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ORIGINAL ARTICLE

A pilot study on pattern B lipoprotein profile in Malaysia

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Abstract

Introduction: Dyslipidaemia is a recognised conventional risk factor for cardiovascular disease (CVD). However, even when traditional lipid parameters are normal, CVD risk can exist. Small dense low-density lipoprotein cholesterol (sdLDL) has appeared as a significant risk marker for CVD. This study aimed to determine the prevalence and associated factors of atherogenic lipoprotein Pattern B in the Malaysian population. *Materials and Methods:* This cross-sectional study included 150 subjects aged 30 years and above who attended a health screening in a Malaysian tertiary institution. Sociodemographics, clinical characteristics and laboratory parameters (lipids, glucose, and sdLDL) were obtained. Lipoprotein subfraction was analysed using the polyacrylamide gel electrophoresis method. *Results:* Malays and females made up the majority of subjects and the median age was 37 years. Normolipidaemic Pattern B was significantly higher in women (p=0.008). Significant independent predictors of Pattern B were gender (p=0.02), race (p=0.01), body mass index (BMI) [p=0.02] and lipid status (p=0.01). Triglyceride was the only independent predictor of sdLDL (p=0.001). *Conclusion:* The prevalence of Pattern B of 33% in this study was comparatively high, of which 6.7% were normolipidaemic. Chinese males with dyslipidaemia and increased BMI independently predicted Pattern B. Differences in triglyceride levels alone among these ethnic groups do not fully explain the differences in the prevalence of Pattern B although it was the only lipid parameter to independently predict sdLDL. Individuals with atherogenic normolipidaemia are at greater risk for a CVD event as they are not included in the protective measures of primary CVD prevention.

Keywords: atherogenic normolipidaemia, cardiovascular risk, small dense low-density lipoprotein cholesterol (sdLDL), Pattern B

INTRODUCTION

In Malaysia, cardiovascular disease (CVD) is estimated to account for 36% of total deaths.[1] The peak incidence of an acute coronary syndrome (ACS) in Malaysia is in the age range 51-60 years, which is comparatively younger than the subjects in the large, multicentre GRACE (Global Registry of Acute Coronary Events) Registry.[2]

The Malaysian National Cardiovascular Disease - Acute Coronary Syndrome (NCVD-ACS) Registry showed that dyslipidaemia, an established risk factor in CVD was present in 55% of patients.[2] However, approximately 50% of individuals with coronary artery disease (CAD) have normal lipid profile results. Besides, only 30% of all myocardial infarctions can be explained on the basis of conventional lipid profile [total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL)]. This indicates the need for a more specific diagnostic tool.[3]

Atherogenic dyslipidaemia, characterised by low HDL, raised TG and small dense low-density lipoprotein cholesterol particles (sdLDL), has emerged as an important marker for increased CVD risk.[4] In prospective studies, a predominance of sdLDL has been linked with

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a 3-7 fold increase in the risk for CAD.[5] Several characteristics link sdLDL to atherogenesis, including enhanced oxidisability, increased plasma resident time and increased endothelial membrane permeability.[6] Numerous methods now exist for measuring lipoprotein subfractions and there is growing evidence for how such tests improve CVD risk prediction.[7] Conventional lipid profile does not convey the CAD risk associated with sdLDL. This risk could exist even when other lipid parameters (TC, TG, LDL and HDL) are normal.[8] Hence, there is an imminent need to identify this risk factor in our multiethnic Malaysian population to further reduce CVD morbidity and mortality.

