2011

Higher Micronutrient Intake is Associated with Human Papillomavirus-Positive Head and Neck Cancer: A Case-Only Analysis

Anna E. Arthur

Sonia A. Duffy

Gloria I. Sanchez

Stephen B. Gruber

Jeffrey E. Terrell

See next page for additional authors

Publication Info

Postprint version. Published in *Nutrition and Cancer*, Volume 63, Issue 5, 2011, pages 734-742.
This is an electronic version of an article published in:
Arthur, A.E., Duffy, S.A., Sanchez, G.I., Gruber, S.B., Terrell, J.E., Hébert, J.R., . . . Rozek, L.S. (2011). Higher Micronutrient Intake is Associated with Human Papillomavirus-Positive Head and Neck Cancer: A Case-Only Analysis. *Nutrition and Cancer*, *63*(5), 734-742. DOI: 10.1080/01635581.2011.570894
Nutrition and Cancer is available online at: www.tandfonline.com.

Author(s)
Anna E. Arthur, Sonia A. Duffy, Gloria I. Sanchez, Stephen B. Gruber, Jeffrey E. Terrell, James R. Hébert, Emily Light, Carol R. Bradford, Nisha J. D'Silva, Thomas E. Carey, Gregory T. Wolf, Karen E. Peterson, and Laura S. Rozek

Published in final edited form as:
Nutr Cancer. 2011 ; 63(5): 734–742. doi:10.1080/01635581.2011.570894.

Higher Micronutrient Intake Is Associated With Human Papillomavirus-Positive Head and Neck Cancer: A Case-Only Analysis

Anna E. Arthur,
Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, USA

Sonia A. Duffy,
Otorhinolaryngology Department, University of Michigan Health System, Ann Arbor, Michigan, USA; University of Michigan School of Nursing, Ann Arbor, Michigan, USA; Psychiatry Department, University of Michigan Health System, Ann Arbor, Michigan, USA; and Ann Arbor Veterans Administration Health System, Ann Arbor, Michigan, USA

Gloria I. Sanchez,
Group Infection and Cancer, University of Antioquia School of Medicine, Medellin, Colombia

Stephen B. Gruber,
Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan, USA; Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA; and Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, USA

Jeffrey E. Terrell,
Otorhinolaryngology Department, University of Michigan Health System, Ann Arbor, Michigan, USA

James R. Hebert,
South Carolina Statewide Cancer Prevention & Control Program, Arnold School of Public Health, University of South Carolina, Columbia, South Carolina, USA

Emily Light,
Biostatistics Unit, University of Michigan Comprehensive Cancer Center, Ann Arbor, Michigan, USA

Carol R. Bradford,
Otorhinolaryngology Department, University of Michigan Health System, Ann Arbor, Michigan, USA

Nisha J. D'Silva,
University of Michigan School of Dentistry, Ann Arbor, Michigan, USA, and Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Thomas E. Carey,
Otorhinolaryngology Department, University of Michigan Health System, Ann Arbor, Michigan, USA, and University of Michigan School of Dentistry, Ann Arbor, Michigan, USA

Address correspondence to Laura S. Rozek, University of Michigan, School of Public Health, Department of Environmental Health Sciences, 6630 SPH Tower, 1415 Washington Heights, Ann Arbor, MI 48109-2029. Phone: 734-763-8095. Fax: 734-936-7283. rozekl@umich.edu.

Gregory T. Wolf,
Otorhinolaryngology Department, University of Michigan Health System, Ann Arbor, Michigan, USA

Karen E. Peterson, and
Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, USA

Laura S. Rozek
Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, USA, and Otorhinolaryngology Department, University of Michigan Health System, Ann Arbor, Michigan, USA

Abstract

No studies have investigated dietary differences between head and neck squamous cell carcinoma (HNSCC) patients with human papillomavirus (HPV)-positive tumors and patients with HPV-negative tumors. This study was designed to investigate the relationship between diet and HPV status in HNSCC patients. Cases of HNSCC were recruited from 2 clinical centers participating in the University of Michigan Head and Neck Specialized Program of Research Excellence (SPORE). HPV tissue genotyping was performed, and epidemiological and dietary data collected. Multivariable logistic regression tested whether pretreatment consumption of 12 selected micronutrients was significantly associated with HPV-positive status in 143 patients newly diagnosed with cancer of the oral cavity or pharynx. After controlling for age, sex, body mass index, tumor site, cancer stage, problem drinking, smoking, and energy intake, significant and positive associations were observed between vitamin A, vitamin E, iron, β-carotene, and folate intake and HPV-positive status ($P_{trend} < 0.05$), suggesting that diet may be a factor in the improved prognosis documented in those with HPV-positive HNSCC. Dietary differences by HPV status should be considered in prognostic studies to better understand the influence of diet on HNSCC survival.

