Comparison of Incident Cervical and Vulvar/Vaginal Human Papillomavirus Infections in Newly Sexually Active Young Women

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Vulvar/vaginal human papillomavirus (HPV) infections may precede cervical infections, and certain low-risk types may display vaginal tropism. We evaluated whether incident infections in young women display site-specific preferences by HPV risk group or phylogenetic species. Although incident infections were more likely to be detected in the vulva/vagina than in the cervix (odds ratio, 4.38 [95% confidence interval, 2.51–7.63]), the majority were first detected at both sites. Low- or undetermined-risk HPV types were more likely than high-risk types to be first detected in the vulva/vagina (P = .03). Site-by-species differences were not statistically significant. Our results suggest that low- or undetermined-risk HPV types preferentially infect vaginal epithelium.

In a previous study of a cohort of young women, we found that human papillomavirus (HPV) infections may be detected in the vulva/vagina before they are detected in the cervix [1]. Furthermore, studies of prevalent infections in older women suggest that certain low-risk HPV types display vaginal tropism [2–4]. In particular, types of the α3/α15 and α1/α8/α10 phylogenetic species (primarily low-risk types) were more prevalent in vaginal than in cervical samples. It is unclear, however, whether these low-risk types preferentially infect or preferentially persist in vaginal versus cervical epithelium. To explore the theory that certain types preferentially infect vaginal epithelium, we determined whether incident HPV infections also display site-specific preferences by HPV risk group or phylogenetic species. To our knowledge, this report is the first to compare HPV types detected in paired, clinician-collected cervical and vulvar/vaginal samples in young women with incident HPV infections.

Methods. From December 2000 through March 2007, we enrolled 18–22-year-old female students attending the University of Washington into a longitudinal study of HPV infection. Recruitment and data collection methods have been described elsewhere [5]. Women were eligible to enroll if they had never had vaginal intercourse with a male partner or had had their first intercourse with 1 male partner within the past 3 months; 250 women were enrolled. All participants provided informed consent, and the protocol was approved by University of Washington’s Institutional Review Board.

Women were followed up with triannual gynecological examinations. At each visit, women self-collected a vaginal sample before the pelvic examination [6]. During the pelvic examination, a nurse practitioner collected separate cervical and vulvar/vaginal samples into transport medium using Dacron-tipped swabs. Samples were tested for HPV DNA by polymerase chain reaction (PCR)–based methods [5]. As described elsewhere, samples testing positive by generic probe were typed using a reverse line blot assay (Roche Molecular Systems) for 38 HPV types [7]. In our statistical analyses, we used PCR results for the clinician-collected cervical and vulvar/vaginal samples only. Analyses were restricted to 161 women who reported their first vaginal intercourse with a male partner during the study or during the 3 months before enrollment and had ≥1 clinical visit after first intercourse. Kaplan-Meier methods were used to estimate the cumulative incidence of HPV infection in the genital tract (combining PCR results for cervical and vulvar/vaginal samples). At-risk time was measured from first intercourse to the first positive result or the last visit, with separate models constructed to estimate the cumulative incidence of infection with any HPV type, any high-risk HPV type (16, 18, 26, 31, 33, 35, 39, 45, 51–53, 56, 58, 59, 66, 68, 73, 82, or IS39) [8], and any HPV type of low or undetermined risk (6, 11, 40, 42, 54, 55, 57, 61, 62, 64, 67, 69, 70–72, 81, 83, 84, or CP6108) [8].

To determine whether infections were more likely to occur first in the vulva/vagina or cervix, we categorized each HPV type within each woman as occurring first in the vulva/vagina only, in the cervix only, at both sites, or at neither site (if infection did not occur or occurred before first intercourse). We then fit a logistic regression model with no covariates to the discordant results.
only (coding vulva/vagina only as 1 and cervix only as 0). The intercept from this logistic regression estimates the log odds that an infection will occur first in the vulva/vagina versus the cervix (i.e., the odds ratio [OR]). Without covariates, this analysis is equivalent to McNemar’s test. We then added covariates to determine whether the OR varied by subgroup (effectively, an adjusted McNemar’s test). Subgroups were compared in 2 separate models. The first compared high-risk types with low- or undetermined-risk types, and the second compared the following phylogenetic species or species groups: α3/α8/α10 (types 6, 11, 40, 42, and 55), α3/α15 (types 61, 62, 71, 72, 81, 83, and 84), α5/α6 (types 26, 51, 53, 56, 66, 69, and 82), α7 (types 18, 39, 45, 59, 68, and 70), and α9/α11 (types 16, 31, 33, 35, 52, 58, 64, 67, and 73). In all cases, we used a robust variance estimate to account for correlation between multiple HPV types within a woman.

