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ORIGINAL ARTICLE

The relationship between Pattern B lipoprotein profile and low-density lipoprotein cholesterol subfractions with Metabolic Syndrome and Framingham Risk Score in adults at health screening in Malaysia

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Abstract

Introduction: Traditional cardiovascular (CV) risk factors are constituents of Metabolic Syndrome (MetS) and Framingham Risk Score (FRS). However, CV risk exists even when these risk parameters are normal and have been attributed to the atherogenic small dense low-density lipoprotein cholesterol (sdLDL). This study aimed to determine the association of Pattern B and LDL subfractions with MetS and FRS among selected Malaysian population. *Materials and Methods:* A cross-sectional study of 380 subjects ≥30 years old at health screening. Sociodemographic factors and clinical characteristics were recorded. Fasting serum lipids, LDL subfractions and plasma glucose were analysed. *Results:* Being older, Malay with Pattern B independently predicted MetS. Being male, Chinese with Pattern B and increased body mass index (BMI) and diastolic blood pressure (DBP) were more likely to be in the intermediate to high risk FRS group. Common independent biochemical predictors include LDL1 and sdLDL: LDL3 in MetS and non-high-density lipoprotein cholesterol in FRS. *Conclusion:* BMI and DBP may provide incremental prognostic value to FRS risk estimates if included. Considering a significant incidence of Pattern B in low FRS risk subjects (13.4%), routine LDL subfraction analysis could identify these individuals that would be overlooked if their risk were predicted solely based on their FRS only. The non-specific lowering of LDL1 by lipid-lowering therapy based on conventional lipid profile might have a negative effect on several physiological processes. Hence, if LDL subfractions are determined, therapy can be targeted towards sdLDL. Recognising asymptomatic individuals who carry high CV risk is pertinent in primary prevention.

Keywords: Cardiovascular (CV) risk, small dense low-density lipoprotein cholesterol (sdLDL), Framingham Risk Score (FRS), Metabolic Syndrome (MetS)

INTRODUCTION

Ischaemic heart disease is the major cause of national mortality, contributing to 17% of all deaths in 2020. It is the principal cause of death in both males and females as well as in the three major ethnicities (Malays, Chinese and Indians) in Malaysia.[1] Traditionally, major cardiovascular (CV) risk factors include age, obesity, dyslipidaemia, smoking, diabetes mellitus (DM) and hypertension.[2]

Metabolic syndrome (MetS), defined by these CV risk factors is also common in Malaysian adults with a prevalence of 25-40% depending on the criteria used.[3] Different authorities such as the International Diabetes Federation (IDF), World Health Organization (WHO) and National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III have dissimilar criteria for diagnosing MetS. The criteria are based on impaired glucose tolerance (IGT), hyperinsulinaemia, low high-density lipoprotein cholesterol (HDL) and elevated triglyceride (TG) levels. In order to provide more consistent care clinically to MetS patients, IDF Task Force on Epidemiology and

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Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity issued a Joint Interim Statement (JIS) in 2009 that provided a "harmonised" definition of MetS.[4] Pajunen *et al*. analysed the predictive value of several criteria of MetS (IDF, NCEP ATP III, WHO, and JIS) and showed that all MetS definitions were significant predictors for CV event and type 2 DM (T2DM). Moreover, the latest JIS criteria was shown to be an improved predictor of CV event than the total of its constituents.[5] MetS is associated with 1.5 to 2 folds increase in CV risk, CV mortality and all-cause mortality, identifying it as a CV risk tool.[6] MetS is characterised by atherogenic dyslipidaemia, which consists of elevated small dense low-density lipoprotein cholesterol (sdLDL) levels, raised TG and reduced HDL levels.[7] The Atherosclerosis and Insulin Resistance (AIR) Study is the first large-scale study to reveal the significant association between the CV risk factors that define MetS (WHO criteria) with sdLDL and increased intima-media thickness of carotid and femoral arteries indicating subclinical atherosclerosis.[8] The increase of sdLDL in MetS depicts an independent predictive CV risk factor. Hence, sdLDL is suggested as a diagnostic and prognostic marker of MetS.[7]

These traditional risk factors also form the Framingham Risk Score (FRS), a coronary prediction tool that estimates a patient's 10-year risk of developing a CV event, which subsequently dictates management. FRS focuses on primary prevention in subjects with several CV risk factors [systolic blood pressure (SBP), age, lipid profile, DM and smoking status] and its calculation categorises these individuals into low (<10%), intermediate (10-20%), high (>20%) and very high CV risk (>30%) groups.[2]

Dyslipidaemia, a recognised CV risk factor, is a component of both MetS and FRS. International guidelines on cardiovascular disease (CVD) recommend low-density lipoprotein cholesterol (LDL) as the primary target of therapy.[9] Although conventional risk factors provide important information regarding prognosis and underlying pathophysiology and further serve as targets of therapy, clinical data continue to display its unreliability.[10] A large analysis by Greenland *et al*. showed that between 85-95% of patient with coronary artery disease (CAD) had at least one conventional risk factor, but similar findings were also seen in participants without CAD, despite after 30 years of follow-up.[11] Despite the widespread practice of FRS, there is mounting curiosity on emerging novel CV risk predictors. A previous study has demonstrated a significant incidence of Pattern B lipoprotein profile in subjects characterised as low CV risk based on FRS.[12] Another study revealed an increased incidence of subclinical atherosclerosis in asymptomatic females in the low CV risk category based on FRS.[13] A possible factor could be sdLDL contributing to the slow progression of atherosclerosis in these asymptomatic subjects categorised as low CV risk where conventional lipid profile does not detect sdLDL.

