ORIGINAL ARTICLE

Prevalence of Ultrasound-diagnosed Non Alcoholic Fatty Liver Disease Among Rural Indigenous Population in Malaysian and Its Association With Biochemical and Anthropometric Measures

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ABSTRACT

Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide, especially in the Western world and Asia-Pacific regions. This study was designed to determine the prevalence of NAFLD detected by sonographic assessment among the rural indigenous population in Peninsula Malaysia and its association with anthropometric and biochemical factors. **Methods:** A cross-sectional survey was carried out from January 2014-February 2016. Subjects were recruited among indigenous peninsula Malaysia population in rural villages, aged ≥18 years old. The survey was questionnaire-based followed by anthropometric and blood parameters measurements. All subjects underwent abdominal ultrasound assessment to screen for the presence of NAFLD. Semi-quantitative visual grading was performed to assess for mild, moderate or severe NAFLD. **Results:** A total of 270 subjects underwent the screening program (mean age 43.3 ±14.0 years). Approximately 53 subjects (19.6 %) were identified with NAFLD. Of those with NAFLD, approximately 83% had moderate grade of fatty liver and the remainder were diagnosed with mild grade. NAFLD was closely associated with age, body mass index (BMI), central obesity, hypertension, total cholesterol (TC), triglyceride/high density lipoprotein-cholesterol (TG/HDL-C) ratio. On multivariate logistic regression, a high BMI (≥23.0 kg/m2), central obesity, and raised TG/HDL-C ratio were independent risk factors for developing NAFLD. **Conclusion:** This pioneer study defines the prevalence of NAFLD among rural indigenous population in Peninsula Malaysia. Lifestyle-related diseases, such as NAFLD can affect both rural and urban communities with equal severity. High BMI, central obesity, and elevated TG/HDL-C ratio were independent risk factors for developing NAFLD.

Keywords: Non-alcoholic fatty liver disease, Ultrasonography, Risk factors, Malaysia, Metabolic syndrome

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common type of hepatic steatosis which develops through three main stages from simple steatosis to non-alcoholic steatohepatitis (NASH) that leads to fibrosis, cirrhosis, and eventually hepatocellular carcinoma (HCC) (1) This spectrum of disease stages is histologically similar to changes observed in alcoholic liver disease but in the absence of significant alcohol consumption (2).

It was first described in 1980 by Ludwig et al (4) and is considered as one of the most common cause of chronic liver diseases in both the Western and Asia-Pacific regions (4). However, the prevalence of NAFLD varies according to the regions. For instance, in the Western countries, Cave et al (5) reported that the prevalence of NAFLD to be estimated at 15-30%. In the Asia-Pacific region, the prevalence of NAFLD has been documented from 9-10% in Japan (6), 18% in Korea (7), 5-24% in China (8,9) and 5-28% in India (10). Chen and his colleagues (11) conducted their study on NAFLD in rural areas in Taiwan and noted a prevalence of 11.5%. In Malaysia, a cross-sectional survey was carried prospectively in suburban area, in which the prevalence of NAFLD was reported to be 22.7% (12) with a slight male predominance.

There is a significant association between NAFLD and hypertriglyceridemia, impaired glucose tolerance, obesity and elevated systolic blood pressure (11,12). Thus, it has been postulated that there is a metabolic predisposition for developing NAFLD (13). Although various imaging modalities such as computed tomography and magnetic resonance imaging have been utilized to diagnose NAFLD (14), ultrasound is the most acceptable, cost-effective imaging tool for mass population screening and a non-invasive method with an excellent sensitivity and specificity of 80% and 99% (15), respectively. It is also easily portable and taken to rural areas as it is a challenge to get the indigenous population to come to the urban clinics or hospitals for health screening. Thus, this study was designed to determine the prevalence of sonographically-detected NAFLD among the rural, indigenous population in Peninsula Malaysia and to determine the associated anthropometric and metabolic risk factors.

