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Research Article

Cancer Epidemiology, Biomarkers & Prevention

Cervical Infection with Cutaneous Beta and Mucosal Alpha Papillomaviruses

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Abstract

Background: Alpha-human papillomavirus (α-HPV) plays a causal role in cervical cancer, but little is known about the epidemiology of genital Beta-human papillomavirus (β-HPV) infection.

Methods: We used Luminex and PCR hybridization to detect β- and α-HPVs prevalence at enrollment and 12-month follow-up in cervical samples from 505 women enrolled in the Ludwig-McGill cohort study. We compared epidemiologic correlates of both β- and α-HPVs and compared genotypes between these genera with respect to co-occurrence and association with cervical cytologic abnormalities.

Results: Infection with β-HPV types was more prevalent than that with α-HPV types at both visits (cumulative prevalences: 27.3% vs. 21.6%, respectively, $P = 0.034$). β-HPVs were mostly transient; however, only 1.98% women retained their original positivity at 12 months, whereas persistence was higher for α-HPVs (5.15%; $P = 0.007$). Age, parity, and sexual activity variables were predictors of α-HPV but not of β-HPV. α- and β-HPV types occurred independently. Increased risk of cervical abnormalities was restricted to women infected with α-9 or α-6 HPV types. We found no epidemiologic correlates for β-HPV infections.

Conclusions: Detection of β-HPV types in the cervix tends to occur as random and transient episodes not explained via the sexual-transmission correlates that characterize infections by α-HPVs.

Impact: Although it is plausible that β-HPVs may play a direct or indirect carcinogenic role, the lack of epidemiologic correlates for detection episodes of these viruses and lack of association with cervical lesions speak against their ancillary role as sexually transmitted agents in cervical carcinogenesis. *Cancer Epidemiol Biomarkers Prev; 26(8); 1312–20. ©2017 AACR.*

Introduction

More than 200 human papillomavirus (HPV) genotypes (types, for short) have been characterized, of which the great majority clusters phylogenetically within three genera of the *Papillomaviridae* family: Alpha (α)-, Beta (β)-, and Gamma (γ)-HPV (1). The α genus contains HPV types that infect mostly mucosal and genital regions, including 25 oncogenic types with an established, probable, or possible role in the etiology of cervical cancer (2). β- and γ genera include HPV types that commonly infect the dry skin; they are commonly referred to as cutaneous HPVs. To date, 52 β- and 82 γ- HPVs have been identified. Thus, in combination, cutaneous HPVs are a more diverse group than α-HPVs (65 types; http://www.hpvcenter.se/html/refclones.html). HPV5 and HPV8 (included in the β-1 species) are recognized as possible etiologic agents in cutaneous squamous cell carcinoma (SCC) in epidermodysplasia verruciformis primarily in sun-exposed areas (3). However, the role of specific β-HPV types in cutaneous SCC among immunocompromised non–epidermodysplasia verruciformis and immunocompetent individuals has proven difficult to demonstrate because of the high viral diversity and ubiquity of multiple types in healthy skin, oral cavity, male anogenital region and condylomas (3–8).

Although most research on oncogenic potential and disease association has focused on α-HPVs, there is interest in identifying a role of non-α-HPV types in the pathogenesis of benign and malignant lesions. It has been hypothesized that, instead of contributing directly to cancer development, β-HPVs may play a role solely at early stages of carcinogenesis, thus allowing the accumulation of mutations and destabilization of the host genome, ultimately driving tumorigenesis (9–13).

The objective of the current study was to describe the prevalence, distribution, and persistence of β-HPV types in DNA from cervical samples among asymptomatic women enrolled in the Ludwig-McGill Cohort Study, and compare these to cervical α-HPV types and species. We examined demographic and behavioral correlates of both β- and α-HPV types, and compared them with respect to their association with precancerous cytologic abnormalities.

Materials and Methods

Study design and participants

The study sample consisted of a subset of women enrolled in the Ludwig-McGill Cohort Study, a longitudinal investigation of the

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Table 1. Characteristics of the study sample at baseline in the Ludwig-McGill Cohort Study ($n = 505$)