Lipoprotein subfractions reveal atherogenic features that are not completely reflected by LDL.[14] Several characteristics link sdLDL to atherogenesis, including enhanced oxidisability, increased plasma resident time and increased endothelial membrane permeability. Subjects with sdLDL will be at an increased CV risk compared with those with marginally normal LDL or within reference interval or with large buoyant LDL (lbLDL).[15] Notwithstanding the recognised role of sdLDL in atherosclerosis, there is no reference method for LDL subfractions to date. Existing methods include density gradient ultracentrifugation, nuclear magnetic resonance, and non-denaturing polyacrylamide gradient gel electrophoresis (PGGE), which are expensive, not user friendly, labour and time intensive.[16] Thus, there is no standardisation among the different methods. However, Food and Drug Administration approved modified PGGE technique named polyacrylamide tube gel electrophoresis (PTGE) is less challenging, operator-friendly, and is more favourable to routine laboratory practice than traditional gel electrophoresis. In this study, PTGE, which has been commercialised by the Lipoprint® System (Quantimetrix, Redondo Beach, CA, USA) was used to quantify and separate LDL particles into seven subfractions (large buoyant LDL1 and 2, and small dense LDL3 through 7) based on size. Characteristically, Pattern A includes lbLDL particles (LDL1 and LDL2 subfractions) and Pattern B, the atherogenic profile has sdLDL particles (LDL3 through LDL7).[17]

To date, there are no data related to the association of Pattern B lipoprotein profile and LDL subfractions with MetS and FRS in Malaysia. Therefore, this study will help identify subjects with an atherogenic profile where LDL levels on conventional lipid profile measurement

are typically normal but there is a higher fraction of sdLDL, which is not detected. In turn, this data could be used to strategise effective prevention and therapeutic programmes to reduce associated CV morbidity and mortality in the Malaysian population.

MATERIALS AND METHODS

Subjects

This is a cross-sectional study of 380 adult subjects (≥30 years old) who attended health screening at the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (FMHS, UPM). This study is an extension of a pilot study on Pattern B, previously described in Thambiah *et al*. (2020).[18] Sociodemographic factors and clinical characteristics were obtained using a Pro-forma. Anthropometric measurements, namely waist circumference (WC), height, weight and blood pressure (BP) measurement were taken. Body mass index (BMI) was calculated using the formula: weight/height squared (kg/m^2). The FRS was calculated using an online calculator.[19] Based on the calculated FRS, each subject was further categorised as: low (<10%), intermediate (10-20%) or high (>20%) 10-year risk of developing CV event.[2] MetS was defined based on the JIS criteria as the presence of at least three out of five risk factors: 1) Central obesity (WC: males ≥90cm and females ≥80cm (Asian cut-off); 2) Elevated TG >1.7 mmol/L; 3) Low HDL: males <1.0 mmol/L and females <1.3 mmol/L; 4) Elevated BP: SBP ≥130 or a diastolic blood pressure (DBP) ≥85 mmHg; and 5) Disorders of glycaemia that include T2DM, IGT or impaired fasting glucose.[4] The Pro-forma was accessible only to researchers. Confidentiality of patient's identification was ensured.

Biochemical analysis

Seven (7) ml of blood was then taken for fasting blood glucose (FBG), fasting serum lipid [FSL: total cholesterol (TC), TG, HDL and LDL] and lipoprotein subfractions, using sodium fluoride and plain tubes, respectively. The collection tubes were transported immediately in an ice-box to the Chemical Pathology Laboratory in FMHS, UPM. Samples for routine biochemistry tests were centrifuged and analysed on the same day for FBG and FSL on the automated biochemistry analyser, Cobas c311 [Roche Diagnostics (M) Sdn Bhd] using UV test enzymatic reference with hexokinase and enzymatic colorimetric methods, respectively. Samples for LDL subfractions were centrifuged and aliquoted on the same day and sera were stored at -80°C until batch analysis was done. Description of the method for LDL subfraction analysis performed on the LipoPrint® Quantimetrix PAGE system (Lipoprint™ LDL System; Quantimetrix, Redondo Beach, CA, USA Inc., Redondo Beach, California) is detailed out in Thambiah *et al*. (2020).[18]

