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Research Article

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Oral Alpha, Beta, and Gamma HPV Types and Risk of Incident Esophageal Cancer

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Abstract

Background: Several studies have examined association between human papillomaviruses (HPV) and esophageal cancer, but results have been inconsistent. This is the first prospective study to investigate associations between α, β and γ HPV detection in the oral cavity and risk of esophageal cancer.

Methods: We conducted a nested case–control study among 96,650 cancer-free participants in the American Cancer Society Cancer Prevention Cohort and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Incident esophageal cancer cases ($n = 125$) were identified during an average 3.9 years of follow-up. Three controls per case ($n = 372$) were selected and matched on age, sex, race/ethnicity, and time since mouthwash collection. α, β, and γ HPV DNA in oral samples were detected using a next-generation sequencing assay. Conditional logistic regression models were used to estimate OR and 95% confidence intervals (CIs), adjusting for smoking and alcohol consumption. Statistical significance was evaluated using permutation test.

Results: Prevalence of oral α, β, and γ HPV was 18.4%, 64.8%, and 42.4% in cases and 14.3%, 55.1%, and 33.6% in controls, respectively. Oral HPV16 detection was not associated with esophageal cancer (OR = 0.54, 95% CI, 0.1–4.84) and none of the esophageal squamous cell carcinoma cases ($n = 28$) were HPV16 positive. Some oral HPV types were more common in cases than controls; however, none of the associations were statistically significant.

Conclusions: Although HPVs in the oral cavity are very common, this study showed no evidence of association between oral HPVs and esophageal cancer.

Impact: Oral HPVs may not contribute to risk of esophageal cancer. *Cancer Epidemiol Biomarkers Prev; 27(10); 1168–75. ©2018 AACR.*

Introduction

Esophageal cancer is the eighth most commonly diagnosed cancer worldwide and is the sixth most common cause of cancer deaths (1–4). These figures include both adenocarcinoma and squamous cell carcinoma (ESCC), the two major histologic types of esophageal cancer. There is a large geographic variation in incidence and mortality rates of esophageal cancer, with the highest incidence rates reported in Iran, China, India, and South Africa (1, 2, 4). ESCC is the most common type in these regions; however, in the last decade there has been an increase in the incidence rates of adenocarcinoma. In contrast, in the Western world including the United States, incidence and mortality rates of esophageal cancer are much lower, with adenocarcinoma being the most predominant type (5–7). Nevertheless, the 5-year survival rate of esophageal cancer in the United States is low and has remained fairly constant over the past decade (5).

The main risk factors for esophageal cancer include increasing age, male sex, cigarette smoking, and alcohol consumption especially for ESCC, whereas gender, cigarette smoking, gastroesophageal reflux disease, and obesity are risk factors for adenocarcinoma (7–13). Infection with oncogenic HPV as a contributor to ESCC was hypothesized over three decades ago (14). However, the International Agency for Research on Cancer (IARC) in a recent review concluded that there was inadequate evidence for HPV carcinogenicity in association with ESCC (15). Several tissue-based studies, which have examined detection of HPV16 and HPV18 in esophageal cancer versus adjacent normal tissue, have yielded conflicting results (16–20). The majority of studies originating from China reported positive associations, whereas studies from Western countries reported no association (16–21). Serologic case–control studies also provide conflicting evidence, with a meta-analysis reporting an OR of 1.89 (95% confidence interval [CI], 1.09–3.29) for HPV16 E6 antibodies and ESCC, but no association for E7 antibodies (22). In contrast, a large cohort study demonstrated no association between HPV16 E6 or E7 antibody seropositivity and risk of esophageal cancer (23).

To date, there has been no prospective study of oral HPV and risk of incident esophageal cancer. Moreover, recent data indicate that the oral cavity contains not only α HPVs, including HPV16, but also a wide spectrum of other HPVs, namely β and γ HPV types (24, 25). We recently reported that in addition to HPV16, detection of several β and γ HPV species and types in the oral cavity were

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positively associated with risk of head and neck cancer independent of HPV16 (25). Therefore, we examined the association of α, β, and γ HPV DNA detected in the oral cavity with subsequent risk of esophageal cancer, as well as with the risks of adenocarcinoma and ESCC types in a nested case–control study within two large prospective cohorts.

Materials and Methods

Study cohorts and data collection

We conducted nested case–control studies amongst participants who provided mouthwash samples in two large prospective cohorts: the American Cancer Society Cancer Prevention Study-II Nutrition Cohort (CPS-II-NC; ref. 26) and the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial (27). The CPS-II-NC enrolled 184,192 men and women ages 50 to 79 years old residing in 21 U.S. states between 1992 and 1993; 53% were women and 97% were Caucasian (26). The PLCO trial enrolled 154,910 men and women ages 55 to 74 years old from 1993 through 2001 at 10 U.S. centers. Participants had no history of prostate, lung, colorectal, or ovarian cancers at enrollment; 50% were women and 86% were Caucasian (27).