To date, there are no studies related to LDL subfraction in the Malaysian multiethnic population. As such, this pilot study aimed to determine the i) prevalence; ii) associated factors and iii) independent predictors of atherogenic lipoprotein profile Pattern B in apparently healthy subjects attending health screening in Malaysia. The semi-automated polyacrylamide gel electrophoresis (PAGE) method was used to measure LDL particle size and quantify LDL fractions. Individuals with large, buoyant LDL (lbLDL) particles (LDL1 and LDL2) are classified as Pattern A whereas individuals with sdLDL particles (LDL3 through LDL7) are classified as Pattern B with a higher risk for CAD.[9]

MATERIALS AND METHODS

Subjects

This was a cross-sectional study that involved 150 subjects aged 30 years and above who attended health screening at a medical faculty of a tertiary institution in West Malaysia. Recruitment was by convenience non-random sampling. Exclusion criteria included TC concentration ≤2.59 mmol/L [to avoid overestimation of very-low-density lipoprotein cholesterol (VLDL)], pregnant women, non-fasting subjects and foreigners. The eligible subjects were given a detailed information sheet on the study and informed consent was obtained. Sociodemographic factors and clinical characteristics were recorded in the proforma which was accessible only to researchers. Anthropometric measurements, namely weight, height, waist circumference (WC) and blood pressure (BP) measurements were taken. Body mass index (BMI) was calculated using the formula: weight (kg)/ height^2 (m^2). Confidentiality of participants' identification was ensured.

Biochemical analysis

Seven (7) mls of blood were taken for fasting blood glucose (FBG), fasting serum lipid (FSL) and lipoprotein subfraction using sodium fluoride and plain tubes, respectively. Samples were centrifuged and analysed immediately for FBG and FSL on an automated biochemistry analyser Cobas c311 Analyser (Roche Diagnostics (M) Sdn Bhd) using UV test enzymatic reference with hexokinase and enzymatic colorimetric methods, respectively. Calibration and quality control for both assays were performed by medical technologists in conjunction with normal laboratory operations.

Samples for LDL subfractions were centrifuged and aliquotted on the same day and sera were stored at - 80°C until batch analysis was done. The method for LDL subfractionation is based on electrophoresis of lipid stained serum (Sudan black) in a non-denaturing gel gradient of polyacrylamide. After each analysis, the Lipoprint system software automatically calculates the amount of cholesterol in each lipoprotein subfraction based on the TC of the sample (value obtained from FSL and manually entered into the system) and determines if the subfraction cholesterol values are within the established reference ranges for each subfraction. The batch analysis was completed in seven days. Analytically, the quality was assured as LDL subfraction analysis was perfomed on The LipoPrint® Quantimetrix PAGE system, which is FDA approved (Lipoprint™ LDL System; Quantimetrix, Redondo Beach, CA, USA Inc., Redondo Beach, California). Quality control material (Liposure) from the manufacturer was run prior to each batch analysis. All samples analysed were serum to achieve uniformity among samples.

Statistical analysis

Statistical analysis was done by the standard statistical software package, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. As all data were not normally distributed, median and inter-quartile range (IQR) were used for the continuous variables. Non-parametric tests (Mann Whitney, Kruskal Wallis and Chi Square) were used to determine the association between lipoprotein profiles and independent variables. Spearman's correlation test was used to report correlations between LDL subfractions and lipid profile parameters. For determination of independent predictors of Pattern B, binary logistic regression and linear regression for categorical and continuous

variables, respectively were used. Statistical significance was considered at a 'p' value of < 0.05 (95% confidence interval).

Ethical consideration

The study was approved by The Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia (JKEUPM).

RESULTS

Among 150 adults, Malays and females made up the majority and the median age was 37 years (IQR=11). Most of them had increased BMI and more than half had abnormal WC. The majority were hypertensive and dyslipidaemic. Prevalence of Pattern B was 33% with 6.7%

TABLE 1: Sociodemographic factors, clinical characteristics and laboratory parameters of the study population

