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Cutaneous viral infections associated with ultraviolet radiation exposure

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Abstract

The complex interplay between ultraviolet radiation (UVR) and cutaneous viral infections in the context of cancer etiology is challenging to unravel, given the limited information on the independent association between UVR and cutaneous viral infections. Using multiple biomarkers of infection with 24 types of cutaneous human papillomavirus (HPV) and 4 types of polyomaviruses (HPyV), we investigated cross-sectional associations with recent UVR exposure, using skin pigmentation measured by spectrophotometer. Age- and sex-adjusted associations between UVR and viral seropositivity, viral DNA present in eyebrow hairs (EBH) and skin swabs (SSW) were estimated using logistic regression. Beta-HPV seropositivity was associated with viral DNA positivity in EBH (OR = 1.40, 95% CI = 1.05-1.88) and SSW (OR = 1.86, 95% CI = 1.25-2.74). Similar associations were observed for Merkel cell polyomavirus. Participants in the highest tertile of UVR exposure were more likely to be seropositive for beta-HPV (OR = 1.81, 95% CI = 1.16-2.38), and have beta-HPV DNA in EBH (OR = 1.57, 95% CI = 1.06-2.33) and SSW (OR = 2.22, 95% CI = 1.25-3.96), compared to participants with the lowest tertile of UVR exposure. UVR exposure was positively associated with three different markers of beta-HPV infection. Therefore, future studies of HPV associated KC development should address more directly the role of HPV and UVR exposure as potential co-carcinogens.

KEYWORDS

cutaneous human papillomavirus, cutaneous human polyomavirus, keratinocyte carcinoma, ultraviolet radiation

Abbreviations: EBH, eyebrow hairs; HPV, human papillomavirus; HPyV, human polyomavirus; KC, keratinocyte carcinoma; MCPyV, Merkel cell polyomavirus; SSW, skin swabs; UVR, ultraviolet radiation; VIRUSCAN, Viruses in Skin Cancer.

1 | INTRODUCTION

Observed associations between cutaneous human papillomavirus (HPV) and/or cutaneous human polyomavirus (HPyV) infection and keratinocyte carcinomas (KC) have varied across epidemiological studies for a number of possible reasons, including sample sizes, the use of different biological markers of viral infection (ie, serology and DNA-based markers), and varying levels of measured (or unmeasured) UVR exposure across studies populations.[1-15] Previously, we observed strong associations between cutaneous HPV and HPyV DNA prevalence in skin swabs (SSW) and eyebrow hairs (EBH), with strong correlations in type-specific prevalence, number of types and the degree of infection across both markers,[16] consistent with a previous study which observed high agreement between HPV DNA in EBH and perilesional skin tissue among a cohort of 21 cutaneous squamous cell carcinoma patients.[17] Some studies found a positive correlation between serological markers of Merkel cell polyomavirus (MCPyV) infection and MCPyV DNA in SSW[18] and skin punch biopsies[19]; however, no studies have examined this association with multiple HPyV types in a large cohort of patients. Four epidemiological studies have examined the relationship between viral DNA positivity and the presence of serum antibodies against cutaneous HPV proteins,[20-23] with two reporting positive associations between seropositivity and viral DNA-positivity in EBH or skin punch biopsies,[20,21] one reporting no association with viral DNA in EBH,[22] and one reporting concordance between seropositivity and DNA positivity in EBH in association with cutaneous SCC among organ transplant recipients.[23] Complicating the interpretation of findings across studies is the possibility that the various study populations may have different underlying exposures to ultraviolet radiation (UVR), which could, in turn, influence cutaneous viral infection.

The association between UVR and cutaneous HPV/HPyV infection has not been clearly established. Findings from epidemiologic studies point to an association between questionnaire-based measures of past and current UVR exposure and markers of cutaneous HPV infection including serology,[24,25] EBH[26] and SSW.[27,28] However, other studies using questionnaire-based data on past and current UVR exposure and markers of HPV infection reported no association with HPV as detected by serology,[26,29,30] EBH[31] and SSW.[32] Self-reported UVR exposure assessment is a limitation of these studies, given that they rely on participant recall and are often imprecise with respect to the timing and the magnitude of the exposure. Of note, there have been no previous epidemiological studies of UVR exposure and cutaneous HPyV infection.

In order to advance the overall field of cutaneous viral infections and carcinogenesis, additional research is needed on the relationship between serology and DNA-based markers of cutaneous viral infections and their associations with recent UVR exposure. Therefore, we aimed to examine the cross-sectional association between baseline spectrophotometer-measured skin pigmentation as an indicator of recent UVR exposure and cutaneous HPV/HPyV infection measured by serology as well as DNA positivity in SSW and EBH corresponding to 17 beta-HPV, 7 gamma-HPV and 4 HPyV types among participants of the prospective Viruses in Skin Cancer (VIRUSCAN) Study. In addition, because natural skin pigmentation has been shown to modify the biological effects of UVR exposure,[33-35] we investigated whether the association between UVR exposure and HPV/HPyV infection was more pronounced among lighter vs darker skinned individuals.

What's new?

Infection with cutaneous human papillomavirus (HPV) or human polyomavirus (HPyV), may influence risk of keratinocyte carcinoma, but it's not known what role ultraviolet radiation (UVR) plays. Here, the authors undertook the first epidemiological study to probe the association between these viral infections and UVR exposure. They used a spectrophotometer to quantitatively measure UVR exposure, and they tested for biomarkers of 24 different HPV types and 4 types of HPyV. People with the highest levels of UVR exposure were the most likely to test positive for beta-HPV, in blood tests, in eyebrow hairs and in skin swabs.