Participants in both cohorts completed self-administered baseline questionnaires, which collected information on demographics and social characteristics, previous cancer diagnoses, current and lifetime smoking history, and alcohol consumption. Follow-up questionnaires were sent every two years to CPS-II-NC and annually to PLCO cohort members to update information on lifestyle exposures, health status, and to ascertain newly diagnosed cancers. Mouthwash samples were collected primarily for genomic DNA from 70,004 CPS-II-NC participants between 2001 and 2002 (26), and from 55,866 participants in the PLCO control arm between 1998 and 2005 (27, 28), who did not provide a blood sample.

Identification of incident cases of esophageal cancer and selection of controls

We designed parallel nested case–control studies among participants who provided informed consent, baseline questionnaire data, and a mouthwash sample. Of the 70,004 CPS-II-NC participants who provided a mouthwash sample, we excluded 16,664 who had a previous cancer diagnosis, 158 whose oral rinse specimens were inadequate, and two whose gender data were missing. Among the remaining 53,180 participants in the at-risk cohort, 51 were diagnosed with a primary incident esophageal cancer between the time of oral sample collection and the end of follow-up (6/30/2009). Of the 55,866 PLCO control arm participants who provided a mouthwash sample, we excluded 5,526 who had a previous cancer diagnosis, and 6,870 whose oral rinse specimens were exhausted or unavailable. Among the remaining 43,470 participants in the at-risk cohort, 74 were diagnosed with a primary incident esophageal cancer between the time of oral sample collection and the end of follow-up (July 31, 2011). Thus, a total of 125 incident cases of esophageal cancer with available mouthwash samples were identified in both cohorts over an average 3.9 years of follow-up.

Three controls were selected for each case from the at-risk cohorts who were alive at the diagnosis date of the case and who had no prior history of cancer at that time. Controls were individually matched to cases on sex, race/ethnicity, date of birth (±6 months), and date of oral sample collection (±30 days for CPS-II-NC, and ±3 months for PLCO trial). A total of 372 controls with available mouthwash samples were used for the analysis (three cases had only two controls in their matched sets).

The current study was reviewed and deemed exempt by the institutional review board (IRB) of the Albert Einstein College of Medicine (Einstein). The original cohort studies received full IRB approval from both the American Cancer Society and the NCI, and written informed consent was obtained from all study participants.

Molecular detection of oral HPV DNA

All HPV testing was performed at Einstein with the laboratory personnel blinded to case–control status of the mouthwash samples as described previously (25). Total DNA was purified from exfoliated oral cavity cells obtained from a Scope mouthwash rinse specimen (24, 25). As described previously (25) HPV DNA detection was performed using three different platforms: (1) The MY09/11 L1-targeted degenerate primer PCR system using AmpliTaq Gold DNA Polymerase (Thermo Fisher Scientific), which preferentially detects α-HPV types (29); (2) a real time (RT)-PCR assay for HPV16 and (3) a multiplexed next-generation sequencing (NGS) method developed to detect and type the diverse and large number of α, β, and γ HPVs present in the oral cavity (30). This method consisted of three separate PCR amplification assays that targeted primer-binding sites within the L1 (NG-S and NG-F assays) and E1 ORFs (NG-E1) (30). Each DNA sample was amplified using sample-specific barcoded primers. Successful amplification of predicted fragment sizes was verified by gel electrophoresis and PCR products were pooled and sequenced on an Illumina HiSeq 2000/2500 (Illumina Inc.) at the Epigenomics Shared Facility at Einstein, using 150-bp paired-end reads. The reads were demultiplexed, filtered for quality, and blasted against a PV reference database (30). We evaluated the sensitivity of the NG-S assay that was designed to specifically detect α-HPVs by serial dilution of an HPV16 plasmid and were able to detect this type at an input of plasmid DNA as low as 10 copies/μL. We evaluated specificity by comparing the NGS assays with MY09/FAP amplicon typing using oligonucleotide hybridization and the overall concordance rate and kappa value was 91.9% and 0.749, respectively.

Definition of HPV type positivity

Oral HPV type positivity was defined as described previously (25). Briefly, we considered a sample being positive for HPV16 if it scored positive in two of three assays. For other α HPV types, we used both the MY09/11 PCR data and the NGS results, whereas for oral β and γ HPV types we relied on results of the NGS assays. Quality control analysis was carried out in 10% randomly selected oral samples for repeat testing; the agreement of the prevalence of HPV types between the two repeats was excellent (kappa $\geq$ 0.90).

