ORIGINAL ARTICLE

Correlations of Estimated Serum Small Dense Low-Density Lipoprotein and Isoprostane with Metabolic Syndrome Criteria between Metabolic Syndrome and Non-Metabolic Syndrome Subjects in Selangor

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ABSTRACT

INTRODUCTION: Metabolic syndrome (MetS) is a global healthcare burden associated with an increased risk of atherosclerosis (ATH). It is well understood that atherogenesis involves the complexity of inflammation, endothelial dysfunction, and oxidative stress. However, the relationship between atherogenic lipoproteins [small dense low-density lipoproteins (sdLDL)] and oxidative stress biomarkers [isoprostane (ISP)] among those with MetS has not been well established, which this study aims to understand. **MATERIALS & METHODS:** This cross-sectional study involved 67 MetS and 43 non-MetS subjects diagnosed by JIS criteria 2009. Demographic details and anthropometric measurements were recorded. Blood samples were collected to analyse direct LDL, sdLDL-c, and ISP. **RESULTS**: Mean serum sdLDL-c were significantly higher in MetS than non-MetS (1.14+0.44 mmol/L vs. 0.87±0.38 mmol/L, respectively, p=0.005). Similarly, mean serum ISP concentration was higher among MetS than non-MetS (884.40+602.69 ng/L vs. 657.89±616.42 ng/L, respectively, p=0.054). sdLDL-c was positively correlated with triglyceride (TG) in MetS (Spearman 0.552, p<.001), while HDL-c was positively correlated with sdLDL-c among non-MetS (Spearman 0.400, p<.005). **CONCLUSION**: This study emphasises the relationship between sdLDL-c and TG in MetS, emphasising the importance of closely monitoring and managing TG in this cohort to reduce the risk of ATH. It was noted that HDL-c showed a positive correlation with sdLDL-c among non-MetS. This discordant finding suggests that HDL-c may not be causally associated with cardiovascular benefits and that HDL-c subfractions may be a better approach to determining the cardioprotective effects of HDL-c.

Keywords
sdLDL-c, atherosclerosis, metabolic syndrome, isoprostane, JIS Criteria.

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Received: 17th Jan 2023; Accepted: 17th May 2023

Doi: https://doi.org/10.31436/imjm.v22i3

INTRODUCTION

The burden of non-communicable disease (NCD) has caused a significant negative impact on the global healthcare systems and economy. NCDs have caused substantial increases in morbidities and mortalities, reduced quality of life, and escalated healthcare expenditures, particularly in low and middle-income countries. The rise in MetS incidence has posed a new challenge to global public health. MetS is a cluster of at least three interconnected cardio-metabolic dysfunctions that occur concurrently. They include hypertension, abdominal obesity, dyslipidaemia, and glucose intolerance. The global epidemic proportions of MetS were estimated to be around 20–25%, with Malaysia having a prevalence of 25-40%. Individuals with these characteristics are predisposed to the development of ATH, an indolent process involving chronic inflammation, endothelial

dysfunction, oxidative stress, and pro-thrombogenesis that forms atherosclerotic plaques and manifests clinically as atherosclerosis cardiovascular disease (ASCVD).[1]

Low-density lipoprotein cholesterol (LDL-c) has been well established to be one of the risk factors for atherosclerosis, and therefore, ASCVD is one of the criteria to define MetS.[2] Although LDL-c is a strong risk factor for ASCVD, LDL-c levels are not always elevated among patients with ASCVD. It is known that LDL-c consists of three subclasses with distinct sizes, densities, and physicochemical compositions. These subclasses include LDL I–III, representing large buoyant, intermediate density, and small dense LDL, respectively. Accumulating evidence has shown that a predominance of sdLDL-c is closely associated with ASCVD.[3]

sdLDL-c levels were associated with elevated triglyceride (TG) and low HDL-c concentrations, constituents of the 'pro-atherogenic lipoprotein phenotype' commonly observed in T2DM and MetS.[4,5] Previous studies reported that subjects with higher sdLDL-c levels were associated with an increased risk for ASCVD.[6–9] Furthermore, high circulating sdLDL-c levels have been linked with obesity, and systemic inflammation, which are key attributes observed in MetS patients.[10,11]

sdLDL-c are highly susceptible to oxidation, and their propensity to oxidation is related to the atherogenic potential of sdLDL-c particles. It is associated with a higher incidence of ASCVD.[12–15] SdLDL-c are more easily taken up within the arterial walls and exhibit a high susceptibility to oxidation due to their low affinity for LDL receptors, prolonged circulation half-life, and low oxidative stress resistance. It may cause macrophages to take up sdLDL-c and foam cells to form, both of which are crucial components of atherosclerotic plaques.[12,14,16]

