THE ROLE OF DIET IN THE PATHOGENESIS AND PROGNOSIS OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

by

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DEDICATION

This dissertation is dedicated to the participants of the University of Michigan Head and Neck Specialized Program of Research Excellence (HN-SPORE). The HN-SPORE participants are extraordinarily generous to provide the research program with high volumes of data and biological specimens during what may arguably be the most stressful and trying times of their lives. I admire these patients and families for their participation in this research project, which is of little benefit to themselves, but potentially of great benefit to future head and neck cancer patients, and for their strength and courage as they battle this difficult and painful disease.

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TABLE OF CONTENTS

DEDICATION……...……...ii
ACKNOWLEDGEMENTS.……...……...iii
LIST OF FIGURES……...…viii
LIST OF TABLES…….....ix
ABSTRACT……...….....x

CHAPTER

1. Introduction…….1
Theme
Epidemiology of head and neck squamous cell carcinoma
Demographic characteristics
Tobacco use and alcohol consumption
Emergence of human papillomavirus
Diet and weight status as risk factors
Dietary challenges of head and neck cancer patients
Diet and head and neck cancer outcomes
Specific aims
Study population: The UM Head and Neck SPORE
Dietary assessment for nutritional epidemiology studies
Conceptual framework
References

2. Pretreatment dietary patterns, weight status, and head and neck squamous cell carcinoma prognosis…….……19
Abstract
Introduction
Methods
Design
Study population
Procedures
Measures
Statistical Analysis
Results
Discussion

3. Higher micronutrient intake is associated with human papillomavirus-positive head and neck cancer: a case-only analysis……...……44
Abstract
Introduction

Methods
Design
Study population
Procedures
Measures
Statistical Analysis

Results
Discussion

4. Diet, proinflammatory cytokine levels, and cachexia…….....61
Abstract
Introduction
Methods
Design
Study population
Procedures
Measures
Statistical Analysis
Results
Discussion

5. Conclusion……...……...……83

References……...88

LIST OF FIGURES

CHAPTER 1

Figure 1.1: Tumor sites of the head and neck……..…….16
Figure 1.2: Five-year survival by year of diagnosis and tumor site……...16
Figure 1.3: Age-adjusted incidence rates by cancer site; 1975 – 2010…….....17
Figure 1.4: Distinct clinicopathology of HPV-positive and HPV-negative HNSCCs……...17
Figure 1.5: Conceptual Framework……..……..18

CHAPTER 2

Figure 2.1: Kaplan-Meier curves of survival and recurrence for dietary patterns……..…42
Figure 2.2: Kaplan-Meier curves of survival and recurrence for BMI category……..……..43

LIST OF TABLES

CHAPTER 2
Table 2.1: Pretreatment characteristics……34
Table 2.2: Factor loading matrix……..36
Table 2.3: Selected characteristics and dietary intakes of subjects by quintile dietary pattern score……...38
Table 2.4: Multivariate HR and 95% CI according to quintile of dietary pattern score for survival and recurrence events……..…….40
Table 2.5: Multivariate HR and 95% CI of pretreatment body mass index >25 kg/m^2 (overweight/obese) for recurrence and survival events…….41

CHAPTER 3
Table 3.1: Baseline characteristics by HPV status……58
Table 3.2: Quartile designations of nutrient intake among HNSCC patients……...59
Table 3.3: HPV (+) status by quartiles of total nutrient intake…….60

CHAPTER 4
Table 4.1: Pretreatment Characteristics……78
Table 4.2: Factor loading matrix for three dietary patterns……..79
Table 4.3: Proinflammatory cytokine levels by quartile of reported dietary variables……80
Table 4.4: Proinflammatory cytokine levels by quartile of serum carotenoid levels……...81
Table 4.5: Presence of cachexia by proinflammatory……...82

ABSTRACT

The varying risk factor profiles among subtypes and unique challenges observed in the development and course of head and neck squamous cell carcinoma (HNSCC) may influence patients' diet quality and ability to eat. Patients' dietary choices and nutritional status likely have consequences for disease prognosis but have not been evaluated with sufficient rigor. The molecular mechanisms by which diet potentially exerts its effects in HNSCC progression and prognosis also have not been thoroughly investigated. This research was conducted in the context of the University of Michigan Head and Neck Specialized Program of Research Excellence (HN-SPORE). Longitudinal data, including epidemiologic, clinical, and molecular characterization were collected from subjects enrolled into the HN-SPORE between 2002 – 2012. Data were collected at baseline and yearly, with a mean follow-up time among study participants of 4.5 years. Pretreatment dietary data were collected from subjects using the semi-quantitative Harvard food frequency questionnaire (FFQ).

The first objective of this dissertation was to identify major dietary consumption patterns in a population of incident cases of HNSCC, and to examine the association of these dietary patterns and weight status with recurrence and survival. Dietary patterns were identified using principal components analysis and Cox proportional hazard models were fit to examine these associations. Two dietary patterns were identified: a "whole foods pattern", characterized by high intakes of vegetables, fruits, fish, poultry and whole grains, and a

"Western pattern", characterized by high intakes of red and processed meats, refined grains, potatoes and French fries. In multivariable analyses, significantly fewer deaths were observed in subjects most adherent to the whole foods pattern (HR 0.56, 95% CI 0.34 – 0.92, P_{trend}=0.01). Subjects classified as overweight or obese had significantly fewer deaths (HR 0.65, 95% CI 0.49 – 0.85, *P*=0.001) and recurrences (HR 0.70, 95% CI 0.52 – 0.95, *P*=0.02) than normal or underweight subjects.

The second aim of this study sought to compare pretreatment dietary intake of vitamins and minerals between newly diagnosed HNSCC patients with HPV-positive tumors to those with HPV-negative tumors. Multivariable logistic regression was used examine the association between reported intake of twelve micronutrients with HPV-positive tumor status. After controlling for potential confounding factors, 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).

Lastly, associations between pretreatment dietary patterns, FFQ reported and serum levels of carotenoids and vitamin E, and presence of cachexia with pretreatment serum proinflammatory cytokine levels were examined. In multivariable analyses, higher whole foods dietary pattern scores were significantly associated with lower levels of IL-6, TNF-α, and IFN-γ (*P* = <0.001, *P* = 0.008, and *P* = 0.03, respectively). Significant downward trends in IL-6, TNF-α, and IFN-γ levels were found across increasing quartiles of total reported carotenoid intake (*P* = 0.006, *P* = 0.04, and *P* = 0.04, respectively). No significant association was reported for the relationships between IL-6 and TNF-α levels and serum carotenoids. There was a significant trend towards decreased IFN-γ levels across increasing

quartiles of serum α-tocopherol levels ($P = 0.03$). No significant association was observed between cytokine levels and cachexia.

In summary, consuming a pretreatment diet rich in vegetables, fruit, fish, poultry and whole grains were associated with lower HNSCC mortality. The reduction of proinflammatory cytokine levels is one potential mechanism underlying this association. The reported differences in micronutrient intake by HNSCC subtype provides support for the hypothesis that differences in lifestyle factors may contribute to the discrepancy in survival rates observed between HPV-positive and HPV-negative HNSCC cases. This research provides a basis for translational studies aimed at developing individualized dietary interventions and therapeutic regimens to improve HNSCC recurrence and mortality rates.

CHAPTER 1

Introduction

Theme

Despite advances in our understanding of the biology of head and neck squamous cell carcinoma (HNSCC), the 5-year survival rate of HNSCC has remained low over the past few decades at approximately 60% or less. These high rates are likely attributable to late detection and high rates of persistent and recurrent disease (1, 2). Few studies to date have focused on the impact of modifiable lifestyle factors, such as diet, on survival from HNSCC. Modifiable risk factors may serve as feasible, low-risk targets for intervention to improve HNSCC prognosis. To address this gap in our knowledge, the theme of this dissertation is the potential impact of diet and nutritional status on HNSCC pathogenesis and prognosis.

The varying risk factor profiles and unique challenges observed in the development and course of HNSCC may influence patients' dietary intake and ability to eat. Patients' dietary choices and level of nutritional status likely have consequences for disease prognosis but these have not been comprehensively evaluated in a meaningful way. The molecular mechanisms by which dietary compounds potentially exert effects on HNSCC progression and prognosis have also not been thoroughly investigated. My dissertation research addresses important knowledge gaps in these areas, setting the ground for translational research aimed at developing individualized dietary interventions and therapeutic regimens to improve HNSCC recurrence and mortality rates. My dissertation work has used data collected as a

part of the University of Michigan Head and Neck Specialized Program of Research Excellence (UM HN-SPORE), one of only five such programs in the United States funded by the National Cancer Institute (NCI) (2P50 CA097248, Dr. Gregory Wolf, P.I.). The goal of the UM HN-SPORE is to translate research into clinical practice and improve the diagnosis, treatment and prevention of HNSCC; thus findings from my dissertation research can be easily integrated into interdisciplinary patient care.

Epidemiology of Head and Neck Squamous Cell Carcinoma

Demographic Characteristics

Although often combined, HNSCC is a heterogeneous disease that includes tumors of squamous cell histologic type arising in the oral cavity, oropharynx, nasopharynx, hypopharynx and larynx (**Figure 1.1**). HNSCC is the eigth most common type of cancer in the U.S. and accounts for approximately 5% of annual new cancer diagnoses worldwide (3). According to the NCI's Surveillance Epidemiology and End Results (SEER) database, approximately 52,610 American citizens were diagnosed with the disease in 2012, of which 70% were male. The 5-year HNSCC survival rate has remained steady since the mid-1970s at about 60% (**Figure 1.2**) (http://www.seer.cancer.gov). An estimated 50% of HNSCC patients experience a disease recurrence within the first two years after diagnosis (2).

Tobacco Use and Alcohol Consumption

Historically, chronic lifetime exposure to tobacco and alcohol has been accepted as the primary factor involved in the pathogenesis of HNSCC, where disease risk increases multiplicatively with intensity and duration of exposure to each (4). Although these

behaviors are positively and independently associated with the development of HNSCC, concurrent tobacco and alcohol use confers the greatest risk (4) and is associated with higher recurrence and mortality rates in those diagnosed with the disease (5-8).

Prevalence of tobacco use in the United States declined rapidly between 1965 and 2006, subsequent to the US Surgeon General's warning regarding the role of tobacco in the development of lung cancer and other diseases (9), resulting in a decline in the incidence of HNSCC (10, 11). SEER data collected from 1975 – 2010 show a dramatic decline in the age-adjusted incidence rates of SCCs of the larynx, floor of mouth, gum, and other mouth during this period. However, contrary to expectation, age-adjusted incidence rates of SCCs of the tongue and oropharynx/tonsil rose rapidly, with the sharpest increase in the past decade-and-a-half (**Figure 1.3**) (http://www.seer.cancer.gov), and among young individuals, men, and white individuals (12). This change in HNSCC epidemiology indicates other factors are involved in HNSCC etiology (10).

Emergence of Human Papillomavirus

The decline in tobacco use in the 1960s coincided with reported sexual behavior changes by birth cohort in the United States, during which there was the emergence and increase in incidence of human papillomavirus (HPV) infection (12). These trends are hypothesized to be responsible for the shift in the epidemiologic landscape of HNSCC observed since the 1970s (10, 12). Although historically associated with cancers of the genitals (e.g. cervix and anus), we now appreciate that oral infection with a high-risk (i.e. oncogenic) strain of HPV, most often HPV-16, is the major etiologic factor responsible for a distinct subset of oropharyngeal HNSCCs.

A recent study using data from the 2009-2010 National Health and Nutrition Examination Survey (NHANES) data reported that approximately 7% and 1% of the U.S. population aged 14 – 69 were infected with any of 37 known high- and low-risk strains of HPV and oral HPV-16, respectively. 10% of men and 3.6% of women were orally infected with any strain of HPV. Risk of oral infection with a high-risk HPV strain increased with increasing number of lifetime sexual partners and current number of cigarettes smoked per day (12). Oral HPV-16 infection confers up to a 50-fold increased risk in disease development (13) and exerts its carcinogenic effects through integration of the viral DNA into the host genome, likely at fragile sites in chromosomes (14). Molecular studies show that integration of HPV results in the upregulation of the oncoproteins E6 and E7 (14), which inactivate the tumor suppressor proteins retinoblastoma (Rb) and protein 53 (p53) (10). E6 zinc-binding motifs form a complex with host cell p53, prompting p53 degradation and preventing the induction of apoptosis in DNA-damaged cells, leading to uncontrolled cell growth (14). E7 complexes with hypophosphorylated forms of the host Rb protein, leading to a decrease in cellular Rb level and release of E2F, a transcription factor involved in cell cycle progression, resulting in overexpression of *p16* (14).

HPV-positive HNSCC is clinically and pathologically distinct from tobacco and alcohol-related, HPV-negative HNSCC (**Figure 1-4**). HPV is currently associated with an estimated 50-85% of oropharyngeal/base of tongue cancer cases (13, 15, 16) but is infrequently associated with other HNSCC sites, such as the oral cavity and larynx (15, 17, 18). Histologically, HPV-positive tumors tend to be poorly differentiated with non-keratinizing, basaloid features, while HPV-negative tumors tend to be more differentiated with keratinized morphology. HPV-positive tumors are typically found in younger

individuals with higher socioeconomic status and less lifetime exposure to tobacco and alcohol when compared to HPV-negative (19). Most significantly, studies report HPV-positivity to be associated with enhanced response to treatment and lower mortality (15, 17-21).

Diet and Weight Status as Risk Factors

Although to a lesser extent than tobacco, alcohol, and HPV, a number of studies indicate poor diet may play a role in the pathogenesis of HNSCC. Results from observational studies of individual foods suggest low consumption of fruits and vegetables (22-26), and high consumption of red and processed meats (22, 27) and dairy products (28) lead to increased risk of disease. In the largest prospective cohort study to date investigating the association between HNSCC risk and dietary fiber and grain intake, an inverse association between these foods and HNSCC incidence was found among women, but not among men (29).

Studies of individual vitamins and minerals provide evidence that low intake of antioxidant micronutrients (A, C, E and carotenoids), minerals with anticancer properties (iron and zinc) and micronutrients involved in one-carbon metabolism (folate, riboflavin, vitamin B12) may also lead to increased risk of disease (30-33). The potential role of vitamin and mineral supplement intake on HNSCC risk was investigated in a pooled case-control study conducted by the International Head and Neck Cancer Epidemiology Consortium (INHANCE), but no strong associations between supplement use and HNSCC risk were observed (34). Dietary pattern analysis studies report reduced odds of developing HNSCC with high consumption of dietary patterns rich in fruits and vegetables and low in high-fat and processed meats and sweets (22).

Similar to other smoking-related cancers such as lung and bladder, weight status, as measured by body mass index [BMI = weight (kg) / height $(m)^2$], measured at diagnosis (28, 35, 36) and two years prior to diagnosis has been reported to be inversely associated with HNSCC (26, 37). Specifically, overweight or obesity (BMI >25kg/m^2) appears to be protective against HNSCC when compared to normal or underweight status (BMI <25kg/m^2).

Dietary Challenges of Head and Neck Squamous Cell Carcinoma Patients

Data suggesting low BMI and inadequate dietary intake of fruits, vegetables, vitamins, and minerals are associated with increased risk of HNSCC development also suggest the nutritional status of HNSCC cases may be inadequate at the time of diagnosis. Inadequate nutritional status after HNSCC diagnosis is of concern due to several factors. First, smoking and alcohol use are highly prevalent in this patient population, and both drugs suppress appetite and the immune system, leading to decreased food intake and increased susceptibility to infection, respectively. Exposure to tobacco and alcohol generates reactive oxygen species in the body, which in turn depletes tissue stores of antioxidant micronutrients (38). Alcohol also interferes with the metabolism of several micronutrients important in cancer prevention and control, such as folate (add ref).

