



# Human papillomavirus in anal biopsy tissues and liquid-based cytology samples of HIV-positive and HIV-negative Thai men who have sex with men



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## ABSTRACT

**Background:** Men who have sex with men (MSM) are at high risk of developing human papillomavirus (HPV)-related anal cancer. We compared HPV genotypes in anal tissues (Bx) and anal liquid-based cytology fluid (LBC) from HIV-positive and HIV-negative MSM.

**Methods:** Bx (32 normal, 41 low-grade squamous intraepithelial lesions (LSIL) and 22 high-grade squamous intraepithelial lesions (HSIL)), along with LBC from the same visit, were selected from 61 HIV-positive and 34 HIV-negative MSM who enrolled into a prospective cohort in Bangkok, Thailand. HPV genotyping was performed on Bx and LBC.

**Results:** Any HPV and high-risk HPV (HR-HPV) prevalence were 63.2% and 60.0% in Bx and 71.6% and 62.1% in LBC, respectively. HIV-positive MSM had higher rates of HR-HPV genotypes detection (70.5% vs. 47.1%,  $p=0.03$ ) in LBC than HIV-negative MSM. HPV16 (27%) was the most common HR-HPV found in HSIL tissue. In HIV-positive MSM, the frequency of HR-HPV detection increased with histopathologic grading in both Bx and LBC samples. HSIL was associated with the presence of any HR-HPV (OR 7.6 (95%CI 1.8–31.9);  $P=0.006$ ) in LBC and in Bx (OR 5.6 (95%CI 1.4–22.7);  $P=0.02$ ).

**Conclusions:** Our data strongly support the integration of HR-HPV screening on LBC samples, along with HPV vaccination, into an anal cancer prevention program.

## 1. Introduction

Anal cancer incidence has increased over the past decades, [1–3] especially among men who have sex with men (MSM) [4]. The major risk factor thought to contribute to the development of such cancers is persistent infection with high-risk human papillomavirus (HR-HPV) [5,6], which can be transmitted by sexual intercourse [7]. Globally, HPV-related cancers contribute to 5.2% of all malignancies [5,6].

HIV prevalence among MSM in Bangkok increased from 17.3% in 2004 to 30.8% in 2007 [8]. The prevalence of anal HPV infection in MSM varies from 13.7% to 98% [9] and is significantly higher in HIV-positive compared to HIV-negative MSM [10,11]. In addition, HIV-positive MSM also have higher rates of persistent HR-HPV in the anus

than HIV-negative MSM [12,13]. As a consequence, the prevalence and incidence of anal high-grade squamous intraepithelial lesion (HSIL), a putative precursor of anal cancer, is higher in HIV-positive versus HIV-negative MSM [14]. It is believed that the higher susceptibility for HPV infection among HIV-positive MSM is because HIV infection induces a chronic inflammatory response in the epithelium, which promotes proliferation of HPV-infected cells [15].

Although there is currently no standard recommendation for screening, digital anorectal examination, HPV DNA testing, cytology, and high-resolution anoscopy (HRA) have been used in some clinical settings to screen for anal pre-cancerous lesions and anal cancers [16]. We aimed to compare HPV detection in paired anal liquid-based cytology fluid (LBC) and biopsy samples (Bx) collected from a cohort

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of HIV-positive and HIV-negative MSM in Bangkok, Thailand. We hypothesized that HPV subtypes within Bx would be found more frequently in samples of increasing grades of histopathology than those detected in LBC, and that HPV prevalence would be more common in HIV-positive versus HIV-negative MSM.

## 2. Methods

### 2.1. Enrollment and follow-up of study participants

The study was approved by the institutional review board of the Faculty of Medicine, Chulalongkorn University in Bangkok, Thailand ([ClinicalTrials.gov](http://ClinicalTrials.gov) identifier [NCT01637298](https://doi.org/10.1186/1745-6215-298)). The cohort has previously been described [14]. Briefly, Thai MSM aged 18 years or older, with documented HIV-negative or HIV-positive status within the previous 30 days, were enrolled at the Thai Red Cross AIDS Research Centre, Bangkok, Thailand. HIV-positive MSM also had CD4 counts and plasma HIV RNA levels measured at these visits. Written informed consent was obtained from all participants. We selected a convenience sample of paired LBC and Bx samples from HIV-positive and HIV-negative MSM collected from December 01, 2009 until May 31, 2012.

