Published in final edited form as:
Otolaryngol Head Neck Surg. 2013 March ; 148(3): 436–442. doi:10.1177/0194599812471938.

HPV prevalence in a multi-ethnic screening population

Kang Mei Chen, MD, **Josena K. Stephen, MD**, **Tamer Ghanem, MD, PhD**, **Robert Stachler, MD**, **Glendon Gardner, MD**, **Lamont Jones, MD**, **Vanessa P. Schweitzer, MD**, **Francis Hall, MD**, **George Divine, PhD**, and **Maria J. Worsham, PhD, FACMG**
Department of Otolaryngology/Head and Neck Research (Drs. Chen, Gardner, Ghanem, Hall, Jones, Schweitzer, Stachler, Stephen, Worsham), Department of Public Health Sciences (Dr. Divine), Henry Ford Hospital, Detroit, MI 48202

Abstract

Objective—The goal was to determine prevalence of high-risk HPV16 using saliva in a screening population in Detroit, MI.

Materials and Methods—Real time quantitative PCR was applied to detect HPV16 in saliva DNA from 349 screening subjects without head and neck cancer (HNC), 156 HNC, and 19 controls. Cut points for HPV positivity were: >0 and >0.001 copy/cell. Proportions were compared between groups using exact chi-square or Fisher's exact tests ($p<0.05$).

Results—At cut point >0, each group had an overall HPV prevalence of over 5%, with a higher prevalence of 30.8**%** in the HNC patient group. At cut point >0.001, the prevalence was lower, 0% in the control, 1.2% in the screening, and 16**.7%** in the HNC group. In the latter, for both cut points, HPV prevalence was different across sites (<0.001) and significantly higher in the oropharynx (OP) than larynx or site as other after Hochberg's adjustment. At >0, females in the screening group had a higher prevalence of HPV than males ($p=0.010$) and at >0.001, prevalence was higher for males in the HNC group than females ($p=0.035$). In the screening group, at >0, only AA had higher prevalence than CA ($p=0.025$).

Conclusions—In the screening group, a 6.9% and 1.2% screening rate was noted at cut-points >0 and >0.001, respectively. The results provide data to inform public health considerations of the feasibility of saliva as a screening tool in at-risk populations with the long term goal of prophylactic vaccination against oral HPV.

Keywords

saliva; tumor; head and neck cancer (HNC); HPV positive; HPV negative

INTRODUCTION

The human papilloma virus (HPV), especially the oncogenic types, is a causative agent for some head and neck squamous cell carcinoma (HNSCC)[1,2] and an independent risk factor for oropharyngeal HNSCC (OPSCC).[3-5] A recent meta-analysis of 5681 HNSCC[6] found that the prevalence of HPV infection was significantly higher among patients with OPSCC (35.6%) than among those with oral (23.5%) or laryngeal (24.0%) SCC.[7] HPV type 16 (HPV16) accounts for approximately 95% of the infections in these HNSCC subgroups.[8]

Corresponding Author: Maria J. Worsham, Ph.D., FACMG, Director of Research, Department of Otolaryngology/Head and Neck Research, Henry Ford Hospital, 1 Ford Place, 1D, Detroit, MI 48202, Phone: 313-874-3350, Fax: 313-874-1079, mworsha1@hfhs.org.

Podium presentation at the 2012 AAO-HNSF Annual Meeting & OTO EXPO, Washington, DC, September 9-12, 2012

The clinical significance of HPV in HNSCC pathogenesis has been well documented for OPSCC. HPV positive OPSCC patients have improved survival and respond better to treatment than HPV negative. Since 1984 an increase in the prevalence, incidence and survival of OPSCC is attributed to HPV infection.[9]

The current focus of HPV prevention efforts has been in cervical cancer. However, the increased future burden of OPSCC from nine SEER registries when compared to the cervical cancer burden, projected that by 2010, the annual incidence rate of OPSCC among men will have surpassed that for cervical cancers.[9] There would be a 4.7% reduction in incidence rate of head and neck cancer (HNC) in the United States if oncogenic HPV infection could be prevented.[10] Majority of HPV positive HNC occur in the oropharyngeal site where the cancer is diagnosed in the late stages.[11] This is due to the difficulty in distinguishing early OPSCC from lymphoid tonsillar and base of tongue tissue. Saliva-based screening tests, similar to non-invasive pap smear screening performed in cervical cancers, could help with early detection of OPSCC. According to Zhao et al[12], for optimal feasibility using saliva rinse screening of HPV for HNC, the prevalence should be 5% or higher in the population screened. The aim of our study was to determine the prevalence of high-risk HPV16 infection in a multi-ethnic screening population in Detroit, MI.

