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Status of Inflammation, Endothelial Activation and Prothrombogenesis among Negritos with Metabolic Syndrome

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-021580
Article Type:	Research
Date Submitted by the Author:	11-Jan-2018
Complete List of Authors:	Mohd Mokhsin, Nurul Atiqah; Universiti Teknologi MARA, Faculty of Medicine Mokhtar, Siti Shuhada; Universiti Teknologi MARA, Faculty of Medicine Mohd Ismail, Aletza; Universiti Teknologi MARA, Faculty of Medicine M. Nor, Fadzilah; Universiti Teknologi MARA, Faculty of Medicine Shaari, Syahrul Azlin; Universiti Teknologi MARA, Faculty of Medicine Nawawi, Hapizah; Universiti Teknologi MARA, Institute of Pathology, Forensic and Laboratory Medicine (I-PPerForM) Yusoff, Khalid; UCSI University, Faculty of Medicine and Health Sciences Rahman, Thuhairah; Universiti Teknologi MARA, Faculty of Medicine Hoh, Boon Peng; UCSI University, Faculty of Medicine and Health Sciences
Keywords:	Lipid disorders < DIABETES & ENDOCRINOLOGY, Hypertension < CARDIOLOGY, EPIDEMIOLOGY

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Status of Inflammation, Endothelial Activation and Prothrombogenesis among Negritos with Metabolic Syndrome

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Word count: 2,985 words

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ABSTRACT

Aims: To determine the prevalence of MS, ascertain the status of coronary risk biomarkers and establish the independent predictors of these biomarkers among the Negritos.

Methods: Diagnosis of MS was made based on the International Diabetes Federation criteria. Serum samples were collected for analysis of inflammatory (hsCRP), endothelial activation (sICAM-1) and prothrombogenesis [lp(a)] biomarkers in Negritos (n=150) from three inland settlements in East Coast Malaysia and Malays in Malaysia (n=1,227) recruited between 2010 and 2015.

Results: MS was significantly higher among the Malays compared to Negritos (21% vs 7%). Amongst 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. Binary logistic regression analysis affirmed that Negritos were an independent predictor for Lp(a) concentration (p<0.001).

Conclusion: This study suggests that there may possibly be an underlying genetic influence other than lifestyle which could explain the lack of increased coronary risk in MS Negritos compared to their non-MS counterpart and for Negritos predicting Lp(a).

Keywords: Orang Asli; Aborigines; Coronary Artery Disease; Hypertension; Hypercholesterolaemia; Lp(a)

ARTICLE SUMMARY

Strengths and limitations of this study

- Metabolic syndrome (MS) is a cluster of metabolic disturbances such as central obesity, glucose intolerance, dyslipidaemia and hypertension, all of which are risk factors for coronary artery disease (CAD) and had established the association with MS and higher levels of biomarkers of atherogenesis which includes inflammation, endothelial activation, oxidative stress and prothrombogenesis.
- Despite MS becoming a major public health concern with extensive data on this syndrome worldwide, report on the prevalence of MS and the status of the biomarkers reflecting inflammation, endothelial activation and prothrombogenesis among Negrito (smallest population and the earliest tribe with a nomadic lifestyle and the least affected by urbanization of indigenous people in Malaysia) remain scarce.
- Interestingly, our research showed no difference in biomarker levels {inflammation (hsCRP), endothelial activation (sICAM) and prothrombogenesis [Lp(a)]} between MS and non-MS Negritos unlike comparison made between these groups among Malays, where biomarkers were higher in MS than non-MS indicating despite isolation from urbanization suggesting healthier lifestyle habits, there was no added risk to CAD among the MS subjects compared to leaner non-MS counterpart. In addition, Regression Analysis showed that Negrito independently predicted Lp(a).
- The possible explanation to this discrepancy is that perhaps genetics are stronger at play in atheroprotection in the face of several assaulting coronary risks and influence surpasses any form of impact by diet and lifestyle which needs further exploration.

Ethics

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Consent from the Department of Orang Asli Development (JaKOA) [JHEOA,PP.30.042.Jld5(17)] and Institutional ethics approval was granted by Universiti Teknologi MARA (UiTM) [600-RMI(5/1/6)] were obtained prior the initiation of this study. All methods were carried out in accordance with relevant guidelines and regulations. Written, informed consent was obtained from all subjects aged 18 years-old and above, prior to recruitment. Communications were done in Bahasa Malaysia with some input from local translators, either JaKOA officers or tribal leaders if and when required.

Funding

1) The Ministry of Higher Education Malaysia under Fundamental Research Grant Scheme [Grant code: 600-RMI/FRGS 5/3 (136/2014)]
2) The Ministry of Higher Education Malaysia under Long Term Research Grant Scheme [Grant code: 600-RMI/LRGS 5/3 (2/2011)-2]

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Financial Interests

The author(s) declare no competing financial interests.

Data Sharing

No additional data available.

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INTRODUCTION

Orang Asli (OA) are the indigenous people and the oldest inhabitants of Peninsular Malaysia. There are 18 OA tribes, categorized under three main groups according to their different languages and customs: Negrito, located the Northern part of the peninsula; Senoi, residing in the Central Region and Proto-Malays (or Aboriginal Malay), in the Southern Region. The OA constitutes 0.5% (150,000) of the total Malaysian population with Senoi representing the largest OA population (54%) followed by the Proto Malays (43%), and the Negrito (3%) [1].

Negrito, known to be the earliest OA tribe to arrive in Peninsular Malaysia, is believed to enter this region of Malaysia about 25,000 years ago [2]. A Majority 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 have 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 misnomer 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 which 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 from 37.1%-42.5% in 2008 [9].

It has also been well established that MS is associated with enhanced inflammation, endothelial activation and prothrombogenesis which are key processes in atherosclerosis. Previous studies have documented the association between MS and enhanced inflammation [10]. Increases in proinflammatory cytokines such as interleukin-6 (IL-6), resistin and C-reactive protein (CRP) are due to the overproduction by monocyte-derived macrophages residing in the expanded adipose tissue mass [11]. NCEP-ATP reported higher soluble endothelial adhesion molecules such as intercellular adhesion molecule-1 (ICAM-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 manifest significantly reduced endogenous clot lysis in plasma ex vivo [14], possibly to its binding to fibrignogen 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

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related to MS globally in Malaysia [16], 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 urbanized Orang Seletar (a Proto-Malay subtribe) having the highest prevalence for central obesity (66.1±5.9%) compared to the other subtribes who are less urbanized (Senoi and Negrito). The study also revealed that the prevalence for 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 urbanization 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: 1) determine the MS components commonly seen among the Negrito, 2) elucidate the status of inflammation, endothelial activation and prothrombogenesis in Negrito and 3) identify the independent predictors for these biomarkers of coronary risk.

