Author's response to reviews

Title: Inverse correlation between serum interleukin-6 and iron levels among Japanese adults: a cross-sectional study

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Author's response to reviews: see over
Dear Dr O'Donovan,

Please find attached a revised version of our manuscript “Inverse correlation between serum interleukin-6 and iron levels among Japanese adults”, which we would like to resubmit for publication as a Research Article in *BMC Hematology*.

The comments from the reviewer were highly insightful and enabled us to greatly improve the quality of our manuscript. In the following pages, we have summarized point-by-point responses to each of the comments from the reviewer.

Revisions in the text are shown using green highlight for additions, and strikethrough (*example*) for deletions. We hope that the revisions in the manuscript will be sufficient to make our manuscript suitable for publication in *BMC Hematology*.

We look forward to hearing from you at your earliest convenience.

Yours sincerely,

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Responses to the comments of reviewer Kishor B Raja
Comments to the author:

MAJOR:

1. My major issue however remains the time of bleeding. The authors have indicated the morning but no actual time frame provided. As the samples from the 280 volunteers were collected over a 5y period (2005-10), there is likely to be discrepancies in the timing, unless the authors have a record of the actual times. Furthermore, how soon was the serum separated, and were 'trace-element free' vacutainers used (to avoid contamination)?

Response: We thank the reviewer for the pointing out this issue. The subjects were those who visited an urban clinic in Nagoya, Japan, for Helicobacter pylori infection test. Because the clinic was open to them only in the morning with not the actual time frame setting and they were not request to fast, we did not have the information on exact time of bleeding as well as whether blood samples were fasted ones or not. It is sure that we collected the data in the morning. Because the serum iron levels were obtained in a different time in the morning, the association might be weaker than the one in an ideal setting. We added the point as a limitation into the discussion part. The serum was separated soon after bleeding. A plastic vacationer was used for the bleeding and temporarily stored in a freezer, and subsequently trace-element free vacationer was used to avoid contamination. Therefore, it was thought that the contamination could not be significant.

We have revised the part as a limitation in the discussion section (page 15, lines1-2).

Before Revision: A limitation of our study is that there was no information of dietary intake that might have affected serum iron levels.

After Revision: A limitation of our study is that there was no information of dietary intake and of the actual time of bleeding from the subjects; both might have affected serum iron levels.
MINOR:

1. Is the 7.4% value correct (line 13 pg 8)? compare with table 1? OR did 2 women have values between 48 and 50?

Response: Thank you so much for your pointing out. The reference range for serum iron was 54–200 µg/dL in men and 48–154 µg/dl in women. It is correct because 7.4% of women (13/175) were with 48 < µg/dl of serum iron level. As you have pointed out, the 2 women having between 48 – 50 µg/dl were not included in the 7.4% value.

2. Line 9 (pg 9) values significant at p=0.073 in men?.

Response: We thank you for the comment. In men, p-value in the analysis was 0.073 and not statistically significant (not p-value < 0.05). Because, as you have pointed out, it might be confusing, we have revised the part as follows (page 9, lines 7-10).

Before Revision: However, this did not reach statistical significance in each sex; it was marginally significant in men (β=-0.059, p=0.073) and not significant in women (β=-0.048, p=0.17).

After Revision: However, this did not reach statistical significance in each sex; β=-0.059 in men (p=0.073) and β=-0.048 in women (p=0.17).

3. Did the authors try comparing log IL-6 with log TIBC/TfSat? The reduction in TSAT with increasing IL6 is not surprising as serum Fe if being reduced.

Response: We appreciate your comment. Because values of serum TIBC levels and TSAT (%) showed a near-normal distribution, the raw values were used in the analysis. The association of logarithms of serum IL-6 levels with serum TIBC levels as well as TSAT were examined using the Pearson correlation coefficient. Serum TIBC levels and TSAT (%) were not correlated with the logarithm of IL-6 levels overall (r=-0.106, p=0.16 in TIBC level and r=-0.036, p=0.55 in TSAT). Regression analysis adjusted for sex and age showed that a lower TSAT (%) was significantly associated with a higher logarithm of serum IL-6 levels in all subjects (β=-3.31, p=0.040). As you have pointed out, TSAT (%) was calculated as serum iron ÷ TIBC × 100, and the reduction in TSAT with the higher log IL-6 levels is reasonable.
1. The authors have highlighted the hypoferrinaemia in their previous study (pg14). This is somewhat surprising as ferritin is a positive acute phase protein and is expected to rise in inflammation.

Response: We thank the reviewer for the pointing out. Ferritin is a positive acute phase protein in response to inflammation. H. pylori infection causes gastric inflammation. Our previous study has reported that serum ferritin levels were significantly lower in H. pylori-infected subjects than in uninfected subjects. Several mechanisms have been proposed to include chronic occult gastrointestinal blood loss, enhanced bacterial iron uptake, and reduced intestinal absorption of dietary iron. Therefore, although ferritin level is expected to rise in inflammation, these factors could affect serum ferritin levels more strongly and result in lower levels.