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Prevalence and correlates of beta human papillomavirus detection in fingernail samples from mid-adult women

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ABSTRACT

Cutaneous human papillomaviruses (HPVs) have not been evaluated in fingernails from healthy individuals. To determine prevalence and correlates of β-HPVs in fingernails from healthy mid-adult women, we tested archived samples collected from 2011 to 2012 using a multiplex PCR combined with Luminex technology for 46 β-HPV genotypes. One hundred thirteen (61.1%) of 185 fingernail samples were positive for β-HPV, and the median number of types detected in positive samples was 2 (interquartile range: 1–4). The most common genotypes detected were HPV-23 (β−2) (13.5%), HPV-38 (β−2) (13.0%), HPV-5 (β−1) (9.2%), HPV-107 (β−2) (8.7%), and HPV-120 (β−2) (8.7%). In multivariate analysis, β-HPV detection was associated with age (prevalence ratio [PR] for women 40–51 years versus 30–39 years = 1.30, 95% CI: 1.05–1.62) and race (PR for non-white versus white race = 0.65, 95% CI: 0.45–0.94). The prevalence of β-HPV in fingernail samples from healthy mid-adult women was similar to the prevalence of β-HPV reported at other cutaneous sites in prior studies. We did not identify any significant health or sexual behavior predictors of β-HPV detection in fingernails. Our results support the hypothesis that fingers may serve as a source of transmission or autoinoculation of cutaneous HPVs to other anatomic sites.

1. Introduction

The majority of human papillomavirus (HPV) types are phylogenetically classified into alpha (α), beta (β), or gamma (γ) genera, with α-HPVs displaying tropism for mucosal epithelium and β- and γ-HPVs displaying tropism for cutaneous epithelium [1,2]. Cutaneous HPVs have also been detected in the anal canal [3–5], genital [6] and cervical [7] epithelia, and in oral [8,9] and nasal [10] mucosa, supporting their wide anatomic distribution. While the oncogenic potential of certain α-HPVs is well-established in the etiology of anogenital and a subset of oropharyngeal cancers [11], less is known about the potential involvement of β-HPVs in carcinogenesis. Mounting evidence suggests a role for β-HPVs in some skin [12–14] and oropharyngeal [15,16] cancers, but potentially through different carcinogenic pathways than α-HPVs [17–19].

As our understanding of the role of β-HPVs in non-cutaneous cancers expands, understanding the epidemiology and transmission routes of these HPVs is important. For example, fingers likely play a role in α-HPV transmission or autoinoculation between anatomic sites, with potential finger-genital or finger-oral routes [20–23], and similar transmission routes for β-HPVs have also been postulated [3,8]. To our knowledge, however, there are no data on β-HPV detection in fingernail samples from healthy individuals.

Using stored fingernail samples from a study of healthy mid-adult women, we sought to determine β-HPV prevalence using a multiplex PCR assay for 46 β-HPV genotypes. We further evaluated demographic, health, and sexual behavior correlates of detecting β-HPVs in the fingernails.

Abbreviations: CI, confidence interval; PR, prevalence ratio

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2. Methods

2.1. Study participants and setting

We used stored fingernail samples collected between June 2011 and August 2012 as part of a study of HPV infections in healthy mid-adult women. Details of the parent study have been reported previously [20,24]. Briefly, 409 women aged 30–50 years who were affiliated with the University of Washington were enrolled and followed for 6 months. The study protocol included self-collected fingernail samples and online demographic, health, and sexual behavior surveys at enrollment and exit visits. To collect the fingernail samples, women rubbed their fingertips and the underside of the tip of their fingernails on both hands with a cytology brush. The brush was stored in 1 mL STM (Qiagen, Gaithersburg, MD). The study protocol was approved by the Institutional Review Board of the University of Washington.

