Incidence of Anal Cytological Abnormalities in a Cohort of Human Immunodeficiency Virus-infected Women

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Abstract

Little is known about the natural history of anal human papillomavirus (HPV) infection in HIV-infected women because, to date, no longitudinal studies have been reported in the scientific literature. This article estimates the incidence of anal cytological abnormalities in a cohort of HIV-infected women. It also examines potential risk factors for the development of anal cytological abnormality. A cohort of HIV-infected women underwent interview, anal cytology, and anogenital HPV DNA testing. Women with a normal baseline anal cytology were followed for the development of an anal cytological abnormality. The incidence of an abnormality was calculated. Survival analyses were performed to examine risk factors for the development of an abnormality. Fourteen of 100 HIV-infected women had an abnormal anal cytology at baseline. Among the 86 women with a normal baseline cytology, the incidence of an abnormality was 22 [95% confidence interval (CI), 14–33] per 100 person-years. In a multivariate analysis, women were at increased risk if, at baseline, they had a CD4+ T-cell count of <500 cells/mm³ [relative hazard (RH) = 4.11; 95% CI, 1.18–14.25], high-risk type anal HPV infection (RH = 2.54; 95% CI, 0.91–7.14) or were cigarette smokers (RH = 3.88; 95% CI, 1.12–13.42). The incidence of anal cytological abnormalities was high among this cohort of HIV-infected women, indicating that they are at high risk of anal squamous intraepithelial lesions. HIV-infected women are likely to be at higher risk than their HIV-uninfected counterparts because immune suppression conferred substantially increased risk. Continued research on the association between smoking and the development of squamous intraepithelial lesions in HIV-infected women is warranted.

Introduction

HPV is thought to be the principle etiologic agent responsible for the development of anogenital squamous cell cancers and their precursors, SILs (1). Cervical cytology, also known as the Pap smear, is in widespread use for cervical SIL and cancer screening. Similarly, anal cytology may be used to screen for HPV-associated anal squamous cell lesions, and among HIV-infected individuals, an abnormal cytology suggests the presence of a squamous cell lesion (2, 3). Cross-sectional studies show that HIV-infected adolescent girls and women are at increased risk of developing anal HPV infection and anal cytological abnormalities (4–7). A study based on data from AIDS and cancer registries in the United States found that HIV-infected women had eight times the risk of in situ anal cancer and seven times the risk of invasive anal cancer when compared with the number of expected cases (8).

The natural history of cervical HPV infection and its resulting squamous cell lesions have been well studied among cohorts of HIV-infected women (9–11). However, little is known about the natural history of anal HPV infection in HIV-infected women because, to date, no longitudinal studies of anal HPV infection in HIV-infected women have been reported in the scientific literature. Because anal cytology is a method of screening for anal SILs, an estimate of the incidence of anal cytological abnormalities would give an indication of the risk of anal SILs faced by HIV-infected women. An estimate of the incidence of anal cytological abnormalities could also shed light on the proportion of HIV-infected women who might require additional evaluation, if screening were to be undertaken in this group. Finally, incidence studies would be helpful to examine risk factors for anal cytological abnormalities. When examining risk factors for a condition, it is preferable to study incident cases so that the analysis is not affected by factors that influence duration. This may be particularly true when studying anal cytological abnormalities because lesions may regress, remain the same, or progress (12), and the factors that affect this underlying process are not well known.

This article provides an estimate of the incidence of anal cytological abnormalities in a cohort of HIV-infected women. It also examines potential risk factors for their effect on the incidence of anal cytological abnormalities, including smoking (13, 14), HPV infection (1, 5, 6, 14–16), and immune function (12, 16).

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3 The abbreviations used are: HPV, human papillomavirus; ASCUS, abnormal squamous cells of undetermined significance; HSIL, high-grade SIL; LSIL, low-grade SIL; SIL, squamous intraepithelial lesion; HAART, highly active antiretroviral therapy.
Materials and Methods

Subjects. The data were collected as part of the GRACE prospective cohort study. This study focused on the prevention of vaginal candidiasis infection and the natural history of anogenital HPV infection in HIV-infected women. The study was conducted in New Haven, Connecticut, and Fall River, Massachusetts, from March 1995 to March 1998. Women were recruited to the study through a program of community outreach. They were eligible to participate in the study if they were HIV infected, at least 18 years of age, English or Spanish speaking, not pregnant, and not on long-term systemic antifungal medications. The Yale University School of Nursing Human Investigation Committee approved the study. Eligible women who were interested in joining the cohort were asked to give informed consent to participate in the study. A detailed description of the enrollment and baseline characteristics of the GRACE cohort has been published previously (17).

