Oral Health and Human Papillomavirus-Associated Head and Neck Squamous Cell Carcinoma

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BACKGROUND: Indicators of poor oral health, including smoking, have been associated with increased risk of head and neck squamous cell carcinoma, especially oropharyngeal squamous cell carcinoma (OPSCC), yet few studies have examined whether this association is modified by human papillomavirus (HPV) status. METHODS: Data from interviews and tumor HPV status from a large population-based case-control study, the Carolina Head and Neck Cancer Study (CHANCE), were used to estimate the association between oral health indicators and smoking among 102 HPV-positive patients and 145 HPV-negative patients with OPSCC and 1396 controls. HPV status was determined by p16INK4a (p16) immunohistochemistry. Unconditional, multinomial logistic regression was used to estimate odds ratios (ORs) for all oral health indicators adjusting for important covariates. RESULTS: Routine dental examinations were associated with a decreased risk of both HPV-negative OPSCC (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 disease (OR, 1.70; 95% CI, 1.18-2.43) slightly more than the risk for HPV-positive disease (OR, 1.45; 95% CI, 0.95-2.20). Ten or more pack-years of cigarette smoking were strongly associated with an increased risk of HPV-negative OPSCC (OR, 4.26; 95% CI, 2.85-6.37) and were associated less with an increased risk of HPV-positive OPSCC (OR, 1.62; 95% CI, 1.10-2.38). CONCLUSIONS: Although HPV-positive and HPV-negative HNSCC differ significantly with respect to etiology and tumorigenesis, the current 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. Cancer 2017;123:71-80. © 2016 American Cancer Society.

KEYWORDS: dental visits, head and neck cancer, human papillomavirus, oral health, oropharyngeal cancer, periodontal disease, smoking.

INTRODUCTION

Cancers of the head and neck comprise a heterogeneous group of malignancies, of which >90% are squamous cell carcinomas (SCCs) arising from the mucosal lining of the upper aerodigestive tract.1,2 Over the last 20 years, the epidemiology of head and neck SCC (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 from 60% to 70% of oropharyngeal SCCs (OPSCCs) in the United States are associated with HPV infection.4 In the United States, the incidence of HPV-positive OPSCC increased by 225% between 1988 and 2004.5 Compared with patients who have HPV-negative OPSCC, those who have HPV-positive OPSCC have distinct risk-factor profiles and improved oncologic outcomes.6 In addition, 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 and Prevention estimate that >15,000 HPV-associated OPSCCs are diagnosed annually in the United States, and the oropharynx is the only head and neck cancer site to have increased in incidence.8 Because the majority of the reported risk factors for HNSCC were established before HPV status was commonly tested, it is imperative to assess the traditional risk factors 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.\textsuperscript{9,13} Two studies reported that periodontitis is associated with an increased risk of OPSCC without taking into account HPV status.\textsuperscript{10,14} Only 2 case-only studies have examined the association between oral health and malignancy by comparing HPV-positive OPSCC with HPV-negative OPSCC. Both of those studies reported a positive association between periodontitis and HPV-positive OPSCC compared with HPV-negative OPSCC.\textsuperscript{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.\textsuperscript{11,12,15}

In the current 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 also explored this association by HPV status in OPSCC to determine whether the association with oral health is modified by HPV. We hypothesized that poor oral health indicators, including smoking, would be associated with an increased risk of OPSCC regardless of tumor HPV status.