2.2. Laboratory methods

During the parent study, fingernail samples were genotyped for the presence of genital HPV types [20]. Briefly, fingernail samples were digested with 20 μg/mL proteinase K at 37 °C for one hour, and DNA was isolated from 200 μL of digested swab samples using the QIAamp DNA blood mini kit (Qiagen, Cat. No. 51104). HPV and β-globin were amplified simultaneously with the MY09/11 system, dotted onto nylon filters, and probed with biotin-labeled HPV generic and β-globin probes. Specimens that were negative for β-globin were considered insufficient, and specimens that were positive for β-globin but negative for HPV were considered negative. Specimens that were positive by both probes were genotyped with the Roche Linear Array. If a sample was HPV-positive by dot blot but negative for β-globin by Roche assay, it was also considered insufficient. Of 649 fingernail samples collected, 140 (21.6%) were insufficient. Of 326 women with at least one sufficient fingernail sample, we randomly selected 204 women for inclusion in the present study. One fingernail sample per woman was selected for testing by multiplex PCR for β-HPV in 2017.

DNA samples were shipped to the International Agency for Research on Cancer in Lyon, France. Samples were tested for the presence of HPVs using type-specific E7 PCR bead-based multiplex genotyping (TS-MPG) assays that combine multiplex PCR and Luminex technology (Luminex Corp., Austin, TX, USA), as described elsewhere [25–31]. The multiplex type-specific PCR method uses specific primers for the detection of 46 β-HPVs (HPV 5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93, 96, 98, 99, 100, 104, 105, 107, 110, 111, 113, 115, 118, 120, 122, 124, 143, 145, 150, 151, 152, 159, 174). Two primers for the amplification of the β-globin gene were included to provide a positive control for the quality of the DNA in the sample [32]. Following multiplex PCR amplification, 10 μL of each reaction mixture was analyzed by multiplex genotyping using the Luminex technology as previously described [26,29].

2.3. Statistical analysis

Prevalence was calculated for any β-HPV, multiple-type β-HPV, species-specific HPV, and type-specific β-HPV. Log-binomial regression was used to estimate univariate prevalence ratios (PRs) for the associations between selected correlates (age, race, ethnicity, marital status, smoking status, current alcohol consumption, current hormonal contraceptive use, current immunosuppressive condition, abnormal Pap test history, genital warts history, pregnancy history, lifetime number of male sex partners, and sex with new male partners within the previous year) and β-HPV detection. Variables found to be statistically significant in univariate analysis ($p < 0.1$) were entered into a final multivariate model.

Table 1
Demographic, health, and sexual history characteristics of mid-adult women (n = 185).

Characteristics	
Age, mean (SD), years	39.1 (5.9)
Lifetime number of male sexual partners,[a] median (interquartile range)	7 (3–17)
	N (%)
Race	
White	145 (78.4)
Asian	25 (13.5)
African American	3 (1.6)
Other[b]	12 (6.5)
Hispanic ethnicity[c]	
Yes	12 (6.6)
No	171 (93.4)
Marital status[c]	
Currently married or living with a partner	113 (61.4)
Unmarried or separated	71 (38.6)
Smoking history[c,d]	
Never	134 (73.2)
Former	38 (20.8)
Current	11 (6.0)
Ever had an abnormal Pap test[c]	
Yes	77 (41.8)
No	107 (58.2)
Ever had genital warts[c]	
Yes	24 (13.0)
No	160 (87.0)
Ever been pregnant	
Yes	119 (64.3)
No	66 (35.7)
Currently using hormonal contraception[c,e]	
Yes	59 (32.1)
No	125 (67.9)
Currently has an immunosuppressive condition[f]	
Yes	4 (2.2)
No	180 (97.8)
Currently consumes alcohol[c]	
Yes	148 (80.4)
No	36 (19.6)
Sex with a new male partner within the past 12[c] months	
Yes	42 (23.6)
No	136 (76.4)

[a] 6 women did not provide data on lifetime number of male partners at the time of the study visit.
[b] Includes individuals indicating the following: American Indian/Alaska Native, Native Hawaiian/Other Pacific Islander, other race, or multiple races.
[c] Numbers may not add up to total due to missing data.
[d] Smoking was defined as smoking at least 1 cigarette a day for 1 month or longer; former smokers reported ever smoking but not currently smoking, and current smokers reported currently smoking.
[e] Includes birth control pills, hormonal patches, vaginal rings, implanted contraception, injectable contraception, and hormonal intrauterine devices.
[f] Includes HIV positivity (n = 1) or currently taking immunosuppressive medications (n = 3).