Sociodemographic Factors & Clinical Characteristics			(n=150) n (%)
Gender	Male		51 (34.0)
	Female		99 (66.0)
Age (years)	< 40		88 (58.7)
	≥ 40		62 (41.3)
Ethnicity	Malay		79 (51.3)
	Chinese		49 (32.7)
	Indian		23 (15.3)
Smoking status	Yes		7 (4.7)
	No		143 (95.3)
Alcohol status	Yes		26 (17.3)
	No		124 (82.7)
BMI*	Underweight		11 (7.3)
	Normal		64 (42.7)
	Overweight and Obese		75 (50.0)
WC**	Normal		62 (41.3)
	Abnormal		88 (58.7)
BP***	Normal		64 (42.7)
	Prehypertension and Hypertension		86 (57.3)
Lipid status******	Normolipidaemia		67 (44.7)
	Pattern A		57 (38.0)
	Pattern B		10 (6.7)
	Dyslipidaemia		83 (55.3)
	Pattern A		44 (29.3)
	Pattern B		39 (26.0)
FBG*******	Normal		144 (96.0)
	Abnormal		6 (4.0)
Lipid parameters	**Median (IQR)**	**Min – Max**	**Reference range**
TC (mmol/L)	5.20 (1.30)	3.30 – 8.30	≤ 5.20
TG (mmol/L)	1.00 (0.90)	0.30 – 10.40	≤ 1.70
LDL (mmol/L)	3.20 (1.18)	1.40 – 5.70	≤ 2.60
HDL (mmol/L)	1.40 (0.50)	0.80 – 2.90	≥ 1.00

*Based on the International Classification of adult underweight, overweight and obesity according to BMI (kg/m^2) by World Health Organization (WHO): underweight (<18.5); normal (18.5-24.9); overweight (25-29.9); obese (≥30).[41] **Based on the Joint Interim Statement definition of WC (Asia cut-off): normal (male < 90, female < 80); central obesity (male ≥ 90, female ≥ 80).[42] ***Based on recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7): normal (SBP: <120 and DBP: <80); prehypertension (SBP: 120-139 and/or DBP: 80-89); hypertension (SBP: ≥140and/or DBP: ≥ 90).[43] ****Based on recommendation NCEP ATP III Guidelines: normolipidaemia (TC ≤ 5.20, TG≤ 1.70, LDL ≤ 2.60, HDL ≥1.10); dyslipidaemia (at least one parameter outside the recommendations).[14] *****Based on Classification and diagnosis of Diabetes Mellitus by American Diabetes Association (ADA): FBG: normal <7.0 mmol/l; abnormal ≥7.0 mmol/l.[44]

being normolipidaemic Pattern B. The median of each of the lipid parameters was within the normal range except for LDL (Table 1). Table 2 shows significant differences in sociodemographic factors, clinical characteristics and lipid parameters (except LDL) between

TABLE 2: The association of sociodemographic factors, clinical characteristics and laboratory parameters with type of lipoprotein profile pattern

Sociodemographic Factors & Clinical Characteristics	Pattern B n=49 n (%)	Pattern A n=101 n (%)	χ^{2}**	p-value*
Gender				
Male	28 (57.1)	23 (22.8)	17.369	<0.001
Female	21 (42.9)	78 (77.2)		
Age (years)	Median age 43 (IQR =19)	Median age 36 (IQR =11)		
< 40	21 (42.9)	67 (66.3)	7.501	0.006
≥ 40	28 (57.1)	34 (33.7)		
Ethnicity				
Malay	15 (30.6)	63(62.4)	14.836	0.002
Chinese	21 (42.9)	28 (27.7)		
India	13 (26.5)	10 (9.9)		
Smoking status				
Yes	5 (10.2)	2 (2.0)	5.016	0.025
No	44 (89.8)	99 (98.0)		
Alcohol status				
Yes	14 (28.6)	12 (11.9)	6.4	0.011
No	35 (71.4)	89 (88.1)		
BMI				
Normal	15 (30.6)	59 (58.4)	10.204	0.001
Overweight & Obese	34 (69.4)	42 (41.6)		
WC				
Normal	14 (28.6)	48 (47.5)	4.888	0.027
Abnormal	35 (71.4)	53 (52.5)		
BP				
Normal	12 (24.5)	52 (51.5)	9.829	0.002
Prehypertension & Hypertension	37 (75.5)	49 (48.5)		
Lipid status				
Normolipidaemia	10 (20.4)	56 (55.4)	16.438	<0.001
Dyslipidaemia	39 (79.6)	45 (44.6)		
FBG				
Normal	44 (89.8)	100 (99.0)	7.294	0.014
Abnormal	5 (10.2)	1 (1.0)		

