Author's response to reviews

Title: Hypoxia and oxidative stress markers in pediatric patients undergoing hemodialysis: cross section study

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Version: 2 Date: 27 June 2012

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

Thanks for your vulnerable comments on my manuscript No.: 1188571067630111; Title: Hypoxia and oxidative stress markers in hemodialysis pediatric patients with chronic kidney diseases: a prospective study. Here are the answers of review queries.

Reviewer's report (1)

Title: Hypoxia and oxidative stress markers in hemodialysis pediatric patients with chronic kidney diseases: a prospective study

Version: 1 Date: 16 December 2011
Reviewer: Yuichi Makino

Reviewer's report:

Major concern

1) **Reviewer:** Advantages of assessing oxygenation status by the means the authors performed in this study is unclear. What is better than simply measuring, for example PO2 and 8-OHdG, in plasma (or urine).

   **Answer:** PO2 was measured and we correlated our biomarkers to it. However, PO2 is not a direct marker of cellular hypoxia – our target, but rather to systemic hypoxia in a very narrow instantaneous temporal manner. Moreover, out biomarkers are related to cellular metabolic activity that relates to local cellular viability in this disease and would be more tissue specific than PO2 and reflect a long-run hypoxic status, cellular dysfunction, tissue damage and pathophysiological state.

2) **Reviewer:** Measuring plasma HIF-1alpha level is not convincing. HIF-1alpha does not have signal peptide sequence to come out of the cells. What is the source of measured HIF-1alpha in plasma and what is a physiological relevance of such HIF-1alpha. They may reflect nothing correlated with hypoxia.

   **Answer:** Several transcription factors including intracellular receptors were detected in the plasma without having a targeting signal peptide sequence. Most famous in this regard was p53 (mutant and wild types) - like others, we had measured it in human plasma tens of years ago. Our worldwide first-time vision was based on the known
induction of the HIF-1α in tissues subjected to hypoxia and hence was very possible to be detected in the plasma. The used antibodies were designed to detect the factor and were highly specific – did not cross react with its HIF-2α isoform. Using same antibodies, western blot analysis highly confirmed the specificity and WB analysis using different set of specific antibodies gave results correlating the ELISA level, and, pooled samples and HIF-1α spiked samples showed recovery that was 102%. Moreover, it is established that hypoxia-induced damage in several models is accompanied with leakage of cytoplasmic proteins larger than our factor.

3) **Reviewer:** Why PO2 in patients is significantly higher than healthy controls? This would meet serious conflict with the conclusion that hypoxic marker is higher in the patient. This would also suggest physiological inappropriateness of the authors methods to assess the oxygen status.

**Answer:** As compensatory mechanism to metabolic acidosis. Our aim cellular hypoxia and PO2 is not a direct marker of cellular hypoxia

**Reviewer's report (2)**

**Title:** Hypoxia and oxidative stress markers in hemodialysis pediatric patients with chronic kidney diseases: a prospective study

**Version:** 1  **Date:** 21 May 2012

**Reviewer:** Ludmila Buravkova

**Reviewer's report:**

Major Revisions

1. **Reviewer:** The level of HIF-1 in plasma was estimated as one of hypoxic markers. However, the mechanism of HIF induction is very complex and its expression is transitory. Thus, the value of HIF-1 in plasma as hypoxic marker is debate. Authors should be very careful during interpretation of clinical data. Please, find and compare (with your data) the results of HIF-1 measurement in blood of healthy human or patients.

**Answer:** This is completely true, we were very cautious and in fact we were surprised by the positive results that is most probably specific – as explained in point 2 of Reviewer
1. We enrolled healthy controls in this study and we already measured this factor in the plasma of diabetics, cancer, thalassemic, obese with and without fatty liver, and, age-dependently as a senescence biomarker – using age-matching healthy controls for each (data submitted for consideration and/or in-preparation for publication).

2. **Reviewer:** Table 2. The differences between min and max values are very big. It demonstrates significant individual differences even in the control group. Min-Max interval and even mean values practically of all estimated markers of healthy subjects and patients are very close. Lots of test subjects made possible to find statistical significant differences between controls and patients. But authors should analyze the data more deeply taking into account age, sex, the causes of CKD etc.

**Answer:** It is true, however, patients and control participants were homogenous for such factors and subdividing both according to sex did not reveal any significant data. Perhaps, our data needs to be confirmed on larger groups of CKD that could be subdivided according to several factors including the disease causation and at several labs. The number of patients we used was the whole group regularly following up at our hemodialysis unit.

**Minor Revisions**

3. **Reviewer:**
   Table (1); It will be useful to see and analyze the characteristic of patient (biochemical data) after dialysis.
   Table 2. Please check TAC in Controls.
   The ref 2 and 33 could be exclude.

**Author:**
- Unfortunately we did not measured data after dialysis.
- TAC in control SD was wrong and it is corrected and highlighted
- References 2 & 3 are excluded.

4. **Reviewer:** I think that the results presented in manuscript should be discussed more carefully using some recent data. For example:


These articles confirmed the induction of HIF-1α and its possible pathogenic implication in CKD - although this did not reflect on EPO level due to accumulation of specific uremic toxins. All of them were considered in the introduction and discussion.

**Reviewer's report (3)**

**Title:** Hypoxia and oxidative stress markers in hemodialysis pediatric patients with chronic kidney diseases: a prospective study

**Version:** 1  **Date:** 10 June 2012

**Reviewer:** Aydin Ece

**Reviewer's report:**

- The title can be as “Hypoxia and oxidative stress markers in hemodialysis pediatric patients” or “Hypoxia and oxidative stress markers in pediatric patients undergoing hemodialysis” since this study is not a prospective study it is a cross-sectional case-control study.

**Author:** The title is changed as reviewer recommended.
Reviewer: - English language should be reviewed and corrected considerably by a native English speaker
Author: It was corrected.

Reviewer: - ABSTRACT should be re-written so that showing a general view of the study content. For example there is no need to give correlation results of control group in results section of Abstract.
Author: It was corrected.

Reviewer: - BACKGROUND: Previously reported alterations of HIF and VEGF in kidney diseases in the literature should be summarized in Background (Introduction)
Author: It is now considered.

Reviewer: - RESULTS: This section is very short and confusing. The authors should give study results under different subheadings such as Oxidative stress alterations and HIF and VEGF changes. Although it is no good to repeat in the Text all data that shown in Tables once again, some numerical data and P values belong to important results should be given in Results section.
Author: It was added and highlighted.

Reviewer: - DISCUSSION: The discussion should be more focused and somewhat shortened. Significant correlations that found between various variables within control subjects either should be discussed or should be deleted.
Author: The correlations of the controls were deleted.

Reviewer: - TABLES: Minimum and maximum values can be deleted form Table 2.
Author: It was deleted.

Reviewer: - Limitations of the study should be given.
Author: It was added.
Please accept my regards.

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