### 2.2. Anal sample collection

MSM had anal samples collected in LBC (Liqui-PREP, LGM International, Inc., Florida, USA) with the use of a moistened, non-lubricated flocked swab (Rovers EndoCervex-Brush, Rover Medical Devices B.V., Netherlands or FLOQSwabs, Copan Italia S.p.A., Italy) to collect cells from the anal canal surfaces. The collected cells in LBC were prepared to perform anal cytology and HPV genotype testing. HRA was performed on the same visit with the use of acetic acid and Lugol's solution to aid visualization of abnormal lesions. Abnormal lesions were biopsied for histologic diagnosis and HPV genotyping. Histologic diagnoses for each biopsy sample were made by three pathologists who were blinded to the diagnoses made by the others. The pathologist used hematoxylin and eosin stain (H & E) to provide histologic diagnoses. In case of diagnostic discrepancy, the slides were reviewed and discussed by all pathologists. At least 2 agreeing diagnoses were required for the final conclusion. The anal epithelial lesions were classified according to the nomenclature described by the Lower Anogenital Squamous Terminology (LAST) Standardization project for HPV-Associated Lesions into 2-tiered diagnoses: LSIL and HSIL, as described by Darragh et al. [17]. Other relevant lesions eg. Condyloma acuminata and immature squamous metaplasia are also described in the same reference [17]. By strictly following such criteria, we were able to classify most anal lesions in our study. In case of equivocal morphologic changes which might pose diagnostic problems distinguishing between precancer (-IN2) and their mimickers (e.g. immature squamous metaplasia, reparative epithelial changes, tangential cutting), p16 immunohistochemistry was performed to clarify the issue. Strong and diffuse block-positive p16 results were classified as HSIL. Other staining patterns were classified as LSIL [17].

### 2.3. DNA extraction from anal biopsy tissues

All Bx were fixed with formalin and embedded in paraffin, then cut into five serial sections. We protected against cross-contamination by changing the blade and using acetone and 95% alcohol to clean the stage when cutting each paraffin block. The first section was stained with H & E for histologic diagnosis and location mapping and the other four sections used for DNA extraction. DNA extraction was performed using the high-heat treatment protocol [18] by the QIAamp DNA FFPE Tissue Kit (Qiagen company Ltd., Hilden, Germany) and kept in 30  $\mu$ L RNase-free water and stored at  $-80^{\circ}$  C.

### 2.4. DNA extraction from anal liquid-based cytology fluid samples

Cells were collected and stored in anal LBC at  $4^{\circ}$  C for processing within 7 days. DNA was extracted according to the LINEAR ARRAY HPV Genotyping Test (Roche Molecular Diagnostics, California, and USA) (LA) protocol and collected in 120  $\mu$ L of elution buffer.

### 2.5. HPV genotyping

Screening for HPV DNA in extracted DNA samples from Bx was made by DNA ELISA Kit HPV SPF10, version 1 (Labo Bio-medical Products B.V., Netherlands). HPV genotypes were identified in positive samples by RHA Kit HPV SPF10-LiPA25, version 1 (Labo Bio-medical Products B.V., Netherlands). The assay used a reverse hybridization line probe for the identification of HPV genotypes 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70 and 74.

Extracted DNA samples from LBC were genotyped using the LA. Extracted DNA samples were amplified for specific HPV genotypes and beta-globin. HPV and beta-globin amplicons were hybridized with oligonucleotide probes for specific HPV and beta-globin and detected by colorimetric determination. The test kit could detect the following 37 HPV genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108.