MATERIALS AND METHODS

A cross-sectional survey was carried out from January 2014 to February 2016. Ethical approval was obtained from the Ethical Committee for Research Involving Human Subjects at University Putra Malaysia. All subjects were adult Malaysians aged ≥18 years old, that lived in indigenous rural villages in Peninsula Malaysia. Informed consent was obtained from all subjects before commencement of this study. Based on an interview and guided by a structured questionnaire, which was used as an instrument to collect information from the volunteers, the subjects were recruited for this study. Some of the subjects were excluded from the study if they had history of chronic liver disease, previous liver surgery, were Hepatitis B or C carriers or sufferers, had significant alcohol consumption (>140 mg/week) or herbal products consumption, and were smokers.

Anthropometric data acquisition

Weighing scale (Tanita BF-310 GS, Tanita Corporation, Tokyo, Japan) was used to measure body weight, whereas a stadiometre (SECA 206, Tanita Corporation, Tokyo, Japan) was used to measure body height, and then the body mass index (BMI) (kg/m^2) was calculated. BMI was classified according to the World Health Organization guidelines for the Asia-Pacific region (16), where a BMI of less than 23.0 kg/m^2, 23.0-24.9 kg/m^2 and ≥ 25.0 kg/m^2 were classified as normal, overweight and obese, respectively. Waist circumference (WC) and hip circumference (HC) were measured using an elastic tape measure. WC was measured at midpoint between the lower costal border and the iliac crest while hip circumference was measured as a circumference around the buttocks. Then, Waist to hip circumference ratio (WHR) was also calculated for all subjects. WHR of more than 0.90 cm in men and more than 0.85 in women was classified as central obesity (16). The subjects also had their blood pressure measurements taken in the sitting position utilizing a standardized sphygmomanometer to check whether they were hypertensive or normotensive. A subject was considered hypertensive if he had been taking antihypertensive medication(s), if he had a self-reported history of hypertension, or if he had systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg (17).

Biochemical measurements of metabolic risk factors

Blood tests were performed to determine the measurement of the triglyceride (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol, fasting blood glucose (FBG) and glycated haemoglobin (HbA1c). These measurements were classified according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III (National Institute of Health, 2002) (18) and the Malay¬sian Diabetes Mellitus Guidelines, 2009 (Ministry of Health Malaysia, 2009) (19). According to these guidelines, a subject was considered to have dyslipidemia if the TG level was ≥ 1.70 mmol/L, HDL-C < 1.04 mmol/L, LDL-C ≥ 2.59 mmol/L, or TC ≥ 5.18 mmol/L. Moreover, FBG of >5.6 mmol/L was indicative of hyperglycemia, and subjects were diagnosed with diabetes if they had HbA1c of ≥ 6.5%.

Ultrasound screening for fatty liver

Ultrasound screening was performed by radiologists with more than 10 years of work experience, using a portable digital ultrasound machine (Mindray DP-50, MINDRAY Medical International Co., Ltd., Shenzen, China). The machine was equipped with a convex probe (3.5 MHz) to scan the abdomen of our study subjects. By using ultrasound gel to improve the contact between the probe and the anterior abdominal wall, ultrasound examination was performed to scan the liver area and categorically visualize all the segments of the liver. The echogenicity of the liver was compared with that of the right kidney and spleen. Fatty liver disease was diagnosed based on the presence two out of three of the following criteria: markedly increased liver echogenicity as compared with the right renal cortex or spleen, poor or non-visualization of the echogenic portal vascular walls and deep attenuation of the ultrasound signal (20). Semi-quantitative visual assessment was performed to grade the severity of fatty liver by assigning subjects with slightly increased echogenicity of the liver parenchyma as compared to the spleen or right renal cortex as having mild fatty liver or grade I; moderately increased echogenicity of the liver parenchyma with obscured portal vascular wall branches as having moderate fatty liver or grade II; and markedly increased echogenicity/ hyperechoic liver parenchyma with poor or non-visualization of the posterior portion of the diaphragm as having severe fatty liver or grade III (21). The images were also reviewed by two radiologists to diagnose fatty liver by consensus.

