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

We are happy to receive the comments by the Editor and the reviewers on our revised manuscript. We have tried to address all the comments, and made several changes in the manuscript as indicated below. We hope that this second 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 the Editor

1. The approval by the ethics committee at our institute is clearly stated in the Subjects section (Please see Change #4 in the list of changes below).
2. We have made some modifications to conform to the journal style (Change #1).
3. The changes are indicated in red.

To the comments by Reviewer #2

Thank you for your comment to construct two multiple regression model simultaneously including all potential confounders. We performed the suggested analysis, and the results are given in Table 6 of the re-revised manuscript (Change #12). The results by these models indicate that TRX was associated with eGFR (P=0.06), sex (P=0.02), and SBP (P=0.08), and that CRP was associated with SBP (P=0.03), non-HDL-C (P=0.03), and HDL (P=0.01), but not with eGFR (P=0.16). It may be that this study was not powered enough to detect the direct association of eGFR with CRP or TRX, because the small variation in eGFR in this study. However, we interpret the results to indicate that mildly reduced eGFR was associated with increased TRX and CRP, at least in part, via the raised SBP and non-HDL-C and lowered HDL-C in such condition. To provide data for this discussion, we present Table 3 in the re-revised manuscript showing the correlations of eGFR with SBP, non-HDL-C, HDL-C, and other clinical variables (Changes #5, #11). To describe and discuss the newly performed results, we have made several modifications in this revision (Changes #2, #3, #5, #6, #7, #9, #10). We appreciate the reviewer’s suggestion that has improved our analysis.

List of changes

<Change #1>
Title page is modified to conform to the journal style.

<Change #2>
The results part of Abstract is modified.
R1: Even after further adjustment for other confounders, eGFR was significantly associated with TRX.
R2: When adjustment was done for eight possible confounders, CRP showed significant association with systolic blood pressure, high density lipoprotein cholesterol (HDL-C) and non-HDL-C, whereas TRX was associated with sex significantly, and with eGFR and systolic blood pressure at borderline significance.
The conclusions part of the Abstract is modified.

R1: Mildly reduced eGFR was associated with increased serum levels of CRP and TRX independent of age and sex, suggesting that inflammation and increased oxidative stress are present in subjects with only slight decline of renal function.

R2: We showed the increased levels of CRP and TRX in subjects with mildly reduced eGFR. The eGFR-CRP link and the eGFR-TRX link appeared to be mediated, at least partly, by the alterations in blood pressure and plasma lipids in these subjects.

We add the description about ethics to the last part of Subjects.

R1, Page 4: This study was carried out in compliance with the Helsinki Declaration, and approved by the ethics committee at Osaka City University Graduate School of Medical School.

We include the univariate correlations between eGFR and other clinical variables in this revision.

R2, Page 5: Correlations between eGFR and other clinical variables
As shown in Table 3, eGFR was significantly associated with age, sex, BMI, systolic BP, non-HDL-C, HDL-C, and smoking status, but not with glucose level.

Simultaneous adjustment is done and included in this version of manuscript.

R2, Page 7: Multiple regression models to simultaneously adjust for potential confounders
To further investigate the eGFR-CRP and the eGFR-TRX links, we included all potential confounders simultaneously in multiple regression models (Table 6). CRP showed significant and independent associations with systolic BP (positively), non-HDL-C (positively), and HDL-C (inversely), but not with eGFR (P=0.16). TRX showed a significant association with male sex, and trend of association with eGFR (P=0.06) and systolic BP (P=0.08) at borderline significance.

The first paragraph of Discussion is modified based on the new analyses.

R1: The aim of this study was to compare the levels of CRP and TRX between subjects with normal and mildly reduced renal function. When the subjects were divided into two groups by eGFR, both CRP and TRX were higher in the subjects with mildly reduced eGFR. Also, eGFR showed significant inverse correlations with CRP and TRX in the total subjects. The inverse associations of eGFR with CRP and TRX remained significant after adjustment for age and sex. These results demonstrate that mildly reduced eGFR was closely associated with increase in CRP and TRX.
**R2:** The aim of this study was to compare the levels of CRP and TRX between subjects with normal and mildly reduced renal function. When the subjects were divided into two groups by eGFR, both CRP and TRX were higher in the subjects with mildly reduced eGFR. Also, eGFR showed significant inverse correlations with CRP and TRX in the total subjects. The inverse associations of eGFR with CRP and TRX remained significant after adjustment for age and sex. When further adjustment was done for 6 additional possible confounders, the inverse associations of eGFR with CRP and TRX became less significant. In such models, CRP was independently associated with systolic BP, non-HDL-C, and HDL-C levels. Also, TRX was associated with sex significantly, and with eGFR and systolic BP at border significance. These results suggest that the increased levels of CRP and TRX in subjects with mildly reduced eGFR were mediated, at least partly, by alterations in blood pressure and lipid levels in mildly decreased kidney function.

<Change #8>

We corrected our careless mistake in the last part of the second paragraph of Discussion.

**R1:** The present study compared CRP and TRX levels between those with normal and mildly reduced eGFR, and showed that mild reduction in eGFR was associated with increased levels of CRP and TRX independent of age and sex using multivariate analyses in 182 subjects. These data provide further evidence supporting the notion that inflammation and oxidative stress are increased in a very early course of renal function loss.

**R2:** The present study compared CRP and TRX levels between those with normal and mildly reduced eGFR, and showed that mild reduction in eGFR was associated with increased levels of CRP and TRX in dependent of age and sex using multivariate analyses in 182 subjects. These data provide further evidence supporting the notion that inflammation and oxidative stress are increased in a very early course of renal function loss.

<Change #9>

We discuss the results of newly performed multiple regression analyses.

**R2, Page 9, 4th paragraph of Discussion:** Furthermore, the present study indicates possible contributions of blood pressure and plasma lipids to the eGFR-CRP link and the eGFR-TRX link. In the fully-adjusted models, eGFR was not significantly associated with either CRP or TRX, whereas CRP was significantly associated with systolic BP, HDL-C, and non-HDL-C levels. TRX was associated with systolic BP at borderline significance. Since both blood pressure and plasma lipids are adversely affected by impaired kidney function, and these are well known risk factors for atherosclerosis, we speculate that the increased levels of CRP and TRX in subjects with mildly reduced eGFR were mediated, at least partly, by alterations in blood pressure, plasma lipids and presumably arterial wall in such subjects.
<Change #10>
The Conclusions section is modified.

**R1, Page 9, Conclusions:** In conclusion, mild reduction in eGFR was closely associated with increased levels of biomarkers for inflammation and oxidative stress.

**R2, Page 10, Conclusions:** In conclusion, subjects with mildly decreased eGFR showed increased levels of biomarkers for inflammation and oxidative stress.

<Change #11>
We add Table 3 to show the univariate correlation of eGFR with various clinical variables, particularly systolic BP, HDL-C and non-HDL-C, in this revision.

<Change #12>
We add Table 6 to show the results of fully-adjusted multiple regression analysis in this revision.

<Change #13>
Following revision, the numbering of Tables and Figures are changed.