**MATERIALS AND METHODS**

**Study Population**

The Carolina Head and Neck Cancer Study (CHANCE) is a population-based case-control study in North Carolina.\textsuperscript{15} Patients were eligible for CHANCE if they had been diagnosed with a first primary SCC of the oral cavity, pharynx, or larynx between January 1, 2002, and February 28, 2006; were ages 20 to 80 years at diagnosis; and resided in a 46-county region in central North Carolina (“cases”). Patients who had benign tumors, carcinomas in situ, thyroid papillary carcinomas, and adenoid cystic carcinomas were excluded. Patients who had lip and hypopharynx cancers, those for whom the hospital would not release tumor blocks, and those who had completed only proxy interviews were excluded from p16 immunohistochemistry. All patients who had oropharyngeal cancers (\(N = 248\)) and a random sample of patients who had nonoropharyngeal cancers (\(N = 244\); because the relevance of HPV in non-oropharyngeal cancer has not been established)\textsuperscript{17} were selected for the evaluation of p16. A sex, age, and race frequency-matched control group was identified through the North Carolina Department of Motor Vehicle records, and those individuals 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 by trained nurse-interviewers using a structured questionnaire during an in-home visit for both cases and controls.\textsuperscript{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 before cancer diagnosis. Self-reported oral health variables included: 1) the self-reported number of natural teeth lost, excluding third molars and teeth extracted for orthodontic reasons; 2) history of self-reported tooth mobility or “teeth loose in their socket” because of disease; 3) 1 or more routine (non-emergency) dental visits during the decade before HNSCC diagnosis; and 4) gum disease diagnosed by a dentist. History of smoking, dichotomized at 10 pack-years, also was included, because it is an important risk factor for poor oral health and OPSCC.

**Questionnaire and Clinical Assessment**

Demographic, lifestyle, diet, and other risk-factor data also were collected during the 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 matching factors (race, sex, and age), confounders obtained from the questionnaire included education, annual income, the number of sexual partners, and alcohol consumption.

Clinical information such as tumor site was abstracted from participants’ medical records and reviewed independently by a pathologist and a head neck cancer surgeon. Tumors were classified by site according to International Classification of Diseases for Oncology, third edition, topography codes for the oral cavity (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), the larynx (C32.0-C32.3, and C32.8-C32.9), the hypopharynx (C13.0, C13.1, C13.2, C13.8, and C13.9), and the 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 performed the p16 immunocytochemistry evaluation according to the protocol provided with the CINtec Histology p16\textsuperscript{INK4a} kit (9511; MTm Laboratories Inc, Westborough, Mass) for qualitative detection of the p16 expression pattern on slides prepared from formalin-fixed, paraffin-embedded tumor samples. Scores for the percentage of stained cells (with a score of 0 for 0% stained cells, 1 for 1%-10% stained cells, 2 for 11%-50% stained cells, 3 for 51%-80%
stained cells, and 4 for 81%-100% stained cells) and for
the intensity of nuclear or cytoplasmic staining (with a score of
0 for no staining, 1 for weak staining, 2 for moderate stain-
ing, and 4 for strong staining) were multiplied to yield a
composite score ranging from 0 to 12. Scores equal ≥ 4
were considered positive for p16 expression. Because p16 is
the most commonly used clinical marker, tumors that
exhibited p16 protein expression were considered HPV-
positive.18 HPV infection also was determined through
dNA extraction and genotyping using Luminex-based
multiplex polymerase chain reaction (PCR) (TS-E7-MPG,
IARC, Lyon, France) for the following genotypes: HPV
type 6 (HPV6), HPV8, HPV11 HPV16, HPV18,
HPV26, HPV31, HPV33, HPV35, HPV39, HPV58, and
HPV59.19-21 For sensitivity analyses, we also considered
tumors to be HPV-positive only if they were positive for
both HPV PCR and p16 protein expression.

Statistical Analysis
Differences in descriptive statistics according to p16 status
were estimated using the 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 patients with p16-positive OPSCC versus controls and comparing patients with p16-negative
OPSCC versus controls, adjusting for the study matching
factors (age, race, and sex) as well as important confounders related to alcohol use and socioeconomic status (such as income, insurance, and education). On the basis of evidence indicating that women are at increased risk from
the carcinogenic effects of tobacco and literature demonstrat-
ing a differential inflammatory response from cigarette smoking among women compared with men,22-25
we explored multiplicative interactions of sex with all oral
health variables (routine dental examination, tooth mobil-
ity, gum disease, number of lost teeth) and smoking. Be-
cause sex was a matching factor, we were unable to
estimate additive interactions with the relative excess risk
because of interaction. All statistical analyses were imple-
mented using the SAS version 9.4 statistical software
package (SAS Institute Inc, Cary, NC), and an α value 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 patients to have markers of
good oral health. Patients with p16-negative tumors were
less likely to have undergone a routine dental examination
in the last 10 years (P < .001) and were more likely to
have lost teeth (P = .001) and tooth mobility (P = .030)
than those with p16-positive tumors. The prevalence of
gum disease did not differ substantially between patients
with p16-positive and p16-negative OPSCC. Smoking
for ≥ 10 pack-years was most common among patients
who had p16-negative tumors (82.9%), followed by those
with p16-positive tumors (63.1%), and controls (44.2%).