### 2.6. Statistical analysis

Statistical analysis was performed using Stata version 13.1 (Statacorp, College Station, TX, USA). The prevalence of each HPV genotype in Bx and LBC was described, by HIV status and by degree of histological abnormality. HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 were considered HR-HPV. A Chi-square test or Fisher's exact test was used to compare demographics and behavioral data between HIV-positive and HIV-negative participants. A McNemar's test was used to compare the discordant proportions of each HPV subtype in the LBC and Bx samples from the same participant. Logistic regression was used to compare the detection of any HPV and any HR-HPV in study participants by HIV status, and by histology grading. Only subtypes common to both assays were included. A multivariate model was developed for covariates significant at  $P < 0.1$  in univariate models.

## 3. Results

### 3.1. Participant characteristics

A total of 95 paired Bx and LBC samples were selected. 61 Bx (15 normal tissues, 28 LSIL, 18 HSIL) came from HIV-positive MSM (one was found to have seroconverted when anal samples were collected) and 34 Bx (17 normal tissues, 13 LSIL, 4 HSIL) came from HIV-negative MSM. Of 95 Bx, 83% were intra-anal lesions and 17% were peri-anal lesions. At baseline, median age was 29 years (interquartile range, IQR, 25–35 years), 68% had bachelor's degree or higher, 84% were employed, and 16% were current smokers. These baseline characteristics were not different by HIV status (Table 1).

### 3.2. HPV detection

In Bx, HPV of any genotypes were detected in 83.2% and HR-HPV genotypes were detected in 60%. There was no significant difference in the detection of any HPV (85.3% vs. 79.4%,  $p=0.57$ ) and HR-HPV (63.9% vs. 52.9%,  $p=0.38$ ) in Bx between HIV-positive and HIV-negative MSM.

In LBC, 71.6% had any HPV and 62.1% had HR-HPV genotypes. HIV-positive MSM had higher prevalence of any HPV (78.7% vs.

**Table 1**  
Baseline demographic characteristics of MSM participants.

Characteristics	HIV-negative N =35	HIV-positive N =60	Total N =95	P-value
<b>Median (IQR) age (years)</b>	30 (24 – 36)	29 (25 – 34)	29 (25 – 35)	0.58
<b>Age (years) min - max</b>	19–53	19–51	19–53	
<b>Relationship status, n (%)</b>				0.17
Single	31 (89)	56 (93)	87 (92)	
Living together/married	4 (11)	2 (3)	6 (6)	
Widowed	0 (0)	2 (3)	2 (2)	
<b>Education, n (%)</b>				0.03
≤ grade 9*	4 (11)	5 (8)	9 (9)	
Finished grade 12/Lower vocational school	2 (6)	11 (18)	13 (14)	
Certificate/Higher vocational school	7 (20)	2 (3)	9 (9)	
Bachelor's degree	19 (54)	31 (52)	50 (53)	
Master's degree	3 (9)	11 (18)	14 (15)	
<b>Income (Baht), n (%)</b>				0.31
< 10,000/month	3 (9)	9 (15)	12 (13)	
10–20 K/month	20 (57)	25 (42)	45 (47)	
20,001–50 K/month	9 (26)	12 (20)	21 (22)	
> 50,000/month	0 (0)	3 (5)	3 (3)	
Don't want to answer	3 (9)	11 (18)	14 (15)	
<b>Occupation, n (%)</b>				0.91
Unemployed	2 (6)	3 (5)	5 (5)	
Student	2 (6)	5 (8)	7 (7)	
Office or factory worker	15 (43)	25 (42)	40 (42)	
Self-employed/Freelance	10 (29)	16 (27)	26 (27)	
Government employee	4 (11)	10 (17)	14 (15)	
Other	1 (3)	0 (0)	1 (1)	
Don't want to answer	1 (3)	1 (5)	2 (2)	
<b>Smoking</b>				1
Never smoked	27 (77)	45 (75)	72 (76)	
Ex-smoker	3 (9)	5 (8)	8 (8)	
Current smoker	5 (14)	10 (17)	15 (16)	

MSM, men who have sex with men; IQR, interquartile range.