Variables	Categories	*n* (%) [a]
Age (years)	18–22	62 (12.3)
	22–29	121 (24.0)
	30–39	186 (36.8)
	≥40	136 (26.9)
Ethnicity	White	318 (63.0)
	Nonwhite	187 (37.0)
Education	<Elementary	125 (24.8)
	Elementary	301 (59.6)
	High school	68 (13.5)
	College/University	9 (1.8)
Smoking	Never	245 (48.5)
	Current	172 (34.1)
	Former	88 (17.4)
Number of pregnancies	0–1	80 (15.8)
	2–3	225 (44.6)
	4–6	147 (29.1)
	≥7	50 (9.9)
Oral contraceptive use	Never	85 (16.8)
	<6 years	261 (51.7)
	≥6 years	159 (31.5)
Condom use	No	185 (36.6)
	Yes	320 (63.4)
Hygienic tampon use	No	454 (89.9)
	Yes	50 (9.9)
Menstrual cloth use	No	313 (62.0)
	Yes	191 (37.8)
Vaginal douching	Never/occasional	475 (94.1)
	Frequent	30 (5.9)
Age at first intercourse	20–50	142 (28.1)
	18–19	113 (22.4)
	16–17	113 (22.4)
	≤ 15	137 (27.1)
Lifetime number of sex partners	0–1	238 (47.1)
	2–3	176 (34.9)
	4+	91 (18.0)
Number of sex partners in the last 5 years	0–1	410 (81.2)
	≥2	95 (18.8)
Number of sex partners in the last year	0–1	483 (95.6)
	≥2	18 (3.6)
Anal sex practiced between visits	No	301 (59.6)
	Yes	204 (40.4)
Lifetime number of anal sex partners	0	319 (63.2)
	1	172 (34.1)
	2–3	14 (2.8)
Received oral sex	Never	239 (47.3)
	Ever	266 (52.7)
Annual frequency of masturbation acts in the last 5 years[b]	0	351 (69.5)
	<1	92 (18.2)
	1–9	27 (5.3)
	10–35	19 (3.8)
	≥36	16 (3.2)
History of sexually transmitted diseases	No	384 (76.0)
	HPV-related	20 (4.0)
	Other	100 (19.8)
Cytology grade[c]	NILM	471 (93.3)
	ASC-US	16 (3.2)
	LSIL	13 (2.6)
	HSIL	3 (0.6)

Abbreviations: HSIL, high squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy

[a]Frequencies may not add up to 505 women because of missing values for some variables.

[b]Question asked at the follow-up visit. Of those practicing masturbation, 147 reported using hands and 6 using objects (1 missing).

[c]Refers to the highest cytologic grade attained during the first year.

natural history of HPV infection and precursor lesions of cervical cancer. A detailed description of the study design and methods can be found elsewhere (14). Briefly, 2,462 women ages 18 to 60 years were recruited from family medicine, gynecology, and family planning clinics in Sao Paulo, Brazil, from 1993 to 1997. Participants were followed up every 4 months in the first year following enrollment, and then twice yearly, for up to 10 years. Questionnaires were administered and biological specimens were collected. The study was approved by ethical review boards of the participating institutions in Brazil and Canada, and informed consent was obtained from all participants. Supplementary Fig. S1 provides an overview of the sample selection strategy. Of the 2,462 women enrolled, only those with complete data (questionnaire and genotyping) at both visits, and whose samples were considered adequate (i.e., β-globin positive) were considered eligible for the current analysis. In addition, the enrollment and follow-up visits had to be within 10 days of one exact year apart (i.e., ≥355 days and ≤375 days) to permit the assessment of 12-month infection persistence. The analysis sample thus included 505 women randomly selected from 1,160 women who had completed the first and fourth visit (referred to hereafter as enrollment and follow up visit, respectively) within the first year. Samples from original cohort visits two and three were not tested.

HPV genotyping

DNA was extracted from exfoliated cervical cells by spin-column chromatography. Mucosal α-HPVs were tested by PCR amplification with MY09/11 and PGMY09/11 primers followed by genotyping via hybridization with HPV type–specific oligonucleotide probes and restriction fragment length polymorphism analysis. In combination, these two techniques allow the identification of potentially more than 40 genital α-HPV types, which were classified as per the following species: α-1: HPVs 32, 42; α-3: HPVs 61, 62, 72, 81, 83, 84, 89; α-4: HPV57; α-5: HPVs 26, 51, 69, 82; α-6: HPVs 53, 56, 66; α-7: HPVs 18, 39, 45, 59, 68, 70; α-8: HPV40; α-9: HPVs 16, 31, 33, 35, 52, 58, 67; α-10: HPVs 6, 11, 44; α-11: HPVs 34, 73; α-13: HPV54; and α-14: HPV71 (15). The presence of cutaneous β-HPVs was determined by a type-specific, multiplex genotyping PCR assay using a mixture of specific biotinylated primers that amplify a 180 to 280 bp fragment of the *E7* gene, followed by genotyping via a bead-based Luminex technology (16). This assay distinguishes 43 β-HPV types (species β-1: HPVs 5, 8, 12, 14, 19, 20, 21, 24, 25, 36, 47, 93, 98, 99, 105, 118 124, 143; species β-2: HPVs 9, 15, 17, 22, 23, 37, 38, 80, 100, 104, 107, 110, 111, 113, 120, 122, 145, 151; species β-3: HPVs 49, 75, 76, 115; species β-4: HPV92; and species β5: HPVs 96, 150). We included negative and positive controls to ascertain the quality of template DNA (17).