All HNSCC Sites
A history of routine dental examinations was significantly
associated with decreased risk across all sites except the hy-
ppharynx, which had a reduced OR similar in magni-
dude to that for the other sites (Table 2). We observed that
tooth mobility because of disease increased the risk of can-
cer across all sites compared with controls. The associa-
tions for sites in the larynx, oral cavity, and oropharynx
were statistically significant. A report of gum disease was
not significantly associated with any sites of HNSCC.
Smoking for ≥ 10 pack-years was significantly associated
with an increased risk of HNSCC across all sites.

Oral Health in OPSCC by HPV Status
Routine dental examinations were significantly associated
with decreased risk of both p16-positive OPSCC (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 because of
disease was significantly associated with an increased risk
of p16-negative OPSCC compared with controls and had
an elevated OR for p16-positive OPSCC. Gum disease
was not associated with either p16-positive or p16 nega-
tive OPSCC. Smoking for ≥ 10 pack-years was strongly
associated with an increased risk of p16-negative OPSCC
(OR, 4.26; 95% CI, 2.85-6.37) 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 patients with
p16-positive OPSCC did not have high-risk HPV DNA
identified by PCR (n = 7). When considering only
tumors that were both HPV-positive by PCR and positive
for p16 protein expression, the point estimates remained
unchanged. However, smoking was no longer significant-
ly associated with HPV-positive OPSCC (OR, 1.47; 95%
CI, 0.95-2.26; P = .082).

Sex Interaction
There was little evidence of an interaction of sex with den-
tal examinations, tooth mobility because of disease, gum
TABLE 1. Descriptive Statistics of Participants in the Carolina Head and Neck Cancer (CHANCE) Study by Control and Human Papillomavirus Status

| Variable                                           | Controls, n = 1396 | p16-Negative, n = 276 | p16-Positive, n = 215 | P* |
|----------------------------------------------------|--------------------|-----------------------|-----------------------|----|
| Routine dental examinations in last 10 y           |                    |                       |                       |    |
| No                                                 | 333 (23.9)         | 160 (58.2)            | 83 (38.6)             | < .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)            | .315 |
| Yes                                                | 368 (26.4)         | 88 (32.1)             | 60 (27.9)             |    |
| Missing                                            | 8                  | 2                     | —                     |    |
| No. of lost teeth                                  |                    |                       |                       |    |
| 0-5                                                | 835 (60.0)         | 109 (40.2)            | 123 (57.2)            | .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)            | .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)             | < .001 |
| ≥10 Pack-years                                     | 614 (44.2)         | 228 (82.9)            | 135 (63.1)            |    |
| Missing                                            | 7                  | 1                     | 1                     |    |
| Alcohol consumption                                |                    |                       |                       |    |
| <1 Drink/wk                                        | 460 (33.4)         | 37 (13.7)             | 36 (17.1)             | .300 |
| ≥1 Drinks/wk                                       | 917 (66.6)         | 234 (86.3)            | 175 (82.9)            |    |
| Missing                                            | 19                 | 5                     | 4                     |    |
| No. of sexual partners                             |                    |                       |                       |    |
| 0-1                                                | 366 (27.1)         | 43 (15.8)             | 16 (7.5)              | .018 |
| 2-4                                                | 359 (26.5)         | 67 (24.6)             | 45 (21.2)             |    |
| 5-14                                               | 345 (25.5)         | 79 (29)               | 69 (32.5)             |    |
| ≥14                                                | 283 (20.9)         | 83 (30.5)             | 82 (38.7)             |    |
| Missing                                            | 43                 | 4                     | 3                     |    |
| Education                                          |                    |                       |                       |    |
| <High school                                       | 219 (15.7)         | 104 (37.7)            | 44 (20.5)             | < .001 |
| 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)             | < .001 |
| $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)            | < .001 |
| Black                                              | 264 (18.9)         | 102 (37.0)            | 21 (9.8)              |    |
| Other                                              | 18 (1.3)           | 4 (1.4)               | 7 (3.3)               |    |
| Age, y                                             |                    |                       |                       |    |
| <50                                                | 158 (11.3)         | 56 (20.3)             | 66 (30.7)             | .002 |
| 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                                                |                    |                       |                       |    |
| Men                                                | 966 (69.2)         | 200 (72.5)            | 175 (81.4)            | .021 |
| Women                                              | 430 (30.8)         | 76 (27.5)             | 40 (18.6)             |    |

