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Changing trends in human papillomavirus–associated head and neck squamous cell carcinoma☆

Xiaohong Iris Wang, MD, PhD, Jaiyeola Thomas, MD*, Songlin Zhang, MD, PhD

Department of Pathology, Louisiana State University Health Science Center, Shreveport, LA 71130, USA

Abstract Head and neck squamous cell carcinoma (HNSCC) continues to be a significant disease with varying rates of incidence and mortality worldwide. Numerous studies have demonstrated that human papillomavirus (HPV) is etiologically linked with a subset of HNSCC, independent of tobacco and alcohol use. This subset of tumor shows increased sensitivity to radiation therapy and association with better outcomes. The study aims to determine the HPV burden and trend among patients with HNSCC in the southern region of the United States over the past 10 years. Of 142 cases from 2000 to 2004, 18 (13%) were positive for high-risk HPV. Nine of these were oropharyngeal tumors, including 4 cases from the tonsil. These constitute 38% (9/24) of all oropharyngeal tumors and 57% (4/7) of tonsillar tumors. Of 35 cases from 2009 to 2010, 14 (40%) were positive for high-risk HPV. Thirteen of these were oropharyngeal tumors, including 9 cases from the tonsil. These constitute 59% (13/23) of oropharyngeal tumors and 64% (9/14) of tonsillar tumors. When data from the 2 periods are combined, the results show that African American patients are less likely to have HPV-associated disease compared with white patients (9% vs 22%). Human papillomavirus–positive and oropharyngeal HNSCC are more likely to be nonkeratinizing ($P < .0001$). In conclusion, the HPV detection rate in oropharyngeal squamous cell carcinoma increased from 38% to 59% between the 2000-to-2004 and 2009-to-2010 periods.
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Keywords: Head and neck; Squamous cell carcinoma; Human papillomavirus; Oropharynx

1. Introduction

Human papillomaviruses (HPVs) are small nonenveloped DNA viruses that infect squamous epithelial cells. Although more than 100 different genotypes of HPV have been identified, only approximately 15 are considered oncogenic in cervical, vulvar, vaginal, anal, penile squamous epithelia, and more recently, in head and neck squamous cells [1,2]. The low-risk HPV subtypes cause benign neoplasms such as papillomas, whereas the high-risk subtypes have the ability to induce squamous cell immortalization in vitro and have been detected in a subset of malignant neoplasms. Although a number of HPV subtypes have been detected in head and neck squamous cell carcinoma (HNSCC) samples, high-risk types 16, 31, and 33 are linked biologically to the development of oropharyngeal squamous cell carcinoma (SCC) [3]. Numerous studies have shown that HPV-positive HNSCC is a biologically distinct entity different from tobacco- and alcohol-associated carcinoma, with high prevalence in oral and oropharyngeal cancers [4]. In addition, certain HPV-related HNSCC has been associated with better patient survival [5-7].

The frequency of HPV-positive tumors in HNSCC varies considerably depending on the population studied, location of the tumors, and the HPV detection methods. Different methods of HPV detection exist with variable levels of sensitivity and specificity [8]. The different HPV detection methods often used in reported series may be partially responsible for the wide variation in the reported incidence of HPV presence in HNSCC. Viral detection can be performed using immunohistochemistry, in situ hybridization (ISH) technique, or polymerase chain reaction (PCR)–based assays. In situ hybridization is thought to reflect one of

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* Corresponding author. Tel.: +1 318 675 5899; fax: +1 318 675 8589.
E-mail address: jtho19@lsuhsc.edu (J. Thomas).

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the most accurate methods because the technique allows for the visualization of HPV localization within the tumor cells.

This study aims to determine, if any, the trend variation of HPV burden among biopsied HNSCC cases in the southern region of the United States over 2 periods within the last 10 years (2000-2004 and 2009-2010) using a well-validated ISH high-risk HPV (hrHPV) probe (HPV INFORM III; Ventana Medical Systems, Tucson, Ariz).

2. Materials and methods

The study materials consist of formalin-fixed, paraffin-embedded tissue sections and blocks of HNSCC cases seen at the Department of Pathology in our institution between January 2000 and December 2004 as well as between January 2009 and October 2010. The institutional review board at Louisiana State University Health Science Center approved this study.

