≥3.4 mmol/L, serum high-density lipoprotein (HDL) <1.0 mmol/L in men or <1.3 mmol/L in women, or serum triglyceride (TG) ≥1.7 mmol/L. A subject was considered to have type 2 diabetes mellitus if he or she was on antidiabetic medication or if the subject had a fasting blood sugar (FBS) of ≥7.0 mmol/L and the 2-h postprandial blood sugar of ≥11.1 mmol/L. A subject was considered to have the metabolic syndrome if three or more of the following were present: hypertension; central obesity; hypertriglyceridemia; low serum HDL; and impaired fasting glucose, impaired glucose tolerance, or diabetes mellitus.[10]

Fibroscan examination. Fibroscan examination was performed using FibroScan® 502 Touch with M-probe (EchoSens, Paris, France) by a certified operator blinded to the clinical data and the results of Doppler ultrasonography of the carotid artery. An examination was successful if 10 valid measurements were obtained and reliable if the interquartile range (IQR)/median was ≤30%. An examination could still be considered reliable when the IQR/median was >30% if the LSM was <7.1 kPa.[12] Subjects with unreliable examination were not included in the data analysis. Advanced fibrosis was diagnosed based on LSM ≥8 kPa.[3,13] Significant hepatic steatosis was diagnosed based on CAP measurement ≥263 dB/m.[4]

Ultrasound carotid Doppler. Ultrasound carotid Doppler was performed using LOGIQ e (General Electric Healthcare, Chicago, IL, USA) with a 12 MHz probe by an experienced operator blinded to clinical data and Fibroscan examination results. The examination was performed according to standard protocol. CIMT measurements were taken at three different angles from the distal 1 cm of the far wall of both common carotid arteries. An average value was taken for both right and left common carotid arteries. An increased CIMT was defined as CIMT ≥0.8 mm.[14]

Statistical analysis. With an estimated prevalence of 20%, (11) the formula $n = Z^2P\ (1 - P)/d_2$ was used for calculation of the sample size (n = sample size, $Z = Z$ statistic for a level of confidence, P = proportion [$P = 0.2$] and d = precision [$d = 0.05$]). A total of 246 subjects were required to estimate the prevalence with 95% confidence. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, USA). Categorical variables were expressed as percentages and analyzed using Pearson Chi-square test or Fisher's exact test where appropriate. Continuous variables were analyzed using Student's t-test or Mann Whitney U test where appropriate. Continuous variables were expressed as means ± standard deviation or median with interquartile range. Factors associated with NAFLD, advanced fibrosis, and increased CIMT were then analyzed using univariate and multiple logistic regression analysis. A P value of <0.05 was considered statistically significant. If the categorical and continuous forms of the variable were both found to be statistically significant, then the categorical form of the variable was chosen for multiple logistic regression analysis.

Results

Study population. Of the 356 subjects who attended the health screening during the study period, 332 subjects consented to participate in the study; 81 subjects were excluded from the study (57 incomplete data, 8 significant alcohol intake, 2 chronic hepatitis B infection, 6 failed Transient Elastography (TE), 8 had unreliable TE). Therefore, 251 subjects were included in the data analysis (Fig. 1). The mean age of the study population was 47.1 ± 12.4 years old, consisting of 74.1% men. Obesity and central obesity were observed in 64.5 and 74.5%, respectively. Hypertension, diabetes mellitus, dyslipidemia, and the metabolic syndrome were observed in 30.3, 9.6, 76.1, and 40.2%, respectively. The study population consisted of 68.1% Malay, 19.9% Chinese, and 33.1% Indians. The prevalence of NAFLD, advanced fibrosis, and increased CIMT in the overall population and according to the different ethnic groups are shown in Table 1.

Prevalence of NAFLD and the associated factors. The prevalence of NAFLD in the overall population was 57.4%

