

BMJ Open Observational study of the status of coronary risk biomarkers among Negritos with metabolic syndrome in the east coast of Malaysia

Atiqah Mokhsin,¹ Siti Shuhada Mokhtar,¹ Aletza Mohd Ismail,¹ Fadzilah M Nor,¹ Syahrul Azlin Shaari,¹ Hapizah Nawawi,² Khalid Yusoff,³ Thuhairah Abdul Rahman,¹ Boon Peng Hoh³

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¹Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia

²Institute of Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia

³Faculty of Medicine and Health Sciences, UCSI University, Cheras, Kuala Lumpur, Malaysia

Correspondence to

Dr Thuhairah Abdul Rahman; thuhairah@salam.uitm.edu.my and Dr Boon Peng Hoh; hohbp@ucsiuniversity.edu.my

ABSTRACT

Objectives To determine the prevalence of metabolic syndrome (MS), ascertain the status of coronary risk biomarkers and establish the independent predictors of these biomarkers among the Negritos.

Settings Health screening programme conducted in three inland settlements in the east coast of Malaysia and Peninsular Malaysia.

Subjects 150 Negritos who were still living in three inland settlements in the east coast of Malaysia and 1227 Malays in Peninsular Malaysia. These subjects were then categorised into MS and non-MS groups based on the International Diabetes Federation (IDF) consensus worldwide definition of MS and were recruited between 2010 and 2015. The subjects were randomly selected and on a voluntary basis.

Primary and secondary outcome measures This study was a cross-sectional study. Serum samples were collected for analysis of inflammatory (hsCRP), endothelial activation (sICAM-1) and prothrombogenesis [I_p(a)] biomarkers.

Results MS was significantly higher among the Malays compared with Negritos (27.7% vs 12.0%). Among the Malays, MS subjects had higher hsCRP ($p=0.01$) and sICAM-1 ($p<0.05$) than their non-MS counterpart. There were no significant differences in all the biomarkers between MS and the non-MS Negritos. However, when compared between ethnicity, all biomarkers were higher in Negritos compared with Malays ($p<0.001$). Binary logistic regression analysis affirmed that Negritos were an independent predictor for L_p(a) concentration ($p<0.001$).

Conclusions This study suggests that there may possibly be a genetic influence other than lifestyle, which could explain the lack of difference in biomarkers concentration between MS and non-MS Negritos and for Negritos predicting L_p(a).

INTRODUCTION

Orang Asli (OA) are the indigenous people believed to be the earliest inhabitants of Peninsular Malaysia. There are 18 OA tribes, categorised under three main groups according to their different languages and

Strengths and limitations of this study

- This study gains access to the earliest and smallest population of the Malaysian aboriginal tribe, the inland dwelling Negrito, whom have completely isolated themselves from influences of modernisation.
- This is one of the first studies that assessed biomarkers of atherogenesis among the inland-dwelling Negritos other than hsCRP.
- This is one of the first studies to determine the independent predictors of the atherogenesis biomarkers among inland-dwelling Negritos.
- The small sample size of Negritos due to difficulties accessing remote tribes and small total population poses a limitation despite achieving a minimum sample size requirement.

customs: Negritos, located in the northern part of the peninsula; Senoi, residing in the Central Region and Proto-Malays (or Aboriginal Malay) living in the southern region. The OA constitutes approximately 0.5% (150 000) of the total Malaysian population with Senoi representing the largest OA population (54%) followed by Proto Malays (43%) and Negritos (3%).¹

Negrito, known to be the earliest OA tribe to arrive in Peninsular Malaysia, is believed to have occupied this region of Malaysia approximately 25 000 years ago.² A substantial group of the Negritos continue to practice nomadic lifestyle for reasons such as illness, food resources and intra-tribal feuds.