MATERIAL & METHODS

Cohort

The cohort of 524 subjects consisted of three groups: 349 screening subjects without HNC (HN screen), 156 HNC patients (Table 1), and 19 control subjects. Of the 156 HNC, 154 were squamous cell carcinoma (HNSCC) and 2 non-HNSCC patients. Of the 154 HNSCC, 52 were oropharyngeal SCC (OPSCC) and 102 non-OPSCC (larynx: 44, oral cavity: 32, hypopharynx: 6, and site as other [lip-external, nose maxilla, nasopharynx, sinonasal]: 22 (Table 2).

Saliva from the screening population was collected over a period of 4 years during one-day screenings of participants in the Ear Nose & Throat (ENT) clinic during Oral, Head and Neck Cancer Awareness Week, held every April, and a 2011 Health Fair at ACCESS (Arab Community Center for Economic & Social Services, Dearborn, MI). The 19 control subjects were ENT clinic and laboratory staff volunteers. Sexual behaviors of cohort subject were not collected for this study.

This study was approved by the Henry Ford Health System Institutional Review Board committee and informed consent was obtained from all enrolled subjects.

DNA Extraction

Two milliliters of saliva were collected from each study subject in Oragene DNA kits (DNA Genotek Inc, Ontario, Canada), and saliva DNA was extracted according to the manufacturer's instructions.

HPV16 Detection by Quantitative PCR (qPCR)

Tumor HPV DNA concentrations were measured using a real-time quantitative PCR system. Real-time PCR reactions were set up in a reaction volume of 20 ul using the TaqMan Universal Master Mix II with UNG (Applied Biosystems, Foster City, CA). Specific primers and probes were designed to amplify a housekeeping gene, *β-globin*[13] (to standardize the input DNA), and the E6 region of HPV16[14] as previously described.[15] DNA amplifications were carried out in a 96-well reaction plate format in an Applied Biosystems 7900HT Sequence Detector (Applied Biosystems, Foster City, CA). Multiple water blanks were

included in every run. Both the HPV and *β-globin* PCR reactions were carried out in duplicate.

To determine HPV16 viral copy number, CaSki cell line (American Type Culture Collection-ATCC, Manassas, VA) genomic DNA, known to have 600 copies/genome equivalent (6.6 pg of DNA/genome), was utilized to develop standard curves using serial dilutions of 50 ng, 5 ng, 0.5 ng, 0.05 ng, and 0.005 ng of DNA. Standard curves were also developed for the *β-globin* housekeeping gene (2 copies/genome) using the same serial dilutions. This allowed for relative quantification of the input DNA level and attribution of the final quantity as the number of viral copies/genome/cell.[16]

Statistical Analysis

All data were analyzed using SAS 9.2. For prevalence, two cut points for HPV positivity were used: >0 and >0.001 copy/cell. Proportions were compared between groups using exact chi-square or Fisher's exact tests as appropriate based on expected cell count. Hochberg's method was used to adjust for multiple comparisons[17]. Statistical significance was set at $p<0.05$.

RESULTS

Oncogenic HPV16 was examined in saliva from 349 screening subjects without HNC (HN screen; African American {AA}: 85, Caucasian American {CA}: 172, Arab American: 57, other: 26, missing race: 9); 156 HNC patients (HN patient; AA: 36, CA: 107, other: 13), and 19 control subjects (AA: 1, CA: 7, other: 11). Age of the cohort ranged from 19 to 89. Gender distribution was as follows: there were twice as many females as males (224 vs 114) in the HN screening group, three times as many males as females (119 vs 37) in the HN patient group and about equal numbers of males and females (8 vs 11) in the control group.