Between 2000 and 2004, 259 diagnosed HSCCC cases were identified, using the departmental LIS Co-Path retrieval system. Tissue sections were available for 174 cases for further histologic review and 142 suitable blocks were available and retrieved for additional ISH study. Cases were excluded if the remaining tissue specimens were too small or no tumor tissue was identified on the section. Thirty-five cases of HNSCC diagnosed between January 2009 and October 2010, on which hrHPV-ISH studies had been done, were retrieved and studied. All the hematoxylin and eosin–stained sections were reviewed; fresh 5-μm sections were cut, as needed, and stained with Ventana HPV Inform III probe for hrHPV using the ISH technique according to the manufacturer's instructions with adequate positive and negative controls. The probe used is capable of hybridizing with hrHPV genotypes 16, 18, 33, 35, 45, 51, 52, 56, and 66. The presence of dark blue punctate (dotlike) or diffuse nuclear signals in confluent groups of tumor cells was interpreted as "positive."

Tumors were grouped by locations into the following sites: oral cavity, larynx, oropharynx, hypopharynx, and nasopharynx. The oral cavity includes the lips, gums, anterior tongue, floor of mouth, hard palate, and inner surface of the cheeks. The larynx consists of the 3 major anatomical areas: the supraglottic larynx, the glottic larynx, and the subglottic larynx. The oropharynx consists of the soft palate, the tonsil, and the base of tongue. The hypopharynx includes the pyriform sinuses, postcricoid area, as well as the posterior pharyngeal wall. The nasopharynx is the uppermost part of the pharynx.

The histologic types were classified independently by a senior pathologist (JOT), without the knowledge of the clinical outcomes and HPV status, into 2 categories: nonkeratinizing squamous cell carcinoma (NKSCC) and keratinizing squamous cell carcinoma (KSCC). Nonkeratinizing squamous cell carcinoma was composed of invasive sheets, nests, or trabeculae of tumor cells with squamous differentiation but no evidence of distinct keratinization (Fig. 1A). Comedo-type necrosis and brisk mitotic activity were often present but were not considered requisite features. Although varying from well to poorly differentiated, KSCC was composed mainly of recognizable squamous tumor cells with pronounced or focal keratinization (Fig. 1B).

The pathology requisition form and clinical information were reviewed for demographic data, history of smoking, and tobacco use as well as ethnicity.

2.1. Statistics

Fisher exact test was used to test differences in clinical and morphological characteristics as well as hrHPV positivity between groups.

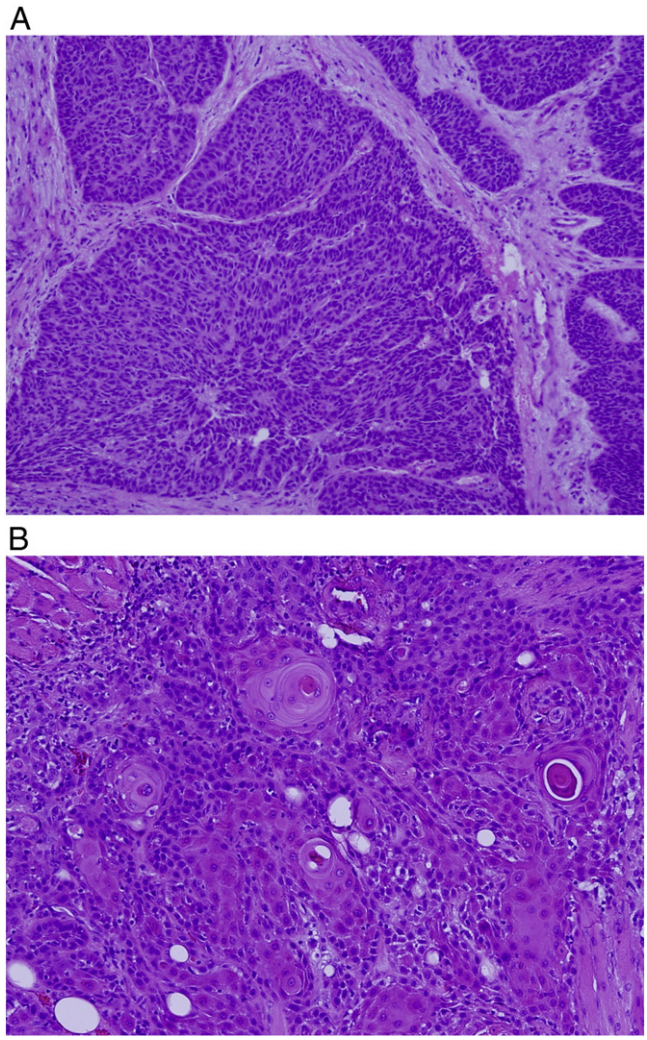

Fig. 1. Nonkeratinizing squamous cell carcinoma composed of invasive sheets, nests, or trabeculae of tumor cells with squamous differentiation but no evidence of distinct keratinization (A). Keratinizing squamous cell carcinoma composed of recognizable squamous tumor cells with pronounced keratinization (B) (original magnification ×200).

