Author’s response to reviews

**Title:** The role of haematological indices in predicting early iron deficiency among pregnant women in an urban area of Sri Lanka

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**Version:** 1  **Date:** 04 Oct 2018

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Title: The role of haematological indices in predicting early iron deficiency in Sri Lankan pregnant women

Manuscript Number: (BHEM-D-18-00018).

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**Version 2:**  **Date:** 4 October 2018
Response letter to Reviewer 1- Philip Crispin

Dear Sir,

I am very much thankful for your constructive remarks, which are meant to improve the manuscript “The role of haematological indices in predicting early iron deficiency in Sri Lankan pregnant women” (BHEM-D-18-00018). The amendments were done in the manuscript according to the comments given by both reviewers and are indicated in the text using track changes.

I have addressed (in italic letters) each of your concerns point by point as outlined below table.

Editorial Remarks 1

The major issue with the paper is the set points for each of the individual indices, which are presumably based on non-pregnant levels. It is unclear whether the a priori cut offs are suitable for this analyser, or for pregnancy.

Response - Based on your valuable remarks, a more comprehensive literature search has been conducted. Confirmed that there is no defined cut-offs for the red cell indices during pregnancy.

Discussions have been carried out with the statistical advisor of the project. Based on your valuable key remarks (highlighted later) we have compared the ROC curves to determine the optimal cutoff values for detecting early iron deficiency in at early stage of pregnancy.

Therefore the tables 2 and 3 were removed in which the prior cutoffs were used to show the number of women who are below the cutoffs and new tables 2 and 3 were included in the revised manuscript.

Further, the figure-01 was removed and included new figure –1 with ROC curves of Haemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular haemogolbin (MCH) and Mean corpuscular haemogolbin concentration (MCHC) in the prediction of iron deficiency.

According to the new tables and the figure the results and the discussion parts were revised and amendments were indicated using track changes.

Location in the text

Methods section, line 12-18, page 9, Results section- line 8-14, Page 12, Please refer Table 2 , line 2-6, Page 12, Refer Table 3, line 1-4, Page 13, Refer Figure 1, attached as supplementary file. Title and the legend of the figure 1 is in line 17-22, Page 25 according to guidelines. Discussion section – Line 4-23, Page 15, Line 1-2, Page 16
Editorial Remarks 2

But can't be determined as the reference (22) appears to be to a book review (although I can't find this review at JAMA on line).

Response - I apologize for the inconvenience caused. The reference (22) is a book review. Based on your valuable remarks I did not used prior cut-off values that are not specific for pregnancy. Therefore, the reference was removed from the reference list.

Location in the text

Reference section, Page 23

Editorial Remarks 3

The results show fairly similar ROC AUCs for MCV, MCH and MCHC, however marked differences in the sensitivity and specificity at the a priori cut-offs despite acknowledging that MCV increases in pregnancy.

The lower sensitivity and higher specificity suggest that the cut-off of 80fL for MCV might be too low. An optimum MCV cut-off based on the ROC is not stated. The stated MCHC optimum is very close to the a priori cut-off.

Do these results therefore suggest the MCV range should be changed in pregnancy for improved detection of iron deficiency rather than the MCHC being vastly superior? Is the mildly better ROC AUC significant?

Response - As mentioned in the first response above, the ROC curves were constructed for the indices (Hb, MCV, MCH and MCHC) which have significant association with serum ferritin. Significant area under the ROC curves were obtained for these indices in the prediction of iron deficiency. Using the sensitivity and the specificity obtained via ROC curves the Youden’s Index [sensitivity+ (specificity -1)] was calculated for each indices. The optimal cut-off values for red cell indices in the prediction of early iron deficiency were selected based on the high value of Youden’s Index.

Location in the text

Please refer Table 2, line 2-6, Page 12, Refer Table 3, line 1-4, Page 13, Refer Figure 1, attached as supplementary file. Title and the legend of the figure 1 is in line 17-24, Page 25 according to guidelines, Discussion section, line 15-23, Page 15

Editorial Remarks 4
It would be useful to compare the ROC curves directly and determine from the data rather than from standard normal ranges the ideal MCV for iron deficiency detection.

Response- I completely agree with your valuable concerns and constructed the ROC curves for the indices which have significant association with serum ferritin. The result and discussion parts were revised accordingly. Please find the amendments indicated using track changes.

Location in the text

Please refer the above locations and the track changes in the manuscript

Editorial remarks

(Minor Issues)

Minor remarks 1

There could be improved clarity between iron deficiency and iron deficiency anaemia, for example on page 7, the validity of haemoglobin for detection of "iron deficiency anaemia" is questioned. This may be reasonable for iron deficiency, but iron deficiency anaemia would require a low haemoglobin

Response - I apologize for the earlier misperception with iron deficiency and iron deficiency anaemia which may not have been clear enough. Now the clarity for iron deficiency and iron deficiency anaemia was checked thoroughly in the entire manuscript and have been made the changes or re-phrased to improve clarity. Please find the amendments indicated using track changes.