2 | MATERIALS AND METHODS

2.1 | Study design and population

The VIRUSCAN Study is a prospective cohort study of cutaneous viral infections, UVR exposure and KC development among skin cancer screening patients in Tampa, FL.[16] The study design and population have been described in detail previously.[16,36,37] Briefly, study participants were individuals, ages 60+ years, undergoing routine skin cancer screening exams at the University of South Florida Dermatology Clinic. Patients were eligible with a history of either BCC or SCC, but were excluded if they had both BCC and SCC. At the enrollment visit, participants underwent a routine total body skin examination during which suspicious skin lesions were biopsied; they also completed a questionnaire and contributed blood, EBH and forearm SSW samples for the measurement of cutaneous viral infection biomarkers.[16,36,37] A spectrophotometer was used to measure the degree of pigmentation of sun-exposed skin relative to sun-unexposed skin as an indicator of recent UVR exposure.[35]

A total of 1303 skin cancer screening patients were enrolled in the VIRUSCAN Study between July 15, 2014 and August 31, 2017, of whom 124 were diagnosed with KC as a result of the initial screening exam and were excluded. Baseline characteristics of cohort participants have been previously described in detail.[37] Participants with the complete complement of available baseline blood samples, EBH and SSW (n = 1090 [92.5%]) were considered for inclusion in the present analysis, whereas 89 participants were removed from analyses due to missing at least one of the baseline samples. Sixteen participants were missing baseline spectrophotometer-based measurements of skin pigmentation and were excluded from the analysis of HPV in relation to UVR exposure.

TABLE 1 Associations between demographic factors and human papillomavirus/human polyomavirus (HPV/HPyV) infection measured by serum antibodies and viral DNA in eyebrow hairs and skin swabs, among VIRUSCAN Study participants who screened negative for keratinocyte cancer at study enrollment

	Baseline demographic characteristics								
		Age in years		Sex			Race		
Viral infection	n (%)	Mean (SD)	P^a	Female n (%)	Male n (%)	P^b	White n (%)	Other/multiple race n (%)	P^b
Seropositivity									
Any β-HPV (n = 17)									
No	252 (25.7)	69.1 (6.3)	.640	141 (28.0)	111 (23.3)	.109	247 (26.1)	5 (14.3)	.127
Yes	729 (74.3)	68.9 (6.3)		363 (72.0)	366 (76.7)		698 (73.9)	30 (85.7)	
Any β_1-HPV (n = 7)									
No	370 (37.7)	68.7 (6.0)	.288	207 (41.1)	163 (34.2)	.026	358 (37.9)	12 (34.3)	.710
Yes	611 (62.3)	69.2 (6.4)		297 (58.9)	314 (65.8)		587 (62.1)	23 (65.7)	
Any β_2-HPV (n = 7)									
No	462 (47.1)	69.0 (6.1)	.713	252 (50.0)	210 (44.0)	.062	449 (47.5)	13 (37.1)	.259
Yes	519 (52.9)	69.0 (6.4)		252 (50.0)	267 (56.0)		496 (52.5)	22 (62.9)	
Any γ-HPV (n = 7)									
No	423 (42.7)	69.4 (6.3)	.130	238 (47.2)	185 (37.8)	.003	407 (42.5)	16 (45.7)	.719
Yes	570 (57.3)	68.8 (6.3)		266 (52.8)	304 (62.2)		550 (57.5)	19 (54.3)	
Any HPyV (n = 4)									
No	13 (1.3)	65.5 (3.9)	.047	4 (0.8)	9 (1.9)	.179	12 (1.3)	1 (2.9)	.609
Yes	981 (98.7)	69.0 (6.3)		505 (99.2)	476 (98.1)		947 (98.7)	33 (97.1)	
Viral DNA positivity in eyebrow hairs									
Any β-HPV (n = 17)									
No	485 (49.4)	68.2 (5.9)	<.001	237 (47.0)	248 (52.0)	.129	463 (49.0)	22 (62.9)	.129
Yes	496 (50.6)	69.8 (6.6)		267 (53.0)	229 (48.0)		482 (51.0)	13 (37.1)	
Any β_1-HPV (n = 7)									
No	663 (67.6)	68.5 (6.0)	<.001	328 (65.1)	335 (70.2)	.086	637 (67.4)	25 (71.4)	.730
Yes	318 (32.4)	70.1 (6.6)		176 (34.9)	142 (29.8)		308 (32.6)	10 (28.6)	
Any β_2-HPV (n = 7)									
No	629 (64.1)	68.4 (6.1)	<.001	314 (62.3)	315 (66.0)	.237	602 (63.7)	27 (77.1)	.106
Yes	352 (35.9)	70.1 (6.5)		190 (37.7)	162 (34.0)		343 (36.3)	8 (22.9)	
Any γ-HPV (n = 7)									
No	934 (94.1)	69.0 (6.3)	.264	480 (95.2)	454 (92.8)	.117	899 (93.9)	34 (97.1)	.565
Yes	59 (5.9)	69.8 (6.4)		24 (4.8)	35 (7.2)		58 (6.1)	1 (2.9)	
Any HPyV (n = 4)									
No	646 (65.0)	68.8 (6.3)	.296	357 (70.1)	289 (59.6)	<.001	622 (64.9)	23 (67.6)	.797
Yes	348 (35.0)	69.2 (6.4)		152 (29.9)	196 (40.4)		337 (35.1)	11 (32.4)	
Viral DNA positivity in skin swabs									
Any β-HPV (n = 17)									
No	131 (13.4)	67.8 (5.7)	.024	67 (13.3)	64 (13.4)	.998	123 (13.0)	8 (22.9)	.094
Yes	850 (86.6)	69.2 (6.4)		437 (86.7)	413 (86.6)		822 (87.0)	27 (77.1)	
Any β_1-HPV (n = 7)									
No	296 (30.2)	68.3 (5.9)	.026	136 (27.0)	160 (33.5)	.025	284 (30.1)	12 (34.3)	.718
Yes	685 (69.8)	69.3 (6.4)		368 (73.0)	317 (66.5)		661 (69.9)	23 (65.7)	
Any β_2-HPV (n = 7)									
No	262 (26.7)	68.3 (6.0)	.042	141 (28.0)	121 (25.4)	.391	250 (26.5)	12 (34.3)	.369
Yes	719 (73.3)	69.2 (6.4)		363 (72.0)	356 (74.6)		695 (73.5)	23 (65.7)	

