DIETARY VITAMIN D INTAKE AND SERUM 25-HYDROXYVITAMIN D LEVEL IN RELATION TO DISEASE OUTCOMES IN HEAD AND NECK CANCER PATIENTS

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ABSTRACT

Background

Low pre-treatment vitamin D status has been associated with worsened disease outcomes in patients with cancer at various sites. Its prognostic significance in head and neck cancer (HNC) patients has not been studied.

Methods

Patients with HNC who participated in a randomized trial were evaluated for: (i) total intake of vitamin D from diet and supplements using a validated food frequency questionnaire (all trial participants, n=540) and (ii) pre-treatment serum 25-hydroxyvitamin D through a radioimmunoassay (n=522). The association of dietary/serum measures of vitamin D status with HNC recurrence, second primary cancer (SPC) incidence, and overall mortality was evaluated using multivariate Cox proportional hazard models.

Results

There was no significant association between dietary or serum vitamin D measures and the three HNC outcomes. The hazard ratios (HR) comparing the highest to the lowest quartile of dietary/supplemental vitamin D intake were 1.10 (95% confidence interval (CI): 0.66-1.84) for recurrence, 1.05 (95% CI: 0.63-1.74) for SPC, and 1.27 (95% CI: 0.87-1.84) for overall mortality. HRs comparing the uppermost to the lowest quartile of serum 25-hydroxyvitamin D levels were 1.12 (95% CI: 0.65-1.93) for recurrence, 0.72 (95% CI: 0.40-1.30) for SPC, and 0.85 (95% CI: 0.57-1.28) for overall

mortality. There was no effect modification by cancer stage, season of initial treatment, or trial arm.

Conclusions

Among patients with HNC, vitamin D status before treatment does not influence disease outcomes. Our results contrast with those from most published studies which suggest prognostic significance of vitamin D status in cancer patients at least in subgroups.

INTRODUCTION

Geographical variations in cancer mortality have been associated with latitude and solar ultraviolet-B radiation[1]. Photosynthesis of vitamin D_3 (cholecalciferol) occurs in the skin by the action of solar ultraviolet–B radiation. The overall vitamin D status in a person depends on both cholecalciferol and on ergocalciferol (vitamin D_2) levels. The 25-hydroxyvitamin D, the major circulating form of vitamin D, reflects well the cumulative effects of exposure to sunlight and dietary intake of vitamin D[2]. It has been reported that cancer specific mortality[3] or all cause mortality[4] was lower for patients with cancer diagnosed during the summer or the fall, the seasons with the highest blood levels of 25-hydroxyvitamin D, than for those diagnosed in the winter.

The prognostic significance of circulating 25-hydroxyvitamin D levels among cancer patients has been examined in a limited number of studies[5-11]. Higher 25-hydroxyvitamin D levels were associated with better overall survival in patients with non-small-cell lung cancer with stage IB-IIB but not in those with stage IA, III or IV[5-6]. In the Nurses' Health Study and the Health Professionals Follow-up Study, 304 participants were diagnosed with colorectal cancer[7]. Higher pre-diagnosis 25-hydroxyvitamin D levels were associated with a significant reduction in overall mortality but not with cancer specific mortality. For breast cancer, lower pre-diagnosis plasma vitamin D levels were associated with a higher risk of distant recurrence and of death in one study[8] while no association was observed with recurrence-free survival in another prospective cohort[9]. In another study, serum 25-hydroxyvitamin D levels were measured in 160 patients with prostate cancer[10]. The men with 25-hydroxyvitamin D serum levels below 50 nmol/L had increased risks of cancer

specific mortality and overall mortality in comparison to those with higher levels. Finally, in a large prospective study of patients with melanoma, higher serum 25-hydroxyvitamin D levels three to six months after diagnosis were associated with significantly lower risks of recurrence[11].

A few studies have examined the relationship of intake of vitamin D from diet and supplements with cancer risk, but there is only one published report investigating specifically the relationship between dietary intake of vitamin D and disease outcomes among cancer patients[5,12]. No association was observed between vitamin D intake and overall survival or disease-free survival in 321 patients with early-stage non-small cell lung cancer. However, among the 151 patients who had surgery in the summer, those with higher vitamin D intake had significantly better outcomes[12].

