Author's response to reviews

Title: Human papillomavirus prevalence and type-distribution in women with cervical lesions: A cross-sectional study in Sri Lanka

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Version: 2 Date: 3 February 2014

Author's response to reviews: see over
Dear Editor,

Please find enclosed a manuscript entitled “Human papillomavirus prevalence and type-distribution in women with cervical lesions: A cross-sectional study in Sri Lanka” which we would like you to consider for publication in BMC Cancer.

Please also find attached the responses to reviewers’ comments. The authors appreciate the constructive comments and have attempted to modify the manuscript as requested within the limits of the study objectives. Changes from the original submitted manuscript are highlighted in track changes.

We hope the revised manuscript is now acceptable for publication in BMC Cancer.

On behalf of all authors,

Yours sincerely,

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Reviewer: Qiang Ruan

Reviewer's report:
- Major Compulsory Revisions
By PCR and reverse hybridization Line Probe Assays, the authors investigated the prevalence and type-distribution of Human papillomavirus in 114 women with cervical lesions from Sri Lanka. However, similar reports (As shown in the References 12, 14 and 20) have been published before. Although this study further confirmed the similar findings by providing some new data (in different population of the same country), the scientific significance is still limited. Based on the content of this manuscript, a brief report would be suitable, if it is accepted by the authors.

Authors agree with the suggestion provided by the reviewer to present this manuscript as a brief report. However, in the Journal website of BMC Cancer, there is no option of Brief report and hence, the manuscript was retained as a full Research Article. Nevertheless, we have kept the manuscript as short as possible.

Minor Essential Revisions
The study is well designed, and the manuscript is well written.

Quality of written English: Acceptable
Reviewer: Cristina Oliveira  
Reviewer’s report:  
This is a study that provides an update on the HPV genotypes distribution in ICC and CIN2/3 cases from Sri Lankan women.

Major Compulsory Revisions:  
1 - Include, in the introduction section, an update on the current organization of the cervical cancer screening and vaccine distribution in Sri Lanka.  
   To the best of Authors’ knowledge, there is no organized "Call and Re Call" cancer screening program offered either in the state or private sector. There is, however, some opportunistic screening done at Well Woman Clinics country wide, but this is limited only to certain areas. Clinic attendance depends on self motivation as well as the enthusiasm of the field midwives. The National Cancer Screening Centre in Colombo offers a walk in facility - again patients often self refer. In the private sector, depending on the practitioner, patients may be offered pap screening annually (US guidelines).  
   The limitation section of the discussion section provides information on the current screening practices in Sri Lanka “In Sri Lanka, structured and organized cervical screening programs are lacking and cervical smears are only collected opportunistically [20]. As a consequence, only the most severe ICC cases are likely to be detected. Furthermore, the ineffective screening programs could result in the relatively lower detection rates of pre-cancerous cases which in turn might have led to small number of CIN 2/3 cases enrolled in this study.” The Authors feel it is important to highlight this aspect and was therefore kept in the limitation section and not included in the Introduction to avoid redundancy.  
   However, information on the vaccination policies was added to the introduction “Although both these vaccines have been licensed in Sri Lanka and are available in the private sector since 2009, they have not been included in the national immunization program [2].”

2 - Discussion section, page 10: Please, explain the sentence “Samarawickerma et al. performed HPV typing on all HPV positive cases[12], which is in contrast to the present study, where HPV type distribution was assessed only on ICC cases.”. Was not Samarawickerma and colleagues study based only on ICC cases?  
   Indeed. The authors thank the reviewer for the constructive feedback and we have now revised the sentence as “In addition to using similar laboratory procedures, Samarawickerma et al. performed HPV typing on all ICC cases [12], which is similar to the present study. However, Samarawickerma study [12] used archival cervical biopsy specimens to assess the prevalence and type-distribution of HPV which is in contrast to the design of the present study. Although this could partly explain the difference in overall HPV prevalence rates between the two studies, the exact reason is uncertain.”

3 - Discussion section, page 11: In the sentence “Since the cervical specimens were prospectively collected, they were more recent and resulted in higher HPV DNA detection rates as compared to archival specimens [15].”, please provide the HPV DNA detection rate obtained in the de Sanjose et al. study  
   The strength of this study was the assessment of HPV prevalence and type-distribution from freshly isolated cervical specimens from women who were prospectively enrolled. Although the reference used was of Sanjose study (which used archival specimens), we are not comparing the detection rates of our study with the Sanjose study. The indicated sentence was modified as “Since the cervical specimens were prospectively collected, they were more recent and might have resulted in higher HPV DNA detection rates as compared to studies which use archival specimens [12,15].”

