Author’s response to reviews

Title: The Longitudinal Effect of Subclinical Hypothyroidism on Urine Microalbumin-to-Urine Creatinine Ratio in Patients with Type 2 Diabetes Mellitus

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Author’s response to reviews:

Dear Editor and Reviewers:

Thank you for your letter and the reviewers’ comments concerning our manuscript entitled “The Longitudinal Effect of Subclinical Hypothyroidism on Urine Microalbumin-to-Urine Creatinine Ratio in Patients with Type 2 Diabetes Mellitus” (ID: BEND-D-18-00384). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and made some corrections. Revised portions are marked in highlight in the paper. The main corrections in the paper and the responses to the reviewer’s comments are as follows:

Editor Darren Byrne:

Comment 1: Under the heading “Ethics approval and consent to participate” in the Declarations, please include information on the consent to participate. In addition, please give the reference numbers for the ethical approval, if available.

Response 1: Thank you very much for your kind reminder, all the authors signed the informed consent document, you can find it at ‘Request for change in authorship’. We have added numbers of Ethical approval in page 7, line 201.
Reviewer #1:

Comment 1: the sample size is small, the data is not clearly presented, and the manuscript lacks a number of relevant detail.

Response 1: The sample size is small that is really one of our flaws. But after revision of the manuscript, we retrieved the electronic medical record system again from April 2017 to April 2018, and now we have added 16 eligible patients, at the same time, the matched control group. And now we have 46 cases in the case group and 96 cases in the control group. You can find it at table 1(page 10). We have done the statistical analysis again and added the relevant details, relevant content can be seen in the methods and statistics section of the article.

Comment 2: The ΔACR is not defined in the abstract, the definition of this measure is also unclear in the methods.

Response 2: I'm very sorry I didn't define UACR accurately, this is a difference from baseline not a proportion of the baseline. In Pages2, line44-45, we adjust the definition of UACR = UACR after one year - baseline UACR, which maybe more appropriate. In Pages3, line72, UACR = UACR after one year - baseline UACR

Comment 3: The prevalence of subclinical hypothyroidism in the general population and in patients with diabetes is cited as 3-15% and 2-17% respectively but the sources for these figures are unclear. A comment (ref 1) and an original article (ref 2) are cited to support these figures but the cited papers do not contain prevalence information. Appropriate reviews with prevalence data should be cited.

Response 3: Thank reviewer for being so careful. In this paper, indirect citations are used in both References 1 and 2. In order to alleviate the concerns of reviewer, direct citations are currently used, you can find them at Ref1 to Ref 6.

Comment 4: In the methods section, why was it necessary to admit the patients overnight for the tests?

Response 4: I'm sorry that I has not express myself clearly. I'm trying to make sure that patient’s samples are fasting blood and morning urine, so as to ensure the uniformity of sampling time in the experiment.
Comment 5: The methods for urine albumin creatinine ratio or thyroid function tests are not described.

Response 5: UACR was measured from a single voided urine sample by Nephropathy Laboratory. Urinary creatinine was detected by enzymatic method(SIEMENS, Germany, BNProSpec); urinary albumin was detected by scattering turbidimetry (Abbot, America, c16000). UACR (mg/mmol) = urinary albumin (mg/L)/ urinary creatinine(mol/L). Detection of TSH, FT3 and FT4 by chemiluminescent particle immunoassay (Abbot, America, i2000SR).

Comment 6: Were the patients with subclinical hypothyroidism treated with levothyroxine?

Response 6: None of the selected patients took levothyroxine tablets, and now we added it to the inclusion criteria (page 3, line 85).

Comment 7: In the results section it states that the ΔACR was higher in SCH compared to the euthyroid group. The opposite is stated in the abstract. This should be corrected.

Response 7: I am ashamed of my carelessness, it be corrected by changing the description that it to the UACR was significantly higher in SCH group than euthyroid group (page 2, line 47).

Comment 8: Table 3. Medians and inter-quartile ranges are presented but it is unclear from the table what is being measured. The measure should be stated in the table.

Response 8: I'm sorry that I didn't make annotation clearly. I've added corresponding annotations under Table 3(page 12, line 324-325).

Comment 9: Reference 1 is repeated as reference 10.

Response 9: I'm still ashamed that I didn't check the references carefully, repeated reference has been deleted.
Reviewer #2:

Comment 1: It is better to show the BMI values in Table 1 not only the status of overweight/obesity.

Response 1: Thank the reviewer suggestion, it will be more visual, we have corrected it, you can find it at table 1.

Comment 2: The authors should discuss why in the multiple linear regression analysis, BMI also significantly associated with ΔUACR.

Response 2: After increasing the sample size, statistical analysis shows that BMI has no difference in multivariate analysis, so we did not add the discussion.

