Vitamin D Intake and Survival and Recurrence in Head and Neck Cancer Patients

Running Title: Vitamin D and Head and Neck Cancer Outcomes

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Abstract

Objective: With an unacceptably low 5-year survival rate and few identified modifiable factors that affect head and neck cancer (HNC) outcomes, HNC survival remains an important public health problem. Vitamin D has been shown to be associated with immune reactivity and improved outcomes for some cancer sites, but findings are mixed and few studies have examined vitamin D in relation to HNC. This study aims to assess the association between vitamin D intake and survival outcomes in HNC patients.

Methods: This prospective cohort study utilized data on 434 HNC patients with valid pre-treatment food frequency questionnaire data who participated in the University of Michigan Head and Neck Specialized Program of Research Excellence (SPORE) epidemiology project. Cox proportional hazard models were used to estimate the associations between total, dietary, and supplemental vitamin D intake and HNC outcomes, while adjusting for other known prognostic factors.

Results: After multivariable adjustment, we found a statistically significant inverse trend between total vitamin D intake and recurrence (Q4 vs Q1 HR=0.47, 95% CI=0.20 - 1.10, p-trend=0.048). We observed no association with dietary or supplemental intake, separately, and no association was observed with all-cause or HNC-specific mortality.

Conclusion: These findings suggest that HNC patients with lower levels of vitamin D intake are at higher risk of recurrence. If borne out in future studies, our results suggest that increased

vitamin D intake through dietary intervention or the use of supplements may be a feasible intervention for prevention of recurrence in HNC patients.

Keywords: vitamin D, head and neck cancer, recurrence, survival

Introduction

Head and neck cancer (HNC) accounts for approximately 3% of all new cancer cases each year in the United States (U.S.) Compared to 25 years ago, incidence has decreased but has remained relatively stable at approximately 11 per 100,000 men and women per year since 2003. HNC is an important public health problem as it has unacceptably low rates of survival; the current 5-year survival rate for HNC is 64%. While smoking history has been shown to be associated with HNC clinical outcomes[1], few other factors have been identified and, currently, there are no evidence-based recommendations for prevention of recurrence and death after treatment.

Vitamin D is well known for the beneficial role it plays in bone metabolism, calcium absorption, and immune function.[2,3] Using National Health and Nutrition Examination Survey data from 2005-2006, Forrest et. al found an overall vitamin D deficiency prevalence of 45% in the United States (U.S.).[4] Vitamin D deficiency is hypothesized to be associated with a number of negative health outcomes, including diabetes, cardiovascular disease, and cancer.[5,6] Laboratory studies have established several biologic actions of vitamin D that may play a critical role in cancer incidence and mortality. It is thought that vitamin D acts through the vitamin D receptor (VDR) to produce its anti-carcinogenic effects and potentially regulate immune cells.[2] This known transcription factor is responsive to binding of vitamin D, is expressed in many normal and malignant tissues, binds target genes that regulate cellular growth and have shown to be deleted or down-regulated in tumor tissue.[7] Pre-clinical studies of the biologically active form of vitamin D, $1,25(OH)_2D$, also support vitamin D-mediated anti-proliferation and induced apoptosis of cancerous cells.[5]

However, epidemiologic studies have produced mixed evidence of the role of vitamin D in risk of cancer. [8] Higher circulating vitamin D levels have been found to lower the risk of

colorectal cancer.[8,9] Few studies, however, have examined vitamin D in relation to risk of HNC. A prospective cohort study in Helsinki, Finland determined that at the time of diagnosis, vitamin D insufficiency was significantly more prevalent in HNC patients compared to the Finnish general population.[10] Furthermore, polymorphisms in the VDR gene have shown to influence risk of HNC, dependent on genotype.[11] Despite our growing knowledge of the effect of vitamin D on cancer risk, relatively little is known about the role vitamin D status has in cancer survival. A few studies have suggested a protective role of vitamin D on survival in cancers of the prostate, colorectum, and breast.[12,13,14] However, the relationship between vitamin D status and survival in HNC has particularly not been well characterized. A recent study found significantly increased survival for HNC patients with higher circulating vitamin D but further research is needed to confirm these findings.[15]

Our aim in this study is to assess the association of pretreatment dietary and supplemental vitamin D intake on survival in HNC patients. We leveraged previously collected food frequency questionnaire and survival and recurrence data on 434 newly diagnosed HNC patients recruited through the Head and Neck Cancer Specialized Program of Research Excellence (SPORE) at the University of Michigan Health System. Given the high prevalence of vitamin D deficiency, further understanding of this relationship is essential to informing clinicians of the potential therapeutic role of vitamin D in providing better outcomes for patients with HNC.

