Original Article

Diet and Proinflammatory Cytokine Levels in Head and Neck Squamous Cell Carcinoma

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BACKGROUND: Proinflammatory cytokine levels may be associated with cancer stage, recurrence, and survival. The objective of this study was to determine whether cytokine levels were associated with dietary patterns and fat-soluble micronutrients in patients with previously untreated head and neck squamous cell carcinoma (HNSCC). **METHODS:** This was a cross-sectional study of 160 patients with newly diagnosed HNSCC who completed pretreatment food frequency questionnaires (FFQs) and health surveys. Dietary patterns were derived from FFQs using principal component analysis. Pretreatment serum levels of the proinflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) were measured using an enzyme-linked immunosorbent assay, and serum carotenoid and tocopherol levels were measured by high-performance liquid chromatography. Multivariable ordinal logistic regression models examined associations between cytokines and quartiles of reported and serum dietary variables. **RESULTS:** Three dietary patterns emerged: whole foods, Western, and convenience foods. In multivariable analyses, higher whole foods pattern scores were significantly associated with lower levels of IL-6, TNF-α, and IFN-γ ($P \leq .001$, $P = .008$, and $P = .03$, respectively). Significant inverse associations were reported between IL-6, TNF-α, and IFN-γ levels and quartiles of total reported carotenoid intake ($P = .006$, $P = .04$, and $P = .04$, respectively). There was an inverse association between IFN-γ levels and serum α-tocopherol levels ($P = .03$). **CONCLUSIONS:** Consuming a pretreatment diet rich in vegetables, fruit, fish, poultry, and whole grains may be associated with lower proinflammatory cytokine levels in patients with HNSCC. ***Cancer*** **2014;120:2704-12.** © *2014 American Cancer Society*.

KEYWORDS: dietary patterns, carotenoids, cytokines, head and neck cancer.

INTRODUCTION

Higher fruit and vegetable intake and levels of serum carotenoids are associated with more favorable prognoses in patients with head and neck squamous cell carcinoma (HNSCC).[1-4] We previously reported that a whole foods pattern, characterized by high intakes of vegetables, fruits, whole grains, poultry, and fish, and a body mass index (BMI) $\geq$25 kg/m^2 at the time of diagnosis were associated with lower recurrence and mortality rates in a large, prospective cohort of patients with newly diagnosed HNSCC.[5] These findings suggest diet as a potential area of intervention to improve HNSCC prognosis. However, research exploring the potential mechanisms underlying these associations is warranted to deepen our understanding of how nutritional interventions may influence patient survival.

It is widely accepted that chronic inflammation can be a driver of cancer development and progression.[6] The proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) are mediators of the immune response and are thought to be involved in malignant transformation, progression, and prognosis.[6] It has been demonstrated that endothelial cells in the tumor microenvironment, and HNSCC cells themselves, secrete high levels of IL-6, inducing tumor

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invasion and metastasis.[7,8] In observational studies, higher IL-6 has been associated with later cancer stage,[9-11] and IL-6[12-15] and TNF-α[16] have been associated with increased HNSCC recurrence and mortality, including within our own HNSCC study population.[17,18]

Dietary intake is known to be involved in the physiologic response to inflammation and oxidative stress.[19] Therefore, we hypothesize that foods and nutrients with antioxidant and anti-inflammatory properties such as carotenoids and vitamin E may mitigate the effects of proinflammatory cytokines in the body, reducing the likelihood of metastasis and prolonging survival. To our knowledge, the relationship between diet and cytokines has not been studied in HNSCC. The objective of this study was to examine associations of dietary patterns, carotenoids, and tocopherols with pretreatment serum levels of proinflammatory cytokines in a cohort of patients with newly diagnosed HNSCC.

MATERIALS AND METHODS

Design, Setting, and Patients

This was a cross-sectional study of patients newly diagnosed with HNSCC and enrolled in the University of Michigan Head and Neck Specialized Program of Research Excellence (HN-SPORE). Institutional Review Board approval was granted from the University of Michigan Health System (Ann Arbor, Mich). Patients were recruited between November 2008 and November 2012. Exclusion criteria included: 1) age <18 years, 2) pregnant, 3) non-English speaking, 4) diagnosed as mentally unstable, 5) diagnosis of another nonupper aerodigestive tract cancer, or 6) any head and neck primary diagnosed within the past 5 years.

