

BMJ Open Multiple types of human papillomavirus infection and anal precancerous lesions in HIV-infected men in Taiwan: a cross-sectional study

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ABSTRACT

Objectives This study aimed to assess the relationship between infection with multiple human papillomavirus (HPV) types and abnormal anal cytology in HIV-infected men.

Design An observational, cross-sectional study.

Setting A regional referral hospital in Taiwan.

Participants In total, 714 HIV-infected men were enrolled between March 2011 and June 2016. Thin preparation anal Pap smears were interpreted according to the 2001 Bethesda System. Thirty-seven types of HPV were detected by reverse line blotting, including 13 oncogenic types and 24 non-oncogenic types.

Outcome measures The relationship between anal HPV infection and abnormal anal cytology in people of Asian ethnicity and the coverage efficacy in HPV-vaccinated HIV-infected men.

Results On anal cytology, 175 (24.5%) subjects had atypical squamous cells of undetermined significance (ASCUS) or higher grades of dysplasia, including 87 (49.7%) with ASCUS, 73 (41.7%) with low-grade squamous intraepithelial lesions (LSILs) and 15 (8.6%) with high-grade squamous intraepithelial lesions (HSILs). A higher proportion of subjects with those without LSIL/HSIL (93.1% vs 67.3%, $P<0.0001$) had multiple HPV types. The odds of having LSIL/HSIL increased with an increasing number of HPV types: the ORs ranged from 1 for no HPV types to 6.96 (95% CI 2.38 to 20.37) for more than five types ($P_{\text{trend}}<0.0001$). Multivariate logistic regression analysis showed a significant association between LSIL/HSIL and the number of HPV genotypes present (OR 1.20; 95% CI 1.02 to 1.42, $P<0.05$). HPV types covered by the nonavalent HPV vaccine (types 6/11/16/18/31/33/45/52/58) were detected in 70.1% of the patients in this study.

Conclusions The odds of having anal LSIL/HSIL are approximately seventimes greater in HIV-infected men with than without six or more types of HPV. Multiple HPV types in HIV-infected patients deserves aggressive follow-up, and HPV vaccination programme require scaling up.

INTRODUCTION

Anal cancers are rare in the general population.¹ In the early era of highly active antiretroviral therapy (HAART), the risk of developing

Strengths and limitations of this study

- This is a large observational study enrolling more than 700 Asian HIV-infected men.
- Anal cytology samples were prepared using thin preparation Pap smears. Liquid-based cytology improves sensitivity and reduces air-dry artefacts, compared with conventional methods.
- The study design precludes generalisation of the findings to other populations and requires the use of ORs, which tend to overestimate the relative risks.
- Although previous reports have documented and validated adequate sensitivity and feasibility of self-collection protocols for HPV samples, self-collected anal swab samples may obscure or bias the results.
- This study did not explore histological data.

anal carcinoma in situ was 60 times greater in HIV-infected men than in the general population, and the risk of developing invasive anal cancer was 38 times greater.² These higher rates did not abate during the years when HAART was used more widely. In the USA, the incidence rate of anal cancer among HIV-infected patients was 30 per 10⁵ person-years for the period 1984–1995, and 137 per 10⁵ person-years for the period 1996–2006.³ The incidence rate of anal cancer is five times greater for HIV-infected men who have sex with men (MSM) than for HIV-uninfected MSM (69 per 10⁵ person-years vs 14 per 10⁵ person-years, respectively).³ Because of HAART, the life expectancy of HIV-infected men is higher than it was in the pre-HAART era; hence, the issue of anal cancer is of great concern.