The overall prevalence of HPV was significantly different between the three groups ($p<0.001$) regardless of cut points (Table 1). At cut point >0, the prevalence of HPV in the controls, HN screen and HN patients was 5.3%, 6.9% and 30.8 % respectively. At cut point >0.001, the prevalence was lower in each group, 0% in the control, 1.2 % in the HN screen, and 16.7 % in the HN patient group. For prevalence by group and gender (Table 1A), females in the HN screen group had a higher prevalence of HPV than males ($p=0.01$) at cut point >0 only. At cut point >0.001, prevalence was higher for males in the HN patient group than females ($p=0.035$).

Prevalence of HPV by race (Table 1B) was significantly different in the screening group ($p=0.031$) only at cut point >0. Pair-wise race comparisons in this group indicated that only AA had higher prevalence than CA ($p=0.025$). In the HN patient group, more CA were HPV positive than AA (33% vs 22% at cut point >0 and 18% vs 8% at cut point >0.001), but this was not significant. HN patients who smoked were significantly less likely to be HPV positive ($p=0.03$, cut point >0; $p=0.045$, cut point >0.001; Table 1C).

Within the HNC group, HPV prevalence was statistically significantly different ($p=<0.001$) across sites for both cut points (Table 2). Pairwise comparisons for each site combination after Hochberg's adjustment (Table 3) for both cut points indicated significantly higher HPV prevalence for OP vs larynx ($p=<0.0001$) and OP vs other ($p=0.0046$ at cut point >0 and $p=0.001$ at cut point >0.001). At cut point >0.001, HPV prevalence in the larynx was significantly higher than in the oral cavity after Hochberg's adjustment (Table 3).

DISCUSSION

In the United States, approximately 52,610 new cases of HNC are expected in 2012 with an estimated 11,500 deaths from cancers of the oral cavity, pharynx, and larynx.[18] In recent years the incidence of HNC has been decreasing in USA due to a decrease in tobacco use.[19] However, the incidence of HPV related OPSCC has been rising.[9] Despite considerable efforts, the 5-year survival rate for HNSCC has not changed significantly. Our goal was to determine the prevalence of high-risk HPV16 infection in the saliva of a screening population, consisting of control subjects, HN patients and HN screening subjects without HNC, in Detroit, Michigan.

Regardless of study cut points, the overall prevalence of HPV was significantly different among the three groups ($p<0.001$). Overall HPV prevalence was over 5% in each of the 3 groups at cut point >0. At cut point >0 and >0.001, HPV prevalence was significantly higher in the HN patient (30.8% and 16.7%, respectively) as compared to the HN screening group (6.9% and 1.2%, respectively). This higher prevalence in the saliva DNA from HN patients is supported by similar studies utilizing comparable cut points[12]. From a community screening study for HNSCC of 92 HN tumor and paired saliva rinse samples and saliva rinse from 604 control subjects, HPV prevalence by qPCR at cut point >0 was 45.6% (42/92) in HNSCC patients. In the screening sample, HPV was detected in 2.8% and 1.3% of samples at cut points >0 and >0.001, respectively. The study measured sensitivity and specificity using cut points of >0, >0.001 and >0.1 copies of HPV, and determined a prevalence requirement of 5% or greater to achieve a positive predictive value close to 70%. A limitation of our study is the lack of HPV data on paired tumor and saliva rinse of the HN patient samples. Though our study achieved a prevalence rate of 6.9% at cut point >0, at cut point >0.001 the prevalence decreased to 1.2% in the screening sample. The study also lacked evaluation in the context of sensitivity and specificity (as well as the positive and negative predictive value) to more comprehensively make a case to argue in favor of saliva screening.

