Author's response to reviews

Title: An epidemiological study assessing the prevalence of human papillomavirus types in women in the Kingdom of Bahrain

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Version: 7 Date: 14 October 2014

Author's response to reviews: see over
Dear Dr. Prof Sophie F Derchain,

Journal Editorial Office
BioMed Central

Please find enclosed a manuscript entitled “An epidemiological study assessing the prevalence of human papillomavirus types in women in the Kingdom of Bahrain” (Manuscript ID: 6792171812874568) which we are resubmitting for consideration by BMC Cancer.

Please also find attached itemized 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.

We look forward to hear the outcome of the review process.

On behalf of all authors,

Yours sincerely,

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Authors Response to Reviews

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Version: 2 Date: 1st September 2014

Author's response to reviews: see over
**Manuscript ID:** 6792171812874568

**Title:** An epidemiological study assessing the prevalence of human papillomavirus types in women in the Kingdom of Bahrain.

**REVIEWER #1**

**Reviewers report**

**General:** This manuscript provides a succinct and clearly presented report on the findings of a study to estimate type-specific prevalence of HPV in women in Bahrain.

**Major Compulsory Revisions**

1. The manuscript should include a table containing information on the characteristics of the study population, e.g., age, nationality, level of education.
   - The authors thank the reviewer for the constructive feedback and have added a table with baseline information of women enrolled in this study (Table 1) (Pg 19).

**Table 1: Baseline characteristics (N = 571)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Parameters or Categories</th>
<th>Value or n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (Years)</td>
<td>N</td>
<td>571</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>35.57</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>11.19</td>
<td>-</td>
</tr>
<tr>
<td>Nationality</td>
<td>Bahraini</td>
<td>464</td>
<td>81.3</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>107</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>African heritage/African American</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Asia – Central/South East Asia</td>
<td>414</td>
<td>72.5</td>
</tr>
<tr>
<td>Race</td>
<td>White – Arabic/North African heritage</td>
<td>147</td>
<td>25.7</td>
</tr>
<tr>
<td></td>
<td>White – Caucasian/European Heritage</td>
<td>5</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

N: Number of subjects enrolled; n: number of subjects in a given category; Value: value of the considered parameter; %: n / N x 100; SD: Standard deviation

2. The statement regarding cross-protective efficacy of the bivalent vaccine at lines 198 – 199 seems gratuitous (noting that 2 of the authors are GSK employees) and should be removed unless it can be given
some context drawn from the results of the study. Similarly, the final sentence in this paragraph stating that this study provides data suggesting the need for a vaccine offering “broader protection” is unsupported and should be reworded unless it can be directly substantiated with reference to specific findings of the study.

- The authors thank the reviewer for the constructive feedback and have removed the information on cross-protective efficacy (Line 228, Pg 10). However, since in this study, we observed HPV types (HPV-52, -31, -51, -6, -70, -74) beyond those in the currently available vaccines, the statement on vaccine offering “broader protection” was retained in the discussion (Line 232, Pg 10).

**Minor comments**

1. The sentence at line 73 in grammatically incorrect. Perhaps it should read “As baseline data on HPV epidemiology and distribution of HPV types in Bahrain…”.

- The authors thank the reviewer for the constructive feedback and have modified the statement as “As baseline data on HPV epidemiology and distribution of HPV types in Bahrain are lacking…” (Line 88, Pg 4).

2. The authors should be more specific about the body that approved the study, given at line 90 as “the local institutional review board”, i.e., what is this body and what sort of approval was sought, was this an ethics approval, etc.

- The protocol was reviewed and approved by the local institutional review board prior to study start. This has been clarified in the Methods section “The study protocol was reviewed and approved by Research Technical Support Team of Ministry of Health. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.” (Lines 107-110, Pg 5).

3. The sentence at line 171 beginning “We found that nearly 10% of women…” should be changed to “The findings from our study suggest that nearly 10% of women…”.  

- The authors thank the reviewer for the constructive feedback and have modified the statement as “The findings from our study suggest that nearly 10%...” (Line 199, Pg 9)

4. It is not clear on lines 151 – 152 whether each of the 3 women infected with HPV-16 were co-infected with all of HPV-31, -45, and -56 or whether each had some combination of co-infection with these types?