Statistical analysis

Data analysis was carried out using Statistical Package for Social Science (SPSS) program version 22.0. Categorical and continuous variables were summarized as percentages and mean ±SD, respectively. Chi-square or Fisher's exact tests were performed for comparison between proportions, where appropriate. A t-test was used to compare the mean ±SD between two groups. *P* value of less than 0.05 is considered statistically significant. Univariate and multivariate analysis using multiple logistic regression analysis was performed to determine predictive risk factors for NAFLD. Variables with *p* value of less than 0.20 on univariate analysis have been subjected to multivariate analysis to adjust for possible confounding factors. Odds ratio (OR) of particular factors for NAFLD were used as a measure of the strength of association with a 95% confidence interval (CI).

RESULTS

Characteristics of the study population

The socio-demographic and health profile characteristics of study population are illustrated in Table I. Two hundred and seventy subjects with mean age of 43.3 ±14.0 years, who met the inclusion criteria were included in the analysis. The study population was predominantly female due to the majority of the males were going to the work at the time of data collection. Approximately two-thirds of the subjects were educated at least to primary school level. More than half of the subjects were obese (54.4%) and had central obesity (53.7%). The mean TC, HDL-C, LDL-C, FBG, and HbA1c were within normal limits. Conversely, triglyceride level was above normal. On sonography, 19.6% of the subjects had NAFLD. Of those with NAFLD, 17.0% of the subjects had mild fatty liver, and 83.0% had moderate fatty liver, whereas; none had a severe grade of fatty liver.

Table I: Socio-demographic, socio-economic and health profile characteristics of the study population (n=270)

Variables	Mean ±SD	n (%)
Age (Years)	43.3 ± 14.0	-
Gender		
Male	-	96 (35.6)
Female	-	174 (64.4)
Salary	699.8 ±578.8	-
Marital status		
Single	-	21 (7.8)
Married	-	239 (88.5)
Widowed	-	10 (3.7)
Educational level		
Illiterate	-	84 (31.1)
Educated	-	186 (68.9)
BMI (kg/m²)		
Normal	-	86 (31.9)
Overweight	-	37 (13.7)
Obese	-	147 (54.4)
Central obesity		
No	-	125 (46.3)
Yes	-	145 (53.7)
TC (mmol/L)	5.2 ±1.2	
TG (mmol/L)	1.9 ±1.3	
HDL-C (mmol/L)	1.4 ±0.3	
LDL-C (mmol/L)	2.9 ±0.9	
TC/HDL-C ratio	4.0 ±1.3	
TG/HDL-C ratio	1.6 ±1.5	
FBG (mmol/L)	4.8 ±2.5	
HbA1c (%)	5.2 ±0.8	
NAFLD		
No	217 (80.4)	
Yes	53 (19.6)	
Fatty liver grades		
Mild	9 (17.0)	
Moderate	44 (83.0)	
Severe	0 (0.0)	

n, Sample size; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein- cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, fasting blood glucose; HbA1c, glycated hemoglobin.

The factors associated with NAFLD

The association between NAFLD and affecting factors is presented in Table II. With regards to age group, our findings revealed a significant association between age and NAFLD (P<0.001). For anthropometric measurements, the prevalence of NAFLD was significantly gradually increased with BMI increases (*P*<0.001), where the prevalence of NAFLD was (1.2%) among normal BMI, (2.7%) among overweight and (34.7%) among the obese subjects, respectively. Similarly, subjects with central obesity had a greater prevalence of NAFLD as compared to those without central obesity, indicating that central obesity was significantly associated with NAFLD (P<0.001). In addition, the prevalence of NAFLD was significantly higher among hypertensive patients (14.3%) as compared to normotensive subjects (24.5%) (*P*=0.036).

With regards to the biochemical measurements, our results revealed that there was a significant association between TC and NAFLD (P=0.030), i.e. the subjects with hypercholesterolemia had much higher prevalence of NAFLD (25.0%) than those with normal cholesterol (14.5%). Moreover, the mean TG/HDL-C ratio was significantly different between subjects with and without NAFLD (P=0.046).

