Author’s response to reviews

Title: Association of vitamin D2 and D3 with Type 2 diabetes complications

Authors:
Lina Ahmed (lha2002@qatar-med.cornell.edu)
Alexandra Butler (aeb91011@gmail.com)
Soha Dargham (srdd03@qatar-med.cornell.edu)
Aishah Latif (alselasih1@gmail.com)
Amal Robay (amr2018@qatar-med.cornell.edu)
Omar Chidiac (omc2002@qatar-med.cornell.edu)
Amin Jayyuosi (aaj2004@qatar-med.cornell.edu)
Jassim Al-Suwaidi (jaa2014@qatar-med.cornell.edu)
Ronald Crystal (rgcryst@med.cornell.edu)
Stephen Atkin (satkin@rcsi-mub.com)
Charbel Abi Khalil (cha2022@qatar-med.cornell.edu)

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Reply to the comments of the Editor and reviewers:

Editor Comments:

The current study reports on the replenish of Vit D deficiency in diabetic and non-diabetic subjects. The results are somewhat novel showing that alterations in Vit D2 could be associated with hypertension in particular in female diabetic patients whereas the decrease in Vit D3 was associated with other negative aspects of diabetes. While the results are of interest to the readers of the Journal, there are several issues that the authors need to be addressed.

Major:
1. Table 1 shows that age, weight and all other markers of diabetes were higher in diabetic patients than the normal controls. How the authors believe that the Vit D deficiency was the cause for the associated complication and not high blood sugar, e.g., for retinopathy?

Thank you for that comment. Table 1 specifically details the differences between the control and T2DM populations, as noted. We are not suggesting that the vitamin D deficiency was causative but only that it was associated; however, that is an excellent suggestion to add the HbA1c and glucose values to the manuscript (Table 2) showing that HbA1c differed significantly for retinopathy. This is addressed in the Results (page 6 line 146) that reads “The relationship of glycemic control for both HbA1c and blood glucose at the time of the visit for T2DM are shown in Table 2, showing that only HbA1c was significantly different in retinopathy (p&lt;0.001) whilst blood glucose was different in hypertension (p&lt;0.02).” This is addressed in the discussion (page 8 line 199) that notes “Retinopathy was associated with poorer glycemic control and with lower vitamin D3 levels, reflecting the literature showing poorer glycemic control is associated with lower vitamin D levels.”

2. Hypertension was associated with high levels of Vit D2 particularly in female diabetics. Was this observed in non-diabetic patients? Was this present before the initiation of the therapy? Was the blood pressure measurement conducted only once or on several office visits or at home?

Thank you for that comment.

High levels of Vit D2 were also observed in non-diabetic patients and this has been added to the results and in Table 4 that details blood pressure and dyslipidemia in the control population. This is detailed in the Results that reads (page 7 line 162) “In control subjects, vitamin D2 was higher in those with both hypertension and dyslipidemia (p&lt;0.02) as was seen for the patients with T2DM, whereas vitamin D3 was lower in controls for dyslipidemia (p&lt;0.001), an association not seen in T2DM subjects (Table 4)”. This is also addressed in the Discussion that reads (page 7 line 177) “The increased prevalence of hypertension and dyslipidemia seen in diabetes would reflect on the elevated vitamin D2 levels seen rather than a causal association and this was also seen in the control population,…..”

Was this present before the initiation of the therapy?

We do not know the answer to that question as this was a cross sectional study.

Was the blood pressure measurement conducted only once or on several office visits or at home?

Blood pressure was measured only at the time of the visit as detailed previously, by the method noted in the “study design” on page 4, line 102). As this was a cross sectional study at a single timepoint, the blood pressure measurement was taken when the subject attended the outpatient clinic. This has been put in the limitations of the study in the Discussion (page 10 line 233) that reads ‘Furthermore, this was a cross sectional study and therefore we did not have serial measurements such as blood pressure’
3. The data should be further divided to male vs. female in each group to see if females are different than males regardless of the health condition or it is the combination of Vit D deficiency plus diabetes that make females develop the other complications, such as hypertension, etc.