Statistical analysis

We calculated descriptive statistics to summarize the baseline characteristics of the study sample and prevalence rates at enrollment and follow-up for all individual HPV types and by grouping them as species within each genus. We constructed scatter plots to display the correlation between prevalence of individual HPV types between enrollment and one-year follow-up visits separately for each genus. We tested the statistical strength and significance of the correlations by calculating nonparametric Spearman's rank correlation coefficients and respective *P* values. We examined the tendency for infections of HPV types of each genus to persist by calculating ratios of observed to expected frequencies and

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Table 2. Prevalence of infection [*n* (%)] with β-HPV types and species in the Ludwig-McGill Cohort Study ($n = 505$)

Type-specific β-HPV	Positivity at enrollment	Positivity at 1-year follow-up	Positivity at enrollment AND follow-up	Positivity at enrollment OR follow-up
HPV5	5 (0.99)	4 (0.79)	0	9 (1.78)
HPV8	3 (0.59)	8 (1.58)	0	11 (2.18)
HPV9	1 (0.20)	1 (0.20)	0	2 (0.40)
HPV12	2 (0.40)	4 (0.79)	0	6 (1.19)
HPV14	0	1 (0.20)	0	1 (0.20)
HPV15	3 (0.59)	0	0	3 (0.59)
HPV17	1 (0.20)	1 (0.20)	1 (0.20)	1 (0.20)
HPV19	1 (0.20)	1 (0.20)	0	2 (0.40)
HPV20	0	0	0	0
HPV21	21 (4.16)	7 (1.39)	0	28 (5.54)
HPV22	8 (1.58)	13 (2.57)	1 (0.20)	20 (3.96)
HPV23	0	2 (0.40)	0	2 (0.40)
HPV24	3 (0.59)	7 (1.39)	0	10 (1.98)
HPV25	0	0	0	0
HPV36	0	10 (1.98)	0	10 (1.98)
HPV37	0	0	0	0
HPV38	14 (2.77)	8 (1.58)	0	22 (4.36)
HPV47	0	2 (0.40)	0	2 (0.40)
HPV49	1 (0.20)	0	0	1 (0.20)
HPV75	0	0	0	0
HPV76	3 (0.59)	6 (1.19)	0	9 (1.78)
HPV80	0	0	0	0
HPV92	0	1 (0.20)	0	1 (0.20)
HPV93	0	0	0	0
HPV96	1 (0.20)	2 (0.40)	0	3 (0.59)
HPV98	0	0	0	0
HPV99	0	0	0	0
HPV100	2 (0.40)	1 (0.20)	0	3 (0.59)
HPV104	0	0	0	0
HPV105	1 (0.20)	1 (0.20)	0	2 (0.40)
HPV107	0	2 (0.40)	0	2 (0.40)
HPV110	3 (0.59)	3 (0.59)	0	6 (1.19)
HPV111	10 (1.98)	2 (0.40)	0	12 (2.38)
HPV113	1 (0.20)	0	0	1 (0.20)
HPV115	0	0	0	0
HPV118	0	0	0	0
HPV120	2 (0.40)	1 (0.20)	0	3 (0.59)
HPV122	0	7 (1.39)	0	7 (1.39)
HPV124	1 (0.20)	0	0	1 (0.20)
HPV143	0	0	0	0
HPV145	0	0	0	0
HPV150	0	0	0	0
HPV151	1 (0.20)	0	0	1 (0.20)
β-HPV species[a]				
β-1	33 (6.53)	39 (7.72)	3 (0.59)	69 (13.66)
β-2	44 (8.71)	37 (7.33)	5 (0.99)	76 (15.05)
β-3	4 (0.79)	6 (1.19)	0	10 (1.98)
β-4	0	1 (0.20)	0	1 (0.20)
β-5	1 (0.20)	2 (0.40)	0	3 (0.59)
Any β-HPV	75 (14.85)	73 (14.46)	10 (1.98)	138 (27.33)

[a]β-HPV species: β-1 (HPVs 5, 8, 12, 14, 19, 21, 24, 36, 47, 105, 124); β-2 (HPVs 9, 15, 17, 22, 23, 38, 100, 107, 110, 111, 113, 120, 122, 151); β-3 (HPVs 49, 76); β-4 (HPV92); β-5 (HPV96).

respective 95% confidence intervals (CI). Expected frequencies were based on the assumption of independence of observations between the enrollment and one-year visits. We also assessed whether infections with β-HPV and α-HPV types tended to co-occur in the same women by comparing observed and expected frequencies based on the period prevalence data for both visits. This analysis was done for individual types and for types grouped within their respective species.

We used unconditional logistic regression to estimate OR and 95% CI for univariate associations between baseline characteristics, as independent variables, and the one-year period prevalence of HPV infection according to genus, as outcome. The referent category consisted of women without the respective genus-specific HPV infection. In separate logistic models, we examined the association between genus-specific HPV species (as independent variables) and cervical cytologic abnormalities (as outcome) using two definitions for an abnormal cytology: atypical cells of undetermined significance (ASC-US) or worse and low-grade squamous intraepithelial lesion (LSIL) or worse. For each, two types of analyses were performed; unrestricted and restricted. In the former, women with infections with types belonging to a given species were compared to all others as

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Table 3. Prevalence of infection [*n* (%)] with α-HPV types and species in the Ludwig-McGill Cohort Study (*n* = 505)