58.8%,  $p=0.057$ ) and any HR-HPV (70.5% vs. 47.1%,  $p=0.03$ ) in LBC than HIV-negative MSM, although this was only statistically significant for HR-HPV. We found a higher prevalence of HPV16 (26.3% vs. 0%), and HPV42 (11.5% vs. 0%) among HIV-positive MSM than HIV-negative MSM ( $p < 0.001$  and  $p=0.047$ , respectively) from LBC.

### 3.3. Comparison of frequency of HPV detection by anal histologic grading

Table 2 shows the frequency of HPV genotypes detected in LBC and Bx by histology grading and by HIV status. The most common HPV subtypes in HIV-positive MSM with HSIL were HPV16 (44.4%), HPV58 (38.9%), and HPV39 (33.3%) in LBC and HPV16 (33.3%), HPV58 (27.8%), and HPV18 (22.2%) in Bx. A McNemar's test identified a significant difference in the detection of HPV6 ( $p < 0.0001$ ), HPV11 ( $p=0.002$ ), HPV39 ( $p=0.0008$ ), HPV42 ( $p=0.008$ ), HPV58 ( $p=0.002$ ), HPV59 ( $p=0.03$ ), HPV68 ( $p=0.005$ ), HPV70 ( $p=0.008$ ), and HPV73 ( $p=0.02$ ) in the LBC and Bx samples from the same participant. Higher proportions of detection were seen in LBC for all of these subtypes except for HPV6 and HPV11.

In Bx, HPV of any genotypes were found in 70.6% of normal histology, 84.6% of LSIL, and 100% of HSIL in HIV-negative MSM and 60% of normal histology, 92.9% of LSIL and 94.4% of HSIL from HIV-positive MSM. HR-HPV genotypes were identified in 58.8% of normal histology, 38.5% of LSIL, and 75% of HSIL from HIV-negative MSM and increasing proportions of 46.7% of normal histology, 60.7% of LSIL and 88.9% of HSIL from HIV-positive MSM. HPV6 and HPV11 were the two most common LR-HPV genotypes found in LSIL from both HIV-negative and HIV-positive MSM.

In LBC, we found HPV of any genotypes in 52.9%, 53.9%, and 100% of LBC from HIV-negative participants with normal histology, LSIL, and HSIL, respectively. In HIV-positive MSM, HPV of any genotypes were found in 53.3%, 85.7% and 88.9% of LBC with normal histology, LSIL and HSIL, respectively. HR-HPV genotypes were found in 41.2%, 38.5%, and 100% of LBC from HIV-negative MSM with normal histology, LSIL, and HSIL, respectively. In HIV-positive MSM, HR-HPV genotypes were found in 40%, 78.6% and 83.3% of LBC with normal histology, LSIL and HSIL, respectively. There was no difference in the HR-HPV detection rates in paired samples.

### 3.4. HPV detection and its associations with histopathology grade and HIV status

We used logistic regression to assess associations with the detection of any HPV and any HR-HPV with increasing histopathology grade and HIV status of the study participants, separately in LBC and Bx (Table 3). In LBC, both HIV status and having LSIL or HSIL versus normal histopathology were associated with a significantly increased detection of HPV or HR-HPV in a univariate analysis. However after adjusting for HIV-status in a multivariate model, only having HSIL versus normal histopathology was associated with increased detection of any HPV (OR 7.3, 95%CI 1.4 – 37.4;  $p=0.02$ ) or any HR-HPV (OR 7.6, 95%CI 1.8–31.9;  $p=0.006$ ).

In Bx, there was no significant association between HIV-status and HPV detection in univariate models, and so only univariate models assessing the relationship with HPV detection and histopathology grade were developed. Compared to participants with normal histopathology, the odds of having any HPV in Bx were 4.9 ((95%CI 1.4 – 17.1);  $p=0.01$ ) in participants with LSIL, and 11.0 ((95%CI 1.3 – 93.0);  $p=0.03$ ) in participants with HSIL. Compared to participants with normal histopathology, there was no significant increase in the odds of having HR-HPV in biopsy samples of participants with LSIL, but a significantly increased odds of HR-HPV in participants with HSIL (OR 5.6, 95%CI 1.4 – 22.7;  $p=0.02$ ).

