

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	An observational study of the status of coronary risk biomarkers among Negritos with Metabolic Syndrome in East Coast Malaysia.
AUTHORS	Mohd Mokhsin, Nurul Atiqah; Mokhtar, Siti Shuhada; Mohd Ismail, Aletza; M. Nor, Fadzilah; Shaari, Syahrul Azlin; Nawawi, Hapizah; Yusoff, Khalid; Rahman, Thuhairah; Hoh, Boon Peng

VERSION 1 – REVIEW

REVIEWER	Kean Ghee Lim International Medical University, Malaysia
REVIEW RETURNED	02-Mar-2018

GENERAL COMMENTS	<p>Metabolic syndrome when first mentioned in Abstract must be given in full not MS. No statement of method of sampling made of both subjects and controls. (Note: Orang Asli, among them Negritos who have left their forest dwellings to live in urban fringes tend to have different in lifestyle and prevalence of non-communicable diseases and 'inland settlements' should be clearly described)</p> <p>Section on strength and limitations DO NOT discuss that and should be re-written</p> <p>No mention of ethics approval</p> <p>The results show the type of MS Negritos have (hardly any with diabetes) is different from the Malays (many have diabetes). The right most column in Tables 1 and 2 is unnecessary (All subjects, merely combines Malays and Negritos and is meaningless) Table 3 could be summed up better in fewer columns</p> <p>The explanation for the results found, no sig difference in biomarkers among Negritos with and without MS is not discussed in relation to diabetes, which is obviously likely to be important, whereas of many genes involved in MS, CDH13 is highlighted without a good reason why.</p>
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REVIEWER	Prof Antonino Tuttolomondo University of Palermo (Italy)
REVIEW RETURNED	09-Mar-2018

GENERAL COMMENTS	Mokhsin and coworkers determined the prevalence of MS and the status of coronary risk biomarkers their independent role as predictors of these biomarkers among the Negritos. 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
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	<p>($p < 0.001$). Authors concluded that 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). This is a interesting study . It seems very original and novel the analysis of MS and coronary pathways in negrito Orang Asli (OA) an indigenous people of Malaysia I have only minor comments:</p> <p>1) Methods Authors should better explain their multiple binary regression analysis</p> <p>2) CONSORT, STROBE or PRISMA checklist Authors should add information about CONSORT, STROBE OR PRISMA Checklist if available</p> <p>3) Discussion authors should add a brief sentence about the role of inflammatory variables in thrombogenesis and endothelial dysfunction and they should add these citations on reference section :</p> <ul style="list-style-type: none"> • Tuttolomondo A, Pecoraro R, Casuccio A, Di Raimondo D, Buttà C, Clemente G, Della Corte V, Guggino G, Arnao V, Maida C, Simonetta I, Maugeri R, Squadrito R, Pinto A. Peripheral Frequency of CD4+ CD28- Cells in Acute Ischemic Stroke: Relationship With Stroke Subtype and Severity Markers. <i>Medicine (Baltimore)</i>. 2015 May;94(20):e81 • Tuttolomondo A, Di Sciacca R, Di Raimondo D, Pedone C, La Placa S, Pinto A, Licata G. Effects of clinical and laboratory variables and of pretreatment with cardiovascular drugs in acute ischaemic stroke: a retrospective chart review from the GIFA study. <i>Int J Cardiol</i>. 2011 Sep 15;151(3):318-22. <p>4) Limitations</p>
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REVIEWER	Nica Borradaile Western University, Canada
REVIEW RETURNED	18-Apr-2018

GENERAL COMMENTS	<p>This is Interesting study with an important rationale for investigating the prevalence of MS in a relatively undisturbed nomadic human population. Although it is clear that sample size issues did not allow for additional statistical comparisons between Malay and Negrito populations, there should be some surrounding this the data. It appears possible that baseline markers of inflammation in Negritos is higher than in Malays. Related to this, the conclusions drawn are overstated based on data obtained from the particularly small group of MS Negritos.</p> <p>Major Points:</p> <p>1) For Table 1:</p> <p>Could be the age difference between Malay and Negrito populations that account for differences in rates of diabetes? Perhaps hypertension and dyslipidemia manifest first, followed by diabetes with aging.</p> <p>Also, the numbers for MS vs. non-MS don't add up to the total numbers for each population.</p> <p>2) For Table 2:</p>
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	<p>As for Table 1, the numbers for MS vs. non-MS don't add up to the total numbers for each population.</p> <p>The lack of ability to detect difference between sCRP in Negrito groups is probably due to small size of MS Negrito group. Conclusions should reflect this limitation.</p> <p>The values for Lp(a) for all subjects is incorrect (same as Negrito).</p> <p>3) For Discussion:</p> <p>'...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...'</p> <p>This comment is an overstatement, given the data provided. The authors are relying on statistical analyses based on a tiny sample (n=17) of Negritos with MS. sCRP could, in fact, be significant given a larger sample. Overall, the Negrito population appears to have higher inflammatory markers than the Malay. Given that this population was significantly younger on average compared to the Malay population, Negritos may actually be at greater risk. All related discussion (and the abstract) needs to be framed accordingly.</p> <p>'...despite having metabolic risks for CAD among the MS Negritos group, the failure to exhibit enhanced atherogenesis...'</p> <p>Evidence of CAD was not provided in this study, and so no claims can be made about atherogenesis in this population.</p> <p>The conclusion that '...MS Negritos do not appear to share similar coronary risks as those of MS Malays...' is not well supported by the data provided.</p> <p>Minor Points:</p> <p>1) Article summary bullets need to more concise. 2) Minor editing for typographical and grammatical errors is required.</p>
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VERSION 1 – AUTHOR RESPONSE