3. Results

The mean age of the 259 patients diagnosed between 2000 and 2004 was 57 years (range, 24-88 years), with male-to-female ratio of 2.5:1. The most common anatomical sites included oral cavity (106 cases; 106/259, or 41%), larynx (95 cases; 95/259, or 37%), and oropharynx (48 cases; 48/259, or 19%), which included 9 (9/259, or 3.5%) cases from the tonsils.

The locoregional distribution of the 142 cases with available suitable tissue blocks (2000-2004 series) are shown in Table 1, with 69 (69/142, or 49%), 44 (44/142, or 31%), and 24 (24/142, or 17%) cases occurring in the oral cavity, larynx, and oropharynx (including 7 cases from the tonsils), respectively. Of these 142 cases, 18 (13%) were positive for hrHPV. When stratified by sites of tumor origin (Table 1), hrHPV was detected in 9 carcinomas arising from the oropharynx, including 4 cases from the tonsil. These constitute 38% (9/24) of oropharyngeal tumors and 57% (4/7) of tonsillar tumors. The other 9 hrHPV-positive cases were from the oral cavity (7/69, or 10%) and larynx (2/44, or 4.5%).

The locoregional distribution of the 35 HNSCC cases diagnosed between 2009 and 2010 series is shown in Table 2, with 23 (66%), 8 (23%), and 3 (6%) cases occurring in the oropharynx (including 14 [40%] tonsillar tumors), oral cavity, and larynx, respectively. Of 35 cases, 14 (40%) were positive for hrHPV, consisting of 13 cases from the oropharynx, including 9 cases from the tonsils. These constitute 57% (13/23) of oropharyngeal tumors and 64% (9/14) of tonsillar tumors. The other hrHPV-positive case was from the larynx (1).

The frequency of smoking history among HNSCC patients remained steady in both groups identified between the 2 periods (70% vs 74%), whereas the frequency of drinking history increased from 48% to 63%. When both series from the 2 periods are combined, the data show African American patients (9%, or 6/64) are less likely to have HPV-positive disease compared with white patients (22%, or 22/99) (Table 3, $P = .036$). Although smoking history is only slightly higher among African American patients (81%) compared with whites (73%), African American patients tend to be more likely to drink compared with whites (73% vs 45%, $P = .006$; data not shown). Although patients with hrHPV-positive tumors tend to have no prior history of tobacco and/or high alcohol use, there is no significant statistical difference observed between hrHPV positivity and history of tobacco and alcohol use (Table 3).

The 177 cases (combined series) with available histologic sections were classified as follows: 121 cases (68%) as KSCC and 56 (31%) as NKSCC. The clinical characteristics grouped by histologic types are shown in Table 4. All tumors were more common in men compared with women. The NKSCC was much more likely to be hrHPV positive than KSCC (Fisher exact test, $P < .0001$, Table 4). The hrHPV-ISH staining pattern of tumor cell clusters varied from focal to diffuse in distribution, as well as in nuclear signals with

Table 1
Head and neck squamous cell carcinoma study population (2000-2004) grouped by hrHPV status

Total	HPV, 142; n (%)	HPV negative, 124; n (%)	HPV positive, 18; n (%)
Sex			
Male	99 (70)	86 (69)	13 (72)
Female	43 (30)	38 (31)	5 (28)
Race			
Black	55 (39)	49 (40)	6 (33)
White	75 (53)	66 (53)	9 (50)
Unknown	12	9	3
Smoking and drinking			
Smoking	98 (70)	86 (69)	12 (67)
Nonsmoking	13 (9)	11 (9)	2 (11)
Unknown	31 (21)	27 (22)	4 (22)
Drinking	69 (48)	62 (50)	7 (39)
Nondrinking	37 (26)	30 (24)	7 (39)
Unknown	36 (26)	32 (26)	4 (22)
Anatomical site			
Oropharynx	24 (17)	15 (12)	9 (50)
Tonsils	7 (5)	3 (2)	4 (22)
Nontonsil	17 (12)	12 (10)	5 (28)
Nonoropharynx	118 (83)	109 (88)	9 (50)
Oral cavity	69 (49)	62 (50)	7 (39)
Larynx	44 (31)	42 (34)	2 (11)
Hypopharynx	4 (3)	4 (3)	0
Nasopharynx	1 (1)	1 (1)	0