Although there has been extensive literature on the role of sdLDL-c in atherogenesis and ASCVD, studies exploring the association between sdLDL-c and MetS with its metabolic components have yet to be fully explored. In addition, the association between sdLDL-c and oxidative stress biomarkers such as ISP remains to be explored. This research addresses these knowledge gaps to better comprehend the role of sdLDL-c in MetS patients and its relationship with ISP as an oxidative stress marker.

MATERIALS AND METHODS

STUDY DESIGN

This cross-sectional study involved 110 participants recruited from a primary care clinic in Sungai Buloh and Selayang regions between July 2020 and December 2021. Demographic information such as marital status, ethnicity, gender, lifestyle habits, age, and occupation was obtained from the participants. Clinical data such as total cholesterol (TC), TG, HDL-c, and fasting plasma glucose (FPG) were retrieved from electronic medical records. The trained nurses measured anthropometric information, such as height, weight, blood pressure, and waist circumference. Blood pressure (BP) was measured using an automated BP reader while the subject was seated and after 5-10 minutes of rest (cuff size 12 x 33cm, Colin press-mate, Japan). The systolic and diastolic blood pressures were measured to the nearest 1 mmHg. BMI was calculated by the formula: BMI=weight (kg)/height^2 (m^2). Waist circumference was measured to the nearest 0.5 cm using a measuring midway between the inferior margin of the last rib and the iliac crest in a horizontal plane.

Study Population

One hundred ten participants were included in this study consisting of 67 MetS and 43 non-MetS subjects aged between 18-65 years old. MetS participants were selected using the Joint Interim Statement (JIS) 2009 criteria.[14] Those who fulfilled any two of the following criteria, which are waist circumference ≥90 cm for males or ≥80 cm for females, systolic blood pressure ≥130 and diastolic blood pressure ≥85 mmHg or on treatment for hypertension, FPG ≥5.6mmol/L or on treatment for elevated glucose, serum TG ≥1.7 mmol/L or on treatment for hypertriglyceridaemia, serum HDL-c <1.0mmol/L for male or <1.3mmol/L for female or on treatment for HDL-c, were included in the MetS group while participants who did not fulfil any of the criteria

were recruited in the non-MetS group. The exclusion criteria included non-Malaysians, pregnant women, established ASCVD, history of life, a renal, endocrine disorder, malignancies that shorten the life span or acute or chronic inflammatory state, and are on any antioxidant supplements.

Sampling Method

This sample was selected using a systematic random sampling procedure to minimise sampling bias until the target sample size was achieved. Every third consecutive patient was screened for eligibility according to the inclusion and exclusion criteria before being invited to participate.

Sample Collection

Blood samples were collected by venipuncture following 8 -10 hours of fasting. Centrifugation separated the plasma and serum for 10 minutes at 3500 rpm. The plasma and serum were transferred into 2.0 ml Eppendorf tubes and frozen at -80^{o}C until further analysis.

Biochemical Analysis

Serum direct LDLs were analysed on an automated analyzer, c501 (Roche Diagnostics, Germany), at an ISO 15189 accredited laboratory (Clinical Diagnostics Laboratory, Hospital UiTM Sg. Buloh). Plasma ISP was measured using a sandwich Enzyme-Linked Immunosorbent Assay principle (ELISA) from Bioassay Technologies.[15]

SdLDL-c concentration was derived by the Srisawasdi formula:[17]

SdLDL-c (mg/dL) = 0.580 (non-HDL-C) + 0.407 (dLDL-C) – 0. 719 (cLDL-C) - 12.05

Ethical Consideration

The study complies with the Declaration of Helsinki. The institution's ethics approval [REC/03/2020 (FB/47)] and informed consent were obtained before data and sample collection.

