Author’s response to reviews

Title: Prevalence of Malaria and Anaemia among HIV infected pregnant women receiving Co-trimoxazole prophylaxis- A cross sectional study in One District in Tanzania.

Authors:

Vicent Manyanga (vcenetz@yahoo.com)
Omary M Minzi (Minziobejayesu@gmail.com)
Billy Ngasala (bngasala@muhas.ac.tz)

Version: 3
Date: 18 March 2014

Author's response to reviews: see over
Biomed Central Editorial,

Dear Sir/Madam,

REF: Response to the comments from the reviewers of the manuscript titled ‘Prevalence of malaria and anaemia among HIV infected pregnant women receiving co-trimoxazole prophylaxis: a cross sectional study in one district in Tanzania’ (MS 137202895113431).

Reference made to the above heading.

Kindly find the responses underneath each of the reviewer’s comments:

Reviewer no. 1: Alexandre Manirakiza

A. Minor Essential Revisions

1. Throughout the article, we find the word spacing errors, (e.g. “avoidsulpha” should be “avoid sulphas”; “policysince2011” should be “policy since 2011” etc.) that should be reviewed.

   Response: Spacing errors have been corrected.

2. Abstract: It seems to me that the number of keywords is high; I suggest “co-trimoxazole, malaria, anaemia, pregnant women”

   Response: ‘HIV’ was also an important keyword because all the subjects in the study were HIV infected (page 3, line 5).

3. Suggest revise the objective of the study as follows: the main objective consisted to “evaluate the prevalence of malaria, anaemia” and then, they should state that “secondly, they assessed the associated factors among HIV infected pregnant women receiving co-trimoxazole prophylaxis....”

   Response: The main objective of the study was to determine the prevalence of malaria and anaemia among HIV infected pregnant women receiving co-trimoxazole prophylaxis. The specific objectives were: to determine the prevalence of malaria and anaemia; and to determine the risk factors...
associated with malaria infection and anaemia among HIV infected pregnant women receiving co-
trimoxazole prophylaxis (page 4, line 6-8)

4. Methods: “anaemia (hemoglobin11g/dl)” that should be “anaemia (haemoglobin<11g/dl)

Response: Correction has been made (page 2, line 12).

5. The study period in the abstract, methods and results is not the same; please review.

Response: The study period has been corrected, now it reads February to April 2013 (page 2, line
11; page 4, line 11 and page 9, line 3).

6. Table 2: Please review the percentages.

Response: The percentages have been corrected (page 26).

B. Major Compulsory Revisions

1. Background: it is not easy to the lecturer to find the interest of the paragraph on malaria rapid
tests, suggest dilating it; or at least putting it in the logic of rational of this study.

Response: The paragraph has been modified to reflect the objectives of the study. The paragraph
was aimed at giving the justification as why MRDT was chosen and not the microscopy in the
diagnosis of malaria. (Page 3, paragraph 4)

2. Methods (Study population): Authors should precise how was made the sample size?

Response: The formula and additional information about the sample size calculation has been
added. Sample size for this study was calculated using the formula for cross-sectional study based on
the study done in Uganda. In that study, the prevalence of malaria infection among HIV infected
pregnant women using co-trimoxazole prophylaxis (x) was found to be 19%. The formula used was:

\[ n = \frac{z^2 x(100-x)}{\varepsilon^2} \]

\( n = \) Minimum sample size, \( z = \) point on standard normal distribution curve corresponding to
significance level of 5% (its value is 1.96), \( x = \) previous prevalence of malaria infection among HIV
infected pregnant women receiving co-trimoxazole prophylaxis (19%), \( \varepsilon = \) margin of error on x (set
at 4%). A total of 420 consented subjects on different trimesters and varied gravidities were enrolled
and entered in the final analysis (page 4, paragraph 4).

3. Methods: The authors need to be precise on criteria of inclusion and non-inclusion. Stating that
“Pregnant women with conditions like haematological disorders e.g. sickle cell disease, vaginal
bleeding and mental disorders were excluded in the study” seems less specific.
Response: Inclusion criteria were: HIV infected and being pregnant, co-trimoxazole prophylaxis use for more than four weeks and provision of consent. Exclusion criteria were: sickle cell disease, bleeding disorders, vaginal bleeding, and other severe medical condition. (Page 4, line 21 and page 5, line 8)

4. Results: The authors stated that they have collected the data on iron supplementation; this data should be considered in the analysis.

Response: Data on the use of iron supplements were collected and included in the analysis (see table 4, page 28). There was no a statistically significant difference in the prevalence of anaemia between the subjects who were using the supplements and those who did not (p=0.567). However, the adherence to the use of iron supplements was not assessed and the information was self-reported; although, antenatal records were used to verify prescriptions of medications.