Lipid Parameters	Pattern B n=49 Median (IQR)	Pattern A n=101 Median (IQR)	z***	p-value*	Reference range
TC (mmol/L)	5.50 (4.7)	5.10 (4.5)	-1.983	0.047	≤ 5.20
TG (mmol/L)	1.90 (10.1)	0.80 (2.0)	-7.739	<0.001	≤ 1.70
LDL (mmol/L)	3.20 (3.9)	3.20 (4.3)	-1.521	0.128	≤ 2.60
HDL (mmol/L)	1.20 (1.1)	1.50 (2.1)	-6.334	<0.001	≥ 1.00

*statistical significance at p <0.05; ** Chi-Square statistical test (χ^{2})*** Mann-Whitney statistical test (z) Footnotes as for Table 1

subjects with Pattern B and Pattern A. Subjects with Pattern B had higher median values of TC and TG with lower median values of HDL compared to Pattern A. Between subjects with normolipidaemic and dyslipidaemic Pattern B, the only significant differences were gender and BP status. There was significant difference in all lipid parameters except HDL between the two groups. As expected, subjects with dyslipidaemic Pattern B had significantly higher

TABLE 3: The association of sociodemographic factors, clinical characteristics and laboratory parameters with normolipidaemic and dyslipidaemic Pattern B

	Pattern B (n = 49)			
Sociodemographic Factors & Clinical Characteristics	**Normolipidaemic n=10 n (%)**	**Dyslipidaemic n =39 n (%)**	**χ^{2}****	**p-value***
Gender				
Male	2 (20.0)	26 (66.7)	7.078	0.008
Female	8 (80.0)	13 (33.3)		
Age (years)	Median age 41 (IQR =32)	Median age 43 (IQR =18)		
< 40	5 (50.0)	16 (41.0)	0.262	0.609
≥ 40	5 (50.0)	23 (59.0)		
Ethnicity				
Malay	4 (40.0)	11 (28.2)	1.805	0.405
Chinese	5 (50.0)	16 (41.0)		
India	1 (10.0)	12 (30.8)		
Smoking status				
Yes	0 (0.0)	5 (12.8)	1.428	0.232
No	10 (100.0)	34 (87.2)		
Alcohol status				
Yes	2 (20.0)	12 (30.8)	0.452	0.501
No	8 (80.0)	27 (69.2)		
BMI				
Normal	5 (50.0)	10 (25.6)	0.223	0.136
Overweight & Obese	5 (50.0)	29 (74.4)		
WC				
Normal	5 (50.0)	9 (23.1)	2.827	0.093
Abnormal	5 (50.0)	30 (76.9)		
BP				
Normal	5 (50.0)	7 (17.9)	4.421	0.035
Prehypertension & Hypertension	5 (50.0)	32 (82.1)		
FBG				
Normal	9 (90.0)	35 (89.7)	0.001	0.981
Abnormal	1 (10.0)	4 (10.3)		

Lipid Parameters	**Normolipidaemic n=10 Median (IQR)**	**Dyslipidaemic n=39 Median (IQR)**	**z*****	**p-value***	**Reference range**
TC (mmol/L)	4.60 (0.90)	5.70 (1.80)	-3.525	<0.001	≤ 5.20
TG (mmol/L)	1.30 (1.00)	2.00 (0.90)	-3.990	<0.001	≤ 1.70
LDL (mmol/L)	2.60 (0.73)	3.70 (1.55)	-2.875	0.004	≤ 2.60
HDL (mmol/L)	1.20 (0.63)	1.20 (0.30)	-0.968	0.333	≥ 1.00

*statistical significance at p <0.05; ** Chi-Square statistical test (χ^{2}); *** Mann-Whitney statistical test (z); Footnotes as for Table 1