Type-specific α-HPV	Positivity at enrollment	Positivity at 1-year follow-up	Positivity at enrollment and follow-up	Positivity at enrollment or follow-up
HPV6/11	5 (0.99)	0	0	5 (0.99)
HPV16	19 (3.76)	12 (2.38)	6 (1.19)	25 (4.95)
HPV18	3 (0.59)	2 (0.40)	0	5 (0.99)
HPV26	0	0	0	0
HPV31	3 (0.59)	5 (0.99)	1 (0.20)	7 (1.39)
HPV32	0	1 (0.20)	0	1 (0.20)
HPV33	1 (0.20)	1 (0.20)	0	2 (0.40)
HPV34	0	0	0	0
HPV35	1 (0.20)	1 (0.20)	0	2 (0.40)
HPV39	0	2 (0.40)	0	2 (0.40)
HPV40	2 (0.40)	2 (0.40)	1 (0.20)	3 (0.59)
HPV42	0	0	0	0
HPV44	3 (0.59)	2 (0.40)	0	5 (0.99)
HPV45	0	1 (0.20)	0	1 (0.20)
HPV51	6 (1.19)	5 (0.99)	1 (0.20)	10 (1.98)
HPV52	5 (0.99)	4 (0.79)	1 (0.20)	8 (1.58)
HPV53	8 (1.58)	7 (1.39)	0	15 (2.97)
HPV54	2 (0.40)	4 (0.79)	1 (0.20)	5 (0.99)
HPV56	1 (0.20)	4 (0.79)	1 (0.20)	4 (0.79)
HPV57	0	0	0	0
HPV58	4 (0.79)	5 (0.99)	1 (0.20)	8 (1.58)
HPV59	2 (0.40)	1 (0.20)	0	3 (0.59)
HPV61	4 (0.79)	4 (0.79)	1 (0.20)	7 (1.39)
HPV62	0	0	0	0
HPV66	4 (0.79)	1 (0.20)	0	5 (0.99)
HPV67	0	0	0	0
HPV68	0	2 (0.40)	0	2 (0.40)
HPV69	0	0	0	0
HPV70	6 (1.19)	2 (0.40)	2 (0.40)	6 (1.19)
HPV71	1 (0.20)	4 (0.79)	0	5 (0.99)
HPV72	0	1 (0.20)	0	1 (0.20)
HPV73	0	0	0	0
HPV81	1 (0.20)	1 (0.20)	0	2 (0.40)
HPV82	1 (0.20)	0	0	1 (0.20)
HPV83	0	1 (0.20)	0	1 (0.20)
HPV84	2 (0.40)	2 (0.40)	0	4 (0.79)
HPV89	0	0	0	0
α-HPV species[a]				
α-1	0	1 (0.20)	0	1 (0.20)
α-3	7 (1.39)	9 (1.78)	1 (0.20)	15 (2.97)
α-4	0	0	0	0
α-5	7 (1.39)	5 (0.99)	1 (0.20)	11 (2.18)
α-6	13 (2.57)	12 (2.38)	1 (0.20)	24 (4.75)
α-7	11 (2.18)	9 (1.78)	2 (0.40)	18 (3.56)
α-8	2 (0.40)	2 (0.40)	1 (0.20)	3 (0.59)
α-9	32 (6.34)	26 (5.15)	9 (1.78)	49 (9.70)
α-10	8 (1.58)	2 (0.40)	0	10 (1.98)
α-11	0	0	0	0
α-13	2 (0.40)	4 (0.79)	1 (0.20)	5 (0.99)
α-14	1 (0.20)	4 (0.79)	0	5 (0.99)
Any α-HPV	69 (13.66)	66 (13.07)	26 (5.15)	109 (21.58)

[a]α-HPV species: α-1 (HPVs 32, 42); α-3 (HPVs 61, 62, 72, 81, 83, 84, 89); α-4 (HPV57); α-5 (HPVs 26, 51, 69, 82); α-6 (HPVs 53, 56, 66); α-7 (HPVs 18, 39, 45, 59, 68, 70); α-8 (HPV40); α-9 (HPVs 16, 31, 33, 35, 52, 58, 67); α-10 (HPVs 6, 11, 44); α-11 (HPVs 34, 73); α-13 (HPV54); α-14 (HPV71).

referent, that is, those who did not have infections with types of that species. In the restricted analysis, we used a fixed referent group of HPV negative women ($n = 396$ for α-species, $n = 367$ for β-species). Statistical analysis was performed using Stata version 13 (StataCorp).

Results

Table 1 presents characteristics of the study sample. The mean age of subjects was 33.5 years (range: 18–57). The majority was white and had at least elementary school education. Slightly more than half of the women reported ever smoking cigarettes. Only 15.8% of women were never or only once pregnant; 83.6% reported at least two pregnancies. Almost half of the women had none or one lifetime sexual partner and most reported no previous sexually transmitted diseases.