Second, development of HNSCC may hinder the ability to eat as a result of side effects related to tumor location and treatment (38). Severe side effects prevalent in HNSCC patients that can impact dietary intake include difficulty swallowing (dysphagia), ulcerations of the oral mucosa (mucositis), altered taste (dysgeusia), decreased salivary production, (xerostomia) and dental problems (39-43). Post-surgical complications such as functional and anatomic chewing and swallowing impairments are also common (43-45).

Third, prevalence of cancer cachexia in the HNSCC population is high, with estimates ranging from 48 – 61% of all patients diagnosed with the disease. Cachexia, a wasting syndrome characterized by progressive skeletal muscle loss (with or without fat loss), functional impairment, and anorexia, is estimated to account for at least 20% of all cancer related deaths (46). The pathogenesis of cachexia is hypothesized to involve competition for nutrients between healthy host cells and tumor cells, leading to increased energy expenditure, protein and fat catabolism. This is facilitated by dysregulation of neuroendocrine hormones, and the release of tumor-derived catabolic factors and pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and interferon (IFN)-γ, resulting in muscle wasting and anorexia. Glucose intolerance and impaired insulin sensitivity are also commonly observed in cachectic patients (38, 46, 47).

Diet and Head and Neck Squamous Cell Carcinoma Outcomes

The nutritional challenges and complications observed in HNSCC patients likely have consequences for survival, and thus nutritional intervention may present an avenue to improve disease outcomes. However, little research has been conducted related to nutritional factors in HNSCC management and survivorship. Studies examining associations between diet and disease outcomes such as survival and recurrence in HNSCC patients are sparse but consistent with studies of diet as a risk factor for disease development, suggesting a positive influence of fruit (5) and vegetable (48, 49) intake and a negative influence of high red meat intake (49). Low pretreatment fruit but not vegetable intake was associated with decreased survival in one study (5) while data from two other studies suggest a protective effect of high vegetable but not fruit intake on risk of second primary cancers (48, 49) and survival (48).

High intakes of meat, liver and retinol are associated with increased risk of second primary cancers (49), but no data on the effect of these dietary factors on survival could be found.

High-dose antioxidant supplementation may be detrimental to HNSCC patients undergoing radiotherapy as those supplemented with high-dose α-tocopherol (400 IU/day), high-dose β-carotene (find amount), or a combination of both had a significantly increased incidence of second primary cancers (50) and decreased overall survival (51) in a randomized controlled trial. A recent case-control study showed that pre- and post-radiotherapy plasma antioxidant (total carotenoids, α-tocopherol and ascorbic acid) levels were significantly lower in HNSCC patients compared to healthy controls and decreased during radiotherapy. Plasma carotenoid levels were inversely associated with long-term survival (52). These data suggest dietary intake and nutrient levels may be associated with patient prognosis, but further research is warranted investigating these relationships between diet and HNSCC prognosis, how these relationships differ by disease subtype, and what molecular mechanisms underlie these relationships. This dissertation research addressed these gaps in knowledge through three Specific Aims.

Specific Aims

Aim 1.1: *To identify major dietary consumption patterns in a population of incident cases of HNSCC, and to examine the association of these dietary patterns and weight status with recurrence and survival.*

As outlined above, few studies of HNSCC have examined diet as an independent prognostic indicator and in those studies only select individual foods and nutrients were investigated, as opposed to overall diet. Most people consume a variety of foods on a daily basis as opposed to large quantities of an isolated food or nutrient. The high collinearity

among nutrients and bioactive compounds found in foods proves disentangling their independent effects challenging; nutrients in food likely interact and work synergistically to produce a stronger effect on a disease outcome than any individually (53). Therefore, measures of overall diet in relation to disease outcomes as opposed to single foods and nutrients may be more useful. Studies suggest HNSCC patients with a BMI greater than 25 kg/m^2 at diagnosis have a survival advantage over those patients with a lower BMI (54, 55), but these studies lacked statistical power and measured BMI two years prior to HNSCC diagnosis. Understanding how overall dietary patterns and weight status influence HNSCC prognosis will have important clinical implications, allowing the development of dietary recommendations that are readily translatable to the clinic. These relationships were examined in the following hypotheses:

Hypothesis 1.1: Diets rich in fruits, vegetables, lean meats, whole grains and poly and monounsaturated fats will be associated with lower recurrence and mortality rates.

Hypothesis 1.2: Diets rich in red meat, saturated and trans fats, and processed foods will be associated with higher recurrence and mortality rates.

Hypothesis 1.3: Higher BMI will be associated with lower recurrence and mortality rates.

Aim 2.1: *To compare pretreatment dietary intake of vitamins and minerals between newly diagnosed HNSCC patients with HPV-positive tumors to those with HPV-negative tumors.*

The basis for improved survival rates in HPV-positive HNSCC compared to HPV-negative has not yet been established. It is unclear if HPV-positive tumors are less aggressive and more responsive to therapy than HPV-negative due to differing tumor biology, whether the prognostic advantage is due to healthier lifestyles exhibited by HPV-positive patients prior to diagnosis, or if it is a combination of both. Understanding variation in lifestyle behaviors between these two HNSCC subtypes may provide insight into mechanisms

underlying the observed prognostic differences, and highlight areas for intervention to improve outcomes in HPV-negative patients. Given the strong epidemiologic differences between patients with HPV-positive cancer and those with HPV-negative cancer, it is reasonable to hypothesize that diet plays a role in the more favorable outcomes of HPV-positive patients. This issue was addressed by the following hypothesis:

Hypothesis 2.1: HPV-positive subjects will report higher pretreatment intake of key micronutrients with known anti-cancer properties than HPV-negative subjects.

Aim 3.1: *Examine associations between pretreatment serum cytokine levels and pretreatment dietary patterns, as well as reported intake and serum levels of carotenoids and vitamin E.*

Aim 3.2: *Compare pretreatment cytokine levels between cachectic and non-cachectic HNSCC cases.*

A previous study reported that the pro-inflammatory cytokine interleukin-6 (IL-6) is positively associated with HNSCC recurrence and mortality. It is hypothesized that IL-6 promotes the progression of HNSCC through mechanisms of carcinogenesis, angiogenesis, and metastasis, possibly through stimulation of cancer stem cell self-renewal. IL-6, TNF-α, and IFN-γ are also hypothesized to be involved in the pathogenesis of cachexia by increasing glucocorticoid production in the body and upregulating ubiquitin preteosome activity, leading to anorexia and increased proteolysis, respectively (47).

Fruits, vegetables, and bioactive compounds found in fruits and vegetables, such as carotenoids and vitamin E are known to play a role in the physiological response to inflammation. It is possible these dietary compounds can mitigate the effects of proinflammatory cytokines in the body, reducing the likelihood of metastasis and cachexia and prolonging survival. If this hypothesis holds true, it would provide insight into a molecular mechanism underlying potential associations between diet and HNSCC survival

and serve as the basis for developing nutritional interventions aimed at preventing cachexia and prolonging HNSCC survival through the reduction of pro-inflammatory cytokines. Consequently, these associations were investigated in the following hypotheses:

Hypothesis 3.1: A dietary pattern characterized by high intakes of fruits, vegetables, lean meats, whole grains and poly and monounsaturated fats, as well as high carotenoid intake and serum levels will be inversely associated with circulating serum cytokine levels.

Hypothesis 3.2: A dietary pattern characterized by high intakes of red meat, saturated and trans fats, and processed foods will be positively associated with circulating serum cytokine levels.

Hypothesis 3.3: Subjects with cachexia six months after HNSCC diagnosis will have higher pretreatment serum cytokine levels compared to non-cachectic subjects.

Study Population: The University of Michigan Head and Neck Specialized Program of Research Excellence

The study population for this dissertation research consists of two consecutive cohorts of HNSCC patients enrolled into the UM HN-SPORE between 2002 and 2012 at the University of Michigan Health System (UMHS) of Ann Arbor, the Veteran's Administration Hospital of Ann Arbor, and the Henry Ford Hospital of Detroit. The first cohort, referred to as SPORE 1, was recruited from all three sites. SPORE 1 data was used for Specific Aims #1 and #2. The second cohort, SPORE 2, was recruited from only the UMHS site and data from this cohort was used for Specific Aim #3.

The HN-SPORE was established in September 2002 through funding by the NCI and is one of only five such programs in the United States. The HN-SPORE's interdisciplinary research team is comprised of physicians, dentists, and basic scientists with training in areas such as head and neck surgery, dental health, otolaryngology, medical and radiation

oncology, epidemiology, molecular biology, and biostatistics. The mission of this comprehensive research program is to significantly improve the diagnosis, treatment and prevention of HNSCC while decreasing morbidity and mortality associated with this disease (http://www2.med.umich.edu/cancer/hnspore).

The HN-SPORE ascertains all newly diagnosed HNSCC patients who present at participating site clinics, with a catchment of ~27% of all new HNSCC cases in the state of Michigan, annually. The HN-SPORE houses an extensive longitudinal collection of information from over 1200 HNSCC patients, including epidemiologic, clinical, and molecular characterization. Eligible patients are recruited into the HN-SPORE at visits to the Otolaryngology, Radiation Oncology, Medical Oncology, and Speech Pathology clinics at UMHS and participating sites by trained HN-SPORE recruiters, nurses, or physicians. Informed consent is obtained and a medical record review is completed for each study participant.

Epidemiologic data are collected from HN-SPORE participants using a self-administered questionnaire collected at baseline and one-year; shortened versions of the questionnaire are collected at half-year intervals to update information. The epidemiologic data collected include: longitudinal data on smoking, alcohol use, diet, exercise, and sleep; clinical data on tumor site and stage, comorbidities, depression, quality of life, and recurrence status; and treatment modalities such as radiation therapy, surgery, chemotherapy, and combination therapy. Blood and saliva samples are collected every six months and tumor tissue as available from biopsies and surgery.

Dietary Assessment for Nutritional Epidemiology Studies

There are many tools for dietary assessment in epidemiologic studies. The food frequency questionnaire (FFQ) remains the preferred method for measuring average dietary intake over a long period of time (typically a year) in large-scale epidemiologic studies due to its ability to rank individuals based on reported intakes at a reasonably low cost (56, 57). The most widely used FFQs include the Harvard FFQ (58) and the Block FFQ (59), both of which have been validated for use in many populations (58-63). However, multiple versions of the FFQ have been developed and differ slightly in design depending on such factors as the study population, gender, age group, and foods and/or nutrients of interest (57).

Other common methods of dietary assessment in epidemiologic studies include 24-hour recalls, food records, diet history and nutritional biomarkers. 24-hour recalls and food records collect detailed information on all foods and beverages consumed over the past 24 hours. Both methods can more precisely quantify foods and nutrients eaten over a short period of time than the FFQ but must be collected at multiple time points to properly estimate usual diet due to variability in day-to-day intake (64). Repeat 24-hour recalls and food records are most valuable for describing mean values of groups and in evaluating the relative validity of FFQs (56). A "diet history" is any dietary assessment tool that asks the respondent to report on past diet (64). A diet history queries meal patterns, eating habits, food preparation practices and recent dietary changes, and results in more qualitative, rather than quantitative data (65). Biochemical measurements of nutrients in the blood can provide valid assessments of adequacy of dietary intake. However, this method of assessment should be

made on a nutrient-by-nutrient basis as not all nutrients have a valid biochemical indicator (56).

For this dissertation research, an FFQ is the sole dietary assessment method used Specific Aims #1 and #2. In Specific Aim #3, biochemical measurements of serum carotenoids and tocopherols are also used in addition to an FFQ. Dietary intake is collected from all UM-SPORE Project 3 participants at baseline (diagnosis) and yearly-post-diagnosis using the self-administered, semi-quantitative Harvard FFQ, validated for use in several populations (56, 58, 61). This FFQ is a feasible instrument well suited to assess the associations of usual dietary intake with relevant lifestyle behaviors such as smoking and alcohol use (66). It has also been used extensively as a measure of dietary exposure in cancer studies (56). FFQ responses are used to rank individuals according to their usual consumption of foods, food groups and nutrients (64). It is relatively low-cost, easy to administer and can be completed on-site by the study participant or at home and returned by mail (67). The FFQ is designed to assess respondents' usual dietary intake from food and supplements over the past year, reflecting seasonal variation in intake. Total energy and nutrient intake is estimated by summing up intakes from each food based on the given standard portion size, reported frequency of consumption and nutrient content of each food item (56).

Conceptual Framework

Figure 1.5 outlines the conceptual framework for this dissertation research. Solid lines represent associations that have been established with prior research; dashed lines represent associations that are investigated herein. As illustrated in the figure, dietary intake and body mass index (BMI) at diagnosis may influence the likelihood of HNSCC survival

and recurrence. The associations between diet and prognosis and BMI and prognosis will be investigated in **Specific Aim 1.1**. Patients with HPV(+) HNSCC have a survival advantage over HPV(-) HNSCC and it is unknown whether diet plays a role in the prognostic discrepancies between these groups. This association will be explored in **Specific Aim 2.1**. A decline in BMI may eventually lead to cancer cachexia, which in turn may increase likelihood of recurrence and mortality. Increased pro-inflammatory cytokine release has been hypothesized to promote tumor progression and to be involved in the pathogenesis of cancer cachexia by inducing skeletal muscle loss and a subsequent decline in BMI. Both of these pathways result in increased recurrence and mortality. As many dietary nutrients and bioactive food compounds have anti-inflammatory properties, it is possible diet can modulate circulating levels of pro-inflammatory cytokines in the body. **Specific Aim 3.1** examines the association between diet and cytokine levels, and **Specific Aim 3.2** examines the association between cytokine levels and cachexia.