INTRODUCTION

While tobacco and concurrent alcohol use accounts for the majority of all cases of head and neck squamous cell carcinoma (HNSCC) in many populations, the oncogenic human papillomavirus (HPV)-16 contributes to a distinct subtype of the disease (1-6). HPV-associated tumors accounted for more than 50% of the squamous cell carcinomas of the oropharynx in the United States prior to 2005 (5). Recent data show that the proportion of HPV-positive oropharynx cancers is continuing to increase. Among a cohort of oropharyngeal cancer patients followed by our group from 1999 to 2002, 64% entered into a clinical trial of induction chemotherapy followed by concurrent cisplatin and radiation had HPV-positive tumors (2). In the consecutive cohort entered from 2002 to 2007 into our subsequent trial of concurrent cisplatin, taxol, and radiation, 82% of the oropharynx cancers contained high-risk HPV (7). The proportion of HPV-positive head and neck cancers at other sites is less clear, with our estimates falling below 20%. The accumulating evidence suggests that these two distinct subtypes of HNSCC are characterized by different risk profiles and clinical outcomes. HPV-positive tumors are typically found in younger individuals with less exposure to tobacco and have been associated with improved long-term survival when compared with HPV-negative, more strongly tobacco-related tumors, independent of treatment modality (7-13). HPV-positive patients with a more extensive smoking history may experience reduced disease-specific survival and increased risk of disease recurrence than their non-smoking counterparts (7,14).

Risk factors for HNSCC other than tobacco use and HPV infection have been identified. Inadequate intake of certain micronutrients and foods may contribute to the development of HNSCC, while adequate intake may be protective (15). Evidence for inverse associations between oral and pharyngeal cancer and dietary consumption of several micronutrients includes associations for the antioxidant vitamins A, C, E, and carotenoids (15-18); minerals such as iron, calcium, and zinc (15,16,18-21); folate (16,22); and riboflavin (19-21). Several studies have reported inverse associations between oral and pharyngeal cancer risk and fruit and vegetable intake (17,19,23-25). Furthermore, low fruit intake may be associated with reduced survival in patients diagnosed with HNSCC (26), and high vegetable intake may reduce the risk of recurrence and mortality in oral cancer patients (27).

Although improved prognoses have been documented in HNSCC patients with HPV-positive tumors, the basis for this survival advantage has not been well established. Whether or not diet plays an independent role in HNSCC survival is unknown. Individuals with HPV-associated tumors tend to lead healthier lifestyles—they are younger, have lower exposure to tobacco and alcohol, and have higher education and socioeconomic status (SES) than individuals with tobacco-related tumors (1). These data suggest that diet may differ between subjects with HPV-positive tumors and subjects with HPV-negative tumors, as diet quality is generally associated with SES and lifestyle (28,29). Hence, this case-only analysis was conducted to determine if there are differences in pretreatment intake of key micronutrients between newly diagnosed HPV-positive and negative HNSCC patients.

METHODS

This was a cross-sectional study of patients enrolled in the University of Michigan Head and Neck Cancer, Specialized Program of Research Excellence (SPORE). The independent variables were total intake from food and supplements of 12 micronutrients: vitamins A, C, D, B12, β-carotene, calcium, iron, zinc, copper, riboflavin, and folic acid. Confounding variables included age, sex, race/ethnicity, educational level, body mass index [BMI = weight(kg)/height(m)^2], smoking, problem drinking, tumor site, and cancer stage. The outcome variable was HPV status (positive or negative).

Study Sample

Newly diagnosed patients with squamous cell carcinoma of the head and neck were recruited to participate in this study. Patients were recruited from the Ann Arbor Veterans Affairs Hospital and the University of Michigan Hospital. Exclusion criteria included: 1) <18 yr of age; 2) pregnant; 3) non-English-speaking; 4) diagnosed as mentally unstable; 5) a diagnosis of another non-upper aerodigestive tract cancers (such as thyroid or skin cancer); or 6) a previous diagnosis and treatment for head and neck cancer. Out of 1,185 patients approached, 934 consented to participate yielding a response rate of 79%.

Tumors with adequate tissue for microdissection were available from 205 patients, and 203 of 205 (99%) had amplifiable DNA as assessed by PCR amplification of beta-globin. Of these 203 patients, 193 had completed a self-administered food frequency questionnaire (FFQ) at presentation (30). No laryngeal tumors contained integrated HPV. Therefore, 50 individuals with larynx tumors were excluded, leaving a total of 143 subjects in the current analysis. Human subjects' approval was received from the institutional review boards at each institution.

Procedures

Research assistants recruited patients to the study in the waiting rooms of otolaryngology clinics. Informed consent was obtained and epidemiologic data were collected using a self-

administered questionnaire, as has been previously described (31). A medical record review was completed for each study participant. Dietary intake was collected using a self-administered, semiquantitative FFQ, validated for use in several populations (30,32,33).

Tumor blocks were recut for uniform histopathologic review and microdissection, with the first and last slides of a series of 12 reviewed by a qualified pathologist to confirm the original diagnosis and to circle areas for microdissection. Percent cellularity was estimated for each tumor, and areas with >70% cellularity of cancer were designated for cancer microdissection.

