Author’s response to reviews

Title: The expression of sirtuins, superoxide dismutase, and lipid peroxidation status in peripheral blood from patients with diabetes and hypothyroidism

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Author’s response to reviews:

Dear Dr. Mockridge,

Please find attached a revision of the manuscript (BEND-D-18-00312R2) entitled” The expression of sirtuins, superoxide dismutase, and lipid peroxidation status in peripheral blood from patients with diabetes and hypothyroidism”. I believe we have addressed all of the comments from the editor and reviewer 3, and have included a detailed response to the comments. All changes entire the revised manuscript have been shown by yellow highlight.

I look forward to hearing your decision relating to our revised manuscript in due course.

Yours sincerely
Dr. Samar Sultan

Technical Comments:

Editor Comments:

BMC Endocrine Disorders operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.
Author response: This has been reviewed

Reviewer reports:

Bo Yan (Reviewer 2): Acceptance is recommended for the manuscript. No further comments.

Author response: No comments to address from this reviewer

Luis Miguel Roman-Pintos, M.D.,Ms.Sc.,Ph.D.,Prof. (Reviewer 3): The present manuscript entitled "The expression of sirtuins and superoxide dismutase in peripheral blood from patients with diabetes and hypothyroidism" was submitted as a research article. The authors aimed to elucidate whether alterations on the expression levels of SIRT1, SIRT3, and SOD2 are related to the observed oxidative stress in type 1 and 2 diabetes, as well as hypothyroidism. The authors concluded that the changes in the expression of SIRT1, SIRT3, and SOD2 they found, may explain the failures of the defense mechanisms against oxidative stress in patients with diabetes and hypothyroidism.

Comments for the authors:

- Authors must be encouraged to follow the STROBE guidelines for cross-sectional studies.

Author response: We found this STROBE guideline checklist very useful

- Please undertake some linguistic editing as there are several errors in all sections of the manuscript.

Author response: The entire manuscript has been proofread and corrected.
In the Introduction section authors avoid the inclusion of TBARS as an objective of the study; however, in Methods, Results and Discussion section, the importance of this variable is evident in the text. Please include as part of the title and aim to support the information mentioned thereafter.

Author response: According to your suggestion, this has now been added to title and objective in (Background section, page 2, line 43)

- Abbreviations should be defined at their first occurrence and used thereafter, specifically glycated hemoglobin and thiobarbituric acid reactive substances. Please, revise all sections.

Author response: This has now been revised throughout the manuscript

- In the Methods section authors do not describe the setting, relevant dates, including periods of recruitment, samples processing, and data collection.

Author response: This has been described in (Methods section, page 3, line 6, 14, 20-25 and 36-35)

- Page 3 line 20. There’s no need to clarify the clinical meaning of HbA1c, since the paper is addressed for readers who are familiar with the terminology.

Author response: This has now been removed.

- The eligibility criteria are not precise, please rephrase and be more specific, e.g. define "severe disease complications" of diabetes and hypothyroidism.
Author response: This has been clarified (Methods section, page 3, line 8 and line 11) and highlighted by yellow color.

- Page 4 line 9. Authors mention a threshold of HbA1c above 7% to diagnose diabetes mellitus; however, the criteria established by the American Diabetes Association is >6.5%. Please correct.

Author response: This has now been corrected.

- Please explain how the study size was arrived at.

Author response: Patients did not match inclusion and exclusion criteria were excluded. The exclusion of subjects was achieved after clinical assessment and biochemical analysis.

- Authors do not specify if the samples were processed immediately or stored, please be specific. Also how much blood was drawned for the analysis.

Author response: Eight milliliters of peripheral blood sample were collected in two tubes (+EDTA and plain) from participants of all four study groups, after overnight fasting. The EDTA sample was divided into equal volumes for RNA extraction and HbA1c analysis. A sample of the extracted RNA was analyzed within 6 hours of collection to avoid RNA degradation. Serum from the plain tubes was separated by centrifugation at 3,000 g for 15 minutes, and split into equal volumes for measurement of biochemical analytes, such as fasting blood glucose, and for other experiments such as ELISA and TBARS. The serum was stored at -80°C until use.