Abbreviation: p16, p16INK4a.  
*P values indicate differences between participants who were negative or positive for human papillomavirus.
### Table 2. Adjusted Odds Ratios of Oral Health Indicators for Each Head and Neck Squamous Cell Carcinoma Site Compared With Controls

| Variable                          | 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                    |
| Routine dental examinations in 10 y | 0.52 (0.24-1.14)     | .101            | .05 (0.39-0.72) | .001                 | 0.65 (0.43-0.98)    | .041                 |
| Yes                               | 0.53 (0.24-1.14)     | .101            | .65 (0.32-0.77) | .002                 | 0.50 (0.35-0.72)    | < .001               |
| Tooth mobility                    | 1.87 (0.95-3.68)     | .069            | 1.40 (1.04-1.87) | .026                 | 1.38 (0.94-2.02)    | .103                 |
| No                                | Ref                  | Ref             | Ref          | Ref                  | Ref                 | Ref                  |
| Yes                               | 1.40 (1.04-1.87)     | .026            | 1.58 (1.05-2.37) | .029                 | 1.42 (1.02-1.98)    | .039                 |
| Gum disease                       | 0.55 (0.26-1.18)     | .125            | 1.04 (0.78-1.39) | .788                 | 1.11 (0.77-1.60)    | .577                 |
| No                                | Ref                  | Ref             | Ref          | Ref                  | Ref                 | Ref                  |
| Yes                               | 1.04 (0.78-1.39)     | .788            | 0.95 (0.63-1.43) | .808                 | 0.89 (0.64-1.23)    | .459                 |
| No. of teeth lost                 | 0.38 (0.12-1.15)     | .086            | 1.57 (1.09-2.26) | .016                 | 0.63 (0.38-1.09)    | .075                 |
| 6-15                              | 0.77 (0.36-1.64)     | .492            | 1.47 (1.04-2.08) | .030                 | 0.72 (0.46-1.13)    | .155                 |
| No                                | Ref                  | Ref             | Ref          | Ref                  | Ref                 | Ref                  |
| Yes                               | 1.47 (1.04-2.08)     | .030            | 0.68 (0.43-1.10) | .118                 | 0.84 (0.57-1.26)    | .402                 |
| Smoking                           | 2.80 (1.25-6.26)     | .012            | 7.46 (5.09-10.95) | < .001               | 1.78 (1.24-2.55)    | .002                 |
| <10 Pack-years                    | Ref                  | Ref             | Ref          | Ref                  | Ref                 | Ref                  |
| ≥10 Pack-years                    | 4.18 (1.12-15.65)    | .034            | 1.55 (1.07-2.24) | .020                 | 2.25 (1.41-3.60)    | < .001               |
| Alcohol                           | <1 Drink/wk          | Ref             | Ref          | Ref                  | Ref                 | Ref                  |
| ≥1 Drinks/wk                      | 1.55 (1.07-2.24)     | .020            | 2.25 (1.41-3.60) | < .001               | 2.28 (1.34-3.88)    | .002                 |
| No. of sexual partners            | 0-1                  | Ref             | Ref          | Ref                  | Ref                 | Ref                  |
| 2-5                               | 0.38 (0.10-1.37)     | .137            | 1.37 (0.95-2.19) | .084                 | 1.11 (0.65-1.89)    | .699                 |
| 6-14                              | 0.79 (0.27-2.31)     | .663            | 1.11 (0.72-1.72) | .623                 | 1.13 (0.66-1.96)    | .65                 |
| ≥14                               | 1.60 (0.54-4.46)     | .367            | 1.23 (0.79-1.91) | .365                 | 1.56 (0.89-2.73)    | .118                 |
| Education                         | <High school         | Ref             | Ref          | Ref                  | Ref                 | Ref                  |
| High school graduate              | 0.46 (0.21-1.03)     | .060            | 0.63 (0.45-0.89) | .009                 | 0.83 (0.52-1.32)    | .436                 |
| Some college and above            | 0.49 (0.21-1.15)     | .100            | 0.46 (0.32-0.66) | < .001               | 0.64 (0.40-1.04)    | .071                 |
| Annual household income           | >$50,000             | Ref             | Ref          | Ref                  | Ref                 | Ref                  |
| <$20,000-$50,000                  | 1.20 (0.49-2.96)     | .686            | 1.27 (0.92-1.77) | .149                 | 1.35 (0.91-2.00)    | .141                 |
| <$20,000                          | 2.56 (0.92-7.10)     | .071            | 1.57 (1.04-2.39) | .033                 | 1.73 (1.02-2.92)    | .041                 |
| Insurance                         | Private              | Ref             | Ref          | Ref                  | Ref                 | Ref                  |
| Medicare/Medicaid                 | 1.42 (0.53-3.76)     | .487            | 1.18 (0.78-1.76) | .434                 | 1.59 (0.95-2.64)    | .075                 |
| None                              | 1.58 (0.58-4.29)     | .375            | 0.83 (0.50-1.36) | .453                 | 1.15 (0.64-2.06)    | .645                 |
| Other                             | 0.82 (0.26-2.66)     | .747            | 1.29 (0.86-1.95) | .222                 | 1.71 (1.03-2.84)    | .039                 |