SUBJECTS AND METHODS

Target population and sample collection

150 Negrito subjects were recruited in this cross-sectional study. They were from Bateq and Mendriq sub-tribe, from three inland settlements in Gua Musang, Kelantan, East Coast Malaysia (4.8843°N, 101.9682°E). 1,192 Malays were also recruited from various national health screening programmes in Malaysia.

The sample size was calculated using PS Power and Sample Size Calculations version 3.0 with a power of study at 99% and prevalence of MS in Negritos and Malays at 15.2%±5% [17] and 38.8%±5% [18] respectively, the minimum sample size for Negrito and Malay are both 125. Due to the significant sample size difference between the two ethnic groups and the relative difficulty in accessing the Negrito's remote locations of habitat, it is relevant to mention that this study conducted a within-ethnic group rather than a between-group comparison of the biomarkers of interest.

History-taking and Topography Measurements

Demographic data was gathered by interview questionnaire. Information such as age, gender, tribe, education and occupation, health-related questions included subjects' past

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medical, social that included smoking status and infectious diseases 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 (WHR). With the subject in a seated position and after 5-10 minute rest, BP was measured by an automated BP reader (cuff size 12 x 33cm, Colin press-mate, Japan). The systolic BP (SBP) and diastolic BP (DBP) were measured to the nearest 1mmHg. BMI was calculated using the formula: BMI=weight(kg)/height^2(m^2). WC was measured to the nearest 0.5cm 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

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 >1.7mmol/L, (2) reduced high-density lipoprotein cholesterol (HDL-c) of <1.0 or 1.3mmol/L in male and female respectively, (3) elevated BP of >140/90mmHg, and a raised fasting plasma glucose (FPG) of ≥5.6mmol/L. Central obesity, using the suggested WC for Asian/South Asians, was defined as ≥90cm and ≥80cm for males and females respectively [19].

Venous blood sampling and on-site biochemical analysis

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

Biochemical Analysis

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

Cardiometabolic parameters tested were fasting serum lipids (FSL) which included total cholesterol (TC), 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 LDL-c concentration was derived by calculation using the Friedewald equation [20].

Analyses of Biomarkers of inflammation, endothelial activation and prothrombogenesis

The biomarkers analysed included high sensitivity of CRP (hsCRP), sICAM-1 and Lp(a). Both hsCRP and Lp(a) were measured using turbidimetric method on an automated

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analyser (Cobas Integra 400 PLUS, Roche Diagnostics, Germany). Serum s-ICAM-1 concentration was determined by enzyme linked immunosorbent assay (ELISA) 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 version 20. A multiple binary regression analysis was used to analyse the independent predictor of biomarkers and components of MS which include age, gender, race, smoking status, glucose and HDL-c concentrations.

RESULTS

Demographic Data

Table 1 summarizes the demographic parameters for the Malays and Negritos. A total of 1,342 subjects were recruited in this study comprising of 1,192 Malays and 150 Negritos. Of these, 326 Malays (27.3%) and 17 Negritos (11.3%) were diagnosed with MS based on the IDF 2006 criteria. On a separate note, 15.5% Malays were diabetic while 1.3% of the Negritos had IFG ≥5.6mmol/L or random plasma glucose of >11.0mmol/L. 44.1% of the Malays and 59.3% of the Negritos were hypertensive while 59.2% Malays and 18.4% Negritos had central obesity.

Among the Negritos, their MS counterpart had higher TC (5.28±1.22 vs 4.49±0.75mmol/L, p=0.02 respectively), TG (2.54±1.20 vs 1.50±0.79mmol/L, p<0.01 respectively), LDL-c (3.18±0.64 vs 2.78±0.62mmol/L, p=0.02 respectively) and lower HDL-c (0.84±0.21 vs 1.02±0.27mmol/L, p=0.01 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.

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Table 1: Demographic and clinical characteristics of the Negritos and Malays

Parameters	Malays (n = 1192)			Negrito (n = 150)			All Subjects (n = 1342)		
	MS (n=326)	Non-MS (n=851)	p value	MS (n=17)	Non-MS (n=124)	p value	MS (n=343)	Non-MS (n=975)	p value
[b] Age (Years)	49.53±11.77	40.00±14.69	<0.001	33.06±12.19	33.52±13.45	NS	48.76±12.28	39.26±14.69	<0.001
[a] Gender (Males/Females)	45.1/54.9	37.3/62.7	0.02	17.6/82.4	60.2/39.8	<0.01	43.7/56.3	40.1/59.9	NS
[a] Diabetes	42.1	5.6	<0.001	0.00	1.4	NS	40.9	5.2	<0.001
[b] Plasma glucose (mmol/L)	8.22±4.38	5.88±2.33	<0.001	5.21±1.33	4.66±1.40	NS	8.14±4.35	5.79±2.30	<0.001
[a] Hypertension	83.2	28.9	<0.001	88.2	55.6	0.02	83.4	32.3	<0.001
[b] SBP (mmHg)	138.22±17.82	120.99±17.86	<0.001	138.26±23.51	130.79±17.26	NS	138.22±18.11	122.25±18.07	<0.001
[b] DBP (mmHg)	83.49±10.63	73.69±11.42	<0.001	82.54±11.12	81.50±13.12	NS	83.44±10.65	74.69±11.93	<0.001
[a] Central Obesity	100	42.9	<0.001	100	3.2	<0.001	100	37.8	<0.001
[b] Waist circumference (cm)	97.19±8.93	82.75±11.24	<0.001	92.21±8.36	71.60±9.68	<0.001	96.94±8.96	81.33±11.66	<0.001
[b] BMI (kg/m²)	30.57±4.90	24.94±4.77	<0.001	26.68±3.06	19.83±2.50	<0.001	30.38±4.90	24.30±4.85	<0.001
[a] Current smoker	16.9	13.2	NS	23.5	46.8	NS	17.2	17.4	NS
[b] Total cholesterol (mmol/L)	5.38±1.00	5.05±0.96	<0.001	5.28±1.22	4.49±0.75	0.18	5.38±1.02	4.98±0.95	<0.001
[b] Triglyceride (mmol/L)	2.70±1.54	1.41±0.82	<0.001	2.54±1.20	1.50±0.79	<0.01	2.69±1.53	1.42±0.82	<0.001
[b] LDL-c (mmol/L)	3.10±0.76	2.99±0.76	0.03	3.18±0.64	2.78±0.62	0.02	3.10±0.75	2.97±0.75	<0.01
[b] HDL-c (mmol/L)	1.08±0.31	1.42±0.37	<0.001	0.84±0.21	1.02±0.27	0.01	1.07±0.31	1.37±0.38	<0.001

Notes: [a] Data expressed as percentage; [b] Data expressed as Mean±SD

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, Body mass index; NS, not significant

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Comparison of the biomarkers of inflammation, endothelial activation and prothrombogenesis in Malay and Negrito subjects (Table 2)

When compared with all subjects, there was significantly higher concentration of hsCRP (mean±SD=1.89±1.17 vs 1.43±1.14mg/L, p<0.001) and sICAM-1 (mean±SD=530.72±183.88 vs 469.17±204.79ng/mL, p<0.01) among MS compared to non-MS respectively, but differences were not observed in serum Lp(a) concentration between these groups (p>0.05).

