Author's response to reviews

Title: The Lipid Profile of HIV-infected patients receiving Antiretroviral Therapy in a Rural Cameroonian Population

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Author's response to reviews: see over
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Title: The Lipid Profile of HIV-infected patients receiving Antiretroviral Therapy in a Rural Cameroonian Population

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Version: 4 Date: 24 January 2014
Reviewer: Michael O Iroezindu

Author's response to reviews:

Thank you for consideration of our manuscript for publication in your journal. We equally thank the reviewer for the pertinent comments.
We have reviewed the above manuscript according to the reviewer’s comments.

Major Compulsory Revisions Discussion
1. There is evidence in the literature that hypertriglyceridaemia associated with HIV disease tends to be more profound at the late stage of the disease when there is severe immunosuppression. Bearing this in mind, how would the authors explain the high proportion of patients with hypertriglyceridaemia in this study despite on-going HAART which would expectedly lead to immune reconstitution as suggested by the fact that 63% of patients had CD4 count > 350. This is another reason why the authors should include the baseline (Pre-ART) CD4 count of the patients.

• In discussion paragraph 6 we highlighted the fact that such changes have been observed in the short term (Kiage et al.) but soon lipid levels rise above pre-seroconversion levels in the long term use of HAART (Riddler et al.)

Minor Essential Revisions
Abstract
1. Background
Line 3: suggest you write “......should be monitored” rather than “......must be monitored”
• “should” now replaces “must” as advised
1. Methods
Line 5: suggest you write “.........was used to assess for factors” rather than “.........was used to screen for factors”
• “assess” now replaces “screen” as advised
2. Results: How was smoking defined? Is it Ever smoked or current smokers? This should be clearly defined in all relevant sections of the manuscript

- **Smoking was defined as current smokers**

Methods:

1. Line 2 under study site: change “2nd” to “second”

- “2nd” has been changed to “second”

2. Ethical aspects: It is not sufficient to have a written consent. The authors failed to specify if it was a written informed consent

- It was a written informed consent. The consent forms and information leaflets were made available to participants. Copies had been sent to BMC when similar concerns were raised earlier by the editors.

3. How did the authors define “Young age”. This needs to be clearly written in the Methodology

- Apparently, we did not use “young age” in methods but it was mentioned in discussion paragraph 4 line 2 as “younger persons” to refer to young adults below the age of 25 years

Results:

1. Actually, a cohort study would have been a very good design to highlight the role of HAART in dyslipidaemia in PLHIV. However, a cross-sectional study with a control arm would also be a good option. Although the authors pointed out the limitation of their study design in terms of not being able to establish causality, I think they could go a step further. In the absence of a control group in this study, I would suggest that the authors find a historical control group which can be a study in rural Cameroon that investigated lipid profile in either HAART-naive HIV-infected patients or Lipid profile in the general population in rural Cameroon. As part of the results, the lipid parameters can then be compared between the patients in this study and the selected historical control provided the study chosen to be the historical control is properly cited/referenced. In this way, it would be possible to make a case for HAART as part of the reason for the high prevalence of dyslipidaemia. But if the picture is the same in the historical control (whether HAART-naive HIV-infected patients or the general population in rural Cameroon, then the high rate of dyslipidaemia reported in this study may simply be a reflection of the overall picture in rural Cameroon. If there are no such studies that could serve as historical control, this should further be pointed out in the limitation.

- We found and included a small study by Lissouk et al. that reported higher levels of TC, TG and LDL-c with lower levels of HDL-c in the urban than in the rural area of a general population of Cameroon (Ref [15])

2. The authors should be specific about the metabolic conditions they identified in the patients. Was it Diabetes mellitus or what?

- It was type 2 diabetes mellitus

3. It will be very good to include the pre-ART (baseline) CD4 cell count of the patients in the results. This should also be shown in Table 1. This is important because HIV disease (without ART) is associated with dyslipidaemia which pattern may be influenced by the degree of immunosuppression.

