Author’s response to reviews

Title: Factors associated with first-line antiretroviral treatment failure in adult HIV-positive patients: a case-control study from Ethiopia

Authors:
Yihienew Mequanint Bezabih (myihienew@gmail.com;yihienew.bezabih@oniris-nantes.fr)
Fekadu Beyene (bsfekadu@yahoo.com)
Woldesellassie Bezabhe (Woldesellassie.Bezabhe@utas.edu.au)

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Author’s response to reviews:

Vicente Estrada/MD, PhD
Editor-in-Chief, BMC Infectious Diseases

Date: May 7, 2019

Dear Dr. Vicente Estrada,
Re: INFD-D-19-00077

Thank you for your email dated on 24 March 2019 enclosing reviewers’ comments. We addressed all their comments and revised the manuscript accordingly. Our responses are given in a point by point manner below. Changes to the manuscript are highlighted.

We hope the revised version is now suitable for publication and look forward to hearing from you.

Sincerely,

Yihienew Bezabih,MD
Master 2 student
ONIRIS, University of Nantes, France
Authors’ Response (highlighted response attached in .docx format)
I would like to extend my deepest gratitude for your reviews. We thoroughly revised the manuscript based on your comments and suggestions. We highlighted our response to each comment as shown below.

Iñigo Sagastagoitia, Ph.D. (Reviewer 1)

1. **General**: In general well written and easy to understand. There are some limitations which may invalidate some conclusions. Before submission revision should be done and several minor limitations included. Discussion should answer an important query.
   We revised the manuscript and resolved the limitations which could have given biased conclusions. We modified the discussion and highlighted the new findings of this study.

2. **Methods**:
   a. Study design is adequate a priori for a case-control study but results show on the multivariate analysis that the highest risk for treatment failure is TB coinfection and this could be biased as only 9 out of 182 patients in the control group were under treatment for TB. Out of 3238 possible controls this should be revised as conclusions could differ if more controls were coinfected by TB. TB coinfection is under-represented in the control cohort.
   It was rightly pointed that TB-coinfection was underrepresented in the control cohort (only 9 TB cases among 182 controls versus 33 TB cases /91 patients with ART failure). We removed the variable TB-coinfection from both the bivariate and multivariate analyses and presented only the descriptive findings. It is highly likely that the high number of tuberculosis disease in the cases group (more than one third of cases had TB) can be because of the ART failure itself. An option to include more TB coinfected controls was not performed due to lack of funding and this could be one of the limitations of this study.
   b. Drug resistance data is not available. This is a major query for an obvious reason and could bias conclusions.
   HIV Drug resistance tests were not available in the study area and were not performed.
   c. Questionnaire was cross checked for consistency, piloted and modified but not validated.
   The questionnaire was validated, and we corrected the sentence. We have highlighted this in the manuscript.

3. **Results**:
   a. Persistent diarrhea was associated with ART failure in the univariate analysis but not in the multivariate. The number of patients with persistent diarrhea is not available in Table 1.
   We included the number of patients with persistent diarrhea in Table 1.
   b. The main factors that have been previously proved to be associated with virological failure are treatment discontinuation, poor absorption, drug interactions and drug resistance. Poor absorption was not determined. This could have been defined by the combination of persistent diarrhea and wheat as main diet (which accelerates bowel transit), as they are both registered.
   Poor absorption was defined as combination of persistent diarrhea and wheat as main diet and included in Table 1. Although we didn’t get enough count per cell to make an odds ratio, it was very interesting that all wheat eaters with persistent diarrhea (3 patients) had ART failure.
4. Discussion: You state that the major limitation is you could not differentiate whether TB coinfection was a risk factor or manifestation of treatment failure but your results show on the multivariate analysis that the risk of treatment failure is higher in the setting of TB coinfection than in those who discontinue treatment and that should be a major issue to be mentioned in the discussion as ART is the Key to success no matter what viral load nor CD4+ count. The variable tuberculosis-coinfection was removed from the discussion as its potential to bias the scientific community. The discussion now underlines the importance of avoiding drug discontinuation.