Participants completed a self-administered health questionnaire before treatment that collected data on demographics, tobacco use, alcohol use, weight status, and comorbidities. Pretreatment dietary intake was assessed using the 2007 self-administered, semiquantitative Harvard Food Frequency Questionnaire (FFQ).[20] Medical records were reviewed to collect data on tumor site and stage.

Peripheral blood samples (30 mL) were collected before 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 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 diet

The validated 2007 Harvard FFQ was designed to assess respondents' usual dietary intake from food and supplements over the past year.[21-23] The FFQ includes standard portion sizes for each item and allows participants to choose their average frequency of consumption over the past year. Total energy and nutrient intakes were 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.[23]

Serum micronutrients

Serum carotenoids and tocopherols were extracted and analyzed using high-performance liquid chromatography (HPLC) as previously described.[24] Briefly, serum was mixed with an equal volume of ethanol containing butylated hydroxytoluene and extracted with hexane. Tocol was used as the internal standard. A YMC C30 reverse-phase column (2 × 150 mm with a 2.0 × 20 mm guard) was used to separate carotenoids and tocopherols (YMC Company, LTD, Kyoto, Japan) using gradient elution at 0.2 mL per minute total flow on a Shimadzu LC-20AT HPLC system (Shimadzu Corporation, Kyoto, Japan). Detection was at 450 to 472 nm for carotenoids. Electrochemical detection was used for Tocol and tocopherols with a Coularray electrochemical detector set (Thermo Scientific, Waltham, Mass) at 310, 390 and 470 mV. Samples were analyzed in 6 batches of approximately 30 samples per batch.

Covariates

Sociodemographic variables were age, sex, and education level. The highest educational level attained was dichotomized as *≤high school diploma or equivalent* and *some college or more*. Smoking data were categorized as *current*, *former*, or *never smoker*, where the status *current* reflects use in the 12 months before cancer diagnosis. Alcohol abuse was measured using the previously validated Alcohol Use Disorders Identification Test (AUDIT).[25] An AUDIT score ≥8 was considered problem drinking. Tumor site was recorded from medical records and categorized into 3 groups: 1) oral cavity, 2) oropharynx, and 3) larynx. To increase the statistical power of stage-wise comparisons, cancer stage was categorized a priori into 3 groups, with stages I and II collapsed, and stages III and IV considered separately. Tumor human papillomavirus (HPV)-status was determined by an ultrasensitive method

using real-time, competitive polymerase chain reaction and matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy with separation of products on a matrix-loaded silicon chip array as previously described.[26] Comorbidities were recorded using the Adult Comorbidity Evaluation-27 instrument and were categorized into *none or mild* comorbidities compared with *moderate to severe* comorbidities.[27]

Serum cytokines

Cytokine levels were determined using standard methodology established in our Cancer Center Immune Monitoring Core using commercially available, paired-antibody enzyme-linked immunosorbent assay kits. All blood samples were obtained in the outpatient clinic and were stored at 4°C until they were transferred to the Tissue Core (within 2 hours), where they were immediately centrifuged, and the sera were separated into 2-μL aliquots and frozen immediately for storage at −80°C. Internal controls were used for all enzyme-linked immunosorbent assay plates, and all patient samples were assayed in the same batch, because the microplate arrays for analysis accommodated 174 duplicate samples at a time. Briefly, serum sample aliquots frozen at −80°C were thawed and then incubated overnight at 4°C in duplicate (25 μL per well) on microtiter plates precoated with monoclonal antibody specific for IL-6, TNF-α, or interferon gamma (IFN-γ). Any unbound substances were washed away, and biotin-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-horseradish peroxidase for an additional hour. After a final wash, substrate solution was added, and color development was 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 1 aliquot was analyzed with the batch of samples. 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 were assessed for missing values and energy outliers using the Rosner method.[23,28] Food consumption data from FFQs were classified a priori into 40 foods and food groups using methods similar to those described in previous studies of dietary patterns and disease,[29,30] and pretreatment dietary patterns were derived by using principal component analysis (PCA), as previously reported.[5] PCA is a data-reduction method that derives dietary patterns by aggregating food variables together for which the intakes are correlated.[31] Pattern factor scores were calculated for each study participant and were categorized into quartiles for analysis with serum cytokine levels.