We also determined whether the number of types detected in cervical and vulvar/vaginal samples differed when HPV was detected at both sites simultaneously. We restricted this analysis to all visits after first intercourse at which HPV was detected in both the cervix and the vulva/vagina (including both incident and prevalent infections). A generalized-estimating-equations approach was used to estimate the mean difference between the number of vulvar/vaginal types and the number of cervical types, with a robust variance estimate to account for correlation between repeated visits by the same woman.

Results. The mean age at first intercourse for the 161 women was 20.0 years (SD, 1.4 years). These women were followed up for a mean of 29.0 months (SD, 20.5 months) after first intercourse, and the median interval between visits was 4.2 months (interquartile range, 3.7–5.3 months). The cumulative incidences of genital HPV infection with any type, high-risk types, and low- or undetermined-risk types from the date of first intercourse are shown in figure 1. Eight infections were detected in 6 women before their reported date of first intercourse.

In total, 299 type-specific HPV infections were detected in 84 women after their first intercourse (mean number of infections per woman, 3.6 [SD, 2.9]). Three incident infections were detected in the vulvar/vaginal sample from 1 woman at a visit at which no cervical sample was collected. Therefore, 296 type-specific incident HPV infections were available for analyses evaluating site-specific preferences of incident infections. The majority of infections (167 [56.4%]) were first detected in both the cervix and the vulva/vagina. Incident infections that were detected at 1 site only were more likely to be detected in the vulva/vagina (OR, 4.38 [95% confidence interval, 2.51–7.63]). Low- or undetermined-risk HPV types were more likely than high-risk types to be detected first in the vulva/vagina (P = .03) (table 1). None of these associations varied significantly by age at the time of incident infection (data not shown). Although site-by-species or species-group differences were observed, none of them reached statistical significance (table 1). At the subsequent 4-month visit, 38.8% of type-specific incident infections detected in the vulva/vagina only were detected in the cervix; the likelihood of subsequent detection in the cervix did not differ by HPV risk group (data not shown). When HPV (incident or prevalent) was detected in both the cervix and the vulva/vagina, the mean number of types detected in vulvar/vaginal samples (2.2 [SD, 1.5]) was greater than that detected in cervical samples (1.9 [SD, 1.1]) (P < .001).

Discussion. In this cohort of newly sexually active young women, the majority of incident infections were first detected in

Figure 1. Cumulative incidence of genital human papillomavirus (HPV) infection with any type (thick black line) (n = 155), high-risk types (thin black line) (n = 157), and low- or undetermined-risk types (gray line) (n = 157). The X-axis shows the no. of months from the date of first vaginal intercourse with a male partner.
both the cervix and the vulva/vagina. We previously reported that, in a cohort of women followed up during the 1990s, the majority of incident infections were first detected in the vulva/vagina only [1]. Changes in HPV DNA assays and study protocols may partially account for this difference. First, the PCR-based methods used in the present study were more sensitive for detecting HPV DNA. With the use of Qiagen columns to isolate DNA rather than ethanol precipitation (a very crude method), the DNA used for PCR was cleaner and contained fewer inhibitors. Furthermore, we used AmpliTaq Gold polymerase (Applied Biosystems) instead of regular Taq polymerase. AmpliTaq Gold ensures a hot start to the PCR, which reduces nonspecific binding and amplification and increases sensitivity. With a less-sensitive assay, infections with low viral levels might have a higher probability of being detected in vaginal samples than in cervical samples, given the increased mucosal surface area, which may enhance the concentration of infected cells and viral loads in collected specimens. The more-sensitive assay used in the present study may have enhanced detection of low-level infections in the cervix. Second, in the present study, women self-collected a vaginal sample before each pelvic examination. Because women were instructed to insert the vaginal swab “as far as it will go without hurting” and rotate it 3 times [6], it is possible that self-swabbing introduced vaginal HPV DNA into the cervix (or cervical HPV DNA into the vagina) and that this DNA was later picked up by clinician-collected sampling. Regardless, our results indicate that a significant portion of new HPV infections are detectable in the entire genital tract.

Although the majority of new infections were detected in both the cervix and the vulva/vagina, it is notable that 44% were detected at 1 site only. Consistent with our previous report [1], these infections were more likely to be detected in the vulva/vagina than in the cervix. Furthermore, even when HPV was detected in paired samples (including both incident and prevalent infections), the mean number of types was higher in vulvar/vaginal samples than in cervical samples. The vagina may be more susceptible to infection than the cervix, given its larger surface area and greater number of potential target cells. Vulvar/vaginal infections may later migrate to the cervix (via intercourse or insertion of tampons, for example). This explanation is consistent with the theory that vulvar/vaginal infections may precede cervical infections [1–4]. Alternatively, rates of infection may be similar in the vulva/vagina and cervix, but HPV detection may be enhanced in the vulva/vagina because of the larger surface area or potential admixing of vaginal and cervical cells in vaginal samples via shedding of cervical cells into the vagina.