TABLE 1 (Continued)

	Baseline demographic characteristics								
		Age in years		Sex			Race		
Viral infection	n (%)	Mean (SD)	P^a	Female n (%)	Male n (%)	P^b	White n (%)	Other/multiple race n (%)	P^b
Any γ-HPV (n = 7)									
No	692 (69.7)	68.8 (6.3)	.115	350 (69.4)	342 (69.9)	.878	664 (69.4)	28 (80.0)	.193
Yes	301 (30.3)	69.5 (6.5)		154 (30.6)	147 (30.1)		293 (30.6)	7 (20.0)	
Any HPyV (n = 4)									
No	181 (18.2)	68.6 (6.2)	.455	115 (22.6)	66 (13.6)	.002	175 (18.2)	6 (17.6)	.991
Yes	813 (81.8)	69.0 (6.3)		394 (77.4)	419 (86.4)		784 (81.8)	28 (82.4)	

[a]*P* values calculated using Wilcoxon rank-sum test.
[b]*P* values calculated using Barnard's test.

2.2 | Data collection

2.2.1 | Cutaneous HPV/HPyV antibody measurement

Baseline peripheral blood samples were collected, and serum aliquots were stored at −70°C until further processing at the German Cancer Research Center. Serum samples were analyzed for antibodies to the major capsid protein L1 corresponding to 17 beta-HPV types [species 1 (types 5, 8, 12, 21, 24, 36, 93), species 2 (types 9, 15, 17, 22, 23, 38, 80), species 3 (type 75), species 4 (type 92) and species 5 (type 96)], seven gamma-HPV types (4, 48, 50, 60, 88, 101 and 103) and four HPyV types (6, 7, trichodysplasia spinulosa-associated polyomavirus and Merkel cell polyomavirus [MCPyV]). Multiplex serological assays were used to allow for measurement of multiple antibodies in one cycle. The antibody detection method was based on Glutathione S-Transferase capture enzyme-linked immunosorbent assay[38,39] in combination with Luminex fluorescent bead technology,[24,40,41] as described in detail previously. A Luminex 200 analyzer (Luminex Corp., Austin, Texas) was used to identify the internal color of the individual beads and quantify their reporter fluorescence, expressed as median fluorescence intensity.[24,40,41]

2.2.2 | Cutaneous HPV/HPyV DNA measurement

At baseline, two to three EBH were plucked from each eyebrow and snap frozen in liquid nitrogen and stored at −80°C. SSW were obtained from the top of forearm using a cotton-tipped Dacron swab, and stored at 4°C until further processing.[37] The EBH and SSW samples were shipped to the International Agency for Research on Cancer for the detection of HPV/HPyV DNA. Multiplex/Luminex PCR-assays were used for the measurement of HPV/HPyV DNA corresponding to 46 beta-HPV, 52 gamma-HPV and 5 HPyV types. Two primers for the amplification of β-globin were included as a positive control for template DNA quality and assay-specific results were considered valid if the particular assay amplified β-globin. Analyses were further restricted to only include participants with HPV/HPyV specific β-globin amplified in both EBH and SSW assays corresponding to beta-HPV (n = 981), gamma-HPV (n = 993) and HPyV (n = 994), respectively. Baseline prevalence for all types measured has been previously described.[37] The present analysis was limited to the 17 beta-HPV, 7 gamma-HPV and 4 HPyV types, which were also measured by serology (see above).

2.2.3 | Measured indicator of recent UVR exposure (UVR indicator)

Skin pigmentation was measured by a portable spectrophotometer during the baseline visit, as described previously.[35] Measurements were obtained from the sun-unexposed inner arm (constitutive pigmentation), and the sun-exposed forearm (facultative pigmentation) using the Commission Internationale de l'Éclair age (CIE) L*a*b* system.[42] This system measures color on three axes, namely L*, representing the brightness ranging from total black (L* = 0) to total white (L* = 100), a*, representing the balance between red and green and b*, representing the balance between yellow and blue, with increasing values on a* and b* axes, indicating saturation of color.[42,43] The instrument was calibrated against a white tile each morning, per manufacturer guidelines. Three readings were obtained at each anatomic site, and the average values were recorded.