Abbreviations: CI, confidence interval; NOS, not otherwise specified; OR, odds ratio; Ref, reference category.

*Analyses were adjusted for matching factors.*
disease, and the number of teeth lost with in patients with either p16-positive or p16-negative OPSCC (Table 4). We observed evidence of an interaction between smoking and sex with p16-positive OPSCC, in which women had an increased risk of HPV-positive OPSCC when their smoking history was ≥10 pack-years, but this association was not observed in men, and the estimates were imprecise.

**DISCUSSION**

The objective of the current study was to provide insights into the correlation between oral health, the frequency of dental examinations, and HNSCC stratified according to disease site and HPV status in patients with OPSCC using data from the CHANCE study. Our findings indicate that routine oral examinations 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 was non-significantly associated with increased risk of HPV-positive OPSCC. Gum disease diagnosed by a dentist was not associated with either HPV-positive or HPV-negative OPSCC. Although HPV-positive and HPV-negative HNSCC differ significantly with respect to etiology and tumorigenesis, our findings suggest that poor oral health and frequency of dental examinations similarly impact the risk of HPV-positive and HPV-negative HNSCC.

The association between oral health and tobacco-associated HNSCC is well established, and the current

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**TABLE 3. Adjusted Odds Ratios of Oral Health Indicators for Patients With Human Papillomavirus (HPV)-Negative and HPV-Positive Oropharyngeal Squamous Cell Carcinoma Compared With Controls**