Odds ratios (ORs) and associated 95% confidence intervals (CIs) were calculated from multivariable logistic regression models to assess the relationships between dietary variables and cytokine levels. Cytokine values were categorized into 2 or 3 levels according to their observed empirical count distribution. IFN-γ and IL-6 were categorized into 3 levels: zero level, nonzero low level, and nonzero high level, in which the medians of the nonzero values were used to separate the latter 2 levels. TNF-α was dichotomized into zero and nonzero levels because of the high proportion of zero values (64%). For the outcomes with 3 levels, an ordinal logistic model with the proportional odds assumption was used.[32] When possible, the proportional odds assumption was imposed (ie, we assumed that the ORs between any 2 neighboring categories were the same). This allowed us to achieve more efficient estimation, especially given the small sample size. Dietary pattern scores and carotenoid and tocopherol levels were categorized into quartiles for use in statistical models. Cochran-Armitage tests for linear trend across quartiles were conducted between cytokines and nutrient quartiles.[33] All final models were adjusted for age, sex, tumor site, cancer stage, smoking, and alcohol problem. Education, BMI, and Adult Comorbidity Evaluation-27 scores were considered as covariates but were excluded from the final models. Stratified analyses were conducted in which associations were examined separately for patients with stage I, II, or III cancers and patients with stage IV cancers to assess potential effect modification by cancer stage. To examine HPV status as a potential confounder, analyses were repeated in the subset of 122 patients who had HPV status available. The SAS statistical software package (version 9.3; SAS Institute Inc., Cary, NC) was used for all analyses. P values $< .05$ were considered statistically significant, and no multiplicity adjustments were performed.

RESULTS

Of 737 eligible patients approached, 670 consented to participate, yielding a response rate of 91%. Additional patients were excluded who did not complete a baseline FFQ (n = 304), withdrew from the study (n = 24), had

TABLE 1. Pretreatment Characteristics of Patients With Newly Diagnosed Head and Neck Cancer (n = 160)

Characteristic	No. of Patients (%)
Age: Mean ± SD [range], y	59.7 ±10.7 [25-93]
Sex	
Men	128 (80)
Women	32 (20)
Race	
White/non-Hispanic	151 (94.4)
Non-white/Hispanic	9 (5.6)
Education	
≤High school	63 (39.4)
≥Some college	97 (60.6)
Site	
Oral cavity	48 (30)
Oropharynx	77 (48.1)
Larynx	35 (21.9)
Stage	
I, II	25 (15.6)
III	24 (15)
IV	111 (69.4)
HPV status[a]	
Positive	47 (38.5)
Negative	75 (61.5)
ACE-27 comorbidity score	
None or mild	114 (71.2)
Moderate or severe	46 (28.8)
Smoking status	
Current	25 (15.6)
Former	93 (58.1)
Never	42 (26.3)
Alcohol problem: AUDIT score ≥8	
Yes	26 (16.2)
No	134 (83.8)
BMI: Mean ±SD, [range] kg/m^2	27.2 ± 5.5 [13.1-54.4]

Abbreviations: ACE-27, Adult Comorbidity Evaluation-27; AUDIT, Alcohol Use Disorders Identification Test; BMI, body mass index; HPV, human papillomavirus; SD, standard deviation.
[a] HPV status was available for 122 study participants.

reported daily energy intake <200 or >5000 kilocalories (kcals) per day (n = 6), or did not have baseline serum levels available for use in the study (n = 175). The final sample size included 160 patients with newly diagnosed HNSCC.

Characteristics of the study population are listed in Table 1. The mean age of study participants was approximately 60 years. The majority of participants were white (94.4%) and were men (80.1%). Approximately 60% of the participants had at least some college education. The most frequent tumor location was the oropharynx (47.8%), and most participants presented to University of Michigan Health System clinics with late-stage (III or IV) cancers (84.9%). About 74% of participants were ever smokers, and <20% reported an alcohol problem (16.2% had AUDIT scores ≥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 US population from 1999 to 2010.[34] Of the 122 patients who had tumor HPV status available, 61.5% were positive, and 38.5% were negative. Correlations between serum and reported antioxidants are displayed in Table 2. Mean cytokine levels by patient characteristics are reported in Table 3.

Three major dietary patterns emerged from 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.