The poverty rate among OA is 76.9% with the majority of OA living in the jungles or rural areas, while a minority have moved into urban areas.³ Although the Malaysian government has taken measures to eradicate the poverty level among the OAs, which subsequently led to the reduction of poverty-associated diseases such as malaria, tuberculosis,

AIDS and dental decay,³ to name a few, disorders such as metabolic syndrome (MS) and coronary artery disease (CAD) are not well addressed. This could most likely stem from the common misconception that these are 'diseases of the rich', when in fact, it is not exclusive to one type of socioeconomic group but transcends all walks of life.^{4,5}

MS, a major public health challenge, is a cluster of metabolic disturbances that result from a complex interaction between genetic and environmental factors.⁶ MS is associated with increased risk of CAD.⁷ The metabolic abnormalities that underlie the definition of MS include insulin resistance, central obesity, dyslipidaemia, hypertension and glucose intolerance.⁸ The prevalence of MS among Malaysians was estimated to be between 37.1% and 42.5% in 2008.⁹

It has also been well established that MS is associated with enhanced inflammation, endothelial activation and prothrombogenesis that are the key processes in atherosclerosis. Previous studies have documented the association between MS and enhanced inflammation.¹⁰ Increases in proinflammatory cytokines such as interleukin-6, resistin and C-reactive protein are due to the overproduction by monocyte-derived macrophages residing in the expanded adipose tissue mass.¹¹ The National Cholesterol Education Program - Adult Treatment Panel (NCEP-ATP) reported higher soluble endothelial adhesion molecules such as intercellular adhesion molecule-1, vascular adhesion molecule-1 and E-selectin among subjects with MS.¹² Furthermore, various studies have also shown elevated lipoprotein (a) [Lp(a)] in MS subjects.¹³ Lp(a) has been found to significantly reduce endogenous clot lysis in plasma *ex vivo*,¹⁴ possibly to its binding to fibrinogen and attenuating fibrin-mediated enhancement of tissue plasminogen activator of plasminogen.¹⁵

Despite MS becoming a major public health concern with extensive data on this syndrome worldwide, reports on its prevalence in Malaysia remain scarce, particularly so among the OA in the country. There have been previous studies addressing factors related to MS globally in Malaysia¹⁶; however, the samples used in these studies were not a representative of the Malaysian population as they focused on the major ethnicities in Malaysia – Malay, Chinese and Indian, while the OA population was poorly represented.

To the best of our knowledge, there is only one recent published study that reported the urbanised Orang Seletar (a Proto-Malay subtribe) having the highest prevalence for central obesity (66.1%±5.9%) compared with the other subtribes who are less urbanised (Senoi and Negrito). The study also revealed that the prevalence of hypertension was highest among the Negritos (43.8%±9.3% and 51.2%±15.3%) who resided in most remote areas and were the leanest among the six OA subtribes investigated.¹

The lack of data on the prevalence of MS among OA, specifically the Negritos, could most likely stem from the preconceived notion that they are not susceptible to the disorder due to their detachment from urbanisation and

their healthier lifestyle. Furthermore, there have been very few studies investigating the status of the biomarkers reflecting inflammation, endothelial activation and prothrombogenesis among the Negritos with MS. In addition, determining the MS components among them will further identify potential modifiable coronary risks such as hypertension, smoking, dyslipidaemia and glucose intolerance through proper education and healthcare services.

Therefore, this study aims to determine the MS components among Negrito, identify their status of inflammation, endothelial activation and prothrombogenesis and ascertain the independent predictors for these biomarkers of coronary risk.

SUBJECTS AND METHODS

Target population and sample collection

One hundred and fifty Negrito subjects were recruited in this cross-sectional study. They were from Bateq and Mendriq sub-tribes, from three inland settlements in Gua Musang, Kelantan, the east coast of Peninsular Malaysia (4.8843°N, 101.9682°E). Also, 1177 Malays were recruited from various national health screening programmes in Peninsular Malaysia. The subjects were randomly selected and on a voluntary basis. These subjects were then categorised into MS and Non-MS groups based on the IDF's consensus worldwide definition of MS.¹⁷ All methods were carried out in accordance with relevant guidelines and regulations.

The sample size was calculated using PS Power and Sample Size Calculations V.3.0¹⁸ with a power of study at 99% and prevalence of MS among Negritos and Malays at 15.2%±5%¹⁹ and 38.8%±5%²⁰ respectively. The minimum sample size calculated for Negritos and Malays are both 125. Due to the significant sample size difference between the two ethnic groups recruited and the relative difficulty in accessing the Negrito's remote locations of habitat, it is relevant to highlight that this study conducted a within-ethnic group rather than a between-group comparison of the biomarkers of interest when subdivided between MS and non-MS.

