Published in final edited form as:

JAMA Oncol. 2017 February 01; 3(2): 169–177. doi:10.1001/jamaoncol.2016.3067.

Differences in the Prevalence of Human Papillomavirus (HPV) in Head and Neck Squamous Cell Cancers by Sex, Race, Anatomic Tumor Site, and HPV Detection Method

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Statistical analysis: D'Souza, Wentz, Fakhry.

Obtained funding: D'Souza, Fakhry.

Administrative, technical, or material support: Westra, van Zante, Kluz, Ryan, Ha, Eisele, Fakhry.

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Conflict of Interest Disclosures: None reported.

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Abstract

IMPORTANCE—Human papillomavirus (HPV) causes an increasing proportion of oropharyngeal squamous cell carcinomas (OPSCCs), particularly in white men. The prevalence of HPV among other demographic groups and other anatomic sites of HNSCC is unclear.

OBJECTIVE—To explore the role of HPV tumor status among women and nonwhites with OPSCC and patients with nonoropharyngeal head and neck squamous cell carcinoma (non-OP HNSCC).

DESIGN, SETTING, AND PARTICIPANTS—Retrospective cohort study at 2 tertiary academic centers including cases diagnosed 1995 through 2012, oversampled for minorities and females. A stratified random sample of 863 patients with newly diagnosed SCC of the oral cavity, oropharynx, larynx, or nasopharynx was used.

MAIN OUTCOMES AND MEASURES—Outcomes were HPV status as measured by p16 immunohistochemical analysis, HPV16 DNA in situ hybridization (ISH), and high-risk HPV E6/E7 mRNA ISH.

RESULTS—Of 863 patients, 551 (63.9%) were male and median age was 58 years (interquartile range, 51-68 years). Among 240 OPSCCs, 144 (60%) were p16 positive (p16+), 115 (48%) were HPV16 DNA ISH positive (ISH16+), and 134 (56%) were positive for any oncogenic HPV type (ISH+). From 1995 to 2012, the proportion of p16+ OPSCC increased significantly among women (from 29% to 77%; $P = .005$ for trend) and men (36% to 72%; $P < .001$ for trend), as well as

among whites (39% to 86%; $P < .001$ for trend) and nonwhites (32% to 62%; $P = .02$ for trend). Similar results were observed for ISH+ OPSCC ($P = .01$ for all). Among 623 non-OP HNSCCs, a higher proportion were p16+ compared with ISH positive (62 [10%] vs 30 [5%]; $P = .001$). A high proportion (26 of 62 [42%]) of these p16+ non-OP HNSCCs were found in sites adjacent to the oropharynx. The proportion of p16+ and ISH+ non-OP HNSCCs were similar by sex. Overtime, the proportion of non-OP HNSCCs that were p16+ (or ISH+) increased among whites ($P = .04$ for trend) but not among nonwhites (each $P > .51$ for trend). Among OPSCCs, p16 had high sensitivity (100%), specificity (91%), and positive (93%) and negative predictive value (100%) for ISH positivity. In non-OP HNSCCs, p16 had lower sensitivity (83%) and positive predictive value (40%) but high specificity (94%) and negative predictive value (99%) for ISH positivity.

CONCLUSIONS AND RELEVANCE—During 1995 through 2012, the proportion of OPSCCs caused by HPV has increased significantly. This increase was not restricted to white men but was a consistent trend for women and men, as well as for white and nonwhite racial groups. Few non-OP HNSCCs were HPV related. P16 positivity was a good surrogate for ISH+ tumor status among OPSCC, but not a good surrogate for non-OP HNSCC.

The epidemiology of head and neck squamous cell cancer (HNSCC) has changed dramatically in recent decades in the United States¹ and other countries.² The incidence of oral cavity and laryngeal squamous cell cancers has declined, while the incidence of oropharyngeal squamous cell carcinoma (OPSCC) has increased.³ The dramatic increase in recent decades in incidence of OPSCC has mostly been observed among white men younger than 60 years.^{4,5} In the United States, during the same time interval, OPSCC incidence decreased among women, older men, and black men and has remained stable in Asians and Hispanics.^{1,4,6} More recently, between 2000 and 2009, OPSCC incidence has increased among white women, as well as white men.⁶ Importantly, the increasing incidence of OPSCC in white men appears to be driven by high-risk human papillomavirus (HPV), particularly HPV16.^{7,8}

