



Published in final edited form as:

Cancer. 2017 January 01; 123(1): 71–80. doi:10.1002/cncr.30312.

Oral health and HPV-associated head and neck squamous cell carcinoma

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Abstract

Background—Indicators of poor oral health, including smoking, have been associated with increased risk of head and neck squamous cell carcinoma, especially oropharyngeal carcinoma (OPSCC), yet few studies have examined whether this association is modified by HPV-status.

Methods—We used interview and tumor HPV-status data from a large population-based case-control study, the Carolina Head and Neck Cancer Study (CHANCE), to estimate the association between oral health indicators and smoking among 102 HPV-positive and 145 HPV-negative OPSCC cases and 1396 controls. HPV-status was determined by immunohistochemistry of p16^{INK4a} expression. Unconditional multinomial logistic regression was used to estimate odds ratios (OR) for all oral health indicators adjusting for important covariates.

Results—Routine dental exams were associated with decreased risk of both HPV-negative [OR: 0.52; 95% confidence interval (CI): 0.35-0.76] and HPV-positive OPSCC (OR: 0.55; 95% CI: 0.36-.86). Tooth mobility (a proxy for periodontal disease) increased the risk of HPV-negative (OR: 1.70; 95% CI: 1.18-2.43) slightly more than HPV-positive OPSCC (OR: 1.45; 95% CI: 0.95-2.20). Ten or more pack-years of cigarette smoking was strongly associated with increased

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There are no conflicts of interest to disclose.

Author Contributions

Conceptualization: JPZ, ALM, AFO; **Investigation:** PB, MCW, DA, BAA, AFO; **Formal Analysis:** ALM; **Writing—Original Draft:** ALM, JMT; **Writing—Review & Editing:** All Authors; **Supervision:** AFO, JPZ, KD

risk of HPV-negative OPSCC (OR: 4.26; 95% CI: 2.85-6.37) and less so with HPV-positive OPSCC (OR: 1.62; 95% CI: 1.10-2.38).

Conclusions—While HPV-positive and HPV-negative HNSCC differ significantly with respect to etiology and tumorigenesis, our findings suggest a similar pattern of association between poor oral health, frequency of dental examinations, and both HPV-positive and HPV-negative OPSCC. Future research is required to elucidate interactions between poor oral health, tobacco use, and HPV in the development of OPSCC.

Precis

Our findings suggest that poor oral health represents a common risk factor for both HPV-positive and HPV-negative oropharyngeal cancers. Future research is required to elucidate interactions between poor oral health, tobacco use, and HPV in the development of oropharyngeal cancer.

Keywords

Human Papilloma Virus; Oral Health; Oropharyngeal Cancer; Dental Visits; Periodontal Disease; Head and Neck Cancer; Smoking

Introduction

Cancers of the head and neck comprise of a heterogeneous group of malignancies in which more than 90% are squamous cell carcinomas arising from the mucosal lining of the upper aerodigestive tract.^{1, 2} Over the last twenty years, the epidemiology of head and neck squamous cell carcinoma (HNSCC) has dramatically changed as a result of its association with human papillomavirus (HPV). Historically, cancers of the head and neck have been most strongly associated with tobacco and alcohol use.³ More recently, evidence has estimated that 60 to 70% of oropharyngeal squamous cell carcinomas (OPSCC) in the US are associated with human papillomavirus (HPV) infection.⁴ In the United States (US), the incidence of HPV-positive OPSCC increased by 225% between 1988 and 2004.⁵ Compared with HPV-negative OPSCC, HPV-positive OPSCC have distinct risk factor profiles and improved oncologic outcomes.⁶ Additionally, HPV-positive OPSCC has a different genetic profile and a different pathway to malignancy than HPV-negative OPSCC,⁷ suggesting that HPV-positive OPSCC is a different disease. Recent data from the Centers for Disease Control estimates that over 15,000 HPV-associated OPSCCs are diagnosed annually in the US and is the only head and neck cancer sites to have increased in incidence.⁸ As the majority of the reported risk factors for HNSCC were established before HPV-status was commonly tested, it is imperative that traditional risk factors be reassessed with reference to HPV-status.