There was no significant differences between MS and non-MS among Negritos for all biomarkers (p>0.05) whereas MS Malays illustrated higher hsCRP (mean±SD=1.88±1.16 vs 1.26±1.13mg/L, p<0.001 respectively) and sICAM-1 (mean±SD=531.73±185.73 vs 465.46±202.08ng/mL, p<0.001 respectively) concentrations compared to their non-MS counterpart.

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

Biomarkers	Malays (n = 1,192)			Negrito (n = 150)			All Subjects (n = 1342)		
	MS (n=326)	Non-MS (n=851)	p value	MS (n=17)	Non-MS (n=124)	p value	MS (n=343)	Non-MS (n=975)	p value
hsCRP (mg/L)	1.88±1.16	1.26±1.13	<0.001	2.19±1.33	1.47±1.28	NS	1.89±1.17	1.27±1.13	<0.001
sICAM-1 (ng/mL)	531.73±185.76	465.46±202.08	<0.001	587.59±322.86	606.38±354.08	NS	530.72±183.88	469.17±204.79	<0.01
Lp(a) (g/L)	0.06±0.04	0.06±0.04	NS	0.28±0.31	0.24±0.23	NS	0.06±0.04	0.06±0.04	NS

Notes: Data expressed as Mean ± SD
Abbreviations: NS, not significant

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Independent predictors of the biomarkers of inflammation, endothelial activation and prothrombogenesis in Malay and Negrito subjects (Table 3)

To further determine the independent predictors of these biomarkers, binary logistic regression analyses were performed with the biomarkers as dependent variables. It was found that BMI (p=0.016), FPG (p<0.001) and DBP (p=0.042) were independent predictors for hsCRP whilst Negrito race determined Lp(a) (p<0.001) when taken account all subjects after adjusted for age, gender, DM, hypertension, smoking status and BMI.

Among the Malays, it was found that male gender (p<0.001), BMI (p<0.001) and FPG concentration (p<0.001) predicted for hsCRP whilst male gender (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.

Table 3: Predictors for hsCRP and Lp(a)

Groups	Variables	Independent predictor	Constant	Beta	SE	Adjusted OR	95% CI Lower, Upper	p value
(a) All Subjects	hsCRP	1) BMI (kg/m^2)	-5.81	0.05	0.02	1.05	1.01,1.10	0.016
		2) Glucose (mmol/L)		0.15	0.02	1.16	1.12, 1.19	<0.001
		3) Diastolic (mmHg)		0.01	0.01	1.01	1.00, 1.02	0.042
	Lp(a)	1) Race (Negrito)	-1.57	1.03	0.20	2.79	1.87, 4.14	<0.001
(b) Malays	hsCRP	1) Gender (male)	-5.76	-0.64	0.15	0.53	0.39, 0.71	<0.001
		2) BMI (kg/m^2)		0.18	0.02	1.20	1.16, 1.24	<0.001
		3) Glucose (mmol/L)		0.08	0.02	1.08	1.04, 1.13	<0.001
	Lp(a)	1) Gender (Male)	-0.87	-0.56	0.20	0.57	0.39, 0.84	0.004
		2) Glucose (mmol/L)		-0.08	0.04	0.93	0.86, 1.00	0.039

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

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DISCUSSION

MS is defined by an aggregation of atherosclerotic factors, mainly visceral obesity, systemic hypertension, glucose intolerance and dyslipidaemia which, in combination, enhance the probability of developing type 2 DM and CAD [21]. In a European studies [22,23], the presence of MS predicted increased CAD mortality. These findings are not unexpected considering MS comprises of 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.

There have been several previous reports determining the prevalence of non-communicable diseases (NCD) such as DM, hypertension, dyslipidaemia and obesity among OA subjects. A previous report on the health status of Lanoh ethnic sub-groups of OA showed that 8.9% were recently diagnosed with hypertension, 6.7% had hypertension and DM and an alarming 26.7% had pre-obesity [24]. A larger population study done identified central obesity and hypertension among OA sampled from 7 different subtribes and 8 settlements [1]. What is more alarming is the report by Aziz et al (2016) which highlighted the higher percentage of OA having high insulin levels, hsCRP and higher percentage categorized as high risk by the Framingham Risk Score which forecasts 10-year risk of CAD [25]. 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 OA in Malaysia, there is scarce research that extended to investigate biomarkers for coronary risk amongst this population. To the best of our knowledge, there is only one other study reported status of inflammatory biomarker among OA in Peninsular Malaysia compared to Malays [25]. 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 to their non-MS counterpart. These findings are expected and in keeping with previous studies denoting the higher CAD risk among MS [19,25]. The association of MS with inflammation and endothelial activation is also well documented [12,26]. The elevated sICAM-1 seen among MS Malays could be attributed to higher prevalence of hypertension, obesity and dyslipidaemia compared to their non-MS counterpart which is parallel with previous reports [26,27].

However and interestingly, there was no difference in the concentrations of biomarkers of inflammation, endothelial activation and prothrombogenesis between MS and non-MS among the Negritos which contradicted previous report underscoring enhanced inflammation among OAs [25]. These inconsistent findings could be explained by population differences where our study targeted mainly inland Negritos in the northern state of Malaysia where majority of them are distributed, while those recruited OAs from a mixture of inland and peripheries of towns where urbanization could have influenced lifestyle which could enhance inflammation and insulin resistance [25]. In addition, as a general index of inflammation, CRP concentrations have been shown to vary by ethnicity and within ethnic groups by fitness, as there were higher in healthy Indian Asians than in

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European white people and were related to greater central obesity and insulin resistance in Indian Asians [28].

Furthermore, findings from this study suggest that despite having metabolic risks for CAD among the MS Negritos group, the failure to exhibit enhanced atherogonesis could possibly be attributed to genetic and/or lifestyle influences which may play a role in attenuating atherogenesis. The Negritos included in this study were located in remote areas of Northern Peninsular Malaysia and to certain extend, still practicing the hunter gatherers lifestyle, and living isolated from urbanization which may have contributed to the differences observed in the biomarkers between the two races when comparing with the MS and non-MS counterparts. This is in keeping 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 [29,30].