## 4. Discussion

We reported for the first time the prevalence of HPV genotypes by anal histopathology grading from Thai HIV-positive and HIV-negative MSM. Our study of HPV genotypes in Bx and LBC is from the largest number of HIV-positive and HIV-negative MSM to date in Thailand. HPV of any genotypes were found in 83.2% of anal tissues and 71.6% of LBC samples while HR-HPV was found in 60.0% of anal tissues and 62.1% of LBC. We also demonstrated that the detection of HPV of any genotypes and HR-HPV genotypes increased in both Bx and LBC from HIV-positive MSM with increasing severity of anal histopathologic diagnosis.

HIV-positive MSM in our study had a higher prevalence of HR-HPV (70.5% vs. 47.1%,  $p=0.03$ ) in LBC than HIV-negative MSM. This finding supports previous reports on the higher prevalence and lower clearance of several HPV genotypes in the anus of HIV-positive MSM compared to HIV-negative MSM [19–21]. Several variables, including CD4 count and use of ART, were reported as independent risk factors for anal HPV infection in MSM [22].

We found that 9% of HSIL tissues in this study contained HPV6 and/or HPV11 without any other HR-HPV subtypes. A few studies also reported a substantial proportion of HSIL tissues in which only HPV6 and/or HPV11 were detected. A study from Slovenia reported 5% of anal cancer specimens to contain only HPV6 [23] and 12–15% of anal cancers in the UK contained LR-HPV genotypes, such as HPV6 and HPV11 [24]. Although HPV6 and HPV11 are considered LR-HPV genotypes which are known to commonly cause benign anogenital warts or LSIL, recent studies have demonstrated the occasional role of HPV6 and HPV11 and their genetic variants in causing or being exclusively associated with HSIL and anogenital carcinoma [25–27]. An abortive infection in which viral gene expression becomes deregulated

**Table 2**  
Frequency of HPV genotypes detected in HIV-negative and HIV-positive MSM, by histology grading, for genotypes detected in both liquid-based cytology fluid samples and anal biopsy tissues.

