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Anal human papillomavirus genotype distribution and its associations with abnormal anal cytology among men who have sex with men

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ABSTRAK

BACKGROUND

Anal HPV is associated with the severity of anal cytologic abnormalities that are precancerous lesions. Knowledge of human papillomavirus (HPV) type distribution in populations at risk for anal cancer is needed. This study investigated anal HPV infections and cytological abnormalities among men who have sex with men (MSM).

METHODS

A cross-sectional study was conducted involving 90 men aged > 30 years with a history of anal sexual intercourse with men. Demographic characteristics and sexual behaviors were collected by using a self-completed questionnaire. Anal cytological results were examined, and HPV genotyping was performed by the Linear Array HPV genotyping test. Descriptive analyses of subject characteristics, prevalence, and 95% confidence intervals (CI) were performed. Logistic regression was used to estimate risk factors and their associations with high-risk HPV infection and cytological abnormalities.

RESULTS

The overall prevalence of abnormal cytology was 32% (24/75), atypical squamous cells of undetermined significance (ASCUS) 17.56% (13/75), 14.66% (11/75) were classified as low-grade SIL (LSIL) and no participant with high-grade SIL (HSIL). Prevalence HPV infection in normal cytology was 86.27% (44/51), ASCUS 92.30% (12/13), and LSIL 100% (11/11). The most common types of anal HPV in participants with cytological abnormalities are HPV 16, HPV 18 for high-risk HPV, and HPV 11, HPV 6 for low-risk HPV.

CONCLUSION

High prevalence HPV infection in MSM with abnormal anal cytology. A routine anal pap smear program and vaccination are needed to prevent HPV infection and anal dysplasia in MSM.

Keywords: Anal human papillomavirus, genotype, anal cytology, men who have sex with men.

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INTRODUCTION

Anal intraepithelial neoplasia (AIN) is a premalignant lesion of the anal mucosa that is a precursor of anal cancer. The risk factors for AIN and anal cancer consist of clinical and behavioral factors associated with the acquisition and persistence of human papilloma virus (HPV) infection. Risky anal intercourse behaviors include receptive anal intercourse,^(1,2) number of sexual partners,^(3,4) and age at first anal intercourse.^(1,5) A recent meta-analysis reported the prevalence of HPV in relation to the severity of anal cytological abnormalities.^(6,7) In addition, it was also reported that the incidence of AIN was higher in patients with HIV than in those without HIV.^(8,9) A study among patients with HIV infection showed that 74.8% (294/393) were negative for intraepithelial malignancies (NILM) and 22.9% (90/393) exhibited squamous intraepithelial lesions (SIL). In the latter category, 13.7% of samples (54/393) contained atypical squamous cells of undetermined significance (ASCUS), 6.9% (27/393) were classified as low-grade SIL (LSIL) and 2.3% (9/393) as high-grade SIL (HSIL).⁽¹⁰⁾ A study in 714 HIV-infected men showed that on anal cytology, 24.5% (175/714) subjects had atypical squamous cells of undetermined significance (ASCUS) or higher grades of dysplasia, including 49.7% (87/714) with ASCUS, 41.7% (73/714) with low-grade squamous intraepithelial lesions (LSILs) and 8.6% (15/714) with high-grade squamous intraepithelial lesions (HSILs).⁽¹¹⁾

A cross-sectional study in China showed that the prevalence of any type HPV was 30.6% for heterosexual men, 74.1% for homosexual and 63.6% for bisexual men among HIV-positive participants, while the prevalence was 8.3%, 29.2% and 23.8% respectively among HIV-negatives.⁽¹²⁾ Anal HPV infection is mainly transmitted through anal intercourse, which is usually considered as a major high-risk sexual behavior in men who have sex with men (MSM).⁽¹³⁾

Among 163 MSM, the overall prevalence of HPV was 66.2%. Cytological findings showed

that 15.3% had atypical squamous cells of undetermined significance, 16.6% had low-grade squamous intraepithelial lesion, 4.9% had atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesions and 17% had high-grade squamous intraepithelial lesions.⁽¹⁴⁾ However, most of the previous studies were mainly confined to HIV-seropositive MSM. Data comparing HPV prevalence among both HIV seropositive and seronegative MSM is limited. Therefore, this study aims first to determine the prevalence of anal HPV among MSM with and without HIV, and second, to determine the prevalence of anal HPV among MSM with and without anal cytological abnormalities in Bali, Indonesia.