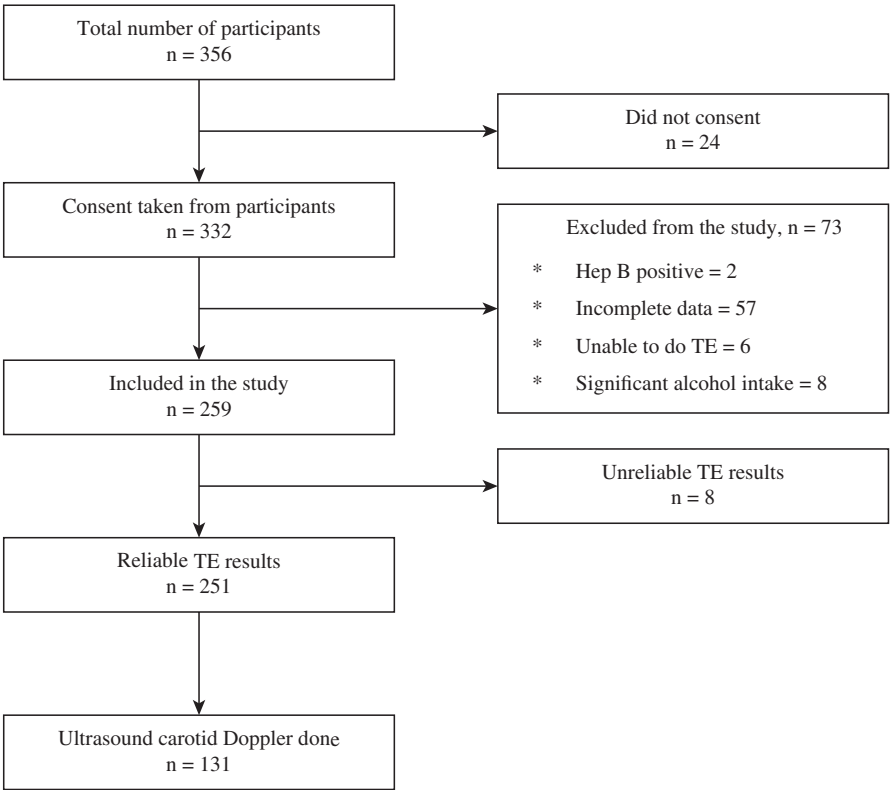

Figure 1 Flow chart of the recruitment process. TE, Transient Elastography.

(144/251). Table 2 shows the characteristics of subjects with and without NAFLD. The subjects with NAFLD were significantly older in age. They were also significantly more likely to be male and to have obesity, central obesity, and hypertension. They were also more likely to have elevated SBP, DBP, serum TC, TG, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) levels. A total of 51.4% (74/144) subjects with NAFLD had metabolic syndrome, whereas 25.2% (27/107) subjects without NAFLD had metabolic syndrome ($P < 0.001$). On multivariate analysis, the independent factors that were associated with NAFLD were waist circumference (odds ratio [OR] = 1.077, 95% confidence interval [CI] 1.038–1.118, $P < 0.001$) and serum ALT (OR = 1.039, 95% CI 1.005–1.074, $P = 0.024$) (Table 3).

Prevalence of advanced fibrosis and the associated factors. The prevalence of advanced liver fibrosis in the overall population was 17.5% (44/251). Table 4 shows the characteristics of subjects with and without advanced fibrosis. The subjects with advanced fibrosis were significantly more likely to be male and were more likely to have dyslipidemia. They had higher BMI; waist circumference SBP; and serum TC, ALT, AST, and GGT levels. A total of 59.1% (26/44) subjects with advanced fibrosis had metabolic syndrome, whereas 36.2% (75/207) without advanced fibrosis had metabolic syndrome ($P = 0.007$). On multivariate analysis, the independent factors associated with advanced fibrosis were serum AST (OR = 1.057, 95% CI 1.003–1.113, $P = 0.036$) and male gender (OR = 4.847, 95% CI 1.369–17.155, $P = 0.014$) (Table 5).

Prevalence of increased CIMT and associated factors. Over half of the study population (131/251, 52.2%) underwent ultrasound carotid Doppler. Increased CIMT was observed in 29.0% (38/131). Table 6 shows the characteristics of subjects with and without increased CIMT. Subjects with increased CIMT were significantly older, and they were more likely males. They

Table 1 Prevalence of NAFLD, advanced fibrosis, and increased CIMT in the various ethnic groups

	Overall population	Malay	Chinese	Indians	*P*-value
Prevalence of NAFLD, % (*n*/*N*)	57.4 (144/251)	56.1 (96/171)	50 (15/30)	66 (33/50)	0.32
Prevalence of advanced fibrosis, % (*n*/*N*)	17.5 (44/251)	17 (29/171)	23.3 (7/30)	16 (8/50)	0.66
Prevalence of raised CIMT, % (*n*/*N*)	29 (38/131)	25 (19/76)	30 (6/20)	37.1 (13/35)	0.42

P values are those comparing the prevalence of NAFLD, advanced fibrosis, and raised CIMT across the different ethnic groups.
CIMT, carotid intima-media thickness; NAFLD, non-alcoholic fatty liver disease.