Statistical analyses

We examined association of α, β and γ HPV with incident esophageal cancer using conditional logistic regression models (CLR) for matched risk-sets to estimate OR and 95% CI (31, 32). For α HPVs, we examined associations for the following exposures: HPV16; other high-risk (HR) oncogenic HPV types (15), which included HPV18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59; other nonHR α HPV types; and any α HPV type. For β and γ HPV species

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and types, we examined associations of any β or of any γ HPV type, different β or γ species groups, and specific β or γ HPV types with risk of esophageal cancer. The associations between the above HPV exposures and risk of esophageal cancer were adjusted for study cohort (CPS-II-NC vs. PLCO), smoking status (current or former smokers vs. never smokers), pack-years of smoking, and alcohol consumption (drinks/week). For the few participants with missing information on pack-years of smoking (3 cases and 9 controls from both cohorts) or alcohol consumption (22 cases and 46 controls from both cohorts), we imputed the missing data using the multiple imputation (MI) method in R-package (33). Because age, sex, race/ethnicity, and time since oral rinse collection were the matching variables, these were not included in CLR models.

A permutation procedure was used to account for multiple comparisons of several HPV exposures and esophageal cancer (34, 35). For each replicate of 10,000 cycles, the matched pairs were permuted by shuffling the case–control status. For each permuted dataset, the CLR models were fit for HPV exposures and the minimum *P* values were kept. This provided an empirical distribution of *P* values under the null hypothesis of no association. The permutation *P* value for an HPV exposure was obtained by comparing their observed *P* values to this empirical distribution. Permutation *P* values can be interpreted as the probability of observing a *P* value less than or equal to what was observed under the null hypothesis of no associations of any of the HPV exposures and risk of esophageal cancer. After this procedure, an HPV exposure was considered to be statistically significantly associated with risk of esophageal cancer if the permuted *P* value was <0.05 (two sided).

We also examined the association of α, β and γ HPV with esophageal cancer by histologic type: ESCC and adenocarcinoma. To account for latency as well as potential for subclinical/undiagnosed cancer, we conducted a sensitivity analysis by excluding incident esophageal cancer cases ($n = 35$) that were identified within the first two years of follow-up in both cohorts and their respectively matched controls ($n = 102$). Finally, we also examined the association between coinfection by multiple types of oral HPV and risk of esophageal cancer. All these statistical models were adjusted for the same variables as the models investigating the overall risk of esophageal cancer. All statistical analyses were carried out in STATA version 14 (Stata Corporation College Station).

Results

Demographic and lifestyle characteristics of incident cases of esophageal cancer and their matched controls are shown in Table 1. In the CPS-II-NC, both cases and controls were on an average 6 years older in comparison with the PLCO cohort. The majority of cases and controls in both cohorts were Caucasian males. Cases were more likely to be current smokers in comparison to controls; however, they were similar with respect to drinking habits. There were no major differences with regard to body mass index (BMI), education, or marital status between the two groups (Table 1). With regard to tumor histology, the majority (64%) of esophageal cancers in both cohorts were adenocarcinoma ($n = 80$), 22.4% ($n = 28$) were ESCC and 13.6% were other histologic types. The distribution of histologic types was similar between the two cohorts.

In both cohorts, the prevalence of any oral HPV was 75.5% in cases versus. 69.4% in controls ($P = 0.26$). The prevalence of any oral α, β, and γ HPV was 18.4%, 64.8%, and 42.4% among the cases and 14.3%, 55.1%, and 33.6% among the controls, respectively.

Associations of HPV16 and other α HPVs with risk of incident esophageal cancer

Among controls from both cohorts, the prevalence of oral HPV16, other high-risk (HR) oncogenic HPVs, and nonHR α HPVs was 1.6%, 4.9%, and 9.7%, respectively (Table 2). Detection of HPV16 DNA in the oral samples was not associated with risk of esophageal cancer (OR = 0.54, 95% CI, 0.10–4.84). There were also no associations of other HR-oncogenic HPVs after excluding HPV16, as well as nonHR α HPVs with risk of esophageal cancer (Table 2).

In the stratified analyses by tumor histology, no oral HPV16 DNA was detected among the 28 cases of ESCC, whereas, one of 83 (1.2%) matched controls was HPV16 positive (Table 2). Interestingly, oral DNA detection of other HR-HPVs (after excluding HPV16) was associated with a higher risk of ESCC (OR = 10.5; 95% CI, 1.01–108.5); however, this result is based on only three HPV positive cases and one HPV positive control and was not statistically significant after adjusting for multiple comparisons. There were no associations of other nonHR HPV types with risk of ESCC. Furthermore, there were no associations of oral HPV16, other HR-oncogenic HPVs, or nonHR α HPV with risk of adenocarcinoma of the esophagus (Table 2).