METHODS

Research design

A cross-sectional study was conducted at Nusa Indah VCT Clinic of Prof,Dr,I.G.N.G Ngoerah General Hospital, which is a HIV and sexual transmitted diseases referral center in Bali, Indonesia, from June 22nd 2011 to February 13rd 2012.

Research subjects

We screened all male patients who came to Nusa Indah VCT Clinic of Prof. Dr. I.G.N.G Ngoerah General Hospital from June 22nd 2011 to February 13rd 2012. Males at least 18 years of age who reported having regular sex with men in the past 6 months were enrolled. Eligibility criteria were as follows: (1) willing to provide blood samples for HIV and anal swab specimens for HPV genotype and cytology examination, and (2) able and willing to provide written informed consent. A total of 90 male patients who came to Nusa Indah VCT Clinic of Prof. Dr. I.G.N.G Ngoerah General Hospital were included in the study.

Data collection

Study subjects were interviewed at study entry using a structured questionnaire. The information collected included demographic

characteristics (age, educational level, and employment), and sexual behavior within the previous year. We used 30 years as cut-off point for age since the median age was 31 years. We used senior high school (grade 9) as cut-off point for education because of the 9-year compulsory education program.

HIV serological determination

Blood samples were collected for HIV serological testing (anti-HIV). Anti-HIV tests were conducted with 3 different rapid test methods i.e. VIKIA® HIV 1/2, HIV 1 & 2 Antibody Rapid Test Oncoprobe, and HIV INTEC ½ rapid test. HIV-positive status was defined by three reactive rapid tests results. 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).

Anal specimen collection for cytology and HPV genotyping

A trained physician collected anal swab samples for HPV genotyping and cytology examination by rotating a moistened and non-lubricated flocked swab in the anal canal without direct visualization. The collected samples were stored in the liquid cytology media (Thin Prep PAP test PreservCyt® solution). Anal swab specimens were then stored at -80° C in the Molecular Biology Laboratory, Faculty of Medicine, Udayana University, Bali, Indonesia. Human papilloma virus genotyping was done using the Linear Array HPV Genotyping Test (Roche Molecular Systems, Inc., Alameda, CA) in the Clinical Pathology Laboratory of Dharmais Cancer Hospital, Jakarta, Indonesia. The test amplified the target DNA within the polymerase L1 region of the HPV genome (450 base pairs) by the polymerase chain reaction (PCR). The test then utilized nucleic acid hybridization to independently identify 37 anogenital HPV DNA 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 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39 and CP6108).

The anal cytology slides were interpreted by two pathologists. The cytological results were interpreted according to the Bethesda system terminology as negative, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), atypical-squamous cells that cannot exclude high grade squamous intraepithelial lesion (ASCH) and high grade squamous intraepithelial lesion (HSIL). HPV prevalence was defined as having a specific HPV genotype at enrollment visit. High risk (HR) HPV types (oncogenic HPV) are HPV with genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.⁽¹¹⁾ Low risk (LR) HPV types (non-oncogenic HPV) are HPV with genotypes 6, 11, 26, 40, 42, 53, 54, 55, 61, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108.⁽¹¹⁾

Statistical analysis

We compared baseline characteristics between HIV seropositive and seronegative MSM and tested differences in categorical variables using Chi-squared or Fisher's exact tests, while continuous variables were analyzed using Student-t or Mann-Whitney U tests. Frequency distributions and descriptive statistics were used to characterize the study sample. Data were analyzed using IBM SPSS Statistics for Windows, version 25.0 (SPSS Inc., Chicago, Ill., USA).

Ethical clearance

The study was approved by the institutional review board of Kerti Praja Foundation with ethical clearance No 027/IRB-YKP/2011.