Data Collection. At the time of enrollment, subjects were interviewed using a standard instrument by trained interviewers. Self-reported data were collected on variables, including demographic characteristics, medications taken, sexual activity, disease history, and cigarette smoking. Information specifically on anal sex was not collected. A complete pelvic exam was performed. If a woman did not have a CD4+ T-cell count performed 6 months before enrollment, blood was drawn at enrollment. Subjects were asked to return for a follow-up visit every 6 months. At each follow-up visit, subjects were interviewed about variables that might change over time. Repeat pelvic exams were performed. Data on CD4+ T-cell count were not systematically collected after enrollment. A brief telephone interview was administered between the study visits at 3-month intervals. This interview included questions on medications currently taken.

During the pelvic exams, specimens were collected for cervical and anal HPV testing by inserting a Dacron swab into the cervical os and a second swab into the anus. The swabs for HPV testing were placed in transport medium (Digene Diagnostics, Inc., Silver Spring, MD). They were immediately frozen and stored in a −70°C freezer. Upon receipt at the laboratory in batches. Upon receipt at the laboratory, they were stored in a −70°C freezer.

After the collection of specimens for HPV testing, anal and cervical cytology smears were obtained. The anal specimen was obtained by inserting a moistened Dacron swab into the anus to the anorectal junction and rotating the swab 360° as it was withdrawn (5). Specimens for cervical cytology were obtained using a cytology brush and Ayre’s spatula (5). For women who had a hysterectomy, vaginal cuff cytology specimens were collected. Cells collected were spread onto glass slides and fixed in 100% ethanol. The resulting slides were read by a pathologist who classified the results using the Bethesda System (18).

Laboratory Methods. The PCR was used to detect the presence of HPV DNA and to determine the genotype(s) of HPV in the anal and cervical specimens. After defrosting, tubes containing the anal and cervical swabs were heated to 56°C for 1 h to inactivate HIV. The PCR assay used MY09/MY11 consensus HPV L1 primers, as well as primers for amplification of the human β-globulin gene (4). After 30 amplification cycles, 6 μl of amplification mixture were applied to a nylon membrane and probed with a biotin-labeled HPV L1 consensus probe mixture. The consensus probe detected DNA sequences common to HPV infections of different types. Therefore, a positive test result using the consensus probe indicated the presence of HPV without indicating a specific type. Additional membranes were prepared as described above with 6 μl of specimen. They were probed for 29 individual HPV types (6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, AE2, Pap 155, and Pap 291), as well as the following 10 types together in a probe mixture: HPV 2, 13, 34, 42, 57, 62, 64, 67, 72, and W13B. Finally, a separate membrane was probed with a biotin-labeled complement to the human β-globulin gene. Specimens negative for β-globulin were considered of insufficient quality and excluded from analysis regardless of HPV result. Negative controls for each PCR reaction consisted of a solution containing all of the PCR components except the target DNA. Positive controls included amplification of cloned HPV DNA. To ensure reproducibility of results, two aliquots of five randomly chosen specimens were amplified individually and blotted separately on two different sections of each membrane. The results of the two tests from the same sample were compared.

Analysis. GRACE cohort members were included in this analysis if they had (a) a study visit with a satisfactory anal cytology smear, a β-globulin-positive anal swab and a β-globulin-positive cervical/vaginal swab (referred to as the baseline visit), (b) at least one subsequent visit with a satisfactory anal cytology smear, and (c) a normal anal cytology smear at the first visit. Cytological specimens were considered abnormal if the result was classified as ASCUS, LSIL, or HSIL according to the Bethesda System. Smears read as ASCUS were categorized as abnormal based on observations as described previously (2, 3, 7). Selected demographic, clinical, and behavioral characteristics of the women included in the analysis were described and compared with those of women not included in the analysis using a χ2 test for heterogeneity.

Baseline anogenital HPV infection and cervical/vaginal cytology results were described for women included in this analysis. The prevalence of HPV, the number of different HPV infection types detected, and the anogenital sites of HPV infection were determined using all available probes (generic and type-specific) and using the probes for high-risk types only (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 73). The incidence of an abnormal anal cytology was calculated as the number of women with an incident abnormal anal cytology detected divided by the total number of person-years at risk of an abnormal anal cytology. For women who had an abnormal anal cytology at one of their follow-up visits, person-years spanned from the baseline visit to halfway between the last visit with the normal cytology and the first visit with the abnormal cytology. For those who did not have an abnormal anal cytology detected during the study, person-years spanned from the first to the last study visit. The incidence of a SIL was calculated as the number of women with incident LSIL or HSIL divided by the person-years at risk of a SIL. In this case, ASCUS results were considered normal when calculating the person-time. The Kruskal-Wallis test was used to examine whether the average interval between satisfactory anal cytology exams was shorter for women who had an incident anal cytological abnormality detected.