On the other hand, the association between gender, TG, HDL-C, LDL-C, TC/HDL-C ratio, FBG, HbA1c, and menopause status and NAFLD were not found to be significant (*P* -values were; 0.960, 0.105, 0.180, 0.131, 0.057, 0.807, 0.196, 0.221, and 0.664, respectively). In the same context, there was no significant association between age, BMI, central obesity, hypertension, TC, TG, HDL-C, LDL-C, TC/HDL-C, TG/HDL-C, HbA1c, FBG, and menopause status with the grading of fatty

Table II: Comparison of different factors among subjects with and without NAFLD

Variables	No NAFLD	NAFLD	*P*-value*
Age			< 0.001
18-35 years	74/79 (93.7)	5/79 (6.3)	
36-53 years	95/133 (71.4)	38/133 (28.6)	
54-71 years	37/47 (78.7)	10/47 (21.3)	
≥ 72 years	11/11 (100.0)	0/11 (0.0)	
Gender			0.960
Male	77/96 (80.2)	19/96 (19.8)	
Female	140/174 (80.5)	34/174 (19.5)	
BMI			< 0.001
Normal	85/86 (98.8)	1/86 (1.2)	
Overweight	36/37 (97.3)	1/37 (2.7)	
Obese	96/147 (65.3)	51/147(34.7)	
Central obesity			< 0.001
No	113/125 (90.4)	12/125 (9.6)	
Yes	104/145 (71.7)	41/145 (28.3)	
Hypertension			0.036
No	109/126 (86.5)	18/126 (14.3)	
Yes	108/143 (75.5)	35/143 (24.5)	
Hypercholesterolemia (mmol/L)			0.030
No	118/138 (85.5)	20/138 (14.5)	
Yes	99/132 (75.0)	33/132 (25.0)	
Hypertriglyceridemia (mmol/L)			0.105
No	133/159 (83.6)	26/159 (16.4)	
Yes	84/111 (75.7)	27/111 (24.3)	
HDL-C (mmol/L)			0.180
Normal	148/179 (82.7)	31/179 (17.3)	
Low	69/91 (75.8)	22/91 (24.2)	
LDL-C (mmol/L)			0.131
Normal	90/106 (84.9)	16/106 (15.1)	
High	127/164 (77.4)	37/164 (22.6)	
TC/HDL-C ratio	3.9±1.3	4.3 ±1.3	0.057
TG/HDL-C ratio	1.5 ±1.2	2.1 ±2.1	0.046
FBG (mmol/L)			0.807
Normal	191/237 (80.6)	46/237 (19.4)	
High	26/33 (78.8)	7/33 (21.2)	
HbA1c (%)			0.196
Normal	212/262 (80.9)	50/262 (19.1)	
High	5/8 (62.5)	3/8 (37.5)	
Menopause			0.664
No	100/123 (81.3)	23/123 (18.7)	
Yes	40/51 (78.4)	11/51 (21.6)	

BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein- cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, fasting blood glucose; HbA1c, glycated hemoglobin.
* *P* value < 0.05 is considered statistically significant.

