Author's response to reviews

Title: Association between Erythrocyte parameters and Metabolic Syndrome in Urban Han Chinese: A Longitudinal Cohort Study

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Author's response to reviews: see over
We appreciate your effort in carefully reviewing of our manuscript titled “Association between Erythrocyte parameters and Metabolic Syndrome in Urban Han Chinese”. We also thank for your timely review and insightful critiques. We have re-organized and carefully checked the manuscript. Specifically, the main changes made in our revision are listed as followings:

a) Re-organized and carefully checked the manuscript to minimize the error.
b) Re-wrote the “Materials and Methods”, clarified the significance and theoretical concept of the GEE model, and selection of confounding factors.
c) Re-wrote the discussion in a clear way.

Response to the reviews:

Reviewer #1:

1) The standard of English in this article is very poor. I being a non-native English speaker could point out lot of mistakes. I strongly suggest authors to have a English proof reading from native English speaker for this manuscript.

Response:
Thanks for the suggestions. We have re-organized and carefully checked the manuscript to minimize the error. We hope the quality is much improved.

2) The very first sentence of the introduction contains the word "hypoglycemia" rather than "hyperglycemia"

Response:
We apologized for the error, and have corrected the spell in the new revision.

3) Please clarify in the introduction section weather low or high red cell indices are associated with MetS and its components. Though the word "enhanced" (second last sentence of the first introduction page) provides some idea, readers still needs clear sentence.
Response:
Thanks for your valuable suggestions. We have rewritten the sentence to “These cross-sectional studies showed that elevated RBC was associated with MetS in Taiwan [2], Israel [3], Korea [4], Japan [5, 6], Hb in Thailand [7] and Japan [6], HCT in Thailand [7] and Japan [6, 8].” (See page 3 line 4-7 in the manuscript).

4) Authors have used Chinese Medical Association definition of MetS. The cut-off Value of HDL-C for both sex is similar as per that definition unlike many other definitions provided. I strongly suggest authors to use IDF definition with waist circumference specific to your population for the standard comparison of your findings with other international findings. The first reference cited could also be used.

Response:
Thanks for your instructions. Unfortunately, in our routine health check-up database, only BMI was used to measure obesity and waist circumference was not measured. Therefore, we could only use the Chinese Medical Association definition of MetS, which is very popular for Chinese population in clinical practice. For your comments, we have added the limitation in discussion (See the section of Discussion paragraph 5, line 3-5).

5) The heart and soul of the results lie on GGE model. Therefore I strongly suggest authors to clarify the significance and theoretical concept behind this model in few lines in statistical analysis paragraph. As far as I know, many other manuscript uses multivariate linear regression analysis to study the independent relationship between two parameters. This test along with logistic regression analysis could be used to associate Mets and its components with increases red cell indices.

Response:
Thanks for your valuable suggestions. We have provide further information about the GEE model in the “materials and methods” for clarifying the model.

Statistically, Multivariate linear regression and logistic regression were not suitable for handling longitudinal repeated measurements data with
auto-correlation, while GEE model can overcome the problem. As our database was the typical longitudinal repeated measurements data, the GEE model was used to detect the association between the erythrocyte parameters and Mets. For more information, please see the “materials and methods” (See the section of “materials and methods” line 13-16).

6) Do all the supplementary table going to be published along with the manuscript. Authors have referred to s1 to s4 in the manuscript. Make sure this tables are published along with this manuscript or this may create confusion.

Response:

Thanks for your suggestions. Taking account to the limited space, we hope to illustrate the table s1-s19 in the supplementary materials online.

7) In the second last sentence of the result, authors have used the term “dose-response existed”. Please clarify this.

Response:

Thanks for pointing this out. The sentence is “Furthermore, for obesity, hypertension and dyslipidemia, all three erythrocyte parameters showed significant association and a dose-response existed”. There “dose-response existed” refers to that a trend of increase of RR was observed from Q2 to Q4 (see table 3 and definition of Q1, Q2, Q3 and Q4 in the section of “materials and methods” line 7-9).

8) In the discussion section, authors has discussed three concepts

a) IR and erythropoiesis

b) Hct and Whole blood viscosity and

c) Hb and Nitric oxide.

