Author's response to reviews

Title: HIV associated hypocalcaemia among diarrheic patients in northwest Ethiopia: A cross sectional study

Authors:

Beyene Moges (beyemoges@gmail.com)
Bemnet Amare (amarebem6@gmail.com)
Timoki Yabutani (yabutani@chem.tokushima-u.ac.jp)
Afework Kassu (afeworkkassu@yahoo.com)

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Response to Reviewers
To: Editorial Manager to BMC Public Health
Article title: HIV associated hypocalcaemia among diarrheic patients in northwest Ethiopia: A cross sectional study
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Point by point Response to Reviewers 1
We made the following major compulsory revisions as you have recommended and edited accordingly,

1. The studies (including those from same facility) showed that hypercalcemia is widespread problem among HIV infected. Why this assumption is raised and studied becomes unclear.

As you have pointed out, we did report on the existence and magnitude of hypercalcemia among pulmonary tuberculosis patients. Since tuberculosis is one of the granulomatous diseases. The association between granulomatous diseases including but not limited to tuberculosis and hypercalcemia is well recognized; however, the incidence in our county Ethiopia was never been reported and because of this reason we conducted a study and published it at the Asian Pacific Journal of Tropical Disease. The current study was done among diarrheic patients with and without HIV infection which is a different scenario.

2. Why was sample size not calculated?
The sample size was originally calculated for the general study that comprises assessment multiple micronutrients statuses of different segment of populations; and, this particular paper was extracted from the pooled data based on clinical background of study subjects, in this cases diarrheic patients with and without HIV patients.
3. How were the clients with hyperparathyroidism, and other calcium related disorders excluded as it may require biochemical testing to know many of these abnormalities?

As we have tried to mention in Q-2, since the data was extracted from the pooled data by setting selection criteria and selection criteria was based on the information obtained from both the questionnaire and experimental studies most of which are done in Japan. In the material and method section we also included how we excluded patients with hyperparathyroidism.

4. Why should they be ART naïve?

From the beginning, the objective of this study was to assess if there is a difference in frequency of serum calcium level alteration in diarrheic patients and would it be affected in the presence of different etiological agents which are directly (IP and enteric bacteria) and indirectly (HIV) and we have only included patients prior to commencing ART drugs because the antiretroviral therapy are meant to inhibit viral replication, to maintain health, and to preserve life therefore we believed that including those patients who are already on ART may not truly reflect the effect of HIV.

5. What was the purpose of stool examination; to confirm which bacteria caused the diarrhea or compare the type of bacterium with calcium level?

High prevalence of diarrhea in developing countries are commonly related to infectious pathogens such as intestinal parasites and enteric bacteria in our case we were able to isolate shigellosis but due to financial constraint we were not able to include as many pathogenic bacteria as possible. Those pathogens causes diarrhea which results removal of essential minerals including calcium and if calcium is not being absorbed the only source of calcium would be demineralization of bone.

6. What is plausibility of testing the association between infectious pathogens and hypocalcemia?

We have tried to address this issue in conjunction with previous question no6

7. Why and how are the multiple statistical tests used in the table shall be clarified. E.g. what was the T-test used for in table 1.

The frequency of patients based on the calcium level together with their mean difference is given in table-1. The mean difference was calculated by both independent T test and ANOVA based on the number of independent variables.

8. Could the association between calcium level and HIV infection be confounded by other factor such as dietary intake,…etc? please explain.

Basically, the calcium bioavailability is affected by many factors because taking calcium rich diet may not ensure its absorption if there is remarkable vitamin D deficiency and in the circulation, over half the circulating calcium is protein bound (albumin) which indirectly measure the nutritional status of the patients that entails mathematical correction before reporting.

Minor essential revision
9. It is unethical to do nothing to those with hypo/hypercalcemia. What was done to them? 2. Were there identifiers of participants used or no? 3. What happened/will happen to the blood samples afterwards?

This information, after publication, will be disseminated to the university community as usual and will be presented in the research conferences to inform physicians about the problem. All the serum sample were not transported to Japan, only measured samples were transported which is only enough to do the stated laboratory tests.

B. Editorial revisions
Revised accordingly

C. Study limitations
A subsection for limitation of the study is included

Reviewer 2

1. Abstract section: the abstract mainly gives emphasis to HIV related figures than the diarrhea associated problems as it has indicated nothing about intestinal parasitosis, shigellosis and salmonellosis. This diverts the objective from the topic. It needs to be modified again!

We accept your concern and the abstract has been modified as per your comments

2. In the Materials and Methods part:

2.1. 1st paragraph: It is well understood that low blood levels of calcium may be caused by low levels of protein (albumin) in the blood, because about half of all calcium in the blood is attached to albumin. For this reason, an ionized calcium level (which is not attached to albumin) and a blood albumin level may also be measured. This may also go to alcoholic behavior of a patient and will be happy if I can see the data on the level of alcohol drinking practices of your study subjects. I ask this question because it is difficult to exclude these individuals like the other patients unless they had a liver damage history.