Quality of written English: Acceptable
Reviewer: Mohammed El Mzibri

Reviewer’s report:

Major compulsory revisions

1) Introduction: Fairly superficial and not enough data is given concerning cervical cancer prevalence and HPV infection in Sri Lanka.

   To the best of Authors’ knowledge, there is no organized "Call and Re Call" cancer screening program offered either in the state or private sector. There is, however, some opportunistic screening done at Well Woman Clinics country wide, but this is limited only to certain areas. Clinic attendance depends on self motivation as well as the enthusiasm of the field midwives. The National Cancer Screening Centre in Colombo offers a walk in facility - again patients often self refer. In the private sector, depending on the practitioner, patients may be offered pap screening annually (US guidelines).

   The limitation section of the discussion section provides information on the current screening practices in Sri Lanka “In Sri Lanka, structured and organized cervical screening programs are lacking and cervical smears are only collected opportunistically [20]. As a consequence, only the most severe ICC cases are likely to be detected. Furthermore, the ineffective screening programs could result in the relatively lower detection rates of pre-cancerous cases which in turn might have led to small number of CIN 2/3 cases enrolled in this study.” The Authors feel it is important to highlight this aspect and was therefore kept in the limitation section and not included in the Introduction to avoid redundancy.

   However, information on the vaccination policies was added to the introduction “Although both these vaccines have been licensed in Sri Lanka and are available in the private sector since 2009, they have not been included in the national immunization program [2].”

   Lastly, information on prevalence of HPV obtained from previous studies was included in the Discussion to compare the results of our study and hence was not included in the Introduction to avoid redundancy.

2) In the methodological section, please give the number of recruited women. Initially, recruitment of 200 women (100 ICC and 100 CIN2/3) was planned, but only 106 were retained. Among them, 98 were diagnosed with ICC and only 8 cases were diagnosed with CIN2/3. This merit more explanations.

   In the Methodology section, we have only presented the number of women planned to be enrolled, the results of which are presented in the Results section. In the results section, the total number of enrolled women and their histological diagnoses has now been edited to provide more clarity.

3) It is widely accepted that HPV type distribution is calculated on the basis of total HPV positive cases. In this paper, HPV16 is detected in 66 cases representing 67.3% of all cases with ICC (66/98) but is present in 79.5% of HPV positive ICC cases (66/83). This frequency is in agreement with reported data showing that HPV16 ranged between 74%-77%.

   The primary endpoint of our study was to assess the prevalence and type-distribution of HPV among ICC cases, based on which the sample size was chosen. Therefore the assessment included all ICC cases in the denominator and not HPV positive ICC cases. In addition, the prevalence of HPV-16 (74%-77%) in previous studies was also estimated among all ICC cases and not HPV positive ICC cases.

4) In the discussion section, the difference between the present study and the previously reported data from Sri Lankan women could be due to sampling bias but also to presence of false negative cases. Indeed, it’s not mentioned that all samples were positive for the internal control and it would be interesting to test all samples for β-globin to be sure that all extracted DNA were able to be amplified by PCR.
During this study the PCR testing was the basic assay performed. DNA extracts of samples were tested only for the presence of HPV DNA. In case of HPV negativity the DNA extract was diluted 10-fold and re-tested.

We did not test for inhibition through PCR test for a human housekeeping gene like B-globin as suggested by the reviewer.

In our experience only very few samples will be human DNA negative at both dilutions tested in our HPV PCR algorithm. In a recent large epidemiological study conducted in China (unpublished) and Europe (Tjalma WA, et al; Int J Cancer. 2013 Feb 15;132(4):854-67.), we observed only 10 out of >700 samples tested which were negative for human DNA. None of these samples showed inhibition as determined through a “spiking” test with a positive control. Our conclusion for these 10 cases was that these samples were inadequate and were excluded for the study. HPV negativity in SCC in our experience is very rarely caused by disruption of our very small PCR target by integration.

Therefore we did not perform any β-globin tests in our study.

**Minor essential revisions**

1) I think it’s difficult to assume that the observed fewer number of multiple infection cases could be due to the lower prevalence of HPV in Sri Lankan women. Many socio-cultural factors may be discussed in this field.

