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Prevalence and Concordance of Cutaneous Beta Human Papillomavirus Infection at Mucosal and Cutaneous Sites

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Background. Cutaneous beta human papillomavirus (HPV) infection across cutaneous and mucosal tissues within individuals has not been examined.

Methods. A subcohort of men (n = 87) participating in the HPV Infection in Men (HIM) study provided eyebrow hairs, forearm skin swabs, genital skin swabs, oral rinse samples, and anal swabs. Beta-HPV DNA in the 5 tissues was detected using a multiplex assay, and site-specific beta-HPV prevalence was examined.

Results. Any beta-HPV was most prevalent in genital skin (81.6%), followed by forearm skin (64.4%), eyebrow hairs (60.9%), oral mucosa (35.6%), and anal mucosa (33.3%). Most prevalent beta-HPV types included HPV-38 (beta-2) in both genital skin (32.2%) and eyebrow hairs (16.1%), HPV-12 (beta-1) in forearm skin (23%) and oral mucosa (9.2%), and HPV-76 (beta-3) in anal mucosa (14.9%). Concordance of any beta-HPV infection was greater (31.0%) across the 3 keratinized tissue sites (genital skin, eyebrow hairs, forearm skin) than across the 2 mucosal sites (anal and oral mucosa, 6.9%).

Conclusions. Prevalence of beta-HPV varied by anatomic site of infection. Biological properties of beta-HPV types detected at mucosal sites and their role in disease pathogenesis should be examined.

Keywords. cutaneous human papillomavirus; prevalence; anatomic sites.

Human papillomaviruses (HPVs) are double-stranded circular DNA viruses with a highly conserved genome organization. Approximately 200 HPV types have been isolated to date, and, based on their genome sequence, are classified into 5 major genera (alpha, beta, gamma, mu, and nu), which are further subdivided into species [1–3]. Genus alpha includes HPV types that infect epithelia of the skin and mucosa at different anatomic sites, including cervix and forearm skin. A subgroup of the mucosal alpha HPV types, referred to as high-risk HPV types, are associated with anogenital cancers, while the low-risk alpha HPV types induce the development of benign skin lesions (reviewed in [4]). Beta-HPV types are subdivided into 5 different species (beta 1–5) and are suspected to be involved in the development of nonmelanoma skin cancer, possibly in conjunction with ultraviolet radiation (reviewed in [5, 6]). Beta-1 and beta-2 HPV infections have been found to be associated with increased risk of cutaneous squamous cell carcinoma [7–10].

Many independent studies have shown that beta-HPV types infect other anatomic regions, in addition to the skin. The presence of beta-HPV DNA has been reported in the oral mucosa, anal mucosa, genital skin, and external genital lesions in men [11–15]. Although there is no clear evidence for a role of beta-HPV types in lesion development at anatomic sites other than the skin, a recent prospective study has shown that presence of some beta-HPV types in the oral cavity is associated with an increased risk of head neck and cancer [16]. The beta-3 species, which includes only 4 HPV types (types 49, 75, 76, and 115), has been found in skin, nasal cavity, anal mucosa, and oral mucosa [13, 17, 18]. Previously, Nunes et al reported a higher prevalence of beta-HPV in male genital skin compared to that in oral and anal mucosa [13]. However, the concordance of beta-HPV infection at cutaneous sites (eyebrow hairs, normal skin), which are comprised of keratinized epithelium, and at mucosal sites (anal mucosa, oral mucosa), within the same individuals, was not examined.

The simultaneous determination of the different beta-HPV types at different anatomic sites within the same individual may provide important insight into the tissue tropism of beta-HPVs and the factors associated with beta-HPV infection at various anatomic sites. In the present study, we examined the prevalence of cutaneous beta-HPV infection in 2 types of mucosal tissues (anal swabs, oral rinse samples) and 3 keratinized tissue samples (genital skin swab, forearm skin swab, eyebrow hairs),

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obtained from a subcohort of men who participated in the HPV Infection in Men (HIM) study [19, 20].