| Variable                              | p16-Negative | | p16-Positive | |
|--------------------------------------|--------------|---------------------------------|--------------|
|                                      | OR (95% CI)  | P                               | OR (95% CI)  | P                               |
| Routine dental examinations in 10 y  |              |                                 |              |                                 |
| No                                   | Ref          | —                               | Ref          | —                               |
| Yes                                  | 0.52 (0.35-0.76) | <.001                           | 0.55 (0.36-0.86) | .009                           |
| Tooth mobility                        |              |                                 |              |                                 |
| No                                   | Ref          | —                               | Ref          | —                               |
| Yes                                  | 1.70 (1.18-2.43) | .004                           | 1.45 (0.95-2.20) | .083                           |
| Gum disease                          |              |                                 |              |                                 |
| No                                   | Ref          | —                               | Ref          | —                               |
| Yes                                  | 0.97 (0.67-1.41) | .889                           | 0.95 (0.63-1.42) | .796                           |
| No. of teeth lost                    |              |                                 |              |                                 |
| 0-5                                  | Ref          | —                               | Ref          | —                               |
| 6-15                                 | 1.00 (0.64-1.58) | .985                           | 1.06 (0.62-1.82) | .833                           |
| 16-28                                | 0.85 (0.55-1.31) | .464                           | 1.24 (0.75-2.04) | .405                           |
| Smoking                              |              |                                 |              |                                 |
| <10 Pack-years                       | Ref          | —                               | Ref          | —                               |
| ≥10 Pack-years                       | 4.26 (2.85-6.37) | <.001                           | 1.62 (1.10-2.38) | .014                           |
| Alcohol                              |              |                                 |              |                                 |
| <1 Drink/wk                          | Ref          | —                               | Ref          | —                               |
| ≥1 Drinks/wk                         | 2.11 (1.34-3.33) | .001                           | 1.51 (0.93-2.44) | .095                           |
| No. of sexual partners               |              |                                 |              |                                 |
| 0-1                                  | Ref          | —                               | Ref          | —                               |
| 2-5                                  | 0.94 (0.57-1.56) | .812                           | 2.52 (1.28-4.94) | .007                           |
| 6-14                                 | 0.89 (0.53-1.49) | .662                           | 3.15 (1.59-6.24) | .001                           |
| ≥14                                  | 0.65 (0.37-1.12) | .122                           | 3.58 (1.77-7.23) | <.001                          |
| Education                            |              |                                 |              |                                 |
| <High school                         | Ref          | —                               | Ref          | —                               |
| High school graduate                 | 0.85 (0.56-1.28) | .429                           | 0.80 (0.47-1.35) | .398                           |
| Some college and above               | 0.42 (0.26-0.67) | <.001                           | 0.81 (0.48-1.37) | .442                           |
| Annual household income              |              |                                 |              |                                 |
| >$50,000                             | Ref          | —                               | Ref          | —                               |
| $20,000-$50,000                      | 1.13 (0.75-1.72) | .554                           | 1.06 (0.71-1.59) | .764                           |
| <$20,000                             | 2.11 (1.27-3.50) | .004                           | 1.29 (0.74-2.26) | .367                           |
| Insurance                            |              |                                 |              |                                 |
| Private                              | Ref          | —                               | Ref          | —                               |
| Medicare/Medicaid                    | 1.00 (0.61-1.63) | .996                           | 1.29 (0.75-2.22) | .364                           |
| None                                 | 0.74 (0.41-1.33) | .31                            | 0.88 (0.47-1.66) | .690                           |
| Other                                | 1.09 (0.65-1.82) | .754                           | 1.19 (0.68-2.06) | .547                           |

Abbreviations: CI, confidence interval; OR, odds ratio; p16, p16INK4a; Ref, reference category.

*Analyses were adjusted for matching factors.
TABLE 4. Sex Interaction Modeled Individually With Each Oral Health Indicatora

