Author's response to reviews

Title: Prevalence and predictors of Pap smear cervical epithelial cell abnormality among women attending gynecological examination in cervical cancer screening unit at Debre Markos referral hospital, East Gojjam, Northwest Ethiopia

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Author's response to reviews:

Dear Editors

The authors would like to thank the Editor and Reviewers for their careful review of our manuscript and providing us with their comments and suggestion to improve the quality of the manuscript. We highlighted the document parts that contain only major changes. The following responses have been prepared to address all of the reviewers' comments in a point-by-point fashion.

The list of abbreviations and authors addresses have provided

Reviewer: Hannah Leslie

Major compulsory revisions

1. This manuscript represents an important addition to the knowledge base on prevalence and risk factors for precursors of cervical cancer in developing countries. However, it suffers from a critical shortcoming in study design and analysis, at least as currently presented. The decision to stratify the sample by HIV status is not explained or justified, and the major implications of this decision on the analysis and study findings are overlooked. Due to the deliberate oversampling of HIV+ and the absence of consideration of this decision in the analysis, the current results are not a valid attempt to depict a hospital-based sample of reproductive-age women. Sampling by HIV would be justified if the purpose of the study were to assess whether HIV contributes to epithelial cell abnormalities (ECA), but the authors note existing research suggesting this association in the background and do not pursue it as a major focus of the manuscript. Another reason for sampling by HIV would be if HIV status is considered a major confounder of the risk factors of interest. However, most of the risk factors analyzed are likely to be antecedents of HIV, making HIV status an intermediate causal variable. Because the sample is fundamentally shaped by the decision to sample by HIV status, the resulting analyses are biased relative to an overall clinic sample, let alone the general population. Aside from an etiologic
investigation, even for the purpose of providing demographic traits associated with ECA to improve targeting of public health programs, the results are skewed by the sampling strategy. The authors must directly address this critical sampling decision, identify the research question(s) that motivated this choice, and take this into account in all analyses and discussion. The sample of 400 as constructed lends itself to analysis of HIV itself as a risk factor and to analysis of any factors that follow it in a causal pathway to ECA or that are specific to HIV-positive women (such as CD4+ T cell count). To obtain results pertinent to an overall clinic sample, the authors should weight the sample based on the selection probabilities or, less optimally, truncate the HIV+ sample at the number who would have been encountered in the time it took to collect 200 HIV- women. The presentation of a causal model, while not required, might help provide a clear depiction of the authors’ key research questions and ensure that these questions form the core of the introduction, results, and discussion.

Response: Thank you for bringing these points to our attention. Authors elaborated the research gap/ research question that indicates the need for doing this research. We also made the sampling procedures detail.

2. The statistical analysis strategy is not described in sufficient detail: how did authors determine what covariates to collect and how to categorize them? For example, how were age categories chosen – did authors assess linearity of the relationship between age and outcome and determine these categories captured a non-linear relationship? Are nulliparous women included in parity <3? Why is 5 years chosen as the breakpoint for OCP use? Were any other hormonal contraceptives assessed? Analysis of OCP in particular should be clarified: how are the women who have never used OCP, the majority of the sample based on Table 3, incorporated in this analysis? The text does not explain which factors are adjusted for in the AORs; the process of developing the adjusted model and deciding what to include (whether that decision process was causal or statistical) should be described.

Response: Thank you for your salient observation and authors described the detail of the analysis parts. The nulliparous women were included in the #2 parity category. The effect of OCP is not merely utilization rather duration of OCP usage were identified as major determinants by different scholars. So women who have never used OCP were included in #5 category. Literatures also supported assessing OCP effect with 5 year interval duration. Regarding age classification, it is also done based of literatures.

3. The response (i.e., counseling, referral to HIV care, follow-up appointment?) if any, for women identified as HIV positive should be noted as part of the study protocol.

Response: As this study was a cross-sectional study conducted for short period of time, we didn’t do anything to the HIV-positive women rather the positive individuals were linked to the hospital ART clinic. The hospital has well established ART clinic and follow-up appointment system as per WHO national guideline.