Authors are requested to further clarify the concept of NO and Hb in the discussion. Authors has used the word “coincidentally” in this paragraph. Please put this paragraph in a clear way in context of your findings.

Response:
Thanks. We have revised the paragraph according to your concern. (Please see the section of “discussion” paragraph 3).

9) Too strong argument has been presented in the conclusion (red blood cell indices as marker for MetS risk appraisal). I strongly suggest authors to discuss how this red cell markers be better or could be used along with others various classic and new inflammatory MetS markers.

Response:

Thanks for your suggestion. We totally agree with you and have modified the conclusion.

10) I again request to improve the standard of English and re-write the first two paragraph of results in a simple way.

Response:

Thanks for the suggestions, again. Following you and the editor’s suggestion, our manuscript has been polished by a native English speaker for improving the language quality.

Reviewer #2:

Introduction

1) Author described that there has been little research on the correlation between hematological parameters and MetS. Is it true? Tabara et al. (Tabara Y, et al.: Association of hematological parameters with insulin resistance, insulin sensitivity, and asymptomatic cerebrovascular damage: The J-SHIP Toon Health Study. Clin Hemorheol Microcirc. 2012 Oct 29. [Epub ahead of print]) have described that hematocrit as an independent determinant of insulin sensitivity ($# = -0.074$, $p = 0.019$) and insulin resistance ($# = 0.115$, $p < 0.001$), but this association was lost after further adjustment for visceral fat area and plasma alanine minotransferase level. Kawamoto et al. (Kawamoto et al: Hematological Parameters are Associated with Metabolic Syndrome in Japanese Community-Dwelling Persons. Endocrine, 2013; 43:334-343. ) demonstrated that hematological parameters were positively associated with
insulin resistance and prevalence of MetS in Japanese dwelling-community persons. Author should quote these papers.

Response:

We apologized for missing the references. In our revised manuscript, we have quoted these two papers (see page 2 line 6-8 in the manuscript).

Materials and Methods:

2) In this study, the distribution of age may be 30 to 55 years. The women should be divided into two groups: pre-menopausal and menopausal. The authors should take into account: i) the use of contraceptive for pre-menopausal women, and ii) hormone replacement therapy for menopausal women.

Response:

We agreed. However, there is no menopausal information in our routine health check-up database, so the specific-menopausal analysis cannot be conducted. We claimed the limitation in our discussion (See the section of Discussion paragraph 5).

3) Please comment on how representative this group is of the target population. It is necessary in aim to understand a bias selection. Individuals with a previous diagnosis of cardiovascular disease, cerebral infarction or gastric cancer, or those who had undergone coronary artery bypass surgery, coronary stenting surgery or gastrectomy, or those who had MetS at baseline should be excluded.

Response:

Thanks for your valuable suggestions. We have excluded the individuals with about disorder based on the health check-up database in the revised manuscript, though some of their information was absence.

4) How did authors measure office blood pressure? Was the appropriate cuff size used for obese individuals? If a standard cuff size was used for large arm, the BP (blood pressure) readings can get seemingly high. Was the BP measured only one time?
Response:

Blood pressure was measured on the right arm from a sitting position following a 5-min rest based on the criterion in clinical practice. We clarified its protocol in detail in the revised paper.

5) How about the medication, especially anti-hypertensive drugs, anti-dyslipidemic drug, and anti-diabetic drug?

Response:

Thanks for your instructions. Unfortunately, the medication record was missed or absented. We, therefore, could not consider this information, as a limitation, it was discussed in the revised manuscript (See the section of Discussion paragraph 5).

6) In MetS diagnosis criteria, raised BP was defined as systolic blood pressure (SBP) \#130 mmHg and/or diastolic blood pressure (DBP) \#85 mmHg (the criteria of the National Cholesterol Education Program's Adult Treatment Panel (NCEP-ATP) \# report), and/or current treatment for hypertension. In addition, visceral obesity is defined as waist circumference. Author should describe reasons why these criteria is used.