Although our understanding of the role of HPV in OPSCC has evolved over the past 10 years, several areas of uncertainty remain. The lower incidence of OPSCC among women and nonwhites is well recognized; however, the proportion of HPV-related OPSCCs among these groups has not been well examined. First, most studies to date have included only limited numbers of women and nonwhites and thus had imprecise measures of HPV prevalence.⁸⁻¹¹ Second, most studies evaluating trends by sex and race have used cancer registries, which lack HPV tumor status information.^{1,2,4,5,12,13} Last, methods of HPV detection have varied considerably across studies, with many HNSCC studies relying on nonquantitative polymerase chain reaction-based detection strategies known to overestimate the true prevalence of HPV-related tumors. This tendency to overestimate the prevalence of HPV has confounded efforts to clarify the role of HPV as a true etiologic agent in HNSCCs arising outside the oropharynx (OP). Therefore, this study was conducted to better understand the impact of HPV in HNSCCs among women and nonwhites and nonoropharyngeal (non-OP) anatomic sites of HNSCC.

Methods

This was a retrospective study of incident HNSCC cases diagnosed between 1995 and 2012 at 2 comprehensive cancer centers, the Johns Hopkins Hospital Sydney Kimmel Comprehensive Cancer Center (JHH) (Baltimore, Maryland) and the University of California-San Francisco Helen Diller Family Comprehensive Cancer Center and affiliated hospitals (UCSF). This study was approved by the institutional review board at each participating institution. Informed consent was waived by the institutional review board due to the retrospective nature of the study. A database of all diagnosed SCC cases of the OP, oral cavity, nasopharynx, and larynx was created using institutional cancer registries, stratified by sex and race. Cases from each tumor site were randomly sampled (when possible) in sex and race groups of interest to oversample cases occurring in minorities and women. Cases in Asian and Hispanic patients could not be randomly sampled due to the lower overall numbers and were therefore all selected for inclusion.

All medical records were reviewed to ascertain patient demographic characteristics (sex, race, age), tumor site, tumor characteristics including specifically whether OP adjacent or nonadjacent, and lifetime and current tobacco and alcohol use at diagnosis. Race was categorized based on race and ethnicity reported in the institutional cancer registry and/or medical record. Oropharynx adjacent was defined as HNSCC subsites within proximity to the OP including posterior tongue, posterolateral tongue, retromolar trigone, epiglottis, and overlapping lesions including OP. Lifetime use was defined as ever regular (at least weekly) use of any tobacco or alcohol product. Current use was defined as use within the month before diagnosis, as recorded in the medical record.

There were 1345 patients originally sampled including 481 women and 864 men. This included 176 OP, 174 oral cavity, 70 nasopharynx, and 199 larynx cancers at JHH and 163 OP, 198 oral cavity, 198 nasopharynx, and 167 larynx cancers at UCSF. Among these cases, 863 (64%) had a pathologic sample available for testing and were therefore considered eligible for study. The proportion of eligible samples tested was similar for men (64%) and women (66%) and for Asian (54%), Hispanic (68%), black (69%), and white (67%) patients. All eligible samples were tested for HPV-related markers, as described in the next subsection.

Testing Methods

Hematoxylin-eosin slides were reviewed by a head and neck pathologist (W.H.W.) at JHH and UCSF. Histologic subtype was confirmed, and relevant paraffin-embedded slides or paraffin blocks were used for HPV detection. All HPV detection was performed centrally by the JHH pathology laboratory and interpreted by a single head and neck pathologist (W.H.W.). Human papillomavirus status was determined using an algorithm that incorporates both p16 immunohistochemical analysis (MTM Laboratories), HPV16 DNA in situ hybridization (ISH) (Dako GenPoint), and high-risk HPV E6/E7 mRNA ISH (called RNA ISH; RNAscope, Advanced Cell Diagnostics). P16 expression was scored as positive if strong and diffuse nuclear and cytoplasmic staining was present in at least 70% of the tumor.