Table 2 Characteristics of participants with and without NAFLD

	Participants with NAFLD, *n* = 144	Participants without NAFLD, *n* = 107	*P* value
Age, years	49.1 ± 11.0	44.4 ± 13.5	0.002
Gender, *n* (%)			
Male	121 (84)	65 (60.7)	<0.0001
Female	23 (16)	42 (39.3)	
Ethnic group, *n* (%)			
Malay	96 (66.7)	75 (70.1)	0.280
Chinese	15 (10.4)	15 (14.0)	
Indian	33(22.9)	17 (15.9)	
Smoking, *n* (%)			
Yes	33 (22.9)	18 (16.8)	0.235
No	111 (77.1)	89 (83.2)	
Diabetes mellitus, *n* (%)			
Yes	13 (9)	11 (10.3)	0.739
No	131 (91)	96 (89.7)	
Hypertension, *n* (%)			
Yes	55 (38.2)	21 (19.6)	0.002
No	89 (61.8)	86 (80.4)	
Dyslipidemia, *n* (%)			
Yes	116 (80.6)	75 (70.1)	0.055
No	28 (19.4)	32 (29.9)	
BMI, kg per m^2	27.7 ± 3.7	24.7 ± 3.4	<0.0001
Obesity, *n* (%)			
Yes	110 (76.4)	52 (48.6)	<0.0001
No	34 (23.6)	55 (51.4)	
Waist circumference, cm	96.9 ± 9.1	88.6 ± 9.4	<0.0001
Central obesity, *n* (%)			
Yes	125 (86.8)	62 (57.9)	<0.0001
No	19 (13.2)	45 (42.1)	
SBP, mmHg	138 ± 14	128 ± 13	<0.0001
DBP, mmHg	83 ± 10	76 ± 11	<0.0001
FBS, mmol/L	5.5 ± 1.2	5.3 ± 1.4	0.283
TC, mmol/L	5.7 ± 1.0	5.4 ± 1.2	0.049
HDL, mmol/L	1.4 ± 0.3	1.5 ± 0.3	0.049
LDL, mmol/L	3.1 ± 1.2	2.9 ± 1.2	0.087
TG, mmol/L	1.8 ± 1.1	1.5 ± 0.6	0.017
ALT, IU/L	38 (28–43)	28 (21–36)	<0.0001
AST, IU/L	28 (21–38)	24 (19–32)	0.003
GGT, IU/L	54 (31–65)	38 (22–58)	<0.0001
WHO recommendation on physical activity for health achieved (assessed using the GPAQ), *n* (%)			
Yes	114 (79.2)	87 (81.3)	0.674
No	30 (20.8)	20 (18.7)	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FBS, fasting blood sugar; GGT, gamma-glutamyl transferase; GPAQ, Global Physical Activity Questionnaire; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WHO, World Health Organization.

were also more likely to have diabetes mellitus and hypertension. They had greater waist circumference and higher SBP, FBS, LDL, and ALT. A total of 50% (19/38) of the subjects with increased CIMT had metabolic syndrome, whereas 40.8% (49/93) subjects without increased CIMT had metabolic syndrome ($P = 0.17$); 34.5% (29/84) of the subjects with NAFLD had an increased CIMT compared to 19.1% (9/47) of the subjects without NAFLD ($P = 0.063$); 30.4% (7/23) of the subjects with advanced fibrosis had an increased CIMT compared to 28.7% (31/108) of the subjects without advanced fibrosis ($P = 0.868$). On multivariate analysis, the only independent factor associated with increased CIMT was older age (OR = 1.146, 95% CI 1.067–1.231, $P < 0.001$) (Table 7).

Discussion

This study has demonstrated several important observations on NAFLD and CIMT in a community-based setting in Malaysia. We observed an alarmingly high prevalence of NAFLD (57.4%). Malaysia is one of the countries with the highest prevalence of

Table 3 Univariate and multivariate analysis of factors associated with NAFLD

	Univariate analysis				Multivariate analysis			
	β	OR	95% CI	*P* value	β	Adjusted OR	95% CI	*P* value
Age	0.032	1.033	1.011–1.055	0.003	0.021	1.021	0.995–1.048	0.114
Male	1.224	3.399	1.882–6.139	<0.001	0.531	1.701	0.861–3.361	0.126
Waist circumference	0.102	1.108	1.070–1.146	<0.001	0.074	1.077	1.038–1.118	>0.001
ALT	0.050	1.052	1.028–1.075	<0.001	0.038	1.039	1.005–1.074	0.024
AST	0.045	1.046	1.016–1.077	0.002	−0.005	0.995	0.949–1.042	0.819
GGT	0.020	1.020	1.008–1.032	0.001	0.004	1.004	0.989–1.019	0.628
Dyslipidemia	0.570	1.768	0.985–3.171	0.056	0.129	1.138	0.579–2.236	0.707
Hypertension	0.929	2.531	1.412–4.536	0.002	0.354	1.425	0.713–2.848	0.316

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; GGT, gamma-glutamyl transferase; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio.

