Author’s response to reviews

Title: Low vitamin D levels are associated with cognitive impairment in patients with hashimoto thyroiditis

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ANSWER TO REFEREE COMMENTS

Martin Hewison (Reviewer 1): The study by Xu et al describes a simple but interesting study in which the authors have highlighted a link between mild cognitive impairment (MCI) and serum 25-hydroxyvitamin D levels in patients with Hashimoto's disease. As with most studies of this nature, the impact of the investigation is somewhat limited by the fact that the patients, and presumably controls, were analysed for vitamin D at a single time point that may have no relevance to the MCI. Nevertheless, the data are compelling and could provide a fresh perspective on the existing proposed links between vitamin D-deficiency and autoimmune disease.
Specific comments:

1. The differences in 25-hydroxyvitamin D levels between Hashimoto's patients and healthy controls is quite striking. Rather than giving mean ± SD levels the authors should provide median and inter-quartile ranges. In other words, exactly how low is the 25-hydroxyvitamin D in the Hashimoto's patients? What are the lowest values for these patients

Answer: We appreciate you for raising the important issue. We have provided median and inter-quartile ranges of 25-hydroxyvitamin D of Hashimoto's patients and healthy controls in the revised manuscript (Result section, line 158, page 8; Table 1 section, line 397, page 19).

2. The quartile data presented by the authors is very interesting, but should either be extended to a quintile to include patients with serum 25-hydroxyvitamin D less than 25 nmol/L - presumably most of these will have MCI? Or expand the quartiles. Basically it is important to know the levels of MCI in patients who are severely vitamin D-deficient (i.e. less than 25 nmol/L). This is the level that has been proposed as predisposing to rachitic bone disease and thus it would be interesting to know if this level also predisposed to MCI.

Answer: Thanks for your careful review. In the current study, as the raw data of 25(OH)D were skewed, serum 25(OH)D levels in HT patients were divided into four quartiles, the method being widely applied by numerous researchers (Bin Han et al., European Journal of Neurology 2015; A Namri et al., European Journal of Clinical Nutrition 2009). Furthermore, up to now, no study has shown an association of rachitic bone disease with depression in HT patients. Hence, we see no need to extend to a quintile.

3. The median 25-hydroxyvitamin D values should be given for each quartile/quintile.

Answer: Thanks for your careful review and insightful comment. The median 25-hydroxyvitamin D values for all quartiles were 30.8, 36.6, 43.7 and 53.1 nmol/L, respectively. These data have been added to the revised manuscript (Methods section, line 116-117, page 6).

Rowan Hardy (Reviewer 2): The authors report a correlation between reduced vitamin D (25(OH)D) and cognitive impairment measured by the Montreal Cognitive Assessment score (MoCA) in a Hashimoto's thyroiditis (HT) cohort. Whilst findings of this nature have been reported in healthy control patients and in disease states such as CKD and T2D the finding may be of less importance where evidence of attenuated vitamin D in a HT cohort exists. Unfortunately, these data are not well reported and require additional clarification and analysis.
Major

Methods: more details on numbers of HT patients required in methods.

Answer: Thank you very much for raising this important issue. We have stated the detail on numbers of HT patients required in the Methods of revised manuscript (Methods section, line 92, page 5).

Were MoCA scores taken in control cohorts? This is not clear

Answer: Thank you for pointing out our negligence. In fact, MoCA scores were not taken in control cohorts in this study, which has been considered as one of limitations of the study in the revised manuscript (Discussion section, line 215-217, page 10).

Table 1: You have a significant difference in age between MCI + and - HT groups. How can you interpret data where age may be a factor in influencing cognitive function

Answer: Thanks for your careful review. Univariate analysis shown a significant difference in age between MCI + and - HT groups. But after adjusting the potential confounders in the logistic analysis, the difference was not significant any more.

Please provide details of the control healthy cohort in a new table compared to the HT cohort. Please report these data in more detail in a table. The authors state that the patients in this study did not differ from the controls in terms of age, sex, BMI, education, as well as levels of TSH, FT3, and FT4 (all P > 0.05). Please let us see this data. Also any data on MoCA between groups would be relevant if collected.

Answer: Thanks for your careful review and insightful comment. We have provided the details of the control healthy cohort in a new table compared to the HT cohort (Table 1 section, line 397-400, page 19). In addition, MoCA scores were not taken in control cohorts in the current study, which has been considered as one of limitations of the study in the revised manuscript (Discussion section, line 215-217, page 10).

Authors state that "Serum levels of 25(OH)D were markedly lower in patients with HT than in healthy controls". Please include all of this data as discussed above.
Answer: Thanks for your careful review and insightful comment. We have provided the data in a new table in the revised manuscript (Table 1 section, line 397-400, page 19).

Authors state "there was a negative correlation between 25(OH)D levels and MoCA scores (r = 160 0.510, P < 0.001).". Please also show these key correlations in a figure and in a table so that we can fully assess their correlation. In addition, what are the correlations like in the control cohort you collected? This data is essential to help us identify if HT differs meaningfully from healthy controls.