For HPV16 DNA ISH (hereafter ISH16), punctate hybridization signals localized to the tumor cell nuclei defined a HPV16-positive tumor. Among 73 tumors that were p16 positive but ISH16 negative, additional testing was performed using an RNA ISH probe targeting 18 high-risk HPV genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82).¹⁴ Only 26 (36%) of these cases were RNA ISH positive; these 26 RNA ISH-positive cases were combined with DNA ISH16-positive cases into an “ISH-positive” category.

Statistical Analysis

Characteristics of patients at each study site were summarized with descriptive statistics. Race, sex, and age were obtained from institutional cancer registries and confirmed using medical record abstraction for each case. Race and ethnicity were categorized as white non-Hispanic, black non-Hispanic, Asian non-Hispanic, and Hispanic any race, and referred to as white, black, Asian, and Hispanic hereafter. Patients of other races were not sampled due to insufficient numbers.

The prevalence of p16 positivity, ISH16 positivity, and ISH positivity were described by tumor site, sex, and race. Differences in prevalence were calculated using the χ^2 test. Performance characteristics (sensitivity, specificity, positive predictive value, and negative predictive value) of p16 status were compared with ISH status. The benchmark for a tumor to be considered HPV related was ISH positivity, which included being HPV16 positive by DNA ISH testing or high-risk HPV positive by RNA ISH testing. For some analyses, oral cavity, laryngeal, and nasopharyngeal tumors were combined and referred to as non-OP HNSCC. The prevalence of p16-positive and ISH-positive HNSCC was explored over calendar time, by sex and race, and stratified by OPSCC or non-OP HNSCC.

Results

This analysis included 863 HNSCCs, including cancers of the OP (n = 240), oral cavity (n = 253), larynx (n = 245), and nasopharynx (n = 125). Characteristics of OPSCC and non-OP HNSCC cases are presented in Table 1. Patients were 64% male and included white (36.7%), black (32.2%), Asian (19.6%), and Hispanic (11.5%) participants. Median age was 58 years (interquartile range, 51-68 years), and most patients had a history of ever tobacco and alcohol use (Table 1). As expected, the proportion of never smokers (36% vs 6%; $P < .001$) and never drinkers (34% vs 9%; $P < .001$) was higher among patients with ISH-positive than ISH-negative OPSCC, but this difference was not observed among patients with non-OP HNSCC. This study included 437 cases diagnosed at JHH and 426 cases diagnosed at UCSF (eTable in the Supplement).

The Prevalence of HPV Among Women and Nonwhite Patients With OPSCC

Among 240 OPSCCs diagnosed during 1995 through 2012, 60% were p16 positive, 134 (56%) were ISH positive, and 115 (48%) were ISH16 positive ($P = .44$). The majority of OPSCCs in both women (44 [56%]) and men (100 [62%]) were p16 positive ($P = .34$) (Table 2). When considering race, the majority of OPSCC cases were p16 positive among whites (73 [71%]), Asians (18 [86%]), and Hispanics (15 [71%]; $P = .37$).

However, only 38 [40%] blacks with OPSCC were p16 positive, which was significantly lower than among whites, Asians, or Hispanics ($P < .001$). When considering ISH16 positivity, or ISH positivity, analogous sex and race-based patterns were observed. Prevalence of ISH positivity was similar in men and women, and the majority of OPSCCs in white, Asian, and Hispanic patients were ISH positive. However, as with p16, a lower proportion of OPSCCs in black patients were ISH positive when compared with other racial groups ($P < .001$) (Table 2).

Temporal trends in p16 and ISH positivity for OPSCC cases were examined. A notable increase in the proportion of p16-positive OPSCCs was observed over time among cases in both sexes and every race (Figures 1A and 2A). From 1995 to 2012, the proportion of p16-positive OPSCCs increased significantly not only among men (from 36% to 72%; $P < .001$ for trend) but also among women (from 29% to 77%; $P = .005$ for trend) (Figure 1A). During this period, the proportion of OPSCCs that were p16 positive also increased significantly for whites (from 39% to 86%; $P < .001$ for trend) and nonwhites (from 32% to 62%; $P = .02$ for trend) (Figure 2A). There was a roughly 2-fold increase in the proportion of OPSCCs that were p16 positive among blacks (from 27% to 50%), Hispanics (from 33% to 80%), and Asians (from 60% to 100%). Temporal changes were similar when considered for ISH positivity (Figures 1B and 2B), or when combining p16-positive and/or ISH-positive tumors (results not shown).