Table 2
Head and neck squamous cell carcinoma study population (2009-2010) grouped by hrHPV status

	Total, N = 35; n (%)	HPV negative, n = 21; n (%)	HPV positive, n = 14; n (%)
Sex			
Male	27 (77)	14 (67)	13 (93)
Female	8 (23)	7 (33)	1 (7)
Race			
Black	9 (26)	9 (43)	0
White	24 (69)	11 (52)	13 (93)
Asian	1 (3)	1 (5)	0
Unknown	1 (3)	0	1 (7)
Smoking and drinking			
Smoking	27 (74)	19 (92)	8 (55)
Nonsmoking	7 (26)	2 (8)	5 (45)
Unknown	1 (3)		1 (7)
Drinking	22 (63)	16 (76)	6 (43)
Nondrinking	10 (29)	5 (24)	5 (36)
Unknown	3 (9)		3 (21)
Anatomical site			
Oropharynx	23 (66)	10 (48)	13 (93)
Tonsils	14 (40)	5 (24)	9 (64)
Nontonsil	9 (26)	5 (24)	4 (29)
Nonoropharynx	12 (34)	11 (52)	1 (7)
Oral cavity	8 (23)	8 (38)	0
Larynx	3 (6)	2 (10)	1 (7)
Nasopharynx	1 (3)	1 (5)	

Table 3
Clinical characteristics of total HNSCC study population and HPV status

	Total, N = 177; n (%)	HPV negative, n = 145; n (%)	HPV positive, n = 32; n (%)	*P* (Fisher exact test)
Mean age (y)		57		58
Sex				
Male	126 (71)	100 (69)	26 (81)	.1994
Female	51 (29)	45 (31)	6 (19)	
Race				
African	64 (36)	58 (40)	6 (19)	.036
White	99 (56)	77 (53)	22 (69)	
Asian	1 (1)	1 (1)		
Unknown	13 (7)	9 (7)	4 (13)	
Smoking and drinking				
Smoking	125 (71)	105 (72)	20 (62)	.0607
Nonsmoking	20 (11)	13 (9)	7 (22)	
Unknown	32 (18)	27 (19)	5 (16)	
Drinking	91 (51)	78 (53)	13 (41)	.1103
Nondrinking	47 (27)	35 (24)	12 (38)	
Unknown	39 (23)	32 (23)	7 (22)	

dotlike (punctate) and diffuse, nuclear granular patterns, as shown in Fig. 2A and B. The staining intensity with the HPV ISH probe also varied from faint to intense in tumor cell nuclei. The NKSCC was more likely to show diffuse staining of tumor cells rather than focal or scattered distribution.

Table 4
Histologic classification of all HNSCC and associated characteristics

	Total	KSCC, 121; n (%)	NKSCC, 56; n (%)	*P* (Fisher exact test)
Age		57.5	56.5	
Sex				
Male	126	82 (68)	44 (79)	.15
Female	51	39 (32)	12 (21)	
P				
Smoker				
Yes	125	84 (69)	41 (73)	1.00
No	20	14 (12)	6 (11)	
Unknown	32	23 (19)	9 (16)	
Drinker				
Yes	91	60 (50)	31 (55)	.7029
No	47	33 (27)	14 (25)	
Unknown	39	28 (23)	11 (20)	
HR-HPV-ISH (%)				
Positive	31	12 (10)	20 (36)	.0001
Negative	145	109 (90)	36 (64)	
Anatomical site				
Oropharynx	47	20 (17)	27 (48)	
Tonsils	21	9 (7)	12 (21)	.0001 (oropharynx vs nonoropharynx)
Nontonsil	26	11 (9)	15 (27)	
Nonoropharynx	129	101 (83)	29 (52)	
Oral cavity	77	64 (53)	13 (23)	
Larynx	47	34 (28)	13 (23)	
Nasopharynx	2	0	2 (4)	
Hypopharynx	4	3 (2)	1 (2)	
Racial				
African	64	43 (36)	21 (38)	1.000
White	99	67 (55)	32 (57)	
Asian	1	1 (1)		
Unknown	13	10 (8)	3 (5)	