HPV genotyping was performed using the GP5+/GP6- PCR reverse line blot (RLB) method for 37 HPV types (34,35). Adequacy of amplifiable DNA was assessed using PCR for betaglobin as a positive control. All runs included positive and negative controls for HPV, as well as water controls to exclude the possibility of contamination. All HPV assays were repeated at least twice for quality assurance and quality control, with perfect correspondence. A subset of tumors also had frozen tumor available, and independent HPV assays were performed on these tumors.

Measures

The 131-item FFQ was designed to assess respondents' usual dietary intake from food and supplements over the past year. Total energy and nutrient intake was estimated by summing intakes from each food based on the given standard portion size, reported frequency of consumption, and nutrient content of each food item (33). As the FFQ has not been validated in HNSCC populations, reliability of energy reporting using this tool is unknown. Because the majority of patients had advanced tumors (Stage 3 or 4) upon entering the study, self-reported dietary intake may be reflective of recent dietary changes due to disease progression, rather than actual intake over the past year. Therefore, we chose to use a surrogate measure of energy intake in this analysis as opposed to self-reported energy intake from the FFQ. The population-adjusted total energy expenditure (PATEE), developed by Hebert et al., is based on an individual's basal metabolic rate (BMR, as calculated by the Harris-Benedict equation) and is weighted by age group-specific median caloric intakes as reported by the National Health and Nutrition Examination Survey (36,37). PATEE was developed for use in the Women's Health Initiative (37). Originally designed for use as a proxy when reported FFQ energy intake (FFQEI) data are missing (38), the current refinement has been validated in a cohort of subjects on which total energy intake was estimated from doubly labeled water (37). The PATEE variable was calculated for each individual and Pearson correlation coefficients were computed to measure the strength of the linear relationship between FFQEI and PATEE, which was found to be excellent ($b = 0.92$; $SE_b = 0.20$) (37).

Smoking data permitted analysis by "current-past-never" smoking and cumulative pack-years. Cumulative pack-years was coded as ≤30 pack-years or >30 pack-years (median) to avoid problems due to non-normality. Alcohol consumption was measured using the previously validated Alcohol Use Disorders Identification Test (AUDIT) (39). An AUDIT score ≥8 was considered problem drinking. Tumor site was recorded from operative notes and surgical pathology forms. For the purposes of data analysis, tumor site was categorized into oral cavity, pharynx, or larynx. Cancer stage was categorized a priori into 3 groups, with Stages 1 and 2 considered together, and Stage 3 and Stage 4 considered separately, enhancing power of comparisons by stage.

Data Analysis

Descriptive statistics were generated for all variables (means and frequencies), and bivariate analyses were conducted to determine differences in demographic characteristics between individuals with HPV-positive tumors and HPV-negative tumors. Student's *t*-tests were conducted to test whether the two study groups differed significantly by age and BMI. To test whether the two groups differed significantly by sex, race, education, smoking, alcohol problem, and tumor site, chi-square tests were performed. The Wilcoxon rank-sum test was used to determine if the HPV-positive and HPV-negative study groups differed significantly by tumor stage. Multivariable logistic regression models were fit to test whether pretreatment intake of 12 select micronutrients was significantly associated with HPV-positive status. Each micronutrient was categorized into quartiles of intake from foods and supplements and quartiles of intake from foods only. Categorical variables were created for sex (male vs. female), tumor site (pharynx vs. oral cavity), cancer stage (Stage 1 or 2, Stage 3, Stage 4), smoking (current, past, never), and problem drinking (none vs. any). A test for trend across increasing quartiles of total dietary intake from food and supplements was performed with an alpha level of 0.05 considered significant. A similar test for trend was performed for dietary intake from food sources only. Covariates adjusted for included age, BMI, PATEE, sex, tumor site, smoking, and problem drinking. Education was considered as a covariate but did not influence the significance of the model. A sensitivity analysis was conducted for the association between each micronutrient with HPV status, controlling for the remaining micronutrients. To address correlation among micronutrient intakes, a principal components analysis was performed for the remaining micronutrients, and the top 3 factors were included in the multivariable model. In all models, these 3 factors accounted for at least 75% of the variability in the remaining variables (77–81%). All analyses were performed using SAS software (SAS 9.1, SAS Institute, Cary, NC).

RESULTS

The majority of the study population was European American (93%) and male (80%). Ever-smoking and problem drinking were highly prevalent in this population (77% and 31%, respectively). Patients were more likely to have Stage 3 or Stage 4 tumors than Stage 1 or 2 at presentation to clinical centers.

Table 1 compares epidemiologic characteristics of those patients with HPV-positive tumors to those with HPV-negative tumors. The vast majority of HPV-positive cancers were found in the pharynx. Individuals with HPV-positive tumors appeared to have a higher mean BMI than those with HPV-negative tumors (27.9 kg/m^2 and 26.0 kg/m^2, respectively; $P = 0.08$). Distribution of "ever" vs. "never" smoking did not differ significantly by HPV status. HPV-positive patients were less likely to be current smokers ($P = 0.05$), and tended to have smoked for less than the median number of pack-years ($P = 0.07$). A greater percentage of individuals with HPV-negative tumors reported they had an alcohol problem than did individuals with HPV-positive tumors (36.2% and 18.4%, respectively; $P = 0.04$). Distribution of HPV status did not differ significantly with respect to age, sex, cancer stage, race, or highest education attained.