Based on the study design and the results observed, the Authors are unable to relate any socio-cultural factors to fewer multiple infections observed. Also, we don’t have data on age of sexual debut in this study or other socio-cultural factors. Therefore, what we reported in the manuscript is the observation from our study. The Authors feel that they cannot offer an explanation to fewer multiple infection based on the results we observed.

**Quality of written English:** Not suitable for publication unless extensively edited

The Manuscript was reviewed and language editing was performed by a native English speaker.
Reviewer: Iacopo Baussano
Reviewer’s report:
The data reported in the paper deserve to be reported in the literature. However, I would suggest to concentrate the paper exclusively on invasive cervical cancers. The data on CIN2/3 are so limited, and below the expected that they cannot be interpreted. A few methodological issues should be clarified in a raised version of the manuscript:

a) How, and according to which criteria, was calculated the required sample size?

During the protocol stage, the prevalence of HPV-16 and -18 were assumed based on published literature. It was based on these prevalence rates and assuming 10% of subjects were non-evaluable (due to drop-out, did not meet inclusion criteria, or other reasons), we planned to enroll 100 ICC and 100 CIN 2/3 cases. The following modification was made to the section on estimation of sample size: “At the time of enrollment, the prevalence of HPV-16 and -18 were assumed to be 50% and 16%, respectively in ICC cases and 30% and 7%, respectively in CIN2/3 cases [10]. Assuming 10% of non-evaluable cases, an enrollment of 200 women (100 ICC and 100 CIN 2/3 cases) was planned.”

b) The authors should provide more details on histological diagnosis quality control. Some information has been provided in the discussion section. These should be reported in the method section and should be more systematically and extensively described.

The following modifications were made in the Methods section “Cervical biopsy or excision specimens obtained during routine clinical diagnostic/operational procedures were fixed in 10% formalin solution and embedded in paraffin as tissue blocks. The review of excision specimen and verification of initial histopathological diagnosis was performed by the site pathologist and classified as ICC or CIN 2/3. Sectioning of tissue blocks was undertaken by sandwich technique, whereby tissue sections for polymerase chain reaction (PCR) were flanked by tissue sections for histopathological review at DDL Diagnostic Laboratory, the Netherlands. A review and final diagnosis was performed by the pathologist at DDL Diagnostic Laboratory to confirm the diagnosis made by the site pathologist. If there was a disagreement between the two diagnoses, a third opinion was sought from a pathologist at DDL Diagnostic Laboratory. The final diagnosis was made by simple majority and the samples were confirmed to be ICC or CIN 2/3.”

c) Were women also tested for HIV infection? If so would it be possible to present the data stratified by HIV status?

We did not look at HIV infection rates among women in this study. Further, this study was not designed to assess the relation between HIV and HPV.

d) Please provide a detailed and evidenced-based explanation of the reasons for the very low number of CIN2/3 lesions identified as compared to the expected 110 cases.

Indeed, the low number of CIN2/3 cases in our study was a limitation and hence no conclusion was drawn on the relation between CIN and HPV. We have detailed this limitation in the Discussion section (Secondly, the number of CIN 2/3 cases enrolled was lower than planned (100 CIN 2/3 cases because the recruiting centers were tertiary hospitals which mainly treated invasive cancers. In Sri Lanka, structured and organized cervical screening programs are lacking and cervical smears are only collected opportunistically [20]. The ineffective screening programs could result in the relatively lower detection rates of pre-cancerous cases which in turn might have led to small number of CIN 2/3 cases enrolled in this study.) and clarified the reason why we were unable to enroll the planned number of CIN2/3 cases.
Quality of written English: Not suitable for publication unless extensively edited

The Manuscript was reviewed and language editing was performed by a native English speaker.
Reviewer: Megan Clarke
Reviewers report:

1. Title
Major Compulsory Revisions:
1.1 – This is not really a “prospective study”. As you say in the Methods section, it is really more like a cross-sectional study. The title is therefore misleading.

The title was modified as “Human papillomavirus prevalence and type-distribution in women with cervical lesions: A cross-sectional study in Sri Lanka”

2. Abstract
Discretionary Revisions:
2.1 – The mention of PCR in the abstract is unnecessary and somewhat distracting, as it was only used for amplification (according to Methods section) and was not used for HPV typing per se.

