Author’s response to reviews

Title: Vitamin D deficiency among newly diagnosed tuberculosis patients and their household contacts: a comparative cross-sectional study

Authors:

Meseret Workineh (mwmesi@gmail.com)
Biniam Mathewos (fikinbinny@yahoo.com)
Beyene Moges (beyemoges@gmail.com)
Addisu Gize (konjoaddisu@gmail.com)
Sisay Getie (sisaygetie@yahoo.com)
Olle Stendahl (olle.stendahl@liu.se)
Thomas Schön (thomas.schon@ltkalmar.se)
Ebba Abate (ebbaabate@yahoo.com)

Version: 1 Date: 16 Feb 2017

Author’s response to reviews:

Author's response to reviews

Vitamin D deficiency among newly diagnosed tuberculosis patients and their household contacts in Gondar, North-West Ethiopia: a comparative cross-sectional study

Meseret Workineh, Biniam Mathewos, Beyene Moges, Adissu Gize, Sisay Getie, Olle Stendahl, Thomas Schön, Ebba Abate.

Email addresses

Ebba Abate: ebbaabate@yahoo.com
Biniam Mathewos: fikinbinny@yahoo.com
With regards!

From: Meseret Workineh
Correspondence Author (mwmesi@gmail.com)

Author's response to reviews: see over

February 16, 2017

To: Archive of Public Health, Editorial Board

Subject: Submitting a revised version of manuscript

Object: Manuscript ID= AOPH-D-17-00020: Vitamin D deficiency among newly diagnosed tuberculosis patients and their household contacts in Gondar, North-West Ethiopia: a comparative cross-sectional study

We would like to thank the reviewer and editor for sharing their view and experience. The comments are very important which will further improve the clarity of our manuscript. The point-by-point responses for each of the comments and the revised manuscript are provided in the attached documents.
POINT BY POINT RESPONSES

We would like to take this opportunity to thank the reviewers and the editor for sharing their view and constructive comments. The comments are very important which will further improve the quality of the manuscript. The point-by-point responses for each of the comments are provided in the following pages.

Point by point Response

Reviewer #1

1. As stated by the Authors, differences in assays can lead to different results and it is quite difficult to interpret the results of this study when compared to others. Singularly, the performance of the assay in the hands of the authors is missing (coefficient of variation, etc.). I would suggest to use another assay or try to collaborate for future research.

• Answer: Thank you dear reviewer for your comment. A 25(OH)-vitamin D3 direct Enzyme Linked Immunosorbent Assay has a very good performance as reported by the assay manufacturer. It has an inter-assay and intra-assay % coefficient of variations of 7 which are generally acceptable. Besides, we will work with international collaborators for our future researches.

2. I disagree with the conclusions from the household. Since they also suffer from poor conditions of living, like the patients (malnutrition, lower incomes...) this impacts their 25OHD concentrations. Now, I agree that they should be followed-up.

Answer: We have accepted the comment. Yes, we also agree with your idea. In this study, we have found high prevalence of vitamin D deficiency in household contacts of TB patients. As a limitation, the present study was a cross-sectional design and therefore, it is not possible to say whether low vitamin D levels were associated with progression to active TB disease in household contacts, although we strongly suspect that this is the case. Therefore, we suggest
every house hold contact of TB patients should be followed and screened for TB. Thus, we will collaborate for future to follow up household contacts.

Reviewer#2

1. It is surprising that authors choose to perform a student T test instead of an ANOVA (to allow directly a three-group comparison).

• Answer: Thank you dear reviewer for your constructive comments. We have accepted the comment. An ANOVA test was used to determine whether there are any significant differences in the mean serum levels between TB patients, healthy controls and household contacts. We have mentioned it in the statistics part (page 8) and presented the results (Table 2, page 22).