Statistical analysis

The statistical software package, IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp was used for statistical analysis. The data was non-parametric. In descriptive analysis, median and interquartile range (IQR) were presented for skewed distribution; whereas, count (n) and percentage (%) were presented for categorical variables. The association between two categorical variables (with and without MetS groups, and FRS risk groups) were analysed using Chi-Square test. Mann-Whitney and Kruskal Wallis tests were used for comparison of skewed continuous data between subjects with and without MetS and between FRS risk groups, respectively. The correlation between LDL subfractions with components of MetS and FRS was analysed using Spearman correlation test. All variables that were significantly associated and correlated with MetS and FRS were included in binary logistic regression analysis to determine their independent predictors, respectively. For categorical variables, because there is more than one explanatory variable in the model, the interpretation of the adjusted odds ratio (AOR) for one variable depends on the values of other variables being fixed. The AOR with 95% confidence interval (95% CI) indicates the odds of the condition, (e.g., MetS and intermediate to high FRS) between categories. Meanwhile for continuous variables, regression coefficient (B) value indicates the direction of the analyte, i.e., negative and positive B values indicate decrease and increase in the analyte, respectively, in MetS and intermediate to high FRS compared with Non-MetS and low FRS groups, respectively. Statistical significance at p-value <0.05 was considered.

Ethical consideration

The study was approved by The Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia (JKEUPM) in their letters ref: UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F2 dated 30/03/2016 and UPM/TNCPI/RMC/1.4.18.2(JKEUPM) dated 26/11/18 and 13/02/2019.

RESULTS

A total of 380 subjects were included in this study with majority being females and Chinese, followed by Malays and Indians. The median age was 54 years (IQR: 23). Most of them were in the overweight/obese category with an abnormal WC in both genders. Dyslipidaemia was found in 85.8% of the study population. The median of biochemical parameters was within reference interval except for TC, LDL and non-HDL. Data for LDL5 and LDL6 were removed because of its small numbers (LDL5 = 6; LDL6 = 1) and there were no subjects with LDL7. Prevalence of Pattern B in the study population was 45% with 3.9% being normolipidaemic Pattern B (Table 1).

Prevalence of MetS in the study population was 16.6% (Table 1). There were significant associations in all variables between subjects with and without MetS, except for alcohol status, normolipidaemic Pattern B, TC, LDL

TABLE 1: Sociodemographic factors, clinical characteristics and biochemical parameters of study subjects

Sociodemographic factors and clinical characteristics		**N= 380 n (%)**
Gender	Male	140 (36.8)
	Female	240 (63.2)
Age (years)	< 50	143 (37.6)
	≥ 50	237 (62.4)
Ethnicity	Malay	117 (30.8)
	Chinese	184 (48.4)
	Indian	79 (20.8)
Smoking status	Yes	19 (5.0)
	No	361 (95.0)
Alcohol status	Yes	65 (17.1)
	No	315 (82.9)
♦BMI (kg/m²)	< 23	137 (36.1)
	≥ 23	243 (63.9)
♥WC (cm)	Male: ≤ 90	65 (46.4)
	> 90	75 (53.6)
	Female: ≤ 80	77 (32.1)
	> 80	163 (67.9)
♣SBP (mm/Hg)	< 130	193 (50.8)
	≥ 130	187 (49.2)
♣DBP (mm/Hg)	< 85	264 (69.5)
	≥ 85	116 (30.5)
•Lipid status	Normolipidaemia	54 (14.2)
	Pattern A	39 (10.3)
	Pattern B	15 (3.9)
	Dyslipidaemia	326 (85.8)
	Pattern A	170 (44.7)
	Pattern B	156 (41.1)
#FBG (mmol/L)	< 6.1	336 (88.4)
	≥ 6.1	44 (11.6)
ΔMetS	Absent	317 (83.4)
	Present	63 (16.6)
∅FRS (%)	Low risk	171 (45.0)
	Intermediate risk	119 (31.3)
	High risk	90 (23.7)

Biochemical Parameters	Median (IQR)	Min - Max	Reference Interval
FBG (mmol/L)	4.90 (0.70)	2.20 - 14.10	< 6.10
TC (mmol/L)	5.30 (2.00)	2.00 - 8.00	≤ 5.20
TG (mmol/L)	1.20 (0.80)	0.30 - 10.40	≤ 1.70
LDL (mmol/L)	3.20 (1.30)	0.80 - 6.30	≤ 2.60
HDL (mmol/L)	1.45 (0.60)	0.70 - 2.90	≥ 1.00
non-HDL (mmol/L)	3.80 (1.30)	1.50 - 7.10	< 3.40
LDL1 (mmol/L)	0.80 (0.47)	0.03 - 1.86	≤ 1.47
LDL2 (mmol/L)	0.54 (0.39)	0.00 - 1.55	≤ 0.76
LDL3 (mmol/L)	0.10 (0.26)	0.00 - 1.06	≤ 0.16
LDL4 (mmol/L)	0.01 (0.03)	0.00 - 0.67	≤ 0.01