Association of β and γHPVs with risk of incident esophageal cancer

Among controls from both cohorts, the prevalence of any β HPV was 55.1% and any γ HPV was 33.6%. As shown in Table 3, there was a borderline statistically significant association between any oral β HPV (OR = 1.57; 95% CI, 1.00–2.47) and risk of esophageal cancer in the multivariate-adjusted model. However, there was no association of specific β HPV species or types and esophageal cancer. Similarly, no associations were observed for γ HPV species (Table 3). After accounting for multiple comparisons, there was no association between any β or γ HPV and esophageal cancer (all permutated *P* values were >0.05).

We also investigated the association of β and γ HPV with risk of histologic type of esophageal cancer (Table 4). Neither oral β, nor γ HPV species nor types were associated with risk of ESCC (Table 4A). In contrast, oral detection of any β HPV (OR = 1.84; 95% CI, 1.05–3.23) and any β 1 HPV (OR = 1.74; 95% CI, 1.00–3.04) were associated with risk of adenocarcinoma of the esophagus (Table 4B), although results were no longer statistically significant after accounting for multiple comparisons (all permutated *P* values were >0.05). There were also no associations between γ HPV species and types and risk of esophageal adenocarcinoma (Table 4B).

To account for latency as well as potential for subclinical/undiagnosed cancer, we excluded incident esophageal cancer cases ($n = 35$) that were identified within the first two years of follow-up in both cohorts and their respectively matched controls ($n = 102$). We did not observe any association of oral α, β, or γ HPV types and esophageal cancer in this sensitivity analysis. There were also no statistically significant associations between oral HPV coinfection and risk of esophageal cancer.

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Table 1. Selected characteristics of incident cases of esophageal cancer and their matched controls in each cohort study

Characteristics	ACS CPS-II NC Cohort			PLCO Cohort		
Matching variables	**Cases** ***N*** **= 51**	**Controls** ***N*** **= 153**	***P***	**Cases** ***N*** **= 74**	**Controls** ***N*** **= 219**	***P***
Age at mouthwash collection; mean (SD)	71.4 (6.1)	71.4 (6.0)	0.97	65.2 (5.6)	65.3 (5.5)	0.82
Months from mouthwash collection to diagnosis (cases) or riskset (controls); mean (SD)	35.3 (24.5)	35.3 (24.3)	1.00	52.6 (30.1)	53.3 (29.9)	0.85
Gender; *n* %			1.00			0.97
Female	8 (15.7)	24 (15.7)		15 (20.3)	44 (20.1)	
Male	43 (84.3)	129 (84.3)		59 (79.7)	175 (79.9)	
Race/ethnicity; *n* %			1.00			0.99
Caucasian	50 (98.0)	150 (98.0)		67 (90.5)	198 (90.4)	
African American/other	1 (2.0)	3 (2.0)		7 (9.5)	21 (9.6)	
Unmatched variables						
BMI group (kg/m2); *n* (%)			0.38			0.89
<25	22 (43.1)	50 (32.7)		18 (24.3)	55 (25.1)	
25–29.9	18 (35.3)	63 (41.2)		37 (50.0)	113 (51.6)	
≥30	5 (9.8)	26 (17.0)		17 (23.0)	48 (21.9)	
Missing	6 (11.8)	14 (9.1)		2 (2.7)	3 (1.4)	
Smoking status; *n* (%)			0.01			0.01
Never	11 (21.6)	60 (39.2)		20 (27.0)	99 (45.2)	
Former	32 (62.7)	86 (56.2)		44 (59.5)	108 (49.3)	
Current	8 (15.7)	7 (4.6)		10 (13.5)	12 (5.5)	
Pack-years, former and current smokers; mean (SD)	31.5 (26.7)	31.9 (31.9)	0.94	54.0 (30.4)	36.2 (28.3)	0.0002
Alcohol consumption; *n* (%)						
None	16 (31.4)	55 (35.9)	0.55	23 (31.1)	73 (33.3)	0.72
Drinker	35 (68.6)	98 (64.1)		51 (68.9)	146 (66.7)	
Drinks/week; median (IQR)	5.7 (2.1–18.1)	3.4 (1.0–9.1)	0.10	5.8 (1.1–14.6)	2.4 (1.1–7.0)	0.13
Education; *n* %			0.94			0.41
<12 grade	3 (5.9)	12 (7.8)		2 (2.7)	17 (7.8)	
High school/vocational	15 (29.4)	39 (25.5)		30 (40.5)	77 (35.2)	
Some college	10 (19.6)	33 (21.5)		10 (13.5)	38 (17.4)	
College graduate	10 (19.6)	29 (19.0)		13 (17.6)	39 (17.8)	
Graduate degree	12 (23.5)	39 (25.5)		18 (24.3)	48 (21.9)	
Missing	1 (2.0)	1 (0.7)		1 (1.4)	—	
Marital status; *n* %			0.48			0.85
Married	40 (78.4)	113 (73.9)		60 (81.1)	175 (79.9)	
Separated/divorced	—	2 (1.3)		7 (9.5)	20 (9.1)	
Widowed	2 (3.9)	12 (7.8)		4 (5.4)	18 (8.2)	
Never married	—	—		2 (2.7)	6 (2.7)	
Missing	9 (17.6)	26 (17.0)		1 (1.4)	—	
Tumor histology; *n* (%)						
Adenocarcinoma	31 (60.8)	N/A		49 (66.2)	N/A	
Squamous cell carcinoma	12 (23.5)	N/A		16 (21.6)	N/A	
Other	8 (15.7)	N/A		9 (12.2)	N/A	