Tables 2 and 3 show the prevalence of infection with β- and α-HPVs, respectively, at each visit, as well as the positivity at both enrollment and follow-up (presumed as persistent infections) and positivity at either enrollment or follow-up (used for

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estimating period prevalence). Among the 43 β-HPVs detectable by the assay, 14 viral types were not found. The most prevalent types were HPV21 (β-1 species), HPV22 and HPV38 (both from β-2 species). The point prevalence of infection with any β-HPV type was just under 15% at each of the two visits, whereas the same estimates were just over 13% for any α-HPV. Overall, considering both visits as period prevalence, β-HPVs were more common than α-HPVs: 27.3% versus 21.6%, respectively ($P = 0.034$).

β-HPV infection was mostly a transient finding: 1.98% of women retained their original positivity at 12 months (Table 2). On the other hand, persistence was higher for α-HPVs (5.15%; $P = 0.007$; Table 3). When considering as denominators the women who had at least one of the visits positive for types of the two genera the differences became more pronounced: 7.25% and 23.85%, for β-HPV and α-HPV types, respectively ($P = 0.0005$).

By examining the strength of the correlation between prevalence estimates at enrollment and follow-up it is possible to further assess the relative transience or persistence of type-specific infection episodes by HPV genus. Figure 1 shows the between-visit scatter plots of type-specific prevalence for types that were detected in at least one of the visits (i.e., period prevalence > zero). β-HPV types (Fig. 1, top) had a scattered distribution, with no statistical evidence that the between-visit prevalence estimates were correlated (Spearman's correlation coefficient = 0.344; $P = 0.068$). On the other hand, the equivalent estimates for α-HPVs (Fig. 1, bottom) were significantly correlated (Spearman's correlation coefficient = 0.533; $P = 0.0035$).

On the basis of the assumption that detection of a given HPV type is independent between visits, we compared the observed and expected positivity for the most common HPV types, that is, those that were detected in at least 10 women in either the enrollment or follow-up visits (Supplementary Table S1). For none of the 7 β-HPV types included in the analysis was there evidence against the assumption of independence. On the other hand, among the 3 most common α-HPV types there was strong evidence for HPV16 that visit-specific prevalence estimates were not independent. Positivity for HPV16 in both visits was 13.3 times (95% CI, 4.87–29.02) more frequent than expected by chance. The ratio for HPV51 was 16.84 but largely imprecise (95% CI, 0.42–93.80).

We also tested whether positivity for β-HPV and α-HPV types were associated as coinfections both as individual types (Supplementary Table S2) and as grouped genus-specific species (Supplementary Table S3). None of the pairwise combinations of the most common β-HPV and α-HPV types seemed to be more common than expected (Supplementary Table S2). There was also no evidence that by grouping all type-specific episodes into species between-genus coinfections became observable because of the larger sample sizes (Supplementary Table S3).

Table 4 displays associations between candidate risk factors and the one-year combined positivity for α- and for β-HPVs, separately. Age and number of pregnancies were inversely associated with risk of α-HPVs. As expected, lifetime and recent (last one or five years) number of sex partners were strong predictors of an increased risk of detecting α-HPVs. Women who reported ≥ 2 sex partners in the last year were 5.03 times (95% CI, 1.94–13.11) more likely to be infected with any α-HPV type compared with women with at most one partner during the same period. In contrast, there were no clear

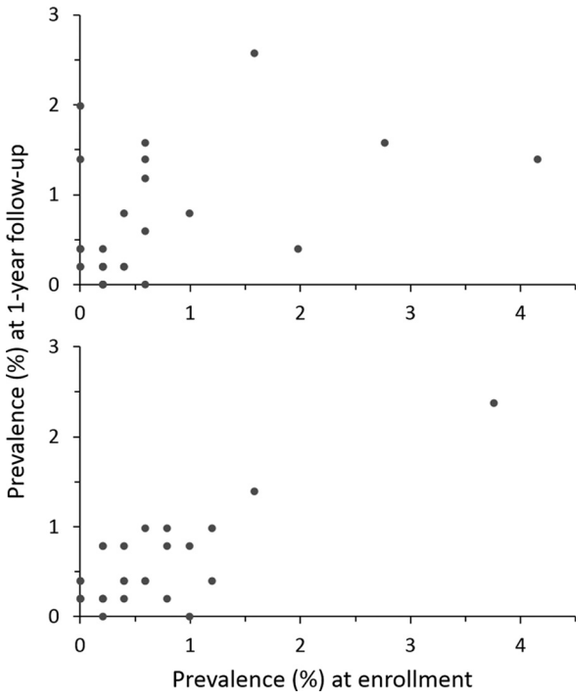

Figure 1.
Correlation between enrollment and one-year follow-up prevalence of type-specific HPV infections in the Ludwig-McGill Cohort Study ($n = 505$). Top, β-HPV types; Bottom, α-HPV types (only types detected in either of the two visits were included).

correlates of β-HPV infection among the same women, except for an implausible reduced risk among women reporting 4 or more lifetime sexual partners.