The Prevalence of HPV in Non-OP HNSCC Cases

Among 623 non-OP HNSCCs, 62 [10%] were p16 positive, 30 [5%] were ISH positive, and 25 [4%] were ISH16 positive. For non-OP HNSCCs, p16 positivity (range, 4%-24%) and ISH positivity (range, 0%-24%) were consistently low among both women and men, and among all racial groups examined (Table 2). Of the non-OP HNSCC cases that appeared to be HPV related, a high proportion of the p16-positive (26 of 62 [42%]) and ISH-positive (12 of 30 [40%]) cases were OP adjacent. Of the 26 p16-positive OP-adjacent non-OP HNSCC cases, most were in the nasopharynx ($n = 10$ [38%]) or larynx ($n = 10$ [38%]), and included T1 or T2 (10 of 25 [40%]) and T3 or T4 (15 of 25 [60%]) cases. When considering the 12 ISH-positive OP-adjacent non-OP HNSCC cases, most were in the nasopharynx ($n = 9$ [75%]), and 6 of 11 (54%) were T3 or T4.

For all non-OP HNSCC anatomic sites, a higher proportion of tumors were p16 positive than ISH positive, including the 253 oral cavity (6% vs 2%; $P = .02$), 245 larynx (13% vs 5%; $P = .002$), and 125 nasopharynx (12% vs 10%; $P = .69$) tumors. While p16 prevalence at each of these anatomic sites was considerably lower than among OPSCC, half (52% [32 of 62]) of the p16-positive non-OP HNSCCs identified were laryngeal.

Among the non-OP HNSCCs, p16 positivity was generally similar by sex and race (Table 2). Similar results were observed when ISH positivity was considered (Table 2). The only sex difference observed was in laryngeal cancer, in which prevalence of ISH positivity (11% vs 2%; $P = .003$) but not p16 positivity (18% vs 11%; $P = .16$) was higher among women than men. The only racial difference observed was in nasopharynx cancer, in which p16 positivity (24% vs 6%; $P = .006$) and ISH positivity (24% vs 4%; $P < .001$) were higher among whites than nonwhites.

In contrast to the increasing proportion of HPV-related OPSCCs over time, among non-OPHNSCCs, p16-positive ($P = .11$ for trend) and ISH-positive ($P = .36$ for trend) prevalence was stable from 1995 to 2012 (Figures 1 and 2). Stable prevalence trends for p16 positivity and ISH positivity were also observed among non-OP HNSCC cases among men, women, and nonwhite patients (Figures 1C, 1D, 2C, and 2D). The notable exception was among non-OP HNSCCs in white patients, in whom the prevalence of p16 positivity (from 6% to 19%; $P = .04$) and ISH positivity (from 2% to 14%; $P = .06$) increased from 1995 to 2012 (Figure 2C and D).

Performance Characteristics of p16 as a Surrogate Marker for ISH-Positivity

Among the 73 p16-positive ISH16-negative samples, only 26 (36%) were positive by RNA ISH. Among OPSCCs, p16 positivity had high sensitivity (100%), specificity (91%), positive predictive value (93%), and negative predictive value (100%) when compared with ISH positivity (Table 3). For non-OP HNSCCs, p16 had reduced sensitivity (83%) and positive predictive value (40%), although specificity (94%) and negative predictive value (99%) for ISH positivity remained high. Results were similar when performance characteristics of p16 for ISH16 were considered, with strong sensitivity and positive predictive value among OPSCCs (100% and 79%, respectively) but not among non-OP HNSCC (78% and 30%, respectively). There was strong agreement between tumor p16 and ISH positivity for OPSCCs ($\kappa = 0.92$; 95% CI, 0.86-0.97), whereas for non-OP HNSCCs agreement was moderate ($\kappa = 0.51$; 95% CI, 0.39-0.64).