Previous studies have examined the association between oral hygiene, dental health, and HNSCC. Oral health indicators including poor dental health, tooth loss, lack of routine dental care by a dentist and a diagnosis of periodontitis have been associated with HNSCC.^{9–13} Two studies reported that periodontitis is associated with an increased risk of OPSCC without taking into account HPV-status.^{10, 14} Only two case-only studies have examined the association between oral health and malignancy by comparing HPV-positive

OPSCC with HPV-negative OPSCC. Both studies reported a positive association between periodontitis and HPV-positive OPSCC when compared with HPV-negative OPSCC.^{15, 16} It has been postulated that the association between poor oral health and cancer risk may be driven by a chronic inflammatory state that alters the natural course of HPV infection, as has been demonstrated in cervical cancer.^{11, 12, 15}

In this study, we used a large population-based head and neck cancer case-control study to evaluate the association between oral health, frequency of dental examinations, and HNSCC. We further explored this association by HPV-status in OPSCC to determine if the oral health association is modified by HPV. We hypothesize that poor oral health indicators, including smoking, will be associated with increased risk of OPSCC regardless of tumor HPV-status.

Methods

Study Population

The Carolina Head and Neck Cancer Study (CHANCE) is a population-based case-control study in North Carolina.¹² Cases were eligible for CHANCE if diagnosed with first primary squamous cell carcinoma of the oral cavity, pharynx, and larynx between January 1, 2002 and February 28, 2006, were aged 20 to 80 years at diagnosis, and resided in a 46-county region in central North Carolina. Benign tumors, carcinomas *in situ*, thyroid papillary carcinomas, and adenoid cystic carcinomas were excluded. Lip and hypopharynx cancers, cases for which the hospital would not release tumor blocks, and cases for which proxy interviews were completed were excluded from p16 immunohistochemistry. All cases of oropharyngeal cancer (N = 248) and, random sample of non-oropharyngeal cancers (N = 244), since the relevance of HPV in non-oropharynx has not been established,¹⁷ were selected for the evaluation of the p16. Sex, age, and race frequency-matched controls were identified through the North Carolina Department of Motor Vehicle records and were frequency-matched with cases on age, race, and sex. The study was approved by the institutional review board at the University of North Carolina at Chapel Hill.

Exposure assessment

Oral health was assessed using a structured questionnaire during an in-home visit for both cases and controls conducted by trained nurse-interviewers.¹² Although the interview was conducted after diagnosis (average time between diagnosis to interview: 5.3 months), for cases the questionnaire specifically asked about dental health and care prior to cancer diagnosis. Self-reported oral health variables included: 1) self-reported number of natural teeth lost excluding third molars and teeth extracted due to orthodontic reasons; 2) history of self-reported tooth mobility or ‘teeth loose in their socket due to disease’; 3) one or more routine (non-emergency) dental visits during the decade prior to HNSCC diagnosis; and 4) gum disease diagnosed by dentist. History of smoking, dichotomized at 10 pack-years was also included since it is an important risk factor for poor oral health and OPSCC.

Questionnaire and Clinical Assessment

Demographic, lifestyle, diet, and other risk factor information were also collected during in-home interview. Confounders to be adjusted for in statistical models were selected *a priori*

based on their potential association with survival and HPV-status. In addition to the race, sex and age matching factors, confounders obtained from the questionnaire included: education, annual income, number of sexual partners, and alcohol consumption.

Clinical information such as tumor site was abstracted from the subjects' medical records and reviewed independently by a pathologist and head neck cancer surgeon. Tumors were classified by site: Oral cavity (ICD-O-3 topography codes: C02.0-C02.3, C03.0, C03.1, C03.9, C04.0, C04.1, C04.8, C04.9, C05.0, C06.0-C06.2, C06.8, and C06.9), larynx (C32.0-C32.3, and C32.8-C32.9), hypopharynx (C13.0, C13.1, C13.2, C13.8, and C13.9) and oropharynx (C01.9, C02.4, C05.1, C05.2, C09.0, C09.1, C09.8, C09.9, C10.0-C10.4, C10.8, and C10.9).