The brightness L* reading from the inner arm has been associated with the melanin index in a previous study,[43] and also shown to be associated with self-reported untanned skin color using VIRUSCAN Study data.[37] The indicator of recent UVR exposure (UVR indicator) was measured by calculating the difference in skin pigmentation between the forearm and the inner arm using the following CIE76 formula: $\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$,[42] and reported by the spectrophotometer directly. A larger UVR indicator value represents a bigger difference in skin pigmentation between the sun-exposed forearm and sun-unexposed inner arm. This method of skin pigmentation measurement was used in a previously reported analysis of 159 VIRUSCAN Study participants, in which an association was

TABLE 2 Association between cutaneous HPV/HPyV seropositivity and prevalence of viral DNA in skin swabs (SSW) and eyebrow hairs (EBH), among VIRUSCAN Study participants who screened negative for keratinocyte cancer at study enrollment

	Association between seropositivity and viral DNA positivity										
	In EBH			in SSW			across EBH and SSW				
							DNA− for both	DNA+ for only 1		DNA+ for both[a]	
Seropositivity	DNA− n (%)	DNA+ n (%)	OR (95% CI)[b]	DNA− n (%)	DNA+ n (%)	OR (95% CI)	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Any β-HPV (n = 17)[a]											
Seronegative	139 (28.7)	113 (22.8)	1.00 (reference)	48 (36.6)	204 (24.0)	1.00 (reference)	42 (36.8)	113 (27.8)	1.00 (reference)	97 (21.1)	1.00 (reference)
Seropositive	346 (71.3)	383 (77.2)	1.40 (1.05-1.88)	83 (63.4)	646 (76.0)	1.86 (1.25-2.74)	72 (63.2)	294 (72.2)	1.57 (1.00-2.44)	363 (78.9)	2.19 (1.39-3.42)
							P-trend[c] < .001				
Any β_1-HPV (n = 7)											
Seronegative	270 (40.7)	100 (31.4)	1.00 (reference)	132 (44.6)	238 (34.7)	1.00 (reference)	125 (44.8)	157 (37.7)	1.00 (reference)	88 (30.8)	1.00 (reference)
Seropositive	393 (59.3)	218 (68.6)	1.51 (1.13-2.01)	164 (55.4)	447 (65.3)	1.54 (1.16-2.04)	154 (55.2)	259 (62.3)	1.36 (1.00-1.86)	198 (69.2)	1.90 (1.34-2.71)
							P-trend < .001				
Any β_2-HPV (n = 7)											
Seronegative	309 (49.1)	153 (43.5)	1.00 (reference)	136 (51.9)	326 (45.3)	1.00 (reference)	131 (53.3)	190 (46.1)	1.00 (reference)	141 (43.7)	1.00 (reference)
Seropositive	320 (50.9)	199 (56.5)	1.28 (0.98-1.67)	126 (48.1)	393 (54.7)	1.30 (0.98-1.72)	115 (46.7)	222 (53.9)	1.31 (0.95-1.80)	182 (56.3)	1.48 (1.05-2.07)
							P-trend = .023				
HPV 8 (β_1)											
Seronegative	647 (71.4)	38 (50.7)	1.00 (reference)	573 (73.6)	112 (55.4)	1.00 (reference)	565 (73.4)	90 (62.1)	1.00 (reference)	30 (45.5)	1.00 (reference)
Seropositive	259 (28.6)	37 (49.3)	2.46 (1.52-3.97)	206 (26.4)	90 (44.6)	2.25 (1.63-3.10)	205 (26.6)	55 (37.9)	1.70 (1.16-2.46)	36 (54.5)	3.35 (2.01-5.62)
							P-trend <.001				
HPV 38 (β_2)											
Seronegative	655 (82.3)	149 (80.5)	1.00 (reference)	428 (82.6)	376 (81.2)	1.00 (reference)	419 (82.6)	245 (81.7)	1.00 (reference)	140 (80.5)	1.00 (reference)
Seropositive	141 (17.7)	36 (19.5)	1.16 (0.76-1.73)	90 (17.4)	87 (18.8)	1.12 (0.81-1.55)	88 (17.4)	55 (18.3)	1.08 (0.74-1.56)	34 (19.5)	1.20 (0.76-1.86)
							P-trend = .437				
Any γ-HPV (n = 7)											
Seronegative	403 (43.1)	20 (33.9)	1.00 (reference)	306 (44.2)	117 (38.9)	1.00 (reference)	301 (44.5)	107 (38.9)	1.00 (reference)	15 (36.6)	1.00 (reference)
Seropositive	531 (56.9)	39 (66.1)	1.45 (0.84-2.57)	386 (55.8)	184 (61.1)	1.27 (0.96-1.68)	376 (55.5)	168 (61.1)	1.27 (0.96-1.70)	26 (63.4)	1.40 (0.73-2.76)
							P-trend = .072				
Any HPyV (n = 4)											
Seronegative	13 (2.0)	0 (0)	1.00 (reference)	5 (2.8)	8 (1.0)	1.00 (reference)	5 (3.0)	8 (1.6)	1.00 (reference)	0 (0)	1.00 (reference)
Seropositive	633 (98.0)	348 (100)	NA	176 (97.2)	805 (99.0)	3.25 (0.96-10.06)	159 (97.0)	496 (98.4)	2.21 (0.65-6.82)	326 (100)	NA
							P-trend = .003				
MCPyV											

TABLE 2 (Continued)