Since the Methods of the Abstract gives an overall view of the entire methods followed, the indicated section was modified as “DNA was extracted from samples with a confirmed histological diagnosis and was amplified using polymerase chain reaction and HPV DNA was detected using Enzyme Immuno Assay.”

3. Introduction:
Major Compulsory Revisions
3.1 – It would be helpful for the reader, and for emphasizing the importance of this study, if the authors could elaborate more on the current cervical cancer screening programs that exist in Sri Lanka, including who is eligible and how they are utilized. Why are the cervical cancer rates so high? Is it due to lack of infrastructure or something else? In the same regard, what is known about the epidemiology of HPV in Sri Lanka?

To the best of Authors’ knowledge, there is no organized “Call and Re Call” cancer screening program offered either in the state or private sector. There is, however, some opportunistic screening done at Well Woman Clinics country wide, but this is limited only to certain areas. Clinic attendance depends on self motivation as well as the enthusiasm of the field midwives. The National Cancer Screening Centre in Colombo offers a walk in facility - again patients often self refer. In the private sector, depending on the practitioner, patients may be offered pap screening annually (US guidelines).

The limitation section of the discussion section provides information on the current screening practices in Sri Lanka “In Sri Lanka, structured and organized cervical screening programs are lacking and cervical smears are only collected opportunistically [20]. As a consequence, only the most severe ICC cases are likely to be detected. Furthermore, the ineffective screening programs could result in the relatively lower detection rates of pre-cancerous cases which in turn might have led to small number of CIN 2/3 cases enrolled in this study.” The Authors feel it is important to highlight this aspect and was therefore kept in the limitation section and not included in the Introduction to avoid redundancy.

However, information on the vaccination policies was added to the introduction “Although both these vaccines have been licensed in Sri Lanka and are available in the private sector since 2009, they have not been included in the national immunization program [2].” and information on prevalence of HPV obtained from previous studies was included in the Discussion to compare the results of our study and hence was not included in the Introduction to avoid redundancy.

In addition, “lack of effective screening programs in lower and middle-income countries, including Sri Lanka, detection of cervical abnormalities is often difficult and leads to higher mortality rates, due to ICC, in these settings” was also added to the Introduction
3.2 – The authors mention the vaccine, but do not say what programs, if any, exist in Sri Lanka for vaccinating adolescents and young adults. Please elaborate here, and to save room, you could remove the sentence regarding the systematic review (this is already re-stated in the Discussion section).

The following sentence was added to the introduction “Although both these vaccines have been licensed in Sri Lanka and are available in the private sector since 2009, they have not been included in the national immunization program [2].” and the statement on the Meta-analysis was removed.

Minor Essential Revisions:
3.3 – Last sentence: the study is not really aiming to provide data on HPV burden in Sri Lanka because it is not population-based, rather it is aiming to quantify HPV positivity and type distribution among a selected (small) sample of women with ICC and CIN2/3.

Indeed, the authors acknowledge and thank the reviewer for the constructive comment and have revised the indicated sentence as “Recent data on the prevalence of HPV infection and its type-distribution are limited in Sri Lanka and therefore this study was undertaken with the primary objective of assessing the prevalence of HPV-16, HPV-18 and other oncogenic HPV types among Sri Lankan women with a diagnosis of ICC and CIN 2/3. Such data is critical for assessing the potential impact of prophylactic HPV vaccines in Sri Lanka.”

Discretionary Revisions:
3.4 – The authors should consider removing the sentence in the second paragraph regarding the meta-analysis, it does not provide additional information beyond what was previously stated above. You could cite the study briefly, and say that findings are similar in Asian countries.

Revised as per suggestion.

4. Methods

Major Compulsory Revisions:
4.1 – Please define the actual age range of participants. Age 21 is very young for cervical cancer, I would expect most cases are among older women in a more narrow age range?

21 years at the time of screening for inclusion into the study was the minimum age at which a woman could be enrolled. However, all women aged 21 years or more (no upper limit specified) were included in the study if they met the inclusion criteria.

However, the actual age range of women enrolled in the study is included in the Results section.

4.2 – A table describing the study population is needed. Do you any have demographic and/or medical record data for these women other than age? If so, please consider making a table with these characteristics.

In this study, we only collected age and ethnic background from all enrolled women and the results were presented. The authors feel this information is better presented in text than in table as the information is not huge.

Minor Essential Revisions:
4.3 – The second sentence under the Statistical Analysis heading should be moved up to the paragraph regarding study design and population.