Discussion

Prevalence estimates of HPV in OPSCC in the United States have been based on study populations consisting primarily of white men.^{7,8} Prevalence and trends of HPV-related OPSCC among women and nonwhites have not yet been well described. This multi-institutional study demonstrates a pronounced increase in the proportion of OPSCC cases caused by HPV in women and nonwhites over almost 2 decades. These data also address a knowledge gap regarding the role of HPV in non-OP HNSCCs. Using a large sample size, the rare detection of HPV in non-OP HNSCCs is demonstrated with a rigorous HPV detection strategy. This analysis suggests that p16 is a reliable surrogate for HPV tumor status only in the OP but not in non-OP HNSCCs. This is one of the first studies to explore the role of HPV in women, nonwhites, and non-OPSCCs in a large sample size in previously underreported groups with centralized testing and data spanning across 2 decades.

Population-based data have consistently demonstrated a lower incidence and prevalence of HPV-related OPSCC among women than men.^{6,8} This study shows that despite distinct incidence trends by sex, the prevalence of HPV among OPSCCs is comparable in women and men. Indeed, a significant increase in the prevalence of HPV in OPSCC over the past 2 decades is shown for women. This suggests that for the first time, HPV is now the primary cause of OPSCCs for both women and men in the United States. This is similar to recent European data, which also reported a large proportion of HPV-related OPSCCs in women, although that study reported that the proportion of OPSCCs that were HPV related was higher in women than men.¹⁵

There is a paucity of literature on HPV-related OPSCCs among blacks that include HPV tumor detection.^{8,10,11,16} Only 2 studies have evaluated OPSCC among Asians or Hispanics in the United States, and neither tested for HPV tumor status.^{9,17} Whereas the incidence of HPV-related OPSCCs remains lower among nonwhites than whites in the United States, the proportion of OPSCCs in Asians and Hispanics that were HPV related in this study was similar to that observed in whites. To our knowledge, this is the first report of the high prevalence of HPV among US Asians and Hispanics with OPSCCs. These data likely reflect that, similar to white men, in whom increasing oral HPV exposure¹⁸ by sexual behavior is responsible for increasing HPV-related OPSCCs,^{2,17} similar exposure to HPV may also be causing increasing HPV-related OPSCCs in Asians and Hispanics in the United States. In contrast, the prevalence of HPV in OPSCCs was notably lower among blacks. Lower prevalence of HPV in black patients with OPSCC may be explained by differences in sexual behavior and tobacco use.^{18,19} This study includes the largest number of black patients with OPSCC to date, all of whom received a diagnosis in recent decades. Only 1 previous study evaluated HPV among black patients with OPSCC with both p16 and ISH,⁸ and included 37 cases diagnosed from 1984 to 2004. Three other studies examined OPSCCs in blacks (each included 50-70 cases) using polymerase chain reaction-based methods.^{10,11,16} This study provides the first stable estimates of HPV prevalence in OPSCCs among blacks, and a first glimpse into the prevalence of HPV in OPSCCs in Asians and Hispanics in the United States.

Temporal changes in incidence of OPSCC among white men have been well documented in the United States^{4,8} and internationally^{2,15,20} and are due to increasing prevalence of HPV-related OPSCC.^{8,20} However, sex- and race-stratified trends in prevalence of HPV-related OPSCC over time have previously been unexplored. Indeed, this study shows that the prevalence of HPV-related OPSCC has significantly increased over time among women, as well as men. In addition, among nonwhites, the prevalence of HPV in OPSCC also appeared to increase over time, although the numbers were small within each period, and in some groups these trends were not statistically significant. The proportion of OPSCCs in blacks that were HPV related increased 2-fold over the 17-year time frame explored, although the trend was not statistically significant.

While the importance of HPV in the epidemiology of OPSCC was clear among all sex and racial groups, the role of HPV in non-OP HNSCC was limited. Indeed, the proportion of HPV-related non-OP HNSCC cases appeared to be low for all anatomic sites and subgroups explored. The subset of non-OP HNSCCs that did appear to be HPV related were primarily OP adjacent, suggesting that HPV-related non-OP HNSCCs may arise from the “ectopic” nests of lymphoid tissue found throughout the aerodigestive tract²¹ and may afford the opportunity for HPV-related tumors to arise in some non-OP sites. Some previous studies have reported HPV-related non-OP HNSCCs,^{15,22,23} although the influence of anatomic site misclassification on the prevalence was unclear. A strength of the present study is that tumor site classification was individually reviewed by clinicians at both institutions to verify the anatomic site and determine whether it was adjacent to the OP, thus reducing this potential bias. However, identification of the originating subsite for tumors involving contiguous anatomic sites can remain difficult. For example, 6 of the 26 OP-adjacent non-OP HNSCCs that were p16 positive were overlapping lesions involving an OP subsite. Consequently, the

possibility of site misclassification cannot be entirely excluded. Prevalence estimates for HPV in non-OP HNSCC in this study are similar to that reported in a recent large international study,¹⁵ although there are differences in the testing algorithm.

