Author’s response to reviews

Title: Serum C-reactive protein and thioredoxin levels in subjects with mildly reduced glomerular filtration rate

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Version: 2 Date: 17 December 2009

Author’s response to reviews: see over
Dear Editor,

We are happy to receive the comments by the three reviewers on our manuscript, and the comments have been helpful in improving the manuscript. We have tried to address all the comments, and made several changes in the manuscript as indicated below. We hope that the revision is satisfactory and the revised manuscript is good enough to be accepted for publication in BMC Nephrology.

With best regards,

Tetsuo Shoji, MD, PhD.
To the comments by Reviewer #1

1. In the revised manuscript, we present a chart to show how the subjects were recruited and the number of subjects who refused (See Changes #3, #11 in the list of changes below).

2. We understand that this reviewer did not request additional measurement of albuminuria in the present study. No revision has been made in response to this comment.

To the comments by Reviewer #2

Thank you for your detailed comments. Before we respond to the comments, we would like to clarify the following two points. First, we did not test a precise etiological hypothesis that CRP and TRX are involved in the pathogenesis of mildly reduced GFR. Because of the cross-sectional design of this study, we examined only the association between GFR and these biomarkers, and also the association does not necessarily indicate causality, as stated in the Discussion section. Second, although the reviewer claimed that we performed only bivariate analysis, the original manuscript presented the results by multiple regression analysis in Tables 3 and 4. We are afraid that the reviewer did not look at these tables.

1. Causal pathway and use of multivariate models: We do agree with the comment that a ‘confounder’ should be distinguished from an ‘intermediate’, the latter being in the causal pathway between an exposure and an outcome. We showed significant univariate (or bivariate) associations among eGFR, CRP, and TRX. These factors showed significant correlation with many variables including age, sex, BMI, SBP, etc. These can be confounders. Since inflammation and oxidative stress are difficult to clearly separate from each other, one of the two (CRP or TRX) can be an intermediate in the relationship between the other (TRX or CRP) and GFR. Therefore, simultaneous inclusion of the two factors would not give reasonable results. Therefore, we chose to separately analyze the association between eGFR and CRP (Table 3), and the association between eGFR and TRX (Table 4), then we entered TRX and CRP, respectively, as an covariate in the 9th model in each set of analysis. As expected, the simultaneous inclusion of TRX and CRP did not give significant results. Therefore, we believe that our way of analyzing the current data using multivariate models is standard and acceptable. Because the discussion is based on the results by multiple regression analysis, we did not make revisions in response to this comment.

2. Problems in the use of English language, in particular the word ‘changes’: As pointed out by the reviewer, our study is not a longitudinal but a cross-sectional one in which the word ‘changes’ is
not appropriate. According to the advice, we have made several changes in the revised manuscript. Because no native English user was available, we asked our co-authors who are good at English for assistance (Changes #1, #2, #5, #7).

3. Regression lines in Figure 1 and Figure 2: Regression lines should be provided when linear regression model (Pearson’s) is applied. However, we analyzed the correlation using nonparametric Spearman’s rank correlation method. We asked two experts in statistics in our institution for suggestions, and both persons advised us not to draw a line in this case. Then, we decide not to draw regression lines.

To the comments by Reviewer #3

1. Recruitment of subjects and selection process: Following the advice, a chart is newly presented as Figure 1 (Changes #3, #11).

2. Detection limits for CRP and TRX: These are indicated in the revised Methods (Change #4).

3. Exactness of variables in Table 1: We have made changes as suggested (Change #8).

4. Possible use of stepwise regression models: Thank you for your advice. We did not aim at building the best model, but we wanted to examine whether the GFR-CRP and GFR-TRX associations were independent of other relevant factors. Because our purpose was achieved by using the current models, we have made no changes in response to this comment.

5. Tables 3 and 4: We have made changes as suggested (Changes #9, #10).

List of changes

<Change #1>

Original, Page 2, Abstract, Background: We examined the possible changes in serum biomarkers for inflammation and oxidative stress in subjects mildly reduced glomerular filtration rate (GFR).