3. Results

Nineteen (9.3%) of 204 samples were determined to be insufficient for HPV testing as they tested negative for beta-globin, leaving 185 sufficient samples for analysis. Demographic, health, and sexual history characteristics of women whose samples were selected were similar to those of the overall parent study cohort [20] (Table 1). A total of 113 (61.1%) fingernail samples were positive for any β-HPV (Fig. 1). Sixty-five (35.1%) were positive for multiple β-HPV types, and the median number of types detected in positive samples was 2 (interquartile range: 1–4). Species-specific prevalence was 34.1% for β−1, 46.5% for β−2, and 13.5% for β−3. β−4 was not detected, and β−5 was detected in one sample. The most common individual types detected were HPV-23 (β−2) (13.5%), HPV-38 (β −2) (13.0%), HPV-5 (β −1) (9.2%), HPV-107 (β −2) (8.7%), and HPV-120 (β −2) (8.7%).

In univariate analysis, β-HPV detection in fingernails was positively

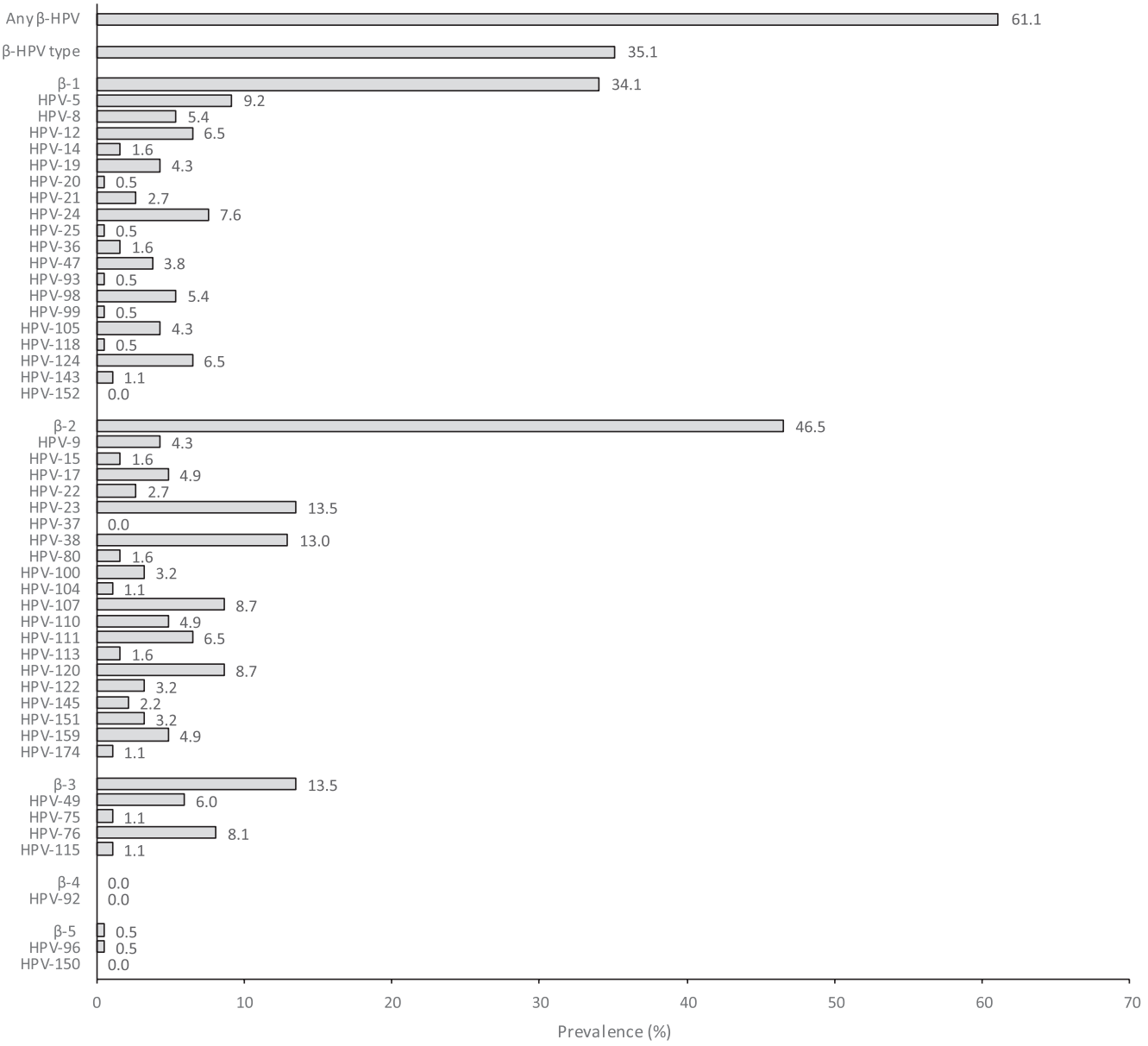

Fig. 1. β-HPV prevalence in fingernail samples from mid-adult women (n = 185).

associated with older age and white (versus non-white) race (Table 2). In the final multivariate model, β-HPV detection remained significantly associated with older age (prevalence ratio [PR] for women 40–51 years versus 30–39 years = 1.30, 95% CI:1.05–1.62) and race (PR for non-white versus white race = 0.65 (0.45–0.94)).

4. Discussion

Using a multiplex PCR assay for 46 β-HPV genotypes, we detected β-HPVs in more than 60% of fingernail samples from healthy mid-adult women. In comparison, we previously detected α-HPVs in only 3.8% of fingernail samples from the same cohort using the Roche Linear array assay [20]. These findings were expected, given that β-HPVs have a tropism for cutaneous epithelium [33]. Prior studies estimating prevalence of β-HPV at other cutaneous sites have reported