Revised as per suggestion.
5. Results

Minor Essential Revisions:

5.1 – For consistency, and because they are etiologically different, please report the % HPV positive and type distribution for adenocarcinoma in your results.

We only present the prevalence of HPV infection among women with ADC was included in the Results section. However, type distribution was performed only among all ICC cases and not by individual diagnoses of ICC this is because the number of ADC cases (n=12) is very small.

Discretionary Revisions:

5.2 – The first sentence in this section is awkwardly phrased and should be rewritten. Consider the following:

“Of the 114 women enrolled in this study, 106 (93.0%) were included in the histologically confirmed cohort, with a mean age of 52.6 years.”

The indicated section was modified as “A total of 114 women were enrolled in this study, of whom 106 (93.0%) were included in the histologically confirmed cohort.” and the mean age was moved to the following sentence along with other demographic characteristics.

5.3 – In the last paragraph of the Results section, the authors switch between calling CIN2/3 cases HPV positive, and women HPV positive. Consider the following: “Co-infection of HPV-16 and HPV-59 was observed in a single case (1.5% [95% CI: 0.0–8.2]) of ICC. All eight CIN 2/3 cases were HPV positive (100.0% [95% CI: 63.1–100.0]), with HPV-16 being the most predominant type detected (50.0% [95% CI: 15.7–84.3]) followed by HPV-33 (25.0% [95% CI: 3.2–65.1]), HPV-52 and HPV-56 (12.5% [95% CI: 0.3–52.7], respectively) (Figure 1).

Revised as per suggestion.

6. Discussion

Minor Essential Revisions

6.1 – The authors suggest that there is selection bias in this study, but don’t actually articulate it. If women are screened opportunistically then are the most severe cases (i.e., ICC) most likely to be detected? If so, this sample is non-representative as you say, because of selection bias, which should be stated. What does the literature say about HPV type distribution among CIN2/3 cases in this population?

As per the suggestion, the indicated limitation section was modified as “In Sri Lanka, structured and organized cervical screening programs are lacking and cervical smears are only collected opportunistically [20]. As a consequence, only the most severe ICC cases are likely to be detected. Furthermore, the ineffective screening programs could result in the relatively lower detection rates of pre-cancerous cases which in turn might have led to small number of CIN 2/3 cases enrolled in this study. Therefore, because of selection bias, the selected sample size is non-representative of the population and further studies with sufficient cases will be required to better understand the prevalence and type distribution of HPV infection among precancerous lesions.”

There is very little information available on HPV in Sri Lanka, so we could not rely on past literature for the distribution of CIN 2/3 cases in Sri Lankan women.

6.2 – The authors did not calculate rates in this study, please change to prevalence or proportion.

All instances of ‘rate’ was removed from the manuscript and changed to prevalence
Discretionary Revisions:

6.3 – I would restructure the Discussion so that your first paragraph is a summary of your main findings – overall HPV positivity and type distribution. Then move onto how your findings are in line/conflict with the evidence in a coherent paragraph (i.e., try aggregating some of the studies that have similar or different findings than you and citing the references at the end of the sentence, instead of individually highlighting each one).

   Revised as per suggestion.

6.4 – The reference regarding the low HPV prevalence in Sri Lanka (fourth paragraph) is somewhat contradictory to the burden of cervical cancer in Sri Lanka on which the importance of this paper is based. I would caution the authors to make more thoughtful statements regarding these other research findings, particular since so few studies have been done, rather than simply citing what each found.

   The study we conducted does not make it possible to relate the lower multiple infection with any factors. Hence the indicated section was modified and the indicated reference was removed from the manuscript.

6.5 – I am not clear on the last point made in the limitations paragraph. One would expect to see a difference in type distributions between SCC and ADC.

   What the limitation means is: since the number of SCC and ADC cases was low, we cannot conclude on the difference in HPV prevalence and type-distribution among these types of ICC. The study was designed only to assess the prevalence and type distribution of HPV among all ICC cases only and not by its sub-categories of histological diagnoses.

6.6 – The authors should consider deleting the last sentence in the third paragraph regarding the need for a broader vaccine. This is already well established with the nonavalent vaccine in clinical trials.

   By “vaccines with broader protection” the Authors do not necessarily mean vaccines with more valents. For example, the bivalent vaccine (Cervarix™) offers protection beyond HPV-16/18 due to its cross-protection against other HPV types. Therefore the indicated sentence was retained.

Quality of written English: Acceptable