- We did not collect data on baseline CD4 because we could not obtain the corresponding baseline lipid profile for comparison given the cross-sectional design of our study. We have provided findings on the effect of low CD4 on ART-naïve HIV-infected persons as cited in Ref [19,22,23]
4. In a few places the authors did not write the result section in reported speech e.g “86 (72.8%) are females”. This should read “86 (72.8%) were females.

- All sentences have now been written using the past tense.

5. The authors should write absolute figures before the percentages quoted in all the places.

- Such omissions have now been included where appropriate.

6. Last line: insert the word “antiretroviral” in the sentence “Having ever interrupted treatment”.

- “antiretroviral” has now been inserted where indicated.

7. The significant association between smoking and dyslipidaemia is well known. Considering that the number of smokers in this study was quite few (6 out of 114) the significant association between smoking and dyslipidaemia should be reported (as well as interpreted) in the light of the small population of smokers.

- We have discussed our findings in the light of a possible information bias associated with under-reporting of undesirable lifestyles but also with regards to the big odd ratio that is unlikely to be a chance finding.

Discussion

1. The finding that alcohol use was associated with a healthier lipid profile was not exhaustively discussed. The major limitation here is that the quantity of alcohol consumed was not captured. The relationship between alcohol and cardiovascular disease is said to be J-shaped so excessive alcohol consumption would actually be associated with dyslipidaemia. Therefore, the authors should clarify in the discussion that it is possible that the observation that alcohol was associated with a more healthy lipid profile may be so if the majority of their patients had low-to moderate alcohol use. This is plausible in a study where ¾ of the patients were women especially in rural African population where women are not likely to engage in excessive alcohol use.

- In discussion paragraph 10, we did acknowledge the fact that the amount of alcohol intake per person was not measured but we have now nuanced our interpretation of the findings in the light that heavy drinking is rare in a population made up of mostly women in a rural (poorer) African setting as suggested.

Minor issues not for Publication

Discussion

1. There were few typographical errors e.g Discussion paragraph 6 last line: “it might be therefore be.....”

- One of the “be” has been deleted Also see Discussion paragraph 10 line 1

Also Discussion penultimate paragraph last line: “thus defaulting”. I guess the authors meant to write “those defaulting”.

- “thus” has been replaced by “those”.

2. Discussion Paragraph 9 last line: the word “fallacy” appears to be inappropriate. The authors should find a better word.

- “fallacy” has been replaced by “selection bias”.

3. Table 1:

- specify that the stated CD4 count is post-ART CD4

- Include pre-ART CD4 count.
As stated earlier, data on pre-ART CD4 was not collected so the stated CD4 count is post-ART for all participants as indicated in the title of the table.

4. Table 2: the authors should CROSS-CHECK the odds ratio (95% CI) statistics for the association between Age and LDL-C, CD4 and LDL-C and NRTI regimen and LDL-C.

The ORs and 95%CI are exactly as they stand. The p-values stated are for the associations between LDL-c and the variables (from likelihood ratio test). We did not present the p-values (Wald test) for the different categories of the variables.

Discretionary Revisions

Introduction

1. The last 3 lines of the introduction should be modified to possibly read “It is hoped that the findings of this study would inform……………”

• Modifications done as advised

Methods

1. The authors pointed out that the HIV clinic offers VCT. In line with current trends regarding HIV testing/counselling, I should think that Provider-initiated HIV counselling and testing (HCT) would be a more appropriate initiative

• PICT is gradually being instituted but still has a milestone to cover
Reviewer: Tazoacha Assonganyi

Author's response to reviews:

Thank you for consideration of our manuscript for publication in your journal.

We equally thank the reviewer for the pertinent comments.

We have reviewed the above manuscript according to the reviewer's comments.

Introduction:

(Major compulsory) Highlight reasons why rural and urban changes may be different by including differences in diet, etc.