In the present study, we prospectively examined the relationship of dietary intake and serum level of vitamin D with disease outcomes in a large cohort of patients with localized (stage I and II) head and neck cancer.

PATIENTS AND METHODS

Study population

Between October 1, 1994, and June 6, 2000, 540 patients with stage I or II head and neck cancer (HNC) were recruited in five radiation therapy centers in the province of Quebec, Canada to participate in a randomized controlled trial[13]. The institutional review

board of each participating center approved the study protocol. All patients gave written informed consent prior to randomization. Patients with an average over the preceding year daily supplement intake of beta carotene or vitamin E above 6.0 mg and 50 IU, respectively were not eligible for the trial. Patients were randomly assigned to receive a daily supplementation consisting of vitamin E (one capsule of 400 IU *dl*-α-tocopherol) and β-carotene (one capsule of 30 mg) or placebos during radiation therapy and for 3 years after radiation therapy ended. Concerns about adverse effects of β-carotene supplementation prompted the investigators to halt the use of β-carotene after the first 156 patients had been enrolled. The trial was continued with alpha-tocopherol alone. The main results of the trial were an increased incidence of second primary cancers (SPC) during the period of supplementation[13] and a decreased overall survival[14] in the supplementation arm.

Baseline data collection

Baseline data and biospecimen collection was completed before randomization and the initiation of radiation therapy. Research nurses administered several questionnaires on patients' characteristics, including socio-economic data, height and weight, alcohol consumption, smoking, dietary intake and vitamin and mineral supplement use. In order to assess the dietary intakes over the year preceding randomization, an 84 item semi quantitative food frequency questionnaire had been designed and validated previously in a population of 73 patients with head and neck cancer[15]. This food frequency questionnaire enabled us to find that severe acute adverse effects of radiation therapy were significantly less frequent in patients with

higher dietary beta-carotene intake[16]. Average daily intakes over the year preceding randomization were calculated using the 2007 Canadian food composition table (http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/index-eng.php). A detailed questionnaire collected information (name, dose, frequency) on the use of all vitamin and mineral supplements taken during the year preceding randomization. Average daily intakes from supplements were calculated using various sources including the compendium of pharmaceuticals and specialties and manufacturer documentation[17]. Total vitamin D intake was calculated by summing dietary and supplement sources. All 540 trial participants provided data for the analysis of vitamin D intake from diet and supplements. Blood samples were collected at the time of randomization from all participants. After processing, serum specimens were frozen and stored at -80ºC. For the measurement of serum 25-hydroxyvitamin D levels, samples were available for 522 HNC patients. Measurement of 25-hydroxyvitamin D levels was done in duplicate with a radio-immunoassay kit (Diasorin laboratories, Stillwater, Minnesota) that detects both 25-hydroxylated derivatives of vitamin D_2 and D_3. The inter-assay variability of the control samples had a coefficient of variation of 8.3%.

Follow-up

Follow-up information was obtained by the collaborating radiation oncologists and the study nurses every 6 months during the 3 years following the end of radiation therapy and then once a year until the end of the study. At each follow-up visit, the radiation oncologist assessed the recurrence of the initial tumor and the occurrence of any SPC. Follow-up schedule and investigations for recurrence or SPC were similar in the two arms of the trial. Blinding was maintained until the end of clinical follow-up on

June 30, 2003. We used recognized criteria to distinguish SPCs from local or distant recurrences of the initial HNC[13]. Record linkage with the Quebec mortality files was performed using the unique Quebec health insurance identifier from enrollment until December 31, 2006 for all but 10 participants who did not consent to this record linkage. All death certificates were obtained from the Institut de la statistique du Québec. Three main separate outcomes were studied: cancer recurrence, SPC incidence, and overall mortality.

