Presence of High-Risk Human Papillomavirus Genotype and Human Immunodeficiency Virus DNA in Anal High-Grade and Low-Grade Squamous Intraepithelial Lesions

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Abstract

Human immunodeficiency virus type 1 (HIV)-infected individuals are at risk for anal cancer, which is caused by human papillomavirus (HPV). The relationship between HIV and HPV that leads to anal cancer remains unclear. Recent data, however, suggest that the continued persistence of HIV DNA in patients treated with combined antiretroviral therapy leads to progression of HIV disease and other HIV-associated complications. Therefore, we investigated the relationship among anal low- and high-grade squamous intraepithelial lesions (LGSIL/HGSIL), high-risk HPV genotypes, and high HIV DNA copy numbers. Anal cytology specimens were assayed for HPV genotype and HIV DNA copy number. High-risk HPV genotypes (odds ratio OR: 3.73; 95% confidence interval CI: 1.08–12.91; $p=0.04$) and high HIV DNA copy numbers (OR_{per 100 HIV DNA copies}: 1.13; 95% CI: 1.01–1.27, $p=0.04$) were both associated with LGSIL/HGSIL. When considering both high-risk HPV genotypes and HIV DNA copy numbers in predicting LGSIL/HGSIL, HIV DNA copy number was significant (OR_{per 100 HIV DNA copies}: 1.09; 95% CI: 0.96–1.23, $p=0.04$) but not high-risk HPV genotypes (OR: 2.30, $p=0.28$), which did not change when adjusted for nadir CD4 cell count and HIV RNA levels. The findings warrant further investigation of HIV DNA and its relationship with HPV in LGSIL/HGSIL pathogenesis.

Introduction

HUMAN IMMUNODEFICIENCY VIRUS type 1 (HIV)-infected individuals are at increased risk for human papillomavirus (HPV) infection. Data have suggested that HPV may increase the risk of acquiring human immunodeficiency virus type 1 (HIV).¹ With HIV-seropositive patients at increased risk of anal cancer, which is one of the leading non-AIDS-defining malignancies,² the direct link between HIV and HPV in causing anal cancer is unclear.^{3,4} The immune system typically eliminates most HPV infections in immunocompetent individuals over time. However in HIV-infected men who have sex with men (MSM), anal HPV persists in more than 90% of individuals with infections from multiple HPV types, which increases the risk of anal cancer.^{3,4} Most experts feel that high-grade anal dysplasia is potentially precancerous, although the rate of progression to anal cancer is unknown. Coinfection with HIV and HPV in

the anal canal may act synergistically, facilitating progression to cancer; however, the mechanistic relationship between HIV and HPV that leads to anal cancer remains unclear. Recent data suggest that the continued persistence of HIV DNA in circulating monocytes in patients treated with combined antiretroviral therapy (cART) leads to progression of HIV disease itself and other HIV-associated complications, which may include HPV infection and associated anal neoplasia.^{5–8} The objective of the present study was to evaluate anal cytology specimens for HIV DNA copy numbers and HPV genotypes in relationship to anal low-grade and high-grade squamous intraepithelial lesions (LGSIL/HGSIL) from patients participating in an anal cancer screening study. We hypothesized that a relationship between high-risk HPV genotypes and the presence of HIV DNA would be associated with anal LGSIL/HGSIL. Additionally, we hypothesized that an independent association of HPV and HIV DNA with anal LGSIL/HGSIL would exist.

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Materials and Methods

Patient population and specimen collection

During a 12-month period in 2010, 38 men and 8 women, 18–65 years of age, were recruited for an anal cancer screening project in collaboration with the Hawaii Center for AIDS (HICFA) University of Hawaii (UH) and UH Cancer Center in accordance with UH Institutional Review Board policy. This prospective study utilized outreach community education efforts focusing on HIV-associated anal dysplasia/cancer screening and informative sessions with community health-care providers who take care of HIV-infected patients. Consecutive participants, referred by their healthcare providers or self-referred, underwent the informed consent process for participation. Anal cytology specimens were collected with a Dacron swab and stored in CytoThinPrep collection medium (Cytoc Corp., Boxborough, MA). The specimens were processed by a CLIA-certified clinical laboratory with the cytopathology reviewed and reported by the same experienced cytopathologist (J.K.) according to the Bethesda system with anal cytology evaluated by using modified criteria and terminology.^{9–12}

Specimen cytologies were categorized as (1) negative if no cellular changes could be detected or if cellular changes were caused by inflammation or reparative process; or (2) LGSIL/HGSIL if abnormal cytological changes were seen indicating HPV infection or evidence of squamous intraepithelial lesions. Differentiation between LGSIL and HGSIL was not available from the cytopathology reports. Additional anal specimen swabs were collected for HPV DNA detection and genotyping and HIV DNA assays and placed into viral transport medium. HIV RNA levels and the nadir CD4 cell count were obtained from the participants with the HIV RNA levels within the last 6 months of study entry. A previous study of participants from the HICFA validated self-reported nadir CD4 cell counts with historical medical records.¹³