METHODS

Study Population

Between 2005 and 2009, the HIM study [20–22] enrolled men into a multinational prospective study of HPV natural history at genital, oral, and anal anatomic sites. Study participants included men aged 18–70 years, who reported no prior history of penile or anal cancer, genital or anal warts, or human immunodeficiency virus/AIDS, and were not currently being treated for sexually transmitted diseases. HIM study men were followed every 6 months for up to 7 years. At each visit, anal swabs, oral rinse samples, and genital skin swabs were collected. The study has been described in detail previously [20–23].

A subset of HIM study participants (n = 209) from the US site in Tampa, Florida, were included in a study of cutaneous HPV natural history [19]. From these 209 men, eyebrow hairs and swabs from sun-exposed skin of the forearm were collected at baseline and at each follow-up visit. The findings from the substudy have been described previously [19]. For the present analysis, HIM study participants with available archived anal swabs, genital skin swabs, and oral rinses, collected at the same visit during which eyebrow hairs and forearm skin swabs were also collected, were identified (n = 123). The parent HIM study and cutaneous HPV substudy protocols were approved by the institutional review board at the University of South Florida [19, 20].

Data and Sample Collection

HIM study men were asked to complete a comprehensive self-administered questionnaire on demographics, sexual history, and lifestyle factors [20–23]. Additional questions on history of blistering sunburns and skin's reaction to sun exposure were included as part of the cutaneous HPV substudy [19]. At each visit, participants were examined by a clinician, and samples of exfoliated cells from the genital skin, anal canal, and oral mucosa were collected [22–24]. In brief, genital skin was sampled using 3 different prewetted Dacron applicators (1 for coronal sulcus and glans of the penis, 1 for the shaft of the penis, and 1 for the scrotum) [22]. A separate swab was used to sample the anal canal between the anal verge and the dentate line [23]. Participants were asked to rinse and gargle with a mouthwash to collect oral samples, which were centrifuged to obtain cell pellets [24]. All samples were maintained at −70°C until HPV genotyping was conducted.

HPV DNA Extraction and Genotyping

Beta-HPV genotyping results were available for eyebrow hairs and sun-exposed forearm skin swabs from the cutaneous HPV substudy of HIM [19]. In brief, using a polymerase chain reaction (PCR) multiplex assay, eyebrow hairs and forearm skin swabs were examined for the presence of beta-HPV DNA corresponding to 25 types (beta-1 [types 5, 8, 12, 14, 19, 20, 21, 24, 25, 36, 47, 93], beta-2 [types 9, 15, 17, 22, 23, 37, 38, 80], beta-3 [types 49, 75, 76], beta-4 [type 92], and beta-5 [type 96]) [19]. Archived genital skin swabs, oral rinse samples, and anal swabs obtained from 123 men included in the cutaneous HPV substudy were shipped to the International Agency for Research on Cancer in France, where beta-HPV DNA genotyping was conducted for the present analysis. Cutaneous beta-HPV genotyping was performed with a bead-based multiplex PCR Luminex assay, using beta-HPV type-specific probes, as described in detail previously [19, 25]. The sensitivity [25] and reproducibility [26] of the assay have been described previously. Whereas in the previous cutaneous HPV substudy [19] 25 beta-HPV types in eyebrow hairs and forearm skin swabs were measured, the coverage of the multiplex assay subsequently expanded to allow genotyping of an additional 21 beta-HPV types (98, 99, 105, 118, 124, 143, 152, 100, 104, 107, 110, 111, 113, 120, 122, 145, 151, 159, 174, 115, and 150). Thus, using the newly available advanced multiplex assay, DNA from genital skin swabs, anal swabs, and oral rinse samples was genotyped for a total of 46 beta-HPV types, including the 25 types assessed in the previous cutaneous HPV substudy of eyebrow hairs and forearm skin swabs.

