Author's response to reviews

Title: Does vitamin D play a significant role in type 2 diabetes?

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Author's response to reviews: see over
To,
Professor Timothy Shipley
Senior Executive Editor
BMC Endocrine Disorders
BioMed Central
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Sub: Manuscript resubmission

Dear Sir,
This communication is in regard to the manuscript entitled, “Does vitamin D play a significant role in type 2 diabetes?” having MS-ID 1763111112147570 was recently submitted to your esteemed journal “BMC Endocrine Disorders”.
The said manuscript was sent for revision as per reviewers comments which we have incorporated in the manuscript and highlighted with yellow paint and also answered all questions in the cover letter as follow:

**Reviewer's report 1**
**Reviewer:** Elizabeth Ramos Lopez
**Reviewer's report:**
Sheth et al. investigated the association between vitamin D levels and HbA1c and HOMA-IR in Indian patients with T2DM
Minor Essential Revisions
The authors analyzed a large group of patients and control, so that the manuscript aport fabled findings in this field at least for the Indian population.
Following points should be addressed:

1-The authors could discuss the differences between this study and studies realized in Indians-T2DM-patients living in others countries as UK, USA, etc.

**Author compliance:**
In the revised manuscript, we have now included this point in the discussion as under:

**A study performed on Indian subjects residing in New Zealand (4) has shown a significant correlation between insulin sensitivity and IR and decrease in FI with vitamin D supplementation while there was a significant negative correlation between HbA1c and vitamin D levels due to supplementation in South Asian subjects in UK (5). Though, both the studies were carried out on relatively smaller populations as compared to the one under report.**

(Section- Discussion, Page- 8, Paragraph-2, Line-11)

2-According to recent randomized controlled trials, the supplementation with VD seem to be offer little or no effects on T2DM (in others populations), this recent studies could be addressed (Elkassaby et al. 2014; Sadiya et al. 2014)

**Author Compliance:**
In the revised manuscript, we have now included this point in the discussion as under:

An Australian study by Elkassaby et al. (XXX) recently observed a transient improvement in glycemia in T2DM with oral D3 supplementation without change in either HbA1c or beta cell function and concluded that high dose D3 has a little or no therapeutic benefit. A similar study from UAE (XXX) has also reported no significant change in HbA1c levels after six month of supplementation with vitamin D3 in vitamin D-deficient obese T2DM patients of Emirati population.

(Section- Discussion, Page- 8, Paragraph-2, Line- 7)

Reviewer's report 2
Reviewer: Ambika Ashraf

Reviewer's report:
The manuscript by Sheth et al includes a large number of subjects with T2DM form western India. It is well written. I have the following comments.

The methodology used to measure 25-hydroxy vitamin D (25OHD) assay is ELISA which is inferior to LCMS/MS and hence may not be reflective of both 25hydroxy vitamin D2 and D3 which constitute the total concentration of 25- hydroxy vitamin D, which indicates vitamin D status. This has to be mentioned under the limitation section.

Author Compliance:
Appropriate changes of mentioning 25OHD instead of vitamin D3 has been made throughout the manuscript.

We have estimated serum levels of 25-hydroxyvitamin D (25OH-D2 and 25OH-D3) using commercially available ELISA kit obtained from Demeditec Diagnostics GmbH, Germany.

(Section- Materials & methods, Subsection- Biochemical investigations, Page- 5, Paragraph-1, Line-1)

Abstract:
1. Background: Instead of ‘found to be associated’, it will be better to use ‘reportedly is associated’.

Author compliance:
- This change is incorporated in the section ‘background’.

Vitamin D deficiency reportedly is associated with type 2 diabetes (T2DM).

(Section- Abstract, Subsection- Background, Page- 2, Paragraph-1, Line-1)

2. Type 2 DM is represented as T2DM and T2D. Authors need to be consistent in the abstract and throughout the manuscript.

Author compliance:
- T2D is replaced with T2DM throughout the manuscript

3. Methods: explain F1, PPBS as they are being used for the first time.

Author compliance:
- Full form of the abbreviations is given in the manuscript as it appears for the first time. In addition, the list of abbreviation is included in the manuscript. F1 is FI which stands for Fasting Insulin and PPBS is Post Prandial Blood Sugar.
- Abbreviations are used in ‘Abstract’ section due to word limit constraint.