Demographic data was gathered by interview questionnaire. Information such as age, gender, tribe, education and occupation, health-related questions such as subjects' past medical history and social history including smoking status were recorded. Family history of cardiometabolic and infectious diseases were also recorded.

Topography measurements included blood pressure (BP), body mass index (BMI), waist circumference (WC) and waist-to-hip ratio. With the subject in a seated position and following 5–10 min of rest, BP was measured by an automated BP reader (cuff size 12×33 cm, Colin press-mate, Japan). The systolic BP and diastolic BP were measured to the nearest 1 mm Hg. BMI was calculated using the formula: BMI=weight(kg)/height²(m²). WC was measured to the nearest 0.5 cm using a measuring tape at midway between the inferior margin of the last rib and

the iliac crest in a horizontal plane. Hip circumference measurement was taken around the pelvis at the point of maximal protrusion of the buttocks. Any visible stigmata of dyslipidaemia and diabetes mellitus were documented.

Defining MS (IDF Criteria, 2006)

An individual was classified as having MS if central obesity was exhibited along with at least two of the following: (1) elevated triglyceride (TG) concentration of >1.7 mmol/L, (2) reduced high-density lipoprotein cholesterol (HDL-c) of <1.0 or 1.3 mmol/L in male and female respectively, (3) elevated BP of $>140/90$ mm Hg, and a raised fasting plasma glucose of ≥ 5.6 mmol/L. Central obesity, using the suggested WC for Asian/South Asians, was defined as ≥ 90 cm and ≥ 80 cm for males and females respectively.¹⁷

Venous blood sampling and on-site biochemical analysis

Venous blood samples were collected following a non-traumatic venepuncture between 0800 hours and 1500 hours. Serum and plasma were separated by centrifugation at 3500 rpm for 10 min within 1 hour and stored at -20°C before analyse.

Biochemical analysis

Serum samples were sent to Centre for Pathology Diagnostic & Research Laboratories of Faculty of Medicine, Universiti Teknologi MARA, Selangor, Malaysia. All clinical chemistry tests analysed were MS ISO 15189:2014 accredited.

Cardiometabolic parameters tested were fasting serum lipids including total cholesterol, TG and HDL-c, which were measured by enzymatic reference methods. Plasma glucose was analysed by hexokinase method. All methods were run on an automated analyser (Cobas Integra 400 PLUS, Roche Diagnostic, Germany) except for low-density lipoprotein cholesterol concentration, which was derived using the Friedewald equation.²¹

Analyses of biomarkers of inflammation, endothelial activation and prothrombogenesis

The biomarkers analysed included hsCRP, sICAM-1 and Lp(a). Both hsCRP and Lp(a) were measured using turbidimetric method on an automated analyser (Cobas Integra 400 PLUS, Roche Diagnostics, Germany). Serum s-ICAM-1 concentration was determined by enzyme-linked immunosorbent assay based on the measured optical density (eBioscience Bender MedSystems, Vienna Austria).

Data analysis

The associations of all three biomarkers were compared between two groups of MS and non-MS, using independent t-test, SPSS V.20. Binary logistic regression was performed on the independent variables (coronary risk biomarkers) to assess their impact on the likelihood that subjects would fall into each of the MS component (obesity, hypertension, diabetes mellitus, and low HDL-c or high TG concentrations). The specific model estimated from the data was: logit (coronary risk biomarkers)

$= \alpha + b_1(\text{age}) + b_2(\text{ethnicity}) + b_3(\text{gender}) + b_4(\text{smoking status}) + b_5(\text{BMI}) + b_6(\text{WC}) + b_7(\text{SBP}) + b_8(\text{DBP}) + b_9(\text{glucose concentration}) + b_{10}(\text{HDL-c concentration}) + b_{11}(\text{TG concentration})$, where the dependent variable is logit coronary risk biomarkers, α is the estimate for the intercept and b_1, b_2, \dots, b_{11} are estimates for the coefficients of the 11 predictors.

The variables were represented by two dummy variables to reflect the number of responses and reference categories. They were: ethnicity=Malay (1), Negrito (0), Negrito=reference group; gender, female (1), male (0)=reference group; smoking status, smoking (1) and not smoking (0)=reference group; While for age, BMI, WC, SBP, DBP, glucose, HDL-c and TG concentrations were continuous variables.