The RHs of individual hypothesized risk factors for the development of a new anal cytological abnormality were calculated using SAS software PROC PHREG. The baseline variables considered potential risk factors for the development of an abnormal anal cytology were CD4+ T-cell count (within the period 6 months before and 2 weeks after the baseline visit), cigarette smoking, detectable anal HPV infection (any type, high-risk type, other type, and multiple types), age, recent sexual activity (within 6 months of the baseline visit), and HPV
infection detected in both the cervix/vagina and anus. A multivariate Cox proportional hazards model was then used to estimate the RHs of hypothesized risk factors simultaneously. Two-way interactions of the main effect terms were entered into the model one-by-one to determine whether any improved the fit of the model. PROC LIFETEST was used to plot the log(-log) survival curves associated with hypothesized risk factors to examine visually whether the assumption of proportional hazards was met. In the case of the multivariate model, the survival curves were plotted for each variable after controlling for the effects of the other variables in the model. Finally, available measures of socioeconomic status (educational level and race) were added individually to the multivariable model to examine whether they affected the smoking parameter estimate. HAART for HIV infection was introduced during the course of the study. Because HAART might affect the association between baseline CD4+ T-cell count and the risk of developing an abnormal anal cytology, HAART was examined as a time-dependent covariate in the Cox proportional hazards model. HAART was defined as a regimen containing three or more antiretroviral medications (protease inhibitors, nucleoside reverse transcriptase inhibitors, and nonnucleoside reverse transcriptase inhibitors). The HAART start date was estimated as the midpoint between the last interview at which a woman reported taking less than three antiretroviral drugs and the first interview at which the woman reported taking three or more antiretroviral drugs.

Results

There were 225 women enrolled in the GRACE study of whom 164 (73%) returned for at least one follow-up study visit. One hundred (61%) of these 164 women had a study visit with a satisfactory anal cytology smear, a β-globin-positive anal swab and a β-globin-positive cervical swab, and at least one subsequent visit with a satisfactory anal cytology smear. Of these 100 women, 14 (14%) had an abnormal anal cytology smear at the first visit (10 had ASCUS and 4 had LSIL). The remaining 86 women were included in this analysis. At enrollment, 15% (13 of 86) of these women were ages 20–29 years, 47% (40 of 86) were ages 30–39 years, and 38% (33 of 86) were ages 40–59 years. Almost half identified themselves as black or African American (42 of 86), whereas 35% (30 of 86) identified themselves as white, 14% (12 of 86) as Hispanic, and 2% (2 of 86) as another race/ethnicity. Seventy percent (60 of 86) had earned a high school diploma or GED. Seventy-four percent (64 of 86) were smokers. Sexual activity in the last 6 months was reported by 60% (52 of 86). A history of gonorrhea, syphilis, and anal warts was reported by 35% (30 of 86), 15% (13 of 86), and 6% (5 of 85), respectively. Twenty-six percent (22 of 86) of the women had a recent (within 6 months before and 2 weeks after enrollment) CD4+ T-cell count of <200 cells/mm³, 41% (35 of 86) had a count of 200–400 cells/mm³, and 33% (28 of 86) had a count of ≥500 cells/mm³. The women included and not included in this analysis were very similar with respect to race/ethnicity, years of education, recent sexual activity, current cigarette smoking, age, enrollment CD4+ T-cell count, and history of sexually transmitted diseases (data not shown). Thirty-five (41%) of women were on HAART at some point during the study, with a total of 25.7 person-years of observation during which HAART was taken.

The 86 women at risk of anal cytological abnormalities contributed at total of 113.5 person-years of observation with a median of 1.43 (range: 0.23–2.6) person-years. There were 25 incident anal cytological abnormalities detected during the study: 17 ASCUS; 7 LSILs; and 1 HSIL. The incidence of a newly detected anal cytological abnormality was 25 of 113.5 or 22 (95% CI, 14–33) per 100 person-years. The incidence of a newly detected SIL (LSIL or HSIL) was 11 of 128.18 or 9 (5–16) per 100 person-years. Of the 25 women with an incident cytological abnormality, 6 remained abnormal at the subsequent smear or smears, 2 had at least one additional abnormal smear and then at least one normal smear, 5 had normal subsequent smears, and 12 had no additional smears. Women who had an incident anal cytological abnormality did not have shorter average intervals between anal cytology exams than women who did not (Kruskal Wallis χ² test: 0.85, P = 0.36).