♦Based on the WHO expert consultation on BMI in Asian populations: normal (<23 kg/m^2); overweight/obese (≥ 23 kg/m^2).[31] ♥Based on the JIS definition of WC (Asian cut-off): normal (male <90, female <80); central obesity (male ≥ 90, female ≥ 80).[4] ♣Based on the CPG Management of Hypertension (2018): normal (SBP: <130 mmHg and DBP: <85 mmHg); hypertension (SBP: ≥130 mmHg and/or DBP: ≥85 mmHg).[32] •Based on CPG Management of Dyslipidaemia (2017): normolipidaemia (TC ≤5.20, TG ≤1.70, LDL ≤2.60, HDL ≥1.00, non-HDL <3.40); dyslipidaemia (at least one parameter outside the CPG recommendations).[20] # Based on the CPG Management of Type 2 Diabetes Mellitus (2016): FBG: normal <6.1 mmol/l; abnormal ≥6.1 mmol/l.[33] ΔBased on the JIS definition of MetS.[4] ∅Based on the CPG Primary and Secondary Prevention of Cardiovascular Disease (2017): Calculated FRS[19] estimates the 10-year risk of developing CHD by categorising subjects into: low (risk < 10%), intermediate (risk 10 - 20%) and high CV risk (> 20%) groups.[2]

and non-HDL. Most subjects with MetS were older, non-Malay, males with median age of 58 years old, with abnormal BMI, WC and BP. Majority of them had Pattern B lipoprotein profile with dyslipidaemia. MetS subjects had significantly higher values of TG, FBG, LDL2, LDL3 and LDL4 and lower values of HDL and LDL1 compared with subjects without MetS (Table 2).

Prevalence of FRS was 23.7%, 31.3% and 45% in low, intermediate and high CV risk groups, respectively (Table 1). There was no category for very high CV risk (>30%) as none of the subjects fulfilled the criteria. Significant associations were found for all variables except alcohol status, WC and normolipidaemic Pattern B between FRS risk categories. An increasing trend was noted in TG, non-HDL, FBG, LDL2 and LDL3, whereas a decreasing trend was seen in HDL and LDL1 as the FRS risk category increased. Median values for LDL4 were 0.01 with varying IQR for all groups (Table 3).

A Spearman's rank-order correlation between LDL subfractions (LDL1 to LDL4) with MetS and FRS individual components and total FRS showed significant correlations between most parameters with LDL subfractions. However, moderate, positive correlations (r = 0.4-0.6) were noted only for the following parameters: TG with LDL2, LDL3 and LDL4, respectively, and TC with LDL1 and LDL2, respectively (Table 4).

Variables included in the criteria for MetS (WC, BP, HDL, TG, FBG) and BMI (as closely correlates with WC) were omitted. For MetS, significant variables included in binary logistic analysis were gender, age, ethnicity, smoking status and lipoprotein profile. However, only age, ethnicity and lipoprotein profile were found to be independent predictors of MetS. Subjects ≥50 years old and Pattern B are three and nine times as likely to have MetS, respectively. Chinese are 0.4 times at less risk compared with Malays for MetS. For significant continuous variables of MetS, LDL1, LDL2 and LDL3 were included in binary logistic analysis. However, only LDL1 and LDL3 were found to be independent predictors of MetS. After adjusting for age, MetS subjects showed a significant decrease in LDL1 by 0.06 mmol/L and an increase in LDL3 by 0.1 mmol/L compared with non-MetS subjects (Table 5).

Variables included in the criteria for FRS (age, SBP, smoking status, HDL, TC, FBG) were omitted. For FRS, significant variables included in binary logistic analysis were gender, ethnicity, DBP, BMI, lipid status and lipoprotein profile. However, only gender, ethnicity, DBP, BMI and lipoprotein profile were found to be independent predictors of FRS. Males with DBP ≥85 mmHg, BMI ≥23 kg/m^2 and Pattern B, respectively, are twice as likely to be in the intermediate to high risk FRS category. Chinese are thrice as likely compared with Malays to be

TABLE 2: Association of MetS with sociodemographic factors, clinical characteristics and biochemical parameters in study subjects

Sociodemographic Factors and Clinical Characteristics	MetS Yes (N = 63) n (%)	MetS No (N = 317) n (%)	χ^{2}**	p-value*
Gender:				
Male	33 (52.4)	107 (33.8)		
Female	30 (47.6)	210 (66.2)	7.837	0.005
Age (years):	Median: 58 (IQR: 11)	Median: 53 (IQR: 26)		
< 50	12 (19.0)	131 (41.3)		
≥ 50	51 (81.0)	186 (58.7)	11.113	0.001
Ethnicity:				
Malay	19 (30.2)	98 (30.9)		
Chinese	22 (34.9)	162 (51.1)		
Indian	22 (27.8)	57 (18.0)	10.106	0.006
Smoking status:				
Yes	7 (11.1)	12 (3.8)		
No	56 (88.9)	305 (96.2)	5.938	0.015
Alcohol status:				
Yes	12 (19.0)	53 (16.7)		
No	51 (81.0)	264 (83.3)	0.201	0.654
♦BMI (kg/m^{2}):				
< 23	5 (7.9)	132 (41.6)		
≥ 23	58 (92.1)	185 (58.4)	25.895	< 0.001
♥WC (cm):				
Male				
≤ 90	1 (3.0)	22 (20.6)		
> 90	32 (97.0)	85 (79.4)	5.645	0.018
Female				
≤ 80	0 (0.0)	77 (36.7)		
> 80	30 (100.0)	133 (63.3)	16.196	< 0.001
♣SBP (mm/Hg):				
< 130	4 (6.3)	189 (59.6)		
≥ 130	59 (93.7)	128 (40.4)	59.674	< 0.001
♣DBP (mm/Hg):				
< 85	30 (47.6)	234 (73.8)		
≥ 85	33 (52.4)	83 (26.2)	17.008	< 0.001
•Lipid status:				
Normolipidaemia	4 (6.3)	50 (15.8)		
Dyslipidaemia	59 (93.7)	267 (84.2)	3.828	0.050
Lipoprotein profile:				
Pattern A	9 (14.3)	200 (63.1)		
Pattern B	54 (85.7)	117 (36.9)	50.581	< 0.001
Normolipidaemic Pattern B:				
Yes	4 (7.4)	11 (9.4)		
No	50 (92.6)	106 (90.6)	0.184	0.668