4. Clarify ‘vitamin D3 level’- are the authors measuring 25- hydroxy vitamin D3 or 1, 25 di hydroxyl vitamin D3.
Author compliance:
- We have measured 25OHD (25-hydroxy vitamin D2 + 25-hydroxy vitamin D3).
- Corrections with this effect have been made throughout the manuscript.

5. Results: vitamin D3 deficiency: what does that mean- is it based on vitamin D status indicator which is 25- hydroxy vitamin D.
Author compliance:
- Yes, it based on vitamin D status indicator which is 25- hydroxy vitamin D.Appropriate correction has been made throughout the manuscript.
(Section- Abstract, Subsection- Results, Page- 2, Paragraph-1, Line-20)

6. Results: clarify the sentence, “There was no association of its deficiency on HbA1C of …..” Do the authors mean “There was no association between serum 25- hydroxy vitamin D and HOMA IR or HbA1C?
Author compliance:
- The deficiency of 25OHD had no association with HbA1c and HOMA IR.
- Following correction is made accordingly as follows:
  - There was no association of serum 25OHD deficiency with HbA1c or HOMA-IR in T2DM cases (p=0.057 & p=0.257 respectively) and in control subjects (p=0.675 & p=0.647 respectively).
(Section- Abstract, Subsection- Results, Page- 2, Paragraph-1, Line-21)

7. Conclusion: ….deficiency of vitamin D3 is incorrect. It is either vitamin D deficiency or low serum level of 25-hydroxy vitamin D3.
Author compliance:
- Vitamin D deficiency is added in place of vitamin D3 deficiency accordingly all thro’ the manuscript.

Main manuscript:

1. Background: lines 7 and 8: Even though the intake of cholecalciferol (D3) is reportedly superior than intake of D2, when discussing vitamin D deficiency, it is incorrect to state vitamin D3 deficiency. It is vitamin D deficiency. Both D3 and D2 have contributions to the vitamin D status.
Author compliance:
- It has been corrected as vitamin D deficiency instead of vitamin D3 deficiency accordingly all thro’ the manuscript.

2. Background: lines 11, 16 and 20: it is 25- hydroxy vitamin D (the term vitamin D is loosely used in the manuscript. Whenever serum concentration is mentioned it has to be 25- hydroxy vitamin D), as that indicates vitamin D status.
Author compliance:
- It has been corrected as serum 25-hydroxy vitamin D (25OHD) instead of vitamin D accordingly all thro’ the manuscript.

Author compliance:
- Correction incorporated as follows:
4. Subjects: study design: is it prospective or cross sectional.

**Author compliance:**
- It is cross sectional study which is a type of prospective study. However, the word ‘prospective’ is changed to ‘prospective cross sectional’ in the manuscript.

(Section - Materials & methods, Subsection - Subjects, Page 3, Paragraph 2, Line 27)

5. Page 4, lines 6, 8, 18, 25, 33: clarify ‘vitamin D3 levels’?

**Author compliance:**
- The authors meant the following stratification of serum 25OHD (vitamin D) levels.
- Serum levels of 25OHD were stratified into normal (≥30 ng/ml), insufficient (≥20 to <30 ng/ml) and deficient (<20 ng/ml).
- Appropriate corrections have been made at indicated places in the revised manuscript

(Section - Materials & methods, Subsection - Subjects, Page 3, Paragraph 2, Line 25)

6. Page 5, lines 12: clarify ‘vitamin D3 deficiency’?

**Author compliance:**
- Serum 25OHD levels of <20 ng/ml are considered as vitamin D deficiency and ≥20 ng/ml as non-deficiency.
- Appropriate correction has been made at the indicated place in the revised manuscript

(Section - Materials & methods, Subsection - Subjects, Page 4, Paragraph 2, Line 9)

7. Page 5, line 15: groups division could be 25OHD3 <20 ng/ml/ >20 mg/ml instead of using the confusing terminology vitamin D3 deficient

**Author compliance:**
- Appropriate correction has been made at the indicated place in the revised manuscript

(Section - Materials & methods, Subsection - Subjects, Page 4, Paragraph 2, Line 9)

8. Table 1: instead of using column title as ‘vitamin D3 non-deficient/ vitamin D3 deficient’, may use ‘serum 25OHD3 >20 ng/ml/ 25OHD3 <20 ng/ml’.

**Author Compliance:**
- Column title of Table 1 has been changed to ‘Serum 25OHD <20 ng/ml’ and ‘Serum 25OHD ≥20 ng/ml’ instead of ‘vitamin D3 deficient’ and ‘vitamin D3 non-deficient’ respectively, as suggested.