There have been previous reports identifying the gene variants of *CDH13* which have been shown to influence metabolic outcome and possibly provides atherogenesis resistant [31–33]. The *CDH13* gene encodes for 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 vasculature [34] and cardiac myocytes [35]. Adiponectin plays a crucial role in the metabolic regulation of obesity, insulin sensitivity and atherosclerosis and several studies have indicated its anti-atherogenic properties [36]. 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 [32]. One study highlighted the presence of a strong signal of *CDH13* in Negritos which also showed a profoundly different genetic variation of this gene compared to the other OA subtribes [37]. 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 to their non-MS counterpart. 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 have shown that Negrito race independently predicts Lp(a) in this study. This finding imply that despite having a healthier lifestyle by being hunter gatherers and relatively isolated from modernization, 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 have shown significant difference in Lp(a) levels among various races including Asian Indian, Chinese, Non-Hispanic and blacks [38,39]. Studies have demonstrated the presence of *LPA* gene polymorphisms primarily determining levels of Lp(a), without significant dietary or environmental effects [40]. Further studies are warranted to identify common polymorphisms in *LPA* gene among Negritos and other OA tribes which would further validate these inferences.

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CONCLUSION

This study highlights several key findings which provide further insights into the metabolic differences between the inland-living aboriginal group, Negritos, in Malaysia and urbanized Malays in Malaysia. Firstly, MS Negritos do not appear to share similar coronary risks as those of MS Malays and MS in general as depicted by several studies which have established that MS is associated with enhanced inflammation, endothelial activation and prothrombogenesis. This warrants further study to understand the mechanism behind this discrepancy which could be due, in part, to genetics, lifestyle or a combination of both. Secondly, 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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ACKNOWLEDGMENTS

The authors would like to express their appreciation to the Centre for Pathology Diagnostic and Research Laboratories (CPDRL) of Faculty of Medicine, Universiti Teknologi MARA for providing the facilities to conduct this research.

CONTRIBUTORSHIP STATEMENT

A.M collected the subjects, performed the experiments, analyzed the samples and data, prepared the tables, wrote the manuscript and reviewed drafts of the paper.
S.S.M collected the subjects, analyzed the samples and reviewed drafts of the paper.
A.M.I collected the subjects, validated results and reviewed drafts of the paper.
F.M.N, S.A.S and K.Y collected the subjects and reviewed drafts of the paper.
H.N collected the subjects, contributed reagents and materials and reviewed drafts of the paper.
T.R conceived and designed the experiments, collected the subjects, contributed reagents and materials, wrote the manuscript and reviewed drafts of the paper.
B.P.H conceived and designed the experiments, collected the subjects, validated results, contributed reagents and materials and reviewed drafts of the paper.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Check
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	✓
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓
Objectives	3	State specific objectives, including any prespecified hypotheses	✓
Methods			
Study design	4	Present key elements of study design early in the paper	✓
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	✓
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	✓
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	✓
		(*b*) Describe any methods used to examine subgroups and interactions	✓
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	✓
		(*e*) Describe any sensitivity analyses	

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Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	✓
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓
		(*b*) Report category boundaries when continuous variables were categorized	✓
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	✓
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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BMJ Open

An observational study of the status of coronary risk biomarkers among Negritos with Metabolic Syndrome in East Coast Malaysia.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-021580.R1
Article Type:	Research
Date Submitted by the Author:	15-Jun-2018
Complete List of Authors:	Mohd Mokhsin, Nurul Atiqah; Universiti Teknologi MARA, Faculty of Medicine Mokhtar, Siti Shuhada; Universiti Teknologi MARA, Faculty of Medicine Mohd Ismail, Aletza; Universiti Teknologi MARA, Faculty of Medicine M. Nor, Fadzilah; Universiti Teknologi MARA, Faculty of Medicine Shaari, Syahrul Azlin; Universiti Teknologi MARA, Faculty of Medicine Nawawi, Hapizah; Universiti Teknologi MARA, Institute of Pathology, Forensic and Laboratory Medicine (I-PPerForM) Yusoff, Khalid; UCSI University, Faculty of Medicine and Health Sciences Rahman, Thuhairah; Universiti Teknologi MARA, Faculty of Medicine Hoh, Boon Peng; UCSI University, Faculty of Medicine and Health Sciences
Primary Subject Heading:	Pathology
Secondary Subject Heading:	Diabetes and endocrinology, Epidemiology, Public health
Keywords:	Lipid disorders < DIABETES & ENDOCRINOLOGY, Hypertension < CARDIOLOGY, EPIDEMIOLOGY

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An observational study of the status of coronary risk biomarkers among Negritos with Metabolic Syndrome in East Coast Malaysia.

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Word count: 3,740 words

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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 East Coast Malaysia and Peninsular Malaysia.

Participants: Hundred and fifty (150) Negritos who were still living in three inland settlements in East Coast Malaysia and 1,227 Malays in Peninsular Malaysia. These participants were then categorized into MS and Non-MS groups based on the IDF consensus worldwide definition of MS and were recruited between 2010 and 2015. The participants were random and on 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 [lp(a)] biomarkers.

Results: MS was significantly higher among the Malays compared to Negritos (27.7% vs 12.0%). Amongst 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 race, all biomarkers were higher in Negritos compared to Malays (p<0.001). Binary logistic regression analysis affirmed that Negritos were an independent predictor for Lp(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 Lp(a).

Keywords: Orang Asli; Aborigines; Coronary Artery Disease; Hypertension; Hypercholesterolaemia; Lp(a)

ARTICLE SUMMARY

Strengths and limitations of this study

1. Metabolic syndrome (MS) has become an increasing health problems contributing to non-communicable diseases, mainly due to high fat and carbohydrate diet shift and a sedentary lifestyle. Although few, there have been reports on the prevalence of metabolic diseases and biomarkers of atherogenesis among the indigenous populations from Peninsular Malaysia, known as the Orang Asli (OA). However, there is very scarce information on this among the inland OA settlements due to difficulties in accessing their tribes.
2. The strength of this study is gaining access to the earliest and smallest population of aboriginal or orang asli (OA) tribe in Malaysia, which is the inland dwelling Negrito, to determine the prevalence of MS and biomarkers of atherogenesis among these aboriginal people who have completely isolated themselves from influences of modernization.
3. To the best of our knowledge, this is one of the first study that assessed biomarkers of atherogenesis among the inland dwelling Negritos other than hsCRP.
4. Key findings from this research were that there were no difference in biomarkers of atherogenesis between MS and non-MS Negritos which interestingly contradicts findings among MS and non-MS Malays as well as established reports comparing the two cohorts. Furthermore, regression analysis highlights that Negritos independently predicted Lp(a) suggesting a possible genetic influence of this biomarker.

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5. The small sample size of Negritos due to difficulties accessing remote tribes in addition to small population by nature poses a limitation to this study despite achieving a minimum sample size requirement. This could, in part, lead to the non-significant difference in inflammatory biomarkers between MS and non-MS Negritos.