Patient and public involvement

Patients were not involved in the recruitment or conduct of the study. Written, informed consent was obtained from all subjects aged 18 years and above, prior to recruitment. Communications were done in Bahasa Malaysia with some input from local translators, either the Department of Orang Asli Development (JAKOA) officers or tribal leaders if and when required. The results of this study were disseminated to subjects through the JAKOA officers or using the postal address if provided. A physician was placed in the settings during the health screening programmes to provide an advice or referral letter when necessary.

RESULTS

Demographic data

Table 1 summarises the demographic parameters for the Malays and Negritos. A total of 326 Malays (27.7%) and 18 Negritos (12.0%) were diagnosed with MS based on the IDF 2006 criteria. On a separate note, among the Malays, 15.5% of them had diabetes while 1.5% of the Negritos had IFG ≥ 5.6 mmol/L or random plasma glucose of >11.0 mmol/L. We observed that 43.9% of the Malays and 57.2% of the Negritos suffered from hypertension while 58.7% Malays and 14.0% Negritos had central obesity.

Compared with the non-MS Negritos, their MS counterpart had higher TC (5.30 ± 1.05 vs 4.48 ± 0.85 mmol/L, $P < 0.001$ respectively), TG (2.14 ± 0.79 vs 1.41 ± 0.68 mmol/L, $P < 0.001$ respectively) and LDL-c (3.36 ± 0.69 vs 2.80 ± 0.84 mmol/L, $P = 0.008$ respectively). The most frequent criteria diagnosing MS among the Negritos were hypertension, elevated TG and low HDL-c and none of the Negritos diagnosed with MS had any form of glucose intolerance.

Comparison of the biomarkers of inflammation, endothelial activation and prothrombogenesis in Malay and Negrito subjects

As can be seen from table 2 there was no significant differences between MS and non-MS among

Table 1 Demographic and clinical characteristics of the Negritos and Malays

Parameters	Malays (n=1177)			Negrito (n=150)		
	MS (n=326)	Non-MS (n=851)	P values	MS (n=18)	Non-MS (n=132)	P values
Age (Years)*	49.53±11.77	40.00±14.69	<0.001	30.56±11.11	31.29±11.00	NS
Gender† (Males/Females)	45.1/54.9	37.3/62.7	0.02	5.6/94.4	59.1/40.9	<0.001
Diabetes†	42.1	5.6	<0.001	0.00	1.8	NS
Plasma glucose*(mmol/L)	8.22±4.38	5.88±2.33	<0.001	5.18±1.26	4.61±1.41	NS
Hypertension†	83.2	28.9	<0.001	83.3	53.5	0.02
SBP* (mm Hg)	138.22±17.82	120.99±17.86	<0.001	133.44±23.06	130.25±17.22	NS
DBP* (mm Hg)	83.49±10.63	73.69±11.42	<0.001	81.39±12.87	81.09±13.41	NS
Central obesity†	100	42.9	<0.001	100	2.3	<0.001
Waist circumference*(cm)	97.19±8.93	82.75±11.24	<0.001	90.81±6.88	69.01±15.35	<0.001
BMI* (kg/m ²)	30.57±4.90	24.94±4.77	<0.001	26.31±3.26	19.75±2.55	<0.001
Current smoker†	16.9	13.2	NS	23.5	46.8	NS
Total cholesterol*(mmol/L)	5.38±1.00	5.05±0.96	<0.001	5.30±1.05	4.48±0.85	<0.001
Triglyceride*(mmol/L)	2.70±1.54	1.41±0.82	<0.001	2.14±0.79	1.41±0.68	<0.001
LDL-c*(mmol/L)	3.10±0.76	2.99±0.76	0.03	3.36±0.69	2.80±0.84	0.008
HDL-c* (mmol/L)	1.08±0.31	1.42±0.37	<0.001	0.98±0.38	1.01±0.28	NS

*Data expressed as mean ±SD. p-value was significant at p<0.05, T-test was applied.

† Data expressed as percentage. p-value was significant at p<0.05, chi-square test was applied.

BMI, body mass index; DBP, diastolic blood pressure; NS, not significant; SBP, systolic blood pressure.