Statistical Analysis

The optical density (OD) of ISP obtained from the microplate reader was analysed using a four-parameter logistic curve on log-log graph paper with standard concentration on the x-axis and OD values on the y-axis. The value of R^2 must be more than 0.95 to ensure that the standard curve is the best-fit curve graph. These calculations can be best performed with computer-based curve-fitting software (MyAssay.com). All the statistical analysis was performed using SPSS version 24.0. Continuous data were presented as mean ± standard deviation, while categorical data were described as numbers and percentages. Analysis of normality was performed by using the Kolmogorov-Smirnov test. Pearson correlation and Spearman analysis were used to determining the correlation between sdLDL-c and ISP, with clinical risk factors of MetS. All p-value is two-tailed, and $p < 0.05$ is considered statistically significant.

RESULTS

Demographic characteristics

One hundred ten participants were recruited in this study, of which 67 were MetS and 43 were non-MetS (Mean age: 53.58±11.52 years and 44.60±13.87 years, respectively). The sociodemographic and clinical characteristics of the participants are shown in Table 1. 53.7% of males and 46.3% of females were in the MetS, while 58.1% were females, and 41.9% were males in non-MetS. The mean BMI among MetS was 30.05±5.44, with more than half (49.3%) categorised as obese. The mean BMI for non-MetS was 26.07±4.27, with 23.3% in the obesity categories. Participants from MetS have diabetes mellitus (49.3%,) hypertension (74.5%), and dyslipidaemia (85.1%).

The mean difference of sdLDL-c and ISP between MetS and non-MetS groups

Table 1 Demographic and clinical characteristics among MetS and non-MetS

	MetS (n=67)		Non-MetS (n=43)		*p-value*
	n	%	n	%	
Gender					0.224
Male	36	53.7	18	41.9	
Female	31	46.3	25	58.1	
Age (Mean ± SD)	53.58 ± 11.52		44.60 ± 13.87		0.011
18-30 years old	4	6.0	9	20.9	
31-40 years old	6	9.0	5	11.6	
41-50 years old	13	19.4	15	34.9	
51-60 years old	22	32.8	7	16.3	
>60 years old	22	32.8	7	16.3	
Ethnicity					0.078
Malay	48	71.6	38	88.4	
Chinese	8	11.9	0	0	
Indian	10	14.9	4	9.3	
Others	1	1.5	1	2.3	
Education level					0.002
No education	5	7.5	0	0	
Primary	30	44.8	8	18.6	
Secondary	31	46.3	35	81.4	
Tertiary	1	1.5	0	0	
BMI (Mean ± SD)	30.05 ± 5.44		26.07 ± 4.27		0.009
Underweight (<18.5)	1	1.5	0	0	
Normal (18.5-24.9)	10	14.9	17	39.5	
Overweight (25.0-29.9)	23	34.3	16	37.2	
Obesity (≥30.0)	33	49.3	10	23.3	
Smoking Status					0.936
Smoker	9	13.4	6	14	
Ex-smoker	5	7.5	4	9.3	
Non-smoker	53	79.1	33	76.7	
Alcohol Drinker					0.690
Occasionally	9	13.4	5	11.6	
Daily	1	1.5	0	0	
Never	57	85.1	38	88.4	
Diabetes Mellitus					<0.001
Yes	33	49.3	0	0	
No	34	50.7	43	100	
Hypertension					<0.001
Yes	50	74.5	0	0	
No	17	25.4	43	100	
Dyslipidaemia					<0.001
Yes	57	85.1	0	0	
No	10	14.9	43	100	
Waist circumference					<0.001
Male (Mean ± SD)	102.69 ± 10.37	16.7	83.50 ± 6.76	88.9	
< 90 cm	6	83.3	16	11.1	
≥ 90 cm	30		2		
Female (Mean ± SD)	94.09 ± 8.87		76.52 ± 3.49		<0.001
< 80 cm	0	0	25	100	
≥ 80 cm	31	100	0	0	
Blood pressure					
Systolic pressure (Mean ± SD)	142.76 ± 13.04		121.14 ± 7.22		<0.001
< 130 mmHg	11	16.4	30	69.8	
≥ 130 mmHg	56	83.6	13	30.2	<0.001
Diastolic pressure (Mean ± SD)	82.18 ± 7.88		71.41 ± 8.74		
< 85 mmHg	42	62.7	42	97.7	
≥ 85 mmHg	25	37.3	1	2.3	
Triglycerides (Mean ± SD)	1.63 ± 0.74		0.90 ± 0.29		<0.001
< 1.7 mmol/L	43	64.2	43	100.0	
≥ 1.7 mmol/L	24	35.8	0	0.0	
Fasting Blood Sugar (Mean ± SD)	7.20 ± 3.18		5.05 ± 0.48		<0.001
< 5.6 mmol/L					
≥5.6 mmol/L	24	35.8	41	95.3	
	43	64.2	2	4.7	
HDL-c (Mean ± SD)	1.18 ± 0.25		1.40 ± 0.23		0.027
Male					
< 1.0 mmol/L	27	75.0	8	44.4	
≥ 1.0 mmol/L	9	25.0	10	55.6	0.036
Female	1.38 ± 0.35		1.62 ± 0.28		
< 1.3 mmol/L	13	41.9	4	16.0	
≥1.3 mmol/L	18	58.1	21	84.0	