REVIEWER: 1

REVIEWER NAME: KEAN GHEE LIM

INSTITUTION AND COUNTRY: INTERNATIONAL MEDICAL UNIVERSITY, MALAYSIA

1 (a)] Metabolic syndrome when first mentioned in Abstract must be given in full not MS.

Response:

The word Metabolic Syndrome has been given in full in the Abstract section on page 2.

1 (b)] No statement of method of sampling made of both subjects and controls. (Note: Orang Asli, among them Negritos who have left their forest dwellings to live in urban fringes tend to have different in lifestyle and prevalence of non-communicable diseases and 'inland settlements' should be clearly described).

Response:

Statement on method of sampling has been added in the Abstract section under the Method heading on page 2, as follows:

“This study was a cross sectional study. 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 who were still living in the three inland settlements in East Coast Malaysia and Malays in Peninsular Malaysia (n=1,227) recruited between 2010 and 2015. The subjects were random and on voluntary basis.”

2) Section on strength and limitations DO NOT discuss that and should be re-written

Response:

The section on strength and limitations have been re-written accordingly on page 2.

3) No mention of ethics approval

Response:

The ethics approval statement has already been mentioned in the “Article Summary” under the “Ethics” section on page 3 and not in the main content, in accordance to the Instruction to Authors.

4) The right most column in Tables 1 and 2 is unnecessary (All subjects, merely combines Malays and Negritos and is meaningless) Table 3 could be summed up better in fewer columns

Response:

Thank you for the suggestion. The tables have been adjusted accordingly. Page 8-10

5) The explanation for the results found, no sig difference in biomarkers among Negritos with and without MS is not discussed in relation to diabetes, which is obviously likely to be important, whereas of many genes involved in MS, CDH13 is highlighted without a good reason why.

Response:

We thank the reviewer for his feedback. The possible reason(s) for the non-significant difference in biomarkers between Negritos with and without MS has been included in the Discussion section on page 12, as follows:

“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 (Tang et al, 2014). 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 (Mazidi et al, 2018; Ferreira et al, 2018).”

We thank the reviewer for raising a valid comment. CDH13 was highlighted in the initial version of the manuscript, because several earlier population genetic studies reported earlier a strong and consistent signal of positive natural selection among the Negritos (Deng et al., 2014, Human Genetics; Liu et al., 2015, Human Genetics). In other words, the Negrito people have shown a unique genetic profile of the gene CDH13 as compared to other populations. CDH13, which encodes for the protein T-Cadherin, has been associated with hypertension, cholesterol and adiponectin synthesis is consistent with the biochemical profiles which we observed in the Negrito community (Phipps et al, 2015; Ashari et al, 2016). Owing to these observations, we therefore postulated that despite their isolated lifestyle, the genetic variation of CDH13 among Negritos could partly be a contributing factor. Nonetheless we agree with the reviewer that our justification may have been too scarce, thus we have revised the related statements extensively in the Discussion, page 12-13.

REVIEWER: 2

REVIEWER NAME: PROF ANTONINO TUTTOLOMONDO
INSTITUTION AND COUNTRY: UNIVERSITY OF PALERMO (ITALY)

1) Methods:

Authors should better explain their multiple binary regression analysis

Response:

Multiple binary regression analysis has been explained in Methods, Page 7.

2) CONSORT, STROBE or PRISMA checklist:

Authors should add information about CONSORT, STROBE OR PRISMA

Response:

Checklist if available STROBE checklist was available as appendix.

3) Discussion:

authors should add a brief sentence about the role of inflammatory variables in thrombogenesis and endothelial dysfunction and they should add these citations on reference section :

- Tuttolomondo A, Pecoraro R, Casuccio A, Di Raimondo D, Buttà C, Clemente G, Della Corte V, Guggino G, Arnao V, Maida C, Simonetta I, Maugeri R, Squadrito R, Pinto A. Peripheral Frequency of CD4+ CD28- Cells in Acute Ischemic Stroke: Relationship With Stroke Subtype and Severity Markers. *Medicine (Baltimore)*. 2015 May;94(20):e81
- Tuttolomondo A, Di Sciacca R, Di Raimondo D, Pedone C, La Placa S, Pinto A, Licata G. Effects of clinical and laboratory variables and of pretreatment with cardiovascular drugs in acute ischaemic stroke: a retrospective chart review from the GIFA study. *Int J Cardiol*. 2011 Sep 15;151(3):318-22.

