Author’s response to reviews

Title: Prevalence of anemia before and after initiation of antiretroviral therapy among HIV infected patients at Black Lion Specialized Hospital, Addis Ababa, Ethiopia: a cross sectional study

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Version: 1 Date: 09 Oct 2017

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A point by point response letter

BHEM-D-17-00029

Prevalence of anemia before and after initiation of HAART among HIV infected patients at Black Lion Specialized Hospital, Addis Ababa, Ethiopia: a cross sectional study.

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We would like to express our heartfelt gratitude to all reviewers /editors of this manuscript for their constructive comments which are extremely helpful to improve this manuscript. Here are point-by-point responses for reviewers of this manuscript. We have tried to provide a detailed response to each reviewer and describe the amendments that have been made to the manuscript text. The exact place in the manuscript text where these amendments can be viewed is indicated using sections, page numbers and line numbers. Additionally all changes (grammatical editing and other corrections) to the manuscript are indicated in the manuscript text by highlighting using yellow color.

Response for the reviewer reports:

A) Bamlaku Enawgaw, MSc (Reviewer 1):
We would like to express our deepest gratitude to Bamlaku Enawgaw (Reviewer 1) for your detailed, constructive and insightful comments. You provide necessary information in all sections of the manuscript text and all the given comments are appreciable. Here are our responses for your comments and questions.

1) Even though there are similar studies in the country, most of them didn’t address the prevalence of anemia fully. For example this study is different from most of the previous studies in that it addresses the prevalence of anemia as well as the types of anemia before and after initiation of the treatment. This study also assesses the prevalence of anemia by comparing the changes before and after the treatment in the same subjects (intrapersonal).

This eliminates many confounding variables. The study also supports the previous studies so it helps to strengthen findings from the previous studies.

2) Abstract section, Page 2, line 32: The study design is cross sectional. All information is collected at a point in time. Baseline information is collected from the subject’s medical records (in this case it could be retrospective) and after this, the subjects were sent to the laboratory for determination of RBC parameters at that moment.

3) Before conducting paired T-test, the normality of the data was checked and the data was normally distributed.

4) Page 2, Line 37 and Page 4, 101: “BD FACSCount” is corrected into “BD FACS Count” and other typographical errors are also corrected accordingly such as: Line 56, "disorders(2)" into “disorders (2)”, line 57, "counts(3)" into “counts (3)”, line 59, "survival(4)" into "survival (4)"….and others.

5) Auto spacing between the paragraphs was used.

6) Method section, page 4, Line 84: January 2017 to April 2017 is corrected into "January to April, 2017"

7) Method section, page 4, Line 85: In this study we include only adults. Recent study reports a considerably higher prevalence of anemia among HIV-infected antiretroviral-naïve adult
Ethiopians. However few studies were conducted in Ethiopia on the assessment of anemia, types and associated risk factors among HIV positive adult patients before and after initiation of HAART. Thus, the aim of this study is to determine the prevalence of anemia and related factors among HIV infected adult patients before and after initiation of HAART. This research was also conducted in adults with the aim to fill the gap in the literature about the changes in RBC parameters of HIV patients on HAART in Ethiopia.

8) Method section, page 4, Line 89: Patients transferred in from other health institutions were excluded. Because for patients transferred in from other health institution, we can’t get the baseline (pre ART) information. Their medical records were not available in the study area (Black lion specialized hospital) and it was difficult to compare the changes in RBC parameters with the baseline values.

9) Method section, page 5, Line 109 – 113: in this study, anemia was defined and classified according to the World health organization (WHO) criteria (Reference number 12).

10) Method section, page 5, Line 115: Paired T test was used after checking the normal distribution of the data and the data was normally distributed.


12) Result section: Page 7, Table 3 & page 8, table 4: Patients who had any opportunistic infections like TB were excluded from the current study. For baseline information we had reviewed their medical records carefully for the presence or absence of any opportunistic infections at that time. All the study subjects were starting HAART after they were free from any opportunistic infections. During the data collection period (after Starting HAART), the study subjects were evaluated by medical experts (internist) using history, physical examination and different laboratory investigations. If there is any opportunistic infection, they were excluded from the study.

13) Result section: Page 8, Line 166: table 4; One major chi-square requirement is, at least 80 % of the expected frequencies should exceed 5 and all the expected frequencies should exceed 1. In our study, this requirement is not met in the age categories.
14) Result section, Page 8, table 4: The CD4 count is classified based on the previous studies. In most studies CD4 count is classified as <200, 200 – 349 and ≥350. To give an example; Reference number 4, 11, 18 etc. We also classifies according to those studies for the purpose of comparing our finding with the findings of other studies.

15) Page 18, line 368-369, Figure 1: depending on your comments, the types of anemia before and after the treatment are presented in line graph.

16) Result section, Page 9, Line 171; Figure 1: Thank you for your comments. Paired T-test is not used for categorical variables. Table 5 is canceled and the data is presented using line graph.

