JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Human papillomavirus infection and anal cytology in Taiwanese homosexual men with and without HIV infection

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Abstract

Introduction: Anal cancer screening has not been adopted by Taiwanese care providers. The study aim was to explore the differences of anal cytology and HPV detection among men with and without HIV.

Methodology: In this case-control study, men with HIV who attended one of the outpatient clinics of Taoyuan General Hospital were enrolled as cases. Men who had experienced condomless sex and tested HIV negative were enrolled as controls. Anal swabs were collected for thin-preparation anal cytology and HPV genotyping.

Results: A total of 288 men who had tested positive for HIV and 208 who had tested HIV negative were enrolled; 75% of subjects with HIV and 30.3% of those without HIV had tested positive for various types of HPV ($P < 0.001$). Anal cell dysplasia, including atypical squamous cells with undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSILs), high-grade squamous intraepithelial lesions (HSILs), or atypical squamous cells cannot exclude HSIL (ASC-H), were noted in 20.8% of men with HIV and 4.8% of those without HIV ($P < 0.001$). In multivariate analysis, HIV serostatus, history of sexually transmitted infections, having male sexual partners, and practice of anal sex were correlated significantly with detection of any type of HPV. Additionally, both oncogenic and non-oncogenic HPV types were significantly associated with anal cytology dysplasia.

Conclusions: We strongly suggest that there should be awareness of anal HPV infection and related anal cellular dysplasia in at-risk populations.

Key words: HIV; HPV; ASCUS; men who have sex with men.

J Infect Dev Ctries 2019; 13(4):318-325. doi:10.3855/jidc.11162

(Received 23 December 2018 – Accepted 13 March 2019)

Introduction

Human papillomavirus (HPV) has been estimated to be the most common sexually transmitted infection (STI) worldwide [1,2]. Studies have shown that HPV infection is causally related to several types of cancer [3,4]. Further, the incidence of anal cancers continues to increase among men who have human immunodeficiency virus (HIV) in the era of highly active antiretroviral therapy (HAART), especially among men who have sex with men (MSM) [5,6].

The prevalence of genital HPV infection among HIV-seronegative men has been found to vary between geographic areas and ethnic groups. Reported prevalence rates range from 14% in China to 57% in the United States and 79% in Australia [7-10]. Moreover, among MSM, anal HPV prevalence rates range from 63.9% in HIV-negative cohorts [11] to 80.5% in German non-smokers and 89.0% in smokers [12]. Previous studies in western countries have shown that the rate of anal HPV infection in men who had HIV is in the range of 46–92% [11,13,14]. A study conducted in southern Taiwan showed that 77% of 130 MEM were infected with HIV and had anal HPV infections [15], whereas in northern Taiwan, 90.8% of men who had HIV had also tested positive for anal HPV [16].

Infection with oncogenic types of HPV has been related to more than 80% of cases of anal cancers and nearly 100% of cases of cervical cancers [17-19]. In

these studies, occurrence of anal cancers was 42-100 times higher in men who had HIV than in those without HIV [5,20]. In view of the increasing health burden of anal cancers in the HAART era, some researchers have performed anal cytology screening, similar to cervical Pap smears, for cancer prevention among men with HIV. In men with HIV, rates of abnormal anal cytology range from 23% to 74% [21-23]. Kreuter *et al*. [22] reported rapid progression (median, 8.6 months) from high-grade anal intraepithelial lesions (HGAINs) to invasive cancer in a German prospective study. These findings highlight the importance of anal cancer screening.

By the end of 2017, Taiwan had more than 36,000 reported cases of patients infected with HIV. Among newly diagnosed patients, more than 70% were MSM [24]. In Taiwan, there is a paucity of data on the prevalence of HPV among MSM, and HPV prevalence among HIV-seronegative MSM has never been reported. Therefore, establishing HPV prevention strategies that include providing vaccinations for men, making timely diagnoses, and providing appropriate treatment, as well as establishing a better understanding of the prevalence of HPV infection in MSM, especially seronegative men and those infected with HIV in Taiwan, is critical for the prevention of HPV transmission and progression. Thus, the aim of this study was to explore the differences between HPV detection and presentations, as shown through anal cytology, in the above targeted populations.