similar prevalence estimates [31,34]. For example, using a similar multiplex PCR assay for 43 β-HPV types (versus 46 β-HPV types in the present study), β-HPV was detected in 64% of forearm skin samples and 61% of eyebrow hair samples from 87 men [34], and in 67% of palmar surface hand samples from 42 adults [31]. Our data on β-HPV prevalence in fingernail samples support the hypothesis that fingers or fingernails may serve as a source of transmission or autoinoculation of β-HPV to the oral cavity, genitals, or anal canal. Plausibility for finger involvement in transmission or autoinoculation of HPV between anatomic sites has been supported by previous studies indicating detection of α-HPVs in fingernails of patients with genital warts [22], sequential detection of α-HPVs in hands and genitals in heterosexual couples [23], and associations between concurrent type-specific genital and fingernail α-HPVs in male [35] and female [20] populations.

Detection of β-HPV in the fingernails was associated with older age and white (versus non-white) race. Associations between older age and cutaneous β-HPV prevalence have been reported previously, with age-related immune waning postulated as a potential mechanism [30,34]. We did not identify any health or sexual behavioral correlates of β-HPVs. In our previous report on α-HPVs in this same cohort of women, we similarly did not identify any health or behavioral correlates of α-HPV detection in fingernails, although the rarity of α-HPV detection in fingernails limited our power to detect significant associations [20]. Other studies of cutaneous HPVs in anogenital sites have not reported strong associations with sexual behavior [3,5,7,25,36].

Table 2
Prevalence ratios (PRs) for the associations between selected risk factors and detection of β-HPV detection in fingernail samples from mid-adult women.