We further explored whether incident HPV infections display site-specific preferences by HPV risk group or phylogenetic species. Previous studies have suggested that certain low-risk types display tropism for the squamous epithelium of the vagina compared with the metaplastic, squamo-columnar epithelium of the cervix [2–4]. It is unclear, however, whether these types preferentially infect or preferentially persist in vaginal versus cervical epithelium. In a study from Guanacaste, Costa Rica, Castle et al. [4] reported that types of the α3/α15 and α1/α8/α10 phylogenetic species (which primarily contain low-risk types) were more prevalent in vaginal samples from women who had undergone hysterectomy than in cervical samples from women who had not. Furthermore, in paired samples collected from a subset of nonhysterectomized women participating in the same study, α3/α15 types were twice as common in vaginal samples as in cervical samples [3]. These patterns were strongest in women <50 [3]

### Table 1. Odds ratios (ORs) for the likelihood of first detecting incident type-specific human papillomavirus (HPV) infection in the vulva/vagina vs. the cervix, by HPV risk group and HPV phylogenetic species or species group.

| HPV species or species group | Risk groupa (n = 129)b | First detection site, no. of infections | Vulva/vagina | Cervix |
|-----------------------------|------------------------|----------------------------------------|---------------|
| OR (95% CI)                 |                        |                                        |               |
| α1/α8/α10                   | 12.00 (0.48–4.65)      | 13                                     | 1             |
| α3/α15                      | 8.00 (2.82–22.66)      | 32                                     | 4             |
| α5/α6                       | 3.14 (1.33–7.42)       | 22                                     | 7             |
| α7                          | 3.00 (0.92–9.75)       | 15                                     | 5             |
| α9/α11                      | 3.00 (1.29–6.96)       | 21                                     | 7             |

**NOTE:** CI, confidence interval.

* P = .03, for the interaction term for HPV risk group (high risk vs. low or undetermined risk) by genital site of incident infection (vulva/vagina vs. cervix).

b Total no. of type-specific infections detected in the cervix only and the vulva/vagina only.

α1/α8/α10 and α3/α15 primarily include low-risk types; α5/α6, α7, and α9/α11 primarily include high-risk types.
and in those <55 [4] years of age, presumably because of the physiology of the aging cervix (whereby cervical epithelium more closely resembles vaginal epithelium after migration of the squamocolumnar junction into the endocervix) [3, 4]. In contrast, the prevalence of high-risk HPV did not differ between cervical and vaginal samples [3, 4]. To explore these patterns further, Castle et al. related the amount of cervical ectopy (exposed columnar epithelium) to species-specific cervical HPV prevalence. Older age and decreasing cervical ectopy were associated with a higher prevalence of α3/α15 types relative to α9 types [2].

In our study of newly sexually active young women, we found that, although both high-risk and low- or undetermined-risk HPV infections were more likely to first be detected in the vulva/vagina than in the cervix, the effect was stronger for low- or undetermined-risk types. Differences by phylogenetic species were also observed, with α3/α15 and α1/α8/α10 types having a greater likelihood than α5/α6, α7, or α9/α11 types of being detected in vulvar/vaginal samples than in cervical samples. Although site-by-species differences did not reach statistical significance, the number of incident infections within each species or species group was small, thus limiting our power to detect a significant difference. Our data support the theory that low- or undetermined-risk types preferentially infect vaginal epithelium.

A limitation of our study is that combined sampling of vaginal and vulvar sites precluded an analysis restricted to vaginal versus cervical HPV types. However, although the vulva is a distinct anatomical site with a higher rate of cancer than the vagina, both vulvar and vaginal cancers are far less common than cervical cancers [9]. Furthermore, we previously observed 83% perfect or partial type-specific concordance between clinician-collected vulvar/vaginal samples and self-collected vaginal samples [6]. Therefore, it seems unlikely that a significant number of additional HPV types were detected with the inclusion of vulvar sampling.

In conclusion, although the majority of incident HPV infections were simultaneously detected in cervical and vulvar/vaginal samples, infections detected only at a single site were more likely to be detected in the vulva/vagina than in the cervix. Furthermore, consistent with previous reports of prevalent infections in older women [2–4], the latter effect was stronger for low- or undetermined-risk types than for high-risk types. To our knowledge, our study is the first to compare HPV types detected in clinician-collected paired samples from young women with incident HPV infections, and our results support the hypothesis that low- or undetermined-risk types display tropism for vaginal epithelium.

References