4. Population summary information (Results, paragraph 2) is not very meaningful
given that HIV-positive individuals can be expected to differ systematically from the HIV-negative women in terms of both demographic and behavioral traits. As in critique #1, the authors should clarify what population they are trying to assess and how their sample (or a weighted version of the sample) represents this target population.

Response: As we indicated previously this concern is addressed.

5. Statistical tests should be applied to any meaningful comparisons. This includes a reliability statistic such as a kappa test for consistency of examinations (results paragraph 1) and chi square or t tests as appropriate for comparisons between the HIV-positive and HIV-negative strata (Results paragraph 3).

Response: -Thank you for your critical review. We conducted a quality control assessment by testing the consistency of examinations between trained laboratory technologist and pathologist on 30 pap smears only and there were no discrepancies in the descriptive analysis. Hence, we couldn’t use kappa test. We also only highlighted the descriptive measures about the patient characteristics of HIV-positive and HIV-negative strata and we have assessed the effect of HIV on development of ECA by multivariate analysis by simultaneous control of confounders.

6. The first paragraph of the discussion focuses on the utility of Pap smears and a recommendation for extending this method; this does not appear to be a focus of the article up to this point. The manuscript does not present itself as a feasibility study of this method, making the choice to emphasize this element at the start of the discussion misleading.

Response: The authors completely agree with the reviewer comment and the document is modified as per your suggestion.

7. The representativeness of this hospital population to the overall population of women should be discussed, particularly when findings are put in context of existing studies of ECA (Discussion paragraph 3), since presenting at the hospital for gynecological investigation could clearly be related to disease state. It is inappropriate to compare the prevalence documented in this sample to other studies without considering the deliberate oversampling of HIV+ women.

Response: Most of the study findings that we compared with our results are from HIV-positive women and we have tried to elaborate the discussion among those population groups.

8. The conclusion that ‘This study signifies that large numbers of these ECA positive women are at increased risk of cervical cancer’ is not correct – the study does not include future assessment of cervical cancer.

Response: Thanks and the document is edited accordingly.

Minor essential revisions

1. Paragraph 4 of the Background overstates the evidence for risk factors such as oral contraceptive pills (OCP) – while the named variables are clearly associated with cervical cancer, causality has not yet been established due to confounding by behavior.
Response: Authors completely agree with the reviewer concern and the document is modified accordingly.

2. In keeping with the first major compulsory revision, the sampling strategy is inadequately described, including number of women approached for involvement, number of women excluded due to pregnancy etc., and response rate. This information should be broken out by HIV status if possible. Other details to help contextualize the patient population would be useful, such as typical number of women presenting with gynecological problems, type of problems most often seen in this patient population. Estimated population prevalence of HIV should be presented, particularly for women in this age group if known. Did sampling take longer for the HIV+ population to achieve the desired sample size?

Response: we the authors would like to thank you for your thorough observation. The sampling method is revised and detailed. The study subject response rate is 100%. However, the excluded women were not counted and only the indicated study variables were collected.

3. The sample size calculation is unclear (Methods, end of first paragraph)– a reference or explanation of the two-population proportion formula should be provided. If the proportion of expected ECA cases was used in this calculation, that estimate and the basis for it should be explained, since one purpose of this article is to estimate prevalence of ECA in this population.

Response: The sample size calculation procedure is modified accordingly

4. The first paragraph of results includes information that should be incorporated within methods. The identity of the ‘investigator’ is unclear – is this the data collector, the laboratory technologist?

Response: This paragraph is critically modified. But we didn’t move the document to the method part because in the method part we mentioned the plan how the quality of the test was assured and here we described the quality assessment results.

5. COR is not defined in the text, only in the table legend. OR is more typically used to refer to a crude odds ratio.

Response: Thank you, the information is added

6. Citation 21 (Lippincott & Wilkins) includes errors in the authors and title. Please re-check references in case of other such errors.

Response: we completely agree with reviewer’s comment and the reference is modified.

Discretionary revisions

1. I was surprised to see an area of 2 million individuals described as a town (Methods, first paragraph); it would be helpful to see an estimate of the hospital catchment area by population and geographical size. There is no reference provided for the central statistical agency report.