Materials and Methods

Study Population

This was a prospective cohort study of 1031 newly diagnosed HNC patients recruited into the University of Michigan Head and Neck SPORE between 2008 and 2014. Patients were identified and recruited through the University of Michigan Health System with approval through

the Institutional Review Board at the University of Michigan Medical School. All enrolled participants gave signed, informed consent to their information being used in research studies. The dataset of 1031 HNC patients had previously excluded those that were less than 18 years of age, pregnant, non-English speaking, diagnosed with mental instability, or diagnosed with another primary cancer. We further excluded participants who had incomplete food frequency questionnaire (FFQ) information (n=449), those with implausibly high (calories/day > 5000, n=7) nutrient intake values, participants who were incorrectly diagnosed or dropped out of the study (n=16), or with missing survival time (n=8), and participants with rare cancer sites (i.e. “skull bone”, “nasopharynx” or “salivary gland”, n=15). Finally, participants consuming an abnormally low number of calories were excluded (calories/day <1200, n=102) in order to avoid reverse causation (i.e. that the symptoms of advanced disease would prevent patients from eating normally). Thus, our analytic cohort consisted of 434 participants. Recurrence analyses were further restricted to only participants without persistent disease (N=389). Further analyses included assessing the association between total vitamin D intake and HNC outcomes when stratifying by stage, sex, and BMI independently.

Exposure

Vitamin D intake data was calculated from validated semi-quantitative Harvard Food Frequency Questionnaires (FFQ) that were self-administered by the patients at the time of diagnosis.[16] Our main exposure variable, total vitamin D intake, included both dietary and supplemental vitamin D intake and was categorized into quartiles (Q1 <200, 200≤Q2<465, 465≤Q3<675, Q4≥675) for analysis. Dietary and supplemental vitamin D intake individually and duration of supplemental vitamin D intake were further analyzed as exposure variables.

Covariates

After diagnosis, each participant was approached by a trained interviewer who completed an initial evaluation and provided participants with a self-administered questionnaire which included information demographic variables (age and sex), education level (Less than High School, High School Diploma, Some College, 4 years of College, More than College) and detailed information on history of tobacco (current, former, never) and alcohol use (current, former, never). Total caloric intake data was collected from the administered food frequency questionnaire at baseline. HPV status was assessed by either PCR technique or in-situ hybridization for patients with available tumor tissue (positive, negative, missing). Site (Laryngopharynx, Oral Cavity, Oropharynx, Unknown) and stage (Stage 1, 2, 3, and 4) data were collected during initial tumor evaluation at the time of diagnosis. Hypopharynx cases (n=11) were included with larynx cases in the laryngopharynx category. The baseline health questionnaire as well as medical record reviews were used to collect comorbidity data and scored using the Adult Comorbidity Evaluation-27 (ACE score 0, 1, 2, and 3).[17] In many clinical studies of head and neck cancer, treatment is heterogeneous and the chosen treatment for any given disease site and stage may differ from physician to physician. However, at the University of Michigan, uniform treatment approaches are implemented across the patient population, within strata of disease site and stage, minimizing the potential for confounding by treatment effectiveness. All patients were evaluated by our multidisciplinary team and discussed at our tumor board where standardized treatment recommendations were made.[1]

Outcomes

Outcome variables included death from any cause (n=108), HNC-specific death (n=66), and recurrence of disease (n=99). Survival and recurrence times were measured from time of diagnosis to time of event. The mean survival time was 3.37 years (range: 0.11 - 7.16 years). The mean time to recurrence was 2.59 years (range 0.003 - 7 years). Follow-up to determine recurrent or persistent disease was conducted through patient visits, medical record reviews

and surveys according to the National Comprehensive Cancer Network guidelines.[18] Death events were collected through annual medical record reviews, family notification, Lexis Nexis, and through the Social Security Death Index. LexisNexis is a public record database which compiles information from various sources, including the Social Security Death Master File. We utilized the database to obtain information on vital status for all SPORE subjects who were still considered to be alive at the time of the final survival update (April 2016). The database receives routine data updates from its various sources, so we conservatively estimated the information obtained was valid within six months of the time of the search. Censoring dates varied by participant and were designated as either their last medical record review date or Lexis Nexis search, ranging from April 15, 2016 to May 25, 2016.