Table 4 Characteristics of participants with and without advanced liver fibrosis

	Participants with advanced fibrosis, *n* = 44	Participants without advanced fibrosis, *n* = 207	*P* value
Age, years	50.2 ± 12.9	46.4 ± 12.2	0.083
Gender, *n* (%)			
Male	41 (93.2)	145 (70)	0.001
Female	3 (6.8)	62 (30)	
Ethnic group, *n* (%)			
Malay	29 (65.9)	142 (68.6)	0.827
Chinese	7 (15.9)	23 (11.1)	
Indian	8 (18.2)	42 (20.3)	
Smoking, *n* (%)			
Yes	12 (27.3)	39 (18.8)	0.207
No	32 (72.7)	168 (81.2)	
Diabetes mellitus, *n* (%)			
Yes	5 (11.4)	19 (9.2)	0.584
No	39 (88.6)	188 (90.8)	
Hypertension, *n* (%)			
Yes	18 (40.9)	58 (28)	0.091
No	26 (59.1)	149 (72)	
Dyslipidemia, *n* (%)			
Yes	39 (88.6)	152 (73.4)	0.032
No	5 (11.4)	55 (26.6)	
BMI, kg per m^2	27.6 ± 4.0	26.2 ± 3.8	0.048
Obesity, *n* (%)			
Yes	33 (75)	129 (62.3)	0.110
No	11 (25)	78 (37.7)	
Waist circumference, cm	97.6 ± 10.7	92.4 ± 9.7	0.004
Central obesity, *n* (%)			
Yes	36 (81.8)	151 (72.9)	0.220
No	8 (18.2)	56 (27.1)	
SBP, mmHg	139 ± 15	133 ± 14	0.024
DBP, mmHg	82 ± 10	79 ± 11	0.085
FBS, mmol/L	5.4 ± 0.9	5.4 ± 1.4	0.994
TC, mmol/L	6.0 ± 1.1	5.4 ± 1.1	0.005
HDL, mmol/L	1.4 ± 0.3	1.4 ± 0.3	0.913
LDL, mmol/L	3.3 ± 1.4	3.0 ± 1.2	0.130
TG, mmol/L	1.8 ± 0.8	1.7 ± 1.0	0.224
ALT, IU/L	40 (32–43)	32 (23–39)	<0.0001
AST, IU/L	36 (24–40)	25 (20–34)	0.001
GGT, IU/L	59 (35–73)	43 (26–59)	0.001

Table 4 (Continued)

	Participants with advanced fibrosis, *n* = 44	Participants without advanced fibrosis, *n* = 207	*P* value
WHO recommendation on physical activity for health achieved (assessed using the GPAQ), *n* (%)			
Yes	35 (79.5)	166 (80.2)	0.922
No	9 (20.5)	41 (19.8)	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FBS, fasting blood sugar; GGT, gamma-glutamyl transferase; GPAQ, Global Physical Activity Questionnaire; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WHO, World Health Organization.

Table 5 Univariate and multivariate analysis of factors associated with advanced fibrosis

	Univariate analysis				Multivariate analysis			
	β	OR	95% CI	*P* value	β	Adjusted OR	95% CI	*P* value
Age	0.026	1.026	0.998–1.055	0.070	0.013	1.014	0.982–1.046	0.409
Male	1.765	5.844	1.744–19.585	0.004	1.578	4.847	1.369–17.155	0.014
Waist circumference	0.055	1.057	1.020–1.095	0.002	0.028	1.028	0.987–1.071	0.178
ALT	0.009	1.009	0.996–1.022	0.160	–0.025	0.975	0.946–1.005	0.103
AST	0.028	1.028	0.999–1.058	0.057	0.055	1.057	1.003–1.113	0.036
GGT	0.009	1.009	0.999–1.018	0.066	0.005	1.005	0.989–1.021	0.557
Dyslipidemia	1.038	2.822	1.058–7.526	0.038	0.794	2.212	0.760–6.443	0.146
Hypertension	0.576	1.779	0.907–3.487	0.094	0.251	1.285	0.598–2.762	0.520