HPV status

The International Agency for Research on Cancer (IARC) performed the p16 immunocytochemistry evaluation according to the protocol provided with the CINtec Histology p16^{INK4a} Kit (9511, mtmlabs) for the qualitative detection of the p16 expression pattern on slides prepared from formalin-fixed, paraffin-embedded tumor samples. The percentage of stained cells (0%=0, 1-10%=1, 11-50%=2, 51-80%=3, 81-100%=4) and the intensity of the nuclear or cytoplasmic staining (none=0, weak=1, moderate=2, strong=3) were multiplied to yield a composite score ranging from 0 to 12. Scores equal to or greater than 4 were considered positive for p16 expression. Since p16 is the most commonly used clinical marker, tumors with p16 protein expression were considered HPV-positive.¹⁸ HPV infection was also determined through DNA extraction and genotyping by Luminex-based multiplex PCR for the following genotypes: HPV6, HPV8, HPV11 HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV58, and HPV59. As a sensitivity analysis, we also considered tumors to be HPV-positive only if they were positive for both HPV through PCR and p16 protein expression.

Statistical Analysis

Differences in descriptive statistics by p16 status were estimated using a chi-square test. HNSCC site-specific adjusted odds ratios (ORs) and p16-positive and p16-negative OPSCC-specific ORs were estimated with unconditional multinomial logistic regression models comparing p16-positive OPSCC with controls and comparing p16-negative OPSCC with controls adjusting for the study matching factors, age, race and gender, as well as important confounders related to alcohol use and socioeconomic status (such as income, insurance, and education). Based on evidence indicating that women are at increased risk from the carcinogenic effects of tobacco and literature demonstrating a differential inflammatory response from cigarette smoking in women compared with men,¹⁹⁻²² we explored multiplicative interactions of gender with all oral health variables (routine dental exam, tooth mobility, gum disease, number of lost teeth) and smoking. Since gender was a matching factor, we are unable to estimate additive interactions with the relative excess risk due to interaction. All statistical analyses were implemented using SAS 9.4 (SAS Institute, Cary, NC) and alpha of 0.05 was used.

Results

Descriptive Statistics

Most oral health variables differed by tumor p16 status (Table 1). Controls were more likely than both p16-positive and p16-negative cases to have markers of good oral health. Cases with tumors that were p16-negative were less likely to have a routine dental exam in the last 10 years (p-value<0.001), and more likely to have lost teeth (p-value=0.001) and tooth mobility (p-value=0.030) than p16-positive cases. The prevalence of gum disease did not differ substantially between p16-positive and p16-negative OPSCC. Smoking 10 pack-years was most common in p16-negative cases (82.9%), followed by p16-positive cases (63.1%) and controls (44.2%).

All HNSCC Sites

History of routine dental exams was significantly associated with decreased risk across all sites except hypopharynx, which had a reduced OR of similar magnitude as the other sites (Table 2). We found that tooth mobility due to disease increased the risk of cancer across all sites compared with controls. The associations for larynx, oral cavity, and oropharynx were statistically significant. Report of gum disease was not significantly associated with any sites of HNSCC. Smoking 10 pack-years or greater was significantly associated with increased risk of HNSCC across all sites.

Oral health in OPSCC by HPV-status

Routine dental exams were significantly associated with decreased risk of both p16-positive (OR: 0.52; 95% confidence interval (CI): 0.35-0.76) and p16-negative OPSCC (OR: 0.55; 95% CI: 0.36-0.86) (Table 3) compared with controls. Tooth mobility due to disease was significantly associated with increased risk of p16-negative OPSCC compared with controls and has an elevated odds ratio for p16-positive OPSCC. Gum disease was not associated with either p16-positive or p16-negative OPSCC. Smoking 10 pack-years was strongly associated with increased risk p16-negative (OR: 4.26; 95% CI: 2.85-6.37) OPSCC and to a lesser extent with p16-positive OPSCC (OR: 1.62; 95% CI: 1.10-2.38) compared with controls.

In the sensitivity analysis, 4.3% of our p16-positive OPSCC did not have presence of high-risk HPV DNA through PCR (n = 7). When considering only tumors that are both HPV PCR positive and positive for p16 protein expression as HPV-positive, the point estimates remained unchanged. However, smoking was no longer significantly associated with HPV-positive OPSCC (OR: 1.47; 95% CI: 0.95-2.26; p-value=0.082).

Gender interaction

There was little evidence of gender interaction with dental exams, tooth mobility due to disease, gum disease and number of teeth lost with either p16-positive or p16-negative OPSCC (Supplemental table S1). We found evidence of an interaction between smoking and gender with p16-positive OPSCC, in which women had increased risk of HPV-positive OPSCC when smoking was 10 pack-years, but this association was not seen in men and the estimates were imprecise.