RESULTS

There were 90 participants enrolled in this study; 11 out of 90 subjects did not attend when the specimen collection was performed. Out of 79 specimens, 2 (2.5%) were invalid and 2 (2.5%) specimens were un-isolated DNA specimens, i.e. their DNA could not be isolated, and 75 (95.0%) were adequate specimens for data analysis. The mean and median ages of the participants were

Table 1. Demographic characteristics of HIV-infected and HIV-uninfected MSM (n=75)

Characteristics	HIV infected (n=47)	HIV uninfected (n=28)	p value
Age (years)			
< 30	17 (48.6 %)	18 (51.4%)	0.017
≥ 30	30 (75 %)	10 (25%)	
Education level			
Low (< 9 years)	20 (76.9%)	6 (23.1%)	0.052
High (≥9 years)	27 (55.1%)	22 (44.9%)	
Age at first time anal sexual debut (years)			
< 18	30 (57.7%)	22 (42.3%)	0.149
≥18	17 (73.9%)	6 (26.1%)	
Number of sex partners in the last 6 months (Median (min-max))	4 (1- 60)	3 (1 - 75)	0.03
Receptive anal intercourse in the last 6 months			
Yes	9 (52.9%)	8 (47.1%)	0.25
No	38 (65.5%)	20 (34.5%)	
Condom use in the last 6 months			
Yes	22 (88%)	2 (12%)	0.001
No	25 (50%)	25 (50%)	
HPV			
Positive	21 (31.3%)	46 (68.7%)	0.03
Negative	26 (87.5 %)	1 (12.5%)	
Cytology			
Normal	30 (63.8%)	17 (36.2%)	0.229
Abnormal	21 (75.0%)	7 (25.0%)	

Note: HPV = human papilloma virus, MSM : men who have sex with men

Table 2. Anal intraepithelial neoplasia among 75 participants

Characteristics	Normal cytology (n=51)	Abnormal cytology (n=24)	p value
Age (years)	31.75 (± 7.82)	30.54 (±7.49)	0.53
Positive HIV status (%)	30 (63.8%)	17 (36.2%)	0.229
Education level			
High (≥ 9 years)	31 (63.3%)	18 (36.7%)	0.17
Partnership status			
Single/Fixed partner	10 (55.6%)	8 (44.4%)	0.157
condom use	35 (70%)	15 (30%)	0.392
HPV DNA detection	44 (65.7%)	23 (34.3%)	0.202
Number of detected high-risk (mean)	1.61 (±1.297)	1.50 (± 1.31)	0.739
HPV high risk genotypes			
HPV16	15 (75.0%)	5 (25.0%)	0.312
HPV18	8 (50%)	8 (50%)	0.077
HPV 31	1 (50%)	1 (50%)	0.54
HPV 33	2 (40%)	3 (60%)	0.18
HPV 39	3 (42.9%)	4 (57.1%)	0.14
HPV 45	4 (80%)	1 (20%)	0.48
HPV 51	11 (57.9%)	8 (42.1%)	0.28
HPV 52	12 (70.6%)	5 (29.4%)	0.522
HPV 56	5 (100%)	0 (0%)	0.136
HPV 58	7 (70%)	3 (30.0%)	0.59
HPV 59	8 (88.9%)	1 (11.1%)	0.146
HPV 68	6 (85.7%)	1 (14.3%)	0.27

Data presented as n (%) except for age and number of detected high-risk men (Mean ± SD)

Note: HIV = Human immunodeficiency virus; HPV = Human papillomavirus

31.36 \pm 7.69 and 31 years, respectively. The demographic characteristics of the participant are shown in Table 1, comprising data on age (<30 years), number of sex partners in the last 6 months, condom usage in the last 6 months, and HPV infection status as predictors for HIV infections.

Overall, 75 participants received anal cytology examinations. Figure 1 shows the anal cytology findings. Normal cytology findings were found in 51 participants (68%), ASCUS in 13 participants (17.33%), and LSIL in 11 participants (14.66%). No HSIL and ASCH was found.