Statistical Analysis

Descriptive statistics (mean(sd) for continuous variables, frequency (%) for categorical variables) were generated for all covariates with potential to be included in our final model. Covariate association with total vitamin D intake was assessed using chi-square association tests for categorical variables and t-tests for continuous variables. Our final model included adjustments for the following variables known or hypothesized to be associated with both the outcome and the exposure: age, site, stage at diagnosis, education level, HPV status, smoking status, drinking status, total calorie intake, and ACE score. Adjusting for these covariates, the association between vitamin D intake exposures and survival was assessed using cox proportional hazard models to generate hazard ratios (HR) and their corresponding 95% confidence intervals (CI). We examined the associations stratified by sex, BMI and stage at diagnosis. Statistical interaction was assessed using the likelihood ratio test. SAS 9.4 (SAS Institute Inc.) was used to perform all statistical analyses.

Results

Characteristics of the study participants by quartiles of total vitamin D intake for the analyzed cohort can be found in Table 1. Most participant characteristics were similar across quartiles of vitamin D intake. However, compared to participants with lower vitamin D intake, those with higher intake were more likely to be older, consumed more calories, and were more educated (Table 1).

We found no association between increasing levels of total vitamin D intake and all-cause or HNC-specific mortality. However, we found a significant inverse trend across quartiles of vitamin D intake for recurrence (Q4 vs. Q1 HR=0.47, 95% CI=0.20 - 1.10; p-trend=0.048; Table 2). Upon stratifying by stage, the association between total vitamin D intake and survival time appeared similar in both those diagnosed at early and late stages but the inverse association between total vitamin D intake and recurrence appeared stronger in individuals with stage 4 disease (Supplemental Tables 1 and 2). When we examined dietary intake of vitamin D and supplemental intake separately, we found no association with both all-cause survival, HNC-specific survival, or recurrence (Supplemental Tables 3 and 4). Upon stratifying by sex, a nonsignificant inverse association was found between total vitamin D intake and recurrence in men. In women, however, hazard ratios were uninterpretable due to small sample size (Supplemental Tables 4 and 5). We observed no interaction between BMI and total vitamin D intake (p-interaction=0.95).

Discussion

In this prospective cohort study of HNC patients, although we found no association between vitamin D intake and all-cause or HNC-specific mortality we did observe a lower risk of recurrence with higher levels of total vitamin D intake.

To our knowledge, no previous studies have examined vitamin D intake in relation to HNC recurrence among HNC patients. However there have been two previous studies assessing the relationship between vitamin D and HNC survival, which have reported mixed findings.[15,19] A study conducted by Meyer et al. found no association between combined dietary and supplemental vitamin D intake pre-treatment and overall mortality and recurrence in HNC cases.[19] In contrast, a study by Fanidi et al. found low circulating vitamin D concentrations to be associated with a higher risk of all-cause deaths in HNC patients.[15] It should be noted that Fanidi et al. measured circulating 25(OH)D levels while both the present analysis and the study by Meyer et al. examined vitamin D intake. Another distinction is that Fanidi et al. evaluated vitamin D status pre-diagnostically while both our study and that by Meyer et al. assessed vitamin D intake in HNC patients prior to treatment. Difference in timing of exposure assessment relative to diagnosis and the use of dietary intake vs. blood measurements may contribute to the differing results between these studies.

Vitamin D has multiple biologic actions that are thought to contribute to its protective role in cancer incidence and progression including roles in regulating cell proliferation and differentiation, reducing invasion, being pro-apoptotic, and anti-angiogneic.[20] One possible biologic mechanism of particular interest in head and neck cancer is the regulatory activity of vitamin D on immune cells within HNC tumors. Previous studies have demonstrated that the pattern of immune cell populations within tumors can be used as a prognostic factor to predict recurrence and survival. The effect of infiltrating immune cells on tumor progression varies by tumor histology, stage and microenvironment.[21] In a previous study conducted in the University of Michigan Head and Neck SPORE, Nugyen et al. found that quantifying subsets of tumor infiltrating lymphocytes (TILs) could be used to predict HNSCC (Head and Neck Squamous Cell Carcinoma) outcomes. Specifically, patients with tumors that had higher levels of CD4 and CD8 TILs individually showed increased overall survival but the effect of TIL level on survival differed

by HNSCC site.[22] While the effect of vitamin D status on survival by TIL status has not been characterized in HNC, one study conducted in colorectal cancer patients reported that higher plasma 25(OH)D vitamin D status was associated with better survival , but only for tumors that had high levels of TILs.[23] This suggests that the effects of vitamin D depend on tumor immune status and may partly explain the results we obtained from our study. The unknown immune statuses of tumors in our cohort along with our outcome comprising a group of unique tumor sites may explain the lack of the effect we observed of vitamin D on survival. Unfortunately, our analysis was underpowered to examine individual sites separately. Future studies are necessary to elucidate how vitamin D interacts with the tumor immune system to affect HNC tumor initiation and progression and whether its influence differs by tumor site.