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; GGT, gamma-glutamyl transferase; OR, odds ratio.

obesity in Asia.[15] The latest National Health and Morbidity Survey has estimated that 47.7% of the adult Malaysians were overweight or obese.[16] This could explain the high prevalence of NAFLD seen in the current study. In a study conducted on subjects who participated in health checks in a suburban medical facility in Malaysia approximately 10 years ago, the prevalence of NAFLD was 22.7%.[8] The previous local study used ultrasonography, which lacks sensitivity in the assessment of mild fatty liver compared with the CAP.[8] It is also possible that a true increase in the prevalence of NAFLD partly accounted for the observed difference. A higher percentage of NAFLD was observed in the Indians (66.0%) and Malays (56.1%) compared with the Chinese (50.0%), consistent with previous studies on NAFLD in our multiethnic population.[8,17,18] However, the difference was not statistically significant, likely due to the smaller number of subjects in the different ethnic groups in the subanalysis. We did not find an association between diabetes mellitus and NAFLD in our study due to the relatively large number of patients with diabetes mellitus who did not have NAFLD in our study population. The majority of these patients were nonobese males who had a relatively well-controlled diabetes mellitus despite being on little or no medications. We hypothesize that these patients could have undergone intensive lifestyle modification following the diagnosis of diabetes mellitus, which could have attenuated any NAFLD, or CAP was not sensitive enough to diagnose NAFLD in these patients.

This study provided information on the prevalence of advanced liver fibrosis in Malaysia. The high prevalence (17.5%) of advanced fibrosis is certainly worrying. Currently, there are limited data from population-based studies on the prevalence of advanced fibrosis. In a population-based study conducted in Hong Kong, the prevalence of advanced liver fibrosis was found to be 3.7%, much lower than that observed in our study population.[19] The relatively high prevalence of advanced fibrosis in our study is partly due to the use of a sensitive cut-off (i.e. 8 kPa) for LSM for the diagnosis of advanced fibrosis. This cut-off value was based on the paper by Yoneda *et al.*,[13] which is one of the earliest papers that provided optimal cut-off values for LSM for the diagnosis of fibrosis stage in NAFLD patients. In a highly cited study on 246 biopsy-proven NAFLD patients,[20] the cut-off value for advanced fibrosis to yield above 90% sensitivity, maximum sum of sensitivity and specificity, and above 90% specificity was 7.9 kPa, 8.7 kPa and 9.6 kPa, respectively. In other words, the 8 kPa cut-off value we used falls within the range of accepted cut-off values for advanced fibrosis but favored sensitivity over specificity. Even when a higher cut-off value was used (i.e. 9.6 kPa, which provided specificity of over 90%), the prevalence of advanced fibrosis in our study population was still much higher at 9.2%. This likely reflects a truly higher burden of advanced fibrosis in our study population. Differences in genetic and environmental factors, including dietary habits, are the likely explanation for this. Our study participants were comprised of a large proportion of Malays and Indians who may be more genetically susceptible to NAFLD.[21] Our study population also had a worse metabolic profile and comprised a much larger proportion of subjects with NAFLD. In the Asia-Pacific area, Malaysia has the second highest prevalence rate of the metabolic syndrome.[22]