Table 5 presents the association between HPV infection status and lesion outcome based on the worst lesion grade observed by cervical cytology during the first year of follow up. Not surprisingly, women with HPV types of the α-9 species (includes HPVs 16 and 31) were at a higher risk of developing LSIL or worse compared with HPV negative women (restricted analysis, OR, 25.72; 95% CI, 7.70–85.95) or with those not harboring a viral type of α-9 species (unrestricted analysis, OR, 19.69; 95%CI, 6.79–57.12). Strong associations were also observed for women infected with HPV types of α-6 species (includes HPVs 53, 56, and 66). In contrast, no significant association was found between women infected with any β-HPV species and cervical lesion risk, regardless of grade and analysis type.

Discussion

We found that cervical infection with mostly cutaneous HPV types of the β-HPV genus were relatively common among low-income Brazilian women attending an opportunistic screening program and enrolled in our cohort study. In fact, β-HPV infections were more common than those by mucosotropic α-HPV types but appear to be mostly transient episodes. Interestingly, we found no epidemiologic correlates of cervical infections with β-HPV types. For construct validity, we conducted the same analyses with α-HPV types, which also served the purpose of assessing whether infection with the latter types, individually and

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Table 4. Univariate associations between subject characteristics and one-year period prevalence of HPV infection by genus in the Ludwig-McGill Cohort Study ($n = 505$)

		α-HPV positivity (n=109)		β-HPV positivity (n=138)	
Variable	**Categories**	***n* (%)**	**OR (95% CI)**	***n* (%)**	**OR (95% CI)**
Age, y	18–22	24 (22.02)	1	17 (12.32)	1
	22–29	24 (22.02)	0.39 (0.20–0.77)	37 (26.81)	1.17 (0.59–2.30)
	30–39	43 (39.45)	0.48 (0.26–0.88)	45 (32.61)	0.84 (0.44–1.62)
	≥40	18 (16.51)	0.24 (0.12–0.49)	39 (28.26)	1.06 (0.54–2.08)
Ethnicity	White	67 (61.47)	1	90 (65.22)	1
	Nonwhite	42 (38.53)	1.09 (0.70–1.68)	48 (34.78)	0.87 (0.58–1.32)
Education	<Elementary	21 (19.27)	1	32 (23.19)	1
	Elementary	65 (59.63)	1.36 (0.79–2.35)	81 (58.70)	1.07 (0.67–1.72)
	High School	21 (19.27)	2.21 (1.10–4.44)	23 (16.67)	1.49 (0.78–2.83)
	College/University	2 (1.83)	1.41 (0.27–7.29)	2 (1.45)	0.83 (0.16–4.20)
Smoking	Never	48 (44.04)	1	67 (48.55)	1
	Current	39 (35.78)	1.20 (0.75–1.94)	41 (29.71)	0.83 (0.53–1.30)
	Former	22 (20.18)	1.37 (0.77–2.43)	30 (21.74)	1.37 (0.81–2.32)
No. pregnancies	0–1	27 (24.77)	1	21 (15.22)	1
	2–3	49 (44.95)	0.55 (0.31–0.96)	65 (47.10)	1.14 (0.64–2.03)
	4–6	19 (17.43)	0.29 (0.15–0.57)	44 (31.88)	1.20 (0.65–2.21)
	≥7	13 (11.93)	0.69 (0.31–1.51)	7 (5.07)	0.46 (0.18–1.17)
Oral contraceptive use	Never	20 (18.35)	1	26 (18.84)	1
	<6 years	60 (55.05)	0.97 (0.54–1.73)	74 (53.62)	0.90 (0.53–1.53)
	≥6 years	29 (26.61)	0.73 (0.38–1.38)	38 (27.54)	0.71 (0.40–1.28)
Condom use	No	30 (27.52)	1	54 (39.13)	1
	Yes	79 (72.48)	1.69 (1.06–2.70)	84 (60.87)	0.86 (0.58–1.29)
Hygienic tampon use	No	89 (81.7)	1	128 (92.8)	1
	Yes	20 (18.3)	2.73 (1.48–5.04)	10 (7.2)	0.64 (0.31–1.31)
Menstrual cloth use	No	74 (67.9)	1	89 (64.5)	1
	Yes	35 (32.1)	0.72 (0.46–1.14)	49 (35.5)	0.87 (0.58–1.30)
Vaginal douching	Never/occasional	98 (89.9)	1	132 (95.7)	1
	Frequent	11 (10.1)	2.23 (1.03–4.83)	6 (4.3)	0.65 (0.26–1.62)
Age at first intercourse	20–50	27 (24.77)	1	38 (27.54)	1
	18–19	22 (20.18)	1.03 (0.55–1.93)	31 (22.46)	1.03 (0.59–1.80)
	16–17	27 (24.77)	1.34 (0.73–2.44)	39 (28.26)	1.44 (0.84–2.47)
	≤ 15	33 (30.28)	1.35 (0.76–2.40)	30 (21.74)	0.77 (0.44–1.33)
Lifetime number of sex partners	0–1	41 (37.61)	1	73 (52.90)	1
	2–3	39 (35.78)	1.37 (0.84–2.23)	53 (38.41)	0.97 (0.64–1.49)
	4+	29 (26.61)	2.25 (1.29–3.91)	12 (8.70)	0.34 (0.18–0.67)
Sex partners in the last 5 years	0–1	71 (65.14)	1	115 (83.33)	1
	≥2	38 (34.86)	3.18 (1.96–5.16)	23 (16.67)	0.82 (0.49–1.37)
Sex partners in the last year	0–1	96 (88.07)	1	134 (97.10)	1
	≥2	10 (9.17)	5.03 (1.94–13.11)	3 (2.17)	0.52 (0.15–1.83)
Anal sex practiced between visits	No	61 (56.0)	1	81 (58.7)	1
	Yes	48 (44.0)	1.21 (0.79–1.86)	57 (41.3)	1.05 (0.70–1.58)
Lifetime number of anal sex partners	Never	67 (61.5)	1	86 (62.3)	1
	1	37 (33.9)	1.03 (0.65–1.62)	48 (34.8)	1.05 (0.69–1.59)
	2–3	5 (4.6)	2.09 (0.68–6.44)	4 (2.9)	1.08 (0.33–3.55)
Received oral sex	Never	47 (43.1)	1	64 (46.4)	1
	Ever	62 (56.9)	1.24 (0.81–1.90)	74 (53.6)	1.05 (0.72–1.56)
Annual frequency of masturbation acts in the last 5 years	0	80 (73.4)	1	97 (70.3)	1
	<1	13 (11.9)	0.56 (0.29–1.05)	25 (18.1)	0.98 (0.58–1.64)
	1–9	6 (5.5)	0.97 (0.38–2.48)	8 (5.8)	1.10 (0.47–2.60)
	10–35	5 (4.6)	1.21 (0.42–3.46)	6 (4.3)	1.21 (0.45–3.27)
	≥36	5 (4.6)	1.54 (0.52–4.56)	2 (1.4)	0.37 (0.08–1.68)
History of sexually transmitted diseases	No	81 (74.31)	1	105 (76.09)	1
	HPV-STD	8 (7.34)	2.49 (0.99–6.31)	4 (2.90)	0.66 (0.22–2.03)
	Other STD	20 (18.35)	0.94 (0.54–1.62)	28 (20.29)	1.03 (0.63–1.69)