5. Minor:
   a. Background: Switching to second line therapy does not necessarily mean a less effective therapy, as a WHO first line could include AZT and EFV plus a third drug and a second line for that patient in case of failure would include Tenofovir and a PI which is a better therapy in terms of genetic barrier and toxicity. The sentence which stated second-line ART as a less effective therapy is modified.
   b. Results: AZT based regimen was associated with treatment failure in the univariate analysis but not in the multivariate (close to statistical significance p-value 0.07). Actually in Ethiopia one of the recommended ART first line regimens is AZT+3TC+NVP as it has been associated with low treatment failure. Table 1 does not include the difference between groups in AZT exposure. Is it well represented? AZT based regimen was associated with treatment failure in the univariate analysis. We now included AZT exposure among cases and controls in Table 1 and it is well represented. The recorded ART regimen for the cases group was their first-line ART regimen and not of their current second-line regimen. As it is common to replace AZT by TDF (and vice versa) during second-line ART this could be the source of difference with other studies.
   c. Discussion: TB coinfection indicates AIDS; as in your study it has been associated with treatment failure in high and low income cohorts. ART must be tailored in such setting in order to avoid drug interactions (we assume this was done), so this possibly has not been determinant as safe regimes have been published in the different guidelines. Treatment adequacy could be revised. We included the number of patients by their WHO stage in Table 1.

Nang Thu Thu Kyaw (Reviewer 2)
The study conducted by Bezabih et al aimed to identify risk factors associated with first-line ART failure among patients receiving care at an ART clinic of a hospital in Ethiopia. There are some risk factors associated with first-line ART failure already being identified in the global literature including in studies from Ethiopia, but there had been no study from Ethiopia that identified risk factor for first-line ART failure diagnosed using viral load. Hence, this study has potential to contribute to identify risk factors associated with first-line treatment failure in HIV population in Ethiopia. However, there are major concern on the methods, the results of the study and the presentation of the results in the manuscript.

Major comments:

1. The author should follow one of the guidelines for reporting observational studies, for example, STROBE guideline.
We addressed all your comments and revised the manuscript. We now followed the STORBE guideline
for presentation of the findings. We analysed the data with checking of multicollinearity issues and incorporation of matching factors in the adjusted analysis. We removed the variable, TB-confection, from bivariate/multivariate analysis as it is a common manifestation of ART failure itself, and not necessarily the cause for it.

2. Background: The author mentioned that the main contribution from this study was that the first-line ART was diagnosed in this study using viral load. The author should explain the potential weakness of using clinical or CD4 to diagnosed first-line ART failure in identifying the risk factors associated with it or the potential advantage on using viral load in this study to present a convincing rationale of this study.

We provided a better justification for the rational of this study by describing the fact that the use of a viral load as a better method to identify risk factors for ART failure. This method is more accurate than using clinical or immunologic criteria which have lower sensitivity to detect ART failure and thus the risk factors.

3. Methods:
   a. The variables used in the study were not clearly defined. There was no detail on the measurements of the variables which made reader difficult to interpret the results and assess the internal validity of the study.

   For example, the author mentioned that the controls were matched by age, sex and treatment duration. Is the treatment duration the duration on first-line ART? Was the ART interruption/discontinuation time during follow-up excluded from this duration?

   The variables used in the study are now defined in a separate heading “variables and measurements”. Controls were matched with cases by age, sex and ART treatment duration. We now included the definition of “Duration on ART”.

   b. In addition, it is not clear that whether the "TB co-infection" factor which is related to the main finding, was the patient having active TB disease during follow-up or active TB disease at the time of ART failure or patients having TB infection. Using the term" TB infection" can give wrong impression as people might interpret as having latent TB infection.

   TB co-infection implied an active tuberculosis disease and definition included.

   c. The author needs to be explicit on which variables were measured at baseline and which ones were measured at the time of ART failure or during the follow-up.