Although we observed an inverse association for HNC recurrence, we observed no association for overall or HNC-specific mortality. This lack of association may potentially be explained by differences in liver and kidney function. Vitamin D_3 taken in through the diet is converted to 25(OH)D_3 in the liver by vitamin d hydroxylases. This is the major form of circulating vitamin D which is then converted to the hormonally active form, 1,25$(OH)_2$D, in the kidney.[24] Thus, the health of an individual's liver and kidneys can affect the bioavailability of ingested dietary and supplemental vitamin D.[25] Individuals with a high intake of vitamin D may not experience the full effect if their liver and kidneys are not functioning properly, and liver and kidney impairment would be expected to be strongly associated with mortality, but not HNC recurrence. Although adjusting for ACE score did not alter our findings, it is possible that residual confounding by liver or kidney function could exist. Alternatively, if a protective association for vitamin D is specific to HNC, we might have expected to see protection for HNC recurrence and HNC-specific survival, but not overall survival. Despite the fact that we had a fairly large sample size of 434 patients with this rare cancer, we may have been underpowered to observe an association for HNC-specific mortality. Future studies in large populations of HNC

patients and randomized controlled trials are needed to establish whether vitamin D intake may improve HNC outcomes.

Our study has some limitations including the lack of sunlight exposure data for our cohort. As sunlight exposure is known to increase blood vitamin D levels, measurement of vitamin D intake alone may not accurately reflect vitamin D status. However, because the exposure was assessed prospectively, any errors in vitamin D measurements due to missing sunlight exposure data are likely to be non-differential with respect to the outcomes of interest. Thus, this source of measurement error would have attenuated our results toward the null. Another limitation in our study design was the use of FFQ data which are self-reported and subject to inaccurate recall. However, a strength of using an FFQ is that it assesses usual vitamin D intake in the year prior to treatment while circulating levels are typically assessed at one time point. Therefore, using these vitamin D intake measurements may be more representative of vitamin D status over a longer period compared to a one-time serum level measurement. Finally, we had limited power to conduct stratified analyses by other potentially effect modifying factors such as sex, disease site, and HPV-status.[26]

The strengths of our study include its prospective design and large sample size for a rare cancer type of 434 HNC cases. To our knowledge, this is the first prospective cohort study of HNC patients that has collected and analyzed FFQ data. Information was available for a number of potential confounders to be adjusted for in our final models. All patients in our cohort were evaluated and treated through the University of Michigan Health System, decreasing potential inconsistencies in clinical data reporting and differences in outcomes due to differences in treatment.

In conclusion, in this large prospective cohort study of HNC patients, we found that higher vitamin D intake was associated with a lower risk of HNC recurrence. If borne out in

future studies, our results suggest that increased vitamin D intake through dietary intervention or the use of supplements may be a feasible intervention for prevention of recurrence in HNC patients. Further study is required to determine when in the natural history of HNC vitamin D may be most effective and if particular subgroups of HNC patients may be more likely to benefit from increased vitamin D intake.

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Table 1: Cohort Characteristics by Quartiles of Vitamin D Intake

Total Vitamin D Intake Quartiles (IU/day)