Abbreviations: IQR: interquartile range; SD: standard deviation.

Discussion

This is the first prospective study to examine the associations of molecularly detected α, β, and γ HPVs in the oral cavity with risks of esophageal cancer overall and by histologic type. Our results show that in general α, β, and γ HPV detected in oral samples were neither associated with risk of overall esophageal cancer nor with risks of ESCC or adenocarcinoma types. Although, oral high-risk α HPVs (excluding HPV16) were associated with a 10-fold higher risk of ESCC, the number of cases and controls with positive test results was very small, the 95% CI was wide and permutated *P* values were not significant. In addition, the lack of association of any HPV type with esophageal cancer after excluding cases and their corresponding matched controls ascertained in the first two years of follow-up, further supports the null findings.

Among sampled controls from both cohorts, oral prevalence of HPV16, other high-risk oncogenic HPVs, and any α HPV were 1.6%, 5.4%, and 14.3%, respectively, with HPV16 being the most common α HPV type in the oral cavity. These prevalences were similar to those observed in our recent study of oral HPVs and head and neck cancers using a different sample of controls from the same cohorts (25). In the NHANES cross-sectional data, Gillison and colleagues (36) also reported oral prevalence of HPV16, high-risk oncogenic HPVs, and any α HPVs of 1.0%, 3.7%, and 6.9% among 5,579 men and women aged 14 to 69 years in the US (2009–2010). In that study, the prevalence of any α HPV was 11% and 4% among participants ages 55–64 and 65–69 years, respectively, and was higher in men in comparison to women, which is consistent with our data. Finally, oral HPV16 prevalence in our controls was also similar to the HPV16 prevalence reported in the HPV Infection in Men study (37) as well as to the pooled HPV16 prevalence of 1.3% reported among 4,581 healthy individuals from 18 different studies (38).

Results of several tissue-based studies that examined the relationship between HPV16 and HPV18 DNA detection in esophageal cancer versus adjacent normal tissue, or tissues from individuals without cancer (controls) have been inconsistent (16–18, 20, 21). A number of studies from China have reported a positive association between oncogenic HPVs and esophageal cancer (17, 18). For example, Zhang and colleagues (17) in their

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Table 2. Associations of HPV16, high-risk (HR) oncogenic HPV, and other α HPV types with risks of esophageal cancer and histologic type

All esophageal cancers	**Cases ($n = 125$)**	**Controls ($n = 371$)[a]**	**Adjusted model[b]**		
α HPV type	***N* (%)**	***N* (%)**	**OR[a] (95% CI)**	***P***	**Permutated *P*[d]**
HPV16	1 (0.8)	6 (1.6)	0.54 (0.10–4.84)	0.58	>0.999
HR-HPVs[c] excluding HPV16	8 (6.4)	18 (4.9)	1.21 (0.49–2.99)	0.68	>0.999
NonHR HPV types	16 (12.8)	36 (9.7)	1.47 (0.77–2.81)	0.24	0.801
Any α HPV	23 (18.4)	53 (14.3)	1.39 (0.78–2.48)	0.26	0.823
ESCC					
α HPV type	**Cases ($n = 28$)**	**Controls ($n = 83$)**	**OR[a] (95% CI)**	***P***	**Permutated *P*[d]**
HPV16	0 (0)	1 (1.2)	—	—	>0.999
HR-HPVs[c] excluding HPV16	3 (10.7)	1 (1.2)	10.52 (1.01–108.5)	0.048	0.236
Non-HR HPV types	4 (14.3)	11 (13.3)	1.68 (0.41–6.91)	0.48	>0.999
Any α HPV	6 (21.4)	13 (15.7)	2.21 (0.61–8.05)	0.23	0.772
Esophageal cancer: adenocarcinoma					
α HPV type	**Cases ($n = 80$)**	**Controls ($n = 238$)**	**OR[a] (95% CI)**	***P***	**Permutated *P*[d]**
HPV16	1 (1.3)	5 (2.1)	0.60 (0.10–5.85)	0.66	>0.999
HR-HPVs[c] excluding HPV16	3 (3.8)	14 (5.9)	0.52 (0.14–1.96)	0.33	>0.999
NonHR HPV types	8 (10.0)	21 (8.8)	1.21 (0.51–2.86)	0.66	>0.999
Any α HPV	12 (15.0)	35 (14.6)	0.98 (0.46–2.08)	0.96	>0.999