TABLE 4: Correlation between LDL subfractions and lipid parameters

Lipid Parameter	LDL1		LDL2		LDL3		LDL4	
	r**	p-value*	r**	p-value*	r**	p-value*	r**	p-value*
TC (mmol/L)	**0.605**	<0.001	**0.582**	<0.001	0.349	<0.001	0.241	0.003
TG (mmol/L)	-0.173	0.035	**0.566**	<0.001	**0.572**	<0.001	0.216	<0.001
LDL (mmol/L)	**0.719**	<0.001	**0.627**	<0.001	0.317	<0.001	0.124	0.133
HDL (mmol/L)	0.306	<0.001	-0.368	<0.001	-0.392	<0.001	-0.277	0.001

*statistical significance at p <0.05; ** Spearman correlation (r); **bolded r** indicates moderate to strong correlations (r > 0.5)

median values of TC, TG and LDL compared to normolipidaemic Pattern B (Table 3).

There were significant correlations between lipid parameters and LDL subfractions. However, significant strong correlations (r = 0.6-0.79) were noted for TC with LDL1 and LDL with LDL1 and LDL2. TC correlated moderately (r = 0.4-0.59) with LDL2. TG was the only lipid parameter that had a moderate correlation with LDL3. Correlation data between lipid parameters with LDL5 and LDL6 were removed because of small numbers (only six samples with LDL5 and one with LDL6) [Table 4]. Binary regression analysis revealed males were three times as likely to have Pattern B compared with females. Non-Malays were almost three times as likely to have Pattern B than Malays, with Chinese being three times as likely to have Pattern B compared to Indians (p = 0.011) [data not shown]. Subjects with abnormal BMI (preobese and obese) and dyslipidaemia were approximately three times as likely to have Pattern B, respectively, compared to normal levels (Table 5). Multivariate regression showed that only TG was an independent predictor for sdLDL, specifically LDL3. There were no significant independent predictors for LDL4 (Table 6).

DISCUSSION

In this first multiethnic study in Malaysia to determine LDL subfraction using PAGE, the majority of subjects were females. The high number of Malays reflects the ethnic majority in Malaysia but with a higher percentage of Chinese and Indians compared to overall Malaysian

TABLE 5: Independent predictors of Pattern B

Variable	AOR **	95% CI	p value*
Gender			
Female	1		
Male	2.63	1.14-6.05	0.02
Ethnicity			
Malay	1		
Non-Malay	2.99	1.33-6.74	0.01
BMI			
Normal	1		
Overweight and obese	2.7	1.21-6.08	0.02
Lipid status			
Normolipidaemia	1		
Dyslipidaemia	3.2	1.31-7.61	0.01

*statistical significance at p <0.05; **binary logistic regression analysis; AOR: adjusted odds ratio; CI: confidence interval

TABLE 6: Independent predictors for sdLDL (LDL3 and LDL4)

Lipid parameter	LDL3			LDL4		
	β-coefficient**	95% CI	p-value*	β-coefficient**	95% CI	p-value*
TC	-1.032	-0.467-0.081	0.167	0.374	-0.140-0.216	0.675
TG	0.892	0.116-0.363	<0.001	0.448	-0.015-0.146	0.110
LDL	1.157	-0.027-0.532	0.076	-0.207	-0.206-0.157	0.790
HDL	0.295	-0.114-0.404	0.271	-0.023	-0.175-0.162	0.942

*statistical significance at p <0.05; **linear regression analysis; CI: confidence interval

statistics[10] probably because subjects were mainly from an urban area. The median age of subjects was 37 years old consistent with the working age group. These subjects were representative of the Malaysian population with regards to some of the traditional CVD risk factors such as increased BMI, abnormal WC and dyslipidaemia.[11]