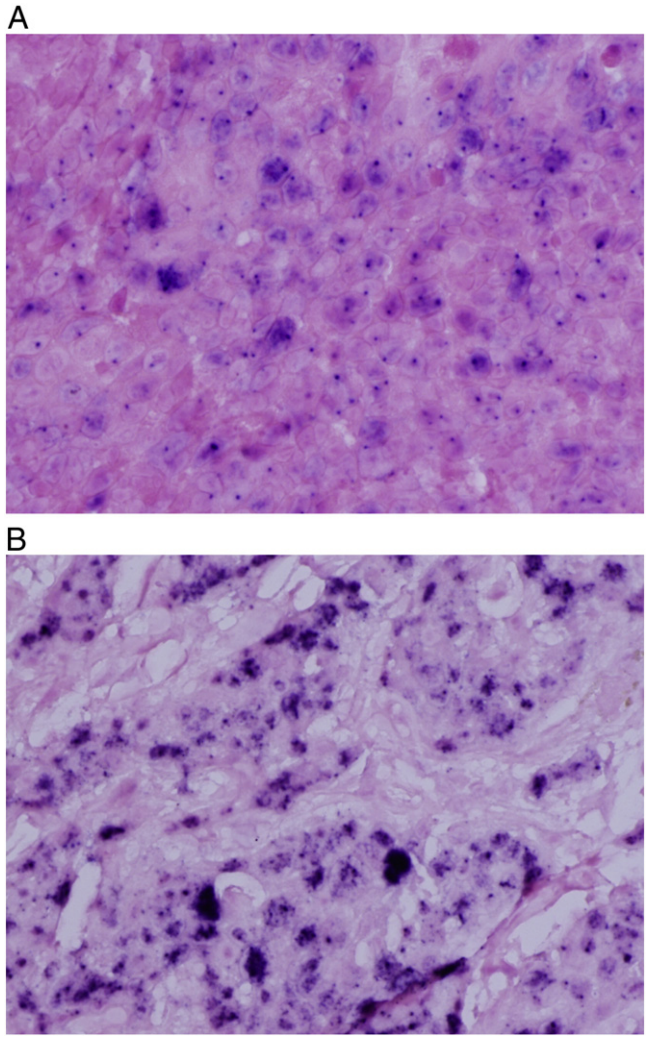

Fig. 2. Dark blue punctate (dotlike, A) or diffuse nuclear staining patterns (single or multiple intranuclear spots of variable size, B) of hrHPV-positive tumor cells (original magnification ×400).

4. Discussion

Human papillomavirus has been associated with SCCs of different sites including head and neck, and it is estimated that 85% of humans will have HPV infection of any subtype during their lifetime [9]. The frequencies of identified HPV-positive tumors among HNSCC, however, show considerable variation in published studies ranging from 0% in oral and laryngeal carcinomas to 93% in oropharyngeal carcinomas [4,10]. This variation is likely related to the tumor sites, geographic areas, and population groups as well as HPV detection methods. With regard to the detection methods, PCR is the most commonly used method, but more recent studies have used ISH or reverse transcriptase PCR [11]. Although PCR is considered more sensitive, it is more

expensive and often not routinely available in most laboratories. Human papillomavirus detection by ISH, on the other hand, is more amenable to routine use than the more sensitive but generally less specific PCR detection method. The use of ISH allows for the determination of signaling pattern as well as localization of HPV DNA in a specimen. Furthermore, the physical status of the HPV DNA can be visually determined with episomal DNA usually identified as diffuse nuclear signal and integrated DNA as punctate (dotlike) nuclear signal. Studies have also shown that ISH has a high specificity but a relatively low sensitivity compared with PCR-based assay [12]. Our findings also demonstrate that HPV ISH probe can be used to accurately visualize and localize HPV DNA within the tumor cells in biopsy specimens of HNSCC even in archival tissue. In addition, hrHPV probe in this study (Ventana INFORM III) is capable of identifying at least 9 hrHPV genotypes, if present, in tissue.

The patients' demographics in this study are consistent with other studies with HNSCC being more common in men than in women with most patients presenting after 45 years of age [13,14]. Chernock et al [15] reported 54% smoking rate in 118 patients with oropharyngeal carcinomas. Similarly, the use of tobacco and alcohol was also widespread in our patient cohort (71% smoking and 51% drinking, Table 3). Although some studies indicate that patients with HPV-positive tumors tend to be younger, no age difference is observed in our study population (Table 3).