Characteristics	N	n	Univariate PR (95% CI)	Multivariate[a] PR (95% CI)
Age, years				
30–39	106	57	1.00 (ref)	1.00 (ref)
40–51	79	56	1.32 (1.05–1.65)	1.30 (1.05–1.62)
Lifetime no. male sexual partners (median split)				
0–7	90	54	1.00 (ref)	
8+	89	57	1.07 (0.85–1.34)	
Race				
White	145	96	1.00 (ref)	1.00 (ref)
Other[b]	40	17	0.64 (0.44–0.94)	0.65 (0.45–0.94)
Marital status				
Unmarried or separated	71	45	1.00 (ref)	
Currently married or living with a partner	113	67	0.94 (0.74–1.18)	
Smoking history[c]				
Never	134	82	1.00 (ref)	
Former	38	22	0.95 (0.70–1.28)	
Current	11	8	1.19 (0.81–1.75)	
Ever had an abnormal Pap test				
No	107	60	1.00 (ref)	
Yes	77	52	1.20 (0.96–1.51)	
Ever had genital warts				
No	160	98	1.00 (ref)	
Yes	24	14	0.95 (0.66–1.36)	
Ever been pregnant				
No	66	41	1.00 (ref)	
Yes	119	72	0.97 (0.77–1.24)	
Currently using hormonal contraception[d]				
No	125	73	1.00 (ref)	
Yes	59	39	1.13 (0.89–1.43)	
Currently has an immunosuppressive condition[e]				
No	180	109	1.00 (ref)	
Yes	4	3	1.24 (0.69–2.21)	
Currently consumes alcohol				
No	36	21	1.00 (ref)	
Yes	148	91	1.05 (0.78–1.43)	
New male partner within the past 12 months				
No	136	83	1.00 (ref)	
Yes	42	24	0.94 (0.70–1.26)	

[a] The multivariate model included age and race.
[b] Includes individuals indicating the following: Asian, African American, American Indian/Alaska Native, Native Hawaiian/Other Pacific Islander, other race, or multiple races.
[c] Smoking was defined as smoking at least 1 cigarette a day for 1 month or longer; former smokers reported ever smoking but not currently smoking, and current smokers reported currently smoking.
[d] Includes birth control pills, hormonal patches, vaginal rings, implanted contraception, injectable contraception, and hormonal intrauterine devices.
[e] Includes HIV positivity (n = 1) or currently taking immunosuppressive medications (n = 3).

Limitations to our study should be noted. First, although β-HPVs were detected frequently in fingernail samples, it is unclear whether these detections represent DNA deposition or true infection. While either scenario could contribute to transmission or autoinoculation, established infection is more likely to be a significant source of spread. Second, our data were cross-sectional, and therefore we were not able to evaluate persistence or acquisition of β-HPV in fingernails. Third, we did not collect data on finger-oral or finger-genital contact to further inform potential transmission routes. Fourth, due to limited resources, we were only able to test a subset of stored samples from the original cohort for β-HPV types, thus limiting our power to detect potentially significant associations with select health and sexual behavior variables. In addition, 9% of samples that were sufficient by PCR analysis in 2011–2012 were deemed insufficient when evaluated by multiplex PCR in 2017, further limiting study power. Insufficiency of fingernail samples for PCR analysis was likely due to low levels of genomic DNA [20]. Finally, because the original study was designed to evaluate mucosal (and not cutaneous) HPV types in the genitals, oral cavity, and fingernails, data were not collected on variables such as duration and pattern of sun exposure, history of sunscreen use, or history of use of UV nail dryers in salons that may be particularly relevant as predictors of cutaneous HPVs.

In conclusion, detection of β-HPVs in fingernail samples from healthy mid-adult women was common, suggesting a potential source of transmission or autoinoculation from fingers to other anatomic sites. At the same time, we did not find evidence that detection of cutaneous HPVs in fingernails was associated with sexual behavior. The prevalence of β-HPV in fingernail samples from healthy mid-adult women was similar to the prevalence of β-HPV reported at other cutaneous sites in prior studies. Future reports from this cohort of mid-adult women will focus on detection of β-HPVs in oral rinse samples and associated risk factors to further expand our understanding of the epidemiology and transmission dynamics of cutaneous HPVs. We will also describe concordance between oral and fingernail samples for detecting β-HPVs to further explore potential transmission or autoinoculation between these two anatomic sites. Given our expanding knowledge of the involvement of cutaneous HPVs in carcinogenesis, continued efforts to increase our understanding of the epidemiology of β-HPVs are warranted.

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Conflict of interest statement

The authors of the manuscript “Prevalence and correlates of beta human papillomavirus detection in fingernail samples from mid-adult women” declare that they have not conflicts of interest to disclose.

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