Levels of IL-6, TNF-α, and IFN-γ decreased significantly across increasing quartiles of whole foods pattern score (Table 4). IL-6, TNF-α, and IFN-γ levels also had a significant, inverse association with total reported carotenoid intake. Significant inverse associations were observed across increasing quartiles of lycopene intake for IL-6 and total vitamin E intake for IFN-γ. No significant associations were reported between IL-6 and TNF-α and serum carotenoids (Table 5). There was a significant trend toward decreased IFN-γ levels across increasing quartiles of serum α-tocopherol and β-cryptoxanthin levels. The results did not differ significantly when stratified by cancer stage or when analyses were performed that included HPV status as a covariate for the subset of 122 patients who had HPV status available.

DISCUSSION

High whole foods dietary pattern scores and reported intake of total carotenoids before treatment were associated with significantly lower serum levels of the proinflammatory cytokines IL-6, TNF-α, and IFN-γ, independent of other factors known from previous research to modulate cytokine levels.[35] In addition, 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.

To our knowledge, this is the first study to examine the correlation of dietary patterns and carotenoids with serum proinflammatory cytokine levels in patients with HNSCC. Our results support findings from prior

research examining this correlation in other study populations. In a prospective analysis conducted among a population of older adults as part of the Health ABC Study, a *healthy* dietary pattern (consistent with our whole foods dietary pattern) was associated with lower IL-6 levels than a dietary pattern high in sweets and high-fat dairy products.[36] An intervention trial conducted among men with metabolic syndrome indicated that those randomized to a Mediterranean diet, which was rich in the foods characteristic of our whole foods dietary pattern, had significantly lower IL-6 and TNF-α levels after 5 weeks compared with the control group.[37]

TABLE 2. Spearman Correlations Between Food Frequency Questionnaire-Reported and Serum-Measured Nutrients (n = 160)

Nutrient	Correlation Coefficient	*P*
Total carotenoids	0.24	.004[a]
α-Carotene	0.29	<.001[a]
β-Carotene	0.30	<.001[a]
Lycopene	0.10	.21
β-Cryptoxanthin	0.38	<.001[a]
Lutein	0.97	<.001[a]
Zeaxanthin	0.87	<.001[a]
α-Tocopherol	0.27	<.001[a]
γ-Tocopherol	0.02	.78

[a] *P* values < .05 indicate a significant difference.

Our current results demonstrate a statistically significant, inverse association between IL-6 levels and reported total carotenoids. Similarly, higher serum concentrations of total carotenoids were correlated with lower IL-6 and TNF-α levels in patients who had recent hip fractures.[38] In contrast, a case-control study of breast cancer risk indicated no association between IL-6 levels and reported intake of β-carotene.[39] Low α-tocopherol plasma concentrations were associated with increased expression of the gene encoding IFN-γ in another study conducted among patients with breast cancer.[40] These results are consistent with our findings that high reported intake of total vitamin E and serum levels of α-tocopherol were associated with lower levels of IFN-γ in patients with HNSCC.

One mechanism by which the whole foods dietary pattern, carotenoids, and tocopherols may help to

TABLE 3. Mean and Standard Deviation Cytokine Levels (pg/ml) by Patient Characteristics (n = 160)

Characteristic	No. of Patients	Mean ± SD		
		IL-6	TNF-α	IFN-γ
Sex				
Men	128	118.7 ± 448.5	187.9 ± 785.2	538.4 ± 838.5
Women	32	17.1 ± 66.8	15.9 ± 74.7	359.3 ± 789.8
Race				
White/non-Hispanic	151	103.4 ± 415.4	162.5 ± 725.8	508.3 ± 834.8
Non-white/Hispanic	9	14.0 ± 15.9	2.1 ± 6.3	407.0 ± 777.6
Education				
≤High school	63	82.1 ± 399.6	145.8 ± 709.5	404.6 ± 767.1
≥Some college	97	108.9 ± 408.5	158.5 ± 707.2	566.2 ± 865.9
Site				
Oral cavity	48	73.6 ± 261.8	85.1 ± 311.3	459.5 ± 787.7
Oropharynx	77	134.9 ± 538.1	222.6 ± 964.1	570.5 ± 910.5
Larynx	35	52.1 ± 122.6	95.2 ± 312.5	412.2 ± 697.9
Stage				
I, II	25	129.9 ± 360.8	158.6 ± 426.9	407.9 ± 717.9
III	24	46.4 ± 67.3	63.9 ± 126.4	641.0 ± 946.8
IV	111	102.5 ± 453.6	171.7 ± 821.6	493.9 ± 829.9
HPV status[a]				
Positive	47	126.9 ± 523.5	188.5 ± 845.5	675.1 ± 955.0
Negative	75	69.2 ± 223.7	98.2 ± 326.5	470.9 ± 774.6
Smoking status				
Current	25	21.9 ± 48.5	31.7 ± 105.7	356.2 ± 735.1
Former	93	37.4 ± 85.3	55.2 ± 197.3	402.0 ± 696.9
Never	42	278.8 ± 754.9	443.6 ± 1312.1	812.3 ± 1062.6
Alcohol problem: AUDIT score ≥8				
Yes	26	26.8 ± 52.1	34.0 ± 105.0	315.7 ± 677.1
No	134	112.3 ± 439.8	176.7 ± 768.3	538.8 ± 853.6

Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; HPV, human papillomavirus; IFN-γ, interferon gamma; IL-6, interleukin-6; SD, standard deviation; TNF-α, tumor necrosis factor alpha.
[a] HPV status was available for 122 study participants.

TABLE 4. Multivariable Odds Ratios and 95% Confidence Intervals for Proinflammatory Cytokine Levels by Quartile of Food Frequency Questionnaire-Reported Dietary Patterns and Nutrient Intake (n = 160)[a]

	IL-6: OR (95% CI)[b]			
Pattern/Nutrient Intake	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)	< .001[c]
Western pattern	1.19 (0.49-2.87)	0.48 (0.19-1.22)	0.70 (0.27-1.77)	.20
Convenience foods pattern	0.90 (0.36-2.23)	1.19 (0.49-2.90)	0.80 (0.32-1.98)	.80
Total vitamin E, μg/d	0.81 (0.32-2.01)	0.90 (0.36-2.26)	0.54 (0.22-1.34)	.23
α-Carotene, μg/d	0.48 (0.20-1.18)	0.49 (0.19-1.24)	0.61 (0.24-1.60)	.33
β-Carotene, μg/d	0.57 (0.23-1.42)	0.55 (0.21-1.43)	0.59 (0.22-1.59)	.31
Lycopene, μg/d	1.01 (0.39-2.58)	1.09 (0.40-2.97)	0.35 (0.13-0.94)	.03[c]
β-Cryptoxanthin, μg/d	0.30 (0.11-0.81)	0.64 (0.25-1.63)	0.40 (0.15-1.04)	.27
Lutein + zeaxanthin, μg/d	0.67 (0.28-1.64)	0.57 (0.23-1.43)	0.45 (0.17-1.20)	.11
Total carotenoids, μg/d	0.42 (0.17-1.06)	0.59 (0.23-1.53)	0.19 (0.23-1.53)	.006[c]
	TNF-α: OR (95% CI)[d]			
Pattern/Nutrient Intake	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)	.008[c]
Western pattern	0.91 (0.34-2.45)	0.62 (0.21-1.81)	0.63 (0.21-1.83)	.31
Convenience foods pattern	1.21 (0.43-3.43)	1.18 (0.42-3.25)	0.44 (0.14-1.33)	.16
Total vitamin E, μg/d	0.92 (0.32-2.63)	1.09 (0.38-3.14)	0.76 (0.27-2.16)	.69
α-Carotene, μg/d	0.42 (0.15-1.22)	0.60 (0.21-1.72)	0.72 (0.25-2.10)	.67
β-Carotene, μg/d	0.36 (0.12-1.05)	0.30 (0.10-0.95)	0.60 (0.20-1.83)	.44
Lycopene, μg/d	0.45 (0.15-1.37)	0.99 (0.32-3.11)	0.24 (0.07-0.84)	.08
β-Cryptoxanthin, μg/d	0.52 (0.17-1.59)	0.61 (0.21-1.77)	0.69 (0.24-2.02)	.67
Lutein + zeaxanthin, μg/d	0.48 (0.17-1.36)	0.68 (0.25-1.90)	0.31 (0.10-0.99)	.09
Total carotenoids, μg/d	0.38 (0.13-1.09)	0.64 (0.22-1.88)	0.22 (0.07-0.72)	.04[c]
	IFN-γ: OR (95% CI)[b]			
Pattern/Nutrient Intake	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)	.03[c]
Western pattern	0.90 (0.37-2.17)	0.75 (0.29-1.92)	1.33 (0.52-3.42)	.64
Convenience foods pattern	1.34 (0.53-3.36)	1.59 (0.64-3.94)	0.84 (0.34-2.12)	.79
Total vitamin E, μg/d	0.53 (0.21-1.36)	0.32 (0.12-0.84)	0.27 (0.10-0.70)	.004[c]
α-Carotene, μg/d	0.76 (0.31-1.88)	0.79 (0.31-2.01)	0.79 (0.31-2.07)	.67
β-Carotene, μg/d	0.87 (0.35-2.20)	0.46 (0.17-1.23)	0.61 (0.22-1.65)	.21
Lycopene, μg/d	0.85 (0.33-2.21)	1.17 (0.43-3.21)	0.60 (0.22-1.62)	.38
β-Cryptoxanthin, μg/d	0.16 (0.06-0.47)	0.51 (0.20-1.35)	0.27 (0.10-1.35)	.14
Lutein + zeaxanthin, μg/d	1.08 (0.44-2.68)	0.58 (0.23-1.47)	0.56 (0.21-1.49)	.14
Total carotenoids, μg/d	0.45 (0.18-1.16)	0.69 (0.26-1.81)	0.29 (0.10-0.78)	.04[c]