| Variable                          | No. of Controls | Women | OR (95% CI) | No. | OR (95% CI) | No. | Int P | No. | OR (95% CI) | No. | OR (95% CI) | No. | Int P |
|-----------------------------------|-----------------|-------|-------------|-----|-------------|-----|-------|-----|-------------|-----|-------------|-----|-------|
|                                   | Women           | Men   |             |     |             |     |       |     |             |     |             |     |       |
| Dental examination in last 10 y   |                 |       |             |     |             |     |       |     |             |     |             |     |       |
| No                                | 45              | 154   | Ref         | 5   | Ref         | 32  | .382  | 8   | Ref         | 24  | .596        |     |       |
| Yes                               | 338             | 727   | 0.25 (0.06-1.09) | 7   | 0.50 (0.24-1.03) | 29  | .475  | 4   | 1.88 (0.96-3.69) | 28  | .475        | 92  | .503   |
| Tooth mobility                    |                 |       |             |     |             |     |       |     |             |     |             |     |       |
| No                                | 196             | 822   | Ref         | 8   | Ref         | 33  | .113  | 9   | Ref         | 90  | .652        |     |       |
| Yes                               | 157             | 116   | 1.06 (0.24-4.65) | 4   | 1.88 (0.96-3.69) | 28  | .475  | 19  | 1.72 (0.68-4.37) | 9   | .113        |     |       |
| Gum disease                       |                 |       |             |     |             |     |       |     |             |     |             |     |       |
| No                                | 294             | 644   | Ref         | 10  | Ref         | 39  | .113  | 19  | Ref         | 90  | .652        |     |       |
| Yes                               | 89              | 237   | 0.28 (0.05-1.71) | 2   | 1.30 (0.65-2.58) | 22  | .113  | 9   | 1.16 (0.47-2.86) | 9   | .113        |     |       |
| No. of teeth lost                 |                 |       |             |     |             |     |       |     |             |     |             |     |       |
| 0-5                               | 231             | 528   | Ref         | 7   | Ref         | 22  | .295  | 14  | Ref         | 80  | .80         |     |       |
| 6-15                              | 66              | 129   | 0.11 (0.01-1.58) | 1   | 2.44 (1.12-5.31) | 20  | .028  | 2   | 0.61 (0.12-3.01) | 17  | .364        |     |       |
| 16-28                             | 86              | 224   | 0.35 (0.08-1.61) | 4   | 0.85 (0.37-1.96) | 9   | .295  | 12  | 1.76 (0.62-4.96) | 24  | .240        |     |       |
| Smoking                           |                 |       |             |     |             |     |       |     |             |     |             |     |       |
| <10 Pack-years                    | 285             | 421   | Ref         | 3   | Ref         | 6   | .997  | 8   | Ref         | 55  | <.001       |     |       |
| ≥10 Pack-years                    | 98              | 420   | 5.31 (1.16-24.31) | 9   | 5.33 (2.10-13.51) | 55  | .997  | 20  | 5.98 (2.32-15.43) | 56  | <.001       |     |       |

Abbreviations: CI, confidence interval; Int P, interaction P value; OR, odds ratio; p16, p16INK4a; Ref, reference category.  
*aAnalyses were adjusted for matching factors, other oral health indicators, alcohol, insurance, income, and education.
study replicates these results.\textsuperscript{11-13} Although those studies did not take HPV status into consideration, they did identify a positive association between poor oral health and HNSCC in sites not commonly associated with HPV, such as the larynx and hypopharynx.\textsuperscript{11-13} Our study is one of the first to demonstrate an association between poor oral health indicators and the 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\textsuperscript{26,27}; it is plausible that the increased risk of HPV-negative OPSCC is caused by the microbial dysbiosis and chronic inflammatory state associated with periodontitis and poor oral health in general.\textsuperscript{28,29}

The association between oral health and the 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 who had base of tongue cancers and observed a 3-fold increased risk (OR, 3.96; 95% CI 1.18-13.36) of HPV-positive tumor status for every 1 mm of alveolar bone loss compared with HPV-negative tumor status.\textsuperscript{16} In a later study published by Tezal and colleagues that had a larger sample size and included all head and neck sites (N = 124), those authors demonstrated a similar trend but a weaker association (OR, 2.61; 95% CI, 1.58-4.30) with HPV-positive versus HPV-negative head and neck tumors.\textsuperscript{15} Although we did not specifically assess periodontitis in our current study, we used tooth mobility caused by 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; furthermore, 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.\textsuperscript{12,30} The results from our study, which had a much larger sample size of patients with OPSCC (N = 372), do not support the strong association between periodontitis indicators and HPV-positive OPSCC reported by Tezal and colleagues. The weaker associations we observed may have been the result of using tooth mobility as a proxy for a clinically determined diagnosis of periodontitis. A previous study indicated that patient self-report of gum disease was only moderately correlated with periodontitis,\textsuperscript{31} which could explain the null association observed with gum disease. However, Hashim and colleagues observed no association with gum disease in a pooled analysis of 1855 patients who had oropharyngeal cancer and 7939 controls, suggesting that there may not be an association between self-reported gum disease and oropharyngeal cancer.\textsuperscript{32}