The prevalence of HPV infection in participants with normal and abnormal cytology was 86.27% (44/51) and 23 (34.3%), respectively. Among the latter, there were 92.30% (12/13) ASCUS and 100% (11/11) LSIL (Table 2). The 6 commonest low risk anal HPV types seen in each cytology result group were as follows: Normal cytology: HPV-70 (7/51; 13.72%), HPV-62 (6/51; 9.8%), HPV-6 (5/51; 9.8%), HPV-53 (5/51; 9.8%), HPV-55 (5/51; 9.8%), and HPV-

61 (5/51; 9.8%). Low risk HPV infection in ASCUS: HPV-11 (3/13; 23.07%), HPV-54 (3/13; 23.07%), HPV-61 (3/13; 23.07%), HPV-6 (2/13; 20.37%), HPV-62 (2/13; 20.37%), HPV-72 (2/13; 20.37%) and HPV-73 (2/13; 20.37%). Low risk HPV infection in LSIL: HPV-62 (6/11; 54.54%), HPV-6 (5/11; 45.45%), HPV-11 (4/11; 36.36%), HPV-54 (3/11; 27.27%), HPV-72 (3/11; 27.27%) and HPV-84. (3/11; 27.27%).

In addition, the 6 commonest high risk anal HPV types seen in each cytology result group were as follows: Normal cytology: HPV-16 (15/51), HPV-52 (12/51: 25.33%), HPV-51 (11/51: 68%), HPV-18 (8/51: 21.33%), HPV-59 (8/51: 13.33%), and HPV-58 (7/51:12%) The prevalence of low risk HPV infection in ASCUS was as follows: HPV-16 (3/13;23.07%), HPV-18 (3/13;23.07%), HPV-51 (3/13;23.07%), HPV-52 (3/13; 23.07%), HPV-39 (1/13; 7.69%), and HPV-45 (1/13; 7.69%). The prevalence of low risk HPV infection in LSIL was as follows: HPV-18 (5/11; 45.45%), HPV-51 (5/11; 45.45%), HPV-39 (3/11; 27.27%), HPV-35 (3/11; 27.27%),

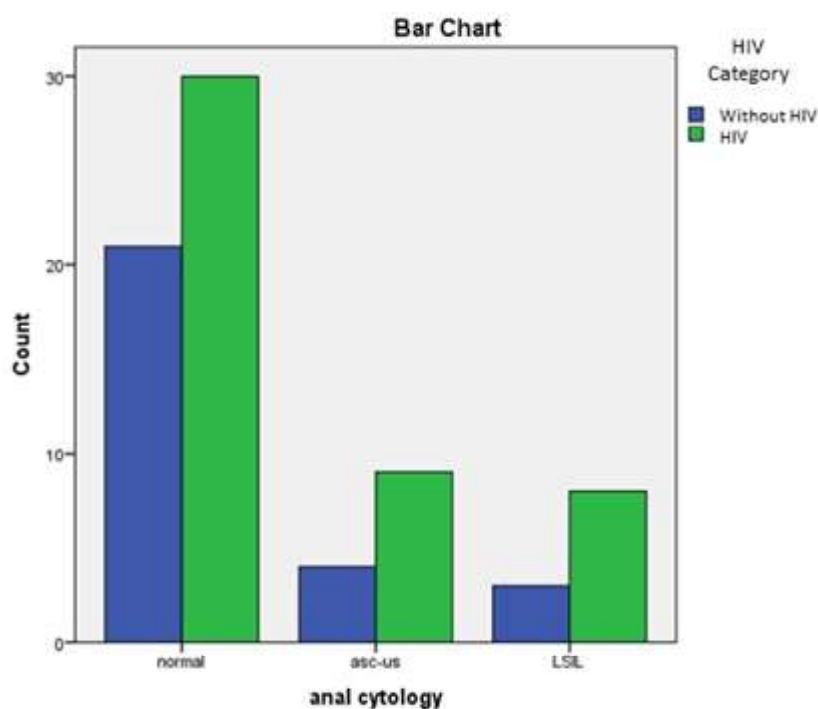


Figure 1. Prevalence of anal cytology results among men who have sex with men with and without human immunodeficiency virus infection

Note: ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion

HPV-16 (2/11; 18.18%), HPV-52 (2/11; 18.18%) and HPV-58 (2/11; 18.18%). As can be seen in Table 2, no characteristic can be used to determine abnormal cytology.