Ethics

Consent from the Department of Orang Asli Development (JaKOA) [JHEOA,PP.30.042.Jld5(17)] and Institutional ethics approval was granted by Universiti Teknologi MARA (UiTM) [600-RMI(5/1/6)] were obtained prior the initiation of this study. All methods were carried out in accordance with relevant guidelines and regulations. Written, informed consent was obtained from all subjects aged 18 years-old and above, prior to recruitment. Communications were done in Bahasa Malaysia with some input from local translators, either JaKOA officers or tribal leaders if and when required.

Funding

1) The Ministry of Higher Education Malaysia under Fundamental Research Grant Scheme [Grant code: 600-RMI/FRGS 5/3 (136/2014)]
2) The Ministry of Higher Education Malaysia under Long Term Research Grant Scheme [Grant code: 600-RMI/LRGS 5/3 (2/2011)-2]

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Financial Interests

The author(s) declare no competing financial interests.

Data Sharing

No additional data available.

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INTRODUCTION

Orang Asli (OA) are the indigenous people believed to be the earliest inhabitants of Peninsular Malaysia. There are 18 OA tribes, categorized under three main groups according to their different languages and customs: Negrito, located the Northern part of the peninsula; Senoi, residing in the Central Region and Proto-Malays (or Aboriginal Malay), in the Southern Region. The OA constitutes 0.5% (150,000) of the total Malaysian population with Senoi representing the largest OA population (54%) followed by the Proto Malays (43%), and the Negrito (3%) [1].

Negritos, 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 have 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 which 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 between 37.1%-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 (IL-6), resistin and C-reactive protein (CRP) are due to the overproduction by monocyte-derived macrophages residing in the expanded adipose tissue mass [11]. NCEP-ATP reported higher soluble endothelial adhesion molecules such as intercellular adhesion molecule-1 (ICAM-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 Malaysia [16], however, the samples used in these studies were

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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 urbanized Orang Seletar (a Proto-Malay subtribe) having the highest prevalence for central obesity (66.1±5.9%) compared to the other subtribes who are less urbanized (Senoi and Negrito). The study also revealed that the prevalence for 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 urbanization 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: 1) determine the MS components commonly seen among the Negrito, 2) elucidate the status of inflammation, endothelial activation and prothrombogenesis in Negrito and 3) identify the independent predictors for these biomarkers of coronary risk.

SUBJECTS AND METHODS

Patient and Public Involvement

All methods were carried out in accordance with relevant guidelines and regulations. Written, informed consent was obtained from all subjects aged 18 years-old and above, prior to recruitment. Communications were done in Bahasa Malaysia with some input from local translators, either JaKOA officers or tribal leaders if and when required.

150 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, East Coast Malaysia (4.8843°N, 101.9682°E). 1,177 Malays were also recruited from various national health screening programmes in Malaysia. The subjects collected were randomly selected and was on a voluntary basis. These subjects were then categorized into Metabolic Syndrome (MS) and Non-Metabolic Syndrome (Non-MS) groups based on the IDF consensus worldwide definition of MS [17].

The sample size was calculated using PS Power and Sample Size Calculations version 3.0 [18] 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

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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, social history including smoking status and 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 (WHR). With the subject in a seated position and following 5-10 minutes of rest, BP was measured by an automated BP reader (cuff size 12 x 33cm, Colin press-mate, Japan). The systolic BP (SBP) and diastolic BP (DBP) were measured to the nearest 1mmHg. BMI was calculated using the formula: BMI=weight(kg)/height^2(m^2). WC was measured to the nearest 0.5cm 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.

The results of this study were disseminated to participants through the JaKOA officers or using the postal address if provided. A physician was placed in the settings to provide an advice or referral letter when necessary.

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 >1.7mmol/L, (2) reduced high-density lipoprotein cholesterol (HDL-c) of <1.0 or 1.3mmol/L in male and female respectively, (3) elevated BP of >140/90mmHg, and a raised fasting plasma glucose (FPG) of ≥5.6mmol/L. Central obesity, using the suggested WC for Asian/South Asians, was defined as ≥90cm and ≥80cm 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 and 1500h. Serum and plasma were separated by centrifugation at 3500rpm for 10 minutes within 1 hour and stored at -20°C before analyse.

Biochemical Analysis

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

Cardiometabolic parameters tested were fasting serum lipids (FSL) which included total cholesterol (TC), TG and high density lipoprotein cholesterol (HDL-c) which were measured by enzymatic reference methods. Plasma glucose was analyzed by hexokinase method. All methods were run on an automated analyzer (Cobas Integra

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400 PLUS, Roche Diagnostic, Germany) except for low density lipoprotein cholesterol (LDL-c) concentration which was derived using the Friedewald equation [21].

Analyses of Biomarkers of inflammation, endothelial activation and prothrombogenesis

The biomarkers analyzed 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 (ELISA) 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 version 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) = α+b1(age) + b2(race) + b3(gender) + b4(smoking status) + b5(BMI) +b6(WC) + b7(SBP) + b8(DBP) + b9(glucose concentration) + b10(HDL-c concentration) + b11(TG concentration), where the dependent variable is logit coronary risk biomarkers, α is the estimate for the intercept and b1, b2, ...,b11 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: race = Malay (1), Negrito (0), Negrito = reference group; gender, female (1), male (0) = reference group; smoking status, not smoking (1) and not smoking (0) = reference group; While for age, BMI, WC, SBP, DBP, glucose, HDL-c and TG concentrations were continuous variables.

RESULTS

Demographic Data

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

Among the Negritos, their MS counterpart had higher TC (5.30±1.05 vs 4.48±0.85mmol/L, p<0.001 respectively), TG (2.14±0.79 vs 1.41±0.68mmol/L, p<0.001 respectively) and LDL-c (3.36±0.69 vs 2.80±0.84mmol/L, p=0.008 respectively). The most frequent criteria diagnosing MS among the Negritos were hypertension, elevated

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TG and low HDL-c and none of the Negritos diagnosed with MS had any form of glucose intolerance.

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Table 1: Demographic and clinical characteristics of the Negritos and Malays

Parameters	Malays (n = 1,177)			Negrito (n = 150)		
	MS (n=326)	Non-MS (n=851)	p value	MS (n=18)	Non-MS (n=132)	p value
[b] Age (Years)	49.53±11.77	40.00±14.69	<0.001	30.56±11.11	31.29±11.00	NS
[a] Gender (Males/Females)	45.1/54.9	37.3/62.7	0.02	5.6/94.4	59.1/40.9	<0.001
[a] Diabetes	42.1	5.6	<0.001	0.00	1.8	NS
[b] Plasma glucose (mmol/L)	8.22±4.38	5.88±2.33	<0.001	5.18±1.26	4.61±1.41	NS
[a] Hypertension	83.2	28.9	<0.001	83.3	53.5	0.02
[b] SBP (mmHg)	138.22±17.82	120.99±17.86	<0.001	133.44±23.06	130.25±17.22	NS
[b] DBP (mmHg)	83.49±10.63	73.69±11.42	<0.001	81.39±12.87	81.09±13.41	NS
[a] Central Obesity	100	42.9	<0.001	100	2.3	<0.001
[b] Waist circumference (cm)	97.19±8.93	82.75±11.24	<0.001	90.81±6.88	69.01±15.35	<0.001
[b] BMI (kg/m²)	30.57±4.90	24.94±4.77	<0.001	26.31±3.26	19.75±2.55	<0.001
[a] Current smoker	16.9	13.2	NS	23.5	46.8	NS
[b] Total cholesterol (mmol/L)	5.38±1.00	5.05±0.96	<0.001	5.30±1.05	4.48±0.85	<0.001
[b] Triglyceride (mmol/L)	2.70±1.54	1.41±0.82	<0.001	2.14±0.79	1.41±0.68	<0.001
[b] LDL-c (mmol/L)	3.10±0.76	2.99±0.76	0.03	3.36±0.69	2.80±0.84	0.008
[b] HDL-c (mmol/L)	1.08±0.31	1.42±0.37	<0.001	0.98±0.38	1.01±0.28	NS