grouped as species, was predictive of β-HPV detection. Although we observed the expected reproductive health and sexual activity correlates for α-HPV infections, none of the sociodemographic, lifestyle, behavioral, and reproductive health variables that we collected via questionnaire was predictive of β-HPV infections. Likewise, infections with α-HPV species that include carcinogenic types were associated with cervical precancerous abnormalities, whereas none of the β-HPV species was statistically associated

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Table 5. Associations between positivity for HPV species by genus and cytologic abnormalities[a] in the Ludwig-McGill Cohort Study ($n = 503$)

		≥ASC-US ($n = 32$)			≥LSIL ($n = 16$)		
		Unrestricted analysis[b]		Restricted analysis[c]	Unrestricted analysis[b]		Restricted analysis[c]
Genus	Species	n	OR (95% CI)	OR (95% CI)	n	OR (95% CI)	OR (95% CI)
Alpha[d]	α-3	0	0.00 (0.00–4.19)	0.00 (0.00–7.90)	0	0.00 (0.00–9.12)	0.00 (0.00–42.26)
	α-5	2	3.42 (0.71–16.55)	5.63 (1.12–28.35)	1	3.18 (0.38–26.46)	9.78 (1.00–95.51)
	α-6	5	4.66 (1.61–13.51)	7.04 (2.30–21.51)	5	11.84 (3.72–37.67)	27.15 (6.72–109.79)
	α-7	1	0.86 (0.11–6.69)	1.49 (0.19–11.95)	0	0.00 (0.00–7.42)	0.00 (0.00–34.83)
	α-9	13	8.52 (3.89–18.69)	9.41 (4.15–21.35)	10	19.69 (6.79–57.12)	25.72 (7.70–85.95)
	α-10	1	1.66 (0.20–13.49)	2.81 (0.33–23.67)	0	0.00 (0.00–14.62)	0.00 (0.00–65.55)
Beta[e]	β-1	4	0.91 (0.31–2.68)	0.93 (0.31–2.79)	0	0.00 (0.00–1.64)	0.00 (0.00–1.61)
	β-2	6	1.34 (0.53–3.39)	1.30 (0.51–3.30)	2	0.81 (0.18–3.64)	0.69 (0.15–3.10)
	β-3	1	1.66 (0.20–13.49)	1.66 (0.20–13.65)	0	0.00 (0.00–14.62)	0.00 (0.00–12.52)

[a]Cytologic outcome defined at two cut-off points (ASC-US and LSIL). Two women had missing data on cytology.
[b]Unrestricted analysis compared women with a genus-specific species infection against a floating referent group of all women who did not have that particular species infection.
[c]Restricted analysis compared women with a genus-specific species infection against a fixed referent group of HPV-negative women (α referent group n=396, β negative group n=367).
[d]β-HPV species: β-1 (HPVs 5, 8, 12, 14, 19, 21, 24, 36, 47, 105, 124); β-2 (HPVs 9, 15, 17, 22, 23, 38, 100, 107, 110, 111, 113, 120, 122, 151); β-3 (HPVs 49, 76).
[e]α-HPV species: α-3 (HPVs 61, 62, 72, 81, 83, 84, 89); α-5 (HPVs 26, 51, 69, 82); α-6 (HPVs 53, 56, 66); α-7 (HPVs 18, 39, 45, 59, 68, 70); α-9 (HPVs 16, 31, 33, 35, 52, 58, 67); α-10 (HPVs 6, 11, 44).

with such findings. We also did not find any evidence that α-HPV and β-HPV types occur preferentially as coinfections.