The multi-ethnic screening population in this study consisted of AA, CA, Arab Americans and Other. This is the first study to have an Arab American screening component. Of the 57 Arab Americans in the screening population, 5 were HPV positive (8.8%) and 52 were HPV negative at cut point >0, but at cut point >0.001, none remained HPV positive. With respect to race, HPV prevalence was significantly different among the four ethnic groups only for the HN screen group and at cut point >0 only. Pair-wise race comparisons demonstrated that at this cut point, AA had a higher HPV prevalence when compared to CA (12.9% vs 3.5%; $p=0.025$). In the Gillison et al[20] study of 5,579 screening oral rinse samples from the 2009-2010 National Health and Nutrition Examination Survey (NHANES), a higher HPV prevalence in AA compared to CA was observed, however, this difference did not reach statistical significance. It is important to note that they detected HPV by multiplex PCR using primer pools followed by line-blot hybridization, which is not as sensitive as qPCR and can result in cross contamination. For years ranging between 1984 and 2004, HPV positive patients were more likely to be younger, male and CA or other races.[9] Data from our prospective study as well as the recent rise in incidence of HPV-related OPSCC appears to support an increase in HPV incidence in the AA population as well.

There were twice as many females in the screening group than males, likely contributing to the higher prevalence of HPV positive females (at cut point >0 only). This may reflect a better response to health care screening on the part of women. Turner et al[21] demonstrated at cut off >0.001 a higher prevalence of HPV in non-White Hispanic women. Their study of 151 healthy adults had a roughly equal distribution of females and males (52.3% and 47.7%, respectively). The Gillison et al[20] NHANES study reported a 5-fold higher prevalence of

HPV16 in men over women. This increased prevalence is consistent with higher rates of HPV positive OPSCC in men. The latter observation is supported by data from our study, which shows that at cut point >0.001 in the HN patient group, more males than females were HPV positive (20.2% vs 5.4%, respectively, p=0.035). Within the HNC group, HPV prevalence was significantly different across sites (larynx, OC, OP, HP, other), with the highest prevalence for the OP site vs larynx and OP vs other, regardless of cut points and after multiple comparisons.

The recognition of HPV as an etiologic agent for development of OPSCC, in addition to smoking and heavy alcohol consumption likely reflects the rise in OPSCC in the US and internationally,[22,23] despite a decrease in tobacco use in recent years. HPV may also be the primary oncogenic factor inducing carcinogenesis in patients without a history of tobacco or alcohol use. Non-tobacco and non-alcohol related OPSCC are six times more likely to harbor HPV than case-matched controls.[24] In our study, we found a higher HPV positive rate in non-smokers compared to smokers (38.2% vs 22.1%, p=0.03, cut point >0 and 22.4% vs 10.4%, p=0.045, cut point >0.001) in the HN patient group. The latter concurs with other studies where HPV is regarded as the main etiologic agent for OPSCC non-smokers.[12,24]

Also, HPV prevalence remained higher in larynx when compared to the oral cavity at cut point >0.001 after pair wise site adjustment. Laryngeal squamous cell cancer (LSCC), the largest subgroup of head and neck cancers share common risk factors to include smoking, and alcohol consumption. The prevalence of oncogenic HPV types in LSCC has a wide distribution, ranging from 0%[25] to 69%,[26], attributable mainly to differences in methods of HPV detection as well as selection bias due to small sample size or poor quality of cancer specimen[27]. In a pooled meta analysis study[7] using conventional PCR based assays, prevalence of HPV16 in LSCC was 17%.[7] In a recent study, HPV16, detected by real-time quantitative PCR, had a reported prevalence of 27%, supporting a role for HPV in LSCC[28].

HPV prevalence and its detection in saliva DNA in the screening population is of high clinical significance because of HPV's recognition as a causative agent for some HNSCC. Paradoxically, HPV positive HNSCC, particularly OPSCC patients show improved survival,[3] due in part to a better response to chemoradation.[29] In addition to studies reporting improved survival in HPV positive OPSCC never-smokers,[30] recent studies show that HPV positive OPSCC smokers had improved prognostic outcomes when compared to HPV negative OPSCC smokers.[31] The latter points to a putative role of HPV as a modulator of the malignancy process in smoking and alcohol related OPSCC.[21]

