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: 2 Date: 13 Dec 2017

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BHEM-D-17-00029R1

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 the changes to the manuscript are indicated in the manuscript text by highlighting using Green color.

Response for the reviewer reports:

A) Bamlaku Enawgaw, MSc (Reviewer 1):

Depending on your comments, we have tried to include some points in the main manuscript which were addressed in the response section but not on the manuscript text such as:
1) Methods section, Page 4, line 90-96: Inclusion and exclusion criteria are included.

2) Statistical analysis section, page 5, line117: The statement which states about normality of data is included in the manuscript text.

3) Methods section, Page 4, line 94-95: The statement "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." is included in a summary way.

4) Definition of variables, page 5, line 111-115. For the definition and severity assessment of anemia, we have used the suggested references (12) and for the type of anemia based on morphological classification, we have used other reference (13).

5) Result sections, page 7&8, Table 3 & 4: For chi-square analysis of age group, some of the categories were merged to fulfill chi-square requirements. For ease of comparing the status of anemia among different age categories before and after HAART initiation, we use the same age categories before and after HAART initiation.

B) Anthony Nnaemeka Ikefuna, MBBS(Ibadan, Nigeria); FMCPaed(Nig); FRCP Edin (UK) (Reviewer 3)

1) Abstract section, page 2, line 41: Corrected accordingly.

2) Methods section, page 4, line 108: Corrected accordingly.

3) Discussion section, page 9, line 194 &195; Result section, page 6, Table 2: As noted, the improved hemoglobin concentration after HAART initiation could be explained by the positive effect of HAART on the survival of erythrocytes. Additionally after HAART initiation, there is less variability in the size of red blood cell which is explained by the decrease in the mean value of Red cell distribution width (RDW). RDW measures the variability in the size of red blood cells. Because of this fact, there is improved hemoglobin concentration and reduction in RDW after HAART initiation.
C) SCOTT DRYDEN-PETerson (Reviewer 4):

1) Methods section, page 5, line 111-115: Normal values for hemoglobin vary by sex. Not only the normal values but also the definition and severity of anemia are different among men and women. Based on WHO criteria, anemia was defined and it was further classified into mild, moderate and severe for both sexes using a different hemoglobin values. Because of the different definition and severity assessment of anemia used in men and women, important differences may not be obscured.

2) Result section, page 18, Figure 1 and line 168-171: The figure shows the types of anemia among HIV positive patients at baseline and after six months of HAART initiation. The percentage is calculated from the total anemic HIV infected patients at baseline as well as after six months of HAART initiation accordingly. The total sum of the percentage is 100% at the baseline and also after six months of HAART initiation. From the total anemic HIV infected individuals at the baseline (n=107), 71% had normocytic-normochromic anemia followed by 14.9%, 6.5%, 4.7% and 2.8% had microcytic-normochromic, Microcytic-hypochromic, Macrocytic-normochromic and Normocytic-hypochromic anemia respectively. Similarly after six months of HAART initiation, the types of anemia from the total anemic HIV infected patients after HAART initiation (n=29) were calculated and presented in line graph. We preferred to present the data using line graph because the line graph is especially useful for the study of variables according to the passage of time. It is suitable for depicting a consecutive trend of a series over a period of time. Box and Whisker Plot is another way to display information when the objective is to illustrate certain locations (variability) in the distribution. In this particular figure, our objective is to determine the effect of HAART on the types of anemia. Initially we have tried to present the data using tables but depending on the comment of other reviewer, it was changed into line graph presentation.

3) Discussion section, page 11, line 229-231: Azidothymidine (AZT) therapy is probably the most common cause of anemia in HIV-infected patients and megaloblastic maturation has developed as a result of AZT therapy. The effect of AZT is modest when taken as HAART than administered as a single dose. Different studies showed that there is an increase in macrocytosis after Zidovudine therapy. In agreement with these findings, this study also showed that the average MCV for patients on HAART were significantly higher compared to their HAART
naïve patients. The degree of macrocytosis is more to the group receiving zidovudine. HIV Patients on AZT based therapy showed higher mean values of MCV than patients who were on non AZT based therapy.

4) We have reviewed the English language

5) Background section, line 71-75: The statement “Azidothymidine (AZT) therapy is probably the most common cause of anemia in HIV-infected patients” is removed.

6) “ART” is now the most accepted term. “HAART” is removed and replaced by “ART” in the title and text.

D) Rupak Shivakoti (Reviewer 5):

1) Despite the presence of few reports on the hematological parameters of HIV positive individuals in Ethiopia, studies conducted to assess the prevalence of anemia both before and after receiving highly active antiretroviral therapy are limited. This study provides further information about the prevalence, risk factors and types of anemia before and after initiation of the treatment. It also compares the changes in Red blood cell parameters and CD4+ T cell counts after the treatment with the baseline values.

A finding from this study supports most of the previous studies and it helps to strengthen the results of those studies. Moreover, this study can serve as a reference material for further researches with regards to hematological profiles in HIV infected adult patients.

2) Method section, page 4, line 105-106: Baseline information of the study subjects were collected from their medical records. The instruments and the procedures used for analysis of blood cell counts were the same during the two time points. Even for the purpose of similarity, patients transferred from other health institutions were excluded. Because for patients transferred from other health institution, baseline information was recorded in a different way and also blood cell counts were determined by a different instrument.

3) The prevalence of anemia in the overall population of HIV-infected adults initiating ART in the hospital at baseline was unknown. One of our objectives is to determine the prevalence and
risk factors of anemia at the baseline. The population is too large for us to consider collecting information from all its members. Among those on ART, a total of 255 HIV infected patients taking ART for at least six months were selected randomly and included in this study. Baseline (pre ART) values of blood cell counts were collected from their medical records. Based on this information the prevalence of anemia at the baseline was determined, which is about 41.9%. Since we have used simple random sampling methods, we can generalize to the whole populations.

4) Methods section, page 4, line 84-85: Black Lion Specialized hospital is selected based on the availability of patients from all parts of the country as it is referral and specialized teaching hospital in Ethiopia as well as the ease of access to standardized facilities and sufficient availability of the data. Our study population will be the representative of HIV-infected adults in the region because they were not from a more specific population.

5) Result section, page 7&8, Table 3&4: The chi-square is also useful in making statistical inferences about categorical data in which the categories are two and above. Anemia by various characteristics was presented using a chi square (χ2) distribution at baseline and after HAART initiation. Because of budget constraint other variables which were listed in your comments such as viral load, hypoalbuminemia were not assessed.

6) Result section, page 8, table 4: As it is indicated in the table title, CD4 count which was used in table 4 is the mean values after six months of HAART initiation. WHO staging were not shown in table 4 because after initiation of the treatment, the patients are on treatment stages. According to the treatment guidelines, HIV infected patients after initiation of the treatment are classified by the type of treatment not by WHO clinical staging.

7) Result section, table 3&4: p-values of 0.000 were removed and replaced with < 0.001

E) Alauldeen Mudhafar Zubair, M.D. (Reviewer 6):

1) As you said, age and sex matched healthy controls are important. Having a control group is also very important to exclude confounding variables. But age and sex matched healthy controls will not be mandatory for this study because this study is conducted before and after the
treatment on the same subjects (intrapersonal). This eliminates many confounding variables. The objective of this study is also to determine the effect of HAART on the prevalence of anemia. For this purpose HAART naïve patients (study subjects at baseline) were taken as a control group and we have determined the changes in RBC parameters, prevalence of anemia and risk factors after six months of HAART initiation.