Higher levels of TC, TG and LDL-c with lower levels of HDL-c in the urban than in the rural area of a general population of Cameroon due to differences in diet have been reported by Lissouk et al. in an article that has now been included and cited in ref[15]

2) (Major compulsory) The several studies in Cameroon in the literature on the same subject are omitted. The following articles (plus any others that may be got through literature search) should be consulted and included:
   a) Danwe C et al. (2005), J. Med. Sci. 5: 78-82

- Articles on the effect of HIV on blood lipids in HAART-naïve persons and on the effects of HAART on blood lipids in HIV-infected persons have been consulted where possible and we have now included the following: Ngondi JL et al. (2006), Teto G et al. (2013), Ngogang J et al.(2008) and Nguemaim NF et al. (2010) and cited in ref[9-12]
• We equally updated reference 1 using the UNAIDS report for 2013 in place of the 2012 report.

Methodology:
1) (Major compulsory) The general set-up of the experiments does not allow the conclusions drawn from the results on “those reported using alcohol”, smokers, and existing metabolic conditions. Proper case-control studies are needed for such conclusions to be drawn. Amount of alcohol consumed by subjects, and number of cigarettes smoked a day, length of consumption, etc are not indicated. Therefore discussion of results related to these should be nuanced.
   • In discussion paragraph 10, we did acknowledge the fact that, the amount of alcohol intake per person was not measured but we have now nuanced our interpretation of the findings in the light that heavy drinking is rather rare in a (study) population made up of mostly women in a rural (poorer) African setting.
   • We have now discussed our findings in the light of a possible information bias associated with under-reporting of undesirable lifestyles like smoking but also with regards to the big odd ratios that is unlikely to be a chance finding
   • We have been careful not to draw definite conclusions (such as causality) about the associations found given the observational cross-sectional approach we have used.

2) (Major compulsory) Methodology does not indicate that subjects were interviewed/questioned
   • we have now included that, a structured questionnaire was used to collect data

3) (Major compulsory) There were “only” 6 smokers; as compared to 9% (10) that “reported being on diet”, 4.5% (5) that interrupted treatment, and “<10%” (<11) who had metabolic/cardiovascular problems. This is to say that if the study bases analyses on 6 smokers, these other parameters are important enough to have affected the results. The normal approach is to exclude subjects with conditions that might affect changes in lipid metabolism like infections with Hepatitis B/C, diabetes, abnormal thyroid hormones, use of hormonal contraceptives, obesity, hypertension, family history of dyslipidemia, smoker, consumption of alcohol, etc. Therefore discussion of outcomes should be nuanced.
   • We did not want to be very restrictive in selecting participants for fear of obtaining a sample that is not representative at all. This trade-off was taken into account at statistical analysis by using multiple regression methods.

4) (Major compulsory) The “enzymatic-linked colorimetric methods” have to be briefly described. Are they ELISA based or the usual colorimetric enzyme assays?
   • Usual colorimetric enzymatic techniques were used. We also made use of the Friedewald formula. A paragraph on a brief description of the laboratory technique has been included.

5) (Major compulsory) Nkongsamba, the site of the study is not a “rural” area. It is one of the big cities with Yaounde, Douala, Limbe, Kumba, Bafoussam, and others that are managed by Government Delegates.. The authors should justify
why they considered it a “rural” area, beyond just stating that most of them engage in subsistence farming.

- We have now added that users of the clinic come from city of Nkongsamba obviously but massively constitute of referrals from the rural municipalities of Melong, Bare-Bakem, Nlonako, Manjo, Loum, Njombe-Penja, Mbanga, Bonalea and Dibombari that make up the Mounngo Division; equally from neighbouring rural areas of Bangem and Tombel in the South-West Region and from the rural municipalities of Kekem and Santchou in the West Region of Cameroon.

Results:
- 1) (Discretionary) Report “3000 cycles per minute” as g/min
  - “g/min” now replaces “cycles per minute”

- 2) (Major compulsory) It is not clear whether the “yes” and “no” in Table 1 for “heart condition” and “metabolic condition” are based on interview responses or clinical examinations.
  - These conditions were confirmed by data from their medical records

Discussion:
- 1) (Major compulsory) Inclusion of the articles cited above, that the authors seem not to know about would greatly modify the discussion.
  - The articles have been included where appropriate

- 2) (Major compulsory) Comparison of results to those of subjects from South Ethiopia and Tanzania should indicate whether they were from rural or urban areas of these countries.
  - We have now indicated that they were carried out in urban areas