Figure 1.1: Tumor sites of the head and neck

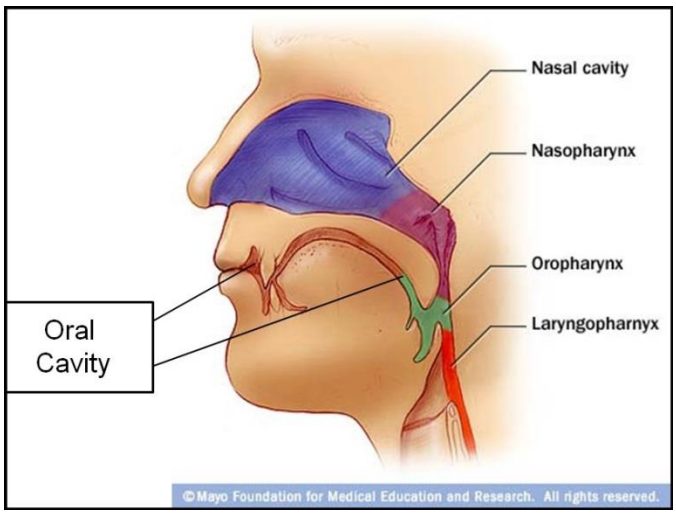


Figure 1.2: Five-year survival by year of diagnosis and tumor site

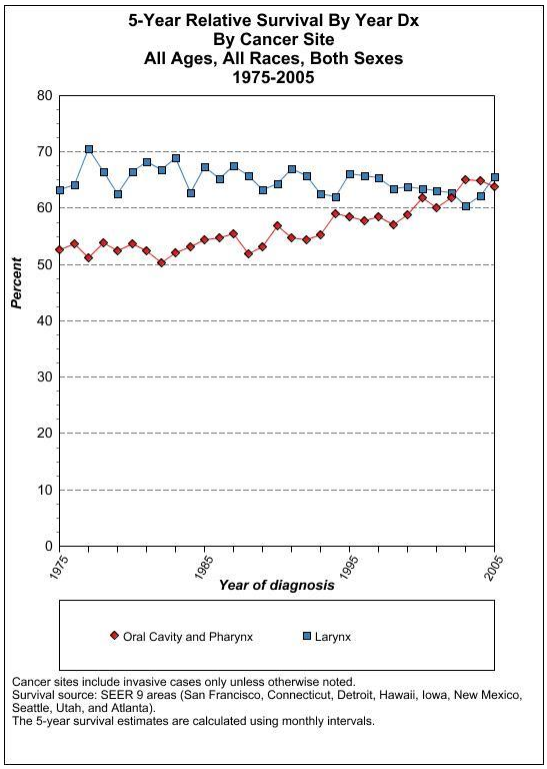


Figure 1.3: Age-adjusted incidence rates by cancer site; 1975 – 2010

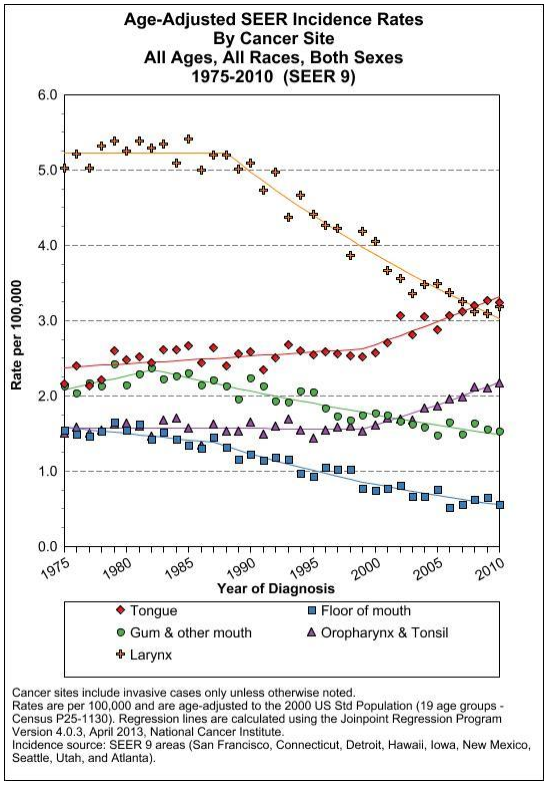
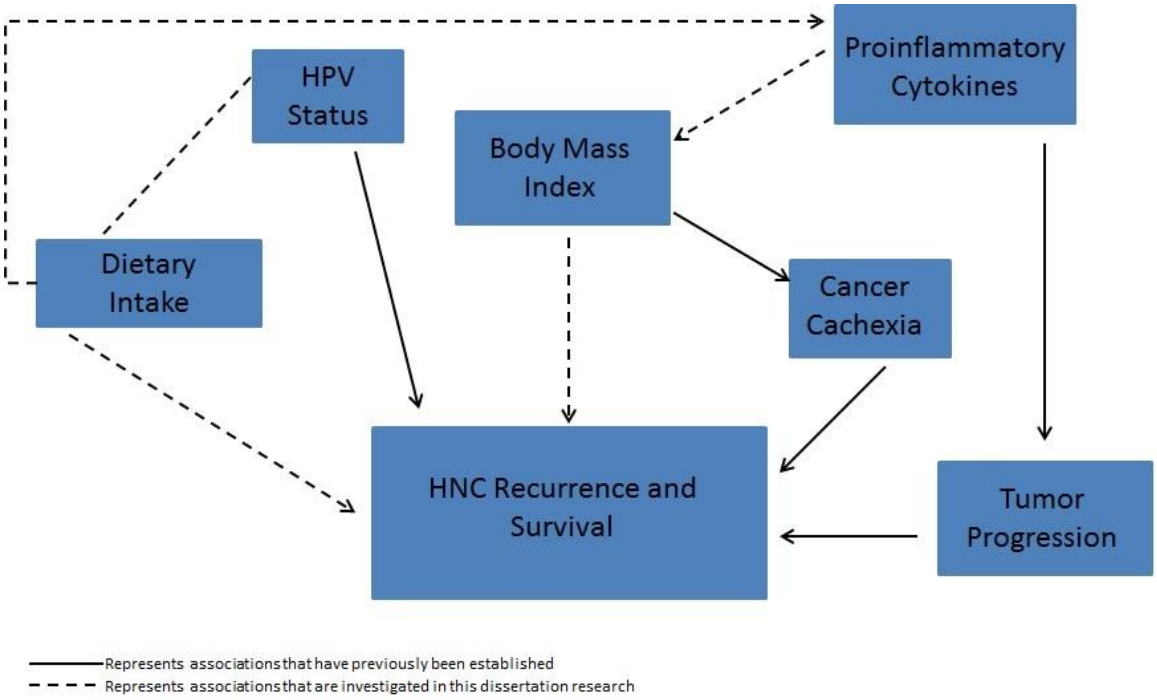


Figure 1.4: Distinct clinicopathology of HPV-positive and HPV-negative HNSCCs

Characteristic	HPV-Positive	HPV-Negative
Anatomic site	Oropharynx/base of tongue	All sites
Histology	Poorly differentiated	More differentiated
Age	Younger	Older
Gender	3:1 men	3:1 men
SES	High	Low
Risk factors	Sexual behavior	Alcohol/tobacco
Incidence	Increasing	Decreasing
Survival	Improved	Worse

Figure adapted from Vidal et al, Hematol Oncol Clin North Am. 2008 Dec;22(6):1125-42, vii.

Figure 1.5: Conceptual Framework



CHAPTER 2

Pretreatment dietary patterns, weight status, and head and neck squamous cell carcinoma prognosis

Abstract

Background: Few studies have evaluated the association of diet and weight status with head and neck cancer (HNC) outcomes.

Objective: The purpose of this study was to determine if pretreatment dietary patterns and weight status are associated with HNC prognosis.

Design: This was a longitudinal study of 542 newly diagnosed HNC patients who completed food frequency questionnaires (FFQ) and health surveys prior to treatment. Clinical data were abstracted from medical records and the Social Security Death Index. Dietary patterns were identified using principal component analysis. Cox proportional hazard models were used to examine the association of derived dietary patterns (fit by quintiles of exposure) and weight status with time to recurrence and survival, controlling for covariates.

Results: During the study period there were 229 deaths and 184 recurrences. Two dietary patterns were identified: a "whole foods pattern", characterized by high intakes of vegetables, fruits, fish, poultry and whole grains, and a "Western pattern", characterized by high intakes of red and processed meats, refined grains, potatoes and French fries. In multivariable

analyses, significantly fewer deaths were observed in subjects most adherent to the whole foods pattern (HR 0.56, 95% CI 0.34 – 0.92, P_{trend}=0.01). Subjects classified as overweight or obese had significantly fewer deaths (HR 0.65, 95% CI 0.49 – 0.85, *P*=0.001) and recurrences (HR 0.70, 95% CI 0.52 – 0.95, *P*=0.02) than normal or underweight subjects.

Conclusions: Consuming a diet rich in vegetables, fruit, fish, poultry and whole grains and being overweight prior to diagnosis with HNC appears to result in better prognosis.

Introduction

Although often combined into a single category, head and neck squamous cell carcinoma (HNSCC) is a heterogeneous disease that includes squamous cell cancers of the oral cavity, oropharynx, nasopharynx, hypopharynx and larynx. Five-year HNSCC survival rates have remained steady at approximately 60%, likely due to late detection and high rates of persistent or recurrent disease. An estimated 50% of patients develop recurrence within the first two years after diagnosis (1, 2). Evidence suggests poor nutritional status and low consumption of foods and nutrients with chemopreventive properties increases the risk of developing HNSCC (4), but little research has investigated the roles of diet and weight status in HNSCC prognosis. Modifiable lifestyle factors such as diet and nutritional status may present a feasible approach for improving head and neck cancer survival and recurrence rates.

Few studies have examined the association of HNSCC prognosis with individual food groups (e.g., fruits and vegetables) (5, 48) or nutrients (68-71). Most of these studies had small sample sizes (48, 68-70), yielded inconclusive or null results (5, 48, 69, 71) and none

examined overall dietary patterns. Our group previously reported that low pretreatment fruit intake is negatively associated with survival in univariate analysis (5). It may be more informative to examine the combined, rather than individual, effects of dietary exposures on HNSCC prognosis as it is likely the many nutrients and nutritive compounds in food interact and work synergistically to produce a stronger effect than any individually (53). Examining overall dietary patterns is advantageous as it allows for the ability to relate usual intake of a range of nutrients to disease prognosis, and can be used as the basis for translational research aimed at developing population-specific nutritional interventions and dietary recommendations.

Weight status as measured by Body Mass Index [BMI=weight(kg)/height(m)^2] may be inversely associated with mortality, where patients classified as overweight or obese (BMI >25 kg/m^2) at the time of HNSCC diagnosis have longer survival time when compared to similar patients with a lower BMI (54, 72-74). Previous studies investigating this association either had a small sample size (54), measured weight status several years prior to diagnosis (72) or did not consider recurrence as an outcome (72, 73).

The objective of this study was to characterize pretreatment dietary patterns, and determine if dietary patterns and weight status predict recurrence and survival among HNSCC patients, controlling for other known prognostic factors such as demographics, smoking, problem drinking, cancer site and stage, comorbidities, and treatment. The hypotheses were that diets high in fruits, vegetables, lean proteins and unprocessed foods, as well as overweight or obese status would be associated with better prognoses, while diets high in fat, processed foods and red meat, and normal or underweight status would predict worse prognoses.

Materials and Methods

Design

This was a prospective study of patients enrolled in the University of Michigan Head and Neck Cancer, Specialized Program of Research Excellence (UM HN-SPORE). The independent variables of interest were pretreatment dietary patterns and weight status. Control variables were age, sex, cancer site and stage, treatment, comorbidities, smoking and total energy intake. The dependent (outcome) variable was disease recurrence and all-cause survival.

Study Population

Patients newly diagnosed with HNSCC were recruited to participate in this study as part of the UM HN-SPORE. Institutional Review Board approval was granted from the University of Michigan Health System (Ann Arbor, MI) the Veterans Affairs Healthcare System (Ann Arbor, MI), and the Henry Ford Health System (Detroit, MI). Patients were recruited between January 2003 and December 2008. Out of 1,185 patients approached, 934 consented, yielding a response rate of 79%. Exclusion criteria included: 1) <18 years of age; 2) pregnant; 3) non-English speaking; 4) diagnosed as mentally unstable; or 5) a diagnosis of another non-upper aerodigestive tract cancer. Patients who did not complete a baseline FFQ (n = 300), had previously diagnosed HNSCC (n = 40) and did not have a signed informed consent on file (n = 3) were excluded. The Rosner method was used to detect statistically influential outliers based on reported daily energy intake (75); n =5 participants were identified and excluded. Comparative analyses of participant characteristics (chi-squared tests for categorical and t-tests for continuous variables) indicated that women reporting >3500 kcals per day and males reporting >4000 kcals per day were more likely to report be

current smokers and have an alcohol problem than the rest of the study population. Since problem drinkers may be unreliable reporters, they were excluded from analyses (n = 44) to avoid potential systematic confounding. Though conservative, these upper bounds are considered reasonable for making exclusions based on energy intake (56). We did not exclude *a priori* based on a lower energy bound because the majority of participants presented to clinic with advanced cancers that may have hindered dietary consumption before diagnosis. Nevertheless, the lowest reported daily energy intake was 519 kcals/day, above the usual lower bound of 500 kcal. The final sample size included 542 newly diagnosed HNSCC patients.

Procedures

Participants completed a self-administered health questionnaire at baseline that collected data on demographics, tobacco use, alcohol use, physical activity, sleep, comorbidities, depression and quality of life. A medical record review was completed at baseline and annually for each study participant from which data on tumor site and stage, recurrence status, treatment modalities and survival were abstracted.

Measures

Predictors: Dietary Intake and Weight Status

Dietary intake was collected using the self-administered, semi-quantitative Harvard FFQ, designed to assess respondents' usual dietary intake from food and supplements over the past year. The reproducibility and validity of the 131-item questionnaire has been reported previously (56, 58, 61). The FFQ includes standard portion sizes for each item [e.g., 1 apple or 3 oz (85 grams) chicken], which allowed participants to choose their average

frequency of consumption over the past year from a list of nine choices ranging from "almost never" to "≥6 times per day". Total energy and nutrient intake was estimated by summing intakes from each food based on the selected standard portion size, reported frequency of consumption, and nutrient content of each food item. Daily food group servings were estimated by summing the frequency weights of each food item based on reported daily frequencies of consumption (56). Weight status was classified as overweight/obese (BMI >25 kg/m^2) or normal/underweight (BMI ≤ 25 kg/m^2) based on self-reported height and weight.

Covariates

Demographic variables were age, sex, race, level of education and marital status. Smoking data was categorized as current/former versus never" smoking and alcohol abuse was measured using the previously validated Alcohol Use Disorders Identification Test (AUDIT); an AUDIT score ≥8 was considered problem drinking (76). Tumor site was recorded from operative notes and surgical pathology forms and categorized into three groups: 1) oral cavity, 2) pharynx and 3) larynx. In order to increase statistical power of stage-wise comparisons, cancer stage was categorized *a priori* into three groups, with TNM Stages 1 and 2 collapsed, while Stage 3 and Stage 4 were considered separately. Comorbidities were measured using the Adult Comorbidity Evaluation-27 and categorized into none or mild comorbidities versus moderate to severe comorbidities (77). Depression was measured using the 5-item Geriatric Depression Scale-Short Forms (GDS-SF) (78). Treatment was categorized into six groups to allow individual treatment approaches, including multimodal therapy, to be estimated separately: 1) surgery only, 2) radiation only,

3) surgery and radiation, 4) radiation and chemotherapy, 5) all treatment (surgery, radiation and chemotherapy), or 6) unknown treatment or none.

Outcome: Survival and Recurrence

Outcome variables were recurrence and overall survival. Follow-up was made with subjects every three months, allowing for tracking of patient status (alive or deceased). Each participant was censored from their last annual chart review as alive or deceased and recurrence or no recurrence as of February, 2012. Death and recurrence data were obtained from medical records and the Social Security Death Index (SSDI). Research assistants abstracted recurrence dates from medical records. Time to recurrence was calculated as the number of days from date of diagnosis to date of first recurrence. Patients with persistent disease were assigned a recurrence time of one day if they had completed treatment, but were never diagnosed as disease-free.

Statistical Analysis

Descriptive statistics (means and frequencies) were generated for all demographic, epidemiologic and clinical variables. A Pearson's correlation analysis was conducted between self-reported weights and weights abstracted from medical records among a random sample of 27 participants, showing excellent correlation between self-reported and clinical measures of weight ($r = 0.98$). Dietary intake data was assessed for missing values and energy outliers using standard techniques (56). Food consumption data derived from the FFQ were classified *a priori* into 39 foods and food groups using methods similar to those described in previous studies of dietary patterns and disease (53, 79). Pretreatment dietary

patterns present in the study population were derived by principal component analysis (PCA) using the orthogonal rotation procedure. In establishing the number of factors to retain in the final analysis, eigenvalues (≥3.0), Scree test, percentage of variability explained and interpretability of factors were considered (80). Pattern factor scores were calculated for each study participant by summing reported intakes of the factor food variables weighted by factor loadings. Dietary pattern scores were categorized into quintiles for analysis with survival and recurrence.

Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the associations of survival and recurrence with each retained dietary pattern and weight status. Previous evidence suggests differential associations of BMI and HNSCC mortality between smokers and non-smokers (72). As such, analyses also were conducted in which we stratified by smoking status (current/former versus never smokers). To avoid instability in estimating regression coefficients, potential covariates were assessed for collinearity by estimating Pearson's correlation coefficients for continuous variables, Chi-squared tests for categorical variables, or logistic regression between a dichotomous and a continuous variable. Exclusions of covariates in final models were made if they were highly correlated with other variables. Confounding was assessed by examining the association of each covariate individually with overall survival in univariate models. Any covariate with a $p<0.10$ was included in the next phase of modeling. Covariates were added to multivariable models one at a time, starting with the lowest p-value in univariate analysis, and included in the final models if addition of the variable altered the parameter estimate by >10% or if it had biological significance to the outcome. Covariates considered for final models were age, sex, race, education, marital status, cancer site, stage, treatment, comorbidities, smoking, alcohol

problem, multivitamin use and depression. Final models for survival and recurrence included age, sex, site, stage, treatment, comorbidities, and smoking. HR and CI were estimated for each quintile (Q) of dietary pattern score versus the lowest, Q1, and a test for trend across increasing quintiles of intake was performed. Dietary pattern and weight status variables were included in the same multivariable models to investigate the independent association of each with survival and recurrence. All statistical analyses were performed in SAS 9.2 or 9.3.

Results

During longitudinal follow-up there were 229 death events (42.2%) and 184 recurrence events (33.9%). Median follow-up time was 2199 days (≈6.0 years; Range 19 days – 8.3 years). Overall epidemiological characteristics of the study population are displayed in **Table 2.1**. The mean age of study participants was 59 years. The majority of participants were White (92.6%), male (78.6%) and married (61.9%). Just over half of the participants were college graduates. The most common tumor location was the pharynx (56.3%) and most participants presented to our clinics with late-stage (III or IV) cancers (65.1%). Over 75% of participants were ever smokers, with about one quarter reporting an alcohol problem (24% with AUDIT score ≥ 8). Approximately 60% of patients were classified as overweight or obese at the time of diagnosis, slightly less than the proportion observed within the general U.S. population (68%) from 1999 – 2008 (81).

Two major dietary patterns were identified with PCA. The first pattern, termed the "whole foods pattern", was characterized by high intakes of vegetables, fruits, legumes, fish, poultry, whole-grains, fruit juice, olive oil, nuts and garlic. The second pattern, termed the "Western pattern", was characterized by high intakes of red and processed meats, refined grains, French fries, potatoes, condiments, high-fat dairy, margarine, butter, eggs, coffee,

desserts, snacks, mayonnaise and regular beverages. The factor-loading matrix for the two dietary patterns is presented in **Table 2.2**.

Table 2.3 shows select characteristics of the study participants according to quintile of factor score for each dietary pattern. Patients in the highest quintile of whole-foods pattern score were more likely to have a higher BMI, be older, female, never smokers and more highly educated, and were less likely to have an alcohol problem than those in the lowest quintile. Patients with the highest whole-foods pattern scores also consumed more servings per day of fruits, vegetables, whole-grains, fish and poultry, and less total, saturated and *trans* fat. Conversely, patients in the highest quintile of Western pattern score were more likely to be younger, male, less educated, have an alcohol problem, and less likely to be never smokers than those in the lowest quintile. Patients in the highest quintile tended to consume more servings per day on average of red and processed meats, refined grains, potatoes and French fries, as well as more total fat than patients with low Western pattern scores.

Figure 2.1 shows the Kaplan-Meier curves for the association between each dietary pattern categorized by quintiles, survival and recurrence. Hazard ratios and 95% CI for survival and recurrence in association with the whole foods and Western dietary patterns are displayed in **Table 2.4**. There was a significant and increasing trend towards decreased risk of mortality across increasing quintiles of whole foods pattern score in multivariable analyses. Although there appeared to be a trend towards decreased risk of recurrence across increasing quintiles of whole foods pattern scores, this association did not reach statistical significance in multivariable analysis. There were no significant associations between Western dietary pattern scores and survival or recurrence.

In multivariable analyses, participants classified as overweight or obese prior to treatment had significantly decreased risk of mortality and recurrence than patients classified as normal or underweight. Results shown in **Table 2.5** are from the multivariate model that includes the whole foods dietary pattern as a covariate. The significant association between weight status and survival persisted in stratified analyses for both ever smokers and never smokers. The significant inverse association between recurrence and pretreatment BMI persisted for ever smokers, but was no longer significant for never smokers. Kaplan-Meier curves for the association between BMI and survival and BMI and recurrence for the total population, and stratified by smoking status, are displayed in **Figure 2.2**.

Discussion

This study showed that high whole foods dietary pattern score prior to treatment was associated with lower risk of recurrence and enhanced survival among HNSCC patients, independent of other factors known to influence prognosis. Being overweight or obese at the time of diagnosis was also associated with better prognosis, independent of diet. These results support and build on the work of prior studies. A small prospective cohort study in Spain reported significantly reduced risk of recurrence, overall mortality and cancer mortality in participants consuming high levels of vegetables before and after diagnosis of oral cancer (48). Similarly, our prior work showed a trend of increased risk of death among HNSCC patients with low fruit intake, though this was not statistically significant in multivariable analysis. Other studies have reported significant inverse associations between plasma carotenoids, specifically lutein, α-carotene, β-carotene (68) and lycopene (69) and HNSCC survival. Our findings support the results of these studies as higher plasma carotenoid levels may reflect higher fruit and vegetable intake consistent with the whole-foods pattern.

The foods that characterize the whole-foods pattern are rich sources of vitamins, carotenoids and polyphenols with known anticancer functions. Individually, many of the nutritive compounds abundant in these foods have been shown to reduce inflammation and oxidative stress in the body, maintain normal cellular processes such as cell growth, proliferation and apoptosis, inhibit angiogenesis and stimulate phase II detoxification enzymes to promote the excretion of carcinogenic compounds from the body (82, 83). All of these functions are shown to inhibit tumor growth, potentially explaining the associations between higher whole foods pattern score and reduced HNSCC mortality and recurrence. In a subset of this same HNSCC population, our group recently reported an association between hypomethylation of tumor suppressor genes and increased intakes of folate, vitamin B12, vitamin A and cruciferous vegetables (84). It is possible high consumption of foods characterizing the whole foods dietary pattern can alter the tumor DNA methylation profile in these patients, allowing tumor suppressor genes to continue being expressed and thus contributing to survival.

There was no association between HNSCC prognosis and Western dietary pattern **score**. This result was unexpected, as high consumption of red and processed meats has previously been associated with cancer development (85) and mortality (86). It has been hypothesized high consumption of these foods is involved in tumor development and progression due to the carcinogenic actions of heterocyclic amines and aromatic hydrocarbons produced during cooking of red meat, and of nitrates and nitrites present in processed meats (87). One possible explanation for the lack of association between the Western pattern and prognosis is that many patients with high baseline Western pattern scores may have changed their dietary intake after diagnosis with HNSCC. Evidence

suggests many attempt to make positive lifestyle changes, including changes in diet, after a cancer diagnosis (88, 89). If patients decreased their consumption of foods included in the Western pattern and increased their consumption of foods included in the whole foods pattern, the potential adverse effect of a Western diet may have been countervailed by the protective effect of the foods with anticarcinogenic (and anti-metastatic) activities. Future studies should address whether patients' dietary patterns change after HNSCC diagnosis, throughout treatment and in the years following and how these potential changes affect prognosis.

The finding that overweight and obese pretreatment BMI was associated with decreased risk of mortality and recurrence in the total study population supports findings of data collected from the larger Cancer Prevention Study-II cohort and the Nutrition cohort case-control study (72), as well as evidence from two smaller cohort studies of HNSCC patients (54, 73). The mechanisms underlying the association of higher BMI with lower recurrence and mortality are unclear. It is estimated over 50% of patients with advanced HNSCC experience significant weight loss and cachexia during the course of disease (46). Marked weight loss and cachexia is associated with increased susceptibility to infection and treatment-related toxicity, and with a reduction in quality of life and likelihood of survival (90). The increased fat and energy stores of overweight and obese patients may help them to withstand deterioration in nutritional status resulting from cachexia and rigorous treatment regimens (54).

To our knowledge, this is the largest study to date examining the association between diet and HNSCC prognosis and the first to relate overall dietary patterns to HNSCC outcomes. The long-term follow-up time and ability to control for multiple confounding

factors are strengths of this study. While the observational study design allows for the determination of an association between dietary patterns and weight status with disease prognosis, it does not demonstrate causality. Our work has previously suggested significant differences in survival by cancer site, where patients with laryngeal cancer had the best survival (5, 91), however, in this analysis we report no significant difference in survival by cancer site perhaps due to the longer follow up time as we continue this longitudinal study.

The heterogeneous nature of the study population in regard to tumor site is a limitation, but we were able to control for site in the final models and the large numbers of patients allowed for good statistical power. While we did not have biochemical verification for smoking status, our work (92) and the work of others (93, 94) has shown greater than 90% sensitivity and specificity for self-reported versus biochemical validation of smoking status, with low misclassification rates, thus, self-reported smoking status was thought to be sufficient; especially since it is a covariate and not the main variable of interest. Although BMI was based on self-reported measures of height and weight, several studies show strong correlation between self-reported and clinical measures of these variables (95-97). A random sampling of 27 of our own participants showed excellent (r=0.98) agreement between self-reported and clinically measured weights.

There was insufficient statistical power to examine HPV as a confounding or effect modifying factor, but controlling for site could be considered a reasonable surrogate for HPV status, as it is likely up to 80% of oropharyngeal cases were HPV(+) (12). While the FFQ used to measure diet is susceptible to measurement error leading to misclassification bias, this type of bias is likely to attenuate associations towards the null (56). Because patients reported their usual pre-diagnosis dietary intake *after* their cancer diagnosis, recall bias

related to social desirability, stress of the diagnosis, or concerns about contributing to their own disease could have occurred. While recall bias is a concern, previous studies of diet and breast cancer indicate either little evidence of recall bias (98, 99), or that the measures of association are attenuated when recall bias occurs (100). Future research in HNSCC populations should be conducted to determine if dietary recall bias exists and how it may influence study results. Despite recruitment efforts at an inner city and Veterans Affairs hospital, the study population consisted predominantly of White males thus the results may not be generalizable to racially diverse HNSCC populations.

In conclusion, consuming a diet high in vegetables, fruits, fish, poultry, and whole grains, as well as overweight or obese status at diagnosis with HNSCC is associated with reduced mortality and recurrence. Patients consuming low amounts of foods included in the whole foods dietary pattern, or with normal or underweight status may benefit from more aggressive surveillance throughout the disease trajectory. Results of this study highlight the need for randomized controlled trials aimed at optimizing the likelihood of a favorable disease prognosis via early nutritional intervention.

Table 2.1: Pretreatment Characteristics of Newly Diagnosed Head and Neck Cancer Patients

Characteristic	No. of Patients (n = 542)	%
Follow-up time, days		
Median	2199	
Range	19 - 3067	
Age, years		
Mean	59	
SD	11	
Range	21 - 92	
Sex		
Male	426	78.6
Female	116	21.4
Race		
Non-Hispanic white	502	92.6
Non-white/Hispanic	40	7.4
Education		
High School or Less	248	45.9
Some College or More	292	54.1
Marital Status		
Married	335	61.9
Not Married	206	38.1
Site		
Oral Cavity	118	21.8
Pharynx	305	56.3
Larynx	119	21.9
Stage		
1,2	112	20.7
3	77	14.2
4	353	65.1
Treatment		
Surgery only	77	14.2
Radiation only	47	8.7
Surgery + radiation	66	12.2
Radiation + chemotherapy	214	39.5
All treatment	124	22.9
Unknown treatment or none	14	2.5
ACE-27 Comorbidity Score		
None or mild	364	67.2
Moderate or severe	178	32.8
Smoking		
Current	117	21.4
Former	306	56.1
Never	123	22.5

Alcohol Problem (AUDIT ≥8)		
Yes	130	24.0
No	412	76.0
BMI (kg/m^2)		
Underweight (<18.5)	19	3.5
Normal weight (18.5 - 24.9)	195	36.0
Overweight (25 - 29.9)	205	37.8
Obese (≥30)	123	22.7
Multivitamin Use		
Current	265	48.9
Past	105	19.4
Never	172	31.7
Depression		
Depressed	270	49.8
Not depressed	257	47.4
Unknown	15	2.8
Fruit and Vegetable Intake (servings/day)		
0 – 1	43	7.9
>1 – 3	249	45.9
>3 – 5	149	27.5
>5	101	18.6

Table 2.2: Factor Loading Matrix†

Food Group	Whole Foods	Western
Green Leafy Vegetables	**0.74**	-0.05
Other Vegetables	**0.69**	0.19
Dark Yellow & Orange Vegetables	**0.66**	-0.01
Fruit	**0.64**	-0.13
Cruciferous	**0.63**	-0.05
Legumes	**0.60**	0.12
Tomatoes	**0.52**	0.09
Fish	**0.47**	0.10
Poultry	**0.42**	0.14
Whole Grains	**0.41**	0.09
Salad Dressing	**0.37**	0.13
Olive Oil	**0.37**	-0.13
Fruit Juice	**0.34**	-0.04
Nuts	**0.33**	0.09
Garlic	**0.30**	0.07
Low-fat Dairy	0.28	-0.20
Cereal	0.28	-0.03
Wine	0.24	-0.12
Tea	0.21	-0.05
Beer	-0.22	0.17
Red Meats	0.01	**0.62**
Refined Grains	-0.03	**0.59**
Processed Meats	-0.06	**0.52**
French Fries	-0.13	**0.48**
Potatoes	0.02	**0.47**
Condiments	-0.03	**0.43**
High-fat Dairy	0.04	**0.42**
Margarine	-0.08	**0.38**
Butter	-0.15	**0.36**
Eggs	0.14	**0.35**
Coffee	0.07	**0.33**
Desserts	-0.01	**0.32**
Snacks	0.16	**0.31**
Mayonnaise	0.14	**0.30**
Regular beverages	-0.16	**0.30**
Creamy Soups & Chowder	0.06	0.25
Pizza	0.05	0.24
Organ Meats	0.08	0.10

Diet Beverages	0.07	0.09
Liquor	-0.04	0.08

†Bolded numbers signify factor loadings ≥0.30 and considered to be major contributors to the overall pattern

Table 2.3: Selected Characteristics and Dietary Intakes of Subjects by Quintile (Q) Dietary Pattern Score (n = 542)[†]