Methodology

Study Subjects

Between 2013 and 2014, HIV-infected men who attended one of the outpatient clinics of Taoyuan General Hospital, Taiwan, were enrolled voluntarily as cases in this study. HIV-negative men who had experienced condomless sex and had been counseled about HIV testing were enrolled as controls for comparison in this case-control study. Taoyuan General Hospital is a 1,000-bed regional referral hospital in northern Taiwan. After providing written informed consent, the subjects completed a self-administered questionnaire that addressed the following: their education level; marital status; substance use (current use of alcohol or tobacco and/or use of 3,4-methylenedioxy-N-methylamphetamine, amphetamine, ketamine, marijuana, flunitrazepam, or heroin within the previous 6 months); sexual behavior (heterosexuality or homosexuality, lifetime number of sexual partners, number of new sexual partners within the previous 6 months, frequency of receptive anal sex [always, often, occasionally, seldom, or never], frequency of condom use during anal sex [always, often, occasionally, seldom, or never], frequency of chemsex [always, often, occasionally, seldom, or never], participation in a sex party or internet-initiated sex [yes or no]); self-reported sexually transmitted infections (STIs) within the previous 6 months (syphilis, gonorrhea, chlamydial urethritis, condyloma acuminata, amebic colitis/liver abscess, or other clinical diagnoses of STIs), and circumcision status. Data were collected at the time of anal sampling.

This study was performed at and approved by the institutional review board of Taoyuan General Hospital (IRB No TYGH102054).

HIV Serologic Determination

HIV-1/2 antibody testing was performed using a chemiluminescence microparticle immunoassay (Architect HIV Ag/Ab combo; Abbott Laboratories, Abbott Park, North Chicago, IL, USA). Positive samples were run in duplicate and verified by western blot HIV-1 and HIV-2 assays (New LAV Blot-I and II; Bio-Rad Fujirebio, Tokyo, Japan) or HIV viral loads (Cobas AmpliPrep/Cobas TaqMan HIV-I test; Roche Molecular Systems, Branchburg, NJ, USA).

Anal Pap Smears

After receiving instructions, the subjects inserted saline-wetted Dacron swabs (Amplicor STD Swab Specimen Collection and Transport Set; Roche Molecular Systems) approximately 5 cm beyond the anal verge. Rectal swabs were rinsed immediately in a vial containing PreservCyt solution (Cytyc, Marlborough, MA, USA). Anal cytology samples were prepared by using thin-preparation Pap smears (ThinPrep; Hologic, Marlborough, MA, USA) and sent to a certified laboratory for interpretation blindly by two cytopathology technicians and two pathologists. The results were classified according to the 2001 Bethesda System [25]. We considered the following to be anal cellular dysplasias: atypical squamous cells of undetermined significance (ASCUS); low-grade squamous intraepithelial lesions (LSILs); high-grade squamous intraepithelial lesions (HSILs), and atypical squamous cells cannot exclude HSIL (ASC-H). The cells were preserved in PreservCyt solution and stored at -70°C for DNA testing.

HPV Genotyping

HPV genotyping was performed by a reverse line blotting method (Linear Array HPV Genotyping Test; Roche Molecular Systems). This method uses

biotinylated primers to amplify HPV polymorphic L1 consensus regions by polymerase chain reaction. Thirty-seven types of HPV were detected, including oncogenic types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; and non-oncogenic types, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108. Amplicons were denatured and hybridized to the oligonucleotide probe on the strips for visual interpretation. ß-globin was used as a positive control.

Statistical Analyses

Demographic data were presented as mean ± standard deviation (SD) for continuous variables and percentiles for discrete variables. Distributions of cytology grading were calculated, and HPV genotype results were analyzed. Chi-square tests were used to compare categorical variables, and Student's *t*-tests were used to compare continuous variables.

Covariates with $P < 0.2$ in the univariate analyses were included in the multivariate logistic regression analyses to determine which covariates predicted HPV detection and anal cytology results revealing ASUCS or higher grade (ASCUS+). The odds ratio (OR) and 95% confidence interval (CI) were estimated, and $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using SAS 9.3 software (SAS Institute, Inc., Cary, NC, USA).

Results

Subjects' demographics

In total, 496 subjects were enrolled in this study; of these, 288 subjects had HIV infection and 208 had tested negative for HIV. The subjects' mean age was

Table 1. Demographic data, detection rates of HPV, and anal cytology among 288 men who had HIV infection and 208 men who were HIV-negative in Taiwan.