The overall point prevalence of β-HPV infections in the cervix in the present investigation (15%) was considerably lower than those we observed using the same assay methodology in a study of healthy men for the anal canal (54.3%), genitals (77.7%), external genital lesions (61.1%), as well as the oral cavity (29.3%; refs. 5, 18, 19). Moreover, infection by two or more β-HPVs was not detected in the same cervical specimen despite the fact that multiple β-HPVs are commonly observed in the skin of healthy individuals and organ transplant recipients (20, 21), as well as in male genitals where we detected up to 19 viral types simultaneously (3, 19, 22).

A limitation of the current study is that we examined 43 out of the 52 β-HPV types described up until now and did not access γ-HPV genomes. Because new HPV cutaneous types are continually characterized, this analysis possibly underestimated the frequency of untested and unknown HPVs. In addition, short-term persistence of β-HPVs could not be evaluated as samples tested were collected 12 months apart.

Some studies have reported co-detection of α- and β-HPVs in the oral cavity and in penile cancer specimens (5, 23). However, a possible role of β-HPVs as carcinogenetic co-factors augmenting that of α-HPVs at these anatomic sites remains unclear. It is conceivable that cutaneous HPV infections could affect the acquisition and/or clearance of mucosal HPVs. With this hypothesis in mind, we examined the co-occurrence of α- and β-HPVs in our cohort in an attempt to identify a propensity for some types to occur more frequently as joint infections. Our findings do not support this hypothesis, as we observed that α- and β-HPV types and species occurred mostly independently of each other. This is in contrast with the recent report of a significant association between β-HPV detection and HPVs 16/18 infections at the anal canal among HIV-negative men who have sex with men (24). Similarly, in one study, detection of α-HPVs in the oral cavity significantly increased the odds of β-HPV detection (25).

Clinical manifestations of infections with certain β-HPV types include common, plantar, planar, and genital warts (26). Although recent functional in vitro studies reveal that some β-HPV types have intrinsic oncogenic potential (12, 27), they tend to cause neoplastic disease at anatomic sites typically exposed to ultraviolet radiation. However, β- and γ-HPV DNA have also been detected in condylomas and other genital lesions, and seem to play a carcinogenic role in head and neck cancers (23, 28, 29).

Although β-HPVs were more common than α-HPVs, the latter were more likely to persist and be associated with markers of sexual activity and with cervical lesions in the current study. In contrast, β-HPVs infections were not associated with cervical abnormalities or any of a long list of putative predictors. We had observed the same lack of epidemiologic or lesion correlates in a study of anogenital specimens of men (6, 7). It is reasonable to suppose that β-HPVs detection in mucosal surfaces, including cervicovaginal, may reflect deposition of virions shed from cutaneous body sites and introduced by hand as part of genital hygiene. Because hair follicles are potential reservoirs of persistent HPV infection, it cannot be ruled out that some instances of β-HPV detection in our subjects may have resulted from skin/anogenital hair contamination during specimen sampling or from recent sexual activity.

In summary, detection of cervical β-HPVs was more common than α-HPVs, but detection episodes were transient, seemed to occur at random, and were not associated with risk for cervical lesions. There was no correlation between β-HPV and α-HPV detection, either at the individual type level or as grouped species. In contrast with α-HPV, which were correlated with known sexual activity markers, we found no predictors of cervical β-HPV detection. Detection of cutaneous HPVs in the cervix may be unrelated to an active infectious process and may merely represent deposition of virions or contamination during cervical sampling.

Disclosure of Potential Conflicts of Interest

L.L. Villa reports receiving commercial research support and speakers bureau honoraria, and is a consultant/advisory board member for Merck, Sharp & Dohme. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The funders of the study had no involvement in study design; in the collection, analysis, and interpretation of data; neither in the writing of the report; and in the decision to submit the article. The corresponding author had

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full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Development of methodology: E.L. Franco, L.L. Villa
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Sichero, E.M. Nunes, S. Ferreira, E.L. Franco
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Sichero, M. El-Zein, E.M. Nunes, S. Ferreira, E.L. Franco, L.L. Villa
Writing, review, and/or revision of the manuscript: L. Sichero, M. El-Zein, E.M. Nunes, S. Ferreira, E.L. Franco, L.L. Villa
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. El-Zein, S. Ferreira, E.L. Franco, L.L. Villa
Study supervision: L. Sichero, E.L. Franco, L.L. Villa
Other (the PI for the parent study and conceived the strategy for this secondary study): E.L. Franco

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