5. Discussion: comparison of the effect of a strategy based on data from a cross-sectional study has limitations. The authors should discuss some of these limitations.

Response: The study limitations have been mentioned. We pointed out three limitations. First, the potential residual confounding effects of the unmeasured factors like the presence of other parasitic infections or pathological conditions. Second, the nature of the study design in which there has been just one point of measurement of both the outcomes and exposure and hence no causal inferences can be made from the findings of the study. Third, the potential for information bias because measurement of adherence to the medications was done by a self-reported method and no drug levels in the blood were measured (page 15, line 10-19). In addition prevalence of malaria from peripheral blood was tested using mRDT with persistent positivity after clearance of infections.

C. Discretionary Revisions

1. Methods (Study design and area): what is the burden of malaria in Kinondoni municipality?

Response: Kinondoni municipality is categorized as an area with stable malaria transmission (holoendemic), with transmission occurring during the entire year. Malaria is the leading cause of both the outpatient visits and inpatient admissions (page 4, line 16-19).

2. Methods: The authors stated that “The targeted subjects were HIV infected pregnant women receiving co-trimoxazole prophylaxis for more than four weeks”. Why the authors chose this cut-off at more than four weeks for co-trimoxazole prophylaxis?

Response: The four weeks was chosen because it is the duration that is considered necessary before the prophylactic effect against malaria infection can be assessed. This gave time to rule out the chances of previous infections because it takes about two to three weeks to develop
malaria symptoms after getting infected by the Plasmodium parasites.

Quality of written English: Needs some language corrections before being published
Response: Language corrections have been made in the whole manuscript.

Reviewer 2: Peter Aduloju

1. Background

i. Line 6- experiences
Response: corrected (page 3, line 11)

ii. Line 11- avoid sulphur-related adverse reactions
Response: the word ‘sulpha’ has been omitted (page 3, line 16)

iii. Line 12- sulphur
Response: the word ‘sulpha’ has been omitted (page 3, line 16).

iv. Line 13- policy since 2011
Response: changed to ‘policy since the 2011’ (page 3, line 7)

v. Line 14- anaemia among
Response: a space has been added between the words (page 3, line 19).

vi. Line 18- targeting histidine
Response: a space has been added between the words (page 4, line 2).

vii. Line 21- MDRT can
Response: the phrase has been omitted.

2. Methodology

The authors should write briefly about the geographical location of the site of research and seasons in the area with respect to rainfall pattern and yearly transmission of malaria infection.

Response: Information about the study area has been expanded. Kinondoni has a hot and humid tropical climate with two rainfall seasons: an intense one observed from the month of March to May, and a mild one in November and December. The average temperature ranges from a minimum of 18.1°C to a maximum of 32.1°C. The average annual rainfall is 1,115 mm. Kinondoni municipality is categorized as an area with stable malaria transmission (holoendemic) with transmission occurring during the entire year. Malaria is the leading cause of both the outpatient visits and inpatient admissions (page 4, line 11-19)
3. Data collection
   i. Line 1- used to
      Response: changes have been made (page 6, line 4).
   ii. Line 7- was collected
      Response: a change has been made (page 6, line 10).

4. Adherence to co-trimoxazole prophylaxis
   Line 2- Morrisky medication
   Response: No change was made because the correct spelling is Morisky (page 6, line 13).

5. Data analysis
   Line 1- called IBM
   Response: A space has been added between the two words (page 7, line 18).

6. Discussion
   Last paragraph, line 4- or worm
   Response: No change has been made because the correct spelling is de-worming (page 15, line 6).

7. Conclusion
   Line 1- this study
   Response: The change has been made (page 15, line 21).

Quality of written English: Needs some language corrections before being published
Response: Language corrections have been made in the whole manuscript.

Reviewer 3: Gilbert Kongola

Minor essential revisions

Typographical errors

1. Background
   1.1 Line 5 …this population experiences the s is missing
      Response: the letter ‘s’ has been added to the word experience, now it reads ‘experiences’ (page 3, line 11)
   1.2 Line 10 ……advised to avoid sulpha the two words are joined in the script
      Response: the word ‘sulpha’ has been omitted and a space has been added (page 3, line 16).
   1.3 Line 11……adverse reactions ….. the two words are joined in the script
1.4 Line 13….effects particularly anaemia among …. The two words are joined in the script.

Response: a space has been added between those two words (page 3, line 16).

1.5 Line 18 ….. MRDT are made up of specific… the of is missing in the script.

Response: the phrase has been omitted.

1.6 Line 26. The study reports the prevalence of malaria, anaemia and the associated factors among HIV infected pregnant women. What are the associated factors?

