Author's response to reviews

Title: Determination of Hematological and Immunological parameters among HIV positive patients taking HAART and HAART naive in the ART clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: a comparative cross-sectional study

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Version: 3
Date: 3 January 2014

Author's response to reviews: see over
Dear BMC Hematology editorials,

All the comments and suggestions given by each reviewers to our manuscript entitled “Determination of Hematological and Immunological parameters among HIV positive patients taking HAART and HAART naïve in the ART clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: a comparative cross-sectional study” are very important and valuable to make the paper scientifically sound. We the authors tried to correct and include all the comments given by each reviewer’s point-by-point. In addition, the hard copy of this revised manuscript had been edited by English language expertise. For the convenience purpose, we attached/uploaded the point-by-point reply to each reviewers and the revised manuscript.

With regards
Author’s Response to Reviews

Determination of Hematological parameters among HIV positive patients taking HAART and HAART naïve in ART clinic of Gondar University Hospital, Gondar, North West Ethiopia: Comparative cross-sectional study

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Version: 3 Date: 31December 2013

Author’s Response to Reviews: See over
Reviewer's report

Title: Determination of Hematological parameters among HIV positive patients taking HAART and HAART naive in ART clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: Comparative cross-sectional study

Version: 2 Date: 26 November 2013

Reviewer: William Kwame BoakyeAnsahOwiredu

Reviewer's report:

REVIEWER’S REPORT ON: DETERMINATION OF HAEMATOLOGICAL PARAMETERS AMONG HIV POSITIVE PATIENTS TAKING HAART AND HAART NAÏVE IN ART CLINIC OF GONDAR UNIVERSITY HOSPITAL, GONDAR, NORTH-WEST ETHIOPIA: COMPARATIVE CROSS-SECTIONAL STUDY’

Minor Corrections:

Title: Could be changed to ‘Haematological and Immunological changes among HAART-Naïve HIV patients and those on HAART at the Gondar University Hospital, North West Ethiopia’

- Accepted and done as per the given comment

Affiliation: 1 Department of Hematology & Immunohematology, School of Biomedical and Laboratory Sciences, College of Medicine and Health sciences, University of Gondar, Ethiopia

- Accepted and done as per the given comment

2 Tropical and Infectious Disease Expert. This should be substituted with Departmental Affiliation i.e Department of Clinical Microbiology

- Accepted and done as per the given comment
ABSTRACT

Background: Line 3 should read: …commonest hematological abnormalities resulting from HIV infection. Lines 5-6 does not read well.

- Line 3: Accepted and done as per the given comment
- Line 5 – 6 Accepted and corrected as: Therefore, this study aimed to compare hematological and immunological parameters in HIV positive patients taking antiretroviral therapy and those treatment naïve patients in Gondar University Hospital.

Methods: This should include the number of study participants. This section should be re-written for clarity

- Accepted and re-written by including study participants

Results: First sentence should be expunged.

- Accepted and done as per the given comment

Conclusion: Line 4 should read……investigated for hematological and immunological changes following which appropriate therapeutic interventions could be adopted in their management.

- Accepted and done as per the given comment

INTRODUCTION

Paragraph 1 line 3 should read ….different types of hematological abnormalities are common

- Accepted and done as per the given comment

Paragraph 3 lines 1 should read: Thrombocytopenia is the second most frequent complication of human immunodeficiency virus infection ……Accepted and done as per the given comment

Paragraph 3 line 3 should read: Thrombocytopenia correlates with low CD4 cell count

……..Accepted and done as per the given comment
Paragraph 4 lines 1-3 should read: Neutropenia is the most common leucopenia occurring in HIV infected individuals. It may occur in 10-30% of HIV patients with advanced disease (Ref).

- Accepted and done as per the given comment

Paragraph 4 line 7 should read: ……including cytomegalovirus, tuberculosis, histoplasmosis and leishmaniasis may cause leucopenia

- Accepted and done as per the given comment

Paragraph 5: Mention should be made of the following paper: Ferede G and Wondimeneh Y: Prevalence and related factors of anemia in HAART-naïve HIV positive patients at Gondar University Hospital, Northwest Ethiopia. BMC Hematology 2013 13:8

- Accepted and recent data is included

Paragraph 5 line 10-11 should read: This study, therefore, assessed the haematogical parameters ………Accepted and done as per the given comment

METHODS

Paragraph 1 lines 1-2 should read: This cross-sectional study was conducted at the Gondar University Hospital which is a teaching and referral hospital ……..