When the performance characteristics of p16 and ISH tumor testing were evaluated, the sensitivity and specificity of p16 for ISH positivity was high among OPSCC cases, and trends were comparable when either test was used for OPSCC cases. In contrast, among non-OPSCCs, p16 had lower sensitivity and a poor positive predictive value for ISH positivity. Prior studies have shown that prevalence of p16 is higher than ISH in non-OPSCC, a discordance that is greater in non-OPSCC than OPSCC.²⁴ Among non-OPSCC cases, p16 lacks specificity for ISH positivity. For non-OP HNSCC, it is possible that elevated levels of p16 may reflect the biologic characteristics of the tumor itself rather than the HPV status.

Limitations

This study has several limitations and strengths. First, demographic and clinical information was abstracted retrospectively from hospital records; therefore, we cannot exclude the possibility that race or ethnicity could have been misclassified in some participants. Second, overall and site-specific estimates of HPV prevalence may not be representative of cases at those anatomic sites because we oversampled cases occurring in minorities and women for inclusion. However, sex- and race-based estimates of HPV are accurate. Strengths of this study are that HPV detection assays were performed centrally, and oversampling of minorities allowed testing of larger numbers of cases in these previously poorly studied groups. Methods of HPV detection have evolved during the study period, which is reflected by the use of RNA ISH and DNA ISH testing. While RNA ISH would ideally have been performed on all samples, we believe that the algorithm used is rigorous, yet practical. This research adds to our understanding of the prevalence of HPV among women and US minorities. Our results confirm that both p16 and HPV ISH tests are accurate tests for identifying HPV-related OPSCC.¹⁴ It also emphasizes the importance of clinical awareness that HPV is an important cause of OPSCC among women and nonblack minorities in the United States.

This research demonstrates that HPV is an important but previously underappreciated cause of OPSCC among women and minorities in the United States. These results also inform clinical practice by suggesting that both p16 and HPV ISH tests are accurate tests for identifying HPV-related OPSCC.

Conclusions

Human papillomavirus is an increasingly important cause of OP cancer, not just among white men, but among women and nonwhites as well. Human papillomavirus caused a small proportion of non-OP HNSCC and was not a good surrogate for HPV among non-OP HNSCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding/Support: This study was supported by grant P50DE019032 from the National Institute of Dental and Craniofacial Research.

Role of the Funder/Sponsor: The National Institute of Dental and Craniofacial Research had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Key Points

Question Does human papillomavirus (HPV) cause a similar proportion of cancers among women, Asians, Hispanics, and blacks, as it does among men and white oropharyngeal cancers, and what is its role in nonoropharyngeal head and neck cancers?

Findings In this cohort study of 863 patients with newly diagnosed squamous cell cancer of the oral cavity, oropharynx, larynx, or nasopharynx, HPV is the cause of most oropharyngeal cancers in women as well as men, whites, Asians, Hispanics, and blacks. From 1995 to 2012 the proportion of oropharyngeal (but not nonoropharyngeal) head and neck cancers caused by HPV increased among all sex and race groups.

Meaning HPV is an important cause of oropharyngeal cancer, not just among white men, but among women and nonwhites as well.

Figure 1. Prevalence of Human Papillomavirus When Measured by p16 Immunohistochemical Positivity or by In Situ Hybridization (ISH) Positivity Among Patients With Oropharyngeal Squamous Cell Cancer (OPSCC) and Nonoropharyngeal Head and Neck Squamous Cell Cancer (non-OP HNSCC) (Oral Cavity, Larynx, and Nasopharynx Carcinoma) Over Time, by Sex

A, *P* for trend for p16 positivity among OPSCC cases was .005 for women and <.001 for men. B, *P* for trend for ISH positivity among OPSCC cases was .01 for women and <.001 for men. C, *P* for trend for p16 positivity among non-OP HNSCC cases was .33 for women and .20 for men. D, *P* for trend for ISH positivity among non-OP HNSCC cases was .75 for women and .12 for men.