HPV genotyping

DNA from the anal specimen was extracted using commercial reagents (Qiagen Inc., Valencia, CA) and analyzed for the presence or absence of HPV DNA by PCR using a modified version of the PGMY09/PGMY11 primer system.¹⁴ β -Globin-positive and HPV DNA-positive specimens were genotyped using a reverse line blot detection method for 36 different HPV types (Roche Molecular Systems, Pleasanton, CA).¹⁴ We defined the risk (oncogenic potential) associated with various HPV types using the definition by Castle *et al.*¹⁵

HIV DNA analysis

DNA from the anal specimens was extracted from the cell pellet after centrifugation of the CytoThinPrep suspension. HIV DNA copy numbers were measured by real-time PCR as previously published.⁶ Primers and probes included HIV gag (forward 5'-GAC ATC AAG CAG CCA TGC AA-3'; reverse 5'-CTC ATC TGG CCT GGT GCA AT-3') and β -globin (forward 5'-AGG GCC TCA CCA CCA ACT TC; reverse 5'-TCA CTA GCA ACC TCA AAC AGA CAC C-3') primers, and VIC-labeled HIV gag (5'-ACC ATC AAT GAG GAA GCT GCA GAA TGG GA-3') and FAM-labeled β -globin (5'-CTC CTG AGG AGA AGT CTG CCG TTA CTG CC-3') probes. Negative- and positive-control DNA were included for all

reactions, which were set up in triplicate. Analyses were completed using SDS 2.3 software (Applied Biosystems, Foster City, CA). The copy numbers of each sample gene were analyzed against the standard curves and the HIV DNA copy numbers per 1×10^6 cells were extrapolated based on previously published methods.⁶ The sensitivity of the HIV DNA assay is 10 HIV DNA copies per 1×10^6 cells with the HIV DNA copy number dichotomized ($>$ or $\leq$) at the median (129.5 copies per 1×10^6 cells).

Statistical analyses

Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the associations of high-risk HPV genotypes (yes or no) and HIV DNA copy number with anal cytology results (normal or LGSIL/HGSIL). For the analysis, non-high-risk HPV genotypes included specimens that were either negative for HPV or low-risk HPV genotypes. Potential predictors of risk, including CD4 nadir and HIV RNA level, and the interaction of high-risk HPV genotypes and HIV DNA copy number were considered in adjusted models. The likelihood ratio test (LRT) was utilized to determine the significance of a predictor, and the Hosmer–Lemeshow statistic was used to test the model goodness-of-fit. The distributions of HIV DNA copy number among participants with normal and LGSIL/HGSIL were compared using the Kruskal–Wallis test.

Results

The 46 participants were from diverse ethnic backgrounds, reflecting the multiethnic population of Hawaii (Table 1). The mean ages of men (48.4, SE=8.5) and women (49.7, SE=4.8) were similar ($p=0.66$). All of the participants were on cART with 41 (89%) participants having undetectable HIV RNA levels. Nadir CD4 cell counts were available from 43 (93%) of the participants; three participants did not know their nadir CD4 cell counts. The median nadir CD4 cell count was 245 cells/mm³ (5–661 cells/mm³).

All of the anal specimens as reviewed by the cytopathologist were adequate for cytology assessment with 26 of 46 (57%) having LGSIL/HGSIL cytology results. Assessment for genomic DNA by β -globin PCR found that all of the specimens had sufficient DNA for HPV and HIV DNA analysis. High-risk HPV genotypes were detected in 22 of the 46 specimens (48%). At least one high-risk HPV genotype was found in 16 of 26 (62%) specimens that had LGSIL/HGSIL compared to 6 of 20 (30%) normal cytological specimens (Table 1). The presence of a high-risk HPV genotype was significantly associated with LGSIL/HGSIL (OR: 3.73; 95% CI: 1.08–12.91; $p=0.04$). There was also an association of LGSIL/HGSIL with HIV DNA copy numbers (OR_{per 100 HIV DNA copies}: 1.13; 95% CI: 1.01–1.27; $p=0.04$) (Table 1).

When patients with undetectable HIV RNA levels were analyzed separately ($n=41$), an increase of 100 HIV DNA copies in the anal specimen resulted in a 1.11-fold increase in the odds of having LGSIL/HGSIL (95% CI: 0.99–1.25) (data not shown). When both high-risk HPV genotype and HIV DNA copies were included in the same model as predictors of LGSIL/HGSIL, the likelihood ratio test showed a significant association with HIV DNA copy number (OR_{per 100 HIV DNA copies}: 1.09; 95% CI: 0.96–1.23, $p=0.04$) but no association with high-risk HPV genotype (OR: 2.30, $p=0.28$). The associations of LGSIL/

