Author's response to reviews

Title: High genital prevalence of cutaneous human papillomavirus DNA on male genital skin: The HPV Infection in Men Study

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Date: 4 November 2014

Author's response to reviews:

November 3rd, 2014

Dear Prof. Rachel Winer,

Associate Editor - BMC Infectious Disease

Re: # 1446586435135677 - revised manuscript

Following up on your e-mail of October 16th, 2014 we had the opportunity to revise the aforementioned manuscript. We are very thankful to the referees for their constructive criticisms.

All suggestions were incorporated into the manuscript as described below. All modifications are clearly marked in the text. It follows a detailed response to both reviewers' comments.

Comments from Reviewers:

Reviewer #1:

Reviewer's report
Title: High genital prevalence of cutaneous human papillomavirus DNA on male genital skin: The HPV Infection in Men Study

Version: 1
Date: 12 October 2014

Reviewer: Chris JLM Meijer
Reviewer's report:

This is a inventorising study in which the authors describe the prevalence of individual HPV types among genital HPV unclassified specimens collected earlier in men participating in the “HPV infection in men study” running in USA, Mexico and Brasil. Typing was done by 1. sequencing PCR amplimers or cloned amplicons of PGMY09/11 primers 2. Nested PCR using GP5+/6+ primers on PGMY9/11 negative amplimers followed by sequencing of the amplicons and 3. nested PGMY09/11-GP5+/6+ PCR negative samples using amplification using FAP59/64 primers followed by direct sequencing of the amplimers. Results were related with socio-demographic and behavioral characteristics. The results show that 66% of the originally unclassified specimen showed readable sequences with characterized (77%) and yet uncharacterized (23%) HPV types. Most of the characterized types were beta HPV types (73%) followed by alpha HPV types (20%) and gamma HPV types. Alpha HPV types were associated with smoking. No other socio-demographic or behavioral characteristics could be demonstrated. Among the alpha HPV types, HPV types were present which should have been found in the original typing procedure. They were probably missed due to very low viral load as these were only demonstrated by nested PCR.

Comments

The paper is well written, techniques well described, and conclusions carefully drawn.

1. As the authors describe the significance of these beta HPV types on the penis is not clear. This might have been partly cleared if the authors had used in these men a non-genital skin control for beta HPV types detection or had presented data on the prevalence of beta HPV types in a subset of specimen of the original study which showed a positive HPV typing result. These beta HPV prevalence data might have shed light on the significance of the present findings. The lack of relationship with socio-demographic and behavioral characteristics underlines this.

If these data can be provided it would strengthen the paper.

Level of interest: An article whose findings are important to those with closely related research interests.

The presence of cutaneous Beta-HPVs in the genital area of immunocompetent men is a novel finding. We agree with the reviewer that the significance of the detection of #-HPV types in the skin and the role of these viruses in tumorigenesis is not fully elucidated at present.

Alterations were made in the Discussion section (p.10) to better clarify this question.

Reviewer #2:

Reviewer's report
Title: High genital prevalence of cutaneous human papillomavirus DNA on male genital skin: The HPV Infection in Men Study

Version: 1 Date: 25 August 2014
Reviewer: Maria Alejandra A Picconi

Reviewer's report:

The manuscript presents new and valuable information on the HPV Infection in Men (HIM) study, which examines the natural history of genital HPV infection in men. The main goal of the present work was to identify and assess the prevalence of individual HPV types among unclassified genital HPV specimens collected in the study population at enrollment, and examine associations with socio-demographic and behavioral characteristics.

These data complement previous publications by this group. I do believe that the additional information included in this manuscript warrants a new publication as an Original Article (Research) in BMC Infectious Diseases.

Below are some suggestions/recommendations that could enhance the manuscript.

Background.
- 2nd paragraph, line 4. The authors might consider adding a new reference on HPV type classification by Bouvard et al (2009), the most recent and currently widely used and accepted classification. (Minor essential revisions)

The reference was included as suggested.

Methods. Typing of unclassified samples:

Why did authors analyze the FAP amplicons only by direct sequencing without cloning? An explanation would be appropriate, since the FAP system was designed to analyze cloned DNA by sequencing, but it is less successful for direct sequencing of amplicons (references 14 and 24) (Discretionary revisions)

We performed direct sequencing of FAP amplicons because we were successful in obtaining readable sequences in most cases.

Results.
- 2nd paragraph, line 4

It would be better to indicate just the result. I suggest the following changes:
“HPV type distribution of previously unclassified HPV infections is shown in Table

The suggestion was incorporated in the manuscript.

2. Overall, 18 samples (4.5%) were determined to be HPV negative using all primers sets (PGMY09/11, PGMY09/11 nested to GP5+/6+, and FAP59/64)”. The part of this phrase that explains the observation should be moved to the Discussion section (2nd paragraph, line 1). (Minor essential revisions)
Discussion

- 2nd paragraph, line 1. The authors might consider discussing here the result. I suggest the following changes: “Among the 404 samples analyzed employing three PCR systems to detect a wide spectrum of #-, #-, and #-HPV DNA, 4.5% of samples were determined to be HPV negative. The result suggests that a small proportion of initially unclassified HPV detection presumably represents spurious amplification.” (Minor Essential Revisions)

We considered the suggestions and made changes where appropriate.

- 2nd paragraph, line 2.

The authors might consider inserting quotes number 7 and number 8 related to their previous publication (J Clin Virol 2013 and Virology 2013) to clarify these comments. I suggest the following changes: (Full stop) “In 30.0% of the specimens, unreadable sequences were generated after direct sequencing of FAP54/69 PCR products; among these, the presence of more than one HPV type is suspected (insert references 14 and 24). This hypothesis has been confirmed by sequencing a number of clones from each sample whenever overlapping peak patterns were observed, and also using the Luminex technology (insert quotes 7 and 8).” (Minor Essential Revisions)

Changes were performed as suggested.

- 4th paragraph, line 4: In order to emphasize the idea that alpha, beta and gamma HPV coinfections in the total number of samples of the HIM study may have been underestimated, I suggest the following changes: (Full stop) “As with all studies, there are limitations that could influence the types that were grouped together as unclassified HPV types at enrollment in the HIM Study. For this reason, the analysis excluded specimens for which an HPV type had already been assigned by Linear Array, although these specimens may have also harbored beta and gamma -HPV types. Therefore, an even greater rate of alpha, beta and gamma HPV coinfections would be expected in the total number of samples of the HIM study. Co-detection . . . “ (Discretionary revisions)

We considered the suggestions and made changes where appropriate.

Minor issues not for publication:

Background:

- 1st paragraph, line 2: change by 8,000 bp
- 3rd paragraph, line 3: change by 4,000

Methods

- Study population, line 6: change by 3,105
- Statistical analysis, line 6. Insert comma after "beta-HPV species".

Discussion

- 1st paragraph, line 4, insert comma after "skin specimens"
-1st paragraph, line 9, insert comma after "knowledge"
-3rd paragraph, line 3, insert semicolon after "habits" and insert comma after "however"
-3rd paragraph, line 12, Insert period, same line, after "HPV types [26]"

We considered the suggestions and made changes where appropriate.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare I have no competing interest

We thank you in advance for the opportunity of submitting this manuscript to BMC Infectious Disease and look forward to hearing from you

Sincerely yours,

Laura Sichero

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