The mean serum calculated sdLDL-c concentration was more significant than the cut-off value of 0.8 mmol/L and was significantly higher in the MetS group than those without MetS (mean±SD: 1.09±0.45 mmol/L vs. 0.86±0.36, p=0.005). Mean serum ISP concentration was significantly higher in the MetS group compared to the non-MetS (mean±SD: 861.11± 593.58 vs. 630.98±634.39, p=0.050) as summarised in Table 2.

Table 2 Mean difference of sdLDL-c and ISP between MetS and non-MetS group

Variables	Mets (n=67) Mean (SD)	Non-MetS (n=43) Mean (SD)	Mean diff. (95% CI)	t-statistics (df)	*p-value*
sdLDL-c (mmol/L)	1.09 (0.45)	0.86 (0.36)	0.23 (0.071,0.393)	2.86 (108)	0.005
ISP (ng/L)	861.11 (593.58)	630.98 (634.39)	230.13 (-6.05,466.31)	1.93 (108)	0.050

Correlation between serum sdLDL-c and ISP concentrations with JIS criteria between MetS and non- MetS groups

Table 3 summarises the correlations between the JIS criteria for MetS and sdLDL-c.

In the MetS group, only triglycerides are positively correlated with sdLDL-c level (R =0.552, p <0.001). Diastolic and systolic blood pressure positively correlated with sdLDL-c; however, the correlation was insignificant. Other risk factors of MetS, such as waist circumference, fasting blood sugar, and HDL, were not significantly correlated with sdLDL-c, as shown in Table 3. HDL-c showed a strong positive correlation with sdLDL-c level among the non-MetS (R=0.422, p <0.010). Other MetS criteria were negatively correlated but did not achieve statistical significance. Diastolic blood pressure showed a positively strong correlation with ISP in non-MetS. However, no correlations were observed between ISP and MetS criteria, as depicted in Table 3.

DISCUSSION

By comparing the BMI, 49.3% of the Mets subjects had BMI >30 kg/m^2; in non-MetS, only 23.3% had a BMI of >30 kg/m^2. This study is consistent with previous studies reporting that body weight plays a significant role in MetS.[18,19,20] Obesity or overweight increased the risk of MetS by seven-fold and 14.5 in women and men.[21] These consistent findings denote the significance of lifestyle modifications in MetS prevention, mainly using BMI as the monitoring tool of management progress.

A previous study reported that out of 86 hypertensive patients with obesity, 51 (59.3%) had MetS, which involved 47.1% of men and 52.9% of women.[23] Our study is in keeping with these findings, where 74.5% of MetS subjects were hypertensive, and there was no hypertension in the non-MetS subjects. This study also denoted a significantly higher WC among MetS in males and females compared to non-MetS. The MetS group also had higher FPG concentrations (>5.6 mmol/L) compared to the non -MetS counterpart (64.2% vs. 4.7%, respectively). Similarly, with TG where 35.8% of MetS subjects had high concentrations (TG>1.7 mmol/L) in contrast with the non-MetS group, which had none elevated above the cut-off. This is concordant with a previous study that found TG, FPG, and WC significantly higher among patients with MetS.[23] One study reported the prevalence of MetS to be generally higher among females, increased age, in urban areas, and among the Indian population.[24] However, this study observed the prevalence of MetS

Table 3 Correlation between sdLDL-c, ISP, and JIS Criteria in the MetS and non-MetS group