Discussion

We aimed to provide insights into the relationship between oral health, frequency of dental examinations, and HNSCC stratified by site and with HPV-positive and HPV-negative OPSCC using CHANCE. Our study demonstrated that routine oral exams are associated with a decreased risk of both HPV-positive and HPV-negative OPSCC. Furthermore, tooth mobility was associated with a significantly increased risk of HPV-negative OPSCC. Tooth mobility and HPV-positive OPSCC also had a non-significantly elevated OR. Gum disease diagnosed by a dentist was not associated with either HPV-positive or HPV-negative OPSCC. While HPV-positive and HPV-negative HNSCC differ significantly with respect to etiology and tumorigenesis, our findings suggest poor oral health and frequency of dental examinations impact the risk of HPV-positive and HPV-negative HNSCC similarly.

The association between oral health and tobacco-associated HNSCC is well established, and the present study replicates these results.¹¹⁻¹³ Although these studies did not take HPV-status into consideration, they did find a positive association between poor oral health and HNSCC in sites not commonly associated with HPV such as the larynx and hypopharynx.¹¹⁻¹³ Our study is one of the first to demonstrate an association between poor oral health indicators and risk of OPSCC in HPV-negative patients. Periodontitis is a disease typified by bacterially induced chronic inflammation most often associated with gram-negative anaerobic rods;^{23, 24} it is plausible that the increased risk of HPV-negative OPSCC is due to the microbial dysbiosis and chronic inflammatory state associated with periodontitis and poor oral health in general.^{25, 26}

The association between oral health and risk of HPV-positive OPSCC has not been studied as extensively. Tezal and colleagues examined the association between HPV-status and periodontitis in a small sample of 30 patients with base of tongue cancers and found a 3-fold (OR: 3.96; 95% CI 1.18-13.36) increased risk of HPV-positive tumor status for every 1 mm of alveolar bone loss compared with HPV-negative tumor status.¹⁶ In a later study published by Tezal and colleagues, with a larger sample size including all head and neck sites (N=124), the authors demonstrated a similar trend but a weaker association (OR: 2.61; 95% CI: 1.58-4.30) with HPV-positive compared with HPV-negative head and neck tumors.¹⁵ Although our study did not specifically assess periodontitis, we used tooth mobility due to disease and report of gum disease as proxies for periodontitis. Tooth mobility is a result of alveolar bone destruction and loss of periodontal attachment, which are associated with periodontitis; further, the assessment of self-reported or clinically-determined tooth mobility is commonly used in the periodontal assessment and has been used in previous studies as a marker for periodontitis.^{12, 27} Our study, which had a much larger sample size of OPSCCs (N=372), does not support the strong association between periodontitis indicators and HPV-positive OPSCC found by Tezal and colleagues. Our weaker associations could be due to using tooth mobility as a proxy for clinically-determined periodontitis diagnosis. A previous study found self-report of gum disease only moderately correlates with periodontitis,²⁸ which could explain the null association seen with gum disease. However, Hashim and colleagues found no association with gum disease in a pooled analysis of 1,855 oropharynx cases and 7,939 controls, suggesting that there may not be an association between self-report gum disease and oropharyngeal cancer.²⁹

Oral HPV infection is necessary for the development of HPV-positive OPSCC. Poor oral health can affect cancer development by either increasing the risk of HPV infection or by increasing the carcinogenicity of HPV. Previous studies have demonstrated a relationship between oral HPV infection and poor oral health.^{30, 31} Research on HPV-associated cervical cancer has shown that co-infection with bacterial species such as *Chlamydia* and HPV exhibit synergistic effects and result in increased risk of cervical cancer.³² The biological pathways underlying this association may involve increased levels of inflammatory cytokines such as interleukins and tumor necrosis factor-alpha that modulate HPV gene expression.^{33, 34} Because periodontitis is a disease characterized by a polymicrobial dysbiosis, similar mechanisms between the inflammatory cascade, HPV gene expression, and cancer risk may explain the associations demonstrated between HPV-status and periodontitis. Further, a similar mechanism may also play a role in the pattern found in our study between HPV-positive OPSCC and poor oral health in general. Future studies to elucidate the mechanisms behind these associations with HPV are warranted.