	Whole Foods Pattern					Western Pattern				
	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
n	108	109	108	109	108	108	109	108	109	108
Clinical Characteristic										
Age (mean)	57.0	59.4	59.1	59.7	61.1	61.3	59.2	61.1	58.6	56.2[‡]
BMI (kg/m^2, mean)	25.2	26.9	27.0	27.0	27.7[*]	26.0	27.2	26.7	26.9	26.9
Female (%)	13.9	17.4	22.2	25.7	27.8	29.6	23.8	19.4	18.3	15.7
Stage 4 (%)	71.3	70.6	62.0	65.1	56.5	63.9	71.6	53.7	67.0	69.4
≤ High School/GED education (%)	67.3	45.4	40.7	45.9	30.6[‡]	30.6	47.7	38.7	60.5	51.8[‡]
Never smokers (%)	15.7	19.3	24.1	22.9	32.4[‡]	28.7	27.5	23.1	18.3	16.7[*]
Alcohol Problem (%)	45.4	24.8	21.3	18.3	10.2[‡]	14.8	23.8	23.1	26.6	31.5
Current Multivitamin Use (%)	35.2	49.5	51.8	53.2	54.6	64.8	55.0	41.7	43.1	39.8[‡]
Depressed (%)	50.9	45.9	51.8	53.2	47.2	53.7	50.5	52.8	55.0	37.0
Mean Food Intake (servings per day, energy-adjusted)										
Total fruit	0.4	0.8	1.0	1.4	1.7[‡]	1.5	1.1	1.0	0.9	0.7[‡]
Total vegetables	1.2	1.6	2.1	2.7	4.3[‡]	2.9	2.5	2.3	2.0	2.1[‡]
Whole-grains	0.5	0.9	1.1	1.2	1.3[‡]	1.2	1.1	1.2	0.9	0.8[‡]
Fish	0.1	0.2	0.2	0.2	0.3[‡]	0.2	0.2	0.2	0.2	0.2
Poultry	0.2	0.3	0.4	0.4	0.5[‡]	0.4	0.4	0.4	0.3	0.4
Red and processed meats	1.2	1.1	1.1	0.9	0.8[‡]	0.8	0.9	1.0	1.2	1.3[‡]
Refined grains	1.3	1.4	1.3	1.0	0.9[‡]	0.9	1.0	1.1	1.2	1.7[‡]
Potatoes and French fries	0.4	0.4	0.4	0.3	0.3[‡]	0.3	0.4	0.4	0.4	0.5[‡]
Mean Nutrient Intake (grams per day, energy-adjusted)										
Total energy (kcals)	1789	2020	2136	2357	2590[‡]	1414	1815	2155	2451	3057[‡]
Total carbohydrate	235	252	255	268	268[‡]	267	266	264	244	238[‡]
Total protein	77	83	89	86	96[‡]	87	85	85	86	89[‡]
Total fat	83	84	82	80	79	77	76	80	86	89[‡]

Saturated fat	31	30	29	27	25‡	26	27	27	31	31‡
Monounsaturated fat	31	32	31	30	31	31	29	30	33	34‡
Polyunsaturated fat	13	14	14	15	14‡	13	13	14	14	15‡
trans fat	3.4	3.5	3.4	3.1	2.6‡	2.8	3.0	3.3	3.6	3.4‡

†*P*-values were derived by using a chi-square test for categorical variables and by using the Kruskal-Wallis test for continuous variables
**P* < 0.05
‡*P* < 0.01

Table 2.4: Multivariate HR and 95% CI According to Quintile (Q) of Dietary Pattern Score for Survival and Recurrence Events

	Q1	Q2	Q3	Q4	Q5	*P**
n	108	109	108	109	108	
Survival						
Whole Foods Pattern						
Univariate	1.0	0.88 (0.60, 1.3)	0.81 (0.55, 1.19)	0.70 (0.47, 1.05)	0.56 (0.37, 0.86)	0.004
Multivariate†	1.0	0.81 (0.54, 1.23)	0.88 (0.58, 1.35)	0.65 (0.42, 1.03)	0.56 (0.34, 0.92)	0.01
Western Pattern†						
Univariate	1.0	0.98 (0.65, 1.49)	1.11 (0.74, 1.67)	1.05 (0.70, 1.57)	0.98 (0.65, 1.48)	0.99
Multivariate†	1.0	1.11 (0.71, 1.72)	1.02 (0.65, 1.60)	1.03 (0.62, 1.70)	0.90 (0.49, 1.68)	0.70
Recurrence						
Whole Foods Pattern†						
Univariate	1.0	0.88 (0.57, 1.36)	0.87 (0.56, 1.30)	0.75 (0.48, 1.17)	0.60 (0.38, 0.97)	0.03
Multivariate†	1.0	0.87 (0.55, 1.38)	0.98 (0.61, 1.57)	0.78 (0.47, 1.29)	0.66 (0.38, 1.16)	0.13
Western Pattern†						
Univariate	1.0	1.39 (0.89, 2.17)	1.08 (0.68, 1.73)	1.02 (0.64, 1.64)	0.98 (0.61, 1.58)	0.48
Multivariate†	1.0	1.51 (0.95, 2.41)	1.01 (0.60, 1.69)	0.91 (0.52, 1.61)	0.82 (0.42, 1.61)	0.24

†Adjusted for age, sex, tumor site, cancer stage, treatment, ACE-27 comorbidities, smoking, BMI and total energy intake
**P*-value represents a trend test across quintiles of dietary pattern. Each individual's dietary pattern score was set to the median score for that quintile, and treated as a continuous variable in the cox regression models. $P < 0.05$ considered significant.

Table 2.5: Multivariate HR and 95% CI of Pretreatment Body Mass Index >25 kg/m^2 (overweight/obese) for Recurrence and Survival Events

	Survival			Recurrence		
	HR	95% CI	*P**	HR	95% CI	*P**
Total population†	0.65	0.49, 0.85	0.001	0.7	0.52, 0.95	0.02
Ever smokers‡	0.60	0.45, 0.81	<0.001	0.66	0.48, 0.92	0.01
Never smokers‡	0.35	0.13, 0.91	0.03	0.54	0.22, 1.36	0.19

†Adjusted for age, sex, tumor site, cancer stage, treatment, ACE-27 comorbidities, smoking, whole foods dietary pattern and total energy intake

‡Adjusted for age, sex, tumor site, cancer stage, treatment, ACE-27 comorbidities, whole foods dietary pattern and total energy intake

*$P < 0.05$ considered significant

Figure 2.1: Kaplan-Meier curves of survival and recurrence for dietary patterns. (A) Survival by quintile of whole foods pattern score; (B) Recurrence by quintile of whole foods pattern score; (C) Survival by quintile of western pattern score; (D) Recurrence by quintile of western pattern score.

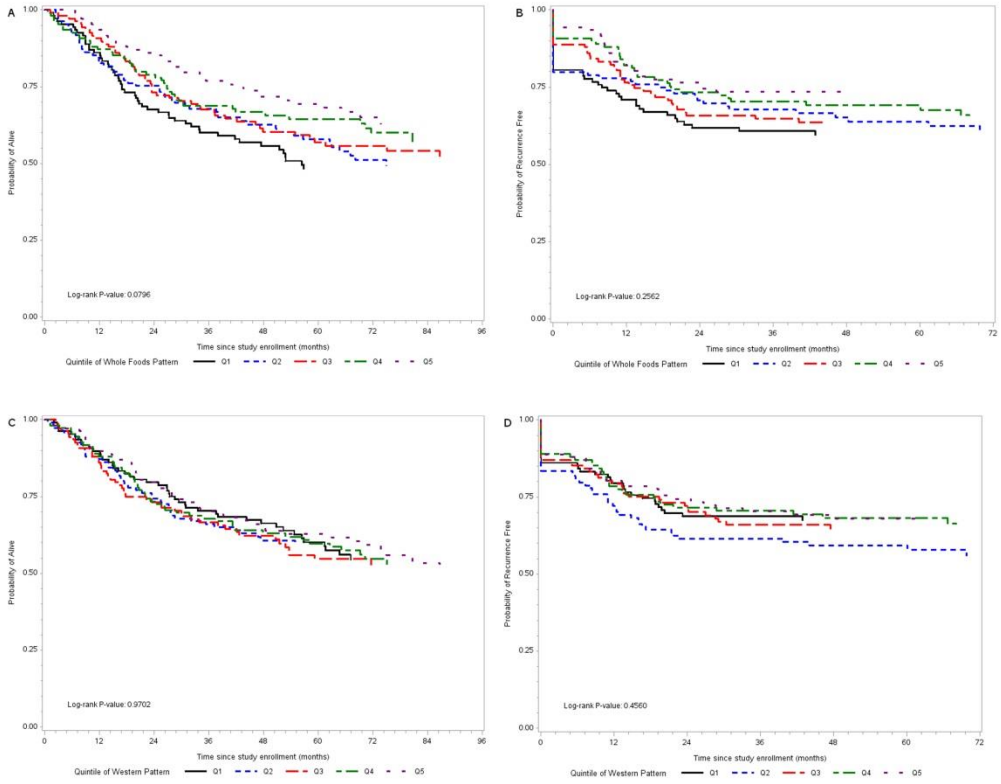
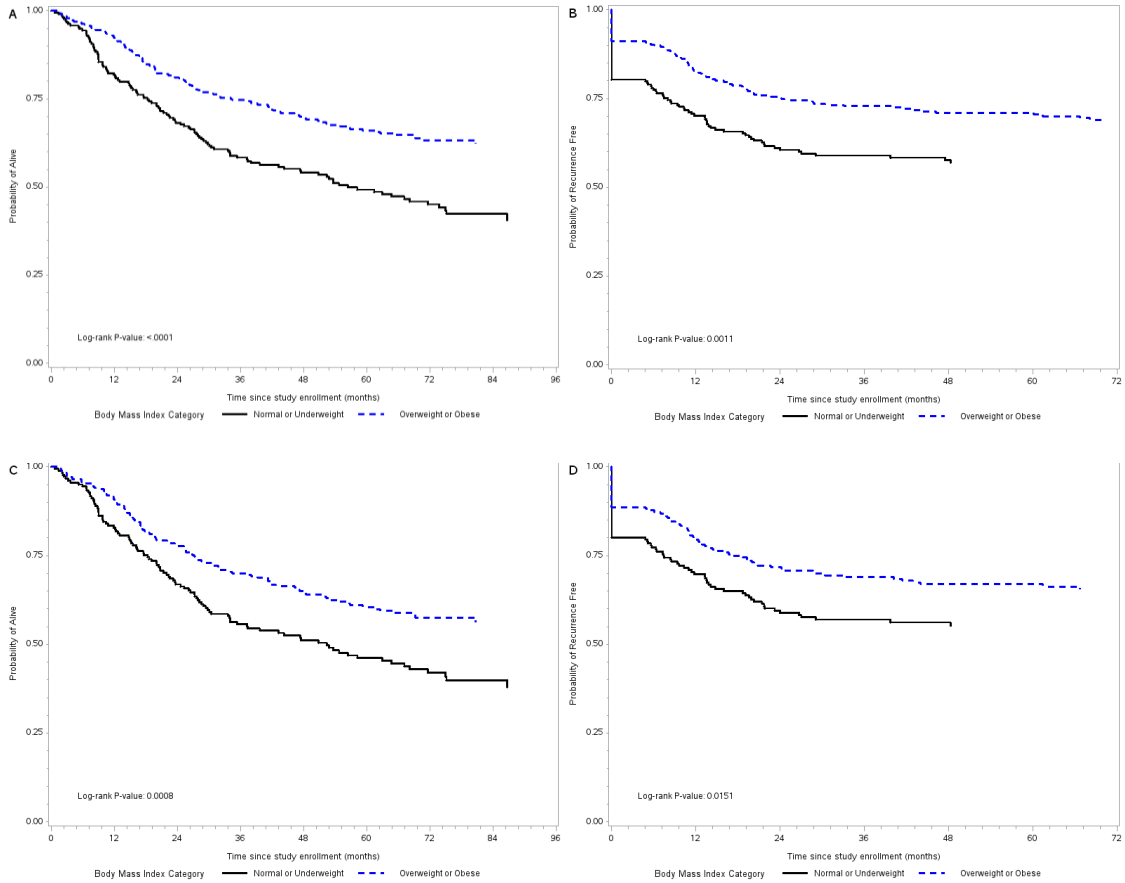


Figure 2.2: Kaplan-Meier curves of survival and recurrence for BMI category. (A) Survival by BMI category of overall population; (B) Recurrence by BMI category of overall population; (C) Survival by BMI category of ever smokers; (D) Recurrence by BMI category of ever smokers; (E) Survival by BMI category of never smokers; (F) Recurrence by BMI category of never smokers.



CHAPTER 3

Higher micronutrient intake is associated with human papillomavirus-positive head and neck cancer: a case-only analysis

Abstract

Background: 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.

Objective: This study was designed to investigate the relationship between diet and HPV status in HNSCC patients.

Design: Cases of HNSCC were recruited from two clinical centers participating in the University of Michigan Head and Neck Specialized Program of Research Excellence (SPORE). HPV tissue genotyping was performed and epidemiologic 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.

Results: 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 ($P_{trend}<0.05$).

Conclusions: HPV-positive HNSCC cases report higher intake of key micronutrients with anti-cancer and anti-metastatic properties compared to HPV-negative cases. These results support the hypothesis that differences in lifestyle characteristics may be a factor in the survival discrepancies observed between these two HNSCC subtypes. 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 (13, 101-105). HPV-associated tumors accounted for more than 50% of the squamous cell carcinomas of the oropharynx in the United States prior to 2005 (104). Recent data show that the proportion of HPV-positive oropharynx cancers is continuing to increase. Among a cohort of oropharyngeal cancer cases followed by our group from 1999-2002, 64% entered into a clinical trial of induction chemotherapy followed by concurrent cisplatin and radiation, had HPV-positive tumors (101). In the consecutive cohort entered from 2002-2007 into our subsequent trial of concurrent cisplatin, taxol and radiation, 82% of the oropharynx cancers contained high-risk HPV (15). HPV-associated head and neck cancers are infrequently associated with other sites, such as the oral cavity and larynx. 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 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 (15, 17, 18, 106-109). HPV-positive cases with a more extensive smoking history may experience reduced disease-specific survival and increased risk of disease recurrence than their non-smoking counterparts (15, 110).

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 (111). 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 (32, 111-113); minerals such as iron, calcium and zinc (30, 32, 111, 113-115); folate (31, 32); and riboflavin (30, 114, 115). Several studies have reported inverse associations between oral and pharyngeal cancer risk and fruit and vegetable intake (23, 112, 114, 116, 117). Furthermore, low fruit intake may be associated with reduced survival in cases diagnosed with HNSCC (5), and high vegetable intake may reduce the risk of recurrence and mortality in oral cancer cases (48).

Although improved prognoses have been documented in HNSCC cases with HPV-positive tumors, the basis for this survival advantage has not been well established. The discrepancy in survival rates by HNSCC subtype may be due to differences in tumor biology, differences in lifestyle characteristics, or a combination of both. Epidemiologic studies indicate individuals with HPV-associated tumors tend to exhibit healthier lifestyle

characteristics—they are younger, have lower exposure to tobacco and alcohol, and have higher education and socioeconomic status (SES) than individuals with tobacco-related tumors (13). 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 (118, 119). Hence, this case-only analysis was conducted to determine if differences exist in dietary intake of key micronutrients with known anti-cancer and anti-metastatic properties between HPV-positive and HPV-negative HNSCC cases.

Methods

Design

This was a cross-sectional study of cases 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 Population

Newly diagnosed cases with squamous cell carcinoma of the head and neck were recruited to participate in this study. Cases were recruited from the Ann Arbor Veterans Affairs Hospital and the University of Michigan Hospital. Exclusion criteria included: 1) < 18 years 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 cases approached, 934 consented to participate yielding a response rate of 79%.

Tumors with adequate tissue for microdissection were available from 205 cases, and 203/205 (99%) had amplifiable DNA as assessed by PCR amplification of beta-globin. Of these 203 cases, 193 had completed a self-administered food frequency questionnaire (FFQ) at presentation (58). 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 cases 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 (120). A medical record review was completed for each study participant. Dietary intake was collected using a self-administered, semi-quantitative FFQ, validated for use in several populations (56, 58, 61).

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 (121, 122). Adequacy of amplifiable DNA was assessed using

PCR for beta-globin 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 (56). As the FFQ has not been validated in HNSCC populations, reliability of energy reporting using this tool is unknown. Because the majority of cases 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 (123, 124). PATEE was developed for use in the Women's Health Initiative (124). Originally designed for use as a proxy when reported FFQ energy intake (FFQEI) data are missing (125), the current refinement has been validated in a cohort of subjects on which total energy intake was estimated from doubly labeled water (124). 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) (124).