Table 6 Characteristics of participants with and without raised CIMT

	Participants with increased CIMT, *n* = 38	Participants without increased CIMT, *n* = 93	*P* value
Age, years	55.1 ± 6.6	46.3 ± 10.5	<0.0001
Gender, *n* (%)			
Male	34 (89.5)	65 (69.9)	0.018
Female	4 (10.5)	28 (30.1)	
Ethnic group, *n* (%)			
Malay	19 (50.0)	57 (61.3)	0.152
Chinese	6 (15.8)	14 (15.1)	
Indian	13 (34.2)	22 (23.6)	
Smoking, *n* (%)			
Yes	6 (15.8)	15 (16.1)	0.962
No	32 (84.2)	78 (83.9)	
Diabetes mellitus, *n* (%)			
Yes	10 (26.3)	5 (5.4)	0.001
No	28 (73.7)	88 (94.6)	
Hypertension, *n* (%)			
Yes	20 (52.6)	24 (25.8)	0.003
No	18 (47.4)	69 (74.2)	
Dyslipidemia, *n* (%)			
Yes	31 (81.6)	68 (73.1)	0.367
No	7 (18.4)	25 (26.9)	
BMI, kg per m^2	27.7 ± 3.8	26.8 ± 3.7	0.203
Obesity, *n* (%)			
Yes	29 (76.3)	68 (73.1)	0.705
No	9 (23.7)	25 (26.9)	
Waist circumference, cm	98.7 ± 7.2	94.4 ± 9.2	0.006
Central obesity, *n* (%)			
Yes	36 (94.7)	78 (83.9)	0.150
No	2 (5.3)	15 (16.1)	
SBP, mmHg	142 ± 16	133 ± 14	0.005
DBP, mmHg	83 ± 10	79 ± 11	0.075
FBS, mmol/L	6.0 ± 1.7	5.3 ± 1.1	0.004
TC, mmol/L	5.7 ± 1.4	5.3 ± 0.9	0.098
HDL, mmol/L	1.3 ± 0.3	1.4 ± 0.3	0.757
LDL, mmol/L	3.3 ± 1.4	2.7 ± 1.2	0.029
TG, mmol/L	1.7 ± 0.6	1.7 ± 1.3	0.636
ALT, IU/L	38 (25–47)	30 (23–39)	0.026
AST, IU/L	26 (21–36)	25 (20–35)	0.711
GGT, IU/L	49 (27–65)	42 (23–59)	0.197
NAFLD, *n* (%)			
Yes	29 (76.3)	55 (59.1)	0.063
No	9 (23.7)	38 (40.9)	
Advanced fibrosis, *n* (%)			
Yes	7 (18.4)	16 (17.2)	0.868
No	31 (81.6)	77 (82.8)	
WHO recommendation on physical activity for health achieved (assessed using the GPAQ), *n* (%)			
Yes	31 (81.6)	76 (81.7)	0.985
No	7 (18.4)	1**7** (18.3)	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CIMT, carotid intima-media thickness; DBP, diastolic blood pressure; FBS, fasting blood sugar; GGT, gamma-glutamyl transferase; GPAQ, Global Physical Activity Questionnaire; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WHO, World Health Organization.

There were more subjects with NAFLD with an increased CIMT compared to those without NAFLD. However, the difference was not statistically significant, likely due to the small number of subjects with ultrasound carotid Doppler in our study. In a large study on male subjects undergoing health screening in South Korea, persistent NAFLD based on ultrasonography was

Table 7 Univariate and multivariate analysis of factors associated with increased CIMT

	Univariate analysis				Multivariate analysis			
	β	OR	95% CI	*P* value	β	Adjusted OR	95% CI	*P* value
Age	0.127	1.136	1.067–1.209	<0.001	0.136	1.146	1.067–1.231	>0.001
Male	1.298	3.662	1.187–11.298	0.024	1.202	3.327	0.928–11.929	0.065
Waist circumference	0.059	1.061	1.012–1.112	0.014	0.039	1.040	0.975–1.110	0.233
ALT	0.003	1.003	0.991–1.016	0.612	0.004	1.004	0.987–1.021	0.650
Diabetes mellitus	1.838	6.286	1.981–19.945	0.002	0.960	2.611	0.657–10.370	0.173
Hypertension	1.161	3.194	1.452–7.026	0.004	0.510	1.666	0.617–4.496	0.314
NAFLD	0.800	2.226	0.947–5.232	0.066	0.855	2.352	0.768–7.206	0.134

ALT, alanine aminotransferase; CI, confidence interval; CIMT, carotid intima-media thickness; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio.

reported to be associated with the development of subclinical carotid atherosclerosis.[23] Similar reports demonstrating the association between NAFLD and CIMT have been published.[6,7,24–28] However, negative studies on the association between NAFLD and CIMT have also been reported.[29,30] These studies were conducted among patients with diabetes mellitus. In a previous study on patients with diabetes mellitus at our center, NAFLD diagnosed by ultrasonography was reported not to be associated with ischemic heart disease.[31] We hypothesize that the association of NAFLD and cardiovascular disease is more attenuated when there are more established risk factors for cardiovascular disease that are closely related to NAFLD.