[a]One control had missing α HPV exposure and thus was excluded from these analyses.
[b]OR and 95% CI were estimated from CLR models adjusted for smoking, alcohol consumption, and study cohort.
[c]High-risk (HR) oncogenic HPVs include HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59.
[d]Permutated *P* values were calculated to account for multiple comparisons (see Material and Methods).

meta-analysis of 10 studies, which included 1,442 esophageal cancer cases and 1,602 controls, reported a pooled OR of 6.36 (95% CI: 4.46–9.07) for HPV16 DNA detection in cancer versus adjacent normal tissue. In another meta-analysis of tissue DNA detection of HPV16 and HPV18 and risk of esophageal cancer, Wang and colleagues (18) reported a pooled OR = 1.62 (95% CI, 1.33–1.98). Methodologic issues of some of the studies included in these meta-analyses were that HPV16 DNA detection in paraffin-embedded tissues was examined in cancer versus. adjacent normal tissues in the same subject, and there was substantial heterogeneity with respect to geographic region, control group selection, and various HPV detection methods. In contrast similar studies that examined the relationship between HPV16 and other high-risk HPVs DNA detection in tissue with ESCC in Western countries reported modest or no association (16, 20, 21). A meta-analysis of serologic case–control studies reported an OR of 1.89 (95% CI, 1.09–3.29) between HPV16 E6 antibodies and risk of ESCC, but there was no association for E7 antibodies (22). However, a recent large prospective cohort study, where antibodies were measured in the blood before cancer, demonstrated no association between HPV16 E6 or E7 antibody seropositivity and risk of esophageal cancers (23).

Table 3. Associations of β and γ HPV species and types with risk of esophageal cancer

	Cases ($n = 125$)	**Controls ($n = 372$)**	**Adjusted model[a]**		
β HPV Species[b]	**N (%)**	**N (%)**	**OR[a] (95% CI)**	**P**	**Permutated *P*[c]**
Any β HPV	81 (64.8)	205 (55.1)	1.57 (1.00–2.47)	0.048	0.538
Any β 1 HPV	59 (47.2)	146 (39.3)	1.49 (0.96–2.31)	0.08	0.716
Any β 2 HPV	64 (51.2)	160 (43.0)	1.34 (0.88–2.05)	0.17	0.951
Any β 3 HPV	24 (19.2)	60 (16.1)	1.26 (0.73–2.18)	0.41	>0.999
Specific β HPV types[b]					
β 1 HPV5	22 (17.6)	42 (11.3)	1.75 (0.97–3.16)	0.07	0.655
β 1 HPV12	11 (8.8)	27 (7.3)	1.24 (0.58–2.65)	0.58	>0.999
β 1 HPV20	22 (17.6)	46 (12.4)	1.53 (0.87–2.70)	0.14	0.908
β 1 HPV36	15 (12.0)	33 (8.9)	1.20 (0.61–2.35)	0.60	>0.999
Clade of β 1 HPVs 5, 36, 47, & 143	26 (20.8)	59 (15.9)	1.34 (0.79–2.27)	0.28	0.995
β 1 HPV105	14 (11.2)	29 (7.8)	1.31 (0.65–2.63)	0.45	>0.999
β 1 HPV124	10 (8.0)	28 (7.5)	1.01 (0.46–2.21)	0.99	>0.999
β 2 HPV23	12 (9.6)	26 (7.0)	1.34 (0.64–2.81)	0.44	>0.999
β 2 HPV37	8 (6.4)	28 (7.5)	0.97 (0.42–2.27)	0.95	>0.999
β 2 HPV38	24 (19.2)	57 (15.3)	1.28 (0.75–2.18)	0.37	>0.999
β 2 HPV107	17 (13.6)	29 (7.8)	1.85 (0.94–3.62)	0.08	0.703
γHPV Species[b]					
Any γ HPV	53 (42.4)	125 (33.6)	1.38 (0.89–2.13)	0.15	0.924
Any γ 7 HPV	20 (16.0)	49 (13.2)	1.11 (0.62–2.01)	0.72	>0.999
Any γ 8 HPV	13 (10.4)	38 (10.2)	1.02 (0.52–2.01)	0.95	>0.999
Any γ 10 HPV	13 (10.4)	28 (7.5)	1.33 (0.66–2.71)	0.42	>0.999
Any γ 12 HPV	8 (6.4)	19 (5.1)	1.34 (0.56–3.20)	0.51	>0.999
Any γ 15 HPV	13 (10.4)	28 (7.5)	1.39 (0.66–2.89)	0.39	>0.999