Response: The word ‘risk’ has been added and the phrase reads as ‘The study reports the prevalence of malaria, anaemia and the associated risk factors among HIV infected pregnant women’ (page 4, line 6-8).

2. Methods

2.1 Study design and study area

2.2 Line 1 The design was a cross sectional study and not across as it appears in the manuscript.

Response: change has been made (page 4, line 11).

2.3 Line 4…… in-patient admissions the s is missing in the text.

Response: The letter ‘s’ has been added (page 4, line 19).

2.4 Sampling procedure

2.5 Line 3 health facilities to be included the words to be are missing in the script.

Response: The words ‘to be’ have been included in the script (page 5, line 13).

2.6 Data collection methods

2.7 Line 1… schedule was used to obtain…. the words are joined in the text.

Response: A space has been added between the words (page 6, line 4).

2.8 line 2 these includes the s should be omitted.

Response: The letter ‘s’ has been omitted (page 6, line 5).

3. Results

4. Discussion

4.1 Line 14 …..pattern showed in our study……. the word showed should be replaced by seen.

Response: The word ‘showed’ has been replaced by ‘seen’ (page 11, line 3).

4.2 Line 28 …. Kapito-Tembo et al reported a similar findings ….. the letter should be omitted.

Response: change has been made (page 12, line 6-8).
4.3 Line 39..replace the phrase “this variation are not clear” by these variations are not clear

Response: change has been made (page 12, line 17).

4.4 Line 60…..other infections were therefore more vulnerable to becoming anaemic [3,4] this phrase should replace the phrase other therefore they were more vulnerable to anaemia infections [3,4] which appears at the beginning of the same line.

Response: The new phrase reads as ‘…other infections were therefore more vulnerable to became anaemic’ (page 13, line 11-13)

4.5 Line 65……therefore another cause of anaemia could also be ….. The two words are joined in the text. Still on the same line …..chronic illness should read chronic illnesses.

Response: A space has been added between the two words and the letter ‘s’ has been added to the word illness (page 13, line 17-19).

5. Conclusion-

Minor essential revisions

Comments

1. The question posed by the authors has not been well defined. Use of insecticide treated bed nets (ITN) could have contributed to the low prevalence of malaria in the study group, but the authors have put a lot of emphasis on prophylactic use of co-trimoxazole. Have the authors disregarded the contribution of ITN in preventing malaria infection?

Response: The contribution of the ITN was not disregarded in this study because its data were included in the analysis as seen on the table 3 (page 27). We agree sleeping under the ITN could have contributed to the low prevalence of malaria because 90.5% (380/420) of the subjects reported to be using ITN. Among them only 3.9% had a positive malaria test as compared to 10% (4/40) of those not sleeping under the ITN tested positive for malaria infection. However, this finding was not statistically significant (p=0.0960 because our study was not statistically powered to detect this difference. The methods used were appropriate, but inclusion and exclusion criteria for study participants were not defined and hence the low prevalence of malaria and the high prevalence of anaemia could not be adequately explained not knowing the criteria for participants. Other parasitic infections could have caused anaemia and the ITN could have been the reason for the low prevalence of malaria.

Response: The inclusion and exclusion criteria have been mentioned clearly. The inclusion criteria were: HIV infected and being pregnant, co-trimoxazole prophylaxis use for more than four weeks and provision of consent. Exclusion criteria were: sickle cell disease, bleeding disorders, vaginal bleeding and any severe medical condition (page 4, line 21 and page 5, line 8)
3. No stool examination was carried out to rule out other parasitic infestations

4. Response: This is one of the limitation of this study, it was not possible to rule out all possible causes of anaemia like stool examinations for helminth. This made it difficult to collect stool samples at the same time. Nonetheless, de-worming drug (specifically mebendazole) is usually given routinely as part of antenatal service; therefore data on the use of this drug were collected and entered in the analysis. Response to the comments on ITN was made on the part (1) above (page 11, line 5-16).

5. The data as presented is sound

6. The manuscript has adhered well to the standards for reporting and data disposition

7. The role of insecticide treated bed nets has not been well discussed

Response: See the response in the part (1) above (page 11, line 5-16).

8. Limitations of the work have not been well stated. Use of the insecticide treated bed nets and presence of other pathological conditions in the study patients was confounding factors in the study

Response: The study limitations have been mentioned. We pointed out three limitations. First, the potential residual confounding effects of the unmeasured factors like the presence of other infections or pathological conditions. Second, the nature of the study design in which there has been just one point of measurement of both the outcomes and exposure and hence no causal inferences can be made from the findings of this study. Third, the potential for information bias because measurement of adherence to the medications was done by a self-reported method and no drug levels in the blood were measured (page 15, line 10-19).