Statistical Analyses

Samples that were β-globin negative and negative for all beta-HPV types examined were considered invalid and were excluded. Thus, of the 123 men with archived genital skin swabs, oral samples, and anal swabs, 6 men with invalid anal swab (n = 4), genital skin swab (n = 1), or oral sample (n = 1) were excluded. Of the remaining 117 men, 30 men with invalid eyebrow hair samples (n = 1) or forearm skin swabs (n = 29) were excluded. Therefore, the final sample size for the present analysis comprises 87 men with a valid beta-HPV test for all 5 specimen types, corresponding to 5 anatomic sites. To compare beta-HPV prevalence across the 5 anatomic sites, analyses were restricted to the 25 beta-HPV types that were genotyped in all 5 samples using the 2 multiplex assay panels. Prevalence of beta-HPV, overall, by species, and by type, was defined as the proportion of men, out of the total study population, who were positive for any, species-specific, or type-specific beta-HPV DNA, respectively. Prevalence of beta-HPV was determined for each of the 5 anatomic sites, overall and stratified by demographic, lifestyle, and skin cancer risk factors. Fisher exact or χ^2 test was used to determine the significance of differences in HPV prevalence by demographic and skin cancer risk factors, at each of the anatomic sites. Concordance of any and species-specific beta-HPV in mucosal specimens (oral and anal samples) was defined as the proportion of men who were positive for any or species-specific beta-HPV infection of the same type, respectively, in both oral and anal samples. Similarly,

concordance of any and species-specific beta-HPV infection in keratinized tissue samples (genital skin swabs, eyebrow hairs, and forearm skin swabs) was defined as the proportion of men who were positive for any or species-specific beta-HPV infection of the same type, respectively, in samples from all 3 keratinized tissues. Finally, concordance of beta-HPV infection at all 5 sites was described as the proportion of men with the same beta-HPV type(s) in all 5 samples.

RESULTS

Of the 87 HIM study participants included in the present analyses, approximately 59% of men were 18–44 years of age; 77% were white and 90% non-Hispanic. As seen in Table 1, the prevalence of any beta-HPV was highest in genital skin (81.6%), followed by forearm skin (64.4%), eyebrow hairs (60.9%), oral mucosa (35.6%), and anal mucosa (33.3%). When examined by species, the prevalence of beta-1 and beta-2 HPV DNA was higher in genital skin, forearm skin, and eyebrow hairs, compared with that in oral mucosa and anal mucosa (Table 1). Interestingly, the prevalence of beta-3 HPV infection was highest in genital skin (42.5%), followed by anal mucosa (20.7%), with a low prevalence (between 8.0% and 17.2%) in eyebrow hairs, forearm skin, and oral mucosa (Table 1). When examined within each tissue, beta-1 and beta-2 HPVs were more prevalent than beta-3 HPV in genital skin, eyebrow hairs, and forearm skin. In contrast, in anal mucosa, the prevalence of beta-3 HPV (20.7%) was greater than that of beta-1 (11.5%) and beta-2 HPV (18.4%).

The distribution of beta-HPVs across the 5 anatomic sites also varied by HPV type, although some HPV types were frequently detected at all 5 sites. Overall, beta-HPV types 5, 12, 17, 22, 23, 24, 38, and 76 were among the 10 most frequently

Table 1. Species- and Type-Specific Beta Human Papillomavirus Prevalence and Concordance Across Anatomic Sites Among 87 Men in the HPV Infection in Men Study