We also found an association between risk of OPSCC and smoking in both HPV-positive and HPV-negative OPSCC. Although most HPV-positive cancer cases involve some form of tobacco use, this association is often less pronounced than in HPV-negative cancer.^{1, 35} This was confirmed in our study where smoking was more strongly associated with the risk of HPV-negative than HPV-positive OPSCC. The relationship between oral HPV infection and smoking is well established.^{36, 37} Since smoking is further upstream in the tumorigenesis pathway (i.e. smoking increases the risk of oral HPV infection which leads to HPV-positive oropharynx cancer),³⁸ the diminished association between smoking and HPV-positive OPSCC is expected. In an exploratory analysis, we examined the interaction of smoking with gender. Females who smoked appeared to be at higher risk of HPV-positive OPSCC compared with female nonsmokers, but this relationship was not seen in males. However, there were very few women with HPV-positive OPSCC (n=28) in our study, and these sparse data produced imprecise estimates.

There are a few limitations to our study. All oral health variables were self-reported and thus they may be considered as less valid oral health indicators than clinically-diagnosed disease.³⁹ However, previous studies have also found high correlation between self-reported tooth loss and a clinical examination.⁴⁰ The current study is in agreement with previous work demonstrating that routine dental visits are associated with decreased risk of developing head and neck cancers. Although our study is large, we are unable to replicate the results for HPV-positive base of tongue cancer found by Tezal and colleagues since some strata were very sparse. Further studies are needed in this area to clarify potential associations and effect sizes. Although we adjusted for smoking in the model, we did not have adequate power to further examine the oral health and smoking interaction with HPV status.

Importantly, it has been shown that oral health, frequency of dental examinations, as well as HNSCC are strongly associated with socioeconomic factors and risk behaviors.^{41, 42} Although we included indicators of socioeconomic status such as number of sexual partners, education, annual household income and insurance status in our final adjusted model, there still is potential for residual confounding. However, this inverse association could also be

due to routine oral examinations and interventions facilitating a healthier oral ecology and microbiome with less pathogenic microflora and lower levels of inflammation.

This study has several notable strengths. We used CHANCE, which is a large population-based case-control study with a diverse population. We are able to ascertain detailed information on smoking, oral health indicators and demographics from interviews conducted by trained nurses. Additionally, the current study is the largest study of oral health and risk of OPSCC stratified by HPV-status using p16 immunohistochemistry, the most commonly used clinical marker of HPV-status, as well as HPV status through PCR.

In conclusion, in this population-based case-control study we found a modest positive association between oral health indicators and risk of both HPV-positive and HPV-negative OPSCC. Routine dental visits almost halve the risk of both types of OPSCC, while smoking appears to have a weaker association with HPV-positive OPSCC than HPV-negative OPSCC. These findings underscore the importance of oral health surveillance and routine dental examinations for HNSCC prevention regardless of HPV-status. Further research into the relationship between oral health, HPV infection, and risk of OPSCC is warranted to clarify possible mechanisms and optimize prevention strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported in part by the National Cancer Institute (R01 CA90731) and the Lineberger Cancer Control Education Program (R25 CA57726).

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Table 1

Descriptive statistics of CHANCE study participants by control and HPV-status

| | Control (n=1396) | p16-negative (n=276) | p16-positive (n=215) | p-value* |
|---------------------------------------|---------------------|-------------------------|-------------------------|----------|
| | n (%) | n (%) | n (%) | |
| Routine dental exams in last 10 years | | | | |
| No | 333(23.9) | 160(58.2) | 83(38.6) | <0.001 |
| Yes | 1063(76.1) | 115(41.8) | 132(61.4) | |
| Missing | -- | 1 | -- | |
| Gum disease | | | | |
| No | 1022(73.6) | 186(67.9) | 155(72.1) | 0.315 |
| Yes | 366(26.4) | 88(32.1) | 60(27.9) | |
| Missing | 8 | 2 | -- | |
| Lost teeth | | | | |
| 0-5 | 835(60.0) | 109(40.2) | 123(57.2) | 0.001 |
| 6-15 | 210(15.1) | 49(18.1) | 27(12.6) | |
| 16-28 | 347(24.9) | 113(41.7) | 65(30.2) | |
| Missing | 4 | 5 | -- | |
| Tooth mobility | | | | |
| No | 1071(76.9) | 164(60.3) | 150(69.8) | 0.030 |
| Yes | 321(23.1) | 108(39.7) | 65(30.2) | |
| Missing | 4 | 4 | | |
| Smoking | | | | |
| <10 pack-years | 775(55.8) | 47(17.1) | 79(36.9) | <0.001 |
| 10 pack-years | 614(44.2) | 228(82.9) | 135(63.1) | |
| Missing | 7 | 1 | 1 | |
| Alcohol consumption | | | | |
| <1 drink/week | 460(33.4) | 37(13.7) | 36(17.1) | 0.300 |
| 1 drink/week | 917(66.6) | 234(86.3) | 175(82.9) | |
| Missing | 19 | 5 | 4 | |
| Number of sexual partners | | | | |
| 0-1 | 366(27.1) | 43(15.8) | 16(7.5) | 0.018 |