	<200	[200-465)	[465-675)	>=675	p-value
N	108	108	109	109	
Age in years at Diagnosis (SD)	58.6(12.7)	58.5(11.1)	60.0(9.5)	63.1(10.4)	.0014
Sex Male	86(80.4%)	86(80.4%)	87(80.6%)	80(73.4%)	.493
Smoker Current Former Never	44(40.7%) 37(34.3%) 27(25.0%)	44(40.7%) 38(35.2%) 26(24.1%)	37(33.9%) 36(33.0%) 36(33.0%)	40(36.7%) 45(41.3%) 24(22.0%)	.531
Drinker Current Former Never	77(71.3%) 26(24.1%) 5(4.6%)	79(73.2%) 24(22.2%) 5(4.6%)	86(78.9%) 19(17.4%) 4(3.7%)	69(63.3%) 31(28.4%) 9(8.3%)	.288
Cancer Site Laryngopharynx Oral Cavity Oropharynx Unknown	23(21.3%) 43(39.8%) 38(35.2%) 4(3.7%)	29(26.9%) 28(25.9%) 42(38.9%) 9(8.3%)	21(19.3%) 35(32.1%) 46(42.2%) 7(6.4%)	24(22.0%) 39(35.8%) 42(38.5%) 4(3.7%)	.476
Stage 1 2 3 4	20(18.5%) 12(11.1%) 17(15.7%) 59(54.6%)	17(15.7%) 13(12.0%) 14(13.0%) 64(59.3%)	20(18.4%) 11(10.1%) 13(11.9%) 65(59.6%)	17(15.6%) 12(11.0%) 16(14.7%) 64(58.7%)	.996
Ace Score 0(none) 1(mild) 2(moderate) 3(severe)	30(27.8%) 56(51.6%) 19(17.6%) 3(2.8%)	31(29.0%) 47(43.9%) 19(17.8%) 10(9.4%)	33(30.3%) 52(47.7%) 17(15.6%) 7(6.4%)	24(22.0%) 54(49.5%) 22(20.2%) 9(8.3%)	.638
HPV Status Negative Positive Unknown	38(35.2%) 16(14.8%) 54(50.0%)	32(29.6%) 23(21.3%) 53(49.1%)	30(27.8%) 23(21.3%) 55(50.9%)	33(30.3%) 19(17.4%) 57(52.3%)	.819
BMI (kg/m^2)	27.8 (5.9)	27.4 (6.0)	27.4 (5.8)	27.8 (5.6)	0.44
Average Daily Calorie Intake (SD)	1942(573)	2232(701)	2106(697)	2324(738)	<.0006

Average Hours Walked Per Week	4.7(6.0)	4.8(5.9)	4.6(5.2)	4.3(5.1)	.620
Average Hours Sitting Per Week	16.6(8.9)	16.5(9.2)	16.7(9.0)	18.4(8.3)	.166
Average Hours of Light Exercise Per Week	1.3(2.9)	1.3(3.8)	0.9(2.7)	0.9(2.6)	.275
Average Hours of Moderate Exercise Per Week	0.3(1.2)	0.2(0.9)	0.2(1.1)	0.2(1.1)	.754
Average Hours of Strenuous Exercise Per Week	0.4(2.8)	0.6(2.9)	0.7(2.1)	0.6(1.8)	.654
Highest Education Level More than Coll 4 year College Some College HS Diploma Less than HS	 9(8.4%) 12(11.2%) 41(38.3%) 36(33.6%) 9(8.4%)	 17(16.0%) 13(12.3%) 39(36.8%) 27(25.5%) 10(9.4%)	 22(20.4%) 13(12.0%) 43(39.8%) 24(22.2%) 6(5.6%)	 23(21.1%) 16(14.7%) 36(33.0%) 28(25.7%) 6(5.5%)	 .007

N=434

Table 2: Cox Proportional Hazard Models of Total Vitamin D Intake Quartiles

	Total Vitamin D Intake(IU/day)				*p-value for trend*
	<200	[200-465)	[465, 675)	>=675	
HNC-specific Death					
# Deaths	18	12	15	21	
17,592.14 p-mos	4277.45	4623.77	4533.09	4157.83	
HR (95% CI) †	1.0(ref)	0.60(0.29, 1.25)	0.73(0.37, 1.46)	1.01(0.54, 1.90)	.814
HR (95% CI) ‡	1.0(ref)	0.55(0.25, 1.20)	0.65(0.32, 1.35)	0.79(0.40, 1.56)	.707
Overall Death					
# Deaths	27	24	25	32	
17,592.14 p-mos	4277.45	4623.77	4533.09	4157.83	
HR (95% CI) †	1.0(ref)	0.82(0.47, 1.42)	0.82(0.47, 1.41)	1.00(0.60, 1.67)	.967
HR (95% CI) ‡	1.0(ref)	0.71(0.39, 1.27)	0.73(0.41, 1.29)	0.84(0.48, 1.47)	.656
Recurrence					
# Recurring	14	19	16	10	
13,115.27 p-mos	3049.76	3509.75	3443.55	3112.21	
HR (95% CI) †	1.0(ref)	1.16(0.58, 2.32)	0.94(0.46, 1.93)	0.57(0.25, 1.28)	.139
HR (95% CI) ‡	1.0(ref)	1.19(0.57, 2.49)	0.90(0.42, 1.92)	0.47(0.20, 1.10)	.048

†:age-adjusted

‡:adjusted for age, smoking status, alcohol status, hpv status, highest education level, ACE score, total calorie intake, disease site, and stage.

N=434, Recurrence analysis N=389