The prevalence of dyslipidaemia in this study population (55.3%) was relatively higher compared to that which was reported by NHMS in 2015 (47%).[11] From a worldwide perspective, the prevalence of dyslipidaemia in our study population was comparable to the USA (53%)[12] but higher than China (41.9%).[13] These discrepancies could be attributable to the way dyslipidaemia was defined. In this study, dyslipidaemia was defined as at least one lipid parameter (TC, TG, LDL or HDL) outside the reference range[14] whereas the NHMS 2015 solely based its definition on one parameter, which was hypercholesterolaemia.[11] Toth *et al*. (2012) defined dyslipidaemia based on NCEP ATP III but included only TG, LDL and HDL[12] whereas Huang *et al*. (2014) estimated the prevalence of dyslipidaemia by calculation based on the random effect model from a pool of 38 observational studies conducted previously, covering most of the regions in China, and representing the adult Chinese population.[13]

The novel observation in this study population was a prevalence of 33% for Pattern B. This figure is relatively high compared to the Western population; 24% and 15% in the United States[15] and a Mediterranean population, respectively.[16] In Asians, the prevalence ranged from 7% in Mongolians[17] to 53.2% in Indians[18], with Japanese (21%) Koreans (36%)[17] and Thais (48.4%)[19] midway. This high prevalence of Pattern B is consistent with the markedly growing rates of metabolic syndrome in Malaysia, reported as approximately 25-40% of Malaysian adults depending on the criteria used[20] and the increasing trend of type 2 diabetes mellitus for the past two decades with a current prevalence of 17.5%.[21]

There were significant differences in all sociodemographic factors and clinical characteristics between Pattern B and Pattern A. However, independent predictors of Pattern B include gender, race, BMI and lipid status. Similar to previous studies, Pattern B was more prevalent in males than females.[22,23] In this study, males were three times as likely to have Pattern B with significantly higher values of TC (z=-2.333, p=0.02), TG (z=-4.779, p<0.001), LDL (z=-2.473, p=0.013), and lower HDL (z=-3.494, p<0.001), compared to females (data not shown). The more atherogenic profiles in males can be explained by gender-specific differences in fat distribution[24], lipid metabolism[25] and sex hormones.[26]

Males have less total body fat, on average but higher central/intra-abdominal adipose tissue, while females tend to have more body-fat, reduced visceral white adipose tissue, and higher subcutaneous adipose (abdominal and gluteofemoral regions).[24] Females also show higher rates of mobilisation of TG from adipose tissue stores compared to males. This is perhaps because under conditions of high energy demands (exercise) they are more reliant on free fatty acids as an energy source. Simultaneously, they are more efficient in handling free fatty acids and, hence, preserve their insulin sensitivity.[25] Sex hormones are required to regulate adipocyte metabolism and also influence the sex-specific remodelling of particular adipose depots.[26] Androgens have been linked with an atherogenic lipid profile in some studies. Oestrogens reduce body fat and improve insulin sensitivity, hence, exerting beneficial metabolic effects. However, the role of androgens in regulating insulin action,

especially under conditions of various diets, remains controversial.[24] Higher sex hormone binding globulin (SHBG) levels in females are also associated with a less atherogenic lipoprotein profile. SHBG binds with higher affinity to androgens than oestrogens. Androgens are known to stimulate hepatic TG lipase. Therefore, increased SHBG would result in low free androgen, which maintains reduced hepatic TG lipase activity, hence, slowing the transformation of LDL particle from lbLDL to sdLDL.[27]

Differences in prevalence of sdLDL have been reported in various Asian ethnicities.[15,17,18,28] In this study, being non-Malay had a 3-fold increase in having Pattern B, with Chinese being three times as likely to have Pattern B compared to Indians. This was rather an unexpected finding as most Asian studies showed that Indians are associated with a higher CVD risk compared to other races. Ghazali *et al*. (2015) found that Indians in Malaysia were independently associated with having three or more CVD risk factors.[29] Another study in Singapore with similar main races as Malaysia, showed that Indian males had greater CVD risk than both Chinese and Malay males.[30] Bilen *et al*. (2016) reviewed data on South Asians (subjects whose ancestors originate from the Indian subcontinent, i.e., India, Pakistan, Bangladesh, Sri Lanka, and Nepal) and found that they have a high prevalence of early-onset CHD compared with other ethnic groups.[31] Similarly, Mulukutla *et al*. (2008) also showed a higher prevalence of sdLDL in Asian Indians compared with blacks and whites (53.2% vs. 18.2% vs. 29.9%, $p<0.05$).[18]