	Beta-HPV Prevalence					Beta-HPV Concordance		
HPV Type	Genital Skin	Oral Mucosa	Anal Mucosa	Eyebrow Hairs	Forearm Skin	Men With HPV Infection at Both Mucosal Sites	Men With HPV Infection at All 3 Keratinized Sites	Men With HPV Infection at All 5 Sites
Any beta	71 (81.6)	31 (35.6)	29 (33.3)	53 (60.9)	56 (64.4)	6 (6.9)	27 (31.0)	4 (4.6)
Any beta-1	45 (51.7)	16 (18.4)	10 (11.5)	30 (34.5)	41 (47.1)	4 (4.6)	17 (19.5)	4 (4.6)
HPV-5	18 (20.7)	5 (5.8)	4 (4.6)	10 (11.5)	20 (23.0)	2 (2.3)	7 (8.1)	2 (2.3)
HPV-8	12 (13.8)	3 (3.5)	1 (1.2)	10 (11.5)	5 (5.8)	1 (1.2)	4 (4.6)	1 (1.2)
HPV-12	14 (16.1)	8 (9.2)	4 (4.6)	13 (14.9)	20 (23.0)	1 (1.2)	7 (8.1)	1 (1.2)
HPV-14	3 (3.5)	1 (1.2)	0 (0.0)	2 (2.3)	5 (5.8)	0 (0)	2 (2.3)	0 (0)
HPV-19	3 (3.5)	1 (1.2)	1 (1.2)	2 (2.3)	1 (1.2)	1 (1.2)	1 (1.2)	1 (1.2)
HPV-20	5 (5.8)	0 (0.0)	1 (1.2)	5 (5.8)	3 (3.5)	0 (0)	1 (1.2)	0 (0)
HPV-21	7 (8.1)	1 (1.2)	4 (4.6)	3 (3.5)	4 (4.6)	1 (1.2)	2 (2.3)	1 (1.2)
HPV-24	15 (17.2)	3 (3.5)	1 (1.2)	8 (9.2)	13 (14.9)	0 (0)	5 (5.8)	0 (0)
HPV-25	1 (1.2)	0 (0.0)	0 (0.0)	1 (1.2)	1 (1.2)	0 (0)	0 (0)	0 (0)
HPV-36	1 (1.2)	0 (0.0)	0 (0.0)	6 (6.9)	6 (6.9)	0 (0)	0 (0)	0 (0)
HPV-47	6 (6.9)	2 (2.3)	1 (1.2)	4 (4.6)	5 (5.8)	0 (0)	4 (4.6)	0 (0)
HPV-93	7 (8.1)	0 (0.0)	1 (1.2)	5 (5.8)	6 (6.9)	0 (0)	1 (1.2)	0 (0)
Any beta-2	54 (62.1)	16 (18.4)	16 (18.39)	39 (44.8)	35 (40.2)	3 (3.5)	18 (20.7)	1 (1.2)
HPV-9	9 (10.3)	3 (3.5)	0 (0.0)	8 (9.2)	9 (10.3)	0 (0)	3 (3.5)	0 (0)
HPV-15	6 (6.9)	1 (1.2)	1 (1.2)	5 (5.8)	5 (5.8)	0 (0)	2 (2.3)	0 (0)
HPV-17	13 (14.9)	3 (3.5)	3 (3.5)	6 (6.9)	6 (6.9)	0 (0)	4 (4.6)	0 (0)
HPV-22	19 (21.8)	3 (3.5)	2 (2.3)	12 (13.8)	7 (8.1)	1 (1.2)	4 (4.6)	1 (1.2)
HPV-23	23 (26.4)	2 (2.3)	6 (6.9)	12 (13.8)	6 (6.9)	1 (1.2)	2 (2.3)	0 (0)
HPV-37	9 (10.3)	0 (0.0)	3 (3.5)	7 (8.1)	7 (8.1)	0 (0)	4 (4.6)	0 (0)
HPV-38	28 (32.2)	6 (6.9)	7 (8.1)	14 (16.1)	13 (14.9)	2 (2.3)	5 (5.8)	1 (1.2)
HPV-80	5 (5.8)	0 (0.0)	0 (0.0)	4 (4.6)	4 (4.6)	0 (0)	0 (0)	0 (0)
Any beta-3	37 (42.5)	7 (8.1)	18 (20.7)	14 (16.1)	15 (17.2)	2 (2.3)	8 (9.2)	1 (1.2)
HPV-49	21 (24.1)	4 (4.6)	5 (5.8)	2 (2.3)	0 (0.0)	1 (1.2)	0 (0)	0 (0)
HPV-75	7 (8.1)	0 (0.0)	4 (4.6)	1 (1.2)	2 (2.3)	0 (0)	1 (1.2)	0 (0)
HPV-76	22 (25.3)	4 (4.6)	13 (14.9)	13 (14.9)	14 (16.1)	2 (2.3)	7 (8.05)	1 (1.2)
Beta-4								
HPV-92	3 (3.5)	0 (0.0)	1 (1.2)	1 (1.2)	1 (1.2)	0 (0)	1 (1.2)	0 (0)
Beta-5								
HPV-96	1 (1.2)	0 (0.0)	0 (0.0)	2 (2.3)	5 (5.8)	0 (0)	0 (0)	0 (0)

