Author's response to reviews

Title: HIV and pre-neoplastic and neoplastic lesions of the cervix in South Africa: a case-control study

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Author's response to reviews: see over
Re: Re-submission of paper

On behalf of my co-authors, I submit the revised manuscript “The relationship between HIV and pre-neoplastic and neoplastic lesions of the cervix in South Africa: a case-control study.” A point–by-point response to the reviewer’s comments is provided below.

**Reviewer:** Denise Jamieson

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**Major Compulsory Revisions**

1. *page 4, first 2 lines of abstract:* clarify what the exposure is for the adjusted Ors presented. I assume that it is HIV status?
   
   The exposure is HIV. The sentence has been amended to clarify this and now reads “The adjusted odds ratios associated with HIV infection were: 4.4 [95% CI 2.3 – 8.4] for ASCUS, 7.4 (3.5 – 15.7) for LSIL, 5.8 (2.4 – 13.6) for HSIL and 1.17 (0.75 – 1.85) for invasive cervical cancer”

2. *page 7:* define racial categories
   
   Definition added
3. **Page 7: define what is meant by series matching.**
We have changed “series matching” to the more commonly used term “frequency matched” and have included information on how this was done - “They were frequency-matched to the cases for decade of age, ethnic group and area of residence at a ratio of 3:1 controls per case”

4. **Clarify that IRB approval was obtained for anonymous HIV testing which I assume was not part of the original protocol? Was informed consent obtained for the overall study? For the HIV testing?**
Yes IRB consent was obtained for the original protocol and for the anonymous testing. Informed written consent was obtained from the participants. This information has been added to the paper – methods section.

5. **Throughout the presentation of results, including more information such as denominator data would be helpful. It was hard to follow the presentation of results.**
There were 524 cervical cancer cases, 6% (31) of which were HIV+?
There were 1541 controls without cervical cancer, 5.7% (88) of which were HIV+? If so, I couldn’t reconcile numbers in first part of results, please clarify.
There were 524 original cases but HIV data was available on, 93% (486) of these original cases. Of the 486 HIV tested cases 6% (29) were HIV positive. HIV data was available on 89% (1365) of the original 1541 controls. Of these 1 365 HIV tested controls 5.7% (78) were HIV positive. We have amended this section of the results to read as follows “Overall 5.7% (78) of the HIV tested controls (1365) and 6.0% (29) of the tested cases (486) were HIV positive”

Table 1 – please include either totals or HIV-negative group so that you can see how the odds ratios were calculated. Clarify in title and results what these are odds of.
Changes made as suggested by reviewer. Note this is now Table 2.

6. **The abstract mentions that interaction was found but this is not adequately described in the methods or results.**
We have added the following paragraph to the methods section “An additional analysis which took into account joint HIV/HPV status was conducted. In this analysis women were stratified women according to whether they were HPV positive or negative and HIV positive or negative. In this analysis we controlled for age and ethnicity.” The interaction is presented in the results section.
Reviewers: Gary M Clifford
Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The question concerning to what extent HIV increases the risk for cervical cancer is especially important in sub-Saharan Africa given the size of the HIV epidemic. Indeed, some previous studies from HIV endemic regions show a lack or little excess of cervical cancer in HIV positive patients (Hawes et al, Sitas et al, Mbulaiteye et al). This is in contrast to the much larger excess seen in developed settings. The described study is a very well powered case:control study for assessing risk of cervical cancer in South Africa and particular concerning HIV as a risk factor. However, this important message is lost by an inappropriate analysis of the case:control data. The authors have stratified the large set of matched controls by PAP results, substituting women with normal cytology as a reference group and turning controls (e.g. ASCUS/LSIL/HSIL) into cases, which presumably ruins all the careful matching that was done. Unfortunately, this approach is highly confounded by HPV prevalence. It is well established, and shown also in the present article that HIV greatly increases HPV prevalence, and that SIL is a largely (but not entirely) HPV related phenomena. Thus, controls with normal cytology will, by definition, have very low rates of HIV, and are not the correct comparison group. The correct, and powerful, matched case:control study should compare HIV prevalence in cases (6.0) with that in ALL matched population controls (5.7%). This would give an OR of not much more than one and a confirmation that there is little excess risk for cervical cancer in this population. I personally believe that this lack of excess is largely due to co-AIDS-mortality in the absence of widespread HAART treatment, but this is an issue for the discussion.

While it is of interest to see the proportion of HIV+ve stratified by PAP result, for issues of screening as the authors rightly note, this is not the principal power of the case:control study design. Many previous cross-sectional studies from Africa and elswhere have established the association of HIV and SIL. I recommend the authors to focus more on cancer risk as few such studies exist.

We agree with the reviewer that the study is well powered for assessing risk of cervical cancer and have conducted the analysis comparing HIV prevalence in cases with that in all matched controls as was recommended. The analysis showed that there was no excess risk of cervical cancer among HIV positive women in our study [OR 1.17 (95% CI -.75 – 1.85)].We have amended the text accordingly. We have outlined the possible reason for this in the discussion. We have also re-oriented the presentation of the results and discussion so that the results and initial discussion focus on invasive cervical cancer. We have retained the analysis and discussion pertaining to the risk of SIL among HIV positive women as we feel it underscores the importance of developing and implementing appropriate cervical screening guidelines for HIV positive women in developing countries.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

At multiple points in the paper the authors speak of their study providing "information on the prevalence and risk of SILs among HIV positive women". This is not strictly true. We cannot really estimate the prevalence or risk of SIL among HIV positive women from this case:control study, but we can accurately estimate the risk of cervical cancer for HIV positive women. The authors themselves draw the caveat that the prevalence of SIL among HIV positive women is higher than other studies - this is maybe because the controls were matched to look like cervical cancer cases.