Abbreviations: CI, confidence interval; IFN-γ, interferon gamma; IL-6, interleukin-6; OR, odds ratio; Q, quartile; TNF-α, tumor necrosis factor alpha.
[a] The model was adjusted for age, sex, tumor site, cancer stage, smoking, and alcohol problem; ORs were calculated by comparing the upper quartile versus the lowest quartile (Q1) for each pattern or nutrient intake.
[b] ORs were calculated by comparing higher versus lower cytokine values for cytokines categorized into 3 levels.
[c] *P* values < .05 indicate a significant difference.
[d] ORs were calculated by comparing nonzero versus zero values for cytokines categorized into 2 levels.

reduce circulating cytokine levels in patients with HNSCC is through modulation of the nuclear factor-kappaB (NF-κB) pathway. Several epidemiologic and clinical studies have implicated NF-κB as a driver of cancer progression, indicating that this pathway is a potential target for therapeutic intervention.[41] IL-6, TNF-α, and IFN-γ all can stimulate the NF-κB pathway.[41,42] It also has been reported that oxidative stress activates the NF-κB family of transcription factors, further up-regulating the expression of proinflammatory cytokines.[39,43] Nutrients that can reduce oxidative stress and inflammation in the body such as carotenoids and tocopherols—both of which are abundant in foods that characterize the whole foods dietary pattern—have demonstrated the ability to suppress NF-κB in laboratory studies.[44]

We observed several significant associations between reported dietary variables and cytokine levels but did not

TABLE 5. Multivariable Odds Ratios and 95% Confidence Intervals for Proinflammatory Cytokine Levels by Quartile of Nutrient Biomarker Levels (n = 151)[a]