Comment 3: Related to #2, is there a collinearity between BMI and TSH values? It may affect the results of the multiple linear regression analysis.

Response 3: Thank for the reviewer’s valuable comment. We did not put them in the same analysis model in multivariate analysis, so maybe there no need to discuss it.

Comment 3: Are there any significant changes in BMI, TSH, fT3, fT4, eGFR after a year? And the association between ΔUACR and ΔBMI, ΔTSH, ΔfT3, ΔfT4, ΔeGFR?

Response 3: Statistical analysis showed that there was no difference in ΔBMI, ΔfT3, ΔfT4 and ΔeGFR between the two groups, so no further statistical analysis was carried out. As shown in the table:

<table>
<thead>
<tr>
<th></th>
<th>ΔBMI</th>
<th>ΔfT3</th>
<th>ΔfT4</th>
<th>ΔeGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann-Whitney U</td>
<td>2128.00</td>
<td>2182.50</td>
<td>2041.50</td>
<td>2059.00</td>
</tr>
<tr>
<td>Sig</td>
<td>0.73</td>
<td>0.91</td>
<td>0.47</td>
<td>0.52</td>
</tr>
</tbody>
</table>
Reviewer #3:

Comment 1: In "Abstract": The result mentioned in the abstract "the ∆UACR was significantly higher in the euthyroid group than in SCH group, as shown by univariate analysis" is inconsistent with the result mentioned in the manuscript text: "Univariate analysis: The ∆UACR in the SCH group was higher than in the euthyroid group". !!!!

Response 1: Thank the reviewers for being so careful and I am ashamed of my carelessness, and now it has been corrected to ‘the UACR was significantly higher in SCH group than euthyroid group’ (page 2, line44-45).

Comment 2: In "Materials and Methods": 1- Controlled blood sugar and controlled lipid profiles must be defined and included as inclusion criteria.

Response 2: The reviewer's suggestions will make the experiment more rigorous. However, in our study, the controlled glycemic level and blood lipid level of the two groups were basically balanced, which would not affect the results of the study.

Comment 3: 2- The uncontrolled hypertension and congestive heart failure as the causes of albuminuria should be consider in exclusion criteria.

Response 3: Thank you for your reminder that uncontrolled hypertension and congestive heart failure have been ruled out by our team in data collection, which we neglected in writing. We have added it to the exclusion criteria(page3, line88).

Comment 4: 3- There is no description about sample size determination.

Response 4: In this study, two independent sample mean tests were used to estimate the sample content. N=/(2+)/ (Q1-1+Q2-1), N:required sample size; assignment is 0.05, assignment is 0.1, /2 =1.96 ; =1.282 ; : Population mean ; : the absolute value of the mean difference; Q1=n1/n1+n2; Q2=n2/n1+n2. We calculated the required sample size of 7 cases.
Comment 5: 4- The SCH must be defined accurately. Was the diagnosis of SCH based on a single TSH measurement? What was the method for TSH measurement? Were all of the measurements done in one laboratory and same method (kit)? Have the authors considered the duration of SCH?

Response 5: Subclinical hypothyroidism was defined as persistently elevated TSH values (at least twice, at least three months apart) with FT4 levels within the reference range, exclusion of previous thyroid disease. Detection of TSH, FT3 and FT4 by chemiluminescent particle immunoassay (Abbot, America, i2000SR), testing in the Central Laboratory of our hospital. All the patients we just included who diagnosed for the first time in our hospital, and followed up for one year after diagnosis. Their medical history can be traced back.

Comment 6: In "Results": 5- Table 2 is unclear. Needs revision.

Response 6: This is Univariate analysis for UACR in patients with T2DM. I'm sorry for my unclear expression. We have made corrections.

Comment 7: 6- Table 3: Control condition of DM (Well vs. Poor) is not defined accurately in the manuscript text.

Response 7: According to the guidelines for good glycemic control, the glycosylated hemoglobin (HbA1c) level should be controlled at a level of 7 % for most patients with T2DM (Nathan DM, Buse JB, Davidson MB et al. Medical management of hyperglycaemia in type 2 diabetes mellitus: a consensus algorithm for the initiation and adjustment of therapy. DIABETOLOGIA 2009, 52(1):17 ; Chinese Geriatric Diabetes Diagnosis and Treatment Measures Expert Consensus (2018 Edition). Chinese Journal of Internal Medicine 2018, 57 (9): 626-641.)

Comment 8: 7- Did you analyze the linear correlation between serum TSH levels and urine albumin levels?

Response 8: I tried to do a linear relationship between them, but the linear relationship between them is not obvious.
Comment 9: 8- The results of this study should be compared with previous researches on association between SCH and albuminuria in type 2 DM.

Response 9: Thank you for your advice. We have compared it with previous studies, you can find it at page6, line160-163.

With kind regards,

Juan Xie