Interestingly, we found that advanced liver fibrosis was not associated with an increased CIMT. This lack of association was regardless of whether the 8 kPa or 9.6 kPa cut-off value was used for the diagnosis of advanced fibrosis (data not shown). One possible explanation is that the development of cardiovascular disease and the progression of liver disease in subjects with NAFLD occur through separate pathways, which may be differentially activated in each individual, thus accounting for the varied outcome. Cusi described the current understanding of the pathophysiology of NAFLD, metabolic syndrome, and cardiovascular disease.[32] To the best of our knowledge, this is the first population-based study that evaluated the association between advanced liver fibrosis and subclinical atherosclerosis in the context of NAFLD. In the present study, the factor associated with increased CIMT was older age in concordance with previous community studies.[33,34]

There were several strengths to this observational study. First, it was conducted among health-screening subjects and not among healthcare-seeking adults. Hence, the data are a close estimate of prevalence in a population-based setting. Second, as mentioned before, a more sophisticated and accurate method of detecting NAFLD and liver fibrosis with transient elastography was utilized for the first time in this study, compared to other population-based studies on NAFLD. The CAP and LSM are reliable in the evaluation of significant hepatic steatosis and advanced fibrosis, respectively, and, more importantly, are practical and acceptable to the subjects for the purpose of this study.[3,4]

This study had several limitations. First, due to the sample size of the patients with raised CIMT, we could not draw conclusions on the association of NAFLD with increased CIMT. A larger number of study subjects would be needed to address this limitation. Moreover, the study participants were predominantly middle-class income people, and therefore, this study may not be representative of the general population. The reliability criteria for CAP were not yet established at the time this study was conducted and completed, and therefore, it was not applied in this study. In addition, NAFLD and raised CIMT share common risk factors, such as hypertension, dyslipidemia, and type 2 diabetes. These can be confounding factors in the analysis of the association between NAFLD and raised CIMT. Furthermore, the cross-sectional study design only enabled us to study an association between epidemiological factors with NAFLD and CIMT, rather than causation, which would have been better evaluated using a longitudinal study design.

In conclusion, the prevalence of NAFLD and advanced liver fibrosis was alarmingly high in this middle-aged multiethnic Malaysian population that attended a health screening. NAFLD, advanced liver fibrosis, and increased CIMT were found to be associated with traditional risk factors. NAFLD and advanced liver fibrosis appeared not to be associated with increased CIMT. However, a larger sample size is needed to demonstrate whether there is any association.

References

1 Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer MJH. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016; **64**: 73–84.

2 Wong RJ, Aguilar M, Cheung R *et al*. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology*. 2015; **148**: 547–55.

3 Chan WK, Nik Mustapha NR, Mahadeva S. A novel 2-step approach combining the NAFLD fibrosis score and liver stiffness measurement for predicting advanced fibrosis. *Hepatol. Int.* 2015; **9**: 594–602.

4 Chan WK, Nik Mustapha NR, Mahadeva S. Controlled attenuation parameter for the detection and quantification of hepatic steatosis in nonalcoholic fatty liver disease. *J. Gastroenterol. Hepatol.* 2014; **29**: 1470–6.

5 Wang D, Wang J, Jin C *et al*. Asymptomatic extracranial artery stenosis and the risk of cardiovascular and cerebrovascular diseases. *Sci. Rep.* 2016; **6**: 33960.

6 Li X, Xia M, Ma H *et al*. Liver fat content is associated with increased carotid atherosclerosis in a Chinese middle-aged and elderly population: the Shanghai Changfeng study. *Atherosclerosis*. 2012; **224**: 480–5.