HPV	Bx										P (McNemars)						
	LBP					Bx											
	HIV-negative					HIV-positive											
	All (n=34)	Normal (n=17)	LGAIN (n=13)	HGAIN (n=4)	All (n=61)	Normal (n=15)	LGAIN (n=28)	HGAIN (n=18)	All (n=34)	Normal (n=17)	LGAIN (n=13)	HGAIN (n=4)	All (n=61)	Normal (n=15)	LGAIN (n=28)	HGAIN (n=18)	
HPV 6	3 (8.8)	0 (0)	3 (23.1)	0 (0)	10 (16.4)	1 (6.7)	8 (28.6)	1 (5.6)	13 (38.2)	4 (23.5)	7 (53.9)	2 (50)	18 (29.5)	3 (20)	13 (46.4)	2 (11.1)	< 0.0001
HPV 11	7 (20.6)	1 (5.9)	5 (38.5)	1 (25)	9 (14.8)	2 (13.3)	5 (17.9)	2 (11.1)	13 (38.2)	5 (29.4)	6 (46.2)	2 (50)	18 (29.5)	5 (33.3)	10 (35.7)	3 (16.7)	0.002
HPV 16	0 (0)	0 (0)	0 (0)	0 (0)	16 (26.3)	0 (0)	8 (28.6)	8 (44.4)	5 (14.7)	4 (23.5)	1 (7.7)	0 (0)	10 (16.4)	2 (13.3)	2 (7.1)	6 (33.3)	0.8
HPV 18	5 (14.7)	4 (23.5)	1 (7.7)	0 (0)	10 (16.4)	1 (6.7)	6 (21.4)	3 (16.7)	3 (8.8)	3 (17.7)	0 (0)	0 (0)	9 (14.8)	2 (13.3)	3 (10.7)	4 (22.2)	0.41
HPV 31	0 (0.0)	0 (0)	0 (0)	0 (0)	5 (8.2)	0 (0)	3 (10.7)	2 (11.1)	0 (0.0)	0 (0)	0 (0)	0 (0)	4 (6.6)	0 (0)	2 (7.1)	2 (11.1)	0.32
HPV 33	1 (2.9)	0 (0)	1 (7.7)	0 (0)	3 (4.9)	0 (0)	0 (0)	3 (16.7)	0 (0.0)	0 (0)	0 (0)	0 (0)	1 (1.6)	0 (0)	0 (0)	1 (5.6)	0.08
HPV 35	1 (2.9)	1 (5.9)	0 (0)	0 (0)	1 (1.6)	1 (6.7)	0 (0)	0 (0)	0 (0.0)	0 (0)	0 (0)	0 (0)	1 (1.6)	1 (6.7)	0 (0)	0 (0)	0.32
HPV 39	3 (8.8)	2 (11.8)	0 (0)	1 (25)	14 (23.0)	3 (20)	5 (17.9)	6 (33.3)	2 (5.9)	2 (11.8)	0 (0)	0 (0)	2 (3.3)	1 (6.7)	1 (3.6)	0 (0)	0.0008
HPV 40	1 (2.9)	1 (5.9)	0 (0)	0 (0)	3 (4.9)	1 (6.7)	2 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (4.9)	1 (6.7)	2 (7.1)	0 (0)	0.56
HPV 42	0 (0.0)	0 (0)	0 (0)	0 (0)	7 (11.5)	0 (0)	3 (10.7)	4 (22.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.008
HPV 45	1 (2.9)	0 (0)	1 (7.7)	0 (0)	8 (13.1)	2 (13.3)	3 (10.7)	3 (16.7)	1 (2.9)	0 (0)	1 (7.7)	0 (0)	3 (4.9)	0 (0)	2 (7.1)	1 (5.6)	0.06
HPV 51	2 (5.9)	0 (0)	2 (15.4)	0 (0)	10 (16.4)	2 (13.3)	5 (17.9)	3 (16.7)	7 (20.6)	4 (23.5)	2 (15.4)	1 (25)	10 (16.4)	3 (20)	5 (17.9)	2 (11.1)	0.28
HPV 52	2 (5.9)	1 (5.9)	0 (0)	1 (25)	7 (11.5)	1 (6.7)	5 (17.9)	1 (5.6)	6 (17.7)	3 (17.7)	1 (7.7)	2 (50)	8 (13.1)	0 (0)	6 (21.4)	2 (11.1)	0.23
HPV 53	2 (5.9)	0 (0)	1 (7.7)	1 (25)	7 (11.5)	0 (0)	4 (14.3)	3 (16.7)	1 (2.9)	1 (5.9)	0 (0)	0 (0)	3 (4.9)	0 (0)	2 (7.1)	1 (5.6)	0.06
HPV 54	1 (2.9)	0 (0)	1 (7.7)	0 (0)	3 (4.9)	0 (0)	3 (10.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (6.6)	0 (0)	1 (3.6)	3 (16.7)	1
HPV 56	0 (0.0)	0 (0)	0 (0)	0 (0)	5 (8.2)	1 (6.7)	2 (7.1)	2 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.6)	0 (0)	1 (3.6)	0 (0)	0.05
HPV 58	3 (8.8)	0 (0)	2 (15.4)	1 (25)	13 (21.3)	0 (0)	6 (21.4)	7 (38.9)	0 (0)	0 (0)	0 (0)	0 (0)	5 (8.2)	0 (0)	0 (0)	5 (27.8)	0.002
HPV 59	1 (2.9)	0 (0)	0 (0)	1 (25)	7 (11.5)	2 (13.3)	3 (10.7)	2 (11.1)	2 (5.9)	1 (5.9)	0 (0)	1 (25)	0 (0.0)	0 (0)	0 (0)	0 (0)	0.03
HPV 66	1 (2.9)	1 (5.9)	0 (0)	0 (0)	5 (8.2)	0 (0)	2 (7.1)	3 (16.7)	1 (2.9)	1 (5.9)	0 (0)	0 (0)	4 (6.6)	3 (20)	1 (3.6)	0 (0)	0.76
HPV 68	1 (2.9)	0 (0)	0 (0)	1 (25)	9 (14.8)	2 (13.3)	2 (7.1)	5 (27.8)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3.3)	0 (0)	0 (0)	2 (11.1)	0.005
HPV 70	4 (11.8)	3 (17.7)	1 (7.7)	0 (0)	3 (4.9)	0 (0)	2 (7.1)	1 (5.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.008
HPV 73	1 (2.9)	0 (0)	1 (7.7)	0 (0)	8 (13.1)	0 (0)	4 (14.3)	4 (22.2)	1 (2.9)	1 (5.9)	0 (0)	0 (0)	1 (1.6)	0 (0)	0 (0)	1 (5.6)	0.02
Any HPV	16 (47.1)	9 (52.9)	7 (53.9)	4 (100)	43 (70.5)	8 (53.3)	24 (85.7)	16 (88.9)	18 (52.9)	12 (70.6)	11 (84.6)	4 (100)	39 (63.9)	9 (60)	26 (92.9)	17 (94.4)	0.03
Any HR-HPV	20 (58.8)	7 (41.2)	5 (38.5)	4 (100)	48 (78.7)	6 (40)	22 (78.6)	15 (83.3)	27 (79.4)	10 (58.8)	5 (38.5)	3 (75)	52 (85.3)	7 (46.7)	17 (60.7)	16 (88.9)	0.86