9. Table 1: The subjects with T2DM who had 25OHD3 >20 ng/ml, had lower HOMA-IR and lower fasting insulin compared to those with 25OHD3 <20 ng/ml. This is also observed in the control group indicating a role of vitamin D in insulin resistance. As the early pathophysiology of T2DM involves insulin resistance this finding may be of significance.

**Author Compliance:**
- Table 1 shows the mean of the biochemical indices (including fasting insulin and HOMA-IR) of the groups- 25OHD <20 ng/ml and 25OHD ≥20 ng/ml of T2DM and control subjects. The significance (p-value) is of the difference in the mean values of both the groups. However, the regression
analysis between 25OHD and HOMA-IR of each group could not show the significance, indicating no association of HOMA-IR with 25OHD levels.

10. Page 5, line 20--, under results (and based on table 1): Subjects with poorly controlled T2DM of >8 years duration, will have poor beta cell reserve. Hence it will be difficult to find any association between HbA1C and 25OHD levels. Did the authors adjust for duration of diabetes? This could be also commented under discussion page 7.

Author Compliance:
- We have not adjusted for ‘poorly controlled diabetes of >8 years duration since we did not get the accurate information on the degree of glycation control (whether it was ‘better’ or ‘good’ or ‘poor’) of T2DM patients for the period prior to enrolment in the study. However, there were 35% patients with >8 years duration of T2DM.
- We did not find significant association of HbA1c and serum 25OHD levels in patients with >8 years of T2DM ($r^2=0.003$, $p=0.501$) and patients with ≤8 years of T2DM ($r^2=0.008$, $p=0.152$).
- We also did not find significant association of HOMA-IR and serum 25OHD levels in patients with >8 years of T2DM ($r^2=0.014$, $p=0.151$) and patients with ≤8 years of T2DM ($r^2=0.010$, $p=0.093$).
- We prefer not to bring the duration of disease and beta cell reserve in the discussion in the current manuscript in view of the above results. However, the gist of it has been added in the discussion section as follows:

As with the progression in the duration of T2DM, the β-cell reserve attenuates, but in our study we could not observe significant association between HbA1c and HOMA-IR with 25OHD taking into account with the duration of T2DM.

(Section- Discussion, Page- 8, Paragraph-1, Line-6)

11. Is the association between 25OHD levels and HOMA IR adjusted for duration of diabetes and HbA1C?

Author Compliance:
- We have not adjusted for duration of T2DM for the association between 25OHD levels and HbA1C or HOMA IR.

12. Need to include a limitation section: cross sectional study design, use of ELISA which does not measure 25OHD2 and D3 concentrations, inclusion of subjects with poorly controlled T2DM of long standing duration etc.

Author Compliance:
Limitation section has been added to the manuscript after discussion section as follows:

Limitation:
The study was a cross sectional study, carried out on urban metropolitan subjects where rural subjects were not included. Moreover, the method used to measure 25OHD levels was ELISA which is less sensitive as compared to LCMS/MS method.

(Section- Limitation, Page- 9, Paragraph-2, Line-3)
Reviewer's report 3
Reviewer: OHK-HYUN RYU
Reviewer's report:
Summary: Vitamin D deficiency is prevalent in T2DM and non-diabetic subjects in India, but vitamin D has no role in hemoglobin glycation and insulin resistance.
Major Strength: There was no significant association between hemoglobin glycation (and insulin resistance) and vitamin D. This study results are inconsistent with previous observational studies.
Major Weakness:
#1. This study is an observational, cross-sectional study. This study just investigated the relationship between diabetes and vitamin D deficiency.
#2. This study did not investigate the lifestyle factors, such as outdoor physical activity and diet, which influence on vitamin D levels, hemoglobin glycation, and insulin resistance. The authors have to add the lifestyle data at least in T2DM subjects.
Comments
#1. The title of the study (Does vitamin D3 play a significant role in type 2 diabetes?) is not clear.
Author Compliance:
• The title has now been changed to ‘Does vitamin D play a significant role in type 2 diabetes? instead of ‘Does vitamin D3 play a significant role in type 2 diabetes?’