Oncogenic types of human papillomavirus (HPV), especially genotype 16, are found in more than 80% of anal carcinomas.^{4 5} Additionally, the association between oncogenic HPVs and histologically confirmed high-grade anal intraepithelial neoplasia (AIN)

Table 1 Demographic and behavioural characteristics of 714 HIV-infected Taiwanese men

Variable	n (%) or mean±SD
Age (years)	30.66±8.18
MSM	594 (83.2)
Duration since diagnosis of HIV	3.9±5.2
On highly active antiretroviral therapy	367 (51.4)
Current smoker	297 (41.6)
Recreational drug use	128 (17.9)
History of a recent STI	212 (29.7)
Number of new sexual partners in 6 months	2.83±4.27
Practice anal sex*	238 (33.3)
Condom use during anal sex*	405 (56.7)
Find sexual partners via websites*†	117 (36.1)
Have chemsex*	90 (12.6)
Latest CD4+ T-cell count (×10 ⁹ /L)	504.5±245.0
Undetectable HIV viral load	272 (74.1)

*Frequency: every time, always, often.

†Data from 324 cases.

MSM, men who have sex with men; STI, sexually transmitted infection.

has also been confirmed.⁶ A previous study showed that the risk of developing high-grade anal dysplasia was 77% lower in HPV-negative MSM than in HPV-positive MSM.⁷ Thus, oncogenic HPVs play an important role in both anal cell dysplasia and invasive anal carcinoma. However, other research has demonstrated that HPV type 16 is less frequently detected in surgical specimens with AIN and anal carcinoma in the HIV-infected population.^{8,9} Moreover, among HIV-infected patients, less than 50% of AIN sites sampled by laser-captured microdissection were causally related to HPV type 16.¹⁰ Given this, the role of concomitant infections with other HPV types in the development of anal cancer should be considered.

In Taiwan, the cumulative number of HIV infections exceeded 33 000 at the end of 2016. More than 85% of newly diagnosed HIV-infected patients are MSM.¹¹ Free access to HAART has been provided by the Taiwanese government since 1997¹²; thus, with increased life expectancy, a higher chance of developing comorbidities and cancers is expected, now and in the future. Several studies have described the prevalence of anogenital HPVs among HIV-infected MSM in the Asia-Pacific region. Previous studies in Taiwan showed that genital HPV was detected in 45.3% of 305 HIV-infected men¹³ and that 90% of 196 HIV-infected men had anal HPV, 81% of whom had oncogenic anal HPVs.¹⁴ In another study, the authors demonstrated that 77% of 130 HIV-infected homosexual men had anal HPV infection.¹⁵ In Japan, 75% of HIV-infected MSM were shown to harbour anal oncogenic HPVs.¹⁶ In Beijing, China, 82.1% of HIV-infected men had anal HPV infection; 61.3% had oncogenic HPVs.¹⁷ In a study from

Thailand, 85% of HIV-infected MSM had anal HPV infection, 57.5% of which were oncogenic HPVs.¹⁸ In Korea, 82.7% of HIV-infected MSM had anal HPVs, 47.4% of which were oncogenic.¹⁹

Despite these studies, there is still a limited understanding of anal HPV infection and abnormal anal cytology in MSM of Asian ethnicity who live in conservative societies with traditional societal norms, like Taiwan. Moreover, there is a paucity of research exploring the relationship between the number of HPV types present and anal cellular dysplasia. In addition, although HPV vaccination programme have been launched (primarily among the young female population) in Asian regions, there are no data to support HPV vaccination of men, especially MSM.^{20–24} This study aimed to assess the relationship between infection with multiple HPV types and abnormal anal cytology; this information could assist the proposal for an HPV vaccination programme for HIV-infected Taiwanese men.

METHODS

Study subjects

HIV-infected men visiting the outpatient clinics of Taoyuan General Hospital between March 2011 and June 2016 were invited to participate in the study. Taoyuan General Hospital is a 1000-bed regional referral hospital in northern Taiwan that accumulated 2000 cases of HIV infection until June 2016. Annually, approximately 150–200 cases of HIV were referred or newly diagnosed; these patients were enrolled for participation in this study. Subjects provided written informed consent prior to participation in the study.

