Author’s response to reviews

Title: Elevated Inflammatory Cytokines In Aqueous Cytokine Profile in HIV-1 Infected Patients With Cataracts in Uganda

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Reviewer 1

Major questions:

1. Methods: There were the uveitis patients both in case group and control group. These uveitis cases should be deleted in statistics because it could also cause elevated inflammatory aqueous cytokine levels without development of cataracts and cause statistical basis.

Response:

We removed cases with uveitis and intraocular infections from the comparative cytokine analysis. Repeat analysis has been done as shown in tables 2 to 5
2. Why the authors chose these 8 cytokines for examination? Please explain this in introduction.

These 8 cytokines were chosen for investigation in this study because of their documented roles in mediating intraocular infection. This has been explained in background, under subtitle ‘intraocular inflammation and cataracts’, paragraph 3, last sentence.

3. Result: What's the result from table 3? Why there is no word explanation or discussion about it?

Response:

After re running the analysis, we now have 5 tables, and all of them including table 3 have an explanation at bottom summarizing significant results.

4. What's the valuable result of the manuscript? Please state in explicit sentences in the first paragraph of the discussion part.

Response:

This has been mentioned in the first paragraph of the discussion: Our study suggests that there is increased intraocular inflammatory activity among HIV patients with cataracts.

5. Discussion part: the authors just discussed the IL-10 in "IL-10, IFN-g, and TNF-a" part. It's not appropriate and should be revised.

Response:

We have revised and improved the discussion, all cytokines studies and values have been discussed.
Minor question:

6. Introduction: "There is limited published information about the role of these intra ocular inflammatory processes in early development of cataracts among HIV infected individuals in sub-Saharan Africa" is not appropriate here as the authors did not answer the question, either. The manuscript just studied the association of these intra ocular inflammatory with cataract

Response:

We have limited the gap addressed in this study to association between intra-ocular inflammation and cataracts

7. Figure 1 repeated with Table 1. Please delete one and mark the P values in the figure.

Table 1 and Table 2 should be exchanged because table 1 deals with the baselines of two groups.

Response:

We have removed figure 1, and sequenced tables 1 to 5 appropriately

8. Abstract: please delete the background, and rewrite the purpose and method part with concise sentences. What's more, please enrich the result part.

Many of the sentences of the abstract were exactly the same as that in the manuscript. Please revise the abstract with concise words.

Response:

We have improved background, purpose of study highlighted in second paragraph. Results section has been enriched.

9. What's the manufacturer's information of the cytokines assay kits? Please mark it in the method part.
Response:

We have given details of the manufacturers information if the cytokine assay kit in the methods section under section ‘Cytokine analysis’

10. Table 3, category 3. The authors can delete the sub-table since there is no significant cytokine here. Please state "there is no significant difference in each cytokine when we compare stage I, II and Stage III and IV.

Response:

The sub table comparing WHO clinical stages has been deleted, after repeat analysis, we have two tables 4, and 5 that summarize information on ART and CD4+ T cell count.

Reviewer 2: An interesting study, however it has important limitations that need to be fully discussed.

1. It should be discussed in the limitations of the study the fact that the controls were older than the HIV-positive patients and therefore the differences founded could be secondary to the age difference and not the HIV status.

Response:

This has been mentioned in the discussion.

2. Not all the molecules assessed are cytokines. Preferably authors should use cytokines/chemokines.

Response

The 8 molecules that we studied are often defined as cytokines in literature because of their role in inflammation. Some Chemokines are often referred to interchangeably as Cytokines depending on role that they play in inflammation. We have used cytokine (referring to
immunoregulatory and proinflammatory roles of these molecules) GM-CSF, TNF-a and IFN-γ are often referred to as cytokines in literature review.

3. As per inclusion/exclusion criteria the presence of active or past uveitis was allowed. That could be a very important confounder.

Response:

Patients identified with uveitis were excluded from the cytokine comparative analysis.

4. Lower value detected for the technique should be provided.

Response:

The lower value for each cytokine has been mentioned in the methods section.

5. What was the percentage of detection of each molecule for each group. Any significant difference in the percentage of detection?

Response:

Table 2 has been included and summarizes the percentage of detection of each molecule and significant differences in the percentage of detection.

6. When the cytokine/chemokine was not deleted in a sample, was that sample not taken into consideration for the statistical analysis of that molecule or 0 or the lower limit of detection were used instead?
Response:

Any sample with undetectable levels was for replacement with a consecutive sample, however this did not happen to any of our samples. Any value less than the lower limit was regarded as undetectable.

7. Bomferroni correction or other statistical correction should be done to correct for the multiple comparisons and rewrite the discussion according to the new results.

Response:

Bomferroni correction has been done and new results discussed.

8. The affirmation that IL-6 or IL-8 are not detected in normal vitreous should be supported by a reference. There are other stamens in the discussion that are not supported by a reference.

Response:

All Relevant references have been inserted.

9. The statistical analysis done in each case should be clarified. Was the normality of the variable assessed before deciding to use a parametric test?

Response:

This has been addressed in the statistical analysis section.

10. Some of the results discussed in the discussion are not provided in the results section, such as the level of detection.
Response:

We have provided all results in tables mentioned in discussion