- Accepted and done as per the given comment

Paragraph 1 lines 4-5 should read: …follow-up care in the ART clinic during the period. The study was conducted from February to May 2012 on adult HIV positive individuals who were managed at the ART clinic during the study period.

- Accepted and done as per the given comment

Paragraph 2 line 2 should read: ……who were on HAART for a period of 6 months or more.
Data was collected using a structured pre-tested questionnaire. The questionnaire consisted of socio-demographic characteristics of ….

- Accepted and done as per the given comment

4 ml of venous blood was collected by an experienced…..

- Accepted and done as per the given comment

Hematological parameters were determined using the hematology analyzer Cell-Dyn 1800 (abbott Laboratories Diagnostics Division, USA) where as the immunological (CD4+ T cells) were assayed using the BD FACSCOUNT system (Becton Dickenson and Company, California, USA).

- Accepted and done as per the given comment

To ensure good quality data, pre-testing of the assays was done on patients being managed at the Gondar University Hospital before the study. The performance of the hematology nalyzer and the BD FACSCOUNT system were controlled by running quality control material alongside the study participant’s sample. In addition, all flagged specimen were subjected to manual, differential and morphological assays to confirm the results.

- Accepted and done as per the given comment

Ethical Clearance

The ethical clearance should read as follows:

Ethical clearance was obtained from the Institutional Ethics Review Board of the University of Gondar. Permission for the conduct of the study was also obtained from the University Hospital. After informing study participants of the objectives of the study and assuring them of confidentiality of their data, written informed consent was taken from all the participants. To ensure confidentiality, the study participants were identified by codes and the data was not accessible to unauthorized persons. Accepted and done as per given comment
Major Corrections:

RESULTS

1. The data should be analyses by a Biostatiscian
   - The Biostatiscian was consulted and they suggest independent T-test because the objective of the study is comparison of hematological and immunological parameters between HAART naïve and those on HAART HIV positive patients. Also the parameters are normally distributed.

2. Gender differences in the haematological changes should be explored

3. The correlation of the changes with age should be explored.
   - We share the reviewers comment but the objective is comparison of hematological and immunological parameters between HAART naïve and those on HAART HIV positive patients. Age and sex were not considered because primary objective is to determine the effect of HAART on hematological and immunological parameters correlation between age and sex with hematological parameters are not my objective.

Table 1: Note that MPV is statistically lower among patients on HAART compared to those who are HAART-naïve
   - Accepted and done as per comment

DISCUSSION

This section may be affected by the additional information which may be obtained if analysis is re-done: Re-done by considering comments also given by other reviewers

CONCLUSION

This section should be re-done. Currently it is just a re-stating of the results.
   - Re-done by considering comments also given by other reviewers

The limitations of the study have not been stated: it is included after the discussion
Reviewer's report

Title: Determination of Hematological parameters among HIV positive patients taking HAART and HAART naïve in ART clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: Comparative cross-sectional study

Version: 2 Date: 30 October 2013

Reviewer: Romanee Chaiwarith

Reviewer's report:

The authors conducted the study plan to determine hematological parameters among HIV infected patients taking cART and cART naïve in Northwest Ethiopia. There are existing data regarding hematological abnormalities among HIV-infected worldwide. However, the authors would like to fill the gap of lacking the data in Ethiopia.

Major Compulsory Revisions

1. Methods

1.1 There are 9000 people living with HIV and receiving care at the clinic, why were there only 290 patients included in the study? Did the authors do the sample size calculation? And how did the author sampling 290 patients from 9000 people?

- Sample size to be taken from each group was assumed to be equal and it was calculated by using two population proportion formula by Epi Info version 2002. By assuming a 95% confidence level, 80% power and 5% level of significance and by taking 63% in HAART-naïve patients and 46% their counterparts on HAART incidence of anaemia study done Ghana from September 2008 to September 2009. Finally we got 145 from each group.

1.2 Why did the authors exclude only patients on medication i.e. vitamin supplements and tuberculosis from the studies? How about patients taking co-trimoxazole or other medications
for opportunistic infections particularly prophylaxis, as these medications particularly co-
trimoxazole had an effect on hematologic abnormalities:

- We share the author’s comment but it is difficult to exclude and both HAART naïve and
  those on HAART were taking it, has equal effect on both groups.

1.3 Statistical analysis: Are all continuous data normally distributed? The authors stated that
independent T test was used to compare means of each hematologic parameter. If the data are not
normally distributed, did the authors still use independent T test?