Characteristics	HIV(+) N = 288 (%) or mean (± SD)		HIV(-) N = 208 (%) or mean (± SD)		P-value
Basic profiles					
Age (± SD)	32.90	± 7.83	28.31	± 6.00	< 0.01
Education (≥12 years)	166	(57.6%)	158	(76.0%)	< 0.01
Not married	273	(94.8%)	188	(90.4%)	0.07
Current smoker	141	(49.0%)	64	(30.8%)	< 0.01
Current betel-nut use	10	(3.5%)	6	(2.9%)	0.80
Drinking (often, daily)	5	(1.7%)	8	(3.8%)	0.16
Sexual lifestyle					
Number of lifetime sexual partners	31.47	± 87.85	10.21	± 14.50	< 0.01
Number of new sexual partners within the prior 1/2 year	2.86	± 6.36	2.28	± 1.98	0.15
Men who have sex with men	253	(87.8%)	134	(64.4%)	< 0.01
Having receptive anal sex (ever)	249	(86.5%)	130	(62.5%)	< 0.01
Having oral sex (ever)	264	(91.7%)	189	(90.9%)	0.75
Using a condom during anal sex (often, every time)	187	(75.1%)	92	(70.8%)	< 0.01
Attending a sex party (occasionally, often, every time)	24	(8.3%)	2	(1.0%)	< 0.01
Having sex for pay (occasionally, often, every time)	13	(4.5%)	18	(8.7%)	0.09
Meeting sexual partners by internet (occasionally, often, every time)	103	(35.8%)	69	(33.2%)	0.03
Having chemsex (occasionally, often, every time)	37	(12.8%)	14	(6.7%)	0.04
History of STD within the prior 1/2 year	76	(26.4%)	21	(10.1%)	< 0.01
Circumcision	37	(12.8%)	43	(20.7%)	0.03
Recreational drug usage	46	(16.0%)	4	(1.9%)	< 0.01
Heroin usage	10	(3.5%)	0	(0.0%)	0.03
Detection of HPV					
Any type	216	(75.0%)	63	(30.3%)	< 0.01
Oncogenic types	172	(59.7%)	46	(22.1%)	< 0.01
Non-oncogenic types	173	(60.1%)	41	(19.7%)	< 0.01
Anal Cytology					
Normal/Inflammation	213	(74.0%)	195	(93.8%)	< 0.01
ASCUS	28	(9.7%)	4	(1.9%)	< 0.01
LSIL/HSIL/ASC-H	32	(11.1%)	6	(2.9%)	< 0.01
Failure	15	(5.2%)	3	(1.6%)	0.02

ASCUS: atypical squamous cells with undetermined significance; ASC-H: atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; HIV: human immunodeficiency virus; HPV: human papillomavirus; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SD: standard deviation.

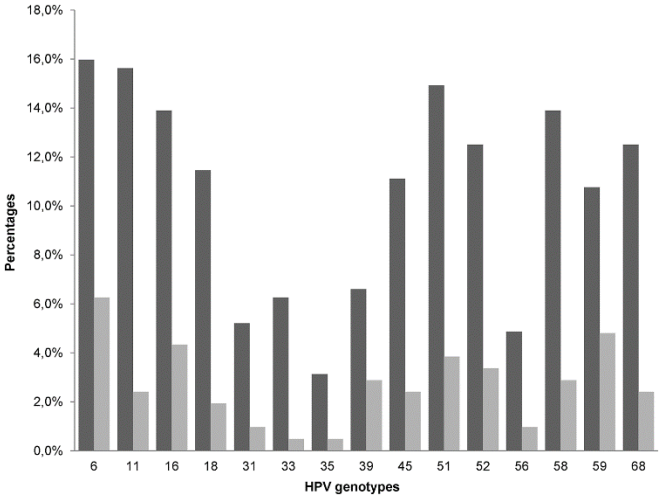
30.57 years. More than 90% of the subjects were unmarried and 78% were MSM.

As shown in Table 1, patients with HIV tended to be older than seronegative subjects, had a greater tendency to be smokers, were less apt to have received at least 12 years of education and were less apt to have been circumcised. Both HIV-positive and HIV-negative subjects were equally apt to be unmarried. Further, the mean number of lifetime sexual partners were higher in patients with HIV than in HIV-negative subjects; however, the mean number of new sexual partners within the previous half year were similar between the two groups. Of the patients with HIV, 87.8% had had sex with men, whereas this percentage was 64.4% in the seronegative group. Further, 86.5% of patients with HIV and 62.5% of seronegative subjects had practiced anal sex; moreover 75.1% of the patients with HIV and 70.8% of the seronegative subjects had used condoms either every time or frequently while having anal sex. In both groups, less than 10% had attended sex parties or had exchanged sex for money; however, more than 30% had met casual sexual partners through the internet. Further, 12.8% of the patients with HIV and 6.7% of the seronegative subjects had engaged in chemsex. The percentage of recreational drug use in the previous 6 months was higher in patients with HIV than in seronegative subjects (16.0% vs. 2.9%). Further, 26.4% of the patients with HIV and 10.1% of the seronegative subjects had had STIs within the previous 6 months. None of the subjects had received HPV vaccination before enrollment in the study.