Table III: Association of different factors with NAFLD grades

Variables	Fatty liver grades**		*P*-value*
	Mild	moderate	
Age			0.083
16-32 years	2/3 (66.7)	1/3 (33.3)	
33-49 years	4/36 (11.1)	32/36 (88.9)	
50-66 years	3/13 (23.1)	10/13 (76.9)	
≥ 67 years	0/1 (0.0)	1/1 (100.0)	
Gender			0.034
Male	6/19 (31.6)	13/19 (68.4)	
Female	3/34 (8.8)	31/34 (91.2)	
BMI			0.809
Normal	0/1 (0.0)	1/1 (100.0)	
Overweight	0/1 (0.0)	1/1 (100.0)	
Obese	9/51 (17.6)	42/51 (82.4)	
Central obesity			0.974
No	2/12 (16.7)	10/12 (83.3)	
Yes	7/41 (17.1)	34/41 (82.9)	
Hypertension			0.466
No	4/18 (22.2)	14/18 (77.8)	
Yes	5/35 (14.3)	30/35 (85.7)	
Hypercholesterolemia (mmol/L)			0.649
No	4/20 (20.0)	16/20 (80.0)	
Yes	5/33 (15.2)	28/33 (84.8)	
Hypertriglyceridemia (mmol/L)			0.467
No	3/26 (11.5)	23/26 (88.5)	
Yes	6/27 (22.2)	21/27 (77.8)	
HDL-C (mmol/L)			0.093
Normal	3/31 (9.7)	28/31 (90.3)	
Low	6/22 (27.3)	16/22 (72.7)	
LDL-C (mmol/L)			0.568
Normal	2/16 (12.5)	14/16 (87.5)	
High	7/37 (18.9)	30/37 (81.1)	
TC/HDL-C ratio	5.03 ±1.67	4.14 ±1.23	0.067
TG/HDL-C ratio	3.41 ±2.87	1.83 ±1.89	0.146
FBG (mmol/L)			0.199
Normal	9/46 (19.6)	37/46 (80.4)	
High	0/7 (0.0)	7/7 (100.0)	
HbA1c (%)			0.420
Normal	9/50 (18.0)	0/3 (0.0)	
High	41/50 (82.0)	3/3 (100.0)	
Menopause			0.970
No	2/23 (8.7)	21/23 (91.3)	
Yes	1/11 (9.1)	10/11 (90.9)	

BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein- cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, fasting blood glucose; HbA1c, glycated hemoglobin.
* *P* value < 0.05 is considered statistically significant.
** There was no patient with severe grade of fatty liver.

liver (Table III). Interestingly, gender was significant associated with the grading of fatty liver (P=0.034).

Risk factors for NAFLD

The OR with 95% CI was adjusted on univariate and multivariate analysis. On univariate analysis (Table IV), the following factors were found to have a significant association with NAFLD: BMI ≥23.0 kg/m^2 (*P*=0.001, OR=33.49, 95% CI=4.543-246.780), central obesity (*P*<0.001, OR=3.71, 95% CI=1.850-7.448), hypertension (*P*=0.038, OR=1.94, 95% CI=1.038-3.643), and hypercholesterolemia (*P*=0.031, OR=1.97, 95% CI=1.062-3.642). Furthermore, the results demonstrated that every one unit increase in the scores

Table IV: Univariate logistic regression analysis for NAFLD

Variables	B	S.E.	Wald	df	Sig	OR	95% CI for EXP (B)	
							Lower	upper
Age								
< 53 years	-	-	-	-	-	1.00	-	-
≥ 53 years	0.269	0.307	0.766	1	0.382	1.31	0.717	2.389
Gender								
Male	-	-	-	-	-	1.00	-	-
Female	-0.016-	0.320	0.002	1	0.960	0.98	0.526	1.841
BMI								
< 23.0 kg/m^2	-	-	-	-	-	1.00	-	-
≥23.0 kg/m^2	3.511	1.019	11.870	1	0.001	33.49	4.543	246.780
Central obesity								
No	-	-	-	-	-	1.00	-	-
Yes	1.312	0.355	13.634	1	<0.001	3.71	1.850	7.448
Hypertension								
No	-	-	-	-	-	1.00	-	-
Yes	0.665	0.320	4.308	1	0.038	1.94	1.038	3.643
Hypercholesterolemia								
No	-	-	-	-	-	1.00	-	-
Yes	0.676	0.314	4.626	1	0.031	1.97	1.062	3.642
Hypertriglyceridemia								
No	-	-	-	-	-	-	-	-
Yes	0.497	0.308	2.605	1	0.107	1.64	0.899	3.008
HDL-C								
Normal	-	-	-	-	-	1.00	-	-
Low	0.420	0.315	1.784	1	0.182	1.52	0.822	2.820
LDL-C								
Normal	-	-	-	-	-	1.00	-	-
High	0.494	0.329	2.248	1	0.134	1.64	0.859	3.125
TC/HDL-C	0.210	0.111	3.536	1	0.060	1.23	0.991	1.534
TG/HDL-C	0.232	0.090	6.689	1	0.010	1.26	1.058	1.503
FBG								
Normal	-	-	-	-	-	1.00	-	-
High	0.111	0.456	0.060	1	0.807	1.12	0.457	2.735
HbA1c								
Normal	-	-	-	-	-	1.00	-	-
High	2.934	0.747	1.562	1	0.211	2.54	0.588	11.000
Menopause								
No	-	-	-	-	-	1.00	-	-
Yes	0.135	0.424	0.101	1	0.751	1.14	0.498	2.627

