Cervical HPV Infection in Romanian Women Infected with HIV During Early Childhood

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Introduction

Cervical Cancer (CC), caused by the human papillomavirus (HPV), is the second most common carcinoma in women worldwide[1]. Romania has approximately 9.4 million women ages 15 years and older who are at risk of developing CC, and has the highest mortality rate for CC in Europe, at 10.5 per 1000 women[2]. In Romania, CC was the most frequent type of cancer among women between 15–44 years of age in a 2006 study[2]. In this same study, it was found that approximately 90% of Romanian women aged 15–44 had never been screened for CC[2]. Systematic screening with cytology or HPV DNA is still not currently available[3], and the HPV vaccine is not currently being administered to Romanian children[4, 5].

HIV-infected women are more vulnerable to HPV infection, with more frequent and more severe infections[6, 7]. Sexual and behavioral risk factors do not explain the entire discrepancy in HPV prevalence between the HIV-infected and uninfected population[8], and so it has become clear that immunodeficiency can affect both HPV prevalence and severity[9]. Current thinking proposes that the increased prevalence of cervical HPV infection in HIV-infected women is due to a combination of increased susceptibility, increased viral persistence, and reactivation of infection[10].

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Most HIV-infected women acquire HPV infection prior to HIV, thus HPV specific immune responses will already be present. The dynamics of HPV infection in women who acquire HIV first may be markedly different, and potentially much worse. Here we examine the prevalence and dynamics of HPV infection in a unique cohort of Romanian women infected with HIV during early childhood[11], who have reached their sexual debut and are now sexually active[12].

Materials and Methods

Study design

We performed a case-control study with an observational component to evaluate the differences in HPV detection, cervical pathology and viral dynamics between young women infected with HIV during early childhood and HIV-uninfected women. Approval for the study was obtained from both the University of California San Diego Human Research Protections Program (#111136) and the Research Review Board of the "Dr. Victor Babes" Hospital for Infectious and Tropical Diseases in Bucharest, Romania (VBH) (#2-4/13/2011).

Study cohort

VBH is an infectious disease hospital in Bucharest, Romania that currently cares for nearly 600 individuals infected with HIV during early childhood. These patients have lived with chronic HIV for more than two decades, and have been treated with combination antiretroviral therapy (cART) since 2000. Approximately 200 female subjects aged 22–25, iatrogenically infected with HIV by a parenteral route (i.e. blood transfusions and medication injections) during early childhood, are followed in the HIV Department of VBH every 6 months. Individuals from this cohort were recruited for the study group. Sixty-five of these female HIV-infected subjects who provided written informed consent to participate at the study were enrolled. In addition, 25 age-matched HIV-uninfected control subjects who attended a gynecology clinic for routine visits at the adjacent Victor Babes Center for Diagnosis and provided written informed consent, were enrolled. Exclusion criteria for both groups included a prior history of cervical cancer, current pregnancy, or a history of receiving the HPV vaccine.

Study Visits

A standard medical history and physical exam including pelvic exam (together with Papanicolau cytology and cervical cell sampling for HPV typing) was performed on all study participants by a study gynecologist. Tests for pregnancy, sexually transmitted infections (STI) (syphilis, chlamydia, gonorrhea, candidiasis, bacterial vaginosis, and trichomoniasis), and standard HIV-related laboratories (viral load, CD4 count and percentage, chemistry panel, liver function test, and complete blood count) were also performed. Enrolled subjects were also given a paper questionnaire, which elicited information about demographics, illicit drug use, alcohol use, smoking, and sexual history. Data on nadir CD4 count and antiretroviral regimen were obtained from the medical charts from the hospital. Control participants received the same evaluation except for the standard HIV-related laboratory testing, but did have HIV testing in order to confirm their

seronegative status. All participants found to have cervical pathology or STIs were offered treatment as per Romanian Ministry of Health guidelines.

Follow up visits

HIV-infected individuals were asked to return for a follow up visit between six months and one year after the baseline visit. At this visit, STI screening, cervical cytology with a Papanicolau smear, and HPV screening were also performed.

Laboratory Testing Methods

Cervical swabs were tested at the "Dr. Victor Babes" Center for Diagnosis Bucharest for HPV typing using the Roche® Linear Array HPV Genotyping Test (Basel, Switzerland). High-risk HPV subtypes were defined using the WHO/IARC guidelines[13]. Cytology samples obtained using ThinPrep® (Hologic, Bedford, MA) were sent off to a specialized reference laboratory for histologic evaluation. Reports were generated using the Bethesda classification system[14].