Oral HPV infection is necessary for the development of HPV-positive OPSCC. Poor oral health can affect cancer development by increasing either the risk of HPV infection or the carcinogenicity of HPV. Previous studies have demonstrated a correlation between oral HPV infection and poor oral health.\textsuperscript{33,34} Research on HPV-associated cervical cancer indicates that coinfections with bacterial species like Chlamydia and HPV exhibit synergistic effects and result in an increased risk of cervical cancer.\textsuperscript{35} The biologic pathways underlying this association may involve increased levels of inflammatory cytokines, such as interleukins and tumor necrosis factor-\textgreek{z}, that modulate HPV gene expression.\textsuperscript{36,37} 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. Furthermore, a similar mechanism may also play a role in the pattern observed 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 observed an association between the risk of OPSCC and smoking in both HPV-positive and HPV-negative patients with OPSCC. Although most HPV-positive cancers involve some form of tobacco use, this association is often less pronounced than that in HPV-negative cancers.\textsuperscript{1,38} This was confirmed in our study, in which smoking was more strongly associated with the risk of HPV-negative than HPV-positive OPSCC. The correlation between oral HPV infection and smoking is well established.\textsuperscript{39,40} Because smoking is farther upstream in the tumorigenesis pathway (ie, smoking increases the risk of oral HPV infection, which leads to HPV-positive oropharyngeal cancer),\textsuperscript{41} a diminished association between smoking and HPV-positive OPSCC is expected. In an exploratory analysis, we examined the interaction between smoking and sex. Women who smoked appeared to be at higher risk of HPV-positive OPSCC compared with women who were nonsmokers, but this correlation was not observed in men. 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 may be considered as less valid oral health indicators than clinically diagnosed disease.\textsuperscript{42} However, previous studies have also reported a high correlation between self-reported tooth loss and a clinical examination.\textsuperscript{43} The current study is in
agreement with previous work demonstrating that routine dental visits are associated with a decreased risk of developing head and neck cancers. Although our study was large, we were unable to replicate the results for HPV-positive base of tongue cancer reported by Tezal and colleagues, because some of our 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 interaction of oral health and smoking with HPV status.

It is noteworthy that oral health, frequency of dental examinations, and HNSCC have been strongly associated with socioeconomic factors and risk behaviors.\textsuperscript{44,45} 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 may be related to routine oral examinations and interventions that facilitate a healthier oral ecology and microbiome with less pathogenic microflora and lower levels of inflammation.

This study has several notable strengths. We used the CHANCE study, which is a large, population-based, case-control study with a diverse population. We were able to ascertain detailed information on smoking, oral health indicators, and demographics from interviews conducted by trained nurses. In addition, the current study is the largest study to date of oral health and the risk of OPSCC stratified according to HPV status using p16 immunohistochemistry, which is 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 observed a modest positive association between oral health indicators and the risk of both HPV-positive and HPV-negative OPSCC. Routine dental visits cut the risk of both types of OPSCC almost in half, whereas smoking appeared 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 the prevention of HNSCC regardless of HPV status. Further research into the correlation between oral health, HPV infection, and risk of OPSCC is warranted to clarify possible mechanisms and optimize prevention strategies.

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AUTHOR CONTRIBUTIONS

Angela L. Mazul: Conceptualization, formal analysis, writing—original draft, and writing—review and editing. James M. Taylor: Writing—original draft and writing—review and editing. Mark C. Weissler: Investigation and writing—review and editing. Paul Brennan: Investigation and writing—review and editing. Devesana Anantharaman: Investigation and writing—review and editing. Behnoush Abedi-Ardekani: Investigation and writing—review and editing. Andrew F. Olshan: Conceptualization, investigation, writing—review and editing, and supervision. Jose P. Zevallos: Conceptualization, writing—review and editing, and supervision.

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