	Association between seropositivity and viral DNA positivity										
	In EBH			in SSW			across EBH and SSW				
							DNA− for both	DNA+ for only 1		DNA+ for both[a]	
Seropositivity	DNA− n (%)	DNA+ n (%)	OR (95% CI)[b]	DNA− n (%)	DNA+ n (%)	OR (95% CI)	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Seronegative	169 (24.6)	20 (6.5)	1.00 (reference)	77 (30.3)	112 (15.1)	1.00 (reference)	70 (30.2)	106 (22.2)	1.00 (reference)	13 (4.6)	1.00 (reference)
Seropositive	518 (75.4)	287 (93.5)	4.61 (2.90-7.71)	177 (69.7)	628 (84.9)	2.41 (1.72-3.36)	162 (69.8)	371 (77.8)	1.50 (1.05-2.14)	272 (95.4)	9.02 (4.98-17.58)
							P-trend < .001				

[a]Prevalence of any β-HPV, any β_1-HPV and any β_2-HPV, any gamma-HPV and any HPyV across multiple markers were restricted to the same type.
[b]Odds ratios and 95% confidence intervals were estimated using logistic regression analysis, adjusted for age and sex.
[c]*P*-trend was calculated using ordinal logistic regression, adjusted for age and sex.

observed between higher values of UVR indicator and higher levels of self-reported UVR exposure in the past week, based on a UV dosimeter-validated questionnaire.[37]

Analyses of associations between the UVR indicator and HPV/HPyV infection were restricted to participants who had spectrophotometer data and also HPV/HPyV specific β-globin amplified in both EBH and SSW assays corresponding to beta-HPV (n = 966), gamma-HPV (n = 980) and HPyV (n = 980), respectively. Within each HPV/HPyV specific subgroups, participants who had a constitutive L* reading lower or equal to the median were considered to have darker natural skin tone, while participants who had a constitutive L* reading higher than the median were considered to have lighter natural skin tone. Finally, the UVR indicator was categorized into three levels based on tertile values within each HPV/HPyV specific subgroup.

2.3 | Statistical methods

The prevalence of any viral infection including beta-HPV, gamma-HPV or HPyV was defined as the proportion of participants whose sample tested positive for at least one virus type and calculated separately for serum antibodies and viral DNA in EBH and SSW. The prevalence of any viral infection across EBH and SSW was defined as the proportion of participants whose samples were positive for any of the same viral type(s) in both EBH and SSW. Analyses were conducted for all beta-HPV types combined and separately for beta species 1 and 2. Three virus types were selected a priori for type-specific analysis, including HPV 8 and 38, as transgenic mice models have shown these two types to interact with UVR exposure in the development of KC,[44,45] and MCPyV, which was shown to be associated with squamous cell carcinoma in at least one study.[46]

Wilcoxon rank-sum test and Barnard's test were used to test for differences in cutaneous viral infection by age, sex and race. Seroprevalence and viral DNA prevalence measured in EBH and SSW were calculated for each of the HPV/HPyV types. Associations between seropositivity and viral DNA positivity in EBH and SSW were estimated using logistic regression, adjusted for age and sex. Additionally, the associations between seropositivity and DNA-positivity across both EBH and SSW were measured by calculating *P* values for trend using ordinal logistic regression, with the ordinal response variable coded as 0 (no DNA positivity), 1 (positive in only 1 DNA marker) and 2 (positive in both DNA markers).

The mean, standard deviation, median and range were described for the UVR indicator and the inner arm brightness reading. A histogram plot was used to visualize the distribution of the UVR indicator and the Kolmogorov-Smirnov normality test was used to examine whether the distribution was normal. Logistic regression was used to estimate the association between tertiles of the UVR indicator and HPV/HPyV measured in each viral marker. *P* values for trend were calculated by creating a dependent variable set to the median value of the spectrophotometer-based measurement of the tertile in which the participant's measurement fell. To understand the potential effect modification of natural skin color on the association between the

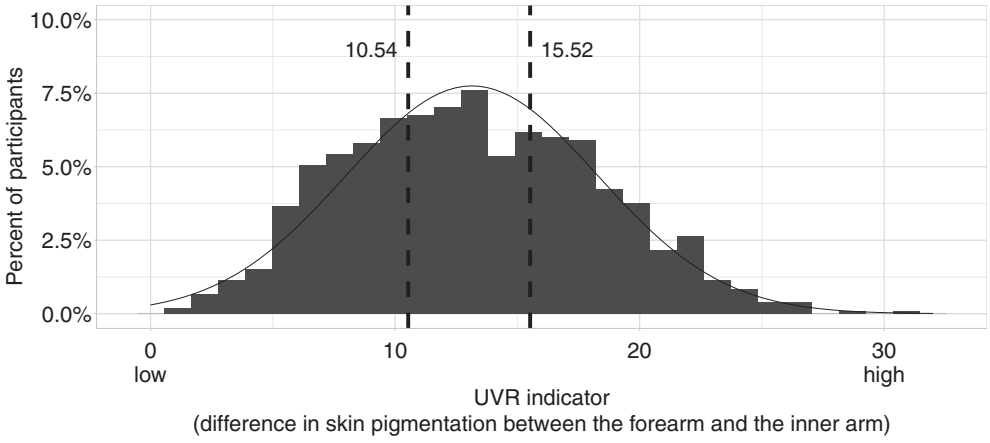

FIGURE 1 Distribution of the indicator of recent UVR exposure (UVR indicator) among participants included in analysis of the association between beta-HPV and the UVR indicator (n = 966). The distribution of UVR indicator, among participants included in beta-HPV analysis, resembles a bell-shaped normal distribution

UVR indicator and cutaneous viral infection, this analysis was further stratified by lighter- or darker-than median natural skin tone (constitutive L* reading). In a separate analysis, an interaction term was included in the logistic model to understand the interaction effect between the UVR indicator and natural skin tone on cutaneous viral infection.