| | Control (n=1396) | p16-negative (n=276) | p16-positive (n=215) | p-value* |
|-------------------------|------------------------|-------------------------|-------------------------|-----------|
| | n (%) | n (%) | n (%) | |
| | 2-4 | 359(26.5) | 67(24.6) | 45(21.2) |
| | 5-14 | 345(25.5) | 79(29.0) | 69(32.5) |
| | 14+ | 283(20.9) | 83(30.5) | 82(38.7) |
| | Missing | 43 | 4 | 3 |
| Education | | | | |
| | Less than high school | 219(15.7) | 104(37.7) | 44(20.5) |
| | High school graduate | 334(23.9) | 95(34.4) | 54(25.1) |
| | Some college and above | 843(60.4) | 77(27.9) | 117(54.4) |
| Annual household income | | | | |
| | >\$50,000 | 590(43.9) | 56(21.3) | 88(42.1) |
| | \$20,000-\$50,000 | 493(36.7) | 89(33.8) | 72(34.4) |
| | <\$20,000 | 261(19.4) | 118(44.9) | 49(23.4) |
| | Missing | 52 | 13 | 6 |
| Race | | | | |
| | White | 1114(79.8) | 170(61.6) | 187(87.0) |
| | Black | 264(18.9) | 102(37.0) | 21(9.8) |
| | Other | 18(1.3) | 4(1.4) | 7(3.3) |
| Age (Years) | | | | |
| | <50 | 158(11.3) | 56(20.3) | 66(30.7) |
| | 50-54 | 164(11.7) | 37(13.4) | 47(21.9) |
| | 55-59 | 208(14.9) | 47(17.0) | 37(17.2) |
| | 60-64 | 207(14.8) | 51(18.5) | 25(11.6) |
| | 65-69 | 252(18.1) | 37(13.4) | 19(8.8) |
| | 70-74 | 235(16.8) | 30(10.9) | 14(6.5) |
| | 75+ | 172(12.3) | 18(6.5) | 7(3.3) |
| Sex | | | | |
| | Male | 966(69.2) | 200(72.5) | 175(81.4) |
| | Female | 430(30.8) | 76(27.5) | 40(18.6) |

* Comparing HPV-negative with HPV-positive

Table 2

Adjusted odds ratio for oral health indicators of each HNSCC site compared with controls