7 Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. *J. Hepatol.* 2008; **49**: 600–7.
8 Goh SC, Ho EL, Goh KL. Prevalence and risk factors of non-alcoholic fatty liver disease in a multiracial suburban Asian population in Malaysia. *Hepatol. Int.* 2013; **7**: 548–54.
9 Goddard E. *Estimating Alcohol Consumption from Survey Data: Updated Method of Converting Volumes to Units. National Statistics Methodological Series*, No. 37. Newport: National Statistics, 2007.
10 Alberti KG, Eckel RH, Grundy SM *et al*. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009; **120**: 1640–5.
11 Surveillance and Population-based Prevention. Prevention of Noncommunicable Diseases Department. *Global Physical Activity Questionaire Analysis Guide*. World Health Organisation, 2006.
12 Boursier J, Zarski JP, de Ledinghen V *et al*. Determination of reliability criteria for liver stiffness evaluation by transient elastography. *Hepatology*. 2013; **57**: 1182–91.
13 Yoneda M, Fujita K, Inamori M *et al*. Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD). *Gut*. 2007; **56**: 1330–1.
14 O'Leary DH, Bots ML. Imaging of atherosclerosis: carotid intima media thickness. *Eur. Heart J.* 2010; **31**: 1682–9.
15 Ng M, Fleming T, Robinson M *et al*. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014; **384**: 766–81.
16 *The National Health and Morbidity Survey 2015. Non-communicable Diseases, Risk Factors & Other Health Problems*, Vol. **2**. Kuala Lumpur: Institute for Public Health, Ministry of Health, 2015. www.iku.gov.my/images/IKU/Document/REPORT/nhmsreport2015vol2.pdf.
17 Chan WK, Bahar N, Razlan H, Vijayananthan A, Sithaneshwar P, Goh KL. Non-alcoholic fatty liver disease in a young multiracial Asian population: a worrying ethnic predilection in Malay and Indian males. *Hepatol. Int.* 2014; **8**: 121–7.
18 Chan WK, Tan AT, Vethakkan SR, Tah PC, Vijayananthan A, Goh KL. Non-alcoholic fatty liver disease in diabetics--prevalence and predictive factors in a multiracial hospital clinic population in Malaysia. *J. Gastroenterol. Hepatol.* 2013; **28**: 1375–83.
19 Wong VW, Chu WC, Wong GL *et al*. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using proton-magnetic resonance spectroscopy and transient elastography. *Gut*. 2012; **61**: 409–15.
20 Wong VW, Vergniol J, Wong GL *et al*. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology*. 2010; **51**: 454–62.
21 Zain SM, Mohamed R, Mahadeva S *et al*. A multi-ethnic study of a PNPLA3 gene variant and its association with disease severity in non-alcoholic fatty liver disease. *Hum. Genet.* 2012; **131**: 1145–52.
22 Ranasinghe P, Mathangasinghe Y, Jayawardena R, Hills AP, Misra A. Prevalence and trends of metabolic syndrome among adults in the asia-pacific region: a systematic review. *BMC Public Health*. 2017; **17**: 101.
23 Sinn DH, Cho SJ, Gu S *et al*. Persistent nonalcoholic fatty liver disease increases risk for carotid atherosclerosis. *Gastroenterology*. 2016; **151**: 481.e1–8.e1.
24 Kim HC, Kim DJ, Huh KB. Association between nonalcoholic fatty liver disease and carotid intima-media thickness according to the presence of metabolic syndrome. *Atherosclerosis*. 2009; **204**: 521–5.
25 Nahandi MZ, Khoshbaten M, Ramazanzadeh E *et al*. Effect of nonalcoholic fatty liver disease on carotid artery intima-media thickness as a risk factor for atherosclerosis. *Gastroenterol. Hepatol. Bed Bench*. 2014; **7**: 55–62.
26 Kang JH, Cho KI, Kim SM *et al*. Relationship between nonalcoholic fatty liver disease and carotid artery atherosclerosis beyond metabolic disorders in non-diabetic patients. *J. Cardiovasc. Ultrasound*. 2012; **20**: 126–33.
27 Targher G, Bertolini L, Padovani R, Zenari L, Zoppini G, Falezza G. Relation of nonalcoholic hepatic steatosis to early carotid atherosclerosis in healthy men: role of visceral fat accumulation. *Diabetes Care*. 2004; **27**: 2498–500.
28 Fracanzani AL, Tiraboschi S, Pisano G *et al*. Progression of carotid vascular damage and cardiovascular events in non-alcoholic fatty liver disease patients compared to the general population during 10 years of follow-up. *Atherosclerosis*. 2016; **246**: 208–13.
29 Guo K, Zhang L, Lu J *et al*. Non-alcoholic fatty liver disease is associated with late but not early atherosclerotic lesions in Chinese inpatients with type 2 diabetes. *J. Diabetes Complications*. 2017; **31**: 80–5.
30 Silaghi CA, Silaghi H, Silaghi AE *et al*. Age, abdominal obesity, and glycated hemoglobin are associated with carotid atherosclerosis in type 2 diabetes patients with nonalcoholic fatty liver disease. *Med. Ultrason.* 2015; **17**: 300–7.
31 Chan WK, Tan AT, Vethakkan SR, Tah PC, Vijayanathan A, Goh KL. Ultrasonography-diagnosed non-alcoholic fatty liver disease is not associated with prevalent ischemic heart disease among diabetics in a multiracial Asian hospital clinic population. *J. Gastroenterol. Hepatol.* 2013; **28**: 1375–83.
32 Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology*. 2012; **142**: 711–25.
33 Wu TW, Hung CL, Liu CC, Wang LY, Yeh HL. Associations of cardiovascular risk factors with carotid intima-media thickness in middle-age adults and elders. *J. Atheroscler. Thromb.* 2017; **24**: 677–86.
34 Weber F. Risk factors for subclinical carotid atherosclerosis in healthy men. *Neurology*. 2002; **59**: 524–8.