[a]OR and 95% CI were estimated from CLR models adjusted for smoking, alcohol consumption, and study cohort.
[b]β and γ HPV species and types presented have a prevalence of 5% or higher either in cases or controls.
[c]Permutated *P* values were calculated to account for multiple comparisons (see methods).

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Table 4. Associations of β and γ HPVs species and types with risk of histologic type of esophageal cancer

	Cases ($n = 28$)	Controls ($n = 83$)	Adjusted model[a]		
A. ESCC	**N (%)**	**N (%)**	**OR[a] (95% CI)**	***P***	**Permutated *P*[c]**
β HPV species[b]					
Any β HPV	14 (50.0)	41 (49.4)	1.05 (0.37–2.97)	0.93	>0.999
Any β 1 HPV	10 (35.7)	27 (32.5)	1.19 (0.42–3.35)	0.75	>0.999
Any β 2 HPV	11 (39.3)	33 (39.8)	0.99 (0.36–2.72)	0.99	>0.999
Any β 3 HPV	3 (10.7)	19 (22.9)	0.35 (0.1–1.48)	0.15	0.792
Specific β HPV types[b]					
β 1 HPV5	5 (17.9)	10 (12.1)	1.56 (0.44–5.53)	0.49	>0.999
β 1 HPV12	3 (10.7)	9 (10.8)	1.24 (0.26–5.79)	0.79	>0.999
β 1 HPV20	3 (10.7)	8 (9.6)	1.10 (0.25–4.81)	0.89	>0.999
β 1 HPV36	4 (14.3)	6 (7.2)	1.72 (0.36–8.14)	0.50	>0.999
Clade of β 1 HPVs 5, 36, 47, & 143	6 (21.4)	12 (14.5)	1.31 (0.39–4.39)	0.66	>0.999
β 2 HPV23	2 (7.1)	6 (7.2)	1.31 (0.20–8.38)	0.78	>0.999
β 2 HPV38	4 (14.3)	16 (19.3)	0.62 (0.18–2.11)	0.45	>0.999
γ HPV species[b]					
Any γ HPV	12 (42.9)	26 (31.3)	1.31 (0.51–3.31)	0.57	>0.999
Any γ 7 HPV	3 (10.7)	6 (7.2)	0.81 (0.15–4.48)	0.81	>0.999
Any γ 8 HPV	3 (10.7)	9 (10.8)	0.61 (0.12–3.11)	0.55	>0.999
Any γ 10 HPV	4 (14.3)	8 (9.6)	1.46 (0.37–5.82)	0.59	>0.999
Any γ 15 HPV	3 (10.7)	8 (9.6)	1.22 (0.29–5.14)	0.79	>0.999
B. Esophageal adenocarcinoma	**Cases ($n = 80$)**	**Controls ($n = 239$)**	**Adjusted model[a]**		
	***N* (%)**	***N* (%)**	**OR[a] (95% CI)**	***P***	**Permutated P[c]**
β HPV Species[b]					
Any β HPV	56 (70.0)	134 (56.1)	1.84 (1.05–3.23)	0.04	0.199
Any β 1 HPV	41 (51.3)	94 (39.3)	1.74 (1.00–3.04)	0.05	0.296
Any β 2 HPV	42 (52.5)	105 (43.9)	1.34 (0.80–2.25)	0.27	>0.999
Any β 3 HPV	18 (22.5)	34 (14.2)	1.86 (0.95–3.63)	0.07	0.363
Specific β HPV types[b]					
β 1 HPV5	14 (17.5)	27 (11.3)	1.78 (0.85–3.72)	0.13	0.605
β 1 HPV12	6 (7.5)	16 (6.7)	1.11 (0.42–2.93)	0.83	>0.999
β 1 HPV20	14 (17.5)	31 (13.0)	1.42 (0.70–2.91)	0.33	>0.999
β 1 HPV36	10 (12.5)	23 (9.6)	1.13 (0.50–2.55)	0.77	>0.999
Clade of β 1 HPVs 5, 36, 47, & 143	17 (21.3)	39 (16.3)	1.36 (0.71–2.58)	0.35	>0.999
β 1 HPV105	11 (13.8)	20 (8.4)	1.62 (0.72–3.63)	0.24	>0.999
β 1 HPV124	7 (8.8)	20 (8.4)	1.06 (0.42–2.67)	0.90	>0.999
β 2 HPV23	9 (11.3)	17 (7.1)	1.67 (0.70–3.97)	0.24	>0.999
β 2 HPV37	8 (10.0)	21 (8.8)	1.21 (0.49–2.97)	0.68	>0.999
β 2 HPV38	15 (18.8)	34 (14.2)	1.48 (0.75–2.92)	0.26	>0.999
β 2 HPV107	10 (12.5)	19 (8.0)	1.69 (0.72–3.98)	0.23	>0.999
γ HPV species[b]					
Any γ HPV	32 (40.0)	77 (32.2)	1.53 (0.86–2.70)	0.15	0.668
Any γ 7 HPV	13 (16.3)	34 (14.2)	1.12 (0.54–2.34)	0.76	>0.999
Any γ 8 HPV	4 (5.0)	21 (8.8)	0.43 (0.14–1.36)	0.15	>0.999
Any γ 10 HPV	7 (8.8)	17 (7.1)	1.38 (0.52–3.66)	0.52	>0.999
Any γ 12 HPV	7 (8.8)	13 (5.4)	1.92 (0.72–5.14)	0.19	>0.999
Any γ 15 HPV	8 (10.0)	14 (5.9)	1.93 (0.69–5.42)	0.21	>0.999