17) Discussion, page 9, line 191-193: The prevalence rate of anemia before and after HAART initiation in this study was compared with different studies. The observed differences might be due to different reasons. One difference might be due to the heterogeneity of study population. This means in some studies, patients with opportunistic infection and on other medication were included (e.g studies conducted by Alamdo et al. 2015(Ref 4), Assefa et al. 2015(Ref 11) and Gedefaw et al.2013 (Ref 12)). Study conducted by Daka et al. 2013 (Ref 13) includes only patients with HAART regimen consists of zidovudine, stavudine and nevirapine.

There is also a difference in the socio demographic characteristics. In other studies, anemia was defined in a different way for example; in study conducted by Assefa et al, 2015 (Ref 11), anemia was defined as Hgb concentration less than or equal to 13.9 g/dl for adult males and less than or equal to 12.2 g/dl for adult females.

18) Discussion section, page 10, Line 200 – 210: The paragraph which deals about the prevalence of anemia across sex groups is modified.

19) Statement and references mismatching is corrected.

20) Discussion section, page 10, Line 222 – 223: the statement is modified according to reference 21.

B) Ebunoluwa A Adejuyigbe (Reviewer 2):
We would like to express our deepest gratitude to Ebunoluwa A Adejuyigbe (Reviewer 2) for your constructive and insightful comments. You provide important comments on this manuscript and all the given comments are appreciable. Here are our responses for your comments and questions.

1) Abstract section, page 2, line 34 and methods, page 4, line 86: The study units were selected using simple random sampling techniques. All of these individuals who fulfill our inclusion criteria had an equal chance to be included in the study. Among those on HAART, a total of 255 HIV infected adults were selected randomly using lottery methods and included in this study.

2) Patients who had any opportunistic infections like TB were excluded from the current study. For baseline information we had reviewed their medical records carefully for the presence or absence of any opportunistic infections at that time. All the study subjects were starting HAART after they were free from any opportunistic infections. During the data collection period (after starting HAART), the study subjects were evaluated by medical experts (internist) using history, physical diagnosis and different laboratory investigations. If there is any opportunistic infection, they were excluded from the study.

3) The interaction between cotrimoxazole and anemia is shown in result section, page 8, table 4. The patients were on cotrimoxazole for more than 3 weeks before they started HAART. Actually, all study subjects were not on cotrimoxazole for the same duration of time.

4) Discussion section, page 10, Line 200-210: Even though the difference is not statistically significant, there is a difference in the prevalence of anemia across sex groups before and after HAART initiation. The prevalence of anemia was higher in males before HAART initiation and in females after HAART initiation. This is explained in the discussion part clearly.

5) Result section, page 7, table3: We have reviewed all the tables in the manuscript. One major chi-square requirement is, at least 80% of the expected frequencies should exceed 5 and all the
expected frequencies should exceed 1. It is quite acceptable for an observed frequency to be 0, provided the expected frequencies meet the criterion. In all tables, we have checked this and other requirements and we used chi-square tests if and only if the criteria are satisfied.

6) References, page 14-16, line 293-356: Even though we have used many references from Ethiopia, we have also used references from other countries which are relevant to the current study. Most of our references (15 references) are from other countries. After your comment, other references which are from other countries and relevant to this study were reviewed by the authors such as Ref 15, 16. Using references from Ethiopia will also help for comparing the findings of other studies with the current study in a similar condition or setup. In the discussion part, we have tried to modify and add additional points in order to make it strong enough. We have reviewed the English language extensively.

C) Anthony Ikefuna (Reviewer 3):

We would like to express our deepest gratitude to Anthony Ikefuna (Reviewer 3) for your constructive and insightful comments. You provide important comments on this manuscript and all the given comments are really appreciable. Here are our responses for your comments and questions.

1) Method section, page 5, Line 109 – 113: Anemia was defined and classified according to the world health organization (WHO) criteria (12). The normal range of Hb in the study area is unknown. There is no strong study conducted in the study area to show the normal values of hematological parameters.

2) Result section, page 6, line 135-137, table 2: Red cell distribution width (RDW) measures the heterogeneity of red blood cell sizes. This study showed that after 6 months of HAART initiation, the mean value of RDW was statistically lower than the baseline values. Lower value of RDW after HAART initiation could be as a result of improved nutritional status of
the subjects and decreases in the underlying inflammatory state that leads to impaired erythrocyte maturation and anisocytosis (Ositadinma IM, Ikponmwosa OS, Okechukwu OC. Haemorheology and red cell indices in HIV positive individuals on antiretroviral therapy in delta state, Nigeria. International Journal of Current Research and Review. 2015; 7(12):24-30). The concentration of hemoglobin was increased after HAART initiation; this might due to HAART ameliorate many of the different factors that lead to anemia.

3) Discussion section, page 11, line 235-237: More subjects had macrocytic normochromic anaemia after 6 months of being on HAART than before treatment. This showed that the average MCV for patients on HAART were significantly higher compared to their before HAART. This is probably due to the effect of HAART particularly AZT which is responsible for the development of macrocytosis.

4) Declarations, page 13, line 288-289: The Funders of the study, Addis Ababa University, is acknowledged.