B, beta coefficient; SE, standard errors; df, degree of freedom; Sig, significant; OR, odd ratio; CI, confidence interval; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein- cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, fasting blood glucose; HbA1c, glycated hemoglobin.

for TG/HDL-C ratio was significant associated with NAFLD (*P*=0.010, OR=1.26, 95% CI=1.058-1.503).

The results of multivariate analysis are as illustrated in Table V, with BMI ≥23.0 kg/m^2 (*P*=0.002, OR=25.74, 95% CI=3.416-193.973) and central obesity (*P*=0.031, OR=2.30, 95% CI=1.079-4.907) as having independent significant risk factors for developing NAFLD. As such, the results showed that an increase in each unit of TG/HDL-C ratio led to increasing the risk for NAFLD (*P*=0.029, OR=1.58, 95% CI=1.048-2.394).

DISCUSSION

Ultrasound is non-invasive, safe, available and easy to transport from one place to another so that it was acceptable to be used in this survey. In addition, as the lifestyle and environment of rural community differ from those in urban, this motivates to carry out this survey (17). The knowledge about NAFLD screening and

Table V: Multivariate analysis of risk factors for NAFLD using multiple logistic regression analysis

Variables	B	S.E.	Wald	df	Sig	OR	95% CI for EXP (B)	
							Lower	upper
Hypertension								
No	-	-	-	-	-	1.00	-	-
Yes	0.387	0.364	1.131	1	0.288	1.47	0.722	3.007
BMI								
< 23.0 kg/m^2	-	-	-	-	-	1.00	-	-
≥23.0 kg/m^2	3.248	1.030	9.936	1	0.002	25.74	3.416	193.973
Central obesity								
No	-	-	-	-	-	1.00	-	-
Yes	0.833	0.386	4.654	1	0.031	2.30	1.079	4.907
Hypercholesterolemia								
No	-	-	-	-	-	1.00	-	-
Yes	0.598	0.472	1.607	1	0.205	1.82	0.721	4.585
Hypertriglyceridemia								
No	-	-	-	-	-	1.00	-	-
Yes	-0.005-	0.428	0.000	1	0.991	1.00	0.430	2.301
HDL-C								
Normal	-	-	-	-	-	1.00	-	-
Low	0.375	0.448	0.699	1	0.403	1.45	0.604	3.499
LDL-C								
Normal	-	-	-	-	-	1.00	-	-
High	0.892	0.525	2.883	1	0.090	2.44	0.871	6.836
TC/HDL-C	-0.601-	0.278	4.673	1	0.081	0.55	0.318	0.945
TG/HDL-C	0.460	0.211	4.755	1	0.029	1.58	1.048	2.394
FBG								
Normal	-	-	-	-	-	1.00	-	-
High	0.123	0.567	0.047	1	0.828	1.13	0.372	3.434
HbA1c								
Normal	-	-	-	-	-	1.00	-	-
High	0.439	0.953	0.212	1	0.645	1.55	0.240	10.035

B, beta coefficient; SE, standard errors; df, degree of freedom; Sig, significant; OR, odd ratio; CI, confidence interval; BMI, body mass index; HDL-C, high-density lipoprotein- cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, Total Cholesterol; FBG, fasting blood glucose; HbA1c, glycated hemoglobin.

assessment is relatively poor among general populations, particularly rural indigenous populations. In clinical practice, identifying NAFLD is significantly required at risk populations where therapeutic interventions would be of greatest value. Furthermore, this study can be directly translated into clinical practice, where non-invasive screening and monitoring protocols can be developed to address the growing epidemic of NAFLD in the rural indigenous population.