Statistical analyses

We calculated the average energy-adjusted dietary intake of vitamin D from the diet and supplements during the year preceding entry into the trial. Energy-adjusted vitamin D intake values were obtained by regressing total vitamin D intake values on total dietary energy and obtaining the residuals from the model[18]. To investigate the relationship of dietary and serum vitamin D on HNC outcomes, we classified the study population into four subgroups according to the quartiles of each of these two vitamin D variables separately. Cox proportional hazard regression models were applied to assess the relationships between quartile-based vitamin D variables and the time to event outcomes such as cancer recurrence, SPC incidence, and overall mortality. Multivariate models were used including potential confounders as covariates[19]. In the models assessing the effect of 25-hydroxyvitamin D levels, season of blood collection was always included as a covariate. Hazard ratios (HRs) and their 95% confidence intervals (CI) were calculated according to quartiles of vitamin D variables with the lowest quartile as the reference category. A two-tailed p-value for linear trend test across categories was calculated. All regression models were stratified according to randomization arm

(i.e. supplements or placebos). The proportionality assumption of the regression models was formally tested using the standardized score process. The overall adequacy of the models was assessed by examining deviance residuals[19]. All statistical tests were two-sided. The analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC).

RESULTS

The baseline personal, clinical, dietary and serologic characteristics of study participants are presented in Table 1. The proportion of trial participants taking supplements containing vitamin D was relatively low (12.4%). Total vitamin D intake from foods and supplements was 248 IU per day on average. The mean serum level of 25-hydoxyvitamin D was 63.6 nmol/L. The Spearman correlation coefficient between vitamin D dietary intake and serum 25-hydroxyvitamin D level was 0.26 ($p<0.0001$). Serum 25-hydroxyvitamin D was higher among those who took supplements with vitamin D in the previous year (73 vs 62 nmol/L). Serum 25-hydroxyvitamin D was not significantly associated with the other variables listed in table 1 except for gender (higher in males), site (higher for laryngeal cancer), and alcohol intake (lower for higher alcohol intake). In particular, there was no association with age or body mass index. During the active follow-up (median 4.4 years) for clinical events, 119 patients had a HNC recurrence and 113 were diagnosed with a SPC (53 were lung cancers). During the longer follow-up for mortality (median 8.0 years), 223 patients died, 62 from their initial HNC and 81 from SPC including 52 from lung cancer.

Table 2 presents the relationships between dietary vitamin D intake and HNC outcomes. No association was observed with HNC recurrence, SPC incidence, or overall mortality. The HRs comparing the uppermost quartile to the lowest quartile were all close to 1.0. Similarly, as documented in table 3, there was no association between serum 25-hydroxyvitamin D level and HNC recurrence (p-value for trend=0.56) or overall mortality (p-value for trend=0.65). There was a tendency for lower incidences of SPC with increasing serum levels of 25-hydroxyvitamin D but this trend did not reach the level of statistical significance (p-value=0.23). The HR of SPC comparing the uppermost quartile to the lowest quartile of 25-hydroxyvitamin D levels was 0.72 (95% CI: 0.40-1.30). This relationship between serum 25-hydroxyvitamin D and SPC was observed not only with incidence data but also with mortality data. In comparison to patients in the lowest quartile of serum 25-hydroxyvitamin D levels, those in the uppermost quartile had a lower mortality from SPC (HR=0.76, 95% CI: 0.38-1.51). There was no effect modification by cancer stage, season of initial treatment, or trial arm. The p-value for interaction between the vitamin D variables and the trial supplementation ranged from 0.67 to 0.99.

The inverse but not significant trend between serum 25-hydroxyvitamin D and SPC occurrence prompted us to explore a possible association with lung cancer, which is the most frequent site of second primaries in HNC patients. When compared to patients in the lowest quartile of serum 25-hydroxyvitamin D, those in the uppermost quartile had a lower lung cancer incidence (HR=0.54, 95% CI: 0.22-1.30) and a lower lung cancer mortality (HR=0.56, 95% CI: 0.22-1.40); none of these associations reached statistical significance level.

DISCUSSION

In this large mature cohort of patients treated for localized HNC, we observed no association between pre-treatment dietary or serum vitamin D measures and disease outcomes. Although this could be viewed as a negative result, our study remains informative as it is in the top of studies of the prognostic significance of vitamin D in cancer patients in terms of number of patients, duration of follow-up, and number of death events[5-12]. Furthermore, few investigators have collected dietary information among cancer patients before initial therapy, in addition to serum samples.