A sensitivity analysis was conducted to determine whether exclusion of the participants with history of KC or organ transplantation changed the main findings. All statistical analyses were performed using R, Version 3.5 (R Foundation for Statistical Computing, Vienna, Austria) and two-sided *P* values < .05 were considered significant.

3 | RESULTS

As described in Table 1, beta-HPV seroprevalence (74.3%) and prevalence of viral DNA in SSW (86.6%) were greater than prevalence of viral DNA in EBH (50.6%), with similar patterns observed for gamma-HPV and HPyV. Type-specific HPV/HPyV prevalence measured by each of the three markers are presented in Table S1. Age was positively associated with the presence of any beta-HPV DNA in EBH and SSW but not with any gamma-HPV or HPyV (Table 1). The only association between age and seropositivity was observed for HPyV. Inconsistent associations were observed for sex, with females being more likely than males to be positive for beta-HPV species 1 in SSW and less likely to be seropositive for beta-HPV species 1. In contrast, males were more likely to be positive for HPyV DNA in both EBH and SSW. No significant associations were observed between race and viral infection across the three viral markers, although the power to detect such findings was low, as only 35 participants were non-White.

Associations between seropositivity and viral DNA prevalence are presented in Table 2. Any beta-HPV seropositivity was associated with viral DNA-positivity in EBH (OR = 1.40, 95% CI = 1.04-1.88) and SSW (OR = 1.86, 95% CI = 1.25-2.74), whereas no associations were observed for any gamma-HPV seropositivity. The association between beta-HPV seropositivity and DNA positivity was particularly pronounced when both EBH and SSW showed positivity (OR = 2.19, 95% CI = 1.39-3.42), with a positive significant trend observed between seropositivity and increasing number (0, 1 or 2) of DNA-based markers positive for beta-HPV (Table 2). A similar trend was observed for beta-HPV species 1 and 2, although seropositivity for beta species 2 was not significantly associated with EBH viral DNA positivity or SSW viral DNA positivity individually. Results for HPV 8 and 38 recapitulated the associations observed for their respective species, with seroprevalence being significantly associated with DNA prevalence for HPV 8 in species 1, while the association for HPV 38 in species 2 was not significant. MCPyV seropositivity was associated with viral DNA positivity in EBH (OR = 4.61, 95% CI = 2.90-7.71) and SSW (OR = 2.41, 95% CI = 1.72-3.36), with a significant positive trend observed between MCPyV seropositivity and increasing number of DNA markers positive for MCPyV.

The UVR indicator and inner arm brightness (L*) reading among each HPV/HPyV specific subgroup are described in Table S2. Among the subgroup of participants included in the analysis of the association between the UVR indicator and beta-HPV, the mean, standard deviation and median of the UVR indicator were 13.14, 5.15 and 12.91 respectively, with range of 1.34-30.85. Among the same subgroup of participants, the corresponding inner arm brightness (L*) reading had a mean of 67.92, standard deviation of 4.2, median of 68.44, and range of 36.01-77.92. Similar summary statistics were observed among subgroups of participants included in the gamma-HPV and HPyV analyses (Table S2). The distribution of the UVR indicator among participants included in beta-HPV analysis resembled a bell-shaped normal distribution, with a nonsignificant Kolmogorov-Smirnov normality test *P* value of .14 (Figure 1). A similar distribution was observed for the UVR indicator among participants included in gamma-HPV and HPyV analyses, respectively (data not shown).

The associations between tertiles of the UVR indicator and HPV/HPyV infection measured by each viral marker are presented in Table 3. After adjusting for age and sex, the highest tertile of the UVR indicator was positively associated with any beta-HPV seropositivity (OR = 1.81, 95% CI = 1.16-2.85) compared to the lowest tertile, with a significant trend observed for increasing levels of the UVR indicator and any beta-HPV seropositivity (*P*-trend = .009). Participants in the highest tertile of the UVR indicator were also more likely to have

TABLE 3 Association between tertiles of the indicator of recent UVR exposure (UVR indicator) and HPV/HPyV infection measured by serum antibodies and viral DNA in skin swabs (SSW) and eyebrow hairs (EBH), among VIRUSCAN Study participants who screened negative for keratinocyte cancer at study enrollment