Although NAFLD is a benign disease, it should not be considered lightly (22). It is known as an important cause of liver cirrhosis, as well is predictive risk factor for hepatocellular carcinoma (HCC) (23), even in the absent of cirrhosis (24). A study was carried out on the etiology of cirrhosis and its association with HCC, identified cryptogenic cause which was thought to result from NAFLD, evident in 15.4% of cirrhotic patients and was a risk factor of HCC (25). Thus, NAFLD poises as the most common cause of chronic liver disease in the Western world, which affects 20 to 40% of the general population (26) In Asia, the prevalence of NAFLD was initial uncommon but now it is growing rapidly, affecting up to 30% of the general population (27). In Malaysia, as the prevalence of diabetes mellitus and obesity are demonstrated to be on the rise (28), it could be to speculate that NAFLD may become a common problem as well. In the present study, the prevalence of NAFLD among the rural Malaysian population has been reported to be 19.6%. However, the prevalence is much lower than that documented by two previous studies in Malaysia (44.2% and 49.6, respectively) (29). This may be attributed to several reasons. Firstly, the villagers depend on fresh fruits and vegetables (cholesterol-free food) as their main food source in their daily lives, which is consider as protecting against fat accumulation in the hepatocytes. Secondly, the villagers are broadly using primitive machinery such as bicycles as mode of transportation from one place to another or prefer walking. Thus, making them more physically active

compared to their urban counterparts. This is in keeping with the theory that active subjects are less likely to have NAFLD particularly severe grade than sedentary subjects (30).

A recent study has showed that men had significantly greater prevalence of NAFLD than women for all ages, where a peak prevalence of NAFLD in men was between 40-49 years old; whilst, a peak prevalence in women was between 60-69 years old (31). Our study documented that age was closely associated with NAFLD. The peak prevalence of NAFLD was observed in the patients aged 36-53 years, and then it is declined after the age of 53 years and even became nil at ≥ 71 years old. These differences in the prevalence of NAFLD among different age groups may be attributed to lifestyle-related protective factors. Nevertheless, logistic regression analysis revealed that the age ≤53 years was not a significant risk predictor for developing NAFLD. Moreover, subgroup analysis for gender demonstrated that the highest prevalence of NAFLD in men was between 54-71 years old, whereas; the highest prevalence was in women was between 36-53 years old. However, we did not find a statistically significant association between gender and NAFLD.

Our study is consistent with previous published literature that confirmed a linear correlation between the prevalence of NAFLD and an increase in BMI, central obesity, blood pressure, TC, and TG/HDL-C. As expected, we also found that high BMI (≥ 23 kg/m^2), central obesity, as well an increase in TG/HDL-C. These were revealed to be independent risk factors for developing NAFLD on both univariate and multivariate logistic regression analyses. We noted that the prevalence of NAFLD was dramatically increased with increases in BMI, i.e. the prevalence of NAFLD was higher among the obese subjects, which corroborates the metabolic syndrome theory. In fact, based on the annual health check results in Asian countries, the prevalence of NAFLD is progressively increased with BMI, where it has been documented to be 10-20% among individuals with normal BMI, around 50% among those with a BMI ranges between 25 kg/m^2 and 30 kg/m^2, and 80% among those with a BMI more than 30 kg/m^2 (32). Furthermore, in the line with a previous study from Malaysia (33), our study confirmed that central obesity to be an independent risk predictor of NAFLD. Although several studies revealed a significant association between hypertension and NAFLD (34), the physiological mechanism between hypertension and NAFLD is still unclear. Notably, hypertension is a major risk factor for inducing cardiovascular disease, and is one of the most common components of metabolic syndrome (35) as well as considered as an independent predictive factor for NAFLD (36). This present study showed that hypertension was closely associated with the occurrence of NAFLD, where double to amount of hypertensive subjects were more likely to have NAFLD compared to normotensive subjects. Nevertheless, multivariate logistic regression analysis did not demonstrate hypertension to be a statistically significant risk factor for developing NAFLD.