Bx, anal biopsy tissues; LBC, liquid-based cytology fluid samples; LSIL, low-grade squamous intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial neoplasia. A McNemar's test was used to compare the discordant proportions of each HPV subtype in the LBC and Bx samples from the same participant.

lated might explain these findings [28]. When considering HPV screening as part of future AIN screening programs it may be worthwhile to include screening for HPV6 and HPV11 in addition to the other HR-HPV types. HPV vaccines which could induce immunity to HPV6 and HPV11 may also be beneficial for anal cancer prevention in addition to the known benefit of preventing anogenital warts.

We found HPV16 (27%), HPV58 (23%), HPV18 (18%), and HPV52 (18%) to be the most common HR-HPV found in HSIL tissue from all participants. This finding supports data from a study in Taiwan which demonstrated that HPV16 (68%), HPV58 (16%), HPV18 (4%), HPV31 (4%), HPV33 (4%), and HPV51 (4%) were the most common HR-HPV in anorectal biopsies of men and women with anal HSIL and squamous cell carcinoma [29]. In the US, HPV16 (77.4%) and HPV33 (6.2%) were found to be the most common HR-HPV genotypes in anal cancer tissues [30], while HPV16 (36%), HPV18 (16%) and HPV58 (16%) were the most common HR-HPV identified in HSIL biopsies of HIV-positive men. In the same study, HPV16 (55%), HPV18 (37%), HPV45 (29%), HPV58 (24%), and HPV33 (21%) were among the most common HPV genotypes found in anal swab samples collected from HIV-positive men with HSIL [31]. Our study identified HPV16 (36%), HPV58 (36%), HPV39 (32%) and HPV68 (27%) to be the most common genotypes in LBC from MSM with HSIL.

We identified from logistic regression models that having HSIL, regardless of HIV status, was significantly associated with increased detection of any HPV or any HR-HPV in LBC. Although discordant detection was seen among certain HR-HPV subtypes in LBC compared to Bx, this was not the case for HPV16 (the most common HPV subtypes in HSIL tissues) and any HR-HPV genotypes. Given that using anal swabs to collect LBC sample is much more convenient and less invasive than anal biopsy, we propose that anal swab could be a preferred and acceptable method for the screening of anal precancerous lesions. Our data also strongly supports the importance of the inclusion of HPV vaccination, which provides protection against common HR-HPV genotypes found in anal HSIL and anal cancer tissues, as well as HPV6 and HPV11, as part of anal cancer prevention program for MSM. Thailand, however, has just started the pilot school-based HPV vaccination program using bivalent HPV vaccine among grade 5 girls in 2014 with an aim to cover the whole country in 2020.