Revised, Page 2, Abstract, Background: We compared the levels of serum biomarkers for inflammation and oxidative stress between subjects with normal and mildly reduced glomerular filtration rate (GFR).
Original, Page 4, 2nd paragraph: In the present study, we measured C-reactive protein (CRP) and TRX as biomarkers for inflammation and oxidative stress, respectively, to examine possible changes in these biomarkers in subjects with mildly to moderately reduced glomerular filtration rate (GFR).

Revised, Page 4, 2nd paragraph: In the present study, we measured C-reactive protein (CRP) and TRX as biomarkers for inflammation and oxidative stress, and compared them between subjects with normal and mildly reduced glomerular filtration rate (GFR).

Original, Page 4, Subjects: The subjects were screened from 241 participants of a health check-up program at the Osaka Health Promotion Center, Osaka, Japan. They gave written informed consent to take part in the study. Among the total participants, we excluded 8 subjects with reduced eGFR < 60 mL/min/1.73m² and 59 subjects taking medications for diabetes mellitus, hypertension, and/or dyslipidemia, to avoid possible influence of these medications to oxidative stress biomarkers. The remaining 182 individuals were the final subjects of this study (Table 1).

Original, Page 4, Subjects: The subjects were recruited from 264 consecutive participants of a health check-up program at the Osaka Health Promotion Center, Osaka, Japan. Twenty-three individuals refused to participate, and 241 subjects gave written informed consent to take part in the study. From the 241 people, we excluded 8 subjects with reduced eGFR < 60 mL/min/1.73m² and 51 subjects taking medications for diabetes mellitus, hypertension, and/or dyslipidemia, to avoid possible influence of these medications to oxidative stress biomarkers. The remaining 182 individuals were the final subjects of this study (Figure 1). Table 1 summarizes the characteristics of the final subjects.

Original, Page 5, Blood collection and measurements: CRP was assayed by a sensitive Latex-immunoassay (Denka Seiken, Tokyo). Serum TRX was quantified using a commercial ELISA kit for human TRX (Redox Bioscience Inc, Kyoto).

Revised, Page 5, Blood collection and measurements: CRP was assayed by a sensitive Latex-immunoassay (Denka Seiken, Tokyo) with a detection limit of 0.01 mg/dL. Serum TRX was quantified using a commercial ELISA kit for human TRX (Redox Bioscience Inc, Kyoto) with a
detection limit of 2 ng/mL.

<Change #5>

Original, Page 7, Discussion, 1st sentence: The aim of this study was to examine the possible changes in CRP and TRX among subjects with mildly reduced renal function.

Revised, Page 7, Discussion, 1st sentence: The aim of this study was to compare the levels of CRP and TRX between subjects with normal and mildly reduced renal function.

<Change #6>

In the revised manuscript, we add another limitation of this study.

Revised, Page 9, Second paragraph: Third, the subjects of this study do not represent the general population although we recruited them from the participants of a health check-up program. They included more women than men, and did not include those taking medications for the three common diseases.

<Change #7>

Original, Page 9, Conclusions: Further studies are necessary whether the observed changes in the biomarkers of inflammation and oxidative stress are predictive of occurrence of CVD.

Revised, Page 9, Conclusions: Further studies are necessary whether the observed deviations in the biomarkers of inflammation and oxidative stress are predictive of occurrence of CVD.

<Change #8>

Table 1: We corrected the exactness of male sex (37.9% to 38%), smokers (37.9% to 38%), and eGFR (85.2 to 85) as suggested.

<Change #9>

As suggested, we add a sentence to the footnote of Table 3 as follows;

The table gives beta coefficients between eGFR and CRP, and coefficients of determination ($R^2$) for whole models.
<Change #10>
As suggested, we add a sentence to the footnote of Table 4 as follows;
The table gives beta coefficients between eGFR and TRX, and coefficients of determination ($R^2$) for whole models.

<Change #11>
A chart is newly shown as Figure 1 to clarify the method of subject recruitment and selection.

<Change #12>
The revised manuscript includes two co-authors (EI, MI) who contributed during the process of revision.