Response: Actually we were talking about the zonal population that may attend the hospital and it was editorial problem. And now we only mentioned the town
population based on the recent unpublished zonal statistical report.

Minor issues not for publication

1. Suggested English language revisions:
   a. Spelling (sever instead of severe, line 68; crud instead of crude, line 557; upon instead of upon, line 194; downward line 216, line 296, line 326; counter parts line 222 and line 322; one or two sexual partner, line 222; corner stone line 241; parities instead of parity lines 319;).
   b. Missing hyphens: for example, ECA-positive women, HIV-positive women, long-term OCP use
   c. Variable spacing around numbers, including references as well as ‘100’ on line 60, ‘4 648’ on line 103, 1.0% line 261,
   d. Unclear sentences / phrases: line 70 – 71, 186 (‘were used condom’), 193 (‘vaginal discharge were observed’), line 225 – 227, lines 235 – 238, 261 – 263, 309 – 310, 322-323
   e. Repetition: lines 83 – 89, severity of disease informing treatment options

2. Suggested stylistic revisions: the background could be slightly reorganized to include the information on Ethiopia specifically at the end, prior to the research question addressed by the manuscript.

Response: Once again thank you for your salient observation and authors have thoroughly edited the document accordingly.

Reviewer: Pam Michelow

Background

Line 62: There are several more high risk HPV types, other than HPV 16 and 18, that may be more prevalent in Africa e.g. HPV 56. The full list of HPV types should be stated here (with references).

What is the prevalence and mortality of cervical cancer in Ethiopia?

Line 68: spelling error “sever”- presumably should be “severe”

Line 81: The Bethesda System 2001 divides squamous cell abnormalities into: atypical squamous cells (1) of undetermined significance (ASCUS) and (2) cannot exclude HSIL (ASCH), LSIL, HSIL, squamous cell carcinoma. ASCH category seems to have been omitted in this manuscript.

Line 86: “For women diagnosed with ASCUS and LSIL follow-up assessment within certain time interval is a management option.” Please state what the follow up assessment is? repeat pap smear? HPV testing? colposcopy and directed-biopsy.

In the abstract, pap smear results are divided into HIV infected and HIV negative women. A brief mention of the prevalence of HIV in Ethiopia, particularly northwest Ethiopia should be provided here (with references). If the HIV prevalence in North West Ethiopia is not available, this should be stated here. If there is a policy within Ethiopia or Debre Markos referral hospital regarding when to start an HIV infected person on antiretroviral therapy, this should be stated
here.

Response: Thank you for your careful observation. The background is modified as per your suggestion. In Ethiopia HIV – positive people start ART based on WHO guideline. But we are not interested to include ART related information since ART was not our study variable.

Study setting and design

Line 116: “All women attending at Debremarkos referral hospital during the study period for any gynecological problem were eligible for the study”. By its name, the hospital is a referral hospital. How are woman referred to Debremarkos referral hospital? Do they have a pap smear at the primary health facility that they are referred from? This may cause bias in the results.

Line 123: Data were collected after obtaining written informed consent from each participant.

Better grammar to state “was” rather than “were”.

Line 129: “After taking the clinical data, cervical swab specimens were collected by trained and experienced gynaecologist. The cervical swabs were smeared onto a glass slides for cytological examination. Briefly, cervical swabs were taken with a wooden applicator stick, smeared on a microscopic slide, immediately fixed with 95% ethanol and were allowed to air dry”. It is best to replace the word “swab” with “smear” throughout the entire manuscript (including abstract) as “cervical smear” is the accepted terminology on a global basis.

Line 136: Again, the category “ASCH” seems to be omitted here

Line 148-150: “were” should be replaced by “was”.

Response: The study setting and design is also modified accordingly. Even though the name of the hospital is referral, it doesn’t mean that the hospital gives service for referral cases only. All people can get service from the hospital. Moreover, the peripheral health facilities don’t provide Pap tests.

Results

Line 171: I’m not sure religious persuasion is of relevance here and I suggest that it be omitted.

Line 216: “down ward” should be spelled “downward”. This error should be corrected in the remainder of the manuscript.