The inclusion criteria were men aged >20 years with confirmed HIV infection. Subjects with acute anal discomfort and/or an anal mass were excluded. Subjects completed a self-administered web-based questionnaire that addressed their education level; marital status; substance use (current use of alcohol or tobacco in addition to the use of 3,4-methylenedioxy-N-methylamphetamine, amphetamine, ketamine, marijuana, flunitrazepam or heroin in the previous 6 months); sexual behaviour (heterosexuality or homosexuality, number of new sexual partners in the previous 6 months, frequency of receptive anal sex (always, often, occasional, seldom or never); frequency of condom use during anal sex (always, often, occasional, seldom or never); chemsex (yes or no) and participation in a sex party or web-based sex (yes or no)); self-reported sexually transmitted infections in the previous 6 months (syphilis, gonorrhoea, chlamydial urethritis, condyloma acuminata, amoebic colitis/liver abscess or other clinical diagnoses of sexually transmitted infections) and circumcision status. Data were collected at the time of anal sampling.

HIV serological determination

Initial HIV-1/2 antibody testing was performed using a chemiluminescent microparticle immunoassay (Architect

Table 2 Detection of human papillomavirus in 714 HIV-infected Taiwanese men

Variable	Total (n = 714)	Normal cytology (n=539)	ASCUS (n=87)	LSIL/HSIL(n=88)	P _{trend}
Oncogenic	509 (71.3)	353 (65.5)	77 (88.5)	79 (89.8)	<0.0001
Non-oncogenic	529 (74.1)	370 (68.6)	80 (91.9)	79 (89.8)	<0.0001
Type 6	140 (19.6)	82 (15.2)	21 (24.1)	37 (42.0)	<0.0001
Type 11	159 (22.3)	84 (15.6)	42 (48.3)	33 (37.5)	<0.0001
Type 16	125 (17.5)	69 (12.8)	26 (29.9)	30 (34.1)	<0.0001
Type 18	99 (13.9)	65 (12.1)	18 (20.7)	16 (18.2)	0.04
Type 31	56 (7.8)	39 (7.2)	8 (9.2)	9 (10.2)	0.32
Type 33	49 (6.9)	29 (5.4)	12 (13.8)	8 (9.1)	0.04
Type 45	92 (12.9)	59 (10.9)	18 (20.7)	15 (17.0)	0.03
Type 51	131 (18.3)	84 (15.6)	21 (24.1)	26 (29.5)	0.0007
Type 52	144 (20.2)	97 (18.0)	26 (29.9)	21 (23.9)	0.05
Type 53	112 (15.7)	68 (12.6)	25 (28.7)	19 (21.6)	0.002
Type 58	108 (15.1)	73 (13.5)	15 (17.2)	20 (22.7)	0.03
Type 59	100 (14.0)	64 (11.9)	20 (23.0)	16 (18.2)	0.02
Type 81	76 (10.6)	49 (9.1)	12 (13.8)	15 (17.0)	0.02
Type 84	96 (13.4)	65 (12.1)	16 (18.4)	13 (14.8)	0.48
16/18	199 (27.9)	121 (22.4)	37 (42.5)	41 (46.6)	<0.0001
6/11/16/18	376 (52.7)	231 (42.8)	70 (80.4)	75 (85.2)	<0.0001
6/11/16/18/31/33/45/52/58	501 (70.1)	337 (62.5)	83 (95.4)	81 (92.0)	<0.0001

ASCUS, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

HIV Ag/Ab combo; Abbott Laboratories, Abbott Park, Illinois, 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).

CD4+ T-cell count and HIV viral load measurements

CD4+ T-cell counts were determined by flow cytometry (BD FACSCanto II; Becton, Dickinson and Company, San Jose, California, USA) and HIV viral loads were quantified (Cobas AmpliPrep/Cobas TaqMan HIV-I test; Roche Molecular Systems, Branchburg, New Jersey, USA) at enrolment and every 3–6 months thereafter. Recent data were recorded for analysis.

Anal Pap smears

After receiving instructions and while at the outpatient clinic, the subjects inserted saline-wetted Dacron swabs (Amplacor STD Swab Specimen Collection and Transport Set; Roche Molecular Systems, Branchburg, New Jersey, USA) approximately 5 cm beyond the anal verge. Rectal swabs were rinsed immediately in a phial containing PreservCyt solution (Cytoc, Marlborough, Massachusetts, USA) and were sent to a certified laboratory within 1 week of being obtained.