No previous study has looked at sdLDL in Malays, Chinese and Indians. Since sdLDL was significantly increased in Chinese, studies on Oriental population were also reviewed. Anuraad *et al*. (2004) reported differing prevalence of sdLDL amongst Koreans, Japanese and Mongolians with 36%, 21% and 7%, respectively.[17] In Van *et al*. (2007) study in the United States, among ethnic Chinese, 8 (44%) of 18 had Pattern B. This was significantly different from the non-Chinese group (Vietnamese, Middle Eastern, southern Asian, Filipinos and Koreans) that had 19% with Pattern B ($p=0.02$).[15] Similar to our study, these researchers also questioned why these apparently healthy Chinese individuals had an increased prevalence of the atherogenic lipid profile. They hypothesised emigration to a Western society from southern (Indian), eastern (Chinese), and south-eastern (Vietnamese) Asia that report lower plasma glucose levels in their motherland, particularly if from a rural area, caused metabolic profiles to markedly deteriorate.[15,17] This explanation is not plausible in our study as ours is a purely Southeast Asian society with no recent emigration.

Since the results are conflicting, we decided to further analyse factors associated with sdLDL pathophysiology (BMI, WC, TG) and their relationship with ethnicity (data not shown). BMI showed significant difference amongst the races. In Indians, 19 (82.6%) of 23 were overweight/obese, which was significantly different from the Chinese (20/49, 40.8%) and Malays (37/78, 47.4%), $\chi^2=11.616$, $p=0.003$. Similarly, WC was highest in Indians and lowest in Chinese, although the difference was not significant ($p=0.238$). Lipid parameters between races were significant with highest values for TC, TG and LDL and lowest value for HDL found in Indians ($p<0.05$). As such, none of the sdLDL-associated factors explained the Chinese being three times as likely to have Pattern B compared to Indians. In fact, these CVD risk factors were highest in Indians, concurring with previous studies.[18,29,30,31]

The reason for the greater propensity to form sdLDL in Chinese is not yet clear and conventional risk factors may not fully explain it. Although genetic influences on Pattern B of about 30-60% have been suggested, the specific genes involved have yet to be determined.[18,28] LDL particle size is influenced by a number of genes including Apo E, hepatic lipase, cholesterol ester transfer protein (CETP), lipoprotein lipase and the apoA1/C3/A4/A5 cluster.[28] During sdLDL formation, hepatic lipase removes core TG and surface phospholipid of LDL and there is core lipid exchange by CETP. Previous research has demonstrated that hepatic lipase and CETP genes have variations in different races, which can significantly influence sdLDL formation.[17] Hence, it may be hypothesised that Chinese have higher hepatic lipase or CETP levels, both implicated as determinant of LDL3 formation along plasma TG. However, TG levels were highest among Indians. The effect of these genes on the Malaysian population is not well elucidated. This is an area that merits further research.

However, the finding of a relatively higher median plasma TG level (although within the reference range) in Chinese 1.2 mmol/L compared to Malays 0.9 mmol/L with Indians still being the highest 1.6 mmol/L ($p<0.001$) was similar to a study of Koreans and Japanese

who were reported to have increased dietary carbohydrate intake. As such, we hypothesised that these Chinese individuals may have higher production of TG-rich VLDL at relatively lower TG concentrations with consequent increase in VLDL and increased sdLDL particles, even with normal TG levels.[17]

Increased BMI being an independent predictor for Pattern B was consistent with other studies that have revealed significant association between obesity with increased sdLDL particles and reduced average LDL particle size.[32,33] Even with normal glucose and lipid homeostasis, obesity was associated with an increase in sdLDL levels and a shift toward a pro-atherogenic lipoprotein distribution.[32] Excess adiposity leads to increased free fatty acid production by adipocytes that are taken up by the liver to produce TG. Increased hepatic TG synthesis leads to increased hepatic production and secretion of VLDL particles that in turn gives rise to an increased number of sdLDL particles. TG in VLDL is traded for cholesterol ester in plasma LDL through the actions of CETP. These LDL particles rich in TG are the preferred substrate for hepatic lipase that converts them into smaller LDL particles.[34]