TABLE 1. PATIENT DEMOGRAPHICS AND ANAL CYTOLOGY CHARACTERISTICS

Characteristic	Total	Cytology results		Odds ratio ^b	(95% CI)	p-value
		Normal	LGSIL/HGSIL ^a			
Sex						
Male	38	15	23			
Female	8	5	3			
Race/ethnicity						
Non-Hispanic white	23	12	11			
Pacific Islander/Native Hawaiian	9	4	5			
Hispanic	6	3	3			
Black/Native American	8	1	7			
HIV RNA levels						
Undetectable (≤ 50 copies/ml)	41	19	22			
High-Risk HPV DNA ^c						
Negative	24	14	10	Reference		
Positive	22	6	16	3.73	(1.08–12.91)	0.04
Nadir CD4 cell count ^d						
> 200 cells/mm ³	23	11	12	Reference		
≤ 200 cells/mm ³	20	8	12	1.00 ^f	(1.00–1.00)	0.62
HIV DNA copy number ^e						
Low	23	12	11	Reference		
High	23	8	15	1.13 ^g	(1.01–1.27)	0.04

^aLGSIL, low-grade squamous intraepithelial lesion; HGSIL, high-grade squamous intraepithelial lesion; HPV, human papillomavirus.

^bOdds ratios were all derived from univariate logistic regression without adjusting other factors.

^cPositive: high-risk (oncogenic) HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; negative: low-risk HPV genotypes or negative for HPV DNA.

^dNadir CD4 cell count obtained from participant; unknown from three participants.

^eHIV DNA copy number dichotomized at the median (129.5 copies/10⁶ cells; $\leq$ or $>$ for Low, High, respectively).

^fOdds ratio was based on a unit increase of nadir CD4 cell count.

^gOdds ratio per 100 copies of HIV DNA.

HGSIL with high-risk HPV genotype and HIV DNA copy numbers were not influenced by adjustment for nadir CD4 counts. The distribution of HIV DNA copy number in anal specimens with LGSIL/HGSIL (median: 792; interquartile range: 38–2,100) and normal cytology (median: 27.5; interquartile range: 10–225) was significantly different (Kruskal–Wallis test, $p < 0.01$).

Discussion

Our study demonstrated an association of HPV with anal LGSIL/HGSIL, which has been previously reported^{16–18}; but this is the first report that HIV DNA copy numbers were also associated with LGSIL/HGSIL. The identification of high-risk HPV genotypes with anal LGSIL/HGSIL is consistent with other published data.^{16,17} We previously described HIV DNA as an important factor in HIV disease progression.^{5–8} The significance of HIV DNA in disease pathogenesis lies in the fact that monocytes harboring HIV DNA persist in patients on cART in spite of having undetectable HIV RNA levels.^{5,7,8} The current study was limited to assessing HIV DNA from the pool of cells from anal cytology specimens. Since monocyte subsets may possibly be key players in the disease process, plans to measure HIV DNA from isolated cellular subsets will be considered for the future. The presence of these quiescent viral reservoirs in monocytes suggests that the cells appear to be more resistant to apoptosis and may be important factors in disease progression.¹⁹ The significance of the association of high-risk HPV genotypes with HIV DNA and LGSIL/HGSIL

remains to be determined. However, the pattern may be similar to reports of HIV-associated disease progression being related to persistence of HIV DNA in circulating cells including monocytes.^{6,20} Limitations of the study are that we did not look at relationships between duration of cART or duration of HIV suppression and the level of HIV DNA in anal specimens, thus the association of HIV DNA that we describe could possibly be related to these factors in anal intraepithelial neoplasia pathogenesis.

The relationship or interaction of HPV and HIV DNA is unclear, but systemic immune activation occurs in the setting of chronic HIV infection, which may be a factor influencing the risk for coinfection by HIV and HPV.²¹ Immune activation and/or recruitment of cytokines could create an inflammatory environment perpetuated by continuous chronic infection. Recent data demonstrated higher levels of inflammatory cytokines from activated monocytes with high HIV DNA copies,⁶ which could establish a persistent inflammatory milieu for HIV and HPV that affects each other synergistically.

Our study was also limited by the number of participants enrolled as well as the cross-sectional design. Study enrollment is continuing as well as follow-up of repeat anal cytology and HPV genotyping and HIV DNA analysis, which will provide additional information on the persistence and importance of both pathogens in LGSIL/HGSIL. The conclusions are also limited because only anal LGSIL/HGSIL was compared to HPV genotypes and HIV DNA with no high-resolution anoscopy (HRA) and anal biopsy results

available.²² HRA and anal biopsies were not part of the initial research study nor was the differentiation between LGSIL and HGSIL available from the cytopathology reports; both of these are planned for follow-up studies.

In the current cART era with effective reduction of HIV RNA to undetectable levels, the significance of persistent HIV DNA in cells may become an important factor in improving chronic HIV infection. Our unique findings of anal LGSIL/HGSIL associated with HIV DNA copy numbers and high-grade HPV genotypes highlight the importance of HIV and HPV in the pathogenesis of LGSIL/HGSIL. Future investigations warrant a study of a larger number of patients with follow-up HRA to determine the significance of HIV DNA and HPV in LGSIL/HGSIL pathogenesis.

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Author Disclosure Statement

No competing financial interests exist.

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