   The variables which were measured at baseline were baseline WHO stage and CD4 count. The rest were measured retrospectively after ART failure occurred during patient follow-up.

   d. There is no description on how the missing data was handled, whether no missing data or the patients with missing data were excluded.

   There were no missing data as we identified and filled them during the study period.

   e. I would suggest including the matching factor in the adjusted analysis because matching does not always remove the confounding of the matched factors. In addition, matching process in a case-control study can introduce bias by changing the association between the matching factor and the outcome and can create an association even if there were none before the matching was conducted.

   We included the matching factors in the adjusted analysis.

   f. Did the author check multicollinearity of factors that were included in the adjusted analysis? Patients who missed ART follow-up might be similar to those who discontinuation of ART.

We checked for multicollinearity for all the variables included in multivariate analysis.
4. Results
   a. The author could mention the factors/variables consistently to make the reader easy to follow. The data in the table are not completed. Some of the rows did not have total for each category and percent should be row or column percent and they need to be consistent. Some factors included in the bivariate analysis (table-2) were not presented in the descriptive table-1.

   The factors/variables in the table are now consistently written. We now used column percentages. Row total also included for all variables. Now all variables in table 2 were included in Table 1.

   b. The tables, their titles and legends should be stand alone. The author used some abbreviation which were not defined.

   We made changes in the tables. The data presented in the table are now complete and stand alone. The definition for all abbreviations used in the tables are now included.

5. Discussion:
   a. The author should identify limitations of studies regarding internal validity and generalizability of the study. The estimate for TB co-infection was very large compared to other studies that author mentioned, and author should comment on potential bias that might contribute to overestimation of the results. For example, imbalance distribution of patients with TB between case and control. The same suggestion for other factors that the study may overestimate or underestimate.

   We included the limitations of our study specifically stating an area where the odds ratio could be overestimated. For example, the odds ratio for repeated/persistent diarrhea could be overestimated as it was disproportionately high among the cases group.

6. Conclusion: I would suggest rephrasing the conclusion based on the results presented and the discussion section and being cautious on the limitation of the study. The author correctly pointed out, the factors that were associated with first-line ART failure can be the consequences of the ART failure. The conclusion on hygiene conditions to prevent diarrheal disease is not based on the results or was not discussed in the discussion.

   The conclusion was rephrased based on the recent adjusted analysis.

7. Minor comments:
   a. Page 1, Line 57-58: History of TB treatment or current TB treatment or had active TB?
      We now corrected that TB implies active disease/requiring curative therapy.

   b. Page 4, Line 6-7: Was failure diagnosed with one-time viral load testing? If that the case, patient might have low adherence or not take ART at the time of VL test and it might not necessarily failure on first-line ART.

      ART failure was determined by two viral load tests done three months apart with adherence counselling in between. As correctly pointed out one-time viral load determination could be as a result of nonadherence.

   c. Page 4, Line 27-28: Does discontinuation of ART mean patients stopped ART for certain duration and retook again or completely stopped ART?
      Discontinuation of ART meant that they discontinued for and resumed ART.

   d. Page 6, Line 34-35: Column title should be properly named.
We renamed the column title for Table 1 with the second column describing a total for each category.

e. Page 6, Line 38-39: The second column should be number of total males. Or add another column for total for each category.

The second column is modified to show number of total males.

f. Page 6, Line 41-42: For age and ART duration variables, the distribution should be assessed and presented accordingly instead of presenting mean. I believe age might not normally distributed.

We now presented the distribution in terms of median with interquartile range for the two variables age and ART duration.

g. Page 6, line 50-54: Why there are no %?

We now included its percentage.

h. The variables in the table-1 should be consistent in term of name, presentation (as continuous or categorical variables) and grouping.

Page 9, Line 20-21: "lead to first-line ART regimen"? It must be typo.

The variables are now written consistently, with all sociodemographic variables in the upper part of the table and the clinical variables in the lower part. We grouped related variables together. We removed the phrase “lead to first-line ART regimen”.