In addition, dyslipidaemia[14] is another independent predictor for Pattern B. Pattern B in this study was associated with a significantly higher level of TC, TG and lower HDL compared to Pattern A similar to other studies.[19,35] There is a common misconception that total LDL levels and LDL particle size or sdLDL levels are related.[15] The median of LDL was above the reference range but similar for both Patterns in this study, which concurs with previous studies.[15,36] Furthermore, LDL strongly correlated only with lbLDL in this study. All lipid parameters correlated significantly with LDL subfractions (LDL1 to LDL4) but at variable strengths. Only TG correlated moderately with and was an independent predictor for sdLDL (LDL3) in line with prior studies.[17,35] Studies on Caucasians indicated TG levels greater than 133 mg/dL (1.5 mmol/L) favours formation of sdLDL.[17] In this study, median TG concentration was 1.9 mmol/L in Pattern B subjects. Under conditions of high TG availability, the liver secretes increased concentrations of large precursor lipoproteins, which are hydrolysed by lipoprotein lipase and hepatic lipase directly to sdLDL.[19]

Individuals with atherogenic normolipidaemia are at increased risk to develop premature atherothrombosis and experience sudden CVD event.[37] There is published evidence that more than 75% of patients with ACS had normal serum values of LDL and/or HDL.[38] Hence, the existence of even 6.7% of normolipidaemic individuals with an atherogenic lipoprotein profile among our clinically healthy subjects puts doubt on the generally accepted theory that normolipidaemia protects against atherosclerosis. Previous studies found slightly lower prevalence of normolipidaemic Pattern B in Japanese[39] and Europeans[38] with 5.4% and 6%, respectively. In both studies, however, the study population was younger (median age of approximately 20 years) with a normal BMI compared to our study.

In contrast to the overall Pattern B subjects (dyslipidaemic and normolipidaemic), normolipidaemic Pattern B were predominantly female. There was no previous data to compare this gender difference as the subjects in the study by Kazumi *et al*. (1998) was only men[39] and Oravec *et al*. (2014) did not study prevalence difference between genders.[38] However, Magkos *et al*. (2008) concluded that there was no sex difference in total LDL particle levels, subclass distribution and average particle size in normolipidaemic subjects.[32] Evidently, more kinetic studies are required to better appreciate the mechanisms responsible for the observed effects of gender on LDL-subfraction – dyslipidaemic versus normolipidaemic.

This study has several limitations. Potential unmeasured confounders were not adjusted for being a cross-sectional study. Thus, the temporal relationship could not be proven. Result comparison with previous studies may be inaccurate as the various methods for LDL subfraction were not identical, hence no harmonisation or standardisation. However, these methods have been found to be highly correlated.[40]

In conclusion, this study is the first in Malaysia to explore the basis of ethnic differences in the prevalence of Pattern B. Prevalence of Pattern B was relatively high compared to previous studies. Being male, Chinese, with dyslipidaemia and increased BMI independently predicted Pattern B in study subjects. Differences in TG levels alone among these ethnic groups do not fully explain the differences in the prevalence of Pattern B although it was the only lipid parameter to independently predict sdLDL. Although discrepancies exist in the association between Pattern B and its related factors, detection of Pattern B should be considered a useful preventative tool of CHD. Individuals with atherogenic normolipidaemia are at greater risk

for a CVD event as they are not included in the protective measures of primary CVD prevention.

Being a pilot study, there are many questions left unanswered. Hence, this study should be extended to determine the influence of genetic factors and therapeutic strategy (therapeutic lifestyle change and lipid-lowering medication) in Pattern B lipoprotein profile. Further prospective cohort studies with larger sample size are warranted to verify these results and to appreciate the optimum approach to diagnosis, risk stratification, and treatment of CVD across various populations.

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