The main focus of prophylactic HPV vaccination has been on cervical cancers. This is justified as the public health burden of cervical precancer and invasive cancer is greater when compared to OPSCC. The incidence rates of cervical cancer would be greater if not for the current screening protocol that is in place.[9] It is estimated that by 2020 the number of HPV positive OPSCC will surpass the number of cervical cancers.[20] Considering the increasing incidence rates in OPSCC seen in recent years, it may be worthwhile to develop a screening process for early detection of these cancers. However, in the absence of a standardized evidence-based screening process, it may be useful to evaluate the efficacy of HPV vaccination in preventing oral HPV infection. The quadrivalent HPV vaccine has been determined to be highly efficacious in preventing extra-cervical infections in women[32] and penile and anal infections[33,34] in men. HPV vaccination is already in place for young boys.[35] Considering that the majority of HPV positive OPSCC cases are in men, prophylactic HPV vaccination in adult males may be a consideration.

The higher prevalence of HPV in AA vs CA in the screening population in our study is difficult to interpret and necessitates additional investigation. Overall, the results of this

study provide HPV prevalence data in understudied AA and Arab American screening groups. Pursuing the feasibility of saliva as a screening tool in higher risk populations is relevant not only to early detection of HNC but also to the long term goal of establishing a rationale for prophylactic vaccination against oral HPV acquisition. Currently, no screening process is available for oral HPV detection. A rationale to justify screening for oral HPV will require assessment of oral HPV prevalence in larger cohorts with sufficient representation of different ethnicities/races in the context of sensitivity and specificity, as well as clear recommendations of interventional strategies for individuals with oral HPV.

Acknowledgments

Drs. Alvin Ko, Werner Roennecke, Ahsan Syed, William Young, Syamal Mausumi, Brandon Musgrave, Robert Deeb; Dr. Adnan Hammad and Mona Farroukh (ACCESS), and Wayne State University Medical Students (Paul Judge, Mathew Smithe, Mark Toma, Carmen Tugulan, Mark Shamoun, Usam Khalid, Haroon Yousul, Lisa Vitale, Hemancher Makker, Victoria Yee, Emin Elezi, Shawn Kumar) contributed to patient recruitment efforts and saliva collection. Meredith Mahan assisted with statistical data analysis.

Dr. Worsham had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. This study was supported by NIH R01 DE 15990 (Dr. Worsham).

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Table 1

Cohort Demographic Characteristics

			Total	HPV Cut Point 0			HPV Cut Point 0.001		
				HPV positive (>0)	**HPV negative (0)**	**p value**	**HPV positive (≥0.001)**	**HPV negative (<0.001)**	**p value**
Overall Prevalence									
	Controls		19	1 (5.3%)	18 (94.7%)	**<0.001**	0 (0%)	19 (100%)	**<0.001**
	HN Patients		156	48 (30.8%)	108 (69.2%)		26 (16.7%)	130 (83.3%)	
	HN Screen		349	24 (6.9%)	325 (93.1%)		4 (1.2%)	345 (98.9%)	
Prevalence by group and									
A: Gender *									
	Controls	Male	8	0 (0%)	8 (100%)	1	0 (0%)	8 (100%)	—
		Female	11	1 (9.1%)	10 (90.9%)		0 (0%)	11 (100%)	
	HN Patients	Male	119	40 (33.6%)	79 (66.4%)	0.168	24 (20.2%)	95 (79.8%)	**0.035**
		Female	37	8 (21.6%)	29 (78.4%)		2 (5.4%)	35 (94.6%)	
	HN Screen	Male	114	2 (1.8%)	112 (98.3%)	**0.01**	1 (0.9%)	113 (99.1%)	1
		Female	224	21 (9.4%)	203 (90.6%)		3 (1.3%)	221 (98.7%)	
B: Race *									
	Controls	AA	1	0 (0%)	1 (100%)	1	0 (0%)	1 (100%)	—
		CA	7	0 (0%)	7 (100%)		0 (0%)	7 (100%)	
		Other	11	1 (9.1%)	10 (90.9%)		0 (0%)	11 (100%)	
	HN Patients	AA	36	8 (22.2%)	28 (77.8%)	0.410	3 (8.3%)	33 (91.7%)	0.153
		CA	107	35 (32.7%)	72 (67.3%)		19 (17.8%)	88 (82.2%)	
		Other	13	5 (38.5%)	8 (61.5%)		4 (30.8%)	9 (69.2%)	
	HN Screen**	AA	85	11 (12.9%)	74 (87.1%)	**0.031**	3 (3.5%)	82 (96.5%)	0.165
		CA	172	6 (3.5%)	166 (96.5%)		1 (0.6%)	171 (99.4%)	