Biochemical Parameters	Yes (N = 63) Median (IQR)	No (N = 117) Median (IQR)	z***	p-value*	Reference Interval
FBG (mmol/L)	6.10 (3.00)	4.80 (0.70)	- 7.483	<0.001	< 6.10
TC (mmol/L)	5.10 (2.00)	5.40 (2.00)	- 1.292	0.196	≤ 5.20
TG (mmol/L)	1.90 (0.70)	1.10 (0.70)	- 8.002	<0.001	≤ 1.70
LDL (mmol/L)	3.00 (1.30)	3.20 (1.20)	- 1.389	0.165	≤ 2.60
HDL (mmol/L)	1.10 (0.30)	1.50 (0.50)	- 8.124	<0.001	≥ 1.00
non-HDL (mmol/L)	3.90 (1.40)	3.70 (1.40)	- 1.413	0.158	< 3.40
LDL1 (mmol/L)	0.67 (0.36)	0.83 (0.44)	- 4.204	<0.001	≤ 1.47
LDL2 (mmol/L)	0.75 (0.41)	0.52 (0.39)	- 4.683	<0.001	≤ 0.76
LDL3 (mmol/L)	0.31 (0.36)	0.08 (0.18)	- 6.975	<0.001	≤ 0.16
LDL4 (mmol/L)	0.03 (0.10)	0.00 (0.00)	- 5.386	<0.001	≤ 0.01

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

in the FRS intermediate to high risk group. For significant continuous variables of FRS, TG, LDL, non-HDL, LDL1, LDL2 and LDL3 were included in binary logistic analysis. However, only non-HDL and LDL1 were found to be independent predictors of FRS. After adjusting for BMI, subjects in the intermediate to high risk FRS category showed a significant increase in non-HDL by 0.9 mmol/L and decrease in LDL1 by 0.09 mmol/L compared with subjects in low FRS risk category (Table 6).

DISCUSSION

In this first study in Malaysia on Pattern B with MetS and FRS, majority were Chinese, females with a median age of 54 years old. The percentage of Chinese and Indians were higher compared with overall Malaysian statistics although Malays are the ethnic majority.[1] This was probably because the subjects were mainly from an urban area. The increasing prevalence of traditional CV risk factors such as dyslipidaemia, obesity, hypertension and diabetes[20] is reflected in this study population by the majority having increased BMI, abnormal WC, raised SBP and dyslipidaemia. Prevalence of Pattern B (45%) in this study population is higher compared with that in the pilot study on Pattern B lipoprotein profile in Malaysia where it was 33%.[18]

Prevalence of MetS in this study population is 16.6% based on the JIS criteria, which is comparatively lower to that reported as approximately 25-40% of adults in Malaysia.[21] Independent predictors of MetS include age, race and Pattern B. Subjects who were more than 50 years old were thrice as likely to have MetS compared with those less than 50 years old. This is consistent with the review on MetS in Malaysia that showed that the prevalence of MetS increases with age. It is estimated that the risk of MetS increased by 3% for every year increase in age.[21] Chinese were less likely than Malays to have MetS whereas there was no significant difference between Indians with Malays and Chinese. This was in contrast to previous studies that demonstrated MetS risk was notably higher among Indians in Malaysia compared with the other ethnic groups. However, in some studies, Malays were shown to have higher incidence compared with Chinese while there was no significant difference from the Malaysia Non-Communicable Disease Surveillance 2005/2006.[21] It is noteworthy that this discrepancy could be due to studies using various criteria of MetS yield different results. Tan *et al*. showed that Chinese males were more likely to exhibit MetS if NCEP ATP III criteria was used instead of the IDF criteria.[22] The disparity in our study could also be due to the uneven numbers in the ethnic groups not representative of the Malaysian population whereby Chinese were overrepresented.