Anal cytology samples were prepared using thin preparation Pap smears (ThinPrep; Hologic, Marlborough, Massachusetts, USA) and were analysed by two cytopathology technicians and two pathologists. The results

were classified according to the 2001 Bethesda System.²⁵ Anal cellular dysplasia, including atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions, were described. The cells were preserved in PreservCyt solution and stored at -70°C for DNA testing. Subjects with findings of ASCUS, LSIL or HSIL were referred for proctoscopy.

HPV genotyping

HPV genotyping was performed using a reverse line blotting method (Linear Array HPV Genotyping Test; Roche Molecular System, Branchburg, New Jersey, USA). This method uses biotinylated primers to amplify HPV polymorphic L1 consensus regions by PCR. 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 hybridised to the oligonucleotide probe on the strips for visual interpretation; β -globin was used as a positive control.

Statistical analysis

Demographic data are presented as the mean \pm SD for continuous variables and as percentiles for discrete

Table 3 Dose effect of an increasing number of HPV genotypes on the frequency of detection of LSIL/HSIL

Number of genotypes	Number of cases	LSIL/HSIL, n (%)	OR	95% CI	P
Any type					
0	105	4 (3.8)	1.00	NA	NA
1	101	2 (2.0)	0.51	0.09 to 2.84	0.237
2	121	14 (11.6)	3.30	1.05 to 10.37	0.066
3	97	13 (13.4)	3.90	1.22 to 12.43	0.007
4	79	11 (13.9)	4.08	1.24 to 13.35	0.008
5	64	12 (18.8)	5.82	1.79 to 18.96	0.001
≥6	148	32 (21.6)	6.96	2.38 to 20.37	<0.0001
Oncogenic type					
0	205	10 (4.88)	1.00	NA	NA
1	201	21 (10.44)	2.27	1.04 to 4.96	0.018
2	148	26 (17.56)	4.16	1.93 to 8.91	<0.0001
3	68	12 (17.64)	4.18	1.71 to 10.17	0.002
4	43	7 (16.28)	3.79	1.35 to 10.61	0.008
5	26	6 (23.07)	5.85	1.92 to 17.78	0.002
≥6	22	6 (27.27)	7.31	2.35 to 22.71	<0.0001

ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NA, not applicable.

variables. Distributions of cytology grading were calculated and HPV genotype results were analysed. The χ^2 (for trend) test was used to compare categorical variables, and Student's t-test was used to compare pairs of continuous variables. Covariates with a P value<0.2 in the univariate analyses were included in the multivariate logistic regression analyses to determine which covariates predict LSIL/HSIL. Adjusted ORs and 95% CI were estimated. A P value<0.05 was considered statistically significant. All

statistical analyses were conducted using SAS V.9.3 (SAS institute).

RESULTS

A total of 714 HIV-infected subjects were enrolled. Their demographic and behavioural characteristics are presented in table 1. The mean age was 30.7±8.2 years and 594 (83.2%) subjects identified as MSM. In the last