- Response: The authors thank the reviewer for the constructive feedback and have modified the statement as “Three of the six women infected with HPV-16 (50%) were co-infected with other HR-HPV types (HPV-16/31, HPV-16/45 and HPV-16/56, respectively).” (Line 178, Pg 8).
1. I am curious about HPV-74 found in 0.5% of women in the study. HPV-74 is not included in the list of low risk types HPV types given on lines 65 – 66 as being associated with disease. Is this an omission or is it unusual that HPV-74 was found in the study population? Is HPV-74 known to be associated with disease?

- The authors would like to clarify that HPV-74 was indeed included in the list of LR-HPV types detected using the DEIA positive-Line probe assay (updated in the methods section) and hence is not an unusual finding. According to literature, “HPV74 is phylogenetically related to HPV types associated with a low risk of cancer development” (Longuet M, Cassonnet P, Orth G: A novel genital human papillomavirus (HPV), HPV type 74, found in immunosuppressed patients. J Clin Microbiol 1996, 34:1859-1862).

2. I think it would be interesting if the authors could provide some comment on the large difference in prevalence and risk of HPV infection between Bahraini and non-Bahraini women in the Discussion. Could this be for example partly due to sample bias?

- The authors would like to clarify that subjects were invited to participate regardless of their nationality. As per the screening log, there were no differences in the rates of rejection to participate among Bahraini and non-Bahraini women. This might suggest a selection bias and has been acknowledged in the limitation section (Lines 248, 249, Pg 11). Furthermore, our objective was to generate overall data on HPV types among women visiting the study centers and did not explore differences in HPV types among women of different nationalities.
Reviewer #2

Reviewers report

Major Compulsory Revisions

General

1. The authors provide a nice summary of the prevalence of HPV among a select population of women living in Bahrain. However, from the authors’ description of the population surveyed, this very select study population is not representative of all women living in Bahrain, and therefore the HPV information obtained from this population cannot be highly generalized, limiting the impact of this work. Specifically, women selected for inclusion in this study were those who had the resources to attend health care services (i.e. mostly native Bahraini), had normal cytology results, a portion were post-natal, and were all married. Over half of the current Bahraini population is estimated to be non-native immigrants who probably have a vastly different socio-demographic and health care access profile than their Bahraini female peers. In the current study population, only 18% reports being non-Bahraini, and probably represents a unique sub-sample of all female immigrants in the country. This fact needs to be addressed before these results can be interpreted further, and to me seems to be the largest gap in this work. In fact, I think a more interesting and higher impact analysis of this work, could stem from the differences in health care access and HPV prevalence between Bahraini and non-Bahraini women.

- The authors would like to clarify that the result can be generalized for Bahraini women and to less extent to “married” non-Bahraini women. For Bahrainis there is no “significant” difference in using the health services, especially for post-natal and screening and there is no evidence of such difference also among non-Bahrainis. Our study provides the estimation among the participating reference centers where all eligible women (as per inclusion-exclusion criteria were eligible to participate) will provide data on the HPV prevalence during the study period. Additionally, we estimated the sample size in advance to generate data on the HPV prevalence which would be representative for the country in the target population under study. One important point need to be considered that majority of expat workers are single males (around 70%) and majority of them are not permanently living in Bahrain. Most of expat females who are staying for a long period of time in Bahrain are “married”, while single females are staying for few years and go back to their country. The sample selection for non-Bahraini female was selected from the more “stable” part of this category.
2. In addition, it seems slightly illogical to ask about HPV knowledge questions to a population of women attending routine cervical screening services (if this information was intended to represent women’s HPV knowledge on a national level). The authors themselves state that only a very selection portion of the population is aware of these health services.

- The authors would like to clarify that although cervical screening is very well known for prevention of cervical cancer, our research question was to find out how many enrolled women had knowledge of ‘HPV infection as necessary cause for cervical disease’. HPV testing was not part of routine screening in the country at the time the study was conducted. However, by providing a questionnaire to women attending to screening, important information towards prevention of HPV infection could be attained.

3. Is there not a more generalizable population of women that could be used for this purpose?

- The authors would like to clarify that the population included in this study was indeed a representative of the entire population in Bahrain giving the reader a fair idea about the current knowledge of women in Bahrain about HPV. The statement on the generalizability of the population in the Discussion was modified as “Furthermore, the primary healthcare centers and the hospital included in this study were recognized by the Ministry of Health as reference hospitals. The population visiting these centers represented 90% of the local population of the Kingdom of Bahrain” (Lines 235-238, Pg 10).