Mean estimated BMR differed significantly between HPV-positive and HPV-negative individuals (mean = 1534 and 1446, respectively; $P = 0.02$), while mean FFQEI and PATEE did not differ significantly by HPV status. FFQEI and PATEE were not well correlated with one another ($r = 0.17$, $r = 0.09$, and $r = 0.19$ for total, HPV-positive, and HPV-negative, respectively). However, controlling for FFQEI instead of PATEE in the multivariable models did not significantly change the quartile-specific odds ratios and P_{trend} for each respective micronutrient (results not shown).

Because micronutrients were modeled as quartiles of intake, the cut point for each quartile is listed in Table 2 for total nutrient intake from food and supplements and for nutrient intake from food sources alone. After controlling for age, sex, BMI, tumor site, cancer stage, smoking, problem drinking, and PATEE, a significant association was observed between HPV-positive status and increasing quartiles of intake from food and supplements for vitamin A (OR = 22.41 for the highest quartile versus the lowest quartile; $P_{trend} = 0.003$), vitamin E (OR = 3.84; $P_{trend} = 0.04$), β-carotene (OR = 3.86; $P_{trend} = 0.02$), iron (OR = 14.45; $P_{trend} = 0.003$) and folate (OR = 8.97; $P_{trend} = 0.02$) (Table 3). Associations between HPV status and total intake of vitamin C, vitamin D, calcium, zinc, copper, riboflavin, and vitamin B12 were not significant. The results did not substantially differ when considering micronutrient intakes from food sources alone. After adjustment for the remaining micronutrients in a sensitivity analysis, vitamin A and iron remained significant predictors of HPV status.

DISCUSSION

In this study of HNSCC patients, we observed a significant and positive association across quartiles of intake and HPV-positive status for total nutrient intake from food and supplements, and intake from food sources alone of vitamin A, vitamin E, β-carotene, iron, and folate. These results indicate that patients with HPV-positive tumors consume significantly higher levels of key micronutrients with anticancer functions compared to patients with HPV-negative tumors. Sensitivity analyses indicated that, regardless of overall micronutrient intake, vitamin A and iron predicted HPV status. HPV-positive head and neck cancer cases generally have lifestyle characteristics that are associated with better overall health than the lifestyle characteristics of HPV-negative patients. For example, HPV-positive patients typically have a higher SES and less lifetime exposure to tobacco and alcohol compared to HPV-negative patients. Higher SES and less tobacco use and alcohol consumption are generally associated with better overall diet quality (28,29). This is also seen in our study, where HPV-positive cases have a less extensive history of smoking and alcohol abuse than do HPV-negative cases.

There are other potential explanations for the observed micronutrient differences between HPV-positive and HPV-negative cases. A previous study reported that HPV-16 seronegative individuals with greater fruit or citrus intake were significantly less likely to develop HNSCC than those with low fruit or citrus intake. On the other hand, though counterintaitive, HPV-16 seropositive individuals with greater citrus fruit consumption were significantly more likely to develop HNSCC than HPV-16 seropositive individuals consuming lower amounts of citrus fruit. The authors hypothesized a mechanism by which citrus fruit and other highly acidic foods may erode the mucosal lining of the gastrointestinal tract, increasing the probability of HPV infection (40). Higher prediagnosis intake of the micronutrients observed to be significantly associated with HPV-positive status in the currents study, or foods rich in the observed micronutrients, could make an individual more susceptible to oral HPV infection and therefore more at risk for developing HNSCC due to HPV.

Another study reported that higher plasma levels of carotenoids were associated with a 43% to 50% reduction in the risk of initial anal HPV infection, but lower levels of carotenoids and retinol were associated with clearance of persistent anal HPV infection (41). This evidence is consistent with our finding that higher intakes of vitamin A and ß-carotene are associated with HPV-positive HNSCC.

Higher intakes of vitamin A, vitamin E, β-carotene, iron, and folate may independently influence the better prognosis of HPV-positive patients and this possibility should be

explored further. There are several mechanisms by which adequate dietary intake of vitamin A, β-carotene, iron, and folate may contribute to better clinical outcomes in HNSCC patients. Two previous studies reported that higher levels of plasma carotenoids in HNSCC patients before and after radiotherapy were associated with improved progression-free survival, as well as with overall survival (42,43). Vitamin A in the form of retinoic acid is believed to regulate epithelial cell differentiation through its effects on gene expression. Homodimer and heterodimer complexes produced during metabolism of vitamin A bind to retinoic acid response elements (RARE) in promoter regions of specific genes, resulting in modification of cellular processes important in controlling carcinogenesis such as apoptosis (44,45). Furthermore, retinoic acid and β-carotene may play a role in preventing uncontrolled cell growth by preserving gap junction communication between cells (45-47). β-carotene also functions as an antioxidant, quenching singlet oxygen species and scavenging peroxyl radicals, thus helping to prevent cellular damage (48).