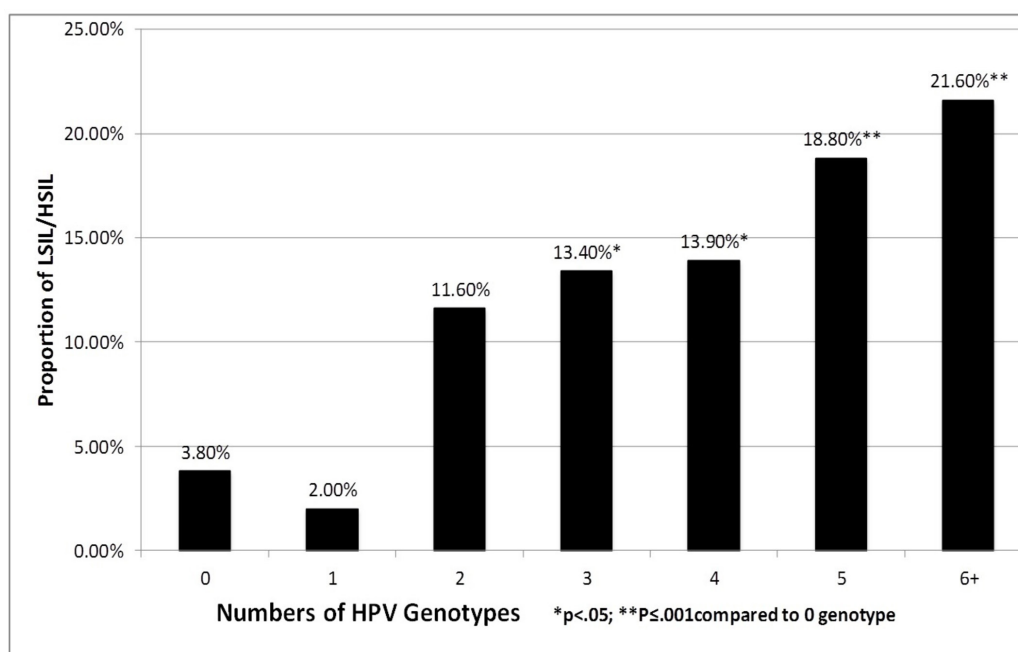


Figure 1 Relationship between the rates of LSIL/HSIL and the number of any HPV genotype. HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

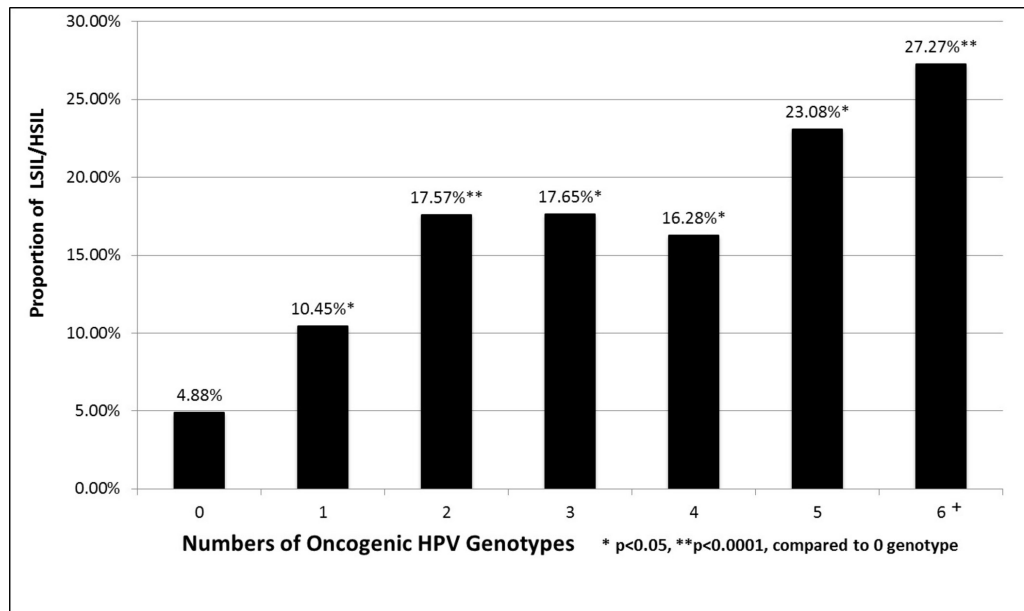


Figure 2 Relationship between the rates of LSIL/HSIL and the number of oncogenic HPV genotypes. HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

6 months, the subjects' mean number of new sexual partners was 2.8 ± 4.3 and 212 (29.7%) subjects had had a sexually transmitted infection. In terms of sexual behaviour, 33.3% practised anal sex, 56.7% of whom used condoms during anal sex, 36.1% had casual sex online and 12.6% had experienced chemsex. The mean duration of HIV infection was 3.9 ± 5.2 years; the mean CD4+ T-cell count was $504.5 \pm 245.0 \times 10^9/L$ and 367 (51.4%) were on HAART, 272 (74.1%) of whom had virological suppression.