| | Hypopharynx (n=70) | | | Larynx (n=481) | | | NOS (n=251) | | | Oral cavity (n=212) | | | Oropharynx (n=372) | | |
|----------------------------------|--------------------|-------|--|------------------|--------|--|-----------------|--------|--|---------------------|--------|--|--------------------|--------|--|
| | OR (95% CI) | p | | OR (95% CI) | p | | OR (95% CI) | p | | OR (95% CI) | p | | OR (95% CI) | p | |
| Routine dental exams in 10 years | | | | | | | | | | | | | | | |
| No | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | |
| Yes | 0.52(0.24-1.14) | 0.101 | | 0.53(0.39-0.72) | <0.001 | | 0.65(0.43-0.98) | 0.041 | | 0.49(0.32-0.77) | 0.002 | | 0.50(0.35-0.72) | <0.001 | |
| Tooth mobility | | | | | | | | | | | | | | | |
| No | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | |
| Yes | 1.87(0.95-3.68) | 0.069 | | 1.40(1.04-1.87) | 0.026 | | 1.38(0.94-2.02) | 0.103 | | 1.58(1.05-2.37) | 0.029 | | 1.42(1.02-1.98) | 0.039 | |
| Gum disease | | | | | | | | | | | | | | | |
| No | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | |
| Yes | 0.55(0.26-1.18) | 0.125 | | 1.04(0.78-1.39) | 0.788 | | 1.11(0.77-1.60) | 0.577 | | 0.95(0.63-1.43) | 0.808 | | 0.89(0.64-1.23) | 0.459 | |
| Number of teeth lost | | | | | | | | | | | | | | | |
| 0-5 | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | |
| 6-15 | 0.38(0.12-1.15) | 0.086 | | 1.57(1.09-2.26) | 0.016 | | 0.63(0.38-1.05) | 0.075 | | 0.76(0.45-1.30) | 0.317 | | 1.30(0.88-1.92) | 0.189 | |
| 16-28 | 0.77(0.36-1.64) | 0.492 | | 1.47(1.04-2.08) | 0.03 | | 0.72(0.46-1.13) | 0.155 | | 0.68(0.43-1.10) | 0.118 | | 0.84(0.57-1.26) | 0.402 | |
| Smoking | | | | | | | | | | | | | | | |
| < 10 pack-years | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | |
| 10 pack-years | 2.80(1.25-6.26) | 0.012 | | 7.46(5.09-10.95) | <0.001 | | 1.78(1.24-2.55) | 0.002 | | 4.76(2.94-7.70) | <0.001 | | 1.90(1.39-2.59) | <0.001 | |
| Alcohol | | | | | | | | | | | | | | | |
| < 1 drink/week | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | |
| 1 drink/week | 4.18(1.12-15.65) | 0.034 | | 1.55(1.07-2.24) | 0.02 | | 2.25(1.41-3.60) | <0.001 | | 2.28(1.34-3.88) | 0.002 | | 1.63(1.08-2.44) | 0.019 | |
| Number of sexual partners | | | | | | | | | | | | | | | |
| 0-1 | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | |
| 2-5 | 0.38(0.10-1.37) | 0.137 | | 1.44(0.95-2.19) | 0.084 | | 1.11(0.65-1.89) | 0.699 | | 1.40(0.80-2.46) | 0.243 | | 1.97(1.11-3.48) | 0.02 | |
| 6-14 | 0.79(0.27-2.31) | 0.663 | | 1.11(0.72-1.72) | 0.623 | | 1.13(0.66-1.96) | 0.65 | | 1.06(0.57-1.95) | 0.853 | | 3.00(1.71-5.25) | <0.001 | |
| 14+ | 1.60(0.58-4.46) | 0.367 | | 1.23(0.79-1.91) | 0.365 | | 1.56(0.89-2.73) | 0.118 | | 1.11(0.59-2.09) | 0.749 | | 3.07(1.72-5.48) | <0.001 | |
| Education | | | | | | | | | | | | | | | |
| Less than high school | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | | Ref | -- | |
| High school graduate | 0.46(0.21-1.03) | 0.06 | | 0.63(0.45-0.89) | 0.009 | | 0.83(0.52-1.32) | 0.436 | | 0.81(0.51-1.29) | 0.372 | | 0.80(0.53-1.21) | 0.291 | |

| | Hypopharynx (n=70) | | Larynx (n=481) | | NOS (n=251) | | Oral cavity (n=212) | | Oropharynx (n=372) | |
|-------------------------|--------------------|-------|-----------------|--------|-----------------|-------|---------------------|-------|--------------------|-------|
| | OR (95% CI) | p | OR (95% CI) | p | OR (95% CI) | p | OR (95% CI) | p | OR (95% CI) | p |
| Annual household income | | | | | | | | | | |
| Some college and above | 0.49(0.21-1.15) | 0.1 | 0.46(0.32-0.66) | <0.001 | 0.64(0.40-1.04) | 0.071 | 0.53(0.32-0.88) | 0.015 | 0.70(0.46-1.06) | 0.092 |
| >\$50,000 | Ref | -- | Ref | -- | | | Ref | -- | Ref | -- |
| \$20,000-\$50,000 | 1.20(0.49-2.96) | 0.686 | 1.27(0.92-1.77) | 0.149 | 1.35(0.91-2.00) | 0.141 | 1.51(0.92-2.46) | 0.1 | 0.89(0.63-1.25) | 0.495 |
| <\$20,000 | 2.56(0.92-7.10) | 0.071 | 1.57(1.04-2.39) | 0.033 | 1.73(1.02-2.92) | 0.041 | 2.56(1.42-4.60) | 0.002 | 1.62(1.03-2.55) | 0.038 |
| Insurance | | | | | | | | | | |
| Private | Ref | -- | | | | | | | | |
| Medicare/Medicaid | 1.42(0.53-3.76) | 0.487 | 1.18(0.78-1.76) | 0.434 | 1.59(0.95-2.64) | 0.075 | 1.18(0.67-2.08) | 0.572 | 1.01(0.64-1.58) | 0.978 |
| None | 1.58(0.58-4.29) | 0.375 | 0.83(0.50-1.36) | 0.453 | 1.15(0.64-2.06) | 0.645 | 1.14(0.60-2.16) | 0.689 | 0.59(0.34-1.00) | 0.05 |
| Other | 0.82(0.26-2.66) | 0.747 | 1.29(0.86-1.95) | 0.222 | 1.71(1.03-2.84) | 0.039 | 1.37(0.76-2.45) | 0.298 | 0.92(0.58-1.45) | 0.71 |