Negritos for all biomarkers (P>0.05) whereas MS Malays illustrated higher hsCRP (mean ±SD=1.88±1.16 vs 1.26±1.13 mg/L, p<0.001 respectively) and sICAM-1 (mean ±SD=531.73±185.73 vs 465.46±202.08 ng/mL, p<0.001 respectively) concentrations compared with their non-MS counterpart. When comparing between Negritos and Malays, Negritos showed significantly higher concentrations of hsCRP (mean ±SD=2.27±2.25 vs 1.41±1.17 mg/L, p<0.001 respectively), sICAM-1 (mean ±SD=684.85±388.03 vs 482.25±200.05 ng/mL, p<0.001 respectively) and Lp(a) (mean ±SD=0.22±0.22 vs 0.06±0.04 mg/L, p<0.001 respectively) compared with the Malays.

Independent predictors of the biomarkers of inflammation, endothelial activation and prothrombogenesis in Malay and Negrito subjects

To further determine the independent predictors of these biomarkers, binary logistic regression analyses were performed with the biomarkers as dependent variables

(table 3). It was found that BMI (p=0.016), FPG (p<0.001) and DBP (p=0.042) were independent predictors for hsCRP while the Negrito determined Lp(a) (p<0.001) when taken account all subjects after adjusting for age, gender, DM, hypertension, smoking status and BMI.

Among the Malays, it was found that male (p<0.001), BMI (p<0.001) and FPG concentration (p<0.001) predicted for hsCRP while male (p=0.040) and FPG (p=0.039) predicted Lp(a) after adjusting for the same parameters. There were no independent predictors for any of these biomarkers among Negritos.

DISCUSSION

MS is defined by an aggregation of atherosclerotic factors, mainly central obesity, hypertension, glucose intolerance and dyslipidaemia, which, in combination, enhance the probability of developing type 2DM and CAD.²² In two European studies,^{23 24} the presence of MS predicted

Table 2 Concentrations of inflammatory, endothelial activation and prothrombogenesis biomarkers in MS and non-MS Malays and Negritos

Biomarkers	Malays (n=1177)			Negrito (n=150)		
	MS (n=326)	Non-MS (n=851)	P values	MS (n=18)	Non-MS (n=132)	P values
hsCRP (mg/L)	1.88±1.16	1.26±1.13	<0.001	4.21±3.20	2.06±2.04	NS
sICAM-1 (ng/mL)	531.73±185.76	465.46±202.08	<0.001	670.06±377.27	688.84±390.97	NS
Lp(a) (g/L)	0.06±0.04	0.06±0.04	NS	0.23±0.26	0.22±0.22	NS

Data expressed as mean ±SD. p-value was significant at p<0.05, T-test was applied.

NS, not significant.

Table 3 Predictors for hsCRP and Lp(a)

Groups	Variables	Independent predictor	Beta	Adjusted OR	95% CI Lower, Upper	P values
(a) All subjects	hsCRP	1. BMI (kg/m ²)	0.05	1.05	1.01 to 1.10	0.016
		2. Glucose (mmol/L)	0.15	1.16	1.12 to 1.19	<0.001
		3. Diastolic (mm Hg)	0.01	1.01	1.00 to 1.02	0.042
	Lp(a)	1. Ethnicity (Negrito)	1.03	2.79	1.87 to 4.14	<0.001
(b) Malays	hsCRP	1. Gender (male)	-0.64	0.53	0.39 to 0.71	<0.001
		2. BMI (kg/m ²)	0.18	1.20	1.16 to 1.24	<0.001
		3. Glucose (mmol/L)	0.08	1.08	1.04 to 1.13	<0.001
	Lp(a)	1. Gender (Male)	-0.56	0.57	0.39 to 0.84	0.004
		2. Glucose (mmol/L)	-0.08	0.93	0.86 to 1.00	0.039

The model reasonably fits well. Model assumptions are met. There are no interaction and multicollinearity problem.

increased CAD mortality. These findings are not unexpected considering MS comprises established risk factors for CAD such as hypertension, impaired glucose tolerance, low HDL-c concentration, elevated TG concentration and obesity, which enhances endothelial activation and inflammation, key processes in atherogenesis. Furthermore, coronary risk biomarkers are strongly associated with endothelial dysfunction²⁵ and thrombosis²⁶ and are elevated in patients with atherosclerosis-related disease such as CAD²⁷ and peripheral artery disease²⁸ in previous reports.