Any type of HPV was detected in 610 (85.4%) subjects; oncogenic and non-oncogenic HPVs were detected in 509 (71.3%) and 529 (74.1%) subjects, respectively. HPV type 16 was detected in 125 (17.5%) subjects. HPV types covered by the readily available bivalent (types 16/18), quadrivalent (types 6/11/16/18) and nonavalent (types 6/11/16/18/31/33/45/52/58) HPV vaccines were detected in 27.9%, 52.7% and 70.1% of study subjects, respectively (table 2).

Cytology showed ASCUS or higher grades of dysplasia in 175 (24.5%) subjects; 15 (8.6%) had HSIL (table 2). A greater proportion of subjects with than without LSIL/HSIL had any type of HPV (93.1% vs 84.1%, $P < 0.0001$) and 93.1% versus 67.3%, respectively, had multiple HPV

types ($P < 0.0001$). The frequency of LSIL/HSIL increased as the number of HPV types present increased from 3.8% in those with no HPVs to 21.6% for those with ≥ 6 HPV types ($P_{\text{trend}} < 0.0001$), and as the number of oncogenic HPV types increased from 4.8% in those with no HPVs to 27.3% for those with ≥ 6 types of HPV ($P_{\text{trend}} < 0.0001$). The odds of having LSIL/HSIL increased as the number of HPV types detected (either any type or oncogenic types) increased (table 3, figures 1 and 2).

The multivariate logistic regression analysis demonstrated a significant association between LSIL/HSIL and both the number of HPV genotypes (OR 1.20; 95% CI 1.02 to 1.42; $P = 0.035$) after adjusting for age, number of new sexual partners, recreational drug usage and history of sexually transmitted infections (table 4).

DISCUSSION

Infection with HPV genotypes is common, both in MSM and in HIV-infected patients. In South Africa, Muller *et al*²⁶ showed that 72% of MSM had any type of HPV, 59% had multiple types and 57% had oncogenic HPVs. In Australia, a mean of 7.1 anal HPV types was detected in

Table 4 Multiple logistic regression analysis to determine the factors related to LSIL/HSIL

Variable	OR	95% CI	aOR	95% CI	P
Number of genotypes (any)	1.23	1.05 to 1.45	1.20	1.02 to 1.42	0.035
Age	0.95	0.91 to 1.00	0.96	0.92 to 1.01	0.164
Number of new sexual partners in 6 months	1.14	0.76 to 1.72	0.92	0.59 to 1.42	0.711
History of STI	1.47	0.78 to 2.27	1.10	0.56 to 2.18	0.769
Recreational drug use	2.14	1.08 to 4.23	1.94	0.95 to 3.95	0.069

aOR, adjusted OR; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; STI, sexually transmitted infection.

HIV-infected MSM and a mean of 4.2 types was detected in HIV-uninfected MSM.²⁷ Regarding individuals of Asian ethnicities, a meta-analysis showed that the pooled prevalence of HPV infection among Chinese MSM was 66%, among whom 33% had multiple types of HPV.²⁸ Another study, from Japan, showed that any oncogenic HPV and multiple oncogenic HPVs were detected in 75% and 54% of Japanese MSM, respectively; the mean number of HPVs detected was 2.4±2.2. Risk factors for acquiring multiple HPVs include young age, MSM, CD4+ T-cell count <100 cells×10⁹/L and multiple episodes (≥2) of sexually transmitted infections.¹⁶

In studies focused on HIV-infected MSM, infection with multiple oncogenic types of HPV was found in 30.9% of cases that had findings less severe than AIN-I (defined by the combined results of biopsy and cytology), in 45.5% of cases with AIN-I, in 76.4% of cases with AIN-II and in 76.3% of cases with AIN-III.²⁹ Up to 14 concurrent HPV types and up to 7 concurrent oncogenic HPV types were detected in individual specimens.²⁹ In a meta-analysis,⁴ infection with multiple HPV genotypes was found to be associated with high-grade AIN in 65% of HIV-infected individuals, compared with 13% of HIV-uninfected individuals (OR 12.60; 95% CI 7.05 to 22.51). Moreover, HPV genotype 16 was associated with high grade AIN in 53% and 76% of HIV-infected and HIV-uninfected individuals, respectively (OR 0.38; 95% CI 0.24 to 0.61). A study from Spain showed that multiple HPV types were present in 76% of HIV-infected patients versus 17% of HIV-uninfected patients with histology-confirmed perianal LSIL, whereas multiple HPV types were present in 75% of HIV-infected patients and in 80% of HIV-uninfected patients with perianal HSIL.³⁰