OR: Odds Ratio; CI: Confidence Interval; p: p-value

* Adjusted for matching factor

Adjusted odds ratios for oral health indicators for HPV-negative OPSCC and HPV-positive OPSCC compared with controls

Table 3

| | p16-negative | | p16-positive | |
|----------------------------------|-----------------|---------|-----------------|---------|
| | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Routine dental exams in 10 years | | | | |
| No | Ref | -- | Ref | -- |
| Yes | 0.52(0.35-0.76) | <0.001 | 0.55(0.36-0.86) | 0.009 |
| Tooth Mobility | | | | |
| No | Ref | -- | Ref | -- |
| Yes | 1.70(1.18-2.43) | 0.004 | 1.45(0.95-2.20) | 0.083 |
| Gum disease | | | | |
| No | Ref | -- | Ref | -- |
| Yes | 0.97(0.67-1.41) | 0.889 | 0.95(0.63-1.42) | 0.796 |
| Number of teeth lost | | | | |
| 0-5 | Ref | -- | Ref | -- |
| 6-15 | 1.00(0.64-1.58) | 0.985 | 1.06(0.62-1.82) | 0.833 |
| 16-28 | 0.85(0.55-1.31) | 0.464 | 1.24(0.75-2.04) | 0.405 |
| Smoking | | | | |
| < 10 pack-years | Ref | -- | Ref | -- |
| 10 pack-years | 4.26(2.85-6.37) | <0.001 | 1.62(1.10-2.38) | 0.014 |
| Alcohol | | | | |
| < 1 drink/week | Ref | -- | Ref | -- |
| 1 drink/week | 2.11(1.34-3.33) | 0.001 | 1.51(0.93-2.44) | 0.095 |
| Number of sexual partners | | | | |
| 0-1 | Ref | -- | Ref | -- |
| 2-5 | 0.94(0.57-1.56) | 0.812 | 2.52(1.28-4.94) | 0.007 |
| 6-14 | 0.89(0.53-1.49) | 0.662 | 3.15(1.59-6.24) | 0.001 |
| 14+ | 0.65(0.37-1.12) | 0.122 | 3.58(1.77-7.23) | <0.001 |
| Education | | | | |
| Less than high school | Ref | -- | Ref | -- |
| High school graduate | 0.85(0.56-1.28) | 0.429 | 0.80(0.47-1.35) | 0.398 |

| | p16-negative | | p16-positive | |
|-------------------------|-----------------|---------|-----------------|---------|
| | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Some college and above | 0.42(0.26-0.67) | <0.001 | 0.81(0.48-1.37) | 0.442 |
| Annual household income | | | | |
| >\$50,000 | Ref | -- | Ref | -- |
| \$20,000-\$50,000 | 1.13(0.75-1.72) | 0.554 | 1.06(0.71-1.59) | 0.764 |
| <\$20,000 | 2.11(1.27-3.50) | 0.004 | 1.29(0.74-2.26) | 0.367 |
| Insurance | | | | |
| Private | Ref | -- | Ref | -- |
| Medicare/Medicaid | 1.00(0.61-1.63) | 0.996 | 1.29(0.75-2.22) | 0.364 |
| None | 0.74(0.41-1.33) | 0.31 | 0.88(0.47-1.66) | 0.690 |
| Other | 1.09(0.65-1.82) | 0.754 | 1.19(0.68-2.06) | 0.547 |

OR: Odds Ratio; CI: Confidence Interval

* Adjusted for matching factors