Notes: [a] Data expressed as percentage; [b] Data expressed as Mean±SD

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, Body mass index; NS, not significant

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Comparison of the biomarkers of inflammation, endothelial activation and prothrombogenesis in Malay and Negrito subjects (Table 2)

There was no significant differences between MS and non-MS among Negritos for all biomarkers (p>0.05) whereas MS Malays illustrated higher hsCRP (mean±SD=1.88±1.16 vs 1.26±1.13mg/L, p<0.001 respectively) and sICAM-1 (mean±SD=531.73±185.73 vs 465.46±202.08ng/mL, p<0.001 respectively) concentrations compared to 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±1.17mg/L, p<0.001 respectively), sICAM-1 (mean±SD=684.85±388.03 vs 482.25±200.05ng/mL, p<0.001 respectively) and Lp(a) (mean±SD=0.22±0.22 vs 0.06±0.04mg/L, p<0.001 respectively).

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

Biomarkers	Malays (n = 1,177)			Negrito (n = 150)		
	MS (n=326)	Non-MS (n=851)	p value	MS (n=18)	Non-MS (n=132)	p value
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

Notes: Data expressed as Mean ± SD
Abbreviations: NS, not significant

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Independent predictors of the biomarkers of inflammation, endothelial activation and prothrombogenesis in Malay and Negrito subjects (Table 3)

To further determine the independent predictors of these biomarkers, binary logistic regression analyses were performed with the biomarkers as dependent variables. It was found that BMI (p=0.016), FPG (p<0.001) and DBP (p=0.042) were independent predictors for hsCRP whilst 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 whilst 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.

Table 3: Predictors for hsCRP and Lp(a)

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

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

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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 2 DM and CAD [22]. In two European studies [23,24], the presence of MS predicted increased CAD mortality. These findings are not unexpected considering MS comprises of 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 dysfunction [25] and thrombosis [26] and are elevated in patients with atherosclerosis-related disease such as CAD [27] and peripheral artery disease [28] in previous reports.

There have been several previous reports determining the prevalence of non-communicable diseases (NCD) such as DM, hypertension, dyslipidaemia and obesity among OA subjects. A previous report on the health status of Lanoh ethnic sub-groups of OA 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 (2016) which highlighted the higher percentage of OA having high insulin levels, hsCRP and higher percentage categorized 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 OA in Malaysia, there is scarce research that extended to investigate biomarkers for coronary risk amongst 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 to 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 to 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 to 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

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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 urbanization 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 to the Malays. This suggests that the Negritos may be at higher risk of CAD at a younger age group hence warrants further investigation.

Furthermore, findings from this study suggest that the MS Negritos despite having coronary risk factor including hypertension and dyslipidaemia, the failure to exhibit enhanced atherogenesis compared to 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 extend, still practicing the hunter gatherers lifestyle, and living isolated from urbanization, thus may have contributed to the differences observed in the biomarkers between the two races when comparing with the MS and non-MS counterparts. This is in keeping 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 were 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 vasculature [40] and cardiac myocytes [41]. Genetic variation of this gene has been shown to influence metabolic outcome and possibly provides atherogenesis resistant [39,42,43]. Adiponectin plays a crucial role in the metabolic regulation of obesity, insulin sensitivity and atherosclerosis and several studies have indicated its anti-atherogenic 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

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to their non-MS counterpart. 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 Negrito race independently predicts Lp(a) in this study. This finding imply that despite having a healthier lifestyle by being hunter gatherers and relatively isolated from modernization, 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 have shown significant difference in Lp(a) levels among various races 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 that are not subjected to urbanization. This along with major physical problems accessing all these remote tribes prevented us from having a larger sample to analyze.

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 urbanized Malays in Malaysia. Firstly, 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. Secondly, 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).

ACKNOWLEDGMENTS

The authors would like to express their appreciation to the Centre for Pathology Diagnostic and Research Laboratories (CPDRL) of Faculty of Medicine, Universiti

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Teknologi MARA for providing the facilities to conduct this research and all of the subjects who were voluntarily participate in this study.

CONTRIBUTORSHIP STATEMENT

A.M collected the subjects, performed the experiments, analyzed the samples and data, prepared the tables, wrote the manuscript and reviewed drafts of the paper.
S.S.M collected the subjects, analyzed the samples and reviewed drafts of the paper.
A.M.I collected the subjects, validated results and reviewed drafts of the paper.
F.M.N, S.A.S and K.Y collected the subjects and reviewed drafts of the paper.
H.N collected the subjects, contributed reagents and materials and reviewed drafts of the paper.
T.R conceived and designed the experiments, collected the subjects, contributed reagents and materials, wrote the manuscript and reviewed drafts of the paper.
B.P.H conceived and designed the experiments, collected the subjects, validated results, contributed reagents and materials and reviewed drafts of the paper.

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STROBE Statement
An observational study of the status of coronary risk biomarkers among Negritos with Metabolic Syndrome in East Coast Malaysia.

Section	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	6-7
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5-6
		(*e*) Describe any sensitivity analyses	

Continued on next page

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STROBE Statement
An observational study of the status of coronary risk biomarkers among Negritos with Metabolic Syndrome in East Coast Malaysia.