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) (76). 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 three groups, with Stages 1 and 2 considered together, and Stage 3 and Stage 4 considered separately, enhancing power of comparisons by stage.

Statistical 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. Students 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 utilized 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 twelve 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 three factors were included in the multivariable model. In all models, these three 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). Cases were more likely to have stage 3 or stage 4 tumors than stage 1 or 2 at presentation to clinical centers.

Table 3.1 compares epidemiologic characteristics of those cases 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 cases 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 3.2** 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 cases, 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 cases with HPV-positive tumors consume significantly higher levels of key micronutrients with anti-cancer functions compared to cases 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 cases. For example, HPV-positive cases typically have a higher SES and less lifetime exposure to tobacco and alcohol compared to HPV-negative cases. Higher SES and less tobacco use and alcohol consumption are generally associated with better overall diet quality (118, 119). 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. Our finding that HPV-positive cases report consuming higher levels of vitamin A, vitamin E, β-carotene, iron and folate support the existing epidemiologic evidence that HPV-positive HNSCC cases exhibit lifestyle characteristics associated with better overall health, and that these healthier characteristics may translate to better survival rates.

Other than supporting previous evidence that HPV-positive and -negative HNSCC cases display different lifestyle characteristics and risk profiles, the observed differences in micronutrient intake between the two groups have other potential implications. Higher intake of the observed micronutrients, or foods rich in the observed micronutrients, could make an individual more susceptible to oral HPV infection. A prior study reported that HPV-16 seronegative individuals with high fruit or citrus intake were significantly less likely to develop HNSCC than those with low fruit or citrus intake, but that the opposite was true for HPV-16 seropositive individuals (i.e. high fruit and citrus intake was associated with greater risk of HNSCC development in HPV-16 seropositive individuals). 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 (126). Another study reported that while higher plasma levels of carotenoids were associated with a 43% to 50% reduction in the risk of initial anal HPV infection, once infected higher levels of carotenoids and retinol were associated with decreased clearance of persistent anal HPV infection (127). This evidence is consistent with our finding that higher intakes of vitamin A and β-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 cases and this possibility should be explored in the future. 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 cases. Two previous studies reported that higher levels of plasma carotenoids in HNSCC cases before and after radiotherapy were associated with improved progression-free survival, as well as with overall survival (52, 68). Vitamin A in the form of retinoic acid is

believed to regulate epithelial cell differentiation through its effects on gene expression. Homo- 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 (128, 129). Furthermore, retinoic acid and β-carotene may play a role in preventing uncontrolled cell growth by preserving gap junction communication between cells (129-131). β-carotene also functions as an antioxidant, quenching singlet oxygen species and scavenging peroxyl radicals, thus helping to prevent cellular damage (132).

Recent findings of our research group show that HPV-positive cases may have enhanced adaptive immunity, which is associated with improved tumor response to chemotherapy and prolonged survival when compared to HPV-negative cases (133). 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 (134). 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 (134). Folate works synergistically with vitamins B6 and B12 to supply one-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 (134). Associations between intake of vitamin A, folate, iron and immune function in HNSCC cases 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.

While there was no significant difference between HPV-positive and HPV-negative cases 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 cases as HPV-negative cases have smoked at some point in their lives, HPV-positive cases 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 cases 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 reported 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 and/or foods abundant in these micronutrients may increase the susceptibility of cases to HPV infection, or decrease the ability of the body to clear a persistent 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 cases compared to HPV-negative cases. This hypothesis should be examined in the future, as it may present an avenue to improve survival in cases with this deadly cancer.

Table 3.1: Baseline characteristics by HPV status (n=143)

Demographics	Total	HPV +	HPV -	p-value
		Number (percent)		
N	143	38 (26.6)	105 (73.4)	
Age (sd)†	57.9	57.89 (7.4)	57.88 (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

Table 3.2: 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	8589	12602	3957	6074	8984
Vitamin C (mg/day)	81.4	147	263	57.1	97.4	155
Vitamin E (mg/day)	6.6	13.4	27.3	4.9	6.9	10.0
β-carotene (μg/day)	1512	2484	4342	1296	2127	3621
Vitamin D (IU/day)	159	345	566	101	171	256
Calcium (mg/day)	620	914	1351	538	770	1117
Iron (mg/day)	10.7	15.9	24.1	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	2.0
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	7.0	10.1
Folic acid (μg/day)	381	587	817	294	400	548

Table 3.3: 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 – 14.16)		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

CHAPTER 4

Diet, proinflammatory cytokine levels, and cachexia

ABSTRACT

Background: No studies to date have examined associations between dietary patterns, carotenoids, and vitamin E in relation to proinflammatory cytokine levels in head and neck squamous cell carcinoma (HNSCC).

Objective: The primary aim of this study was to determine if pretreatment dietary patterns and reported dietary intake and serum levels of carotenoids and tocopherols are associated with pretreatment proinflammatory cytokine levels in HNSCC. The secondary aim of this study was to examine associations between proinflammatory levels and the presence of cachexia.

Design: This was a cross-sectional study of 161 newly diagnosed HNSCC patients who completed food frequency questionnaires (FFQ) and health surveys prior to treatment. Pretreatment serum levels of the proinflammatory cytokines IL-6, TNF-α, and IFN-γ were measured with ELISA and serum carotenoid levels were measured with HPLC. Three dietary patterns were identified using principal component analysis: whole foods, western, and convenience foods dietary patterns. Multivariable logistic regression models were used to examine quartiles of factor scores for dietary patterns derived from FFQ reports and serum levels of carotenoids with cytokine levels, controlling for age, sex, site, stage, smoking, and

alcohol problem. Multivariable logistic regression models also were used to assess associations between cytokine levels and presence of cachexia defined as weight loss >5%, or a baseline BMI of <20 kg/m^2 and weight loss >2% within the timeframe between the baseline and six-month health questionnaire.

Results: Three dietary patterns were identified: a whole foods pattern, Western pattern, and convenience foods pattern. In multivariable analyses, higher whole foods dietary pattern scores were significantly associated with lower levels of IL-6, TNF-α, and IFN-γ (P = <0.001, P = 0.008, and P = 0.03, respectively). Significant downward trends in IL-6, TNF-α, and IFN-γ levels were found across increasing quartiles of total reported carotenoid intake (P = 0.006, P = 0.04, and P = 0.04, respectively). No significant association was reported for the relationships between IL-6 and TNF-α levels and serum carotenoids. There was a significant trend towards decreased IFN-γ levels across increasing quartiles of serum α-tocopherol levels (P = 0.03). No significant association was observed between cytokine levels and cachexia.

Conclusions: Consuming a diet rich in vegetables, fruit, fish, poultry and whole grains and higher intake of total carotenoids prior to diagnosis with HNSCC may be associated with lower proinflammatory cytokine levels.

Introduction

Decreased risk of HNSCC recurrence and mortality has been related to higher fruit and vegetable intake (5, 48) and higher levels of serum carotenoids (68, 69). In a study of dietary patterns and HNSCC prognosis, we reported that higher pretreatment whole foods dietary pattern score, characterized by high intakes of vegetables, fruits, whole grains, poultry, and fish, and a BMI $\geq$ 25 kg/m^2 at the time of diagnosis, are associated with lower recurrence and mortality rates (135). These findings suggest diet as a potential area of intervention to improve HNSCC prognosis. However, research exploring the potential mechanisms underlying the associations between diet and HNSCC disease outcomes is warranted to further understand how nutritional interventions can influence patient survival.

Although inflammation is a protective biologic response by the body to destroy harmful stimuli, it is widely accepted that the inflammatory response also can be a major driver of cancer development and progression (136). The proinflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α are both mediators of the immune response and are thought to be involved in malignant transformation, progression, and prognosis (136, 137). Endothelial cells in the tumor microenvironment, and HNSCC cells themselves, have been shown to secrete high levels of IL-6, inducing tumor invasion and metastasis (138, 139), possibly through regulation of cancer stem cells (140, 141). In epidemiologic studies, IL-6 has been correlated to later cancer stage (142-145) and poorer prognosis of several cancers (139, 143, 144, 146). Specifically, previous studies have reported high levels of IL-6 (147-149) and TNF-α (150) to be associated with increased HNSCC recurrence and mortality. Our prior work identified pretreatment IL-6 as an

important biomarker in predicting recurrence and mortality in a large, prospective cohort of HNSCC cases (91).

Cancer cachexia, a wasting syndrome characterized by progressive skeletal muscle loss (with or without fat loss), functional impairment, and anorexia, is estimated to be present in approximately 48% - 61% of all HNSCC patients and accounts for ~20% of all cancer related deaths (46). IL-6, TNF-α, and interferon (IFN)-γ are hypothesized to play critical roles in the maintenance of body mass during cancer and in the pathogenesis of cachexia (151). Animal studies have shown that, IL-6, TNF-α, and IFN-γ induce cachexia by increasing glucocorticoid production in the body and upregulating ubiquitin proteosome activity, leading to anorexia and increased proteolysis, respectively (47). However, further research is necessary that describes the association between these cytokines and cachexia in humans.

A large cohort study previously conducted by our research group examining associations between health behaviors and pretreatment IL-6 levels among HNSCC cases reported significantly higher IL-6 levels among smokers and subjects with sleep disturbances (152). Dietary intake has long been known to play a role in the physiological response to inflammation, but is yet to be investigated in HNSCC (153). It is reasonable to hypothesize that nutrients with antioxidant and anti-inflammatory properties such as carotenoids and vitamin E can mitigate the effects of proinflammatory cytokines in the body, reducing the likelihood of metastasis and cachexia and prolonging survival. To our knowledge, the potential relationships between dietary factors and cytokines have not been studied in HNSCC. The primary aim of this study was to examine the associations of these dietary patterns, as well as reported and serum levels of carotenoids and vitamin E, with pretreatment

serum levels of proinflammatory cytokines (IL-6, TNF-α, and IFN-γ) in a cohort of newly diagnosed HNSCC cases. A secondary aim was to examine differences in cytokine levels between cachectic and non-cachectic subjects in a subset of the study population.

Subjects and methods

Design

This was a cross-sectional, case-only study of patients enrolled in the University of Michigan Head and Neck Cancer, Specialized Program of Research Excellence (UM HN-SPORE). For the primary study aim, dietary patterns and micronutrients, as reported from a food frequency questionnaire (FFQ) and as measured in serum, were the independent variables. The reported dietary variables included derived dietary patterns (whole foods, Western, convenience foods), carotenoids (α-carotene, β-carotene, lycopene, β-cryptoxanthin, and lutein + zeaxanthin) and total vitamin E. Serum-derived biochemical nutrient measures included α-carotene, β-carotene, total lycopene, β-cryptoxanthin, lutein, zeaxanthin, α-tocopherol, and γ-tocopherol. Pro-inflammatory cytokine levels were the outcome variables. These included IL-6, TNF-α, IFN-γ, and VEGF. Control variables were age, sex, tumor site, cancer stage, smoking, problem drinking, and education. For the secondary study aim, cytokine levels were the independent variables and cachexia was the outcome.

Study population

Patients newly diagnosed with HNSCC were recruited to participate in this study as part of the UM HN-SPORE. Institutional Review Board approval was granted from the

University of Michigan Health System (Ann Arbor, MI). Patients were recruited between January 2008 and November 2012. Out of 737 patients approached, 670 consented, yielding a response rate of 91%. Exclusion criteria included: 1) < 18 years of age; 2) pregnant; 3) non-English speaking; 4) diagnosed as mentally unstable; or 5) a diagnosis of another non-upper aerodigestive tract cancer. Excluded were patients who did not complete a baseline food frequency questionnaire (FFQ) (n = 304), withdrew from the study (n = 24), had reported daily energy intake <200 or >5000 kilocalories (kcals) per day (n = 6), or did not have baseline serum available for use in this study (n = 175). The final sample size included 161 newly diagnosed HNSCC patients.

Procedures

Participants completed a pretreatment, self-administered health questionnaire at baseline and six months that collected data on demographics, tobacco use, alcohol use, weight status, sleep, comorbidities, depression and quality of life. Dietary intake was collected from subjects prior to treatment using the 2007 self-administered, semi-quantitative Harvard FFQ (154). A medical record review was completed at baseline and annually for each study participant from which data on tumor site and stage, treatment modalities, recurrence status, and survival were collected.

Peripheral blood samples (30 mL) were collected prior to beginning treatment using routine venipuncture technique. Sera were collected after centrifugation of blood and stored in 0.5 mL aliquots at -80 °C until testing. To ensure patient confidentiality and to blind laboratory personnel, all serum samples were immediately barcoded and assigned a numerical identifier before being stored in the HN-SPORE Tissue Core.

Measures

Reported dietary intake

The 2007 Harvard FFQ was designed to assess respondents' usual dietary intake from food and supplements over the past year. The reproducibility and validity of the 131-item questionnaire has been reported previously (56, 58, 61). The FFQ includes standard portion sizes for each item (e.g., 1 medium banana or 3 oz chicken) which allowed participants to choose their average frequency of consumption over the past year from a list of 1-9 choices ranging from "almost never" to "≥6 times per day". Total energy and nutrient intake was estimated by summing intakes from each food based on the portion size, frequency of consumption, and nutrient content of each food. Daily food servings were estimated by summing the frequency weights of each food item based on reported daily frequencies of consumption (56).

Serum carotenoids and tocopherols

Serum carotenoids and tocopherols were extracted and analyzed by high pressure liquid chromatography as previously described (155). Briefly, serum was mixed with an equal volume of ethanol containing butylated hydroxytoluene and extracted with hexane using Tocol as the internal standard. A YMC C30 reverse phase column (2X150 mm with a 2.0X20mm guard) was used to separate carotenoids and tocopherols using gradient elution at 0.2 ml/min total flow on a Shimadzu LC-20AT HPLC system. Detection was at 450-472 nm for carotenoids, and electrochemical detection was used for Tocol and tocopherols with a Coularray electrochemical detector set at 310, 390 and 470 mV. Samples were analyzed in six batches of approximately 30 samples per batch.

Cachexia

Cachexia was defined as weight loss >5%, or a baseline BMI of <20 kg/m^2 and weight loss >2% within the timeframe between the baseline and six-month health questionnaire (156). Participants who did not fit either of these criteria were considered to be non-cachectic. Weight in pounds and height in inches were self-reported on the health surveys at baseline and six months.

Covariates

Sociodemographic variables were age, sex, level of education. Smoking data permitted analysis by "current-past-never" smoking and alcohol abuse was measured using the previously validated Alcohol Use Disorders Identification Test (AUDIT) (76). An AUDIT score ≥8 was considered problem drinking. Tumor site was recorded from operative notes and surgical pathology forms and categorized into three groups: 1) oral cavity, 2) pharynx and 3) larynx. In order to increase statistical power of stage-wise comparisons, cancer stage was categorized *a priori* into three groups, with Stages 1 and 2 collapsed, while Stage 3 and Stage 4 were considered separately. Highest educational level attained was dichotomized as "≤ high school diploma or equivalent" and "some college or more".

Serum proinflammatory cytokine levels

Cytokine levels were determined using standard methodology established in our laboratories or alternatively in our Cancer Center Immune Monitoring Core using commercially available paired-antibody ELISA kits. Briefly, serum sample aliquots frozen at – 80 °C were thawed, then incubated, overnight @ 4^{o}C in duplicate (25ul/well) on microtiter plates pre-coated with monoclonal antibody specific for IL-6, TNF-α, or IFN-γ, accordingly.