Our results showing that subjects with Pattern B were found to be nine times as likely to have MetS compared with Pattern A are in line with other studies that have demonstrated an association between MetS and sdLDL.[8,23] MetS subjects also showed a significant decrease in LDL1 (lbLDL) and an increase in LDL3 (sdLDL) compared with non-MetS similar to the study by Gazi *et al*. that used the same method/analyser as in our study but an alternative definition of

TABLE 3: Association of FRS risk categories with sociodemographic factors, clinical characteristics and biochemical parameters in study subjects

Sociodemographic Factors and Clinical Characteristics	FRS Categories Low (N: 171) n (%)	Intermediate (N: 119) n (%)	High (N: 90) n (%)	χ^{2}**	p-value*
Gender:					
Male	42 (24.6)	36 (30.3)	62 (68.9)		
Female	129 (75.4)	83 (69.7)	28 (31.1)	53.027	< 0.001
Age (years):	Median: 39 (IQR: 14)	Median: 58 (IQR: 14)	Median: 65.5 (IQR: 15)		
< 50	132 (77.2)	11 (9.2)	0 (0.0)		
≥ 50	39 (22.8)	108 (90.8)	90 (100.0)	209.194	< 0.001
Ethnicity:					
Malay	70 (40.9)	26 (21.8)	21 (23.3)		
Chinese	67 (39.2)	66 (55.5)	51 (56.7)		
Indian	34 (19.9)	27 (22.7)	18 (20.0)	16.230	0.003
Smoking status:					
Yes	3 (1.8)	4 (3.4)	12 (13.3)		
No	168 (98.2)	115 (96.6)	78 (86.7)	17.623	< 0.001
Alcohol status:					
Yes	28 (16.4)	18 (15.1)	19 (21.1)		
No	143 (83.6)	101 (84.9)	71 (78.9)	1.412	0.494
♦BMI (kg/m^2):					
< 23	77 (45.0)	36 (30.3)	24 (26.7)		
≥ 23	94 (55.0)	83 (69.7)	66 (73.3)	11.152	0.004
♥WC (cm):					
Male					
≤ 90	9 (21.4)	6 (16.7)	8 (12.9)		
> 90	33 (78.6)	30 (83.3)	54 (87.1)	1.327	0.515
Female					
≤ 80	48 (37.2)	20 (24.1)	9 (32.1)		
> 80	81 (62.8)	63 (75.9)	19 (67.9)	3.985	0.136
♣SBP (mm/Hg):					
< 130	129 (75.4)	43 (36.1)	21 (23.3)		
≥ 130	42 (24.6)	76 (63.9)	69 (76.7)	78.939	< 0.001
♣DBP (mm/Hg):					
< 85	138 (80.7)	71 (59.7)	55 (61.1)		
≥ 85	33 (19.3)	48 (40.3)	35 (38.9)	18.533	< 0.001
•Lipid status:					
Normolipidaemia	35 (20.5)	9 (7.6)	10 (11.1)		
Dyslipidaemia	136 (79.5)	110 (92.4)	80 (88.9)	10.515	0.005
Lipoprotein profile:					
Pattern A	120 (70.2)	59 (49.6)	30 (33.3)		
Pattern B	51 (29.8)	60 (50.4)	60 (66.7)	34.394	< 0.001
Normolipidaemic Pattern B:					
Yes	4 (7.8)	5 (8.3)	6 (10.0)		
No	47 (92.2)	55 (91.7)	54 (90.0)	0.182	0.913

Biochemical Parameters	Low (N = 171) Median (IQR)	Intermediate (N = 119) Median (IQR)	High (N = 90) Median (IQR)	H***	p-value*	Reference Interval
FBG (mmol/L)	4.70 (0.60)	5.00 (0.70)	5.20 (1.75)	34.729	< 0.001	< 6.10
TC (mmol/L)	5.10 (1.00)	5.50 (2.00)	5.35 (2.00)	12.180	0.002	≤ 5.20
TG (mmol/L)	1.00 (0.70)	1.30 (0.90)	1.50 (0.60)	45.750	< 0.001	≤ 1.70
LDL (mmol/L)	3.10 (1.10)	3.40 (1.40)	3.10 (1.40)	6.243	0.044	≤ 2.60
HDL (mmol/L)	1.50 (0.50)	1.50 (0.50)	1.30 (0.50)	10.571	0.005	≥ 1.00
Non-HDL(mmol/L)	3.60 (1.20)	4.00 (1.50)	4.00 (1.50)	19.162	< 0.001	< 3.40
LDL1 (mmol/L)	0.91 (0.49)	0.76 (0.36)	0.69 (0.36)	28.414	< 0.001	≤ 1.47
LDL2 (mmol/L)	0.47 (0.39)	0.59 (0.44)	0.59 (0.37)	7.856	0.020	≤ 0.76
LDL3 (mmol/L)	0.08 (0.18)	0.16 (0.26)	0.18 (0.26)	31.966	< 0.001	≤ 0.16
LDL4 (mmol/L)	0.01 (0.00)	0.01 (0.03)	0.01 (0.05)	18.181	< 0.001	≤ 0.01

*statistical significance at p <0.05; **Chi-Square test (χ^2); ***Kruskal Wallis Test (H). Footnotes as for Table 1.