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7-8
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	7-8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	8-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

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An observational study of the status of coronary risk biomarkers among Negritos with Metabolic Syndrome in East Coast Malaysia.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-021580.R2
Article Type:	Research
Date Submitted by the Author:	02-Oct-2018
Complete List of Authors:	Mohd Mokhsin, Nurul Atiqah; Universiti Teknologi MARA, Faculty of Medicine Mokhtar, Siti Shuhada; Universiti Teknologi MARA, Faculty of Medicine Mohd Ismail, Aletza; Universiti Teknologi MARA, Faculty of Medicine M. Nor, Fadzilah; Universiti Teknologi MARA, Faculty of Medicine Shaari, Syahrul Azlin; Universiti Teknologi MARA, Faculty of Medicine Nawawi, Hapizah; Universiti Teknologi MARA, Institute of Pathology, Forensic and Laboratory Medicine (I-PPerForM) Yusoff, Khalid; UCSI University, Faculty of Medicine and Health Sciences Rahman, Thuhairah; Universiti Teknologi MARA, Faculty of Medicine Hoh, Boon Peng; UCSI University, Faculty of Medicine and Health Sciences
Primary Subject Heading:	Pathology
Secondary Subject Heading:	Diabetes and endocrinology, Epidemiology, Public health
Keywords:	Lipid disorders < DIABETES & ENDOCRINOLOGY, Hypertension < CARDIOLOGY, EPIDEMIOLOGY

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An observational study of the status of coronary risk biomarkers among Negritos with Metabolic Syndrome in East Coast Malaysia.

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Word count: 3,885 words

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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 East Coast Malaysia and Peninsular Malaysia.

Subjects: Hundred and fifty (150) Negritos who were still living in three inland settlements in East Coast Malaysia and 1,227 Malays in Peninsular Malaysia. These subjects were then categorized into MS and Non-MS groups based on the IDF consensus worldwide definition of MS and were recruited between 2010 and 2015. The subjects were random and on 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 [lp(a)] biomarkers.

Results: MS was significantly higher among the Malays compared to Negritos (27.7% vs 12.0%). Amongst 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 to Malays (p<0.001). Binary logistic regression analysis affirmed that Negritos were an independent predictor for Lp(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 Lp(a).

Keywords: Orang Asli; Aborigines; Coronary Artery Disease; Hypertension; Hypercholesterolaemia; Lp(a)

ARTICLE SUMMARY

Strengths and limitations of this study

1. 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 modernization.
2. This is one of the first study that assessed biomarkers of atherogenesis among the inland dwelling Negritos other than hsCRP.
3. This is one of the first study to determine the independent predictors of the atherogenesis biomarkers among inland dwelling Negritos.
4. 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.

Ethics

Consent from the Department of Orang Asli Development (JAKOA) [JHEOA,PP.30.042.Jld5(17)] and Institutional ethics approval granted by Universiti Teknologi MARA (UiTM) [600-RMI(5/1/6)] were obtained prior to the initiation of this study. All methods were carried out in accordance with relevant guidelines and regulations. Written, informed consent was obtained from all subjects aged 18 years-old and above, prior to recruitment. Communications were done in Bahasa

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Malaysia with some input from local translators, either JAKOA officers or tribal leaders if and when required.

Funding

1) The Ministry of Higher Education Malaysia under Fundamental Research Grant Scheme [Grant code: 600-RMI/FRGS 5/3 (136/2014)]
2) The Ministry of Higher Education Malaysia under Long Term Research Grant Scheme [Grant code: 600-RMI/LRGS 5/3 (2/2011)-2]

The funders had no role in the study design, data collection, data analysis, decision to publish, or preparation of the manuscript.

Competing Financial Interests

The author(s) declare no competing financial interests.

Data Sharing

No additional data available.

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INTRODUCTION

Orang Asli (OA) are the indigenous people believed to be the earliest inhabitants of Peninsular Malaysia. There are 18 OA tribes, categorized under three main groups according to their different languages and customs: Negritos, located in the Northern part of the peninsula; Senoi, residing in the Central Region and Proto-Malays (or Aboriginal Malay), 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 which 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 between 37.1%-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 (IL-6), resistin and C-reactive protein (CRP) are due to the overproduction by monocyte-derived macrophages residing in the expanded adipose tissue mass [11]. The NCEP-ATP reported higher soluble endothelial adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-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 Malaysia [16], however, the samples used in these studies were

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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 urbanized Orang Seletar (a Proto-Malay subtribe) having the highest prevalence for central obesity (66.1±5.9%) compared to the other subtribes who are less urbanized (Senoi and Negrito). The study also revealed that the prevalence for 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 urbanization 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, East Coast of Peninsular Malaysia (4.8843°N, 101.9682°E). 1,177 Malays were also recruited from various national health screening programmes in Peninsular Malaysia. The subjects collected were randomly selected and on a voluntary basis. These subjects were then categorized into Metabolic Syndrome (MS) and Non-Metabolic Syndrome (Non-MS) groups based on the IDF 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 version 3.0 [18] 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

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medical history, 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 (WHR). With the subject in a seated position and following 5-10 minutes of rest, BP was measured by an automated BP reader (cuff size 12 x 33cm, Colin press-mate, Japan). The systolic BP (SBP) and diastolic BP (DBP) were measured to the nearest 1mmHg. BMI was calculated using the formula: BMI=weight(kg)/height^2(m^2). WC was measured to the nearest 0.5cm 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 (DM) 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 >1.7mmol/L, (2) reduced high-density lipoprotein cholesterol (HDL-c) of <1.0 or 1.3mmol/L in male and female respectively, (3) elevated BP of >140/90mmHg, and a raised fasting plasma glucose (FPG) of ≥5.6mmol/L. Central obesity, using the suggested WC for Asian/South Asians, was defined as ≥90cm and ≥80cm 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 0800h and 1500h. Serum and plasma were separated by centrifugation at 3500rpm for 10 minutes within 1 hour and stored at -20°C before analyse.

Biochemical Analysis

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

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

Analyses of Biomarkers of inflammation, endothelial activation and prothrombogenesis

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The biomarkers analyzed 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 (ELISA) 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 version 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, DM, and low HDL-c or high TG concentrations). The specific model estimated from the data was:
logit (coronary risk biomarkers) = α+b1(age) + b2(ethnicity) + b3(gender) + b4(smoking status) + b5(BMI) +b6(WC) + b7(SBP) + b8(DBP) + b9(glucose concentration) + b10(HDL-c concentration) + b11(TG concentration), where the dependent variable is logit coronary risk biomarkers, α is the estimate for the intercept and b1, b2, ...,b11 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-old and above, prior to recruitment. Communications were done in Bahasa Malaysia with some input from local translators, either the 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 summarizes 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, 15.5% Malays were diabetic while 1.5% of the Negritos had IFG ≥5.6mmol/L or random plasma glucose of >11.0mmol/L. We observed that 43.9% of the Malays and 57.2% of the Negritos were hypertensive while 58.7% Malays and 14.0% Negritos had central obesity.

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Compared to the Non-MS Negritos, their MS counterpart had higher TC (5.30±1.05 vs 4.48±0.85mmol/L, p<0.001 respectively), TG (2.14±0.79 vs 1.41±0.68mmol/L, p<0.001 respectively) and LDL-c (3.36±0.69 vs 2.80±0.84mmol/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.