Variables	Group	Triglycerides	Systolic Blood Pressure	Diastolic Blood Pressure	Waist Circumference	Fasting Blood Sugar	High-Density Lipoprotein
sdLDL-c (mmol/L)	Mets	0.552 (0.000)**	0.163 (0.188)	0.196 (0.112)	-0.124 (0.318)	-0.082 (0.510)	-0.062 (0.617)
	Non-MetS	0.201 (0.196)	-0.018 (0.908)	-0.124 (0.429)	-0.139 (0.375)	-0.032 (0.837)	0.400 (0.008)**
ISP (ng/L)	MetS	0.044 (0.722)	-0.075 (0.546)	0.045 (0.719)	-0.062 (0.620)	-0.147 (0.236)	-0.057 (0.648)
	Non-MetS	-0.012 (0.938)	0.245 (0.113)	0.313 (0.041)*	0.080 (0.608)	-0.044 (0.781)	-0.063 (0.689)

**p-value < 0.05*
***p-value < 0.01*

being equally distributed between males and females, highest among obesity subjects, non-smokers, and non-alcohol drinkers among the Malay population. This highlights the differences in the predominance of risk factors for MetS geographically and could, in part, be attributed to a combination of genetic and lifestyle variations worth exploring.

A growing number of studies in recent years have demonstrated that sdLDL-c is more likely to contribute to atherosclerosis. Several factors make sdLDL-c an atherogenic biomarker. Firstly, sdLDL-c has a longer half -life and is more prone to oxidative modification than large LDL-c particles due to its specific characteristics of low density, small particle size, and more remarkable penetration ability to the artery wall. These characteristics can cause significant damage to endothelial cells, resulting in the chemotaxis of monocytes in blood vessels to form macrophages, increased permeability, and phagocytosis of oxidized LDL-c to develop foam cells, all of which contribute to the formation of ATH.[25] Secondly, the probability of sdLDL-c undergoing oxidation is higher as alipoproteinB-100 (apoB-100) plasma retention time on the surface of sdLDL-c is significantly longer than the apoB-100 on the surface of large buoyant LDL (LDL). The binding of sdLDL-c to the LDL receptors is much reduced compared to LDL-c due to the low binding affinity of apoB100 to the LDL receptor.[26] Thirdly, sdLDL-c may lead to ATH through mitochondrial damage. This is given the observation that when compared to all LDL phenotypes, Lipoprotein-associated phospholipase A2 (Lp-PLA2) prefers to bind with sdLDL-c particles resulting in sdLDL-c granules containing more Lp-PLA2.[25] Lp-PLA2 which produces Lysophosphatidylcholine (LPC) which has been shown to promote the expression of inflammatory mediators, increase oxidative stress, damage arterial relaxation, induce endothelial activation and ATH.[27] This study demonstrated higher serum sdLDL-c concentration among MetS compared to non-MetS groups, keeping with previous studies reporting similar findings.[28] It has been demonstrated that the proportion of patients with MetS gradually increases with rising sdLDL-c levels, and the number of MetS components is associated with a gradual increase in sdLDL-c levels.[29] sdLDL-c with elevated TG levels and low HDL-cholesterol constitute 'atherogenic lipoprotein phenotype (ALP), a form of atherogenic dyslipidaemia that is a feature of T2DM and MetS.[30]

There was a positive correlation between serum-calculated sdLDL-c and TG, concordant with previous studies which reported TG as a significant determinant of sdLDL-c by direct measurement.[31] They observed a positive correlation between sdLDL-c and serum TG. Upon exclusion of subjects with TG > 400 mg/dL, the correlation between sdLDL-c and TG became substantially stronger. Another study reported positive correlations between sdLDL-c with waist circumference, FPG, HDL-c, and TG after adjusting for physical activity, smoking, alcohol, and age.[30] They also noted the strongest correlation between sdLDL-c and TG from their partial Spearman correlation analysis. Hypertriglyceridaemia is characterised by increased VLDL particle production and decreased catabolism. The cholesteryl-ester transfer protein (CETP), which facilitates the exchange of core TG within VLDL and cholesteryl-esters within LDL particles, is also associated with it. This results in TG enrichment of LDLs. The latter are suitable substrates of hepatic lipase (HL) and lipoprotein lipase (LPL), which further converts them into smaller, denser forms.[28] These findings imply that sdLDL-c may be a biomarker of the 'pro-atherogenic phenotype' observed in patients with hypertriglyceridaemia, metabolic syndrome and diabetes, and other high atherosclerotic cardiovascular diseases risk groups and, therefore, worth considering including sdLDL-c in CVD risk assessment.[3,25]