Since the microplate arrays for analysis accommodated 174 duplicate samples at a time, all samples were analyzed in one batch. Any unbound substances were washed away and boitin-linked polyclonal antibody specific for the cytokine was introduced. After incubation for 2 hours at room temperature, the plates were washed and incubated with streptavidin-HRP for an additional hour. After a final wash, substrate solution was then added and color development stopped after 25 minutes at room temperature. A microplate reader was then used to determine colorimetric densities for each sample as calculated from a standard curve. The test sensitivity was determined per manufacturer guidelines. For quality control, multiple plasma aliquots from a single donor were prepared and one aliquot analyzed with each batch of samples. For all assays, frozen aliquots were stored for repetitive testing if necessary.

Statistical analysis

Descriptive statistics (means and frequencies) were generated for all demographic, epidemiologic and clinical variables. Dietary intake data was assessed for missing values and energy outliers using the Rosner method (56, 157). Food consumption data derived from the FFQ were classified *a priori* into 40 foods and food groups using methods similar to those described in previous studies of dietary patterns and disease (53, 79) and as we previously reported. Pretreatment dietary patterns present in the study population were derived by principal component analysis (PCA) using the orthogonal rotation procedure. In establishing the number of factors to retain in the final analysis, eigenvalues ($\geq$3.0), Scree test, percentage of variability explained and interpretability of factors were considered (80). Pattern factor scores were calculated for each study participant by summing reported intakes of the factor food variables weighted by factor loadings. Dietary pattern scores were categorized into quartiles for analysis with serum pro-inflammatory cytokines levels.

Multivariable logistic regression models were used to assess the relationship between dietary variables and cytokine levels. The cytokine counts were categorized into two or three levels according to their observed empirical count distribution. IFN-γ and IL-6 were both categorized into three levels: zero level, non-zero low level and non-zero high level, where the medians of the non-zero values were used to separate the latter two levels. TNF-α was dichotomized into zero and non-zero levels, due to the high proportion of zero-values (64%). For the outcomes with three levels, ordinal logistic model with the proportional odds assumption was employed. For each outcome, known confounders included in final models were selected based on results from a stepwise model selection with the whole foods pattern as the main predictor. For all three cytokines, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to quantify the magnitude of association. Dietary pattern scores and carotenoid and tocopherol levels were categorized into quartiles for use in statistical models and a test for linear trend across quartiles was conducted. All final models for this primary aim were adjusted for age, sex, tumor site, cancer stage, smoking, and alcohol problem. Multivariable logistic regression models were used to quantify the relationship between cachexia and cytokine levels. Final models for this secondary aim were adjusted for age, sex, stage, and smoking. The SAS system Version 9.3 (SAS Institute Inc., Cary, NC) was used for all analyses.

Results

Characteristics of the study population are displayed in **Table 1**. The mean age of study participants was approximately 60 years. The majority of participants were white (94.4%) and male (80.1%). About 60% of the participants had at least some college education or more. The most frequent tumor location was the oropharynx (47.8%) and most

participants presented to UMHS clinics with late-stage (III or IV) cancers (84.9%). About 74% of participants were ever smokers, with about one quarter reporting an alcohol problem (16.2% with AUDIT score ≥ 8). The mean BMI at the time of diagnosis was 27.2 kg/m^2, which is considered overweight but lower than the average BMI of 28.7 kg/m^2 in the United States population from 1999 – 2010 (158). Approximately 57% of the 122 participants who completed six month health questionnaires were classified as cachectic.

Three major dietary patterns were identified with PCA. The first pattern, termed the "whole foods pattern", was characterized by high intakes of vegetables, fruits, legumes, fish, poultry, fruit juice, water, and wine, and low intakes of sugar sweetened beverages and beer. The second pattern, termed the "Western pattern", was characterized by high intakes of red and processed meats, refined grains, condiments, eggs, coffee, butter, and high-fat dairy, and low intakes of fruits, legumes and cereals. The third pattern, termed the "convenience foods pattern" was characterized by high intakes of cereals, pizza, desserts, diet beverages, and energy bars, and low intakes of other vegetables and beer. The factor-loading matrix for the three dietary patterns is presented in **Table 2**.

There was a significant trend towards decreased IL-6, TNF-α, and IFN-γ levels across increasing quartiles of whole foods pattern score (P = <0.001, P = 0.008, and P = 0.03, respectively, **Table 3**). Significant trends toward decreased IL-6, TNF-α, and IFN-γ levels were also found across increasing quartiles of total carotenoid intake (P = 0.006, P = 0.04, and P = 0.04, respectively). Significant inverse associations across increasing quartiles of lycopene intake were observed for IL-6 (P = 0.03) and across increasing quartiles of total vitamin E intake for IFN-γ.

No significant associations were reported for the relationships between IL-6 and TNF-α levels and serum carotenoids (**Table 4)**. There was a significant trend towards decreased IFN-γ levels across increasing quartiles of serum α-tocopherol and β-cryptoxanthin levels ($P = 0.03$ and $P = 0.03$, respectively). Results of multivariable logistic regression models examining the relationships between the IL-6, TNF-α, and IFN-γ and cachexia showed no statistically significant associations. The odds ratios and 95% CIs for these associations can be seen in **Table 5**.

Discussion

The most significant findings of this study were that high whole foods dietary pattern scores and reported intake of total carotenoids prior to treatment were associated with significantly lower serum proinflammatory cytokine levels of IL-6, TNF-α, and IFN-γ, independent of other factors known to modulate cytokine levels (152). Additionally, higher reported intake of lycopene was significantly associated with lower IL-6 levels, and higher reported vitamin E intake and serum α-tocopherol levels were associated with lower IFN-γ levels. We did not find any significant associations between proinflammatory levels and presence of cachexia.

To our knowledge, this is the first study to examine the relationship of dietary patterns and carotenoids with serum proinflammatory cytokine levels in HNSCC patients. Nevertheless, our results support the work of prior research examining this relationship in other study populations. In a prospective analysis conducted among a population of older adults as part of the Health, Aging, and Body Composition (Health ABC) Study, a 'healthy' dietary pattern, (consistent with our whole foods dietary pattern, high in low-fat dairy products, fruit, whole grains, poultry, fish, and vegetables) was associated with lower IL-6

levels than a dietary pattern high in sweets and desserts and high-fat dairy products (159). An intervention trial conducted among males with metabolic syndrome reported that randomization to a Mediterranean diet, rich in foods characteristic of our whole foods dietary pattern, had significantly lower levels of IL-6 and TNF-α after five weeks in comparison to the control group (160).

A cross-sectional study of healthy young adults enrolled into the Toronto Nutrigenomics and Health Study reported strong inverse correlations between biochemical levels of α-tocopherol levels and IFN-γ (161). Results of another study conducted in breast cancer cases indicated that low α-tocopherol plasma concentrations were associated with increased expression of the gene encoding IFN-γ expression (162). This results are consistent with our study of HNSCC subjects, in which we found high reported intake of total vitamin E as well as serum levels of α-tocopherol were associated with lower levels of IFN-γ.

Although we reported a statistically significant inverse association between IL-6 levels and biochemical measures of total carotenoids, a cross-sectional study conducted among patients diagnosed with advanced coronary artery disease reported an inverse relationship between IL-6 and plasma β-carotene levels (163). In agreement with the results of our study, higher serum concentrations of total carotenoids were found to be correlated with lower IL-6 and TNF-α levels in subjects with recent hip fractures (164). A significant inverse relationship also was found between IL-6 and TNF-α levels and serum α-tocopherol concentrations in the same study (164). In contrast, a case-control study of breast cancer risk reported no association between IL-6 levels and reported intake of β-carotene or total vitamin E (165).

A number of epidemiologic and clinical studies have implicated the NF-κB signaling pathway and NF-κB-mediated proinflammatory cytokines as drivers in the development of cancer-related symptoms, making this pathway a potential target for therapeutic intervention (166). While IL-6 and TNF-α are both stimulated by NF-κB (166), macrophages stimulated with IFN-γ activate the NF-κB pathway (167). One mechanism by which the whole foods dietary pattern, carotenoids, and tocopherols may help to reduce circulating proinflammatory cytokine levels in HNSCC patients is through modulation of the NF-κB pathway. Oxidative stress has been reported to activate the NF-κB family of transcription factors, upregulating expression of proinflammatory cytokines (165, 168). Nutrients that can reduce oxidative stress and inflammation in the body such as carotenoids and tocopherols—nutrients found in abundance in foods that characterize the whole foods dietary pattern—have been identified to suppress NF-κB in laboratory studies (169).

Despite findings from several animal studies suggesting roles of IL-6, TNF-α, and IFN-γ in the development of cancer cachexia, we did not find any differences in levels of these cytokines in cachectic versus non-cachectic HNSCC subjects. It is likely that our use of self-reported weight was not sufficient to make valid diagnoses of cachexia and led to misclassification. Evidence from prior studies suggests that classification of cachexia using measures of weight and BMI are challenging, particularly in overweight and obese patients (170). To substantiate the results of animal models of cachexia, future research is warranted that examines the role of proinflammatory cytokines in cachexia pathogenesis in which valid and objective measures of body composition are used, such as clinically measured BMI combined with skinfold measures, dual x-ray absorptiometry (DEXA) and computed tomography (CT)-scans.

Although we found several significant associations between reported dietary variables and proinflammatory cytokine levels, with the exception of α-tocopherol and beta-cryptoxanthin with IFN-γ, we did not find any significant associations between cytokine levels and biochemical measures of carotenoids and tocopherols. This suggests that perhaps there are other dietary constituents driving the associations of reported carotenoid intake with cytokine levels that are highly correlated with carotenoids. These could include bioactive compounds found in fruits and vegetables such as polyphenolic compounds and flavonoids. Flavonoids have been shown to inhibit NF-κB signaling and downregulate the expression of proinflammatory markers and should be examined in relation to proinflammatory cytokines in HNSCC in the future (171).

This study used comprehensive dietary and biochemical data collected from subjects and had the ability to adjust for multiple potential confounding factors which strengthened our conclusions. We were limited by the cross-sectional design, because this study design allowed for the determination of associations between diet and cytokines, but does not prove causation. We were also limited by the use of self-reported weight and height to define cachexia and the small sample size. As with any epidemiologic study involving self-reported recall of diet, measurement error leading to misclassification bias is likely to have occurred. However, this type of bias is likely to attenuate associations towards the null (56). Because patients reported their usual pre-diagnosis dietary intake *after* their cancer diagnosis, recall bias related to social desirability, stress of the diagnosis, or concerns about contributing to their own disease could have occurred. While recall bias is a concern, previous studies of diet and breast cancer indicate either little evidence of recall bias (98, 99), or that the measures of association are attenuated when recall bias occurs (100). Finally, as the study population

consisted predominantly of white males, the results may not be generalizable to racially diverse HNSCC populations.

In summary, diet, particularly a whole foods dietary pattern and total carotenoid intake, appears to be associated with lower systemic inflammation in HNSCC cases, as measured by serum levels of IL-6, TNF-α, and IFN-γ. The ability of carotenoids and other dietary compounds found in foods characterizing the whole foods pattern to decrease levels of proinflammatory cytokines may provide mechanistic evidence for our previous described findings of the association between the whole foods dietary pattern and prolonged survival. In addition to targeting inflammation, it is well accepted that carotenoids and other dietary compounds consistent with the whole foods dietary pattern target other cancer-related mechanisms such as oxidative stress, cell growth, proliferation and apoptosis, angiogenesis, phase II detoxification enzymes, and epigenetic regulation of tumor suppressor genes (82-84). Given its multifaceted properties, along with the low-cost, feasibility, and non-invasive nature of implementing interventions, diet is a particularly attractive target for improving HNSCC progression, symptoms, and outcomes.

The results of this study can serve as the basis for translational intervention research in HNSCC populations, where the aim is to increase the consumption of foods characteristic of the whole foods dietary pattern, with particular focus on foods abundant in carotenoids such as green leafy vegetables, orange and yellow fruits and vegetables, and tomatoes. Before the most optimal dietary interventions can be developed and implemented, further research is necessary that is attentive to the unique challenges of the HNSCC population, investigating potential barriers to access and consumption of these healthy foods, such as

food security, social support, and chewing and swallowing problems related to the course and treatment of disease.

Table 4.1: **Pretreatment Characteristics of Newly Diagnosed Head and Neck Cancer Patients**

Characteristic	No. of Patients (*n* = 161)	%
Age, years		
Mean	59.7	
SD	10.7	
Range	25 - 93	
Sex		
Male	129	80.1
Female	32	19.9
Race		
White/Non-Hispanic	152	94.4
Non-white/Hispanic	9	5.6
Education		
High School or Less	63	39.1
Some College or More	98	60.9
Site		
Oral Cavity	49	30.4
Oropharynx	77	47.8
Larynx	35	21.7
Stage		
1,2	25	15.5
3	24	14.9
4	112	69.6
Smoking		
Current	25	15.5
Former	94	58.4
Never	42	26.1
Alcohol Problem (AUDIT ≥8)		
Yes	26	16.2
No	135	83.8
Cachexia		
Cachectic	69	56.6
Non-cachectic	53	43.4
BMI (kg/m^2)		
Mean	27.2	
SD	5.5	
Range	13.1 – 54.4	

Table 4.2. Factor loading matrix for three dietary patterns (n=161)

Food Group	Whole Foods	Western	Convenience Foods
Dark Yellow Vegetables	**0.75**	***	***
Green Leafy	**0.72**	***	***
Cruciferous	**0.66**	***	***
Tomatoes	**0.65**	***	***
Fruit	**0.54**	**-0.35**	***
Poultry	**0.50**	***	***
Legumes	**0.50**	**-0.32**	***
Fish	**0.47**	***	***
Other Vegetables	**0.44**	***	**-0.31**
Juice	**0.40**	***	***
Wine	**0.31**	***	***
Water	**0.31**	***	***
Whole grains	***	***	***
Mayonaisse	***	***	***
Low-fat dairy	***	***	***
Unsaturated fats	***	***	***
Margarine	***	***	***
Sugar sweetened beverages	**-0.40**	***	***
Beer	**-0.42**	***	**-0.40**
Red Meats	***	**0.59**	***
Processed meats	***	**0.54**	***
Refined grains	***	**0.40**	***
Condiments	***	**0.43**	***
Eggs	***	**0.45**	***
Coffee	***	**0.36**	***
Butter	***	**0.38**	***
High-fat dairy	***	***	***
Liquor	***	***	***
Organ meats	***	***	***
Teas	***	***	***
Cereals	***	**-0.36**	**0.62**
Pizza	***	***	**0.51**
Desserts	***	***	**0.47**
Diet beverages	***	***	**0.50**
Energy bars	***	***	**0.40**
French fries	***	***	***
Artificial sweeteners	***	***	***
Snacks	***	***	***
Potatoes	***	***	***
Chowder	***	***	***

Table 4.3: Multivariable† odds ratios and 95% confidence intervals for proinflammatory cytokine levels by quartile (Q) of FFQ reported dietary patterns and nutrient intake‡ (n=161)

