**Author’s response to reviews**

**Title:** Low concordance of oral and genital HPV infection among male patients with sexually transmitted infections in Vietnam

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**Author’s response to reviews:**

Editor

Comment 1

Please be aware that we have some concerns regarding reviewer 1’s ongoing points regarding the statistical analysis, but would ask you to revise in response to their points before making a final decision.

Response:

Thank you for your comments. We have consulted statisticians, re-analyzed our data according to their advice using the McNemar’s test and Cohen's kappa coefficient, and revised the text in response to the points of you and two reviewers.

Comment 2

In addition, you have an unacceptable text overlap in your manuscript which will need to be addressed. Please rewrite the background and methods sections in original language.

Response:
Thank you for your comment. As for the "Background" section, we have deleted the former second paragraph to avoid the overlap, and also changed the sentence as follows (Page 4, lines 18-19): “The frequency of sexual contact and autoinoculation were reportedly related with oral HPV infection in young men in the United States”.

As for the "Methods" section, we have revised the “Statistical analysis” paragraph in the “Methods” section as follows: "Cohen's kappa value was calculated to check the concordance of HPV infection between paired samples in the same patients. The McNemar’s test was used to assess the difference in the detection rates of HPV DNA and specific HPV genotypes between the paired samples [8]. The association of HPV infection between oral and genital samples was assessed by using binary logistic regression analysis." (Page 8, lines 5-9).

Because we used the same methods for sample collection, DNA extraction, HPV screening and HPV genotyping except urine sample collection as those in our previous studies, we have used similar description to our previous report in some parts (ref. 14).

Dr. David Mesher (Reviewer 1):

Comment

The authors have addressed the majority of points previously raised by reviewers and I feel this is an improved version of the manuscript. However, there is still a major issue with the analysis to compare HPV infection at different sites. In their response, the authors state that they have used Fisher's exact test to compare infections at different sites from the same individual. This test assumes independence which isn't correct here. The authors should get statistical advice on appropriate methods to compare HPV infection paired samples taken from the same individual.

Response:

Thank you for your comment. We have consulted statisticians, re-analyzed our data using the McNemar’s test and Cohen’s kappa coefficient, and revised the text as follows:

(1) In the “Results” part of "Abstracts" section:

After reanalyzing, we found that the prevalence of HPV18 did not significantly differ between the oral cavity and the genitals, and the concordance of HPV infection between the oral cavity and genitals was poor. We have deleted the sentence “HPV18 proportion in HPV-positives significantly differed between these sites (P < 0.01).” (Page 2, lines 18-19). We have also added the sentence “The concordance of HPV infection between the oral cavity and the genitals was poor (kappa = 0.01)” (Page 2, lines 13-14).

(2) In the "Statistical analysis" paragraph in the "Methods" section (Page 8, lines 5-9):
We have changed the sentence as follows: “Cohen’s kappa value was calculated to check the concordance of HPV infection between paired samples in the same patients. The McNemar’s test was used to assess the difference in the detection rates of HPV DNA and specific HPV genotypes between the paired samples [8]. The association of HPV infection between the oral and genital samples was assessed by using binary logistic regression analysis.” Moreover, we have prepared a new Table as Table 1 (Page 20-21) to show the results of statistical analyses, and changed other tables' order accordingly.

(3) In the "Profile of HPV infection" paragraph in the "Results" section (Page 9, line 15-16);

Based on the McNemar’s test, the P value was <0.000001. However, according to the reviewer's comment (two digits after the decimal point), we have not changed the P value (P < 0.01) for comparison of the HPV prevalence between the oral cavity and the genitals. We have also added one sentence to show the comparison of HPV prevalence among the genitals “In the genitals, the HPV prevalence was significantly higher in the penis than in the urethra and the urine (both P < 0.01) (Table 1).”

(4) In the "Profile of HPV infection" paragraph in the "Results" section (Page 10, lines 5-6); We have changed the sentences as follows: “The HPV18 prevalence did not significantly differ between the oral cavity and the genitals (4.5% vs. 5.1%, P = 0.50).”

We have also added two sentences:

“The concordance of HPV infection between the oral cavity and the genitals was poor (kappa = 0.01, Table 1).” (Page 10, lines 7-8), and

“Among the genital sites, the concordance of HPV infection was poor between the penis and the urethra (kappa = 0.20), and fair between the penis and the urine (kappa = 0.21), while the concordance was good between the urethra and the urine (kappa = 0.62) (Table 1).” (Page 10, lines 14-16).

(5) We have deleted the former 5th paragraph of the "Discussion" section (former Page 13 line 18 – Page 14 line 2): After reanalyzing our data using the McNemar’s test, we found that the prevalence of HPV18 did not significantly differ between the oral cavity and the genitals, and decided to delete this paragraph.

Dr. Darron Brown (Reviewer 2):

Comment 1
Title: STI should not be in the title; this should be spelled out.
Response:

Thank you for your comment. We have changed the title as follows:

"Low concordance of oral and genital HPV infection among male patients with sexually transmitted infections in Vietnam"

Comment 2

Methods: Line 32-what STIs occurred?
Response:

We have summarized the characteristics of the study subjects in the first paragraph "Subjects used for the analyses" of the "Results" section (Page 9, lines 1-8) as follows:

"GAPDH DNA was successfully amplified in 96.7% (203/210) of the penile samples, 100% (210/210) of the urethral samples, 99.5% (209/210) of the urinary samples, and 97.6% (205/210) of the oral samples. Overall, 198 male STI patients (median age 31.0 years, range 17–68) showed GAPDH DNA positive in all four samples, and their samples and data were used for further analyses. These patients consisted of 118 cases with urethral discharge, 42 with urethritis, five with gonorrhea, and six with dysuria (urethritis group, n = 171); and 20 with balanitis, three with molluscum contagiosum, two with genital ulcer, one with tuberculosis of testis and one with itching of foreskin (non-urethritis group, n = 27)."

Comment 3

Results: Line 37 HPV types not HPV strains.
Response:

As we have described in the "Profile of HPV infection" paragraph in the "Results" section, " Of the 198 patients, HPV DNA was detected in 69 (34.8%) in at least one of the four (penile, urethral, urinal and oral) samples." (Page 9, lines 11-12), and "Of the 69 patients with HPV infection, 121 HPV strains (102 from genital and 19 from oral samples) and 27 different HPV genotypes (13 high-risk and 14 low-risk) were detected (Table 2, additional file 1: Table S1)." (Page 9, lines 17-19)

Comment 4

Discussion: Line 44-Most parotid gland tumors have no relationship to HPV; this statement is misleading as stated in the manuscript.
Response:

Thank you for your comment.

According to Dr. David Mesher (Reviewer 1)’s comment, we re-analyzed our data using the McNemar’s test, and found that the prevalence of HPV18 did not significantly differ between oral cavity and genitals. So, we have decided to delete the former 5th paragraph of the Discussion section (former Page 13 line 18 – Page 14 line 2): “In HPV positive patients, HPV18 was the most common high-risk HPV genotype in the oral cavity and genitals, but the proportion of HPV18 was significantly higher in the oral cavity than in the genitals (56.3% vs. 17.2%, P < 0.01) among male STI patients in Vietnam. Reportedly, HPV18 is also most frequently detected in benign tumour tissues of the parotid glands [29]. These findings suggest that HPV18 has higher affinity for the parotid glands, and that the current HPV vaccine targeting HPV16 and HPV18 can be very effective in preventing Vietnamese men from oral HPV infection.”