2. Did the authors check the distribution of the data prior performing the statistics? Some data do not seem to have a normal distribution (for example, CD4+, CD8+, CD3+ cells for which SD are very dispersed) and deserve to be presented with median (P25-P75). Non-parametric tests should therefore be applied.

• Answer: The comment is well accepted, and we have checked the distribution of the data prior performing the statistics. For example, the vitamin D data was normally distributed and thus we have presented it with mean±SD and parametric test (ANOVA) was applied to compare means. However, the statisticians have advised us not to include CD4+, CD8+, and CD3+ cells data as it is not our major objective. Therefore, CD4+, CD8+, CD3+ cells data are not included in the revised manuscript.

3. It is not clear why table 1 do not present results of level of education, occupational status, alcohol use and tobacco use for house contacts. If these data were not collected for this population, this should be specified in methods.

• Answer: Thank you, the comment is well accepted! Yes, we do not present results for level of education, occupational status, alcohol use and tobacco use for house contacts. These data were not collected for household contacts and we have stated it in the methods section (page 7).
4. It is not clear with table 2 is only showing results of TB patients and healthy controls and not the results of house contacts. In the method part, authors specified that these laboratory measurements were performed on all subjects.

• Answer: The comment is well accepted, and we have entirely revised table 2(page 22 on the revised manuscript). As it is mentioned above (on comment number 2), we have removed CD4, CD8 and CD3 data and we have focused only on the mean vitamin D levels among the three study groups.

5. Table 2 and table 3 should be merged. Therefore, one unique table should show all laboratory results as well as vitamin D levels and deficiency categories.

• Answer: The comment is well accepted, and we have revised both table 2 and table 3. In table 2(page 22 on the revised manuscript) we have summarized comparison of mean Vitamin D levels between newly diagnosed TB patients, household contacts and healthy controls and in table 3(page 23 on the revised manuscript) we have put proportion of Vitamin D deficiency categories among TB patients, healthy controls and House hold contacts in revised manuscript.

6. Table 2 should not present results of the X² but only p-values. Authors could add a footnote to this table to inform that a student T test was use for continuous variables and a X² was used for categorical variables. It is also absolutely necessary to report this in the statistical part of the methods.

• Answer: The comment is well accepted, and we have removed X² values in the table 3(page 23 on the revised manuscript) we have added on the footnote to this table as Chi-square test was used to test for differences in proportions. We have also mentioned it on the statistical analysis part.

7. A multivariate regression should be performed to assess confounding factors of serum vitamin D level. For example, several models of multivariate analyses could be proposed by including age, sex, BMI, etc. as confounding variables.
• Answer: The comment is well accepted. Serum 25(OH)-vitamin D3 levels were dichotomized into severe vitamin D deficiency (≤25 nmol/L) and not severe vitamin D deficiency (>25 nmol/L). One meta-analysis (by Junli Zeng) reported that only severe vitamin D deficiency (serum Vitamin D concentration <25 nmol/L) was associated with a significantly increased risk of active TB while the range 51–75 was not associated with active TB. However, the range 26–50 nmol/L was not statistically associated with active TB. Therefore, based on the above study in this current study we dichotomized our study participants in to two groups to observe the association of severe vitamin D deficiency and TB. After grouping we have done both bivariate analysis and multivariate logistic regression analysis to identify the independent determinants of severe vitamin D deficiency separately (table 4, page 24).

8. Table 4 is not clear at all. Authors do not report the factor of adjustment for the OR. It is not a classical way to report results of a logistic regression and this table should be entirely revised.

• Answer: The comment is well accepted. As we have mentioned above on comment number 7, we have performed both bivariate and multivariate logistic regression analysis to assess the confounding factors for severe vitamin D deficiency and we have summarized it on table 4. We have also added some additional information on the table 4(Page 24) on the revised manuscript to make it clear.

Editors' comment

1. Please remove "in Gondar, North-West Ethiopia" from the title.

• Answer: Thank you dear editor: We have made corrections accordingly.

Thank you!!!!