Our study has a few limitations. The HPV detection kit used for Bx was different from the one used for LBC which resulted in different ability to detect various LR-HPV genotypes and different assay performance characteristics such as sensitivity and specificity. However, both test kits were able to detect most of the known common LR-HPV and HR-HPV genotypes of the anogenital regions. Also, the small number of HIV-negative MSM partially limited the statistical power to evaluate HPV detection by HIV status.

The major strength of this study is that all Bx and LBC were collected from the same participants at the same visits and therefore provided a valuable opportunity for direct comparisons of HPV genotypes. Furthermore, as HRA was performed on all participants prior to anal cytology and HPV results, biases related to HRA physicians' and pathologists' identification of abnormal lesions or diagnosis of high-grade AIN were eliminated. Anal histologic diagnoses were also made based on agreement by at least two out of three pathologists, which ensured correct grading.

## 5. Conclusions

We demonstrated increasing frequencies of HR-HPV detection, both in LBC and Bx of HIV-positive MSM, with increasing histopathologic grading. Low numbers of HIV-negative MSM with higher grades of pathology limited the power to detect a trend in this group. HR-HPV genotypes in LBC were also detected more frequently in HIV-positive MSM than HIV-negative MSM. Our data strongly support the use of HR-HPV screening on LBC samples, along with HPV vaccination, in an anal cancer prevention program.

**Table 3**

Logistic regression models showing the odds ratio and 95% confidence interval for the detection of any HPV or any HR-HPV subtypes in MSM participants by histopathology grading and HIV-status.

	<b>Univariate OR (95%CI)</b>	<b>P</b>	<b>Multivariate OR (95%CI)</b>	<b>P</b>
<i>Any HPV in LBC</i>				
<b>Histopathology</b>				
Normal	1 (Ref)		1 (Ref)	
LSIL	2.7 (1.01–7.4)	<b>0.047</b>	2.4 (0.9–6.7)	0.09
HSIL	8.8 (1.8–44.2)	<b>0.008</b>	7.3 (1.4–37.4)	<b>0.02</b>
<b>HIV-pos vs. HIV-neg</b>	2.58 (1.03–6.47)	<b>0.043</b>	1.9 (0.7–5.0)	0.21
<i>Any HR-HPV in LBC</i>				
<b>Histopathology</b>				
Normal	1 (Ref)		1 (Ref)	
LSIL	2.8 (1.1–7.3)	<b>0.03</b>	2.5 (0.9–6.7)	0.07
HSIL	9.3 (2.3–37.8)	<b>0.002</b>	7.6 (1.8–31.9)	<b>0.006</b>
<b>HIV-pos vs. HIV-neg</b>	2.7 (1.1–6.4)	<b>0.03</b>	1.9 (0.8–4.9)	0.16
<i>Any HPV in Bx</i>				
<b>Histopathology</b>				
Normal	1 (Ref)			
LSIL	4.9 (1.4–17.1)	<b>0.01</b>		
HSIL	11.0 (1.30–93.0)	<b>0.03</b>		
<b>HIV-pos vs. HIV-neg</b>	1.5 (1.7–8.9)	0.47		
<i>Any HR-HPV in Bx</i>				
<b>Histopathology</b>				
Normal	1 (Ref)			
LSIL	1.02 (0.4–2.6)	0.96		
HSIL	5.6 (1.4–22.7)	<b>0.02</b>		
<b>HIV-pos vs. HIV-neg</b>	1.7 (0.7–4.0)	0.23		

OR, odds ratio; CI, confidence interval, LBC, liquid-based cytology fluid samples; Bx, anal biopsy tissues.

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