Figure 2.

Prevalence of Human Papillomavirus When Measured by p16 Immunohistochemical Positivity or by In Situ Hybridization (ISH) Positivity Among Patients With Oropharyngeal Squamous Cell Cancer (OPSCC) and Nonoropharyngeal Head and Neck Squamous Cell Cancer (non-OP HNSCC) (Oral Cavity, Larynx, and Nasopharynx Carcinoma) Over Time, by Race and Ethnicity (Black Non-Hispanic, White Non-Hispanic, Hispanic of Any Race, and Asian Non-Hispanic)

A, *P* for trend for p16 positivity among OPSCC cases was .16, <.001, .30, and .04 for 95 blacks, 103 whites, 21 Hispanics, and 21 Asians, respectively. B, *P* for trend for ISH positivity among OPSCC cases was .09, .002, .33, and .04 for blacks, whites, Hispanics, and Asians, respectively. C, *P* for trend for p16 positivity among non-OP HNSCC cases was .93, .04, .82, and .27 for 183 blacks, 214 whites, 78 Hispanics, and 148 Asians, respectively. D, *P* for trend for ISH positivity among non-OP HNSCC cases was .60, .06, .61, and .93 for blacks, whites, Hispanics, and Asians, respectively.

Table 1. Characteristics of Study Population of Oropharyngeal Squamous Cell Carcinoma (OPSCC) and Nonoropharyngeal Head and Neck Squamous Cell Carcinoma (Non-OP HNSCC) Cases

Characteristic	No. (%)		
	All (N = 863)	OPSCC (n = 240)	Non-OP HNSCC (n = 623)
Study site			
Johns Hopkins Hospital	437 (50.6)	146 (60.8)	291 (46.7)
University of California-San Francisco	426 (49.4)	94 (39.2)	332 (53.3)
Sex			
Male	551 (63.9)	161 (67.1)	390 (62.6)
Female	312 (36.2)	79 (32.9)	233 (37.4)
Race			
White non-Hispanic	317 (36.7)	103 (42.9)	214 (34.4)
Black non-Hispanic	278 (32.2)	95 (39.6)	183 (29.4)
Asian non-Hispanic	169 (19.6)	21 (8.8)	148 (23.8)
Hispanic (any race)	99 (11.5)	21 (8.8)	78 (12.5)
Age, median (IQR), y	58 (51-68)	57 (51-64)	59 (51-69)
Year of diagnosis			
1995-1999	187 (21.7)	56 (23.3)	131 (21.0)
2000-2004	241 (27.9)	56 (23.3)	185 (29.7)
2005-2009	278 (32.2)	86 (35.8)	192 (30.8)
2010-2012	157 (18.2)	42 (17.5)	115 (18.5)
Anatomic site			
Oropharynx	240 (27.8)	240 (100)	0
Oral cavity	253 (29.3)	0	253 (40.6)
Larynx	245 (28.4)	0	245 (39.3)
Nasopharynx	125 (14.5)	0	125 (20.1)
Tumor stage			
T1	217 (25.1)	67 (27.9)	150 (24.1)
T2	220 (25.5)	72 (30.0)	148 (23.8)
T3	157 (18.2)	45 (18.8)	112 (18.0)