Anal HPV

Among the participants, 75% of patients with HIV and 30.3% of seronegative subjects had tested as HPV positive ($P < 0.001$) (Table 1). Moreover, 59.7% of patients with HIV and 22.1% of seronegative subjects had oncogenic HPV, while non-oncogenic HPV types were identified in 60.1% of patients infected with HIV and in 19.7% of seronegative subjects. HPV types 6, 11, 16, and 51 were the most commonly encountered genotypes (Figure 1). The mean number of HPV types detected was 2.66 (± 2.79) for patients with HIV and 0.65 (± 1.27) for seronegative subjects. In multivariate analysis, HIV infection, history of sexually transmitted infections (STIs), MSM, and practice of anal sex correlated significantly with any HPV detection, after adjustment for the number of lifetime sexual partners (Table 2).

Figure 1. Rates of HPV detection in 288 men who had HIV infection and 208 men who were HIV-negative in Taiwan.



Anal cytology

In total, anal cytology could be interpreted in 478 cases, yielding ASCUS/LSIL/HSIL/ASC-H in 20.8% of men with HIV and 4.8% of those without it ($P < 0.001$) (Table 1). In multivariate analysis, HIV infection, history of STIs, MSM, number of oncogenic HPV types, and number of non-oncogenic HPV types were significantly correlated with anal cytology results of ASCUS/LSIL/HSIL/ASC-H, after adjustment for factors including practice of anal sex and numbers of lifetime sexual partners (Table 2).

Table 2. Multivariate logistic regression analyses for risk factors of HPV detection and anal cytology yielding ASCUS or higher grades (ASCUS+) in Taiwanese homosexual men.

	HPV		ASCUS+	
Variables	**aOR**	**95% CI**	**aOR**	**95% CI**
HIV	5.035	3.211-7.872	2.343	1.046-5.247
History of STDs	1.907	1.032-3.521	2.028	1.096-3.751
MSM	2.622	1.085-6.340	4.580	1.023-20.497
Practice of anal sex	4.894	2.124-11.279	0.586	0.171-2.007
No. of lifetime partners	1.077	0.893-1.299	1.002	0.999-1.006
No. of oncogenic HPVs	-	-	1.354	1.096-1.673
No. of non-oncogenic HPVs	-	-	1.245	1.035-1.496

aOR: adjusted odds ratio; ASCUS: atypical squamous cells with undetermined significance; CI: confidence interval; HIV: human immunodeficiency virus; HPV: human papillomavirus; MSM: men who have sex with men; No.: number; STD: sexually transmitted diseases.

Discussion

This is the first study conducted in Taiwan to compare anal HPV status between men who had HIV infection and HIV-seronegative men who had taken sexual risks. Anal HPVs were detected in 75% of patients with HIV, which is a percentage much higher than that of seronegative subjects, 30.3%. The above differences in prevalence of HPVs between patients with HIV and those without HIV corroborate results of previous reports regarding Chinese ethnicity [26]. However, the methods used for genotyping and the number of genotypic detections varied in these studies. For example, Gao *et al*. detected 19 oncogenic HPVs and 7 non-oncogenic HPVs using suspension bead assays; moreover, 26 types of HPV were detected in 96% of patients with HIV and 58.9% of subjects without HIV residing in Beijing and Tainjin [27]. Hu *et al*. [28] also used suspension bead assays and detected 26 types of HPVs; however, the definition of oncogenic types was not the same as that used by Gao *et al*. Among their cohort in Beijing, 82.1% of patients with HIV and 57.5% of subjects without HIV were found to have various types of HPV. Li *et al*. [29] applied the genechip Hybribio 37 GenoArray in diagnostic kits to detect 37 types (23 oncogenic and 14 non-oncogenic) of HPVs. Among enrolled subjects in Chengdu, Xi'an, and Taiyuan, 82.7% of patients with HIV and 62.8% of subjects without HIV tested positive for HPVs. Zhang *et al*. [30] described using HPV Geno Array chips to detect 21 types (15 oncogenic and 6 non-oncogenic) of HPVs. They reported that various types of HPV were detected in 71.4% of patients with HIV and 33.8% of subjects without HIV in Shengzhen.