To date, the pathogenesis of dyslipidemia in NAFLD has never been well understood. Therefore, there is controversy as to whether the fat accumulation within hepatocytes causes lipid metabolism abnormalities or whether lipid metabolism abnormalities are the precipitating factor for developing NAFLD. The results of this study were compatible with recent reports that confirmed a positive association between hypercholesterolemia and NAFLD (37). Nevertheless, some other recent studies did not identify a significant relationship between hypercholesterolemia and NAFLD (38). Additionally, our findings were also consistent with a recent study from Taiwan by Wu et al. (39) that demonstrated an increase in TG/HDL-C ratio was associated with a greater risk for developing NAFLD. This may due to a positive correlation between high TG/HDL-C ratio and metabolic syndrome (40), where the latter is closely associated with the development of NAFLD (41). Surprisingly, hypertriglyceridemia and low HDL-C did not induce NAFLD in our indigenous population, even though many previous studies had strongly confirmed that hypertriglyceridemia and reduced HDL-C were considered as risk factors for developing NAFLD (12,38,42,43). As with most studies, the present findings also detected that high LDL-C did not catalyze NAFLD (8,12,29).

In terms of diabetes mellitus, both HbA1c and FBG are used to diagnose pre-diabetic and diabetic patients. HbA1c measures the average a plasma glucose concentration over three months; whereas, FBG measures to identify the current fasting plasma glucose. Ma et al. (44) revealed that both HbA1c and FBG were closely associated with the risk for developing NAFLD. Our findings were compatible with recent studies, which demonstrated that there was no significant association of FBG and HbA1c with NAFLD (29,43), respectively. Our results overturned previous findings that demonstrated the female hormones may be protective against fat accumulation (7,8,45). Specifically, our results revealed that the prevalence of NAFLD among premenopausal and postmenopausal women was similar, hence indicating a drop in estrogen may not significantly affect the development of NAFLD.

As for the grades of NAFLD, our study did not identify any of subjects as having severe grade of fatty liver. This explains that NAFLD is very slowly progressing condition among the rural villagers due to the same reasons of the low prevalence of NAFLD among rural population which have been explained early in this section. Importantly, only the gender was significantly associated with the grades of fatty liver in this study. Whereby, the males had a higher prevalence of mild fatty liver than females; whilst; females had a higher

prevalence of moderate fatty liver compared to the males. Nevertheless, Sen et al.(46) detected that the severity of fatty liver was significantly increased with triglyceride elevation; whereas, the severity was decreases when HDL-C was reduced. Cheng et al.(42) found that the severity of NAFLD was positively correlated with an increase in BMI. Williamson et al.(47) identified that there was a significant association between the WC with the grades of fatty liver.

The limitation of this study is the relatively lower number of males due to the predominant female prevalence in these populations. Another limitation was the time and logistics constraints that did not permit sampling of the population in East Malaysia, i.e. Sabah and Sarawak. We propose that future studies may be more comprehensive, and also survey the dietary intake of these populations. This would help to shed light on the protective factors that may prevent or slow down the development of NAFLD.

CONCLUSION

NAFLD is not uncommon among rural indigenous population in Malaysia. This population-based epidemiology study could further add to our understanding of the affecting of anthropometric and biochemical factors on prevalence of NAFLD. NAFLD was independently associated with high BMI, central obesity, and elevated TG/HDL-C ratio. Only, gender was significantly associated with developing NAFLD grades. Although NAFLD is considered as one of the lifestyle-related diseases, it is not just confined to the urban population. The further studies are recommended to better equip clinicians to handle this growing up of this condition.

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