We agree with the reviewer and have amended the points in the paper referring to this so that it now reads “information on the risk of SILS among HIV positive women…”

Discretionary Revisions (which the author can choose to ignore)

I recommend that the authors try to gather information on HPV type distribution among the 29 HIV+ve cases if possible, and even do a larger comparison with that in a selected number of HIV-negative cancer. There are suggestions that HIV positive women are infected with a broader range of types than the general population, but there are no data to show if this is also the case in cervical cancer. This study would be one of the very few that could shed light on this issue.

Unfortunately samples for HPV tests were not taken from the cases.
Reviewer: Stephen E Hawes

General

This article is generally well written. It is quite simple in its presentation of results, and lacks some detail which would be interesting and useful. For example, the authors do not present a standard Table 1 containing the demographic and laboratory characteristics for which they have information. They refer to a previously published paper regarding the study details and design, but this referenced paper also does not contain the relevant information. Please provide more information about the study populations (cases and controls separately).

We have added Table 1 which presents the socio-demographic and reproductive characteristics of the cases and controls.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached).

The authors initially attempt to adjust for a number of measured factors (age, ethnic group, sex partners, education, marital status, contraception, STDs, etc) in the analysis, but 1) do not report the prevalence of these factors and 2) give no justification for attempting to adjust for these. Are they potential confounders? Why did you start with a full model? What about missing data? The statement “Systematic elimination of variables resulted in odds ratio estimates adjusted for age and ethnic group only that were similar to the estimates for the full model” is a bit disconcerting. Did your final model confirm confounding (adjusted OR which differed from the crude OR)? If not, then why did you adjust? Instead of starting with a full model and going backwards, perhaps each factor should have been considered singly to determine if confounding was present.

We have added information on the prevalence of the risk factors as suggested by the author (see new Table 1). We have also amended the methods section to better reflect the statistical analysis that done – “Statistical analysis was carried out using unconditional multiple logistic regression (SAS version 9.1). Initial models contained multiple variables that were considered to be potential confounders (age, ethnic group, number of sexual partners, education level, urban or rural residence, marital status, presence of sexually transmitted infection, number of prior Pap smears, parity, age at coitarche, alcohol use, tobacco use, sterilization, use of oral contraceptives, and use of injectable contraceptives). A backward stepwise regression was performed. Variables were retained in the model if they demonstrated independent associations with the outcome of interest, or if their removal altered the association between HIV infections and the outcome of interest. Systematic elimination of variables resulted in odds ratio (OR) estimates adjusted for age and ethnic group.”

The statement that “subgroups were too small to present data separately…” implies that you though about addressing potential effect modification, but did not carry this out.

With over 500 cases and over 1500 controls, it seems likely that you could have attempted to assess effect modification for at least some of the factors in your study.
We did look at whether ethnic group was an effect modifier but only 25% of the study sample was Black. As shown in Table 3 the numbers in the exposed group of interest were too small – only 18 HIV/HPV positive participants had SIL present. We have eliminated the sentence referred to by the reviewer as we do not wish to imply that we thought of addressing potential effect modification, but did not carry this out.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Abstract: In the result section, you need to specify that the odds ratios are for risk associated with HIV infection. For example, “The adjusted odds ratios associated with HIV infection were:.....”
Amended as suggested by reviewer.

Abstract: “HIV positive women were nearly 5 times more likely.....”
Amended.

Methods: p7 - need a space between 1541 and controls
Done.

Methods: p7 – the use of the term “series-matched” seems non-conventional to me. Do you mean “frequency matched”? Was the ratio meant to be 3:1 controls per case? If so, please specify in the text.
We have changed to the term “frequency matched” and added the ratio of controls to cases.

Methods: p7 - It doesn’t seem relevant that the cervical cancer cases were all considered HPV positive. Since this information is never analyzed, I would omit the statement.
We have opted to leave this information in the text so that the reader is clear as to why no further details on HPV typing of cases has been provided.

Methods: p8 – The 40 cases of HSIL should really be 50 according to the tables and sum of all patients.
Amended to read 50 cases.

Results: p8 – Why the term “original” in the first sentence “Overall, 5.7% of the original controls and 6.0% of the original cases were HIV positive? This is confusing and perhaps not correct. On p7, you state that HIV data were available for only a subset of the cases and original controls. Don’t you mean 5.7% of the tested controls and 6.0% of the tested cases?
We agree with the reviewer and have amended the text to read “Overall 5.7% (78) of the HIV tested controls (1365) and 6.0% (29) of the tested cases (486) were HIV positive.”

Results: p9 – Need to say what your odds ratio is for. “With normal women as the reference category, the adjusted odds ratios associated with HIV infection were:”
Amended as suggested by reviewer.

Results: p9. In the HIV, HPV, and cervical lesions section, you state that 193 of 1287 HIV- women were HPV+. However, in Table 2, there are only 1286 HIV- women listed. The text has been corrected to read ”1286”

Results: p9. As in abstract, “….nearly 5 times more likely…..” when the OR is 4.6. Changes made.

Discussion: p10. Remove 2nd 5% from the Kenya line.
Done.

References: p16 – 2nd reference needs reformatting
Reformatted.

References: p16 – Reference 6 has mistake in title (should be “and” instead of “or”).
Corrected.

Table 2 – Add adjustment variables, as in Table 1
Added.

Thank you for the opportunity to revise and re-submit the manuscript.

Yours sincerely

Dr J Moodley