- Yes it is normally distributed and we can use independent T test to compare means of
each hematologic parameters.

2. Results

2.1 Paragraph just before table 3. The number in text in the paragraph and in table 3 are
difference, please make correction.

- The comment is valuable and it is accepted. Based on this comment the percentages in
  the table are corrected.

3. Discussion

3.1 The discussion should be revised. The discussion mainly mentioned about comparing the data
from this study and the previous studies, but the authors did not explain the possibility why the
results were different. Furthermore, the authors mostly re-state the results section.

- Accepted and done as per the comment

3.2 The third sentence of page 9, ….. pancytopenia (0.7%) was lower than study done in India
6% in 2008 and 1.6%. The question is 1.6% for what year?

- Accepted and corrected as per the comment and it is 2002.

4. Conclusions

4.1 The conclusions must be revised. The authors should conclude the main findings and leave
the readers take home message, NOT just re-state the result section

- Accepted and corrected as per the comment
4.2 The authors left the sentence “Other associated factors for anemia, leucopenia, and thrombocytopenia must be addressed for the future”. However, if the authors collected those factors, it could be done in this study and did not need future studies.

- The reviewers comment is interesting but the intention of this study was comparison of hematological parameters so that other associated factors were not considered.

Minor Essential Revisions

1. Introduction

1.1 References are needed to be quoted, for examples, paragraph 3 and 4: Done as per the comment.

1.2 The authors mentioned that several factors play a role in the development of anemia in patients with HIV, including chronic disease, opportunistic infections, and certain nutritional deficiencies. I think toxicities from medications also play an important role and the authors should mention about this factor. : Done as per the comment

2. Other comments

2.1 Spelling and grammar are needed to be carefully checked: Done as per the comment

2.2 All abbreviations mentioned at the first time must be spell out: Done as per the comment

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests
Reviewer's report

Title: Determination of Hematological parameters among HIV positive patientstaking HAART and HAART naive in ART clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: Comparative cross-sectional study

Version: 2
Date: 15 November 2013
Reviewer: Ivan Gentile

Reviewer's report:

General comment: The authors aimed at evaluating hematological parameters in a cohort of naïve as well as treated HIV-positive patients. The subject matter is fairly interesting. However, the paper is not well written and it needs to be highly improved before recommending publication.

Major Compulsory Revisions

1) The aim of the study must be stated thoroughly. If a comparison between groups has to be done, the authors must state which are the variables to be compared, which are the groups, etc
   - The variables compared here are Hematological parameters (Total and differential WBC, RBC, Hgb, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, and CD4).
   - The groups are HIV positive treatment naïve and those on HAART

2) Statistical analysis needs to be improved. The authors should provide different ways of presentation and different tests in case of Gaussian and non-Gaussian distribution of quantitative data. A test for comparison of qualitative data is lacking! As both HAART status and advanced disease seem to impact on the various rates of hematological abnormalities, this reviewer suggests to include significant variables in a logistic regression model to assess to actual role of each variable.
   - The Biostatistician was consulted and they suggest independent T-test because the objective of the study is comparison of hematological and immunological parameters between HAART naïve and those on HAART HIV positive patients. Also the parameters are normally distributed therefore independent T-test is sufficient to describe the comparisons.

3) A table with demographic data in the two groups is needed. 4) The authors performed a useful comparison between patients receiving and not receiving HAART. However this reviewer would perform at-least a separate analysis between those received AZT-based HAART vs. other types of HAART.
The author thanks the reviewer and demographic data is included. On the other hand the intention (objective) of the study is comparison of hematological and immunological parameters between HIV infected patients who were on HAART and those who are treatment naïve.

4) Too many comparisons are performed. This fact increases the risk of type I error. This reviewer suggests to avoid useless comparisons (e.g. RDW, MCH, etc.).
- These variables are important especially to RDW because this variable is used to assess how red cell varies in size. So that especially patients on HAART expected to have macrocytic anemia in turn results red cell size variation due to fragmentation of cells.

Minor Essential Revisions

1) Level of English language is poor throughout the manuscript. E.g. at the end of introduction: “this study was assess…”; in the abstract, section “method”: “…May 2012 HIV …”; line 4 of statistical analysis: “HAAT”; etc.
- Accepted and corrected as per the comment
2) The abstract is poorly informative and should be totally rewritten
- Re-written
3) P values are lacking in table 2. In table 3 this reviewer suggests to include absolute percentages and not relative ones
- The reviewer comment is appreciated and it is included

4) Conclusion is too long. It should be one or two-sentence long.: Corrected as per the comment

5) References should be updated and complete. This reviewer would add at-least the following:

e. Servais J, Nkoghe D, Schmit JC, Arendt V, Robert I, Staub T, Moutschen M, Schneider F, Hemmer R. HIV-associated hematological disorders are correlated with plasma viral load
and improve under highly active antiretroviral therapy. JAcquire Immune Defic Syndr 2001; 28:221-225.