In the planning of our randomized trial, which evaluated the effects of a supplementation with alpha-tocopherol and beta-carotene, we purposely developed a semi-quantitative food frequency questionnaire to assess in patients with HNC the intake over the previous year of the main energy providing nutrients and micronutrients such as vitamin A, vitamin E, and carotenoids. In this validation study, the correlations between dietary intakes and circulating levels were respectively 0.29 (p=0.02) for beta-carotene and 0.39 (p=0.005) for alpha-tocopherol[15]. In the present study, a highly significant correlation coefficient (r=0.26) was observed between dietary vitamin D and serum 25-hydroxyvitamin D level, a value within the range (r=0.21 - 0.26) observed in the Nurses' Health Study[20] and the Women's Health Initiative[21]. This suggests that the assessment of vitamin D intake in our study was appropriate. The concordance of the results with both diet and serum measures of vitamin D is a further validation of our dietary assessment.

The 25-hydroxyvitamin D circulating levels in our study population ranged from 19 to 135 nmol/L with a mean of 64 nmol/L. These levels are comparable to those reported in breast cancer patients in the Toronto area[8] and in patients with colorectal cancer in the United-States[10]. In the Boston area, patients with lung cancer had markedly lower levels of 25-hydroxyvitamin D[5,6]. The interquartile range in our study (48 to 78 nmol/L) is wider than those observed in most previous studies. There is presently no consensus on adequate or optimal levels of vitamin D measured as serum 25-hydroxyvitamin D levels, and these depend on the health outcome considered[22-23].

Our results do not support the hypothesis of an association between diet or serum vitamin D and cancer recurrence or mortality in patients with head and neck cancer. Although there was some indication of a lower mortality for patients with higher serum vitamin D levels in the crude analysis, this trend was much weaker after controlling for multiple confounders. There is, to our knowledge, no other published report investigating the relation of vitamin D to recurrence or survival among HNC patients.

A critical second look at the published studies of the prognostic significance of vitamin D in cancer patients suggests that the association varies according to cancer site. There is a clear and significant downward trend of overall mortality in patients with colorectal cancer[7]. In patients with melanoma, serum hydroxyvitamin D levels were significantly associated with recurrence free survival but not with overall survival[11]. For breast cancer, two large prospective studies yielded opposite results[8,9]. No significant association with overall survival was observed overall in cohorts of patients treated for

lung cancer, but only in subgroups according to season or stage or by combining exposures[5,6,12]. Thus, the current evidence in favor of a beneficial effect of vitamin D on cancer outcomes remains weak.

In our study, patients with serum 25-hydroxyvitamin D level in the uppermost quartile had almost half the rates of lung cancer incidence and mortality than those in the lowest quartile. HNC patients are at very high risk of new lung cancer presumably because of field cancerization and common smoking exposure. Two prospective studies have examined the relationships between pre-diagnosis 25-hydroxyvitamin D circulating levels and lung cancer risk[24,25]. Among the participants in the Third National Health and Nutrition Examination Survey, there was no association between serum 25-hydroxyvitamin D level and mortality from lung cancer[24]. In a large cohort study in Finland, baseline serum 25-hydroxyvitamin D level was inversely related to lung cancer risk but the overall association was no longer significant in a multivariate model[25]. In this latter study, age and sex were effect modifiers and significant associations were observed in younger participants and in women. It is possible that a true association exists between vitamin D and lung cancer risk, but the studies that have examined this hypothesis, including ours, did not have sufficient power.

In conclusion, our study results do not provide support to the hypothesis that pre-treatment vitamin D status is related to disease outcomes among patients with localized head and neck cancer. Further investigations of the relationships between vitamin D and cancer incidence or outcome should take into account genetic variants of key genes involved in vitamin D metabolism.

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Table 1. Baseline personal, clinical and dietary characteristics of the 540 trial participants.

Age, years – mean (SD)	62.5 (9.8)
Male sex – n (%)	426 (79)
Married – n (%)	399 (74)
Alcohol consumption during 10 previous years, g/day – mean (SD)	29 (49)
Smoking during previous year – n (%)	343 (64)
Body mass index, kg/m^2 – mean (SD)	26.1 (4.7)
Stage II cancer – n (%)	208 (39)
Laryngeal cancer – n (%)	450 (83)
Randomized to supplement arm of trial – n (%)	273 (51)
Dietary energy during previous year, kcal/day – mean (SD)	1982 (672)
Protein intake during previous year, g/day – mean (SD)	73.7 (13.6)
Lipid intake during previous year, g/day – mean (SD)	74.8 (13.9)
Carbohydrate intake during previous year, g/day – mean (SD)	237.2 (33.7)
Dietary vitamin D during previous year, IU/day – mean (SD)	221 (148)
Supplements with vitamin D use during previous year – n (%)	67 (12.4)
Total vitamin D from diet and supplements, IU/day – mean (SD)	248 (179)
Dietary calcium during previous year, mg/day – mean (SD)	740 (384)
Total calcium from diet and supplements, mg/day – mean (SD)	769 (393)
Serum 25-hydroxyvitamin D*, nmol/L – mean (SD)	63.6 (21.1)
Blood drawn in July-November* – n (%)	208 (39.8)

* 18 missing

Table 2. Hazard ratios and 95% confidence intervals of HNC outcomes associated with quartiles of energy adjusted vitamin D intake from diet and supplements.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P trend
N	135	135	135	135	
Vitamin D intake - mean (range), IU/d	87 (<147)	185 (147-220)	257 (220-312)	463 (>312)	
HNC recurrence					
HR from simple model* (95% CI)	1.0	1.14 (0.69-1.90)	0.95 (0.56-1.61)	1.13 (0.68-1.88)	0.83
Multivariate HR** (95% CI)	1.0	1.19 (0.72-1.97)	0.91 (0.54-1.53)	1.10 (0.66-1.84)	0.99
Second primary cancer					
HR from simple model* (95% CI)	1.0	1.18 (0.72-1.93)	0.65 (0.38-1.14)	1.05 (0.63-1.75)	0.62
Multivariate HR† (95% CI)	1.0	1.20 (0.73-1.97)	0.68 (0.39-1.19)	1.05 (0.63-1.74)	0.64
Death from all causes					
HR from simple model* (95% CI)	1.0	1.16 (0.79-1.68)	0.99 (0.68-1.45)	1.29 (0.89-1.87)	0.31
Multivariate HR‡ (95% CI)	1.0	1.24 (0.84-1.81)	0.87 (0.58-1.29)	1.27 (0.87-1.84)	0.53

* Stratified analysis by trial arm

** Stratified analysis by trial arm, adjusting for stage and site

† Stratified analysis by trial arm, adjusting for age, smoking and BMI

‡ Stratified analysis by trial arm, adjusting for age, site, stage, smoking, alcohol consumption and BMI

Table 3. Hazard ratios and 95% confidence intervals of HNC outcomes associated with quartiles of serum 25-hydroxyvitamin D level.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P trend
N	133	129	136	124	
25-hydroxyvitamin D - mean (range), nmol/L	37 (<48)	56 (49-63)	71 (64-78)	91 (>78)	
HNC recurrence					
HR from simple model* (95% CI)	1.0	0.94 (0.56-1.60)	1.02 (0.61-1.72)	1.00 (0.58-1.72)	0.93
Multivariate HR** (95% CI)	1.0	0.99 (0.58-1.69)	1.20 (0.71-2.05)	1.12 (0.65-1.93)	0.56
Second primary cancer					
HR from simple model* (95% CI)	1.0	0.93 (0.56-1.55)	0.83 (0.49-1.39)	0.63 (0.35-1.14)	0.12
Multivariate HR† (95% CI)	1.0	0.97 (0.58-1.61)	0.83 (0.49-1.40)	0.72 (0.40 -1.30)	0.23
Death from all causes					
HR from simple model* (95% CI)	1.0	0.70 (0.48-1.03)	0.79 (0.55-1.14)	0.65 (0.42-0.98)	0.07
Multivariate HR‡ (95% CI)	1.0	0.75 (0.51-1.10)	0.93 (0.64-1.36)	0.85 (0.57-1.28)	0.65

* Stratified analysis by trial arm, adjusting season of blood collection

** Stratified analysis by trial arm, adjusting for season of blood collection, stage and site

† Stratified analysis by trial arm, adjusting for season of blood collection, age, smoking and BMI

‡ Stratified analysis by trial arm, adjusting for season of blood collection, age, site, stage, smoking, alcohol consumption and BMI