	Association between the UVR indicator and HPV/HPyV infection measured by three markers					
	seropositivity		viral DNA positivity in EBH		viral DNA positivity in SSW	
UVR indicator[a]	n (%)	OR (95% CI)[b]	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Any β-HPV (n = 17)						
T1-low (n = 322)	222 (68.9)	1.00 (reference)	157 (48.8)	1.00 (reference)	271 (84.2)	1.00 (reference)
T2 (n = 322)	239 (74.2)	1.30 (0.91-1.87)	162 (50.3)	1.24 (0.90-1.72)	278 (86.3)	1.39 (0.88-2.21)
T3-high (n = 322)	257 (79.8)	1.81 (1.16-2.85)	166 (51.6)	1.57 (1.06-2.33)	288 (89.4)	2.22 (1.25-3.96)
P-trend[c]	.009		.025		.007	
Any β_1-HPV (n = 7)						
T1-low (n = 322)	185 (57.5)	1.00 (reference)	104 (32.3)	1.00 (reference)	226 (70.2)	1.00 (reference)
T2 (n = 322)	193 (59.9)	1.10 (0.80-1.53)	106 (32.9)	1.17 (0.83-1.65)	221 (68.6)	1.13 (0.79-1.61)
T3-high (n = 322)	222 (68.9)	1.59 (1.06-2.38)	100 (31.1)	1.24 (0.82-1.89)	229 (71.1)	1.64 (1.07-2.52)
P-trend	.028		.299		.024	
Any β_2-HPV (n = 7)						
T1-low (n = 322)	159 (49.4)	1.00 (reference)	108 (33.5)	1.00 (reference)	220 (68.3)	1.00 (reference)
T2 (n = 322)	169 (52.5)	1.09 (0.79-1.50)	111 (34.5)	1.22 (0.87-1.73)	233 (72.4)	1.33 (0.94-1.91)
T3-high (n = 322)	183 (56.8)	1.21 (0.82-1.79)	124 (38.5)	1.79 (1.19-2.72)	254 (78.9)	2.13 (1.37-3.33)
P-trend	.326		.006		<.001	
HPV 8 (β_1)						
T1-low (n = 322)	88 (27.3)	1.00 (reference)	19 (5.9)	1.00 (reference)	61 (18.9)	1.00 (reference)
T2 (n = 322)	100 (31.1)	1.17 (0.82-1.66)	26 (8.1)	1.64 (0.87-3.11)	61 (18.9)	1.12 (0.74-1.69)
T3-high (n = 322)	101 (31.4)	1.13 (0.74-1.73)	27 (8.4)	2.16 (1.02-4.65)	77 (23.9)	1.76 (1.09-2.86)
P-trend	.548		.044		.024	
HPV 38 (β_2)						
T1-low (n = 322)	52 (16.1)	1.00 (reference)	54 (16.8)	1.00 (reference)	133 (41.3)	1.00 (reference)
T2 (n = 322)	61 (18.9)	1.14 (0.75-1.75)	55 (17.1)	1.20 (0.78-1.84)	154 (47.8)	1.48 (1.07-2.05)
T3-high (n = 322)	59 (18.3)	1.02 (0.61-1.70)	71 (22)	2.07 (1.25-3.46)	167 (51.9)	2.07 (1.40-3.08)
P-trend	.93		.005		<.001	
Any γ-HPV (n = 7)						
T1-low (n = 327)	180 (55)	1.00 (reference)	15 (4.6)	1.00 (reference)	96 (29.4)	1.00 (reference)
T2 (n = 327)	180 (55)	0.89 (0.64-1.22)	19 (5.8)	1.20 (0.58-2.50)	104 (31.8)	1.16 (0.82-1.64)
T3-high (n = 326)	199 (61)	0.94 (0.63-1.38)	24 (7.4)	1.34 (0.60-3.08)	98 (30.1)	1.10 (0.73-1.68)
P-trend	.717		.484		.627	
Any HPyV (n = 4)						
T1-low (n = 330)	327 (99.1)	1.00 (reference)	102 (30.9)	1.00 (reference)	260 (78.8)	1.00 (reference)
T2 (n = 323)	318 (98.5)	0.89 (0.17-3.98)	109 (33.7)	1.02 (0.72-1.44)	258 (79.9)	0.96 (0.65-1.42)
T3-high (n = 327)	322 (98.5)	1.38 (0.22-7.10)	130 (39.8)	1.09 (0.73-1.64)	283 (86.5)	1.23 (0.74-2.06)
P-trend	.645		.661		.487	
MCPyV						
T1-low (n = 330)	257 (77.9)	1.00 (reference)	93 (28.2)	1.00 (reference)	239 (72.4)	1.00 (reference)
T2 (n = 323)	260 (80.5)	1.15 (0.78-1.71)	95 (29.4)	0.97 (0.68-1.38)	234 (72.4)	0.97 (0.68-1.38)
T3-high (n = 327)	276 (84.4)	1.44 (0.88-2.36)	112 (34.3)	1.04 (0.69-1.58)	256 (78.3)	1.23 (0.79-1.93)
P-trend	.149		.835		.382	

[a]The UVR indicator was measured by calculating the difference in skin pigmentation obtained from the forearm and the inner arm, and categorized into tertiles using the following cut-offs for each viral group: beta-HPV 1.34-10.54, 10.55-15.52, 15.53-30.85, gamma HPV 1.64-10.59, 10.60-15.53, 15.54-30.85 and HPyV 1.34-10.55, 10.56-15.51, 15.52-30.85.

[b]Odds ratios and 95% confidence intervals were estimated using logistic regression, adjusted for age and sex.

[c]*P* values for trend were estimated using logistic regression, using the median UVR indicator of each tertile as the predicting variable.

beta-HPV DNA in EBH (OR = 1.57, 95% CI = 1.06-2.33) and SSW (OR = 2.22, 95% CI = 1.25-3.96) compared to those in the lowest tertile of the UVR indicator with significant trends observed for EBH (*P*-trend = .025) and SSW (*P*-trend = .007), respectively. The main effect for the UVR indicator and beta-HPV infection was virtually unchanged after adjusting for season at enrollment. Similar results were observed for beta-HPV species 1 and species 2 in SSW, and beta-HPV species 2 in EBH. At the type level, the UVR indicator was positively associated with HPV8 and HPV38 DNA positivity in EBH and SSW, whereas no associations were observed for seropositivity. The association between the UVR indicator and cutaneous viral infection did not differ by lighter vs darker skin tone (Table S3). In contrast to cutaneous HPV, no associations were observed between the UVR indicator and any of the three markers of gamma-HPV or HPyV infection. Similar associations were observed after excluding 332 participants with history of KC or organ transplantation in the sensitivity analysis.