Recent findings of our research group show that HPV-positive patients may have enhanced adaptive immunity, which is associated with improved tumor response to chemotherapy when compared to HPV-negative patients (49). As reviewed by Wintergerst et al., vitamin A, iron, and folate each play mechanistic roles in immune function. Vitamin A is necessary for both adaptive and innate immunity and is involved in the anti-inflammatory response of T-helper cell 2 (Th2). A state of vitamin A deficiency is associated with reduced natural killer (NK) cell activity and antigen-specific response by Th1 cells (50). Differentiation and proliferation of NK and Th cells are modulated by cellular iron and therefore do not proliferate as rapidly under a state of iron deficiency (50). Folate works synergistically with vitamins B6 and B12 to supply 1-carbon units during synthesis of nucleotides and proteins. A deficiency consequently results in decreased proliferation and number of circulating lymphocytes. Deficiency also is associated with an increased CD4+/CD8+ ratio (50). Associations between intake of vitamin A, folate, iron, and immune function in HNSCC patients should be investigated, as higher dietary intake from food, supplements, or both may play a key role in the improved immune function and prognosis of individuals with HPV-positive tumors.

Finally, while there was no significant difference between HPV-positive and HPV-negative patients reporting “ever” vs. “never” smoking, there was a marginally significant difference between the two groups when comparing current smoking and pack-years of overall exposure. This suggests that although the same proportion of HPV-positive patients as HPV-negative patients have smoked at some point in their lives, HPV-positive patients were more likely to have quit smoking and thus have less lifelong exposure to tobacco. While speculative, it also could indicate that smoking at an earlier age concurrent with HPV infection is associated with development of HNSCC. We did not have the statistical power to detect an interaction between diet and smoking, but this relationship should be examined in the future.

Findings should be interpreted in light of several limitations. Although a common concern about HNSCC patients is inadequate micronutrient intake at the time of diagnosis—with the exception of vitamin E, vitamin D, and calcium—at least 50% of our study population was consuming adequate amounts of each micronutrient (based on recommended daily allowances and adequate intake values) when considering intake from diet and supplements. However, reported intake may include some misclassification due to difficulty in recalling and quantifying foods consumed over the previous year. Our study does not include controls, preventing the ability to estimate risk of disease due to diet. Nevertheless, we note consistency with case-control studies of diet and HNSCC, where protective micronutrients are associated with HPV-positive status of the tumor.

In conclusion, higher intakes of key micronutrients are associated with HPV-positive HNSCC. When consumed at higher levels these micronutrients may increase the susceptibility of patients to HPV infection. Also, it is possible that higher intakes of the micronutrients observed in this study to be associated with HPV status play an independent role in the improved prognoses of HPV-positive HNSCC patients compared to HPV-negative patients. This hypothesis should be examined in the future, as it may present an avenue to improve survival in patients with this deadly cancer.

Acknowledgments

This study was funded as part of the University of Michigan Head and Neck Specialized Program of Research Excellence, NIH/NCI CA097248. Dr. Hébert was supported by an Established Investigator Award in Cancer Prevention and Control from the Cancer Training Branch of the National Cancer Institute (K05 CA136975). The authors would like to acknowledge the patients, clinicians, and individual project principal investigators of the UM Head and Neck SPORE program who provided access to the longitudinal clinical database and were responsible for recruitment, treatment and followup of patients included in this report. These investigators included Carol Bradford, MD; Thomas Carey, PhD; Douglas Chepeha, MD, MPH; Nisha D'Silva, DDS, PhD; Sonia Duffy, PhD, RN; Avraham Eisbruch, MD; Bhavna Kumar, MS; David Kurnit, MD; Theodore Lawrence, MD, PhD; Sofia Merajver, MD, PhD; Mark Prince, MD; Jeremy Taylor, PhD; Theodore Teknos, MD; Jeffrey Terrell, MD; Shaomeng Wang, MD; Frank Worden, MD; and Gregory T. Wolf, MD, SPORE Program Principal Investigator.

References

1. Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst. 2008; 100:407–420. [PubMed: 18334711]
2. Worden FP, Kumar B, Lee JS, Wolf GT, Cordell KG, et al. Chemoselection as a strategy for organ preservation in advanced oropharynx cancer: response and survival positively associated with HPV16 copy number. J Clin Oncol. 2008; 26:3138–3146. [PubMed: 18474879]
3. Kim SH, Koo BS, Kang S, Park K, Kim H, et al. HPV integration begins in the tonsillar crypt and leads to the alteration of p16, EGFR and c-myc during tumor formation. Int J Cancer. 2007; 120:1418–1425. [PubMed: 17205528]
4. Hobbs CG, Sterne JA, Bailey M, Heyderman RS, Birchall MA, et al. Human papillomavirus and head and neck cancer: a systematic review and meta-analysis. Clin Otolaryngol. 2006; 31:259–266. [PubMed: 16911640]
5. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev. 2005; 14:467–475. [PubMed: 15734974]
6. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst. 2000; 92:709–720. [PubMed: 10793107]
7. Maxwell JH, Kumar B, Feng FY, Worden FP, Lee JS, et al. Tobacco use in human papillomavirus-positive advanced oropharynx cancer patients related to increased risk of distant metastases and tumor recurrence. Clin Cancer Res. 2010; 16:1226–1235. [PubMed: 20145161]
8. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med. 2007; 356:1944–1956. [PubMed: 17494927]
9. Applebaum KM, Furniss CS, Zeka A, Posner MR, Smith JF, et al. Lack of association of alcohol and tobacco with HPV16-associated head and neck cancer. J Natl Cancer Inst. 2007; 99:1801–1810. [PubMed: 18042931]
10. Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, et al. Molecular classification identifies a subset of human papillomavirus—associated oropharyngeal cancers with favorable prognosis. J Clin Oncol. 2006; 24:736–747. [PubMed: 16401683]
11. Licitra L, Perrone F, Bossi P, Suardi S, Mariani L, et al. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J Clin Oncol. 2006; 24:5630–5636. [PubMed: 17179101]

12. Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst. 2003; 95:1772–1783. [PubMed: 14652239]
13. Li W, Thompson CH, O'Brien CJ, McNeil EB, Scolyer RA, et al. Human papillomavirus positivity predicts favorable outcome for squamous carcinoma of the tonsil. Int J Cancer. 2003; 106:553–558. [PubMed: 12845651]
14. Kumar B, Cordell KG, Lee JS, Worden FP, Prince ME, et al. EGFR, p16, HPV Titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. J Clin Oncol. 2008; 26:3128–3137. [PubMed: 18474878]
15. Lucenteforte E, Garavello W, Bosetti C, La Vecchia C. Dietary factors and oral and pharyngeal cancer risk. Oral Oncol. 2009; 45:461–467. [PubMed: 18990606]
16. Negri E, Franceschi S, Bosetti C, Levi F, Conti E, et al. Selected micronutrients and oral and pharyngeal cancer. Int J Cancer. 2000; 86:122–127. [PubMed: 10728605]
17. Zheng T, Boyle P, Willett WC, Hu H, Dan J, et al. A case-control study of oral cancer in Beijing, People's Republic of China. Associations with nutrient intakes, foods, and food groups. Eur J Cancer B Oral Oncol. 1993; 29B:45–55. [PubMed: 8180577]
18. Gupta PC, Hebert JR, Bhonsle RB, Murti PR, Mehta H, et al. Influence of dietary factors on oral precancerous lesions in a population-based case-control study in Kerala, India. Cancer. 1999; 85:1885–1893. [PubMed: 10223226]
19. Petridou E, Zavras AI, Lefatzis D, Dessypris N, Laskaris G, et al. The role of diet and specific micronutrients in the etiology of oral carcinoma. Cancer. 2002; 94:2981–2988. [PubMed: 12115387]
20. Richie JP Jr, Kleinman W, Marina P, Abraham P, Wynder EL, et al. Blood iron, glutathione, and micronutrient levels and the risk of oral cancer. Nutr Cancer. 2008; 60:474–482. [PubMed: 18584481]
21. Hebert JR, Gupta PC, Bhonsle RB, Mehta H, Zheng W, et al. Dietary exposures and oral precancerous lesions in Srikakulam District, Andhra Pradesh, India. Public Health Nutr. 2002; 5:303–312. [PubMed: 12020382]
22. Pelucchi C, Talamini R, Negri E, Levi F, Conti E, et al. Folate intake and risk of oral and pharyngeal cancer. Ann Oncol. 2003; 14:1677–1681. [PubMed: 14581278]
23. Levi F, Pasche C, La Vecchia C, Lucchini F, Franceschi S, et al. Food groups and risk of oral and pharyngeal cancer. Int J Cancer. 1998; 77:705–709. [PubMed: 9688303]
24. Freedman ND, Park Y, Subar AF, Hollenbeck AR, Leitzmann MF, et al. Fruit and vegetable intake and esophageal cancer in a large prospective cohort study. Int J Cancer. 2007; 121:2753–2760. [PubMed: 17691111]
25. Davidson PG, Touger-Decker R. Chemopreventive role of fruits and vegetables in oropharyngeal cancer. Nutr Clin Pract. 2009; 24:250–260. [PubMed: 19321899]
26. Duffy SA, Ronis DL, McLean S, Fowler KE, Gruber SB, et al. Pretreatment health behaviors predict survival among patients with head and neck squamous cell carcinoma. J Clin Oncol. 2009; 27:1969–1975. [PubMed: 19289626]
27. Sandoval M, Font R, Manos M, Dicenta M, Quintana MJ, et al. The role of vegetable and fruit consumption and other habits on survival following the diagnosis of oral cancer: a prospective study in Spain. Int J Oral Maxillofac Surg. 2009; 38:31–39. [PubMed: 18951763]
28. Drewnowski A. Obesity, diets, and social inequalities. Nutr Rev. 2009; 67(Suppl 1):S36–S39. [PubMed: 19453676]
29. Breslow RA, Guenther PM, Juan W, Graubard BI. Alcoholic beverage consumption, nutrient intakes, and diet quality in the U.S. adult population, 1999–2006. J Am Diet Assoc. 2010; 110:551–562. [PubMed: 20338281]
30. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol. 1985; 122:51–65. [PubMed: 4014201]
31. Duffy SA, Khan MJ, Ronis DL, Fowler KE, Gruber SB, et al. Health behaviors of head and neck cancer patients the first year after diagnosis. Head Neck. 2008; 30:93–102. [PubMed: 17685451]

32. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol. 1992; 135:1114–1126. discussion 27–36. [PubMed: 1632423]
33. Willett, W. Nutritional Epidemiology. 2. Oxford University Press; New York: 1998.
34. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, et al. GP5+/6 +PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol. 2002; 40:779–787. [PubMed: 11880393]
35. Jacobs MV, Snijders PJ, Van Den Brule AJ, Helmerhorst TJ, Meijer CJ, et al. A general primer GP5+/GP6(+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. J Clin Microbiol. 1997; 35:791–795. [PubMed: 9041439]
36. McDowell MA, Briefel RR, Alaimo K, Bischof AM, Caughman CR, et al. Energy and macronutrient intakes of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988–91. Adv Data. 1994; 24:1–24.
37. Hebert JR, Patterson RE, Gorfine M, Ebbeling CB, St. Jeor ST, et al. Differences between estimated caloric requirements and self-reported caloric intake in the Women's Health Initiative. Ann Epidemiol. 2003; 13:629–637. [PubMed: 14732302]
38. Toporoff E, Hebert JR. A proxy approach to the determination of total caloric intake for use in cancer epidemiology. Nutr Cancer. 1990; 13:35–49. [PubMed: 2300493]
39. Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption—II. Addiction. 1993; 88:791–804. [PubMed: 8329970]
40. Meyer MS, Applebaum KM, Furniss CS, Peters ES, Luckett BG, et al. Human papillomavirus-16 modifies the association between fruit consumption and head and neck squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2008; 17:3419–3426. [PubMed: 19064557]
41. Shvetsov YB, Hernandez BY, Wilkens LR, Thompson PJ, Franke AA, et al. Plasma micronutrients and the acquisition and clearance of anal human papillomavirus infection: the Hawaii HPV Cohort Study. Cancer Res. 2010; 70:9787–9797. [PubMed: 20935226]
42. Sakhi AK, Russnes KM, Thoresen M, Bastani NE, Karlsen A, et al. Pre-radiotherapy plasma carotenoids and markers of oxidative stress are associated with survival in head and neck squamous cell carcinoma patients: a prospective study. BMC Cancer. 2009; 9:458. [PubMed: 20025747]
43. Sakhi AK, Bohn SK, Smeland S, Thoresen M, Smedshaug GB, et al. Postradiotherapy plasma lutein, alpha-carotene, and beta-carotene are positively associated with survival in patients with head and neck squamous cell carcinoma. Nutr Cancer. 2010; 62:322–328. [PubMed: 20358469]
44. Wolf G. The intracellular vitamin A-binding proteins: an overview of their functions. Nutr Rev. 1991; 49:1–12. [PubMed: 2052162]
45. Gropper, SAS.; Groff, JL.; Smith, JL.; Switzer, BR. Advanced Nutrition and Human Metabolism. 4. Thomson Wadsworth; Belmont, CA: 2005.
46. Bertram JS, Vine AL. Cancer prevention by retinoids and carotenoids: independent action on a common target. Biochim Biophys Acta. 2005; 1740:170–178. [PubMed: 15949684]
47. Sies H, Stahl W. Carotenoids and intercellular communication via gap junctions. Int J Vitam Nutr Res. 1997; 67:364–367. [PubMed: 9350479]
48. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes Control. 1991; 2:427–442. [PubMed: 1764568]
49. Wansom D, Light E, Worden F, Prince M, Urba S, et al. Correlation of cellular immunity with human papillomavirus 16 status and outcome in patients with advanced oropharyngeal cancer. Arch Otolaryngol Head Neck Surg. 2010; 136:1267–1273. [PubMed: 21173378]
50. Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab. 2007; 51:301–323. [PubMed: 17726308]

TABLE 1

Baseline characteristics by HPV status ($N = 143$)

Demographics	Total	HPV + No. (%)	HPV – No. (%)	*P* Value
N	143	38 (26.6)	105 (73.4)	
Age (SD)†	57.9	58.9 (7.4)	57.9 (10.7)	0.99
Sex††				
Male	115	34 (89.5)	81 (77.1)	0.10
Female	28	4 (10.5)	24 (22.9)	
Race††				
White	133	37 (97.4)	96 (91.4)	0.38
Black	4	1 (2.6)	3 (2.9)	
American Indian	6	0 (0)	6 (5.7)	
Highest education††				
≤High school/GED	70	19 (50)	51 (48.5)	0.88
Some college	73	19 (50)	54 (51.4)	
BMI in kg/m2 (sd)†	26.5 (5.8)	27.9 (4.6)	26.0 (6.1)	0.08
Smoking††				
Current	44	7 (18.4)	37 (35.2)	0.15
Former	66	21 (55.3)	45 (42.9)	
Never	33	10 (26.3)	23 (21.9)	
Pack-years (median = 30)††				
≥Median	59	11 (28.9)	48 (45.7)	0.07
<Median	84	27 (71.1)	57 (54.3)	
Alcohol problem††				
Yes	45	7 (18.4)	38 (36.2)	0.04*
No	98	31 (81.6)	67 (63.8)	
Site††				
Oral cavity	35	1 (2.6)	34 (32.4)	<0.01*
Pharynx	108	37 (97.4)	71 (67.6)	
Stage‡				
1, 2	16	1 (2.6)	15 (14.3)	0.17
3	32	9 (23.7)	23 (21.9)	
4	95	28 (73.7)	67 (63.8)	

*Indicates significance at $P < 0.05$.