MetS that is the NCEP ATP III criteria.[23] That study showed that subjects with MetS exhibited significantly higher concentrations of sdLDL than those without MetS and the concentration and relative distribution of sdLDL correlated with the number of components of MetS.[23]

The pathogenesis underlying sdLDL in MetS has been attributed to insulin resistance (IR). LDL concentrations are typically within the reference interval or slightly increased in IR. However, the composition of the LDL particle is usually abnormal giving rise to sdLDL. The underlying pathophysiology for sdLDL is hypertriglyceridaemia. Hepatic TG synthesis is stimulated by increased flux of free fatty acids to the liver from the periphery. In the liver, the assembly and secretion of TG containing very low-density lipoprotein cholesterol (VLDL), as well as apolipoprotein B (ApoB) production are stimulated giving rise to accumulation of VLDL1 (large TG rich VLDL) molecules. LDL particles with altered ApoB conformation that are produced from VLDL1 by lipoprotein lipase have prolonged circulatory residence time as they fail to bind well to LDL receptors. TG replaces cholesteryl esters in these LDL particles by cholesteryl ester transfer protein. sdLDL, associated with increased CV risk is then

TABLE 4: Correlation between LDL subfractions (LDL1 to LDL4) with components of MetS (WC, TG, HDL, SBP, DBP, FBG) and FRS (WC, TC, HDL, SBP, FBG, total FRS, age) in study subjects

	LDL1		LDL2		LDL3		LDL4	
Parameters	**r****	**p-value***	**r****	**p-value***	**r****	**p-value***	**r****	**p-value***
MetS and FRS components:								
WC (cm)	- 0.073	0.155	0.252	<0.001	0.212	<0.001	0.088	0.087
TG (mmol/L)	- 0.264	<0.001	**0.426**	**<0.001**	**0.564**	**<0.001**	**0.444**	**<0.001**
HDL (mmol/L)	0.262	<0.001	- 0.318	<0.001	- 0.398	<0.001	- 0.299	<0.001
SBP (mm/Hg)	- 0.185	<0.001	0.104	0.042	0.159	0.002	0.080	0.119
FBG (mmol/L)	- 0.120	0.020	0.149	0.004	0.151	0.003	0.048	0.348
DBP (mm/Hg)	- 0.042	0.413	0.153	0.003	0.122	0.017	0.083	0.105
TC (mmol/L)	**0.430**	**<0.001**	**0.452**	**<0.001**	0.276	<0.001	0.156	0.002
Total FRS points	- 0.274	<0.001	0.130	0.011	0.288	<0.001	0.219	<0.001
Age (years)	- 0.305	<0.001	0.036	0.486	0.149	0.004	0.057	0.265

*statistical significance at p <0.05; **Spearman correlation (r); bolded r values reflect moderate correlation (r = 0.4-0.6)

TABLE 5: Binary logistic regression analysis for MetS

	MetS				
Parameters	**B**	**S.E.**	**AOR**	**95% CI**	***p-value**
Age (years):					
< 50			1		
≥ 50	0.956	0.377	2.602	1.243 - 5.450	0.011
Ethnicity:					
Malay			1		
Chinese	- 0.834	0.377	0.434	0.207 - 0.909	0.027
Indian	0.460	0.408	1.585	0.713 - 3.523	0.259
Lipoprotein profile:					
Pattern A			1		
Pattern B	2.197	0.399	9.000	4.121 - 19.655	< 0.001
LDL1 (mmol/L)	- 0.058	0.020	0.943	0.907 - 0.982	0.004
LDL3 (mmol/L)	0.129	0.059	1.137	1.013 - 1.277	0.029

*statistical significance at p <0.05; AOR = adjusted odd ratio, B = regression coefficient, S.E. = standard error

TABLE 6: Binary logistic regression analysis for FRS categories

	Intermediate to high risk FRS group				
Parameters	**B**	**S.E.**	**AOR**	**95% CI**	***p-value**
Gender:					
Female			1		
Male	0.663	0.247	1.940	1.194 - 3.151	0.007
Ethnicity:					
Malay			1		
Chinese	0.945	0.267	2.572	1.525 - 4.339	< 0.001
Indian	0.321	0.323	1.378	0.732 - 2.593	0.320
DBP (mm/Hg):					
< 85			1		
≥ 85	0.720	0.260	2.055	1.235 - 3.419	0.006
BMI (kg/m^2):					
< 23			1		
≥ 23	0.595	0.248	1.813	1.115 - 2.949	0.016
Lipoprotein profile:					
Pattern A			1		
Pattern B	0.845	0.235	2.328	1.469 - 3.691	< 0.001
non-HDL (mmol/L)	0.912	0.224	2.489	1.604 - 3.862	< 0.001
LDL1 (mmol/L)	- 0.089	0.017	0.915	0.855 - 0.946	< 0.001

*statistical significance at p <0.05; AOR = adjusted odd ratio, B = regression coefficient, S.E. = standard error

generated from these TG rich LDL by hepatic lipase.[9] It has been noted that sdLDL is not detected until serum TG exceeds 1.5 mmol/l.[18] In this study, the TG in MetS was 1.9 mmol/L and had the highest significant positive correlation with LDL3 ($r = 0.564$, $p < 0.001$).