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Table 1: Demographic and clinical characteristics of the Negritos and Malays

Parameters	Malays (n = 1,177)			Negrito (n = 150)		
	MS (n=326)	Non-MS (n=851)	p value	MS (n=18)	Non-MS (n=132)	p value
[b] Age (Years)	49.53±11.77	40.00±14.69	<0.001	30.56±11.11	31.29±11.00	NS
[a] Gender (Males/Females)	45.1/54.9	37.3/62.7	0.02	5.6/94.4	59.1/40.9	<0.001
[a] Diabetes	42.1	5.6	<0.001	0.00	1.8	NS
[b] Plasma glucose (mmol/L)	8.22±4.38	5.88±2.33	<0.001	5.18±1.26	4.61±1.41	NS
[a] Hypertension	83.2	28.9	<0.001	83.3	53.5	0.02
[b] SBP (mmHg)	138.22±17.82	120.99±17.86	<0.001	133.44±23.06	130.25±17.22	NS
[b] DBP (mmHg)	83.49±10.63	73.69±11.42	<0.001	81.39±12.87	81.09±13.41	NS
[a] Central Obesity	100	42.9	<0.001	100	2.3	<0.001
[b] Waist circumference (cm)	97.19±8.93	82.75±11.24	<0.001	90.81±6.88	69.01±15.35	<0.001
[b] BMI (kg/m²)	30.57±4.90	24.94±4.77	<0.001	26.31±3.26	19.75±2.55	<0.001
[a] Current smoker	16.9	13.2	NS	23.5	46.8	NS
[b] Total cholesterol (mmol/L)	5.38±1.00	5.05±0.96	<0.001	5.30±1.05	4.48±0.85	<0.001
[b] Triglyceride (mmol/L)	2.70±1.54	1.41±0.82	<0.001	2.14±0.79	1.41±0.68	<0.001
[b] LDL-c (mmol/L)	3.10±0.76	2.99±0.76	0.03	3.36±0.69	2.80±0.84	0.008
[b] HDL-c (mmol/L)	1.08±0.31	1.42±0.37	<0.001	0.98±0.38	1.01±0.28	NS

Notes: [a] Data expressed as percentage; [b] Data expressed as Mean±SD

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, Body mass index; NS, not significant

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Comparison of the biomarkers of inflammation, endothelial activation and prothrombogenesis in Malay and Negrito subjects (Table 2)

There was no significant differences between MS and non-MS among Negritos for all biomarkers (p>0.05) whereas MS Malays illustrated higher hsCRP (mean±SD=1.88±1.16 vs 1.26±1.13mg/L, p<0.001 respectively) and sICAM-1 (mean±SD=531.73±185.73 vs 465.46±202.08ng/mL, p<0.001 respectively) concentrations compared to 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±1.17mg/L, p<0.001 respectively), sICAM-1 (mean±SD=684.85±388.03 vs 482.25±200.05ng/mL, p<0.001 respectively) and Lp(a) (mean±SD=0.22±0.22 vs 0.06±0.04mg/L, p<0.001 respectively) compared to the Malays.

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

Biomarkers	Malays (n = 1,177)			Negrito (n = 150)		
	MS (n=326)	Non-MS (n=851)	p value	MS (n=18)	Non-MS (n=132)	p value
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

Notes: Data expressed as Mean ± SD
Abbreviations: NS, not significant

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Independent predictors of the biomarkers of inflammation, endothelial activation and prothrombogenesis in Malay and Negrito subjects (Table 3)

To further determine the independent predictors of these biomarkers, binary logistic regression analyses were performed with the biomarkers as dependent variables. It was found that BMI (p=0.016), FPG (p<0.001) and DBP (p=0.042) were independent predictors for hsCRP whilst 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 whilst 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.

Table 3: Predictors for hsCRP and Lp(a)

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

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

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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 2 DM and CAD [22]. In two European studies [23,24], the presence of MS predicted increased CAD mortality. These findings are not unexpected considering MS comprises of 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 dysfunction [25] and thrombosis [26] and are elevated in patients with atherosclerosis-related disease such as CAD [27] and peripheral artery disease [28] in previous reports.

There have been several previous reports determining the prevalence of non-communicable diseases (NCD) 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 (2016) which highlighted the higher percentage of OA having high insulin levels, hsCRP and higher percentage categorized 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 amongst 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 to 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 to 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 to 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

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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 urbanization 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 to the Malays. This suggests that the Negritos may be at higher risk of CAD at a younger age group hence warrants further investigation.

Furthermore, findings from this study suggest that the MS Negritos despite having coronary risk factor including hypertension and dyslipidaemia, the failure to exhibit enhanced atherogenesis compared to 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 extend, still practicing the hunter gatherers lifestyle, and living isolated from urbanization, 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 keeping 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 vasculature [40] and cardiac myocytes [41]. Genetic variation of this gene has been shown to influence metabolic outcome and possibly provides atherogenesis resistant [39,42,43]. Adiponectin plays a crucial role in the metabolic regulation of obesity, insulin sensitivity and atherosclerosis and several studies have indicated its anti-atherogenic 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

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inflammatory or endothelial activation status when compared to their non-MS counterpart. 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 predicts 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 modernization, 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 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 that are not subjected to urbanization. This along with major physical problems accessing all these remote tribes prevented us from having a larger sample to analyze.

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 urbanized Malays in Malaysia. Firstly, 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. Secondly, 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).

ACKNOWLEDGMENTS

The authors would like to express their appreciation to the Centre for Pathology Diagnostic and Research Laboratories (CPDRL) of Faculty of Medicine, Universiti

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Teknologi MARA for providing the facilities to conduct this research and all of the subjects who were voluntarily participate in this study.

CONTRIBUTORSHIP STATEMENT

A.M collected the subjects, performed the experiments, analyzed the samples and data, prepared the tables, wrote the manuscript and reviewed drafts of the paper.
S.S.M collected the subjects, analyzed the samples and reviewed drafts of the paper.
A.M.I collected the subjects, validated results and reviewed drafts of the paper.
F.M.N, S.A.S collected the subjects and reviewed drafts of the paper.
H.N collected the subjects, contributed reagents and materials and reviewed drafts of the paper.
K.Y. contributed reagents and materials, drafted the manuscript and reviewed the draft of the paper.T.R conceived and designed the experiments, collected the subjects, contributed reagents and materials, wrote the manuscript and reviewed drafts of the paper.
B.P.H conceived and designed the experiments, collected the subjects, validated results, contributed reagents and materials and reviewed drafts of the paper.

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An observational study of the status of coronary risk biomarkers among Negritos with Metabolic Syndrome in East Coast Malaysia

STROBE Statement—checklist of items that should be included in reports of observational studies

Section	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	6-7
		(*c*) Explain how missing data were addressed	
		(*d*) *Case-control study*—If applicable, explain how matching of cases and controls was addressed	5-6
		(*e*) Describe any sensitivity analyses	

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Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7-8
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cross-sectional study*—Report numbers of outcome events or summary measures	7-8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	8-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

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