†Student's *t*-test.

††Chi-square test or Fisher's exact test was used as appropriate.

‡Wilcoxon rank-sum test.

Nutr Cancer. Author manuscript; available in PMC 2012 July 08.

TABLE 2

Quartile designations of nutrient intake among HNSCC patients ($N = 143$)

	Food and Supplements Percentile			Food Percentile		
Nutrient	**25**	**50**	**75**	**25**	**50**	**75**
Vitamin A (IU/day)	5406.2	8589.1	12601.5	3957.3	6074.2	8984.2
Vitamin C (mg/day)	81.4	146.6	262.5	57.1	97.4	154.7
Vitamin E (mg/day)	6.6	13.4	27.3	4.9	6.9	10
β-carotene (μg/day)	1512.4	2483.7	4342.1	1296.3	2127.3	3621.5
Vitamin D (IU/day)	158.5	345.5	585.8	100.7	171	255.7
Calcium (mg/day)	619.5	913.7	1351.1	537.9	769.7	1117.3
Iron (mg/day)	10.7	15.9	24.12	9.8	13.6	18.8
Zinc (mg/day)	9.3	13.9	23.1	8.6	11.7	16.2
Copper (μg/day)	1.1	1.8	3.2	1.1	1.5	2
Riboflavin (mg/day)	2.2	3.2	4.5	1.7	2.4	3.1
Vitamin B12 (μg/day)	6.4	10.7	17.9	4.7	7	10.1
Folic acid (μg/day)	381.3	586.7	816.7	294.2	399.7	547.8

Nutr Cancer. Author manuscript; available in PMC 2012 July 08.

TABLE 3

Adjusted odds of HPV+ status categorized by quartiles of total nutrient intake among oral and pharyngeal HNSCC patients ($N = 143$)[†]

Nutrient	OR (95% CI)	*P* Trend	Nutrient	OR (95% CI)	*P* Trend
Vitamin A			Iron		
Quartile 2	11.18 (1.88–66.32)	0.003*	Quartile 2	4.30 (0.97–19.02)	0.003*
Quartile 3	5.72 (1.00–33.00)		Quartile 3	1.86 (0.39–8.93)	
Quartile 4	22.41 (3.57–140.84)		Quartile 4	14.45 (3.04–68.73)	
Vitamin C			Zinc		
Quartile 2	4.09 (1.02–16.35)	0.261	Quartile 2	1.99 (0.56–7.14)	0.107
Quartile 3	3.38 (0.80–14.24)		Quartile 3	1.34 (0.37–4.88)	
Quartile 4	2.82 (0.66–12.13)		Quartile 4	3.17 (0.94–10.72)	
Vitamin E			Copper		
Quartile 2	2.39 (0.65–8.73)	0.041*	Quartile 2	1.03 (0.30–3.62)	0.344
Quartile 3	3.33 (0.87–12.71)		Quartile 3	2.41 (0.71–8.18)	
Quartile 4	3.84 (1.04–4.16)		Quartile 4	1.35 (0.39–4.70)	
β-carotene			Riboflavin		
Quartile 2	1.18 (0.25–5.53)	0.018*	Quartile 2	1.93 (0.55–6.81)	0.093
Quartile 3	5.89 (1.51–23.01)		Quartile 3	3.12 (0.81–12.05)	
Quartile 4	3.86 (1.00–14.99)		Quartile 4	2.80 (0.79–9.89)	
Vitamin D			Vitamin B12		
Quartile 2	0.52 (0.15–1.79)	0.501	Quartile 2	1.44 (0.44–4.67)	0.229
Quartile 3	1.67 (0.50–5.60)		Quartile 3	2.05 (0.56–7.56)	
Quartile 4	1.15 (0.33–3.98)		Quartile 4	1.95 (0.59–6.45)	
Calcium			Folic Acid		
Quartile 2	0.78 (0.23–2.64)	0.308	Quartile 2	6.07 (1.47–25.07)	0.015*
Quartile 3	0.61 (0.15–2.38)		Quartile 3	3.54 (0.89–14.09)	
Quartile 4	2.09 (0.58–7.56)		Quartile 4	8.97 (1.95–41.19)	

*Indicates test for trend significant at $P < 0.05$ adjusting for age, sex, BMI, tumor site, energy intake, problem drinking, and pack-years.

†Subjects were categorized into quartiles based on reported nutrient intake.