Location in the text

Please refer the track changes in the manuscript

Minor remarks 2

Page 5, L40 the authors assert that functional consequences of iron deficiency are not apparent in the early stages, which is contrary to the later well-developed argument that early iron deficiency without anaemia may have an impact on foetal outcomes. I presume this assertion relates to the effect of anaemia only.

Response
I would like to bring to your kind notice that we have mention the functional consequences of iron deficiency considering the pregnant women. At early stages when they have iron deficiency it not shows any apparent or obvious changes or clinical symptoms in pregnant women. However, Maternal iron deficiency at early stages without anemia have health consequences for the fetus such as, long term effect in the brain development which were discussed later. However, the remarks clearly showed that the reader cannot capture the actual meaning of what we have written. Therefore, we have removed the sentence to make the reader more comfortable and clearer. Please find the amendments indicated using track changes.

Location in the text

Background section, Line 15, Page 4

Minor remarks 3

At L46 "severe" is defined as 2SD below the mean healthy population. This definition is unreferenced and is problematic as ferritin is not normally distributed and a "healthy" population of women includes a high proportion with iron deficiency both in developed and emerging economies.

Response

I atone for not to include the reference for the definition. The definition was taken from the WHO report (reference iron deficiency anaemia. Assessment, Prevention and Control, 2001). Although it is a definition of WHO, I completely agree with you concerns and rephrased the definition.

Location in the text

Background section, Line 16-17, Page 4

Minor remarks 4

It is implied that UK guidelines recommend screening with ferritin, whereas the guidelines (Pavord 2012) recommend ferritin in early pregnancy for high risk women only. This is relevant as there is a reluctance even in high resource settings to undertake universal ferritin screening in pregnancy despite increasing understanding of the relevance of iron deficiency and high prevalence. This increases the relevance of the study.

Response

Discussed the valuable points.
The relevance of marital status with respect to iron deficiency is unclear (Table 1). If there are cultural reasons why this association was suspected they should be stated.

Response

Based on the valuable remarks, as there is no relevance of marital status with iron deficiency and no specific cultural association the variable was removed from the table.

Please see the track changes in the table.

The reference formatting needs to be thoroughly checked - for example, the use of month for book chapter, first author only, follow by et al, is not consistent with journal guidance. Reference 22 also appears to be incorrect.

Response

I have formatted the reference based on your valuable suggestions. The reference 22 was removed from the reference list.

The article would benefit from assistance with English.

Response
English editing has been done by the professional and track changes have been made for indication.

Location in the text

Please refer the track changes

Minor remarks 8

There are occasional changes in form also suggested, for example P8, L21 Celltac E (Nihon Kohden Corp. Tokyo).

Response

Included the correction. Please refer the track changes

Location in the text

Method section, line 7-8, page 8

Response letter to Reviewer 2

Dear Sir/ Madam,

I am very much thankful for your constructive remarks, which are meant to improve the manuscript "The role of haematological indices in predicting early iron deficiency in Sri Lankan pregnant women" (BHEM-D-18-00018). The amendments were done in the manuscript according to the comments given by both reviewers and are indicated in the text using track changes.

I have addressed (in italic letters) each of your concerns point by point as outlined below table.

Editorial Remarks 1

The figure needs a legend and explanation.

Response - Based on the remarks by reviewer 1 the results were reanalyzed and new figure was added. The legend and the explanation was entered at the end of the manuscript (based on the journal guideline). Please refer the track changes in the figure 01
Refer Figure 1, attached separately. Title and the legend of the figure 1 is in line 17-22, Page 25 according to guidelines

Editorial Remarks 2

At the end of the discussion, the authors state that MCHC had the highest sensitivity, specificity and accuracy, when in fact the specificity was the lowest.

Response- I apologize for the earlier misperception. Based on the remarks given by the reviewer 1, the results and discussion parts were revised. Constructed ROC curves and defined optimal cut-off values for red cell indices with high sensitivity while maintain effective specificity. Please refer the track changes in the result, discussion and conclusion part.

Methods section, line 12-18, page 9; Results section- line 8-14; Page 12, Please refer Table 2, line 2-6, Page 12, Refer Table 3, line 1-4, Page 13, Discussion section – Line 4-23, Page 15, Line 1-2, Page 16

Editorial Remarks 3

The capitalisation in the references needs to be consistent.

Response - I have formatted the reference based on your valuable suggestions. Please refer the track changes

Reference section- Page 19-25

Editorial Remarks 4

The document needs to be checked carefully for grammar. There are occasional mistakes, for example, "was" instead of "were" in line 10 of the abstract.

Response- English editing has been done by the professional and track changes have been made for indication. Please refer the track changes

Please refer the track changes.
Editorial Remarks 5

It would be worth validating this study with larger numbers.

Response - The remark was discussed in the conclusion section

Location in the text- Conclusion section. Line 11-13, Page 16