

APPENDIX

METHODOLOGICAL DETAIL

*Participants and procedures*MSM sample recruited from the community

Participants were recruited between October 2015 and May 2016. We put flyers and advertisement for study recruitment in lesbian, gay bisexual, and transgender (LGBT) community centers, saunas, and LGBT-friendly bars, restaurants, and on social media. Eligible participants were 20 years of age or older, had ever had sex (including mutual masturbation, oral sex, or anal sex) with another man, and were willing to give written consent to participate in the study. MSM were seen at baseline and were/will be seen at 6, 12, 24 and 36 months. At each visit, anal and penile swabs were obtained for HPV DNA testing, and men completed a questionnaire regarding their sexual experiences. Men were given options to not answer any question they did not wish to answer. Men were also examined for the presence of anogenital warts. The results from the baseline visits are presented in this paper. Men were offered a gift card worth 5 USD to compensate for their time spent for the research. The study was approved by the Ethics Committee of the National Cheng Kung University Hospital (reference number: A-BR-103-075). All participants received clear information about the study, fully understood the study purpose, and signed a written informed consent.

HPV screening and sample collection:

A trained research nurse collected an anal swab and then a penile swab. In the collection of anal samples, a swab was inserted 3 cm into the anal canal to reach the dentate line and

was scraped along the anal walls by rotating three times clockwise and another three times counterclockwise.¹ In the collection of penile samples, a swab was rolled firmly over the entire penile shaft and glans penis, and over the inner and outer aspects of the foreskin if uncircumcised. The research nurse also documented the presence of visual anogenital warts.

The swabs were immediately placed into 1.5 ml of PreservCyt solution (Cytoc) and sent to the lab for further processing. HPV DNA testing and genotyping were performed by polymerase chain reaction (PCR). To automatically extract nucleic acid, DNA were prepared using cobas[®] 4800 (Roche Molecular Diagnostics, Pleasanton, CA). PCR was performed using three kinds of primers: MP09, MP11, GP6. Positive samples were then used to sequence HPV nucleic acid; the sequencing results were compared with HPV DNA data in the National Center for Biotechnology Information (NCBI) database. HPV genotyping was performed by the linear array HPV genotyping test (Roche Molecular Diagnostics, Pleasanton, CA) to identify the following 37 genital HPV types: (a) 21 high-risk types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73 (MM9), 82 (MM4), and (b) 16 low-risk types: 6, 11, 40, 42, 54, 55, 61, 62, 64, 71 (CP8061), 72, 81 (CP8304), 83 (MM7), 84 (MM8), 82v, 89. The categorization of high- and low-risk carcinogenic HPV types were referenced by previous classification.^{2,3}

Screening results were sent out to the study participants via email. If participants have visible anogenital warts examined by the research nurse, we provided them with a list of local clinics/hospitals and told them to bring our screening result for more checkups by a physician. Participants also received the list of clinics/hospitals and recommendations for

more checkups by a physician when they received their screening result and detected positive.

Survey Measurements

Sociodemographics, sexual behavior and other risk factors were assessed in the survey. Risk factors included the following: circumcision history, lifetime HIV testing, lifetime HIV/STI history, smoking status and recreational drug use (including ecstasy, methamphetamine, heroin, marijuana, ketamine, cocaine or RUSH) in the past year. We included a series of questions assessing participants' sexual behaviors adapted from the literature.³⁻⁵ For sexual behavior, the following questions were asked separately regarding insertive and receptive anal sex: the number of sex partners in the past year, time since first anal sex, frequency of condom use, sex with HIV-positive partners in the past year, and age of the majority of sex partners in the past year. Age of the partners were considered older or younger if they were not in the same age category as the participant himself; age range was categorized as less than 25, 25-34, 35-44 and 45 or higher.

Data analysis

Sample characteristics were compared using descriptive statistics. The numbers and proportions were calculated for all variables. For variables that had missing values more than 5%, we generated a missing category. For prevalence, the denominator is the number of men with a specific type of HPV tested at a specific anatomic site, and the numerator is the number of men infected with a specific type of HPV at a specific anatomic site. Chi-square tests were used to compare the difference in the proportion of HPV detection at the penile and anal sites. Variables with a crude odds ratio (OR) that

was marginally significant (a p-value of less than 0.1) were entered into a multivariable logistic regression model (with adjusted OR and 95% CI) to explore potential factors associated with HPV detection at a given anatomical site.

REFERENCE

1. Yu CT, Chao SC, Lee HC, et al. High prevalence of anal human papillomavirus infection and associated risky behaviors in men infected with human immunodeficiency virus in taiwan. *AIDS Behav.* 2013;17(3):1211-1218. doi: 10.1007/s10461-012-0173-6 [doi].
2. Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: Addressing the limits of epidemiology at the borderline. *Infect Agent Cancer.* 2009;4:8-9378-4-8. doi: 10.1186/1750-9378-4-8 [doi].
3. Zou H, Tabrizi SN, Grulich AE, et al. Early acquisition of anogenital human papillomavirus among teenage men who have sex with men. . 2014;209:642-651.
internal-pdf://0854056280/Zou-2014-Early acquisition of anogenital human.pdf internal-pdf://3494991075/J Infect Dis.-2014-Zou-642-51.pdf;
<http://www.ncbi.nlm.nih.gov/pubmed/24265440>
<http://jid.oxfordjournals.org/content/209/5/642.full.pdf>.

4. Ko NY, Koe S, Lee HC, Yen CF, Ko WC, Hsu ST. Online sex-seeking, substance use, and risky behaviors in taiwan: Results from the 2010 asia internet MSM sex survey. *Arch Sex Behav*. 2012;41(5):1273-1282. doi: 10.1007/s10508-012-9908-8 [doi].

5. Thomas EA, Goldstone SE. Should I or shouldn't I: Decision making, knowledge and behavioral effects of quadrivalent HPV vaccination in men who have sex with men. *Vaccine*. 2011;29(3):570-576. doi: 10.1016/j.vaccine.2010.09.101 [doi].