There have been several previous reports determining the prevalence of non-communicable diseases (NCDs) such as DM, hypertension, dyslipidaemia and obesity among OA subjects. A previous report on the health status of the Negrito subtribe Lanoh showed that 8.9% were recently diagnosed with hypertension, 6.7% had hypertension and DM and an alarming 26.7% had pre-obesity.²⁹ A larger population study done identified central obesity and hypertension among OA sampled from seven different subtribes and eight settlements.¹ What is more alarming is the report by Aziz *et al*, which highlighted the higher percentage of OA having high insulin levels, hsCRP and higher percentage categorised as high risk by the Framingham Risk Score, which forecasts 10-year risk of CAD.³⁰ These reports strongly imply that NCDs such as hypertension, dyslipidaemia, central obesity and DM, which are clusters of metabolic factors associated with MS, have gradually led to the increase in CAD risk among OA.

Although several reports highlighted the increasing prevalence of NCDs among the OA in Malaysia, there is scarce research that extended to investigate biomarkers for coronary risk among this population. To the best of our knowledge, there is only one other study which reported the status of inflammatory biomarker among OA in Peninsular Malaysia compared with Malays.³⁰ Our study further explored other biomarkers reflecting atherogenesis such as endothelial activation and prothrombogenesis. This study showed that biomarkers of inflammation (hsCRP) and endothelial activation (sICAM-1) were enhanced in MS Malays compared with their non-MS counterpart.

These findings are expected and in keeping with previous studies denoting the higher CAD risk among MS.^{17 30}

The association of MS with inflammation and endothelial activation is also well documented.^{12 31} The elevated sICAM-1 seen among MS Malays could be attributed to higher prevalence of hypertension, obesity and dyslipidaemia compared with their non-MS counterpart, which is parallel with previous reports.^{31 32}

However, we were unable to observe any statistical difference in the concentrations of biomarkers of inflammation, endothelial activation and prothrombogenesis between MS and non-MS among the Negritos. This contradicts previous reports underscoring enhanced inflammation among OAs.³⁰ These inconsistent findings could be attributed to the small sample size of Negritos in comparing the biomarkers, although the minimum sample size calculated was achieved. Furthermore, a previous study conducted to determine the prevalence of MS in an OA population found that the prevalence of MS among inland Negritos to be 12.5%, which is consistent with our findings of 12% among our Negrito population.¹⁹ The population differences between the previous study and ours could also have led to these differences, where we recruited mainly inland Negritos in the northern state of Malaysia where majority of them are distributed, while the previous study recruited OAs from a mixture of inland and peripheries of towns where urbanisation could have influenced lifestyle, which could enhance inflammation and insulin resistance.³⁰

In addition, as a general index of inflammation, CRP concentrations have been shown to vary by ethnicity and within ethnic groups by fitness, as it was reported to be higher in healthy Indian Asians than in European white people and were related to greater central obesity and insulin resistance in Indian Asians.³³ Furthermore, none of the Negritos diagnosed with MS had any form of glucose intolerance, which could possibly explain these inconsistent findings as there have been reports on the association between CRP and sICAM-1 with MS, hypertension, and DM.^{34 35} It is also worth highlighting that when comparing these

biomarkers between Negritos and Malays, we observed higher concentrations of all three biomarkers among the younger aged Negrito subjects compared with the Malays. This suggests that the Negritos may be at higher risk of CAD at a younger age group, hence warranting further investigation.

Furthermore, findings from this study suggest that the MS Negritos despite having coronary risk factors such as hypertension and dyslipidaemia, the failure to exhibit enhanced atherogenesis compared with their non-MS counterpart could possibly be attributed to genetic and/or lifestyle influences, which could play a role in attenuating atherogenesis. The Negritos included in this study were located in remote areas of northern Peninsular Malaysia and to certain extent, still practising the hunter-gatherer lifestyle and living isolated from urbanisation thus may have contributed to the differences observed in the biomarkers between the two ethnicities when comparing with the MS and non-MS counterparts. This is in agreement with previous studies, which reported improved inflammatory endothelial activation and prothrombogenesis status in MS subjects, following aggressive lifestyle modification, which included dietary improvement and initiation and maintenance of exercise.^{36 37}