#2. The term vitamin D and vitamin D3 was so confused. If your laboratory separately quantitated circulating 25(OH)D2 and 25(OH)D3 individually, you can use the vitamin D3. But your laboratory couldn’t separate D2 and D3, you have to use the term vitamin D.
Author Compliance:
• We have measured serum levels of 25(OH)D2 and 25(OH)D3 together by ELISA method performed on micro-titer plates using commercially available ELISA kits. (Demeditec Diagnostics GmbH, Germany).
• Appropriate changes reflecting 25OHD have been made throughout the manuscript.

#3. In the section of materials and methods, are there any reason to include the plasma glucose levels#126mg/dl in the inclusion criteria of diabetic subjects. The study results might be influenced by this inclusion criteria.
Author Compliance:
• Plasma glucose cut-off of 126mg/dl for diabetic individuals is recommended by WHO.
• A reference is now mentioned in the manuscript with this effect.

(Section- Materials & methods, Subsection- Subjects, Page- 4, Paragraph-2, Line-31)

#4. In the regression analysis (Fig 1 and Fig 2), the graph was displayed separately into vitamin D deficient group and non-deficient group. Recheck the regression results without subdivision of vitamin D status (deficient vs non-deficient) in each group (T2DM and control subjects). Is there any change in the study result?
Author Compliance:
• We have rechecked the regression results without subdivision.
• T2DM patients: r²=0.005; p=0.133 (95% CI: -0.042 –+0.006)
• Controls: r²=0.000; p=0.746 (95% CI: -0.006–+0.004)
There is no major change in the results and therefore, it is not included in the manuscript.
#5. In the conclusion section, the authors summarized their study finding that “its relationship in glycation control or insulin resistance in T2DM subjects could not be confirmed in our population.” In the regression analysis between vitamin D levels and glycation in non-deficient T2DM subjects, P value is 0.045. It meant that there were marginal significant relationship between vitamin D levels and glycation in non-deficient T2DM subjects. I recommend the additional data for the lifestyle between vitamin D non-deficient and deficient T2DM subjects in the baseline characteristics of subjects.

**Author compliance:**

- We have added additional data in results section related to lifestyle in the form of comparing the BMI, waist circumference (WC) and waist:hip ratio (WHR) between vitamin-D deficient and non-deficient groups as suggested.
- The addition done is as follows:
  - All the patients and controls were city dwellers; majority of them were office workers or housewives and connected to non-strenuous life style with minimal aerobic exercise during the day. Increased BMI (>25 kg/m²) was seen in 64.5% (277/429) of T2DM subjects. Higher BMI was observed in 65.2% (255/391) of vitamin D deficient (low serum 25OHD) patients as opposed to 60.0% (21/35) of non-deficient (normal serum 25OHD) T2DM subjects. Similarly, increased waist circumference (Females >80 cms, Males: >90 cms) was above upper limit of normal in 81.5% (350/429) of subjects; increased in 81.9% (319/390) of vitamin D deficient versus 85.7% (30/35) of non-deficient T2DM subjects. The waist : hip ratio (WHR) also followed a similar pattern of increased WHR (Females: >0.85, Males: >0.90) in 83.2% (357/29) of T2DM subjects; 83.8% (328/391) of vitamin D deficient vs 80.0% (28/35) of vitamin D non-deficient T2DM subjects.

  *(Section- Results, Page- 5, Paragraph-3, Line-22)*

**Minor comments**

#1. In the discussion section, recheck the unit of UV index?

**Author compliance:**

- We have rechecked and a correction in the manuscript has been done as follows:
  - Normal office hours in India are usually from 11 am to 7 pm while maximum sun exposure and absorption is between 11 am to 2 pm with an **UV index of 7-9 required** for conversion of 7-dehydrocholesterol to pre-vitamin D₃.

  *(Section- Discussion, Page- 7, Paragraph-1, Line-5)*

#2. In the discussion section, “further confirming this, Kampmann et al. and Witham et al. showed that improvement in vitamin D status may rise insulin secretion but improve insulin resistance and HbA1c in patients with T2DM.” The meaning is inconsistent with your findings. So the sentence should be changed to “but it did not improve”………..

**Author compliance:**

- A correction in the manuscript has been done as follows:
  - Further confirming this, Kampmann et al. and Witham et al. showed that improvement in vitamin D status may increase insulin secretion **but did not improve** insulin resistance and HbA1c in patients with T2DM.
We now submit the updated manuscript file as suggested. I apologize for the inconvenience. I request you to look into the matter as quickly as possible for the publication.

Thanking you,

Yours sincerely,

Dr. Jayesh J Sheth

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