Line 200-204: Were any women on antiretroviral therapy? If so, it would be relevant to determine ECA in women receiving and not receiving antiretroviral therapy. If this data was not collected, it should be stated and the reason this data was not collected provided.

Weren’t HSIL diagnoses confirmed by colposcopy and directed biopsy?

Response: The result is modified as per your suggestion. Even though there were HIV-positive women who started ART, our focus was HIV positivity and their immune response status based on CD4 cells level. Hence, we didn’t collect such information.

Discussion
First paragraph: It should be mentioned in this paragraph that cervical screening (by any method) is not the only step required to reduce mortality from cervical cancer. There are several links in the screening programme that all need to function properly for a cervical screening programme to be effective including (but not limited to) women educated and willing to be screened, health care professionals willing and well trained on how to screen, adequate referral mechanisms in place, colposcopy and LLETZ/LEEP services in place, treatment services for women found to have invasive carcinoma on screening, evaluation and monitoring of the service and sufficient resources in terms of money and trained personnel.

First paragraph: Successful screening programmes in less developed countries should also be cited here (with references) e.g. Chile, Vietnam.

Line 260: The rates of ASCH should be provided. If the category “ASCH” was included in HSIL, this should be stated.

Line 309: “This difference is may be due to divorced and widowed women may have multiple sexual partners than married women” Better to state “may have multiple sexual partners compared to married women”.

The limitations of the study should be provided e.g. the number of participants is relatively small for an epidemiological study; the results may only be generalisable to Northwest Ethiopia etc

HPV as a primary screening modality is becoming more popular. The use of alternative screening modalities e.g. HPV, VIA should be mentioned. Briefly state the reasons why this study was undertaken using conventional cytology.

What advice could be given to the Ethiopian government regarding implementation of a cervical screening program? Is screening worthwhile based on this study? At what age should screening be started? Should HIV infected women be screened at an earlier age? Should women who are divorced or widowed, or on OCP, have more intense screening?

Response: Thank you for identifying these critical concerns. Though the assessment of consistency of examinations between the pathologist and laboratory technologist is indicated as a measure of data quality management method, this does not appear to be a focus of the article. Based on this finding recommending the Pap smear test could be misleading. Therefore, this paragraph is modified in this context. The category “ASCH” mentioned in the background part but we didn’t observe this finding in our cases that is why we have been trying to omit it from the document. Line 309 is also corrected as per your suggestion. The limitation of the study is included. Conclusions are also modified as per your direction.

References
Some of the references have a volume provided and others not. The references
should be standardised according to the journal requirements.
Response: Based on the journal guideline all references are updated.

Tables
Table 2: It is not customary and probably not relevant to provide study participant's religion and this should probably be removed from the table.
The information in Tables 2+3 is more or less the same as in Table 5. Thus either Tables 2+3 should be omitted or Table 5 omitted.
Response: Religion is omitted. Table 2 and 3 are provided to indicate the description of HIV-positive and HIV-negative women characteristics but table 5 is for ECA risk factor analysis. So we believe that the presence of these tables separately can provide more information than the merged one.

Reviewer: Simona Stolnicu
Reviewer’s comment
1. Background. Please carefully check the Bethesda system in order to correct the following paragraph, since there is one more category ASC-H that has to be included in the results and discussions. The Bethesda System 2001 classifies ECA into four major categories as atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC) which in turn promote specificity in mode of treatment.
Response: Authors thanks for your salience observation. The document is modified accordingly.

2. Background. Please check the following sentences, as ASCUS, LSIL and HSIL are not cancer but precursor lesions and there is no stage of the disease for these lesions. Make separate comments for precursor versus invasive carcinoma of the cervix regarding treatment. The treatment options of patients with cervical cancer depend on the stage of the disease. For women diagnosed with ASCUS and LSIL follow-up assessment within certain time interval is a management option.
Response: This is also modified as per your comment.

2. Please provide data regarding the correlation of the cases with abnormal cytology with the colposcopic and pathologic findings.
Response: We conducted a quality control assessment on 30 pap smears only and there were no discrepancies in the descriptive analysis. That is why we couldn’t provide correlation data.

4. Please carefully correct typing errors
Response: The language of the manuscript is thoroughly edited.