There are indications that the prevalence of HPV-associated head and neck cancers is increasing [10], whereas the incidence of HPV-unrelated HNSCC has stabilized [16,17]. This increase might, however, be caused by increasing awareness and detection rates rather than actual increase in prevalence. The risk factors for HPV-related HNSCC are similar to those of HPV-related uterine cervical SCC, which include a high lifetime number of vaginal or oral sex partners [18,19]. Using the same detection method and technique, our data suggested that HPV-positive HNSCC appears to show an increasing trend in the last decade. In our study, the percentage of HPV-positive cases increased from 13% in the 2000-to-2004 series to 40% in the 2009-to-2010 series. The overall 13% prevalence of HPV-associated HNSCC in the earlier series might suggest a comparatively low prevalence in our population at that time. However, this could also be attributed to the low percentage of tumors from the oropharyngeal sites (17%) and a high frequency of smoking and drinking history in our patients. In spite of the limitations of the study and the small sample size of the 2009-to-2010 series, it would appear that the HPV detection rate in oropharyngeal tumors increased from 38% (9/24) to 57% (13/23) within the 2 time frames, using the same detection technique. The high percentage of oropharyngeal SCC in the later series might be a reflection of the increasing community awareness and screening coupled with the introduction of routine testing for hrHPV DNA in tumors of oropharyngeal origin in our laboratory rather than a true reflection of locoregional statistics and rising trend. However, the fact that the percentage of HPV-positive tumors at nonoropharyngeal sites remained the same at 8% (9/118 and 1/12) in the 2 periods would suggest that the increase in oropharyngeal HPV-positive tumors is real.

Human papillomavirus–associated tumors occur much more frequently in the oropharynx than other head and neck sites. A meta-analysis of HPV studies in tonsillar carcinomas by Syrjanen [20] showed a positive rate of 51% at this site. This is in contrast to other head and neck sites where the HPV prevalence has been reported to be between 20% and 25% [21]. Similar to other reports, our results indicate that HPV is strongly associated with oropharyngeal (including tonsil) SCC and that hrHPV prevalence was significantly higher in oropharyngeal SCC compared with nonoropharyngeal SCC for the 2 periods studied [22-24]. The low rate (8%) of HPV-positive nonoropharyngeal SCC in our study might reflect the smoking and drinking habits in our population.

Some reports have indicated that African Americans have a higher incidence of HNSCC with high mortality rate, which has been attributed to heavy alcohol and tobacco use and a low incidence of the better-prognosis HPV-positive HNSCC [6]. Settle et al [25] reported that HPV positivity was 4% in African Americans compared with 34% in whites among patients with oropharyngeal HNSCC, and a more recent study from Chernock et al [26] showed similar findings. Our results also indicate that HPV-positive HNSCC is less common in African American than in white patients (9% vs 22%, $P = .036$). When compared with whites, African American patients are more likely to smoke (81% vs 73%) and drink (69% vs 45%, $P = .006$). This result probably supports the assertion of Fakhry et al [6] attributing the high mortality in African Americans to the tobacco-associated HNSCC.

El-Mofty et al [27,28] have noted that HPV-related SCC of the oropharynx has unique histologic features with most being of the basaloid, nonkeratinizing subtype of SCCs. Chernock et al [15] showed that nonkeratinizing histologic morphology strongly predicts HPV-association and better patient survival compared with keratinizing SCC. The definition of keratinizing SCC and NKSCC used in this study is slightly different from the definition by El-Mofty et al and Chemock et al. Lack of keratinization was used for the morphological distinction between KSCC and NKSCC because of simplicity, consistency, and objectivity while avoiding criterion such as basaloid in the definition of NKSCC. We felt that lack of keratinization was the only constant morphological feature that distinguishes KSCC from NKSCC. Despite our liberal criterion, our results indicate that HPV-positive HNSCC including oropharyngeal SCC is more likely to be nonkeratinizing (both $P < .0001$, Table 4).

In summary, our results demonstrate an increasing trend of HPV-positive oropharyngeal SCC in the last decade. In

addition, HPV-positive HNSCC appears to be different from HPV-negative HNSCC both in its clinical and histopathologic features with white patients more likely to have HPV-positive tumor compared with African Americans and HPV-positive HNSCC is more likely to be of the nonkeratinizing type.

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