The common HPV genotypes varied among different regions and studies areas. In China, the most common types of HPV were the following: In Beijing and Tainjin, 6, 11, 16, and 52 [27,28]; in Chengdu, Xi'an, and Taiyuan, 6, 18, and 16 [29], and in Shenzhen, 6, 16, and 11 [30]. In Thailand, types 16 and 68 were the most commonly encountered [31]. In this study, HPV genotypes 6, 11, 16, and 51 were predominant. HPV vaccination programs have been launched primarily among the young female population in Taiwan; however, there have been no data reported in support of vaccinations for MSM. Detection of type 51 HPV deserves special attention. This type presents an intermediate-risk because it belongs to the alpha-5 alpha papillomavirus species, which is not covered by commercially-available HPV vaccines: 2- (types 16 and 18), 4- (types 6, 11, 16, and 18), or 9-valent (types 6, 11, 16, 18, 31, 33, 45, 52, and 58); moreover, it is not cross-protected by alpha-9 (types 16, 31, 33, 52, and 58) and alpha-7 (types 18 and 45) [32,33].

In addition to the risk factors associated with HIV serostatus and MSM, the present study also showed that anal HPV detection was related to the practice of anal sex and history of STIs. Nagata *et al*. [34] demonstrated that ≥ 2 episodes of STIs presents a risk factor for acquiring anal HPV. Similarly, Kiviat [35] demonstrated that positive chlamydial serology was associated with anal HPV infection. Having STIs may result in mucosal breaks and implicates engagement in unprotected sexual behaviors. Previous studies have shown that men who have sex with women (MSW) had a low prevalence of anal HPVs [36] but could contract infection from autoinoculation of genital sites [37]. In contrast, the present study supported an association between anal HPV and practice of anal sex among MSM.

Our study demonstrated that HIV infection and MSM are associated with anal cellular dysplasia, echoing the worldwide epidemic of anal cancer occurring primarily in HIV-positive homosexual men [38]. Goldstone *et al*. [39] noted that the risk of high-grade anal dysplasia was 77% higher in HPV-positive than in HPV-negative homosexual men. Our study also demonstrated that numbers of HPV genotypes (both oncogenic [OR = 1.35] and non-oncogenic [OR = 1.25], Table 2) were associated with increased risk of developing anal dysplasia. Previous reports have shown a strong association between number of HPV types and anal SILs [40]. HPV types 16 and/or 18 have been considered the most important genotypes found in anal cancer [22] and have been strongly associated with HGAINs in previous studies [13,17]. While HGAINs were not taken into consideration in the present study due to the relatively few numbers of cases, we emphasized the increase in the number of HPV types as being a significant factor indicating ASCUS/LSIL/HSIL/ACH-H in cytology findings. Increasing numbers of HPV infections have been associated with HIV infection [17], with the potential to become persistent HPV infection and involve pathologic changes [41]. Associations between multiple oncogenic HPV infections and HGAINs have also been reported [13,17]. Briefly, persistent anal infection with multiple types of HPVs, including oncogenic HPVs, is one of the risk factors for the progression of high-grade anal intraepithelial neoplasms [40,42,43], and anal squamous intraepithelial neoplasms are known precursors of anal cancer [44-46]. Currently, HPV vaccines have been approved for young MSM for prevention of HGAIN in western countries [47], and

this strategy could also be considered in other countries if resources are available. There are some doubts about the frequency of HGAIN progressing to anal cancer, despite some case series that have reported rapid progression from HSIL to anal cancer [22,45]. In their meta-analysis, Machalek *et al*. [44] estimated that there was 1 case of anal cancer in 600 HIV-positive subjects with HSIL and 1 case in 4,000 in HIV-negative cases. Therefore, the potential benefits from regular anal cancer screening by cytology and high resolution anoscopy are still intriguing.

This study has some limitations. First, despite the large sample size, this is a cross-sectional study conducted in a regional referral hospital, which limits generalizability of the findings to all MSM in Taiwan. Second, because of the study design, we calculated ORs instead of risk ratios, which could have led to an overestimation of the relative risks. Third, the swab samples were self-collected, which may have obscured or biased the results. However, previous reports have documented and validated adequate sensitivity and feasibility of such self-collection protocols for HPV samples [48]. Fourth, in this study, 4.8% (10/208) of men who were HIV-negative had test results indicating anal cellular dysplasia. Since these 10 participants were MSM having anal sex often without condoms, we could not exclude the possibility of HIV infection acquisition during the detectability window.

Conclusion

Our data indicate that HIV-infected men who are MSM with a history of STIs and are infected with various types of HPV are prone to having anal cellular dysplasia. Since a SIL is a precursor of AIN and invasive cancers, we strongly suggest that there should be awareness of anal HPV infection and related anal cellular dysplasia in at-risk populations, especially MSM.

Acknowledgements

This study was supported by Taoyuan General Hospital grant PTH-10314. The grantor had no role in conducting the research or preparation of the article. The authors thank the study participants.

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Conflict of interests: No conflict of interests is declared.