A study by Richel *et al*¹⁰ regarding HPV detection in whole tissue sections and laser-captured microdissection-selected dysplastic lesions showed that HPV genotype 16 is the causative agent in less than 50% of cases, and more than 50% of anal swabs do not detect lesion-specific HPV types. Similarly, Siegenbeek van Heukelom *et al*³¹ applied the same technique and demonstrated that oncogenic HPVs could not be detected in 40% of cases of high-grade AIN.

Thus, previous research has demonstrated that concurrent anal infection with multiple HPV types is common among MSM, especially HIV-infected MSM and that HPV genotype 16 is not the only factor related to anal dysplasia.^{4 10 26–31} Our data support the finding of multiple HPV types present in anal swabs from HIV-infected patients and importantly, demonstrate a strong relationship and dose effect between the number of HPV genotypes and the frequency of anal cellular dysplasia. We propose that infection with multiple HPV types may contribute to the unique microbiota that is prone to developing cellular dysplasia. Recent research has shown that HPV works synergistically with a cervical microbiota that has less abundant *Lactobacillus* spp to facilitate the development of cervical cancer.^{32–34} Whether anal microbiome dysbiosis related to infection with multiple HPV

types contributes to the development of anal cancers warrants further validation.³⁵

In this research, we found that in HIV-infected MSM, anal dysplasia is associated with the presence of an increasing number of oncogenic HPV types. This brought our attention to coverage of the current bivalent (types 16/18) and quadrivalent (types 6/11/16/18) HPV vaccines, compared with the newly launched 9-valent (type 6/11/16/18/31/33/45/52/58) vaccine. In the present study, HPV types in the available bivalent, quadrivalent and nonavalent HPV vaccines were, respectively, detected in 27.9%, 52.7% and 70.1% of patients in this study. Thus, more than tetravalent HPV vaccines may cover >50% of HPV genotypes for Taiwanese HIV-infected MSM. Since 2006, HPV vaccination programme have been instituted in Taiwan, targeting the young female population aged 9–26 years. Based on the coverage efficacy findings of the present study, HPV vaccination of men, especially MSM, is recommended.

The limitations of this study warrant discussion. First, despite the large sample size, this is a cross-sectional study conducted in a regional referral hospital, which limits generalisability of the findings to all HIV-infected men in Taiwan. Second, because of the study design, we calculated ORs instead of risk ratios, which could lead to overestimation of the relative risks. Third, the swab samples were self-collected, which may obscure or bias the results. However, previous reports have documented and validated adequate sensitivity and feasibility of such self-collection protocols for HPV samples.³⁶ Lastly, a specific HPV genotype has been linked to high-grade AIN in tissue samples, raising doubt about the meaning of multiple HPV genotypes detected in anal swabs.³¹ In this study, we were unable to explore histological data. Nevertheless, determining the effect of the interaction of multiple HPVs is a challenge, and there is still a paucity of data on this topic.

In conclusion, this study demonstrates that the risk of having anal LSIL/HSIL is seven times greater in HIV-infected men with six or more types of any HPV and oncogenic HPV detected on anal swabs than those without HPV. Hence, anal infection with multiple HPV types in this population deserves aggressive follow-up; the contribution of the interaction between multiple HPV and other bacteria for the development of anal cancers warrants further research. In addition, HPV vaccination programme require scaling up.

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Contributors SHC and FYC conceived and designed the study; SHC, CYC and CCW collected the clinical and laboratory data; KSL and FYC interpreted the histology data and the HPV genotyping data; SHC and KSL drafted and revised the manuscript and FYC approved the final version. SHC and KSL contributed equally to the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the institutional review boards of Taoyuan General Hospital, Ministry of Health and Welfare (IRB No: TYGH99034, 10020, 101042, 102054, 103040, 104034). All participants provided informed consent prior to their participation in the study.

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