4 | DISCUSSION

This is the first epidemiological study to examine associations between HPV/HPyV seropositivity and viral DNA measured in two DNA-based markers within the same individuals. Overall, seropositivity to any beta-HPV type was associated with the presence of beta-HPV DNA in EBH and SSW, individually and combined. Similar associations were observed for MCPyV but not for gamma-HPV. The association between HPV seropositivity and DNA positivity in EBH and SSW observed in our study is consistent with two[20,21] previous studies; whereas no previous studies have assessed this association with gamma-HPV. The positive association between MCPyV seropositivity and the DNA-based markers lends support to findings from previous smaller studies,[18,19] however, our study is the first to examine this association using two DNA-based markers and multiple HPyV types. Differences in the associations between seroprevalence and DNA-based markers of infection could suggest that beta-HPV has different immunogenic properties compared to gamma-HPV and HPyV. Differences in seroconversion rates have been observed across other virus genotypes, such as hepatitis B[47] and alpha-HPV.[48] Timing of infections could also account for variations in the association between seroprevalence and viral DNA prevalence, as seroprevalence is a marker of past exposure and antibodies may wane with age, whereas viral DNA prevalence is a measure of recent/current infection.

We observed a positive association between the indicator of recent UVR exposure (referred to here as recent UVR exposure) and beta-HPV MCPyV infection as measured by serology and viral DNA in EBH and SSW. Our findings are consistent with some[24-28,49] but not all[26,29-32] previous studies of UVR and HPV infection, three of which focused on organ transplant recipients.[25,29,31] Past investigations of the associations between UVR exposure and cutaneous HPV infection and/or KC have incorporated self-reported measurements of individual UVR exposures, such as history of time spent in the sun during different life phases, residential history and history of sunburns.[2,4,6,7,11,12] These measures of UVR exposure may be particularly relevant to HPV/HPyV serology, as the presence of antibodies marks infection at any time in the past. However, HPV/HPyV DNA in EBH and SSW are more contemporaneous measures of infection, possibly influenced by most recent UVR exposures. Thus, the use of the spectrophotometer in the current study to measure skin pigmentation as an indicator of UVR exposure as recently as the past week is particularly useful for understanding the association with current HPV/HPyV infection. The lack of association between recent UVR exposure and MCPyV juxtaposed with the positive associations between recent UVR exposure and beta-HPV in the same study population suggests true biological differences in the effect of recent UVR exposure on the acquisition and/or persistence of these cutaneous viral infections. Of note, cumulative vs recent UVR exposures are thought to have distinct contributions to KC etiology.[50] Therefore, future studies should incorporate measurements of both cumulative UVR exposure across the lifespan, as well as very recent UVR exposure, to unravel the complex interplay between UVR and cutaneous viral infections in the context of cancer etiology.

Recent UVR exposure may be associated with beta-HPV infection through an immunosuppressive pathway,[51] although the exact immune mechanism involved is not well understood. Previously, we have shown that recent UVR exposure was positively associated with certain skin-homing T regulatory cells, with associations being stronger among VIRUSCAN participants with lighter skin tone.[35] However, our current findings of recent UVR exposure and beta-HPV infection did not differ by skin tone. Furthermore, we have shown that these same T regulatory cells were not associated with beta-HPV DNA positivity[36] among VIRUSCAN participants. Taken together, our work may suggest that an alternate immune mechanism, other than T regulatory cells, is responsible for the association between recent UVR exposure and beta-HPV infection.

The cross-sectional nature of the analysis limits our ability to establish the temporal relationship between UVR exposure and HPV/HPyV infection. However, prospective follow-up of VIRUSCAN study participants will help enable the assessment of associations between baseline UVR exposure, cutaneous viral infection and subsequent skin cancer risk. Given that VIRUSCAN participants are ages 60 and over and exhibited a higher skin cancer risk profile than the general population,[37] our study findings may be limited in their generalizability. However, individuals at higher-than-average risk for KC are the ones who would benefit from novel KC prevention strategies.

In conclusion, significant positive associations were found between beta-HPV and MCPyV seropositivity and viral DNA positivity in EBH and SSW. Recent UVR exposure was positively associated with beta-HPV infection measured by all three viral markers. Future analyses will assess the temporal association between UVR exposure and viral DNA in EBH and SSW using repeated measures and examine the potential interaction between UVR exposure and cutaneous HPV infection in relation to subsequent KC development.

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CONFLICT OF INTEREST

Dr Anna Giuliano reports grants from Merck & CO, Inc, personal fees (Advisory Board Member) from Merck & CO, Inc, during the conduct of the study. Dr Vernon Sondak is a compensated consultant for Merck. The remaining authors state no conflict of interest.

DATA AVAILABILITY STATEMENT

Datasets related to this article are available upon request from the corresponding author. All requests will require a proposal, outlining specific research questions, methods and timelines for completion. Formal approval will be granted by the corresponding author based on the topic and ability to obtain appropriate ethics committee approval. Applicants will be required to complete a Data Use Agreement form prior to receiving any data.

ETHICS STATEMENT

The study was approved by the USF Institutional Review Board and patients provided written informed consent.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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