Thank you for that comment. We have undertaken that analysis by gender and that is shown in Table 5 with differences between the genders being seen for blood pressure, dyslipidemia and neuropathy for vitamin D2 and retinopathy and dyslipidemia for vitamin D3. This is now in the Results that reads (page 7 line 166)”The relationship of vitamin D2 and D3 to diabetic complications according to gender is shown in Table 5 that shows significant differences between the genders being seen for blood pressure, dyslipidemia and neuropathy for vitamin D2 and retinopathy and dyslipidemia for vitamin D3.” In the Discussion this is addressed (page 8, line 182) that reads “When gender differences were investigated, significant differences were seen in male T2DM for hypertension, dyslipidemia and neuropathy with vitamin D2 and dyslipidemia with vitamin D3, whilst a difference for retinopathy was seen for female T2DM patients not seen in males. This may be of particular importance, as lower vitamin D levels in women have been associated with increased severity of coronary artery disease, but the impact of gender differences in diabetes complication onset or severity with respect to vitamin D deficiency is unknown.

4. Why the subjects were giving Vit D2 but not Vit D3?

Thank you for that comment. Vitamin D2 supplements are commonly given as they are considerably cheaper than vitamin D3 supplements with evidence that, whichever supplementation is given, one is equally good as the other.

Minor:

1. Subjects were administered 50,000U of Vit D2 once a week. Is this dose of Vit D2 in the normal dose range?

Yes, this is an accepted dose in normal clinical practice.

2. How the authors confirmed that everyone is complying and taking their Vit D2? Was blood level of the vitamin recorded in each individual at certain time?

As this was a cross sectional study, then the vitamin D levels were collected at that single point of time and other than asking the patients, their compliance to the vitamin D2 supplementation could not be ascertained.

3. Lines 27, 29 and 34; please change diabetes to diabetics. Also, please make this change in other sections.
Thank you this has been done.

We operate a policy of open peer review for this journal, 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.

Reviewer reports:

Reviewer 1: The study aims at addressing a timely and interesting question, whether vitamin D serum levels correlate with diabetic complications. Despite that the results are very limited, and a number of issues preclude the manuscript to be acceptable for publication in its present form:

- the authors state that all individuals were taking a weekly dose of vitamin D, but for how long before the measurements were taken? If all subjects being studied were taking supplements, and one does not know how long have the subjects have been taking supplementation, no inference as to protection by vitamin D or of mere difference in vitamin D absorption by the different group can be established.

Thank you for your comment. All subjects had been taking vitamin D2 supplementation for a minimum of 6 months and this has been added to the methods (page 4 line 92) that reads “All subjects had been prescribed vitamin D2 supplements 50,000 units weekly that they had been taking for more than 6 months, …” It has been shown that ingestion of such doses of vitamin D gives steady state levels even if given monthly, though vitamin D repletion may not be achieved and may vary between individuals. This has been acknowledged in the Discussion that reads (page 10, line 234)” It has been shown that ingestion of such doses of vitamin D2 as given here results in steady state levels even if given monthly, though vitamin D repletion may not be achieved and may vary between individuals (17); therefore, the timing of the dosing of vitamin D2 supplementation would not be thought to influence day to day vitamin D levels.”

- on the diabetic patients - important information on them is lacking, such as years of diagnosis prior to the enrollment in the study

The mean duration of diabetes was 10.6±6.5. This information has now been added to Table 1.

- no analysis controlling for age seems to have been performed. This is particularly important since controls were family members of the diabetics, who supposedly share the same household/living standards, but differ from them by being younger

Thank you for that comment but as the data was skewed the regression for age could not be undertaken. We have addressed that in the limitations (page 10, line 232) that reads “No analysis for age could be undertaken as the data was skewed”

- on table 2, number of patients starting from row #2 don't add up to 274, which the authors state was the number of diabetic patients. Why?
Thank you for that comment. The actual number of diabetic patients per measure are detailed and may not add up to 274 due to missing values when the vitamin D levels were at the lower limit of detection; then, as they could not be accurately determined, they were excluded from the analysis. This has been addressed in the legend of Table 3.

- A very important study by Pittas et al., NEJM 2019 showed that vitamin D supplementation had no positive effect to reduce the risk for diabetes. However, the study reviewed here does not discuss that, using rather older references to justify their study (for instance in the initial paragraph of Introduction).

Thank you for that comment and this has been included in the manuscript, but it should be noted that this NEJM study specifically addressed prediabetes and the risk of developing diabetes whilst the patients in the study had established diabetes with or without complications. This is addressed on page 3 line 57 that reads “though in a study in prediabetes patients 4000 IU daily of vitamin D3 supplementation did not significantly lower the risk of diabetes (2).”