Data are presented as No. (%).

Abbreviation: HPV, human papillomavirus.

detected HPV types in ≥4 tissues (Table 1), suggesting that these types infect both keratinized and mucosal sites. Beta-2 HPV type 38 was the most prevalent HPV type in genital skin (32.2%) and eyebrow hairs (16.1%), while beta-1 HPV type 12 was the most prevalent type in forearm skin (23.0%) and oral mucosa (9.2%). In anal mucosa, beta-3 HPV type 76 was the most commonly detected type (14.9%).

Among beta-3 HPVs, the prevalence of some HPV types was more common at mucosal sites, whereas other types were more frequently detected at keratinized tissues (Table 1). For example, whereas beta-3 HPV type 49 was detected in approximately 5%–6% of the mucosal (anal and oral mucosa) samples, very few eyebrow hair samples (2.3%), and none of the forearm skin swabs, were positive for HPV type 49. In contrast, beta-3 HPV type 76 was equally distributed (14.9%–16.1%) in eyebrow hairs, forearm skin, and anal mucosa, with a lower prevalence in oral mucosa (4.6%). Beta-3 HPV type 75 was observed in 4.6% samples from anal mucosa, with lower prevalence in eyebrow hairs and forearm skin (1.2%–2.3%).

Concordance of HPV infection across anatomic sites within individuals is presented in Table 1. The concordance of any beta-HPV infection at the 3 keratinized tissue sites was 31.0%, while only 6.9% men had beta-HPV infection of the same type at both of the mucosal sites (Table 1). While the concordance of beta-HPVs, overall and by species, at all 5 sites was <5%, the concordance of infection was greater among keratinized tissues vs mucosal tissues for any beta-1 (19.5% vs 4.6%), any beta-2 (20.7% vs 3.5%) and any beta-3 (9.2% vs 2.3%) HPV. Beta-1 HPV types 5 and 12 and beta-3 HPV type 76 showed highest concordance (8.0%) at the keratinized tissue sites. Only 2.3% of men had concordant infection with beta-1 HPV type 5, beta-2 HPV type 38, and beta-3 HPV type 76 at the 2 mucosal sites.

Finally, factors associated with beta-HPV prevalence by anatomic site were examined (results not shown). Beta-HPV prevalence increased with increasing age for all anatomic sites, an association that was statistically significant for all sites with the exception of the oral mucosa. Current smokers had significantly lower beta-HPV prevalence in anal mucosa compared with former or never smokers ($P < .05$). Men who reported having 1 female sexual partner in the past 6 months had higher beta-HPV prevalence in forearm skin compared with those who reported no recent female sexual partners. None of the other measured factors were statistically significantly associated with beta-HPV infection.

DISCUSSION

In this cohort of men, any beta-HPV infection was highest in genital skin. Any beta-1 and beta-2 HPV infections were more prevalent in genital skin, eyebrow hairs, and forearm skin, whereas beta-3 HPV infection was more prevalent in genital skin and anal mucosa. Beta-HPV types 5, 12, 17, 22, 23, 24, 38, and 76 were frequently detected at multiple anatomic sites, suggesting widespread distribution with these HPV types. Almost a third of the men had concordant beta-HPV infection at the 3 keratinized tissue sites.