Interestingly, this current study also found a significant positive correlation between serum sdLDL-c and HDL-c concentrations in the non-MetS group, which does not align with previous studies' findings.[32,33] These discordant findings could be due to knowledge gaps that still exist n HDL-c's role as a 'good cholesterol.' HDL-c itself is not causally associated with cardiovascular benefits. This was supported by Mendelian randomisation studies, which showed that the risk of myocardial infarction was unaffected by genetic polymorphisms linked to higher HDL-c.[34] Furthermore, despite raising HDL-C levels, a

meta-analysis found no improvement in cardiovascular outcomes.[35]

HDL-c has been shown to have two significant subfractions, which are HDL2 (large buoyant) and HDL3 (small and dense). One study reported that HDL-c and HDL2-c levels were inversely correlated with sdLDL-c, suggesting that subjects with higher HDL2-c concentrations appear to be athero-protective. This is not the case with HDL3-c, where a positive correlation exists between its concentration and sdLDL-c.[32] Another study supports this finding, showing positive correlations between sdLDL-c and small-sized HDL (HDL3).[36] Additionally, HDL-c, as well as HDL2-c and HDL3-c subfractions, are inversely associated with all the factors of MetS.[37] Such findings partly explain the discrepancy of this study, where it can be postulated that the non-MetS cohort in this study could have HDL3-c subfractions which are more atherogenic. Future studies comparing the subfractions to the risk of cardiovascular disease and determining whether differences exist with regards to the risk of heart disease between measurement of HDL-c concentrations and HDL-c function could further establish which HDL-c measuring modalities would more accurately reflect its cardioprotective role.

The mean plasma ISP in MetS was significantly higher than in non-MetS. These findings are also in keeping with previous studies.[38] Other studies also proved that plasma ISP was significantly elevated in the MetS group on univariate analysis[39], and levels of ISP are suggested to be an indicator of oxidative stress in MetS.[40] ISP is one of the prostaglandin-like molecules produced by the free-radical-induced peroxidation of arachidonic acid that occurs independently of the cyclooxygenase (COX). These molecules are biomarkers and mediators of oxidative stress in various disease settings, as oxidative stress is defined by an imbalance between increased free radical exposure, primarily from oxygen, and antioxidant defenses. Many acute and chronic diseases, including cancer, cardiovascular disease, and lung disease, have been linked to oxidative stress.[41] Several clinical studies have revealed correlations between BMI and biomarkers or end -products of free radical-mediated oxidative stress (lipid peroxidation or protein carbonylation products) about obesity-induced oxidative stress.[42] These studies support our findings that ISP levels are higher in the MetS group due to oxidative stress and metabolic syndrome.

We acknowledge some limitations to this study with the main one being that this study was constrained to a small sample size despite fulfilling the minimum sample size. Furthermore, this study was conducted in only two primary care clinics within the same district which may not accurately represent the Malaysian population. Therefore, future research should include a larger and more diverse sample from multiple districts in Malaysia to improve the generalizability of our findings.

CONCLUSION

This study highlights the potential role of sdLDL-c and ISP as early biomarkers of atherosclerosis among MetS patients. With early detection, aggressive interventions to reduce the progression of atherosclerosis can be implemented. Interestingly, we discovered a positive correlation between sdLDL-c and HDL-c in non-MetS. This suggests that HDL-c may not be the optimal biomarker to determine cardioprotection as established by previous studies and adopted in several guidelines, but rather to look at its function or subfractions as a more accurate assessment of cardioprotection. Examining these different functions and subtypes of HDL-c can identify more precise markers of cardioprotection.

ACKNOWLEDGMENT

This research is funded by the 600-IRMI/FRGS-RACER 5/3 (094/2019). The authors gratefully acknowledge and appreciate all the participants, Institute Biotechnology and Molecular Laboratory (IMMB) UiTM Sg Buloh, nurses of Primary Care Clinic UiTM Sg Buloh and Selayang, CPDRL staff UiTM Sg Buloh and Selayang for all the contributions to this study.

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