Author's response to reviews

Title: Effect of Glycemic Control on Soluble RAGE and Oxidative Stress in Type 2 Diabetic Patients

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Version: 2 Date: 1 July 2013

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

We have revised our revised manuscript, which addresses the comments made by the referees. Modifications to the manuscript are shown as blue color. Below we have detailed our response to the points made. We hope this addresses all comments.

Sincerely,
Mohamed O. Mahmoud
Reviewer 1
Reviewer: Barry Hudson

Reviewer's report:
The authors study the relationship between glycemia and sRAGE levels in type 2 diabetic subjects. It is found that sRAGE levels are lower in diabetic compared to controls and appear to be inversely associated with sVCAM-1 levels. Whilst these results seem interesting, there are points the authors need to address:

In the current study, the authors find controls (n=20) compared to poorly controlled (n=42), but not well controlled (n=28) diabetic subjects, display differences in sRAGE levels (804, 600, and 634 pg/ml respectively). Given both the low sample number studied here, and the large variability in sRAGE levels even in healthy subjects (Brown et al (Ann Clin Biochem 2008) and Wittwer et al (Anticancer Res 2012)), the current study is most likely underpowered to detect the differences seen here. The authors need to increase sample numbers studied and perform power analysis.

Response: We agree with the reviewer that sample size of our study is small. However, sample size of many published studies that deal with sRAGE or other plasma chemistries is similar or even smaller than our study sample size e.g.

- Piarulli et al. Atherosclerosis 2013
- Chayanupatkul and Honsawek. Clin Biochem 2010
- Abdin et al. J Diabetes Complications 2010
- Hamed et al. Thromb Res 2010

The power analysis of sample sizes between the main studied groups in our study (GCD, PCD and control) indicated that the number of patients in these groups was satisfactory (power = 95%). Power calculations were done using PS Power and Sample Size Calculations Software, version 3.0.43 for MS Windows (William D. Dupont and Walton D. Vanderbilt, USA).

We have added a paragraph before conclusion section to demonstrate that a limitation of our study is the relatively small sample sizes.
Reviewer 2
Reviewer: Kateřina Kaňková

Reviewer's report:
The MS of Motawi TMK et al. “Effect of glycemic control ... in Type 2 Diabetic Patients“ describes the results of case - control study focusing on the effect of glycemic control on various routine as well as experimental parameters ascertained in well vs. poorly controlled T2DM subjects and healthy controls.

Major Compulsory Revisions:
The basic idea is not novel since sRAGE and parameters reflecting oxidative stress in diabetes are widely studied. Comparison of various parameters between study groups doesn’t constitute the study hypothesis. I am missing clearly stated research hypothesis and aims and, therefore, justification why the study was done. In the current form of the MS there are numbers of observations presented without clear pathogenic sequel. Overall MS is very busy and not transparent.

However, my major concern is the interpretation of the results. With such a large number of comparisons correction for multiple comparisons have to be performed. Although authors claim to use Bonferoni correction they still set the significance level at P<0.05!! Considering only data in Table 2 for example – 9 variables compared between 3 groups – the corrected P level should be <0.003. Following this correction, fewer significances would probably hold than shown currently and the whole Result and Discussion section would be modified. Multiple comparisons obviously stress the problem of large number of parameters being studied without clearly stated purpose.

Discussion is far too long and the reader gets soon lost. This section should be structured according to biological significance of the results – the elaborated research hypothesis would help.

Response:
- The background section has been revised to mention the study hypothesis and aims.

- The whole manuscript has been revised to remove unnecessary observations that do not belong to the aims of the study.

- We agree with the reviewer that level of significance should be corrected according to Bonferroni test. However, the use of Tukey test for multiple comparisons may be appropriate test for interpretation of our results keeping level of significance at p < 0.05 according to reference book " Pharmaceutical Statistics: practical and clinical applications, Stanford Boloton and Charles Bon, eds. 5th edition".

Also, many published clinical studies used Tukey multiple comparisons test as a satisfied test for interpretation for their results e.g.


- Discussion section has been revised to remove unnecessary data.
Minor essential revisions:

1. Abstract
- Result section is misleading – it is not clear which control group authors mean (healthy subjects or well controlled diabetics?)

Response: We mean healthy subjects. The text has been revised.

2. Background
- The statement “RAGE can be blocked by usage of soluble RAGE …” is incorrect since it implies that RAGE binds to RAGE while in fact it competes with RAGE for binding for AGEs (if that is the case with RAGE at all). The word “neutralises” would be preferable.

Response: We agree. The text has been revised.

3. Materials and Methods
- Subjects – since the level of plasma transaminases were used as one of the selection criteria it is not clear why authors compare the ALT and AST levels between the groups later on.

Response: The text has been revised. The parameters of inclusion criteria as transaminase or creatinine have been removed from comparison.

4. Results
- The reason for splitting of parameters between the Table 1 and 2 is not justified.

Response: We agree with the reviewer that there is no need to split parameters between two tables. Tables 1 and 2 have been fused into one table with removal of unnecessary parameters.

5. Discussion
- The whole section needs to be rewritten according to corrected and completed results as suggested above.
- Discussion on the relationship between sRAGE and kidney disease (proteinuria) in diabetics (the 1st paragraph) could benefit from including reference to the paper by Kankova K et al. in Arch Physiol Biochem 2008 describing the dependence of sRAGE on kidney function.
- Conclusions and description of the novelty aspects of the study should be incorporated into Discussion.

Response: Discussion has been rewritten according to biological significance of results.
- Additional reference (Kankova K et al. in Arch Physiol Biochem 2008) has been added to describe the dependence of sRAGE on kidney function.
- Conclusion and the novelty aspects of the study have been added in the last paragraph of discussion section.