FRS is a conventional algorithm for the assessment of 10-year CV risk in asymptomatic, middle-aged subjects. Individuals in Malaysia are risk stratified at the onset using the FRS to determine if they are at high, intermediate or low risk for CVD.[2] To our knowledge, only one published study in Southern Europe investigated the relationship of lipoprotein subfractions with FRS in healthy subjects.[12] In this study, being male and Chinese was twice and thrice as likely to be in the intermediate to high CV risk group, compared with females and other ethnicities, respectively. This is consistent with the Malaysian Cohort Project that demonstrated men have a higher 10-year CV risk when compared with women. This was attributed to the fact that males had consistently higher CV risk factors and comorbidities than females. However, in contrast, their study showed that Malays had the highest prevalence of 10-year CV risk compared with other ethnic groups.[24] Global CV risk scores encompassing these conventional risk factors are designed to predict future CV events. However, limitations exist. Risk-scoring tools developed in one patient population may over or underestimate the risk in a population with different ethnicity, socio-economic background and risk level.[25] Furthermore, as mentioned earlier, Chinese were overrepresented hence the findings of this study may not be comparable to other local studies that reflect the appropriate percentages of the Malaysian ethnic population.

BMI and DBP are not currently included in the calculation of FRS. A study by Mora *et al*. showed that high BMI was an independent predictor of increased CV risk.[26] A large prospective study suggests that isolated diastolic hypertension should not be regarded as a benign entity.[27] These previous studies support our findings of subjects with raised BMI and DBP are twice as likely, respectively, to be in the intermediate to high CV risk group. Hence, these variables if included in FRS may provide incremental prognostic value to FRS risk estimates.

Similar to the study by Vekic *et al*.,[12] there was significant increase in frequency of Pattern B, concentrations of LDL2 and LDL3 and decrease in LDL1 with category of risk in this study. Subjects with Pattern B are twice as likely to be in the intermediate to high risk FRS category compared with Pattern A. Hence, these individuals with Pattern B should be considered suitable candidates for lipid lowering therapy that targets reduction in number and increment in size of LDL particles, considering their increased calculated CV risk. It is important to note that in this study population there was a significant incidence of Pattern B in subjects categorised as low risk according to the calculated FRS (51/380; 13.4%) requiring no further intervention. Michos *et al*. demonstrated an increased incidence of subclinical atherosclerosis in asymptomatic females in the low CV risk category based on FRS.[13] Although the usage of FRS significantly improves the prediction of CV risk, almost 20% of all CV events still occurred in individuals with no traditional major CV risk factors.[28] This indicates that sdLDL could be one of the contributing factors towards the slow progression of atherosclerosis in these asymptomatic subjects categorised as low CV risk based on FRS. Hence, these subjects with Pattern B could benefit from targeted preventive measures if identified early. Subjects in the intermediate to high risk FRS category showed a significant increase in non-HDL and decrease in LDL1 compared with subjects in low FRS risk category. Non-HDL, calculated from TC minus HDL is a measure of cholesterol in all atherogenic lipoproteins. It is advocated in CV risk assessment and the specific therapeutic goal for non-HDL should be 0.8 mmol/L above the corresponding LDL goal.[9]

Comparable independent biochemical predictors of FRS and MetS include a decrease in LDL1 (lbLDL) and an increase in sdLDL; LDL3 in MetS and non-HDL in FRS. Large, buoyant LDL1 and LDL2 subfractions are significant physiological carriers of plasma cholesterol. They are essential for the biosynthesis of vitamin D3, membranes of cells, steroid hormones, subcellular structures, and bile acids. Hence, the non-specific lowering of these lbLDL subfractions by lipid-lowering therapy based on the conventional lipid profile may lead to negative consequences on numerous physiological processes. The non-specific reduction of TC does not give rise to a non-atherogenic lipoprotein profile.[29] Hence, if the levels of specific LDL subfractions are determined, therapy can be targeted towards sdLDL.

This study had several limitations, which include unequal number between ethnicities. Since it was a cross-sectional study, potential

unmeasured confounders were not adjusted for. Thus, temporal relationship could not be established. Lastly, result comparison with previous studies may be inaccurate as the various methods for LDL subfractions were not identical, hence no harmonisation or standardisation. However, these methods have been found to be highly correlated.[30]

This study is the first to investigate the association of Pattern B and LDL subfractions with MetS and FRS in Malaysia. Being older, Malay with Pattern B independently predicts MetS in this study population. Being male, Chinese with Pattern B and increased DBP and BMI were more likely to be in the intermediate to high risk FRS group. Hence, these variables if included in FRS may provide incremental prognostic value to FRS risk estimates. Considering a significant incidence of Pattern B in subjects categorised as low FRS risk in this study (13.4% of study population), performing LDL subfraction analysis routinely could identify these individuals that would be overlooked if their risks were predicted solely based on their FRS only. The non-specific lowering of lbLDL subfractions by lipid-lowering therapy based on the conventional lipid profile might have a negative effect on several physiological processes. Hence, if the levels of specific LDL subfractions are determined, therapy can be targeted towards sdLDL. Recognising asymptomatic individuals who carry high CV risk is pertinent in primary prevention.

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