- The authors gave great full thanks for the reviewer suggestion and some of the literatures are used. Many thanks!!!!

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests
Reviewer's report

Title: Determination of Hematological parameters among HIV positive patientstaking HAART and HAART naive in ART clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: Comparative cross-sectional study

Version: 2
Date: 24 November 2013

Reviewer: Heather Leitch

Reviewer's report:

In this study, Enawgaw et al examine the frequency of cytopenias in patients with HIV infection receiving HAART compared to patients not receiving HAART. Findings include a lower incidence of anemia with HAART but higher incidence of thrombocytopenia and neutropenia.

There are significant issues in this paper with content and reporting of the information. The problem that overrides these issues, however, is: what is the clinical significance of these findings? As a practicing clinician, I’m not interested in how often the neutrophils and platelets are just below the lower limit of the normal range, I’m interested in how often they’re low enough to cause clinical problems requiring intervention and/or how often they’re an indication of another underlying problem such as infiltration of the marrow by infection or malignancy, requiring investigation. In the absence of clinical significance, the authors need to clearly demonstrate that there is purpose and significance to their findings, and this is not done.

- When white blood cells decreased, it reduces the body's resistance to many opportunistic infections and the patient becomes more susceptible to bacterial infections and needs medical attention, the condition may become life-threatening.
- On the other hand if the number of platelets is too low, excessive bleeding can occur. However, if the number of platelets is too high, blood clots can form (thrombosis), which may obstruct blood vessels and result in such events as a stroke, myocardial infarction, pulmonary embolism or the blockage of blood vessels.

Writeup: the paper needs to be re-written by someone who is fluent in English with attention paid to detail including pronouns, lack of capitalization of words that should be capitalized, taking away capitalization that appears in the middle of the sentence, typographical errors, punctuation, clear expression of ideas, etc. For example, on the title page: the ART clinic; a comparative cross-sectional; & Immunohematology; big gap between the third and fourth author. In the rest of the manuscript, the details that need correction are too many to list here.
The Authors thank the reviewer and the possible errors are corrected.

Of 9000 patients attending the clinic, the study population was 290. Which patients were chosen for the study? It can’t have been merely the ones that attended during the period mentioned, as there were 145 cases and 145 controls. This did not happen randomly, there was clearly some matching done. Which characteristics were matched for? What were the baseline demographic and clinical characteristics?

The study populations were the one that attends the clinic during the study period. Being on HAART and being naïve was used for selection of 145 patients on HAART and 145 treatment naïve patients. As indicated by the reviewers’ baseline demographic data is included in the paper.

What medications constitute HAART in Ethiopia? What is the relative access of particular patients to HAART and did this influence the study population and therefore the results?

In Ethiopia specifically in Gondar University hospital ART clinic some of the medication combinations used are the following:

- AZT + 3TC + NVP
- TDF + 3TC + EFV
- TDF + 3TC + NVP
- d4T + 3TC + NVP
- AZT + 3TC + EFV
- d4T + 3TC + EFV
- ABC + ddI + LVP/r
- AZT + 3TC + ABC

One would expect anemia in patients on HAART to be macrocytic, but normocytic anemia was found in nearly 20%, suggesting that there might be a second process contributing to the anemia. What investigations did the authors do to rule out iron deficiency? Hemoglobinopathies? Inherited membrane disorders? Other nutritional deficiencies, for that matter?

The calculation on table was corrected and macrocytic-normochromic anemia was 58.8% and normocytic-normochromic anemia was 29.4% in patients on HAART, thus it is as expected.

As indicated by the reviewer it is important to rule out iron deficiency. Hemoglobinopathies, inherited membrane disorders and other nutritional deficiencies but it is difficult in our resource limited settings and this is mentioned in limitation.
What is the OAUTHC?

- It is in Nigeria and difficult to get full form of the abbreviated word. So that it is removed.

The discussion is a listing of comparisons to other studies without context or discussion of in what way the study populations might differ. The conclusions are a repetition of the results, again without context.

- This comment is accepted and corrected.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests