Human Papillomavirus Infections in Nonsexually Active Perinatally HIV Infected Children

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Abstract

Although human papillomavirus (HPV) infections are common in HIV-infected adults, little is known about children. Our objective was to examine the prevalence of and risks for HPV of the oral mucosal and external genital areas in nonsexually active (NSA) perinatally (P) HIV+ children and compare with HIV-exposed but uninfected (HEU) children. A convenience sample attending a pediatric clinic were enrolled. Samples for HPV were obtained from the oral and anogenital areas and tested for one of 37 HPV types. The mean age of the 48 PHIV+ children was 14.3 ± 3.9 years vs. 6.2 ± 4.8 for the 52 HEU ($p < 0.001$). Of the 23 PHIV+ girls, 30.4% had anogenital and 17% had oral HPV, and of the 27 HEU girls, 2 (7.4%) anogenital and 0 had oral HPV. Of the boys, 4/23 (17.4%) and 1/25 (4%) PHIV+ had anogenital and oral HPV, respectively, and 3/24 (12.5%) and 1/25 (4%) HEU had anogenital and oral HPV, respectively. Rates of HPV did not differ by age among the PHIV+, whereas older HEU were more likely to have HPV than younger HEU ($p = 0.07$). This large age gap precluded statistical comparison by HIV status. The presence of HPV in NSA PHIV+ children may have implications regarding HPV vaccination efficacy.

Introduction

HIV IS A KNOWN RISK FOR HPV-associated cancers, including cervical, anal, vulvar, penile, and oropharyngeal in adults.¹ Although these cancers are rare in HIV-infected children and adolescents, these cancers do occur at younger ages in those HIV-infected adults compared to the general population.² Natural history studies in sexually active persons with HIV show that HPV is more likely to persist than the general population.³ Most of these studies are in individuals who acquired HIV after initiating sexual activity. Only one study to date has reported on HPV-associated morbidity among persons who acquired HIV perinatally. The study found that 47.5% of the sexually active adolescents had abnormal cytology—3–5 times higher than found in non-immunocompromised adolescents.⁴

The primary mode of HPV transmission in adolescents and adults is through sexual activity.⁵ As with HIV, HPV can also be transmitted perinatally.⁶ Fortunately, diseases due to HPV such as genital warts and recurrent respiratory papillomatosis are rare in children. A large study of HPV in HIV uninfected children, known as the Finnish HPV Family study,⁷ show that HPV DNA detection is quite common during early childhood. Twelve to 21% of oral samples and 4–15% of genital

samples were HPV DNA positive at any one point over a 36 months period beginning at birth. Genital warts were not noted in any of the children in the study and persistent detections were uncommon. This study suggests that children are frequently exposed to HPV DNA from a parent either during delivery or infant care. However, as seen in adults with HPV, most of these detections are transient and rarely result in clinical disease.⁸

Unfortunately, little information has been published for perinatally HIV-infected children. Exposure to HPV during birth and perinatally are higher among children born to HIV-infected mother since HIV-infected women have HPV prevalence rates 2–3 times that of HIV negative women⁹ and HPV genital load is greater.¹⁰ Since HIV has been implicated as an important risk for HPV persistence, it is possible that HIV-infected children may be more likely to have HPV persistence than uninfected children and therefore when tested would more likely be positive. One study examined the oral cavity of 3–13 year old children for HPV 6, 11, 16 and 18 and found 12% of HIV infected children had oral HPV compared to 6% in HIV uninfected children.¹¹ No study to date has examined the prevalence of genital HPV in children born to mothers with HIV infections. It remains imperative to study HIV infected children to determine whether persistence

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in oral and external genital areas may place them vulnerable to oral and genital cancers as they age. The objective of this study was to examine the prevalence of and risks for HPV infections of the oral mucosal and external genital areas in non-sexually active perinatally HIV-infected children and compare with HIV-exposed but uninfected children.

Methods

A convenience sample of perinatally HIV infected children and adolescents attending the Children's Diagnostic and Treatment Center in Ft. Lauderdale were recruited into the study according to the Institutional Review Board guidelines of the institution. Since perinatally HIV-infected children are at higher risk for HPV exposure, the comparison group for the nonsexually active group was HIV exposed but uninfected children. Nonsexual activity, including known child abuse, was confirmed through chart review. In addition, subjects were screened for sexual behavior on the day of recruitment by a nonclinician study coordinator. Sexual activity was defined as any sexual contact including vaginal, oral, or anal sex, or genital-genital contact. A chart review for recent CD4 count, HAART regimen, adherence to HAART, and viral load was obtained. Mother's history of genital warts was obtained through chart review and interviewing mother. Tanner staging was performed by the clinician at the time of sampling.

HPV DNA sampling and testing

Samples collected included the tongue and buccal mucosa and a separate Dacron swab for tonsils. For females, vulvar (between vaginal opening and labia majora) and perianal areas were sampled separately. For males, separate swabs were used to collect samples from the corona, foreskin (if present), penile shaft, scrotum, and perianal region. Gloves were changed between sampling oral, genital, and perianal areas. Areas of interest were vigorously swabbed 3–5 times over the area and then placed into 1 cc of normal saline. For male genital samples, areas were first rubbed with fine Emory paper and then a moistened Dacron Swab. The jewelry paper was placed into an empty vial and the swabs were placed into the normal saline. Samples were placed on ice and frozen at -20°C within 4 h of collection.

Buccal/tongue, tonsillar, vulvar, and perianal samples were processed as follows: the tube with the swab was vortexed for 60 sec, incubated at 37°C shaking waterbath, overnight, vortexed and centrifuged for another 60 sec the next day, then the swab was discarded. Two hundred μL of the pelleted cellular material was removed for DNA extraction. For the male genital samples, the swab and saline after the initial vortex and centrifugation were transferred to the empty tube with the Emory paper. After the overnight incubation at 37°C shaking waterbath, the tube was vortexed and centrifuged and then only the swab was discarded. Two hundred μL of the pelleted cellular matter was removed for DNA extraction. Prior experience with prepubertal male and female samples found that combining samples enhanced specimen adequacy. Consequently, prior to DNA extraction, we pooled 150 μL of pelleted material from each foreskin, corona, shaft, scrotum, and perianal areas for the boys, and 150 μL of the material from the vulva and perianal areas for the girls. The pooled samples were vortexed and centrifuged again—200 μL of the pooled pelleted cellular material was

removed for DNA extraction. Qiagen MinElue kit (Qiagen Inc. Valencia, CA) was used for DNA extraction of all the samples, according to manufacturer instructions.

The samples were genotyped for HPV by Roche Linear Array (LA) (Roche Molecular Systems, Pleasanton, CA) which types for 37 different HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51–59, 61, 62, 64, 66–73, 81–84, IS39, and 89, as well as high and low beta-globin concentrations to verify higher and lower limits of sample adequacy. Fifty μL of extracted DNA from each sample was used for amplification in AB 2720 thermocycler (Applied Biosystems, Grand Island, NY) and HPV genotyping was performed using Linear Array HPV Genome Typing Test according to the manufacturer's instructions.

Statistical analysis

Differences by HIV status are descriptive. No statistical comparisons were made because of the age difference between the two groups. Comparisons by HPV DNA status were made using chi-square or Fisher's exact test, when appropriate. To test the differences in CD4 count and viral load, we first log-transformed the data to approximate normal distribution and then used *t*-test for comparison between HPV DNA status. For analysis examining variables associated with HPV DNA detection, the HEU and PHIV+ subjects were combined to increase the sample size. Variables included gender, age, Tanner stage, and maternal history of genital warts and for the PHIV+ subjects, CD4 count and viral load.

Results

A total of 100 nonsexually active (NSA) children were recruited into the study—48 were perinatally HIV infected (PHIV+). There were 23 PHIV+ girls, 25 PHIV+ boys, 27 HIV exposed but uninfected (HEU) girls, and 25 HEU boys. The mean age of the PHIV+ children was 14.3 ± 3.9 years vs. 6.2 ± 4.8 for the HEU ($p < 0.001$). This was not unexpected since younger children's mothers were more likely to receive anti-retroviral therapy (ART) at the time of pregnancy.

After pooling specimens (see Methods), all the girl subjects had adequate samples for HPV DNA; however, two PHIV+ and 1 HEU boys had inadequate anogenital samples. When we examined HPV detection at any site (genital or oral), we saw differences between HIV status among the girls but not the boys. Eight of the 23 (34.8%) of the PHIV+ girls had HPV vs. 2/27 (7.4%) of the HEU girls.

When we examined HPV by site, 7/23 (30.4%) PHIV+ girls had anogenital HPV and 4/23 (17%) had oral HPV vs. 2/27 (7.4%) HEU girls had anogenital HPV and 0/27 had oral HPV. In comparison, 4/23 (17.4%) PHIV+ boys and 3/24 (12.5%) HEU boys had anogenital HPV and 1/25 PHIV+ and 1/25 HEU had oral HPV.

Although no sexual contact was reported by both groups, the influence of pubertal hormones may have affected HPV rates as well as false reporting of sexual contacts. We separated HPV prevalence by age. Figure 1 shows the percent HPV infected at any site (oral or anogenital) by age (≤ 12 and > 12 years). Among the ≤ 12 -year-old girls, 2/6 (33%) PHIV+ and 0/22 HEU were HPV positive ($p = 0.04$). Among the PHIV+ infected, 1 infection was genital and the other oral. Among the > 12 -year-old girls, 6/17 (35.3%) PHIV+ and 2/5 (40%) HIV uninfected were positive for HPV

FIG. 1. Prevalence of HPV at the anogenital and oropharyngeal sites by HIV status and age for boys and girls. Three boys ≤ 12 years of age had inadequate anogenital samples.

($p=1.0$). Among the PHIV+ girls, 3 had both oral and anogenital infection; the other 3 had anogenital infection only. The two infections in the >12 -year-old HEU group were anogenital.

Among boys ≤ 12 years of age, 0/7 of the PHIV+ and 3/20 (15%) of the HEU were HPV positive ($p=0.54$). All three were anogenital infections—no oral infections were found. Among the older group (>12 years), 5/18 (28%) PHIV+ and 1/5 (20%) HEU were HPV positive ($p=1.0$). Four of the HPV infections were anogenital and one oral among the PHEU; the single HPV infection in the HEU was an oral HPV.

HPV types detected

HPV types detected were mostly high risk HPV. Among the 13 PHIV+ children, this included HPV 18, 31, 39, 45, 51, 52, 56, 58, and 68. Among the 6 HEU children, HR HPV 16 and 52 were detected. Other types included 6, 11, 42, 53, 54,

61, 62, 81, 84, and 89. Table 1 shows HPV types detected by site and age.

Factors associated with HPV detection

Overall, gender was not associated with HPV status—20% of girls and 18% of boys were HPV positive. Among the PHIV+, 31% of those older than 12 years were HPV positive vs. 15% for those aged 12 and younger ($p=0.47$). Among the HEU, the 30% of the older group vs. 7% of the younger had HPV ($p=0.07$). The significant association for age and HPV for HEU was driven by the girls. HEU older girls were more likely to have HPV than the younger ones ($p=0.02$), whereas no significant difference was found for the PHIV+ girls nor for PHIV+ or HEU boys (see Fig.1). There was a trend association with anogenital HPV and maternal history of warts. Among those with HPV, 4/16 (25%) mother had a history of warts and among those without HPV, 7/71 (10%) had a history ($p=0.10$). No difference was found by Tanner stage—14/77 (18.2%) children who were Tanner stage 1–3 had HPV detected compared to 5/23 (21.7%) children who were Tanner 4–5. Rates looked similar by HIV status.

Among the HIV infected boys and girls, we examined CD4 counts and HIV viral load (VL). The mean CD4 count was similar for both groups—the median CD4 count among those with HPV at any site was 685 (IQ range 367, 946) and among those without HPV was 628 (319, 945) ($p=0.74$). There was also no difference for VL. Among those with HPV, the log VL was 5.9 (95% CI 3.2, 7.9) and among those without HPV was 4.7 (95% CI 3.2, 8.9) ($p=0.32$). There were too few types to examine low risk HPV vs. high risk HPV or to separate by gender.

Discussion

This pilot study of nonsexually active children with perinatal HIV infection demonstrates that approximately a quarter of boys and girls have HPV detected in either the genital area or oral cavity. Oral infections were rare among the PHIV+ males, whereas oral infections were equally common as genital HPV infections in girls. Antiretroviral

TABLE 1. HPV TYPES IN NONSEXUALLY ACTIVE BOYS AND GIRLS

	Anogenital	Oropharyngeal
Boys < 12		
HIV + (n=0)		
HIV – (n=3)	HPV 16, ^a 52 ^a , 62	
Boys > 12		
HIV + (n=5)	HPV 11, 51, ^a 58, ^a 62, 81	HPV 53 ^a
HIV – (n=1)		HPV 42
Girls < 12		
HIV + (n=2)	HPV 6, 18, ^a 53, ^a 56 ^a	HPV 45 ^a
HIV – (n=0)		
Girls > 12		
HIV + (n=6)	HPV 11, 18, ^a 31, ^a 39, ^a 52, ^a 54, 58, ^a 61, 62, ^b 68, ^a 89	HPV 55, 62 ^b
HIV – (n=2)	HPV 6, 84	

^aHigh risk HPV; ^b2 girls had 62 in both oral and genital samples.

therapy during pregnancy is now the norm, so as expected of this aging cohort, the majority of the PHIV+ children were over the age of 12 years, limiting any conclusions for younger children. We did note that HPV DNA was detected in two PHIV+ girls under the age of 12 years (one oral and one anogenital), however, no PHIV+ boy under 12 years of age had HPV. The greater detection rate of anogenital and oral HPV in those greater than 12 years of age may suggest that many of these individuals were already having some type of sexual contact which was not elicited by the study staff. HPV DNA has been detected from the hand and finger nails of sexually active individuals.^{12,13} Since these studies are based on DNA detection and not confirmation of active infections, it is unknown whether these HPV DNA detections reflect live viral particles which can be transmitted. A recent study of 14- to 17-year-old girls reported that HPV DNA was detected in 10 of 22 girls from vaginal swabs prior to reporting vaginal intercourse.¹⁴ Seven of these girls reported noncoital behaviors such as genital touching or oral sex likely explaining the presence of the HPV DNA.

Several studies have documented the transmission of HPV from mother to child during delivery both to the oral cavity and the genital area.¹⁵ In addition, one study showed that transmission between the mother and child continues during early childhood. However, most of these infections appear transient in non-immunocompromised children. In this study of 36 months, oral HPV infections were acquired by 42% of infants, with only 10% showing persistence defined by detection of HPV in two consecutive 6-month visits. Persistent oral HPV infection was associated with persistent oral HPV infection in the mother. In comparison, 36% of infants had a positive HPV test from the genital samples, with only 1.5% showing persistence. Interestingly, persistent genital HPV infection in the infant was predicted if the mother had a history of genital warts. Smith and colleagues¹⁶ tested the oral cavity and oropharynx from 1235 children and found a bimodal distribution with a prevalence of 2.5% in the less than 1 years old, falling to less than 1% in those 1–4 years of age. The prevalence rose again to 3.3% in the 16–20 year olds similar to that reported for adult females.¹⁷ These data clearly underscore the possibility of HPV transmission to occur between parent and child whether during delivery or during childcare. The question remains whether any of these “detections” reflect an active infection. Although genital warts and recurrently laryngeal papillomatosis do occur in children, they are rare events. Since HIV-infected mothers are more likely to shed HPV than uninfected mothers, it is plausible that children born to these mothers have a higher degree of exposure and hence chance of getting infected. No longitudinal data on immunocompromised children are available to date. However, our data suggest that the rates of HPV are higher in both the oral and genital areas among nonsexually active PHIV+ girls and boys than that reported for non-immunocompromised group. The small number of subjects in our study and the large age gap between the two groups precludes any firm conclusions and warrant future longitudinal studies of this important population.

To return to the observed higher rate in those older than 12 years, it is possible that pubertal hormones play an important role in activating latent infections. Both estrogen and progesterone have been shown to increase HPV replication and transcription,¹⁸ thereby increasing viral load and making HPV easier to detect. Several studies have shown that it is

difficult for most assays to detect low levels of HPV reliably.¹⁹ Consequently, it is plausible that HPV persistence may occur at low levels in children until puberty. Certainly, latency of HPV in HIV-infected women is thought to be common. Incident HPV infections have been reported in HIV-infected women who have been sexually inactive for long periods of time, suggesting reactivation of HPV is common among HIV infected persons.⁹

In comparison, the perinatally exposed but HIV uninfected girls were less likely to have HPV detected than the PHIV+ girls. This was true for the younger but not older girls, although any conclusion is limited by small sample sizes. This suggests that HIV may influence persistence since exposure to HPV during delivery and childhood would be similar between groups. Most studies in adults find higher rates of HPV in those persons with low CD4 counts.^{20,21} Although we found no such association with CD4 counts, our population overall had high CD4 counts limiting any comparisons. The higher prevalence observed in the girls was not completely unexpected since the external genitalia of girls appears more vulnerable to HPV infections, including genital warts. Studies of non-immunocompromised adults show that women have 2–3 times the rate of genital warts than men.²²

These findings have important implications for HPV vaccination in perinatally infected girls and boys. Vaccine efficacy studies in perinatally HIV-infected children are essential since prior infections may dampen efficacy. Our data also suggest that HPV may be commonly acquired nonsexually in HIV-infected children and adolescents.

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Author Disclosure Statement

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