	IL-6: OR (95% CI)[b]			
Biomarker, μg/mL	Q2	Q3	Q4	P_{trend}
α-Tocopherol	0.80 (0.33-1.93)	1.07 (0.44-2.64)	1.21 (0.49-2.98)	.56
γ-Tocopherol	1.80 (0.74-4.37)	0.92 (0.38-2.21)	1.23 (0.51-2.96)	.97
α-Carotene	0.84 (0.34-2.05)	0.97 (0.38-2.48)	0.97 (0.36-2.59)	.98
β-Carotene	0.87 (0.35-2.15)	1.48 (0.58-3.75)	1.25 (0.50-3.15)	.43
Lycopene	1.02 (0.42-2.48)	0.64 (0.26-1.59)	0.78 (0.30-1.99)	.43
β-Cryptoxanthin	0.30 (0.11-0.81)	0.64 (0.25-1.63)	0.40 (0.15-1.04)	.27
Lutein	0.83 (0.34-2.02)	0.52 (0.20-1.35)	0.97 (0.38-2.52)	.73
Zeaxanthin	0.81 (0.33-1.97)	0.77 (0.31-1.91)	0.60 (0.23-1.52)	.29
Total carotenoids	0.63 (0.26-1.55)	1.15 (0.45-2.93)	0.71 (0.27-1.81)	.70
	TNF-α: OR (95% CI)[c]			
Biomarker, μg/mL	Q2	Q3	Q4	P_{trend}
α-Tocopherol	1.39 (0.47-4.06)	2.70 (0.90-8.10)	1.92 (0.65-5.69)	.28
γ-Tocopherol	1.98 (0.61-6.41)	2.10 (0.68-6.50)	1.85 (0.57-6.03)	.33
α-Carotene	2.54 (0.73-8.89)	2.44 (0.71-8.33)	1.51 (0.44-5.19)	.57
β-Carotene	2.45 (0.75-8.02)	3.31 (0.91-12.06)	1.02 (0.26-3.99)	.96
Lycopene	1.28 (0.42-3.88)	0.88 (0.27-2.86)	1.35 (0.41-4.45)	.72
β-Cryptoxanthin	2.25 (0.70-7.28)	1.39 (0.44-4.37)	1.18 (0.34-4.06)	.92
Lutein	1.73 (0.54-5.53)	2.33 (0.68-7.97)	2.35 (0.71-7.80)	.16
Zeaxanthin	1.14 (0.35-3.71)	2.31 (0.76-6.97)	0.80 (0.23-2.77)	.88
Total carotenoids	0.79 (0.26-2.41)	1.21 (0.38-3.87)	0.87 (0.26-2.91)	.93
	IFN-γ: OR (95% CI)[b]			
Biomarker, μg/mL	Q2	Q3	Q4	P_{trend}
α-Tocopherol	0.99 (0.41-2.42)	0.90 (0.35-2.26)	0.62 (0.25-1.57)	.03[d]
γ-Tocopherol	2.68 (0.99-7.27)	1.61 (0.62-4.22)	1.39 (0.51-3.78)	.90
α-Carotene	1.51 (0.54-4.27)	1.51 (0.54-4.21)	1.20 (0.42-3.40)	.76
β-Carotene	0.88 (0.34-2.32)	1.62 (0.55-4.76)	0.91 (0.30-2.78)	.91
Lycopene	0.98 (0.38-2.53)	0.60 (0.22-1.68)	1.25 (0.44-3.52)	.82
β-Cryptoxanthin	0.94 (0.35-2.48)	0.60 (0.23-1.57)	0.35 (0.12-1.01)	.03[d]
Lutein	1.07 (0.42-2.73)	0.94 (0.34-2.64)	0.73 (0.27-1.99)	.51
Zeaxanthin	0.68 (0.26-1.79)	0.72 (0.28-1.85)	0.42 (0.15-1.20)	.14
Total carotenoids	0.76 (0.30-1.95)	0.61 (0.22-1.66)	0.53 (0.18-1.54)	.23

Abbreviations: CI, confidence interval; IFN-γ, interferon gamma; IL-6, interleukin-6; OR, odds ratio; Q, quartile; TNF-α, tumor necrosis factor alpha.
[a] The model was adjusted for age, sex, tumor site, cancer stage, smoking, and alcohol problem; ORs were calculated by comparing the upper quartile versus the lowest quartile (Q1) of each biomarker.
[b] ORs were calculated by comparing higher versus lower cytokine values for cytokines categorized into 3 levels.
[c] ORs were calculated by comparing nonzero versus zero values for cytokines categorized into 2 levels.
[d] *P* values < .05 indicate a significant difference.

observe any significant associations between cytokine levels and serum carotenoids and tocopherols, with the exception of α-tocopherol and β-cryptoxanthin with IFN-γ. This suggests that other dietary variables that are highly correlated with dietary carotenoid intakes may be driving the associations of reported carotenoid intake with cytokine levels. These could include bioactive compounds present in fruits and vegetables, such as polyphenolic compounds and flavonoids. It has been demonstrated that flavonoids inhibit NF-κB signaling and down-regulate the expression of proinflammatory markers and should be examined in relation to proinflammatory cytokines in HNSCC in the future.[45]

For the current study, we used comprehensive dietary and serum data collected from patients and had the ability to adjust for multiple potential confounding factors, which strengthened our conclusions. No causal inferences could be made because of the cross-sectional design, which is a limitation of the study. Random measurement error in dietary reporting may have led to misclassification bias. However, such bias is likely to attenuate associations toward the null.[23] Data on additional variables that may

have influenced results, such as steroid use or autoimmune history of patients, were not readily available, which is study limitation.

In summary, pretreatment diet, particularly a whole foods dietary pattern and total carotenoid intake, may be associated with lower systemic inflammation in patients with newly diagnosed HNSCC, as measured by serum levels of IL-6, TNF-α, and IFN-γ. These associations may provide mechanistic evidence for our previously described findings of the association between a whole foods dietary pattern and prolonged survival.[5] The current results can serve as the basis for translational intervention research in HNSCC populations, in which the objective is to increase the consumption of foods that are characteristic of the whole foods dietary pattern and abundant in dietary carotenoids.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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