Table 1 shows the baseline HPV test results for the 86 women included in the analysis based on the results of all HPV probes (generic and type-specific) and the high-risk HPV probes only. Eighty percent (69 of 86) had a normal cervical/vaginal smear at baseline, whereas 7% (6 of 86) had smears read as ASCUS, 8% (7 of 86) as LSIL, and 5% (4 of 86) as HSIL. Table 2 presents RHs of an incident anal cytology abnormality given selected subject characteristics. Race/ethnicity, age, recent sexual activity, and the detection of multiple anal HPV infections were not found to be risk factors based on inspection of the plotted log(-log) survival curves (data not shown). Because the risks for the two CD4+ T-cell groups appeared to be very similar based on the hazard ratios and overall survival curves, these two groups were combined in subsequent analyses. Table 3 presents the results of a multivariate Cox proportional hazards model of risk factors for the development of anal cytology abnormalities. The RH for cigarette smoking was not affected by the inclusion of race/ethnicity or educational level variables in the model. There were no interactions observed between the variables in the model. The log(-log) survival plots of each variable in the model, adjusting for the remaining variables, suggested that the proportional hazards assumption was appropriate.
However, in this study, HIV-related immune suppression, as demonstrated that HIV-infected women are at increased risk because cytological abnormality is high, it does not, by itself, demonstrate that a substantial proportion of HIV-infected women might have diluted the effect of HPV infection. The risk of concurrent detectable HPV infection of the cervix and anus did not confer increased risk over infection of the anus only as was observed by Melbye et al. (15). These authors did not stratify by HIV or immune status. This finding suggests that multifocal infection might have been a proxy for HIV-related immune suppression in their analysis. It was not possible to examine whether anal HPV infection might have occurred as a result of vaginal or anal sex because anal sex data were not available.

Smoking is associated with an increased risk of anal and cervical cancer (22–26). The possibility of a causal association is biologically plausible because studies have demonstrated an interaction between papillomavirus and chemical agents of carcinogenesis in animal models (27), and smoking has been observed to cause immune dysregulation (28). It is also possible that smoking is a proxy measure for some aspect of socioeconomic status or behavior that increases the risk of HPV infection. In this study, after controlling for baseline HPV infection, women who were current smokers at baseline were four times more likely to develop an abnormal anal cytology. The inclusion of educational level and race in the Cox model did not affect the magnitude of the association between smoking and incidence of an abnormality. It has been suggested that smoking increases the persistence of HPV infection (29). If this is the case, our smoking parameter could be expressing the risk of persistent HPV infection. However, two studies of incident cervical cytological abnormalities (14, 30) found smoking to be a risk factor even after controlling for the persistence of HPV infection.

Some participants in the GRACE study were excluded from natural history of anogenital SIL have not consistently demonstrated a beneficial effect of HAART (19, 20).

In this analysis, women with a high-risk HPV infection detected at baseline had twice the risk of developing an anal cytological abnormality. Although this finding has marginal statistical significance, it is unlikely to have occurred by chance because HPV is thought to be the principal etiologic agent responsible for the development of anogenital SILs (1). Women with other HPV infection types were also at increased risk, although the interpretation of this finding is limited given the very wide confidence interval. Given that HPV is thought to be the etiologic agent of anogenital SILs, the risk of HPV infection was lower than might be expected. Persistent HPV infection has been identified as a possible risk factor for SILs (21). Our use of baseline HPV data may have diluted the effect of HPV infection. The risk of concurrent detectable HPV infection of the cervix and anus did not confer increased risk over infection of the anus only as was observed by Melbye et al. (15). These authors did not stratify by HIV or immune status. This finding suggests that multifocal infection might have been a proxy for HIV-related immune suppression in their analysis. It was not possible to examine whether anal HPV infection might have occurred as a result of vaginal or anal sex because anal sex data were not available.

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Some participants in the GRACE study were excluded from...
this analysis because of lack of follow-up, unsatisfactory anal smears, or \( \beta \)-globin-negative anogenital swabs. Of the anal and cervical/vaginal swabs tested as part of the GRACE study, 80 and 86\% were \( \beta \)-globin-positive, respectively. Seventy-seven percent of the anal cytology smears performed were satisfactory. The exclusion of GRACE cohort members from this study could reduce the generalizability of these results. However, the GRACE cohort members included and not included were similar. The follow-up of women at intervals could have allowed transient abnormalities to be missed. However, the average number of days between satisfactory anal cytology exams was not significantly different for those who did and did not have an incident anal cytological abnormality. In a sample of homosexual and bisexual HIV-infected men, the sensitivity of abnormal anal cytology for a SIL were 70\% when compared with the gold standard of anoscopy and biopsy where appropriate. To address risk factors for incident anal SILs in HIV-infected women directly, future studies should be based on the results of anoscopy and biopsy findings.

In conclusion, the incidence of anal cytological abnormalities was high among this cohort of HIV-infected women, indicating that they are at increased risk of anal SILs. Initiation of anal cytology screening could lead to the evaluation of many HIV-infected women for SILs. HIV-infected women appear to be at increased risk of developing an anal cytological abnormality, and thus anal SILs, as a result of immune suppression. Additional investigation into the relationship between smoking and anogenital squamous cell lesions in HIV-infected women is warranted.

References


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