Response:

We thank the reviewer for his feedback. We have revised the Discussion extensively, and the suggested references have been cited deem appropriate.

4) Limitations:

Authors should add a limitation section on their text

Response:

We thank the reviewer for his comment. Limitations of this study has been included in the discussion section, Page 13.

REVIEWER: 3

REVIEWER NAME: NICA BORRADAILE
INSTITUTION AND COUNTRY: WESTERN UNIVERSITY, CANADA

1) This is Interesting study with an important rationale for investigating the prevalence of MS in a relatively undisturbed nomadic human population. Although it is clear that sample size issues did not allow for additional statistical comparisons between Malay and Negrito populations, there should be some surrounding this data. It appears possible that baseline markers of inflammation in Negritos is higher than in Malays. Related to this, the conclusions drawn are overstated based on data obtained from the particularly small group of MS Negritos.

Response:

We greatly appreciate the positive response from reviewer#3.

We are aware of the small sample size in our study, and agree with reviewer#3 that our conclusion drawn may have been overstated. Thus, we have extensively revised the manuscript.

2) For Table 1:

Could be the age difference between Malay and Negrito populations that account for differences in rates of diabetes? Perhaps hypertension and dyslipidemia manifest first, followed by diabetes with aging.

Also, the numbers for MS vs. non-MS don't add up to the total numbers for each population.

Response:

We appreciate the constructive comment from the reviewer. To address this question, we reanalysed the data by matching the age of the samples, but no difference in the rates of diabetes between Malays and Negritos, in that the prevalence of diabetes remained higher among the Malays (1.5% vs 7.2%, $p > 0.05$; Negritos and Malays respectively).

The results of this analysis has been included in Supplementary Data.

However we wish to reiterate that the main objective of this report was to determine the prevalence of MS collectively rather than as separate entities of the disorder.

We apologize for the mistake. The numbers for MS vs. non-MS have been corrected.

3) For Table 2:

The lack of ability to detect difference between hsCRP in Negrito groups is probably due to small size of MS Negrito group. Conclusions should reflect this limitation.

The values for Lp(a) for all subjects is incorrect (same as Negrito).

Response:

We appreciate the constructive comment from the reviewer. We have revised the manuscript by highlighting the limitation of this study in page 13, Discussion.

We apologize for the mistake. The numbers of subjects has been corrected.

4) '...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...'

This comment is an overstatement, given the data provided. The authors are relying on statistical analyses based on a tiny sample ($n=17$) of Negritos with MS. hsCRP could, in fact, be significant given a larger sample.

Overall, the Negrito population appears to have higher inflammatory markers than the Malay. Given that this population was significantly younger on average compared to the Malay population, Negritos may actually be at greater risk. All related discussion (and the abstract) needs to be framed accordingly.

'...despite having metabolic risks for CAD among the MS Negritos group, the failure to exhibit enhanced atherogenesis...'

Evidence of CAD was not provided in this study, and so no claims can be made about atherogenesis in this population.

The conclusion that '...MS Negritos do not appear to share similar coronary risks as those of MS Malays...' is not well supported by the data provided. Thank you for the feedback. Based on a previous study on OA, they determined the prevalence of MS among Negritos (rural Lanoh tribe) to be 12.5% (Ashari et al, 2016) which is consistent with our findings of 12% among our Negrito population. Nonetheless, we have declared the limitation of this study and have revised the discussion to take into account this limitation when explaining our findings on page 11.

Response:

Thank you for highlighting this point. We have revised our discussion to include this finding on page 12 as follows:

"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 which 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 extent, 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 (Ferreira et al, 2018; Antonio et al, 2016).”

This sentence has been revised accordingly on page 13.

5) Article summary bullets need to more concise.

Response:

The article summary has been re-written as suggested on page 2.

6) Minor editing for typographical and grammatical errors is required.

Response:

The manuscript has been revised accordingly to improve the quality of English by a representative of the English Literature Department in our institution.

VERSION 2 – REVIEW

REVIEWER	Nica Borradaile Western University, Canada
REVIEW RETURNED	29-Jun-2018
GENERAL COMMENTS	Concerns from initial review have been adequately addressed. The manuscript would benefit from a final proof-reading for minor grammatical errors.

VERSION 2 – AUTHOR RESPONSE

REVIEWER: 3

REVIEWER NAME: NICA BORRADAILE

INSTITUTION AND COUNTRY: WESTERN UNIVERSITY, CANADA

1) Concerns from initial review have been adequately addressed. The manuscript would benefit from a final proof-reading for minor grammatical errors.

Response: The manuscript has been revised to improve the quality of English by a representative of the English Literature Department in our institution.