In a previous analysis of the subset of men enrolled from the US site in the HIM study, beta-HPV prevalence was reported to be higher in genital skin (74.8%) than in the anal canal (48.7%) and oral mucosa (21.9%) [13], a trend similar to the site-specific HPV prevalence observed in the present study. An important highlight of our study is the direct comparison of prevalence of cutaneous beta-HPV infection, overall and by species and type, in both cutaneous sites involving keratinized tissues and mucosal sites. Whereas, generally, beta-HPVs are thought to infect cutaneous tissues, our study highlights that some beta-HPV types, particularly those within the beta-3 HPV species, may have higher affinity to mucosal tissues.

Overall, when compared across sites, the prevalence of beta-3 HPV was highest in the genital skin (42.5%) and lowest in the oral mucosa (8.1%). However, when compared within the same site, the ratio of beta-1 or beta-2 HPV infection to beta-3 HPV varied by anatomic site of infection, with beta-3 HPV being more prevalent than beta-1 and beta-2 HPV infection in anal mucosa. These findings suggest that the 3 beta-HPV species have subtle differences in their tropism. As mentioned earlier, the phylogenetic classification of HPV into different species is based on the L1 gene nucleotide sequence and not on the biological properties of these viruses [1, 3]. Furthermore, HPVs have been broadly classified as "cutaneous" or "mucosal" based on their tissue origin [1], with alpha-HPVs mostly involving "mucosal" HPV types and beta-HPVs mostly involving "cutaneous" HPVs. Our findings suggest that within the "cutaneous" group of beta-HPVs, beta-3 HPVs have higher affinity to anal mucosa than to keratinized tissues.

Biological studies using in vitro and in vivo experimental models have revealed that beta-3 HPV type 49 shares some functional similarities with the alpha mucosal high-risk HPV type 16 [27, 28]. Expression of beta-HPV type 49 E6 oncogene in primary human keratinocytes, the natural host of the virus, leads to degradation of p53 via the proteasome pathway, similar to the observation for HPV type 16 E6 oncogene [27]. Transgenic (Tg) mouse models expressing the E6 and E7 oncogenes in the basal layer of the epithelia, under the control of the keratin 14 promoter, provide further evidence for the functional similarities between HPV types 16 and 49. K14 HPV type 49 or HPV type 16 E6/E7-Tg animals were found to be highly susceptible to upper digestive tract carcinogenesis upon initiation with 4-nitroquinoline 1-oxide (4NQO), whereas K14 beta-2 HPV type 38 E6/E7-Tg mice were not affected much by 4NQO treatment [28]. In summary, beta-3 HPVs, although conventionally classified as "cutaneous" HPVs, show some similarities to mucosal HPVs in their biological properties as well as in tissue

tropism. This group of beta-3 HPVs should be examined further to study their role in pathogenesis at the site of infection, including mucosal tissue sites such as the anal canal.

We observed that increasing age was associated with higher beta-HPV infection at all sites, except in oral rinse samples. This could be due to decreasing immunity with age, thus predisposing men to beta-HPV infection. Current smokers were less likely to have beta-HPV infection in anal mucosa than former or never smokers. The underlying mechanism of this observation is not clear. Due to the small sample, risk estimates for the association of demographic and lifestyle factors with site-specific beta-HPV prevalence were not determined.

Notably, this is the first study to compare the prevalence of cutaneous beta-HPV infection at 5 anatomic sites, including mucosal and keratinized tissues, within the same individuals. Our findings provide insight into tissue tropism of beta-HPVs by species and type, particularly of beta-3 HPVs that seem to have stronger affinity toward mucosal epithelium. Further research is required to elucidate the differences in tissue affinity across beta-HPV species, including investigation of the biological properties of beta-3 HPVs and their role in disease pathogenesis at mucosal sites.

Notes

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