In the material and methods section, we tried to show how those study subjects were extracted from our pooled data for example patients with hyperparathyroidism and other known calcium metabolism disorders were excluded from the analysis

2.2. Stool examination and culture: Please describe the following statement “The NLF colonies were further tested through a series of biochemical tests followed to identify Shigella and Salmonella species” in detail as the reference indicated is not the right one.

The non-lactose fermenting (NLF) colonies has been described and the references has been corrected accordingly

2.3. The authors should show who the study subjects are? And how they have been recruited? What inclusion and exclusion criteria employed? This will hopefully solve the reason why they mentioned legal guardians of children in the
We believed that people with the age of 15 and above are not considered as children.

3. Result: Table 2 - It is better that the total number and percentage of individuals with less than 8.5 mg/dl, 8.5-10.5 mg/dl and >10.5 mg/dl be shown in a different table. It can be Table 3. Table 2 will, therefore, contain only the Mean and Median values of Calcium level in each category of patients. Otherwise please use different table format which can show the number (percent) of study subjects with the corresponding mean and median values in two categories within a cell of a table.

Corrected accordingly

4. Discussion section

4.1. Paragraph 1: What could be the critical reason for the big difference among patients in the research done in Germany and in your case. Mainly focusing on their therapeutic profile or nutritional status? Will be good to elaborate more at this point?

Possible explanations have been given for the significant differences in the prevalence of hypocalcaemia among HIV patients.

4.2. Paragraph 2: Please give more clear description of the following statement: “As all the participants of the current study were diarrheic, malabsorption and sepsis might account for part of the hypocalcaemia” showing the actual figures of patients with malabsorption and sepsis.

The statement “As all the participants of the current study were diarrheic, malabsorption and sepsis might account for part of the hypocalcaemia.” has been omitted to avoid confusion for readers.

4.3. Paragraph 3: Better to write HIV-1 protein, Vpr, enhances………

Corrected accordingly

5. Ethical clearance section: what measures have you taken to hypocalcaemia patients identified in your study?

Since the analysis took some time to process calcium level even after it has been transported to Japan, it was not possible to take possible measures regarding the results; however, all the intestinal parasitoses and shigellosis data were reported to the physician looking after the patients without any fee. In addition, following publication, the data will be presented to responsible bodies through conferences or workshops.

Minor Essential Revisions

6. Abstract section: Result part: Please show the actual figure in HIV negative patients comparing with calcium level of HIV diarrheic patients. The last statement of the Result part should be corrected as the values for females and males is exchanged unlike what can be seen in the table.

Thank you for your comments and we have corrected the concerns accordingly
7. Stool examination and culture:

7.1. It is better to put specifications of reagents employed in brackets as shown below: Proper microbiological quality control was employed at each step of the procedure and American Type Culture Collection quality control strains of Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853) were used.

Corrected accordingly

7.2. Standard deviation should be written as SD not “S D” (eg. Result section, paragraph 2). In this same statement please: The mean ±S D serum calcium level (mg/dl) of the participants was 8.08±1.60 mg/dl.

Corrected accordingly

8. Reference:

8.1. Discussion paragraph 4: please italicize et al….Deng et al [59]. This should also be considered in most of the references indicated below.

Corrected accordingly

8.2. References 7, 8, 12, 14, 16, 17, 23 (names in capital letter?), 26, 39, etc please check namings if you have to use abbreviations of family names, also decide using one or two letters(eg. Riccardi D, Brown EM), full names(eg.Bemnet Amare, Solomon Meseret, …), and italicizing et al. Please also use full names of journals. Otherwise, please strictly follow the author’s guide of the journal regarding reference writing.

Corrected accordingly

1. Table 1- percentage value of each category of results should be calculated taking 206 as 100% otherwise it is confusing to compare actual figures and their corresponding percentage values. The total values and percentage values of Normocalcaemia, Hypocalcaemia and Hypercalcaemia are Ok. Please also be consistent using terms “Hypocalcemia” or “Hypocalcaemia” in the table as well as in the manuscript if any. Similarly mg/dl and mg/dL in tables should be written with consistency.

Corrected accordingly

2. Discussion: last paracgrap line 1. Please rephrase the statement.

We are panning the next phase of the project by including common OIs in the area and other possible confounding including HAART

Discretionary Revisions

3. Points indicated as weakness of the current study are well addressed. It would, however, be good if you can at least see some of the OIs in HIV patients to strengthen your conclusions.

Minor comments

Introduction: Paragraph 1-The first statement need some grammatical correction
like commas and the term ‘with’ at the end of the 2nd line is not appropriately introduced for reading. The second statement should be corrected as follows: They are also one of among the important causes of........The last statement of this paragraph should also be re-written in a better way.
Corrected accordingly

13.-As editorial mistakes: leaving Space after commas eg. [20,22,26] and space between a number and unit of measurement eg. 8.5mg/dl; 37oC and 2ml should be well taken into consideration in all parts of the manuscript.
Corrected accordingly