Response:

Thanks for your instructions. Unfortunately, in our routine health check-up database, only BMI was used to measure obesity, and waist circumference was not measured. Therefore we could only use the Chinese Medical Association definition of MetS, which is very popular for Chinese population in clinical practice. For your comments, we have added the limitation in discussion (See the section of Discussion paragraph 5).

7) Authors should describe histories of smoking status and alcoholic status (e.g., non, past, current) in detail in Method section.

Response:

Again, you made a critical point. But in the database, only current smoking status and alcoholic status were recorded (See the section of “Study population” line 2-4).
8) How was blood collecting performed? Were these dates measured under a fasting condition?

**Response:**

We have added the protocol of blood sample collection and test in the revised manuscript (See the section of “Study population” line 6-10).

9) In all analyses, parameters with non-normal distributions (glucose, triglycerides, and GGT) should be used after log-transformation.

**Response:**

Based on law of large numbers, when the sample size is large enough (for example, n>100), normal distribution is usually not needed in t-test, while for GEE model the normal distributions of independent variable is not required (see the 11th-14th references).

10) The author's policy for selection of confounding factors in the multivariate analysis is not well described.

**Response:**

Thanks for your instructions. Simple GEE model was first used to select factors associated with MetS, and multiple GEE model was further adopted to detect the association between erythrocyte parameters and MetS/its single components. Variables which were selected at the significant level of 0.05 (α) in the simple GEE analysis entered the multiple GEE model to adjust the potential confounding. We have rewritten this paragraph (See the section of “Statistical analysis”).

11) I cannot understand GEE model in the analysis of Method section. Logistic regression analyses were used to test significant determinants of MetS, and its components, serving as the dichotomous outcome variable. I cannot understand the handling of missing data.

**Response:**

The GEE model is a general liner regression model, many traditional model, such as logistical model, can be used in the framework of the general liner model by link function, and it was showed in the revised manuscript, for further information please see the 11th-14th references.
The imputation was performed for the missing data by MI Procedure in SAS 9.1.3. More detailed information can be found in the book “Multiple imputation: a primer Stat Methods” by Schafer J.

Results

12) Author should describe the prevalence of subjects with MetS in baseline. In cohort study, subjects with Mets in baseline should be deleted in the analyses.

Response:

In baseline, all the participants with MetS and its single component had already been excluded in our cohort for highlighting the association between erythrocyte parameters and MetS/its single component by follow-up.

13) Author should describe prevalence as categorical variables (e.g., sex smoking, alcohol, exercise, sleep, and diet). The differences among the groups should be analyzed by student’s t-test for continuous variables or the Χ²-test for categorical variables.

Response:

Sorry for the mistake. We re-tested the difference between the groups using the chi-squared test for categorical variables in the revised manuscript Table 1.

14) The author should describe the prevalence of subjects with antihypertensive, Anti-dyslipidemic, and anti-diabetic medication also in Table 1.

Response:

According to your instruction, we have calculated the available prevalence in the revision. But we cannot get this one as the medication information is absence.

15) The author should describe the prevalence of subjects with cardiovascular disease also in Table 1.
Response:

It was calculated in revised manuscript (see page 5 line 6-8 in the manuscript).

16) Author should describe prevalence of Mets and its components in other Table.

Response:

It was also added in the revision (see Table S20 in the manuscript).

17) In Table 2, multivariate adjustment should be performed in a model including the presence of antihypertensive, anti-lipidemic, and anti-diabetic medication as well as known conventional risk factors for MetS.

Response:

Again, we are sorry that we cannot adjust them since the medication information is absence.

18) Author should indicate each value under three-digit number (eg., RR, 1.235 (95% CI, 0.253-1.234); P<0.001)

Response:

Thanks for your advice. We have revised it.

Discussion

19) Some limitations of this study must be considered. First, hematological parameter categories are based on a single assessment of blood, which may introduce a misclassification bias. Second, authors also could not eliminate the possible effect of medication (i.e., anti-hypertensive, anti-dyslipidemic, and anti-diabetic medication) and previous disease (e.g., cardiovascular disease) on the present findings. Therefore the demographics and referral source may limit generalizability. Third, as authors described, the participants are not general population.

Response:
Thanks for your instructions. We have illustrated and discussed all the limitations based on your suggestion (See the section of Discussion paragraph 5).