Characteristic	All (N = 863)	OPSCC (n = 240)	Non-OP HNSCC (n = 623)
T4	221 (25.6)	48 (20.0)	173 (27.8)
Missing or unknown ^a	48 (5.6)	8 (3.3)	40 (6.4)
Nodal stage			
N0	333 (38.6)	35 (14.6)	298 (47.8)
N1	121 (14.0)	30 (12.5)	91 (14.6)
N2a-c	307 (35.6)	149 (62.1)	158 (25.3)
N3	44 (5.1)	17 (7.1)	27 (4.3)
Missing or unknown ^a	58 (6.7)	9 (3.8)	49 (7.9)
Metastatic stage			
M0	818 (94.8)	225 (93.8)	593 (95.2)
M1	24 (2.8)	11 (4.6)	13 (2.1)
Missing or unknown ^a	21 (2.4)	4 (1.7)	17 (2.7)
Current tobacco use			
No, never	160 (18.5)	46 (19.2)	114 (18.3)
No, former	247 (28.6)	75 (31.3)	172 (27.6)
Yes	245 (28.4)	69 (28.8)	176 (28.3)
Missing or unknown ^a	211 (24.5)	50 (20.8)	161 (25.8)
Current alcohol use			
No, never	200 (23.2)	44 (18.3)	156 (25.0)
No, former	125 (14.5)	39 (16.3)	86 (13.8)
Yes	312 (36.2)	102 (42.5)	210 (33.7)
Missing or unknown ^a	226 (26.2)	55 (22.9)	171 (27.5)

Abbreviation: IQR, interquartile range.

^a Characteristics were abstracted from medical record data and institutional cancer registries and were missing or unknown for some cases, as indicated. Excluding these missing data, 76% were ever smokers, 38% were current smokers, 69% were ever drinkers, and 49% were current drinkers at diagnosis. Patients with missing data on tobacco and alcohol use were similar to other patients in terms of sex, age, and stage but were more likely to have received a diagnosis between 1995 and 2004.

Table 2.

Prevalence of p16 and Any Oncogenic Human Papillomavirus (HPV) by In Situ Hybridization (ISH) Among Patients With Oropharyngeal Squamous Cell Carcinoma (OPSCC) and Nonoropharyngeal Head and Neck Squamous Cell Carcinoma (Non-OP HNSCC), by Sex and Race

Characteristic	OPSCC				Non-OP HNSCC			
	p16 Positivity		ISH Positivity		p16 Positivity		ISH Positivity	
	No./Total No. (%)	P Value	No./Total No. (%)	P Value	No./Total No. (%)	P Value	No./Total No. (%)	P Value
Sex								
Women	44/79 (56)		41/79 (52)		24/233 (10)		13/233 (6)	
Men	100/161 (62)	.34	93/161 (58)	.39	38/390 (10)		17/390 (4)	.82
Race and ethnicity								
White non-Hispanic	73/103 (71)		71/103 (69)		29/214 (14)		17/214 (8)	
Nonwhite	71/137 (52)		63/137 (46)		33/409 (8)		13/409 (3)	
Black non-Hispanic	38/95 (40)	<.001 ^a	34/95 (36)	<.001 ^a	16/183 (9)		7/183 (4)	.07 ^a
Asian non-Hispanic	18/21 (86)	.003 ^b	18/21 (86)	.001 ^b	8/148 (5)		3/148 (2)	.03 ^b
Hispanic (any race)	15/21 (71)		11/21 (52)		9/78 (12)		3/78 (4)	
Total	144/240 (60)		134/240 (56)		62/623 (10)		30/623 (5)	
					15/253 (6)		5/253 (2)	
					32/245 (13)		12/245 (5)	
					15/125 (12)		13/125 (10)	
								.002

Abbreviations: NP, nasopharynx; OC, oral cavity.

^a P value to indicate comparison across all race/ethnicity categories.

^b P value to indicate comparison of white vs nonwhite.

Performance Characteristics of p16 Immunohistochemical Analysis (IHC) Tumor Results Compared With In Situ Hybridization (ISH)^a for All Tumor Sites and When Stratified Into Oropharyngeal Squamous Cell Carcinoma (OPSCC) and Nonoropharyngeal Head and Neck Squamous Cell Carcinoma (Non-OP HNSCC)

Table 3.

		% (95% CI)					
ISH						Predictive Value	
p16 IHC		Negative	Positive	Sensitivity	Specificity	Positive	Negative
All HNSCC							
Negative	652	5	97 (94-100)	93 (91-95)	77 (71-83)	99 (99-100)	
Positive	47	159					
OPSCC							
Negative	96	0	100	91 (85-96)	93 (89-97)	100	
Positive	10	134					
Non-OP HNSCC							
Negative	556	5	83 (70-97)	94 (92-96)	40 (28-53)	99 (98-100)	
Positive	37	25					

^aRepresents positivity for any oncogenic human papillomavirus type.