Data management and statistical analysis

Statistical comparisons, using T-tests and Fisher's exact test depending upon the type of variable, were made between the HIV-infected and uninfected individuals, between HIV-infected individuals with at least one high-risk HPV subtype and those with only low-risk HPV subtypes, and between HIV-infected individuals that gained a new HPV subtype on follow-up with those that did not. A longitudinal analysis was also performed for the HIV-infected individuals who returned for follow-up six to fifteen months after the baseline visit (mean 8.5 months).

Results

Patient characteristics

The characteristics of the 65 HIV-infected and 25 HIV-uninfected women included in the analysis are shown in Table 1. There were no significant differences between the HIV-infected and uninfected groups in terms of location of residence (urban or rural); tobacco use; age at sexual debut; and number of partners. The HIV-uninfected individuals were slightly younger (22.5 vs. 23.1 years, p=0.01), had more years of schooling (p<0.001), and were less likely to be on government social support (p<0.001).

All of the HIV-infected individuals had long-standing HIV infection (> 10 years), and all were on cART at the start of the study. Median nadir CD4 T-cell count was 165 cells/μl (IQR: 63–260), with 62% having a nadir count <200 cells/μl. The median current CD4 count at the baseline visit was 513 cells/μl (IQR: 365–905), with 88%% of subjects having a current CD4 above 200 cells/μl. HIV viral load was undetectable in 51 of 65 of the subjects at baseline.

STIs and HPV in HIV-infected and HIV-uninfected subjects

Among the HIV-infected participants 36 women had any vaginal infection, 28 were infected with HPV, nine had vaginal candidiasis, four had bacterial vaginal infections (mainly with

Group B *Streptococcus*), two had trichomoniasis and one chlamydia. In the control group, eleven women had any vaginal infection; eight were infected with HPV, two had vaginal candidiasis, one had Group B *Streptococcus* vaginitis, and one had chlamydia. There were no gonorrhea or syphilis infections in this group. Interestingly, although a greater proportion of the HIV-infected group felt that they were at risk for acquiring a STI (49/65 vs. 12/25, p=0.02), the HIV-uninfected group was more likely to use barrier protection with sexual activity (p=0.056).

Overall 43% (28/65) of HIV-infected and 32% (8/25) of HIV-uninfected subjects were infected with HPV (p=ns), and 32% (21/65) and 24%(6/25) had high-risk subtypes respectively (p=ns). There was also no significant difference in the presence of HPV infection with more than one subtype between HIV-infected (14/28) and HIV-uninfected (5/8) subjects (p=ns). The most prevalent HPV subtypes isolated from the HIV-infected women were HPV-52 and HPV-67 (n=7 for each subtype), HPV-16, HPV-73 and HPV-61 (n=6 for each subtype) and HPV-18 and HPV-CP6108 (n=5 for each subtype). The most common subtypes isolated from the controls were HPV-18 and HPV-73 (3 individuals with each subtype).

We observed a trend towards more abnormal cells visualized on Papanicolaou (Pap) smear in the HIV-infected group (16/63 (25%) vs. 1/22 (5%) p=0.06). In the HIV-infected group, six had a Pap smear showing Atypical Squamous cells of Uncertain Significance (ASC-US), four had a finding of Atypical Squamous Cells, Cannot Rule Out High-Grade Squamous Intra-epithelial Lesion (ASC-H), and six had a finding of Low grade Squamous Intraepithelial Neoplasia (LSIL) at baseline. In the HIV-uninfected group, the single subject with an abnormal Pap had LSIL.

HPV infection and dynamics in the HIV-infected group

There were no significant differences between HIV/HPV co-infected group and the HIV mono-infected group by age, location of residence, education, marital status, age at sexual debut, and use of barrier contraception (see Table 2). The co-infected group trended toward having more partners (p=0.05) and more episodes of intercourse in the prior month prior (p=0.06). There was no difference in the type of sexual intercourse (vaginal vs. vaginal and oral vs. vaginal and anal), or in the STI rates between groups. However, the proportion of individuals with nadir and current CD4 T-cell counts of <200 cells/μl was greater in the co-infected group (nadir: 21/28 (75%) vs. 18/37 (49%), p=0.04; and current: 7/28 (25%) vs. 1/37 (3%), p=0.02). Current median CD4, although above 350 cells/μl, was significantly lower for the HPV/HIV co-infected group than the HIV mono-infected group (356 [IQR: 204–468] vs. 758 [IQR: 506–1000] cells/μl, p<0.0001). The proportion of individuals with an undetectable HIV viral load did not differ between groups.

HIV/HPV co-infected individuals carrying multiple HPV subtypes were significantly more likely to be infected with a carcinogenic subtype (13/14 (93%) vs. 4/14 (29%), p=0.001) and to have an abnormal Pap smear (12/14 (86% vs. 4/14 (29%), p=0.006) compared to co-infected individuals with only a single identified HPV subtype. When correcting for the number of viral subtypes present, multiply infected individuals still showed a trend towards being more likely to have infection with a carcinogenic subtype (28/56 (50%) vs. 4/15

(27%), p=0.06). The groups did not differ by nadir or current CD4 count, but the multiply infected group showed a trend towards having more partners (2.38 vs. 3.0, p=0.06).

Finally, the dynamics of HPV were evaluated in the 42 HIV-infected subjects that returned for follow up visits. We observed an annual incidence of 0.69 HPV acquisition events per subject, and an annual incidence of 0.52 per subject for high-risk subtypes. 30% of all HPV acquisition events were due to vaccine preventable subtypes. The presence of HPV at baseline was strongly associated with new subtype acquisition (p=0.002), and even more likely if a high-risk subtype was present (p<0.001). Individuals that acquired a new HPV subtype also had significantly lower nadir (154 vs. 253 cells/μl, p=0.043) and current (508 vs. 772 cells/μl, p=0.010) CD4 counts. The presence of vaginitis at baseline was associated with HPV persistence and progression of HPV infection (p=0.01). In the HIV-infected group, 9/13 (69%) individuals with abnormal cytology at baseline had cytologic progression or remained stable, including two with high-risk subtypes, while only four regressed. CD4 count was not found to be associated with cytologic progression.

Discussion

We found that the prevalence of genital HPV infection in the HIV-uninfected and HIV-infected women was similar to previously reported rates in Romania[3, 15, 16], and similar to rates reported HIV-infected women in a multicenter European study[17] and other studies from limited resource countries[7, 18]. HIV infection in this group of Romanian women has had a different course in comparison to vertically infected children, with a natural history of 8–10 years of asymptomatic infection, similar to adults. Although 60% of this cohort had a nadir CD4 count <200 cells/μl, most of the subjects now have a CD4 count >600 cells/μl in response to ART. Previous reports have demonstrated that loss of CD4 T-cells in genital tissues can facilitate HPV infection and/or reactivation in simian models[19] and in adults with acute HIV infection[20]. However, the subjects in our study likely had at least partially restored their immune system by the start of their sexual life. A recent report on naïve T-lymphocyte restoration in perinatally infected children reaching adult age, found preserved levels of CD4 naïve cells, implying the ability of these patients to respond adequately to pathogens after 20 years of chronic HIV-1 infection[21]. This finding may explain why the prevalence of HPV infection in our study was similar between study groups.

Despite CD4 recovery, early HIV infection can cause significant and long lasting impairment of the immune system that persists even after immune reconstitution following cART[22]. Thus, in this cohort of women infected with HIV well before HPV exposure, we still expected higher rates of HPV infection. However, we did not find significant differences in the proportions of individuals with HPV infection, high multiplicity HPV infection, or high-risk HPV subtypes. This lack of difference was true even though the HIV-uninfected group reported more frequent use barrier protection. This finding could be explained by similar sexual exposures in both groups, or the lack of sufficient sample size to identify differences in HPV infection.

HIV impairment of the immune system also impacts disease progression of HPV-related cancer. Specifically, decreasing CD4 count has been shown risk factor of progression from

low- to high-grade SIL in HIV positive gay or bi-sexual men[23], and the risk of high-grade anal SIL and cervical abnormalities in HIV-infected women increases as CD4 T cell count decreases and HIV viral load increases[24, 25]. In our cohort of HIV-infected young women, we found higher rates of cervical pathology than in the HIV negative control group, even though the actual rates of HPV infection did not differ. During the course of the study, nearly 70% of these co-infected subjects showed progression or maintenance of pathology, but interestingly 2/9 (22%) subjects with progression only had low-risk subtypes detectable. This finding suggests that the restoration of immunity by cART in the genital tract may not be complete.