Minor Essential Revisions

Background


- The authors thank the reviewer for the constructive feedback and the indicated reference has been updated and the statement was modified as “Globally, cervical cancer (CC) is the second most frequent cancer in women, with an estimated 1.6 million women diagnosed with CC between 2004 and 2008”. (Lines 57, 58, Pg 3).

2. There seems to be some disconnect that CC is the fourth most common cancer, incidence of 4 per 100,000 among 209,616 women and it causes approx. 16 deaths per year. Is the female population estimate accurate?
The authors thank the reviewer for the constructive feedback and have modified the statements as “CC ranks as the third most frequent cause of cancer in women with a crude annual incidence of four per 100,000 women in the Kingdom of Bahrain [2-4]. Twenty two new cases of CC are diagnosed every year and CC causes approximately 5 deaths annually in the Kingdom of Bahrain [2].” (Lines 58-62, Pg 3).

Methods

3. Please state which HPV types you classified as HR and LR for the LipPA25 test.

- The authors thank the reviewer for the constructive feedback and have updated the HPV types detected using the LiPA25 test as “LiPA25 version 1 system (Labo Biomedical Products, Rijswijk, the Netherlands) was also used to genotype 25 HR and LR HPV types (14 HR [HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68] and 11 LR-HPV types [HPV-6, -11, -34, -40, -42, -43, -44, -53, -54, -70 and -74]).” (Lines 121-128, Pgs 5, 6).

4. Why were unmarried women excluded? This needs to be addressed somewhere. Did the authors decide on this, or is there some stigma preventing unmarried women from attending these health screening services (i.e. is it assumed that there is no need for them to attend)?

- The authors would like to clarify that marital status was not a criteria for exclusion. All women ≥20 years of age attending routine cervical screening, women ≥16 years of age presenting for post-natal check-ups and women providing a cervical sample were included in this study. This has been described in the ‘Study design and population’ section under Methods (Lines 96–106, Pgs 4, 5). The following statement was added to the Results to clarify that women who were unmarried were also included “From the data available from 553 women, 11 women were single, 513 were married 7 were divorced or separated and 20 women were widowed”. (Lines 154–156, Pg 7).

5. Why were women with abnormal cytology excluded?

- The authors would like to clarify that to provide an overview of the HPV prevalence at population level studies for women attending regular screening have been recommended (de Sanjosé S et al, Lancet Infect Dis. 2007 Jul;7(7):453–9 and Bruni L et al, J Infect Dis. 2010 Dec 15;202(12):1789–99). However, in this study, we did not exclude women with abnormal cytology. As indicated in the Methods section ‘Women were excluded for: immunosuppression, abnormal cervical samples,…’ only women with abnormal cervical samples were excluded (Lines 104-106, Pg 5).
Discretionary Revisions

Discussion

1. Again, a more detailed description of exactly who is and who is not included in this study population needs to be given

- The authors would like to clarify that the inclusion and exclusion criteria have been defined in the Methods “Women aged either ≥20 years undergoing routine cervical screening or ≥16 years attending post-natal check-ups and willing to provide a cervical sample were enrolled. Women were excluded for: immunosuppression, abnormal cervical samples, heavy menstrual bleeding that would interfere with screening, hysterectomy, previous HPV vaccination or pregnancy.” (Lines 96-106, Pgs 4, 5) and hence were not repeated in the Discussion to avoid redundancy. In addition, the statement in the discussion ‘This selection of women for this study was limited to only include legally married women’ was removed (Pg 11) to avoid confusion of inclusion of married and unmarried women.

2. How are the hospitals/clinics representatives of Bahrain, as stated?

- The authors thank the reviewer for the constructive feedback and have clarified the indicated statement in the Discussion by modifying it as “Furthermore, the primary healthcare centers and the hospital included in this study were recognized by the Ministry of Health as reference hospitals. The population visiting these centers represented 90% of the local population of the Kingdom of Bahrain.” (Lines 235-238, Pg 10).

3. How was it determined that women included in this study had not already received the HPV vaccine?

- The authors would like to clarify that women who had previously received HPV vaccination were excluded from the study. This has been mentioned in the Methods “Women were excluded for: immunosuppression, abnormal cervical samples, heavy menstrual bleeding that would interfere with screening, hysterectomy, previous HPV vaccination or pregnancy” (Lines 104-106, Pg 5). This was verified by the investigators prior to enrollment.