Answer: Thanks for your careful review and insightful comment. We are sorry that we made a mistake when inputting data of MoCA scores into SPSS software. We found the mistake after going through the statistical analysis of the current study. In fact, there was a positive correlation between 25(OH)D levels and MoCA scores (r = 0.828, P < 0.001), which has been shown in Figure 1A in the revised manuscript (Results section, line 159-160, page 8; Figure 1 section, line 375-377, page 18). MoCA scores were not taken in control cohorts in the current study, which has been considered as one of limitations of the study in the revised manuscript (Discussion section, line 215-217, page 10).

"25(OH)D levels were inversely correlated with TPOAbs levels (r = -0.316, P < 0.001)." This is an important correlation. Please add a figure showing the univariate analysis. A table of all the univariate analysis performed in control and HT cohorts would be helpful in interpreting the data as requested above.

Answer: We are truly grateful to the helpful suggestions from you. The important correlation has been shown in Figure 1B in the revised manuscript (Figure 1 section, line 375-377, page 18). In addition, we have provided the details of the control healthy cohort in a new table compared to the HT cohort in the revised manuscript (Table 1 section, line 397-400, page 19).

Table 1: the breakdown of vitD data into quartiles is very confusing and poorly explained. This requires better explanation and rationale as to why this was done, what it adds and the rationale for basing you multivariate analysis on these parameters.

Answer: Thanks for your careful review. In the current study, as the raw data of 25(OH)D were skewed, serum 25(OH)D levels in HT patients were divided into four quartiles, the method being widely applied by numerous researchers (Bin Han et al., European Journal of Neurology 2015; ANanri et al., European Journal of Clinical Nutrition 2009).
The multivariate analysis are not well reported and brief. The legend for this table is limited and uninformative.

Answer: Thanks for your careful review. In the current study, binary logistic regression including age, sex, and the factors with P < 0.10 in the univariate analysis, was performed to examine significant risk factors for cognitive impairment in HT patients. With all HT patients taken as a whole, quartile 2 and quartile 3 taken as the references used for serum 25(OH)D levels, and cognitive impairment taken as a dependent variable in the logistic analysis, serum 25(OH)D levels were significantly associated with cognitive impairment in patients with HT. These statements can be find in the Methods and Results in the original manuscript. In addition, we think the legend for this table is concise and informative.

Greater detail is required within tables 1 and 2 in regards to the legends. Statistical tests used, multivariate, univariate etc. Are these HT patients only. Why cant we see data on control cohort here?

Answer: Thanks for your careful review and insightful comment. We have provided the details of the control healthy cohort in a new table compared to the HT cohort in the revised manuscript (Table 1 section, line 397-400, page 19). Statistical tests used had been stated in the original manuscript (Methods section, line 135-137, page 7). Additionally, we think these legend for this tables are concise and informative.

In their current form, interpretation of the results is complicated. These need to be presented more clearly with a more complete data for a better informed discussion. The discussion should then be re-written following the inclusion of additional data.

Answer: Thanks for your careful review. It is really a pity that you think our discussion is incomplete. But the exact role of vitamin D in the pathophysiology of cognitive impairment in HT patients is not yet clear. A possible explanation is the effect of vitamin D on inflammatory cytokines.

Minor:

Poor structure of sentence. Suggest removal of extremely: "It is well known that vitamin D is extremely essential for human health"
Answer: We appreciate you for raising the important issue. We have removed the sentence from our paper.

Methods. Please include all data on ELISA kits. Manufacturer, cat no etc.

Answer: We appreciate you for raising the important issue. Levels of serum 25-hydroxyvitamin D (25(OH)D) were determined using a competitive protein-binding assay (Roche Diagnostics, Mannheim, Germany). The information has been provided in the Methods of the revised manuscript (Methods section, line 113, page 6).

Line 78: vitamin D may have neuroprotective properties by suppressing inflammation and oxidative stress [6-8]. These references do not identify an association with compromised cognitive function but do refer to inflammatory and mortality actions of vit D. Please find better references or include a reference that links inflammation directly to cognitive impairment.

Answer: Thanks for your careful review. Please see the references 27-31 in the Discussion that links inflammation directly to cognitive impairment. (Discussion section, line 204-207, page 10).

These data support a growing body of literature linking cognitive impairment with reduced vitamin D levels. Previous studies have been mixed with reports of both vit D deficiency and normal vit D in HT cohorts (ie, Yasmeh et al, Endocr Pract. 2016 Hashimoto Thyroiditis Not Associated with Vitamin D Deficiency). This should be considered in the discussion.

Answer: Thanks for your careful review and insightful comment. Yasmeh et al reported that 25(OH)D levels for the HT and controls were significantly different in females but not in males. But no correlation was observed between 25(OH)D levels and sex in the present study. Further studies are needed to examine the sex difference of 25(OH)D levels in HT patients. We have added the important section to the Discussion of revised manuscript (Discussion section, line 184-188, page 9).