Factors associated with the detection of HPV infection in our HIV-infected cohort included having more partners, more frequent sexual intercourse, and a nadir or current CD4 T cell count of <200 cells/μl. HIV-infected women with normal CD4 T-cell counts (>500 cells/μl) were less likely to be co-infected with HPV, reinforcing the fact that the control of HPV infection is mediated primarily through host cell-mediated immune responses.

As expected, individuals with HPV infection at baseline were also more likely to acquire new HPV subtypes with time. This effect was even more pronounced in individuals with high-risk subtypes at baseline. Although new detection could represent acquisition of a new subtype or a missed detection of a subtype at the previous visit, both scenarios are consistent with the presence of multiple subtypes, which increases an individual's risk for cervical pathology[26–28]. Assuming the sensitivity of HPV detection was constant at both the baseline and follow-up time-points, an incident HPV infection occurred every 524 days in our HIV-infected cohort, while an incident HPV infection with a high-risk subtype occurred every 699 days. Most importantly, we found that ~30% of newly identified HPV infections were due to subtypes present in the quadrivalent vaccine.

Our study was limited by the small sample size of both groups. Since none of the participants developed cervical cancer during the study period, the short duration of this study didn't allow us to decipher the impact of infection with HPV on development of cervical carcinoma in this young group. Additionally, the HIV-uninfected group was slightly younger and less likely to be on social support. Given the association of cervical cancer with socioeconomic status, this difference could have also impacted our study[29, 30].

Conclusions

We found a similar prevalence but more severe HPV infection in our cohort of young Romanian women infected with HIV during early childhood, when compared to similarly aged controls. While HPV vaccination is most useful when given prior to sexual debut[31], we found in this cohort of HIV-infected women in their early 20s, approximately 30% of newly identified infections were vaccine preventable subtypes. Given the increased rates of cervical dysplasia in HIV-infected women and the availability of highly safe, immunogenic and effective vaccines, even resource-limited countries should consider providing HPV vaccination for all young women infected with HIV.

Acknowledgments

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Table 1

Comparison of sociodemographic variables between HIV-infected and HIV-uninfected subjects.

		HIV-infected (n=65)	HIV-uninfected (n=25)	p value
Demographics				
Mean Age (y)		23.1 +/− 1.1	22.5 +/− 1.1	0.01[*]
Education	Primary or Secondary	46	2	<0.001
	Post-secondary	19	22	
Sexual Orientation	Heterosexual	65	25	ns
Married		13	1	ns
Work Status	Student	12	18	<0.001
	Employed	12	6	
	Unemployed/Social Support	41	0	
Residence	Urban	42	24	0.06
	Rural	23	1	
Social Behaviors				
Alcohol Use		25	16	0.04
Tobacco Use		42	13	ns
Illicit Drug Use		0	3	0.02
Sexual History				
Mean Age at Sexual Debut (y)		18.1	17.8	ns[*]
Median # of partners		2	2.5	ns[**]
Median # of Episodes of Sexual Intercourse in last month		1	6	0.001[**]
Condom Use		53	25	0.056
Depression episodes		13	0	0.007
HIV History				
Risk Factor for Acquisition	Parenteral (non-IDU)	57		
	Transfusion	5		
	Unknown	3		
On cART		65		
Mean CD4 Nadir		188		
Mean CD4 at Baseline Visit		610		

Legend: All comparisons performed using Fisher's Exact Test unless denoted by an asterisk.

[*] comparison performed using t-test.

[**] comparison performed using Wilcoxon Rank Sum Test.y-years, IDU- injection drug use, cART-combination antiretroviral therapy, ns- not significant.

Table 2

Comparison of characteristics between the HIV-HPV co-infected and HIV mono-infected participants.

		HIV/HPV Co-infection (28)	HIV infection alone (37)	p value
Demographics				
Mean Age (y)		23.3 +/− 1.6	23 +/− 0.8	ns *
Education	Primary or Secondary	19	27	ns
	Post-secondary	9	10	
Married		7	6	ns
Work Status	Employed	7	6	ns
	Student	2	8	
	Unemployed/Social support	19	22	
Sexual History				
Mean Age at Sexual Debut (y)		17.8	18.4	ns *
Median # of Partners (IQR)		2 (1.75–3.25)	2 (1–2.25)	0.05 **
Mean # of Episodes of Sexual Intercourse in last month		1.0	1.7	0.06 *
Condom Use		25	28	ns

Legend: All comparisons performed using Fisher's Exact Test unless denoted by an asterisk.

*
comparison performed using t-test.

**
comparison performed using Wilcoxon Rank Sum Test.y-years, IQR- interquartile range, ns- not significant.