[a]OR and 95% CI were estimated from CLR models adjusted for smoking, alcohol consumption, and study cohort.
[b]β and γ HPV species and types presented have a prevalence of 5% or higher either in cases or controls.
[c]Permutated *P* values were calculated to account for multiple comparisons (see methods).

To our knowledge, no prior study to date has provided information on the potential temporal association between oral HPV detection and subsequent incidence of esophageal cancer (i.e., evidence that oral HPV infection preceded the development of cancer). The lack of data on this issue is due, in part, to the relative rarity of these cancers, particularly ESCC in the US, requiring large sample sizes for prospective collection of mouthwash samples and subsequent follow-up for cancer incidence. We utilized the collection of oral samples originally intended for isolation of genomic DNA in two large prospective cohorts with verified cancer endpoints that provided the opportunity to efficiently determine whether HPV DNA detection in the oral cavity precedes cancer development. This is a critical component to determine whether oral HPV is associated with incident esophageal cancer. Indeed, the temporal relationships of HPV infection with risk of cervical (39) and oropharyngeal cancers (25) are well established.

In addition to α HPV types, the oral cavity contains a large number of β and γ HPV species and types (24, 25), and our study is the first to examine associations of β and γ oral HPV types with risk of esophageal cancer. There was a modest signal of an association between any β HPV and any β 1 HPV and risk of esophageal adenocarcinoma, but we could not identify a specific type responsible for this association, unlike the type-specific associations we reported previously for β 1 HPV5 and γ -11 and 12 species with risk of head and neck cancers in the same cohorts (25). Moreover, results were no longer statistically significant after accounting for multiple comparisons. In addition, we did not observe any

association of HPV DNA detection of other oral β and γ types and species with risk of esophageal cancer in this study.

A major strength of this study is the prospective design to examine associations of incident esophageal cancer with HPV DNA detection of α, β, and γ types in oral specimens collected prior to cancer diagnosis. This is also the first study to examine the full spectrum of β and γ HPVs that might contribute to risk of esophageal cancer after adjusting for smoking and alcohol consumption. Limitations of this study include the modest number of cases, particularly for ESCC, which is a relatively rare type of esophageal cancer in the United States. In addition, sequential oral mouthwash samples were unavailable to evaluate the risk of new infection and/or persistent HPV infections associated with esophageal cancer. Finally, the majority of participants in this study were Caucasian, and therefore it is unclear whether the results can be generalized to other race/ethnicities.

In conclusion, this study demonstrates that HPV16 and other oral α, β, and γ HPVs are not associated with risk of esophageal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: I. Agalliu, R.D. Burk
Development of methodology: I. Agalliu, Z. Chen, R.D. Burk
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.B. Hayes, N.D. Freedman, S.M. Gapstur
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): I. Agalliu, Z. Chen, T. Wang, R.B. Hayes, N.D. Freedman, S.M. Gapstur, R.D. Burk
Writing, review, and/or revision of the manuscript: I. Agalliu, T. Wang, R.B. Hayes, N.D. Freedman, S.M. Gapstur, R.D. Burk
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): I. Agalliu, N.D. Freedman
Study supervision: I. Agalliu

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