9. The authors had very well acknowledged the work of other people upon which they have built their own work.

10. The abstract and title conveys the results that have been obtained from the study, but the interpretation of the findings is made difficult by the limitations pointed out above.

Response: Yes, the above mentioned limitations could have affected the strength of the findings. We attempted to control for those confounding factors in the multivariate analyses (see table 4, page 28), but there could still be residual confounding effects of unmeasured factors. One point should be clear that our findings did not disregard other malaria and anaemia control interventions because we included in the analysis the data on ITN use, iron supplements and de-worming drugs use. The findings showed the additional benefit of good adherence of co-trimoxazole prophylaxis. To our understanding, this is the first study to analyse the benefit of good versus poor adherence of co-trimoxazole prophylaxis among HIV infected pregnant women.

11. The writing is acceptable
Minor essential revisions

1. Methods

Study population

Inclusion and exclusion criteria were not clearly defined and this has given the difficulty in interpreting the results. Can the criteria be defined? Study participants provided blood samples for determination of haemoglobin and evidence of Plasmodium infection, other parasites were not looking for. No stool was collected for examination of intestinal parasites which could be responsible for the observed anaemia. Is there any reason for this omission?

Response: The inclusion and exclusion criteria have been stated clearly. The inclusion criteria were: HIV infected and being pregnant, co-trimoxazole prophylaxis use for more than four weeks and provision of consent. Exclusion criteria were: sickle cell disease, bleeding disorders, vaginal bleeding and any severe medical condition (page 4, line 21 and page 5, line 8).

No stool examination was done because the study was a cross sectional in design and the subjects were met just once making it difficult to collect stool samples at the same time. However, de-worming drug (specifically mebendazole) is usually given routinely as part of antenatal service; data on the use of this drug were collected and entered in the analysis (table 4, page 28). The assumption was that, those who took the drug were more likely to have been covered from the infestations. After analysis, we could not find any association between anaemia and not using the de-worming drug. In the discussion, we pointed out that this could be explained by the complexity and multifactorial aetiology of anaemia apart from worm infestations among HIV infected pregnant women in Sub-Saharan Africa (page 15, line 5-9).

2. Results

(a) Malaria: The results of the study have indicated the prevalence of malaria in the study group to be 4.5%. This low figure is said to be due to co-trimoxazole which was used prophylactically. This is hard to believe because the participants also used insecticide treated bed nets. Which of the two offered protection against malaria?

Response: The contribution of the ITN was not disregarded in this study because its data were included in the analysis as seen on the table 3 and 4(page 27 and 28 respectively). We agree sleeping under the ITN could have contributed to the low prevalence of malaria because 90.5% (380/420) of the subjects reported to be using the ITN. Among them, only 3.9% had a positive malaria test as compared to10% (4/40) of those who did not sleep under the ITN. However, this finding was not statistically significant (p=0.096); therefore another factor(s) apart from the ITN use were responsible for the difference in malaria infection in the study group (page 11, line 5-16).
Anaemia: The prevalence of anaemia in the study group was 54% which is quite high. This anaemia could not all be attributed only to malaria since the prevalence of malaria was quite low (4.5%). Zidovudine is known to cause anaemia and the participants were using zidovudine, is not right to assume that the anaemia was due to the zidovudine? Folate deficiency which is common in pregnancy may also be responsible for the anaemia. How many of these participants had hookworm or other parasitic infections? Can the authors give answers to the questions?

Response: Data on the use of zidovudine were collected and analysed (see table 4). We categorized the use of zidovudine as <3 months, 3 up to <6 months and ≥6 months. In all categories we could not find any significant association between anaemia and the zidovudine use (p=0.253). However we need more data (sample size) to find this association. Similar findings were also reported in India.

Data on the use of iron supplements were also collected and included in the analysis (see table 4, page 28). There was no statistically significant difference in the prevalence of anaemia between the subjects who were using the supplements and those who did not (p=0.567). Adherence with the use of iron supplements was not measured and the information was self-reported; however antenatal records were used to verify prescriptions of medications.

It was difficult to conduct investigations on other parasitic infestations because of the cross sectional design of the study. Subjects were met just once making it difficult to collect stool samples at the same time. However, data on de-worming drug (mebendazole) which is usually provided routinely as part of antenatal service were collected and entered in the analysis (see table 4, page 28). There was no statistically significant difference in the prevalence of anaemia between the subjects who were using the de-worming drug and those who did not (p=0.176). However, the information on the use of de-worming drugs was self-reported.

Regards,

Dr OMS. Minzi

Corresponding Author