DISCUSSION

This study was conducted in a voluntary counseling and testing (VCT) clinic setting in a referral hospital in Bali, Indonesia, that receives a mixed population, some of whose individuals are self-referred, whereas others are referred by their health-care providers for specialist care.

The overall prevalence of HPV DNA, low risk HPV and high risk HPV was 89.3%, 69.33%, and 81.33%, respectively. The prevalence of abnormal cytology was 32%, of ASCUS 17.56% and of LSIL 14.66%, but HSIL was not identified. The absence of HSIL detection in this study may be due to the limited sensitivity/specificity for cytological detection of HSIL,⁽⁴⁾ to the high false negative rate in patients with HIV infection⁽⁵⁾ or to sampling factors. In this study, the proportion of participants with HIV was 62.66% with 70.8% having cytological abnormalities. However, in high-risk groups such as MSM, HIV or the immunocompromised, screening with anal cytology is still recommended.⁽⁵⁾

The prevalence of abnormal cytology found in this study was lower than that of studies in India,⁽¹⁵⁾ Taiwan,^(11,14) Pakistan,⁽¹⁶⁾ Thailand,⁽¹⁷⁾ Australia,⁽¹⁸⁾ and the United States of America.⁽¹⁹⁾ In our study, we found that the prevalence of high-risk HPV was 82.35% for normal cytology, 76.92% for ASCUS and 81.81% for LSIL. These figures are higher than in other studies.^(6,7,11,14,20) In our study, the types of HR HPV most often found in all of the abnormal cytological categories were HPV-16, 18, 51 and 52. Similar results have been reported in other studies, with HPV 16 and HPV 18 being the most common HR HPV found in abnormal cytological categories.^(11,14,15) The prevalence of HPV16 and HPV51 increases with the severity of abnormal anal cytological findings.⁽¹⁴⁾ Of interest in this study was the large

number of HPV 52 in abnormal cytology findings. The prevalence of low-risk anal HPV was 60.78% for normal cytology, 84.61% for ASCUS and 90.90% for LSIL. This is similar to the results of a study in Taiwan.⁽¹¹⁾ In our study, the commonest low-risk types seen in all abnormal cytology categories were HPV-11, 6, 62, 54. This is consistent with other reports stating that HPV 6 and HPV 11 are most often found in cytological abnormalities.^(4,15)

There are limitations to this study that should be considered. Due to the heterogeneity in HPV types, larger sample sizes are needed to further elucidate the influence of specific HPV types or number of anal HPV infections on abnormal anal cytological lesions. Lastly, due to its cross-sectional design, from the study results it may be difficult to infer causality, since we could not establish causal temporality. This study has shown the high prevalence of abnormal cytology in those with high-risk HPV. Therefore, this study has shown the importance of HPV vaccination to prevent abnormal anal cytology, such as was done in Scotland.⁽²¹⁾

CONCLUSIONS

This study demonstrated a high prevalence of HPV infection and abnormal anal cytology in MSM. The most common types of HPV in participants with cytological abnormalities are high risk HPV (HPV 16, HPV 18) and low risk HPV (HPV 11, HPV 6). Therefore, it is advisable to recommend routine anal Pap smears and HPV vaccination in this group using the quadrivalent vaccine type (HPV 6, 11, 16, 18).

CONFLICT OF INTEREST


None to declare.

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CONTRIBUTORS

IKAS: study conception and design, storage and shipping of anal swab specimens, statistical analysis and interpretation, manuscript drafting. TPM: study organization, study conception and design, manuscript review. DDS and IGAAEI: participant registration, interviews, informed consent, manuscript review. IBDP: processing, storage and shipping of anal swab specimens. NWW: cytology examination coordinator and manuscript review. MYOP: collecting the anal swab specimens and manuscript review. All authors have read and approved the final manuscript. 

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