			Total	HPV Cut Point 0			HPV Cut Point 0.001		
				HPV positive (>0)	**HPV negative (0)**	**p value**	**HPV positive (≥0.001)**	**HPV negative (<0.001)**	**p value**
		Arab	57	5 (8.8%)	52 (91.2%)		0 (0%)	57 (100%)	
		Other	26	1 (3.9%)	25 (96.2%)		0 (0%)	26 (100%)	
C: Smoking *									
	Controls	No	14	1 (7.1%)	13 (92.9%)	—	0 (0%)	14 (100%)	—
		Yes	0	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
	HN Patients	No	76	29 (38.2%)	47 (61.8%)	0.03	17 (22.4%)	59 (77.6%)	**0.045**
		Yes	77	17 (22.1%)	60 (77.9%)		8 (10.4%)	69 (89.6%)	
	HN Screen	No	242	16 (6.6%)	226 (93.4%)	1	3 (1.2%)	239 (98.8%)	1
		Yes	81	6 (7.4%)	75 (92.6%)		1 (1.2%)	80 (98.8%)	

HN - head and neck

AA - African American

CA - Caucasian American

* missing; A: 11 in HN screen; B: 9 in HN screen; C: 5 in Controls, 3 in HN patients, 26 in HN screen

** After Hochberg's adjustment of pair-wise race comparisons of race groups, only AA had higher HPV prevalence than CA (p=0.025).

Otolaryngol Head Neck Surg. Author manuscript; available in PMC 2013 July 10.

Table 2

HPV prevalence in the HNC group by sites

	Total	HPV Cut Point 0			HPV Cut Point 0.001		
Sites		**HPV Positive (>0), N=48**	**HPV Negative (0), N=108**	**p-value**	**HPV Positive (≥0.001), N=25**	**HPV Negative (<0.001), N=131**	**p-value**
OP	52	28 (58.3%)	24 (22.2%)	**<0.001**	19 (36.5%)	33 (63.5%)	**<0.001**
Non-OP	104						
OC	32	10 (20.8%)	22 (20.4%)		5 (15.6%)	27 (84.4%)	
Larynx	44	4 (8.3%)	40 (37.0%)		1 (2.3%)	43 (97.7%)	
HP	6	2 (4.2%)	4 (3.7%)		0	6 (100%)	
Other HNSCC Non-HNSCC	22 20 2	4 (8.3%)	18 (16.7%)		0	22 (16.8%)	

OP: oropharynx

OC: oral cavity

HP: hypopharynx

Other: lip-external, nose, maxilla, nasopharynx, sinonasal

HNC: head and neck cancer

HNSCC: head and neck squamous cell carcinoma

Table 3
Multiple Comparisons by Site within the HNC group

Sites	HPV Cut Point >0 p-value	HPV Cut Point >0.001 p-value
Larynx vs HP	0.1457	**1**
Larynx vs OC	0.0139	**0.0770** *
Larynx vs OP	**<0.0001** *	**<0.0001** *
Larynx vs Other	0.4249	**1**
HP vs OC	1	0.5701
HP vs OP	0.4155	0.1634
HP vs Other	0.5806	--
OC vs OP	0.0433	0.0394
OC vs Other	0.2816	0.072
OP vs Other	**0.0046** *	**0.0010** *

OP — oropharynx

OC - oral cavity

HP - hypopharynx

Other-lip-external, nose maxilla, nasopharynx, sinonasal

* significant after Hochberg's adjustment