One possible postulating genetic factor that may contribute to this finding is the genetic variation of the candidate gene *CDH13*. Recent population genomic studies on the Negritos have identified a strong and consistent positive natural selection signal spanning the genomic region which harbours *CDH13*.^{38 39} This implies that the genetic profile of this gene in the Negritos was significantly differentiated from the rest of the populations. *CDH13* encodes for protein T-Cadherin, which belongs to the cadherin superfamily of the transmembrane proteins that mediate calcium-dependent intercellular adhesion, is the receptor for the high-molecular-weight adiponectin expressed in the vasculature⁴⁰ and cardiac myocytes.⁴¹ Genetic variation of this gene has been shown to influence metabolic outcome and possibly provides atherogenesis resistance.^{39 42 43} Adiponectin plays a crucial role in the metabolic regulation of obesity, insulin sensitivity and atherosclerosis and several studies have indicated its antiatherogenic properties.⁴⁴ A recent study reported that minor allele of rs12051272 revealed a considerable association with a more favourable metabolic profile, including higher insulin sensitivity, HDL-c, lower DBP, FPG and TG concentrations.⁴² Given the phenotypic observations and the role of this gene, we postulate a plausibility of *CDH13* regulating the phenotype and could explain the findings observed in our study whereby despite fulfilling criteria for MS, these Negritos subjects did not exhibit augmented inflammatory or endothelial activation status when compared with their non-MS counterparts. Future studies exploring expressions of genetic variants of *CDH13* in these Negritos cohorts

could further shed light on influences of this gene on atherogenesis.

Regression analysis has shown that the Negritos independently predict for Lp(a) in this study. This finding implies that despite hunter-gatherers get more exercise, putting them at a lower risk for heart disease and relatively isolated from modernisation, suggests that serum Lp(a) concentrations are not influenced by diet or lifestyle, but more significantly by variants in the *LPA* gene. This is in parallel with previous studies that exhibited a significant difference in Lp(a) levels among various populations including Asian Indian, Chinese, non-Hispanic and blacks.^{45 46} Studies have demonstrated the presence of *LPA* gene polymorphisms primarily determining levels of Lp(a), without significant dietary or environmental effects.⁴⁷ Further studies are warranted to identify common polymorphisms in *LPA* gene among Negritos and other OA tribes, which would further validate these inferences.

The main constraint of this study was the small sample size of the Negrito group, which prevented us from comparing between ethnicities. Although the sample size included was enough to reject the null hypothesis, a larger sample size could provide a better representation of the Negrito population. However, it should be reiterated that Negrito represents only 3% of the total OA population in Malaysia and an even smaller percentage of that embodies the inland dwellers who are not subjected to urbanisation. This along with major physical problems accessing all these remote tribes prevented us from having a larger sample to analyse.

CONCLUSION

This study highlights several key findings that provide further insights into the metabolic differences between the inland-living aboriginal group, Negritos, in Malaysia and urbanised Malays in Malaysia. First, MS and non-MS Negritos failed to show differences in biomarkers of coronary risks as established by previous reports. Although the small Negrito sample could be a contributor to this observation, genetic or lifestyle influence cannot be ruled out. This warrants further studies to confirm these observations and, if replicated, paves way to future research to understand the mechanism behind this discrepancy. Second, Negritos independently predicting serum Lp(a) concentrations suggests a genetic influence that surpasses any form of impact by diet and lifestyle, which needs further exploration. Therefore, future studies to identify common variants of *LPA* gene among this group and to extend such research to other Aborigine tribes would further improve our understanding of interaction between gene and phenotypic expression of Lp(a).

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Contributors NAMM collected the subjects, performed the experiments, analysed the samples and data, prepared the tables, wrote the manuscript and reviewed drafts of the paper. SSM collected the subjects, analysed the samples and reviewed drafts of the paper. AMI collected the subjects, validated results and reviewed drafts of the paper. FMN and SAS collected the subjects and reviewed drafts of the paper. HMN collected the subjects, contributed reagents and materials and reviewed drafts of the paper. KY contributed reagents and materials, drafted the manuscript and reviewed the draft of the paper. TAR conceived and designed the experiments, collected the subjects, contributed reagents and materials, wrote the manuscript and reviewed drafts of the paper. BPH conceived and designed the experiments, collected the subjects, validated results, contributed reagents and materials and reviewed drafts of the paper.

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