This has now been added (Methods section page 3, biochemical analyses subsection, line 20-25)

- In the data analysis section, authors mention the use of parametric tests (Student’s t test); however, no formal statistical analysis evaluating data distribution is shown as to sustain the use of parametric tests (Shapiro-Wilks, Kolmogorov-Smirnov). Moreover, qualitative variables are not taken into consideration in the writing of the section.
Author response: Normally of distributions was checked by Shapiro-Wilk test, Kolmogorov-Smirnov and QQ plot. Data were compared between two groups using unpaired student’s t-test and the results were expressed as the mean ± SEM. Qualitative variables were expressed as percentages and p-values less than 0.05 were considered statistically significant. Statistical calculations were performed using GraphPad Prism Software Version 8.0 (GraphPad Software, Inc., USA).

This has now been added (Data analysis section, page 3 line 50)

- Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study.

Author response: This has been mentioned (Methods section, page 3, Subjects subsection)

- Page 5 line 2. The first phrase in the Results section is redundant being that obviously HbA1c has to be higher in patients with diabetes compared to healthy subjects.

Author response: As per your suggestion, this has now been removed.

- Page 5 lines 8,9,10. The statement is already mentioned in the Methods section (RNA extraction).

Author response: As per suggestion, this has now been removed from the results section.

- The levels for biochemical determinations and molecular markers should be written in the text and figures.

Author response: This has now been added (Results page 4, line 10, 13,15 and 17)
- In the Discussion section authors declare that "sirtruins and antioxidant enzymes might precede the development of diabetes in patients with HT." because the study was not designed to asseverate this statement. At least a prospective or retrospective study is needed to confirm this hypothesis.

Author response: This has now been removed.

- There are several confounding factors that that have to be considered before jumping into conclusions, such as duration of the diseases and HbA1c (not established for hypothyroidism in table 1), TSH and cholesterol in all groups. This is an important limitation of the study and has to be mentioned in the manuscript.

Author response: We added the duration for HT to Table 1 and as per suggestion the limitation has now been added (Discussion page 5, line 52)

- In table 1 qualitative variables must be expressed as n (%). The use of decimals has to be homologated. When p<0.001 there’s no need to report all the following decimals, p<0.001 should be used.

Author response: according to your suggestion, this has now been changed in Table 1, and p value was changed (Results section page 4, line 14 and 17), figure 2 legend, and figure 3 legend.

- Page 13 is blank, please comment if no information was missed out.

Author response: We confirm this blank page no information on this page was missed out

- Legends for figure 2 and 3 do not mention T1D group but it’s included in the figure.
Author response: This has now added to the legends of figure 2 and 3

- In figure 2, levels of SIRT3 mRNA expression are very similar for T1D and HT groups, however, the statistical significance is very different. Please double check your numbers. The same for figure 4 where levels of T2D and HT are very different but p values are the same.

Author response: We apologize for the mistake in figure 2 and figure 4, this has now been checked and corrected.

- Whether patients with HT were taking medication (thyroid hormone) is not mentioned, and this fact can alter the results. Please clarify.

Author response: All the patients with HT were on thyroxine medication. This added to p3 line 13.

In the current study, although patients with HT were on thyroxine medication, the damaging effect of HT was only partially reversible with TH replacement therapy. A previous study showed that neurocognitive functioning in HT patients remained significantly defective despite thyroxine treatment, compared with that of euthyroid controls without HT[1]. Moreover, TH therapy has been shown to augment mitochondrial oxygen consumption and oxidative stress in patients with HT [2, 3]. In line with this, our study showed that oxidative stress is evident in patients with HT after the administration of thyroxine. Therefore, long-term antioxidant strategies, such as vitamin E therapy and an antioxidant diet, are advised along with the use of this drug, to protect against any complications that might develop.

This has now been added (Discussion page 5, line 53-p6).

References