	IL-6			
	Q2	**Q3**	**Q4**	**P_{trend}**
Whole foods pattern	0.41 (0.16, 1.04)	0.32 (0.12, 0.86)	0.16 (0.06, 0.45)	<0.001*
Western pattern	1.19 (0.49, 2.87)	0.48 (0.19, 1.22)	0.70 (0.27, 1.77)	0.20
Convenience foods pattern	0.90 (0.36, 2.23)	1.19 (0.49, 2.90)	0.80 (0.32, 1.98)	0.80
Total vitamin E (μg/day)	0.81 (0.32, 2.01)	0.90 (0.36, 2.26)	0.54 (0.22, 1.34)	0.23
α-carotene (μg/day)	0.48 (0.20, 1.18)	0.49 (0.19, 1.24)	0.61 (0.24, 1.60)	0.33
β-carotene (μg/day)	0.57 (0.23, 1.42)	0.55 (0.21, 1.43)	0.59 (0.22, 1.59)	0.31
Lycopene (μg/day)	1.01 (0.39, 2.58)	1.09 (0.40, 2.97)	0.35 (0.13, 0.94)	0.03*
β-cryptoxanthin (μg/day)	0.30 (0.11, 0.81)	0.64 (0.25, 1.63)	0.40 (0.15, 1.04)	0.27
Lutein + zeaxanthin (μg/day)	0.67 (0.28, 1.64)	0.57 (0.23, 1.43)	0.45 (0.17, 1.20)	0.11
Total carotenoids (μg/day)	0.42 (0.17, 1.06)	0.59 (0.23, 1.53)	0.19 (0.23, 1.53)	0.006*
	TNF-α			
	Q2	**Q3**	**Q4**	**P_{trend}**
Whole foods pattern	0.41 (0.14, 1.18)	0.29 (0.09, 0.92)	0.19 (0.06, 0.64)	0.008*
Western pattern	0.91 (0.34, 2.45)	0.62 (0.21, 1.81)	0.63 (0.21, 1.83)	0.31
Convenience foods pattern	1.21 (0.43, 3.43)	1.18 (0.42, 3.25)	0.44 (0.14, 1.33)	0.16
Total vitamin E (μg/day)	0.92 (0.32, 2.63)	1.09 (0.38, 3.14)	0.76 (0.27, 2.16)	0.69
α-carotene (μg/day)	0.42 (0.15, 1.22)	0.60 (0.21, 1.72)	0.72 (0.25, 2.10)	0.67
β-carotene (μg/day)	0.36 (0.12, 1.05)	0.30 (0.10, 0.95)	0.60 (0.20, 1.83)	0.44
Lycopene (μg/day)	0.45 (0.15, 1.37)	0.99 (0.32, 3.11)	0.24 (0.07, 0.84)	0.08
β-cryptoxanthin (μg/day)	0.52 (0.17, 1.59)	0.61 (0.21, 1.77)	0.69 (0.24, 2.02)	0.67
Lutein + zeaxanthin (μg/day)	0.48 (0.17, 1.36)	0.68 (0.25, 1.90)	0.31 (0.10, 0.99)	0.09
Total carotenoids (μg/day)	0.38 (0.13, 1.09)	0.64 (0.22, 1.88)	0.22 (0.07, 0.72)	0.04*
	IFN-γ			
	Q2	**Q3**	**Q4**	**P_{trend}**
Whole foods pattern	0.58 (0.23, 1.48)	0.40 (0.15, 1.10)	0.32 (0.12, 0.90)	0.03*
Western pattern	0.90 (0.37, 2.17)	0.75 (0.29, 1.92)	1.33 (0.52, 3.42)	0.64
Convenience foods pattern	1.34 (0.53, 3.36)	1.59 (0.64, 3.94)	0.84 (0.34, 2.12)	0.79
Total vitamin E (μg/day)	0.53 (0.21, 1.36)	0.32 (0.12, 0.84)	0.27 (0.10, 0.70)	0.004*
α-carotene (μg/day)	0.76 (0.31, 1.88)	0.79 (0.31, 2.01)	0.79 (0.31, 2.07)	0.67
β-carotene (μg/day)	0.87 (0.35, 2.20)	0.46 (0.17, 1.23)	0.61 (0.22, 1.65)	0.21
Lycopene (μg/day)	0.85 (0.33, 2.21)	1.17 (0.43, 3.21)	0.60 (0.22, 1.62)	0.38
β-cryptoxanthin (μg/day)	0.16 (0.06, 0.47)	0.51 (0.20, 1.35)	0.27 (0.10, 1.35)	0.14
Lutein + zeaxanthin (μg/day)	1.08 (0.44, 2.68)	0.58 (0.23, 1.47)	0.56 (0.21, 1.49)	0.14
Total carotenoids (μg/day)	0.45 (0.18, 1.16)	0.69 (0.26, 1.81)	0.29 (0.10, 0.78)	0.04*

†Adjusted for age, sex, tumor site, cancer stage, smoking, and alcohol problem
‡Odds ratios are comparing each of the upper quartiles to the lowest quartile 1
*Indicates significance at $P < 0.05$

Table 4.4: Multivariable† odds ratios and 95% confidence intervals for proinflammatory cytokine levels by quartile (Q) of nutrient biomarker levels‡ (n=151)

	IL-6			
	Q2	**Q3**	**Q4**	**P_{trend}**
Biomarker (μg/ml)				
α-tocopherol	0.80 (0.33, 1.93)	1.07 (0.44, 2.64)	1.21 (0.49, 2.98)	0.56
γ-tocopherol	1.80 (0.74, 4.37)	0.92 (0.38, 2.21)	1.23 (0.51, 2.96)	0.97
α-carotene	0.84 (0.34, 2.05)	0.97 (0.38, 2.48)	0.97 (0.36, 2.59)	0.98
β-carotene	0.87 (0.35, 2.15)	1.48 (0.58, 3.75)	1.25 (0.50, 3.15)	0.43
Lycopene	1.02 (0.42, 2.48)	0.64 (0.26, 1.59)	0.78 (0.30, 1.99)	0.43
β-cryptoxanthin	0.30 (0.11, 0.81)	0.64 (0.25, 1.63)	0.40 (0.15, 1.04)	0.27
Lutein	0.83 (0.34, 2.02)	0.52 (0.20, 1.35)	0.97 (0.38, 2.52)	0.73
Zeaxanthin	0.81 (0.33, 1.97)	0.77 (0.31, 1.91)	0.60 (0.23, 1.52)	0.29
Total carotenoids	0.63 (0.26, 1.55)	1.15 (0.45, 2.93)	0.71 (0.27, 1.81)	0.70
	TNF-α			
	Q2	**Q3**	**Q4**	**P_{trend}**
Biomarker (μg/ml)				
α-tocopherol	1.39 (0.47, 4.06)	2.70 (0.90, 8.10)	1.92 (0.65, 5.69)	0.28
γ-tocopherol	1.98 (0.61, 6.41)	2.10 (0.68, 6.50)	1.85 (0.57, 6.03)	0.33
α-carotene	2.54 (0.73, 8.89)	2.44 (0.71, 8.33)	1.51 (0.44, 5.19)	0.57
β-carotene	2.45 (0.75, 8.02)	3.31 (0.91, 12.06)	1.02 (0.26, 3.99)	0.96
Lycopene	1.28 (0.42, 3.88)	0.88 (0.27, 2.86)	1.35 (0.41, 4.45)	0.72
β-cryptoxanthin	2.25 (0.70, 7.28)	1.39 (0.44, 4.37)	1.18 (0.34, 4.06)	0.92
Lutein	1.73 (0.54, 5.53)	2.33 (0.68, 7.97)	2.35 (0.71, 7.80)	0.16
Zeaxanthin	1.14 (0.35, 3.71)	2.31 (0.76, 6.97)	0.80 (0.23, 2.77)	0.88
Total carotenoids	0.79 (0.26, 2.41)	1.21 (0.38, 3.87)	0.87 (0.26, 2.91)	0.93
	IFN-γ			
	Q2	**Q3**	**Q4**	**P_{trend}**
Biomarker (μg/ml)				
α-tocopherol	0.99 (0.41, 2.42)	0.90 (0.35, 2.26)	0.62 (0.25, 1.57)	0.03*
γ-tocopherol	2.68 (0.99, 7.27)	1.61 (0.62, 4.22)	1.39 (0.51, 3.78)	0.90
α-carotene	1.51 (0.54, 4.27)	1.51 (0.54, 4.21)	1.20 (0.42, 3.40)	0.76
β-carotene	0.88 (0.34, 2.32)	1.62 (0.55, 4.76)	0.91 (0.30, 2.78)	0.91
Lycopene	0.98 (0.38, 2.53)	0.60 (0.22, 1.68)	1.25 (0.44, 3.52)	0.82
β-cryptoxanthin	0.94 (0.35, 2.48)	0.60 (0.23, 1.57)	0.35 (0.12, 1.01)	0.03*
Lutein	1.07 (0.42, 2.73)	0.94 (0.34, 2.64)	0.73 (0.27, 1.99)	0.51
Zeaxanthin	0.68 (0.26, 1.79)	0.72 (0.28, 1.85)	0.42 (0.15, 1.20)	0.14
Total carotenoids	0.76 (0.30, 1.95)	0.61 (0.22, 1.66)	0.53 (0.18, 1.54)	0.23

†Adjusted for age, sex, tumor site, cancer stage, smoking, and alcohol problem
‡Odds ratios are comparing each of the upper quartiles to the lowest quartile 1
*Indicates significance at $P < 0.05$

Table 4.5: Multivariable† odds ratios and 95% confidence intervals for cachexia‡ by proinflammatory levels (n=122)

Cytokine	OR (95% CI)	P-value
IL-6		
Below median vs. zero	1.23 (0.47, 3.19)	0.90
Above median vs. zero	1.35 (0.54, 3.35)	0.64
TNF-α		
Non-zero vs. zero	1.55 (0.69, 3.45)	0.29
IFN-γ		
Below median vs. zero	0.99 (0.40, 2.52)	0.82
Above median vs. zero	0.82 (0.32, 2.12)	0.66

†Adjusted for age, sex, tumor site, cancer stage, and smoking

‡Cachexia was defined as weight loss >5%, or a baseline BMI of <20 kg/m^2 and weight loss >2% within the timeframe between the baseline and six-month health questionnaire

CHAPTER 5

Conclusion

This purpose of this dissertation research was to investigate the role of diet in HNSCC progression and prognosis. The objective of Chapter 2 was to examine how diet and weight status are associated with HNSCC prognosis. Chapter 3 aimed to describe differences in micronutrient intake of HNSCC cases by HPV-status of the tumor. The objective of Chapter 4 was to investigate associations between pretreatment proinflammatory cytokine levels with dietary patterns, carotenoids, and presence of cachexia. This research provided insight into how diet can influence HNSCC prognosis, how diets of HNSCC cases differ according to disease subtype, providing one hypothesis for the discrepancy in survival rates between the two groups, and, finally, a potential molecular mechanism by which diet may exert its effects on HNSCC progression and survival.

In Chapter 2 we established that following a more healthful, pretreatment, "whole foods" dietary pattern, as well as being overweight or obese at the time of HNSCC diagnosis is associated with lower recurrence and mortality rates. This was the largest study to date investigating the role of diet in HNSCC recurrence and mortality, and the first to describe the association of HNSCC outcomes with overall diet (i.e. dietary patterns) as opposed to individual foods and nutrients. These findings have important implications for clinical practice, suggesting that patients may benefit from nutritional intervention early in the disease trajectory. More aggressive surveillance may be advantageous in patients presenting with poor reported dietary intake and a BMI ≤ 25 kg/m^2 at the time of diagnosis. The long-

term follow-up time and large sample size were strengths of this analysis. Limitations of this research include the observational study design, as it allowed for the determination of an association, but not the determination of causation, the heterogeneous nature of the study population, the potential for recall and misclassification bias of diet, and the lack of statistical power to consider HPV-status of the tumor as a potential confounding or effect modifying factor.

In Chapter 3 we reported higher intake of key micronutrients with anti-cancer and anti-metastatic properties in HPV-positive HNSCC cases compared to HPV-negative. Although several previous studies have reported differences in epidemiologic characteristics between HPV-positive and HPV-negative HNSCC cases, this is the first to document differences in dietary intake between the two groups. The micronutrients reported to be consumed in greater quantity by HPV-positive subjects compared to HPV-negative—folate, iron, β-carotene, vitamin E, and vitamin A—are abundant nutrients found in many of the foods that characterize the whole foods dietary pattern that was associated with survival in Chapter 2. These results are important because they provide support for the hypothesis that diet may be one factor contributing to the lower survival rates observed among HPV-negative HNSCC cases and this hypothesis should be investigated more thoroughly in the future. Limitations of this analysis include the small sample size, cross-sectional design as it raises the question of temporal ambiguity, and the lack of statistical power to test for potential interactions between diet and smoking status. Nevertheless, based on these results, it may be beneficial to develop interventions targeted at HPV-negative HNSCC cases that aim to increase intake of foods abundant in these beneficial micronutrients.

Chapter 4 provided a possible molecular basis for the relationship between higher whole foods dietary pattern score and improved HNSCC survival, establishing an association of higher pretreatment whole foods dietary pattern score and carotenoid intake with lower pretreatment levels of the proinflammatory cytokines IL-6, TNF-α, and IFN-γ. These cytokines have previously been linked to tumor invasion and metastasis (138, 139), higher cancer stage (142-145), cancer cachexia (46), and poorer prognosis of HNSCC (147-150). These findings are particularly interesting in light of the conclusion that higher IL-6 significantly predicted recurrence and mortality in a previous study of a University of Michigan Head and Neck SPORE cohort (91).

To our knowledge, this was the first study to investigate associations between diet and proinflammatory cytokines in an HNSCC population. We did not find significant associations between pretreatment proinflammatory levels and presence of cachexia, although these associations have been well established in animal models. More rigorous studies of the role proinflammatory cytokines play in the regulation of lean body mass and appetite in human cancer cases should be conducted in the future. These studies should include more comprehensive measures of body mass, using methods such as DEXA and CT scans, as well as longitudinal measures of cytokines and hormones involved in appetite regulation, such as ghrelin, leptin, and neuropeptide Y (NPY), and finally, the influence physical activity may have on these associations.

Although not part of this dissertation, prior work of our group reported an association between diet and tumor DNA methylation status of tumor suppressor genes in HNSCC. Specifically, we showed that higher intakes of folate, vitamin B12, vitamin A, and cruciferous vegetables was associated with decreased methylation across a total of 444

individual CpG sites across 237 genes on the Goldengate Cancer Panel linked to tumor suppression (84). Many of the foods that characterize the whole foods dietary pattern are rich sources of folate, vitamin B12, and vitamin A, and cruciferous vegetables loaded highly on this pattern. This suggests that enhanced expression of tumor suppressor genes via decreased DNA methylation is another possible mechanism underlying the epidemiologic association between the whole foods dietary pattern and survival described in Chapter 2.

Longitudinal studies of dietary consumption patterns in HNSCC patients and how these patterns change throughout the disease course and after remission are warranted. Associations between change in dietary patterns over time with recurrence, long-term survival, and development of second primary cancers should also be investigated. Understanding how diet, in general, and changes in diet after HNSCC diagnosis are associated with variations in demographic, behavioral, environmental, and genetic characteristics would be beneficial in developing individualized interventions that target those patients at high-risk for poor diet. Such interventions should be implemented early in the disease trajectory to optimize the likelihood of favorable disease outcomes.

More broadly, the results of this dissertation research would likely be safe to translate to clinical practice immediately, where treating clinicians can recommend the consumption of higher intake of vegetables, fruits, legumes, fish, poultry, whole-grains, fruit juice, olive oil, nuts and garlic to their HNSCC patients. Intervention studies should be conducted in the future aimed at increasing intake of these foods in HNSCC patients and qualitative or mixed methods studies should also be conducted in which barriers to consumption of these foods are identified, such as issues of food security, social support, and chewing and swallowing

difficulties. Future research should also focus on the development and delivery of nutrition and lifestyle education targeting high-risk populations of HNSCC survivors.

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