

Short Communication

Serum 25(OH)-Vitamin D Concentration and Risk of Esophageal Squamous Dysplasia

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Abstract

Background: Squamous dysplasia is the precursor lesion for esophageal squamous cell carcinoma, and nutritional factors play an important role in the etiology of this cancer. Previous studies using a variety of measures for vitamin D exposure have reached different conclusions about the association between vitamin D and the risk of developing esophageal cancer.

Methods: We measured serum 25-hydroxyvitamin D [25(OH)D] concentrations in a cross-sectional analysis of 720 subjects from Linxian, China, a population at high risk for developing esophageal squamous cell carcinoma. All subjects underwent endoscopy and biopsy and were categorized by the presence or absence of histologic squamous dysplasia. We used crude and multivariate-adjusted generalized linear models to estimate the relative risks (RR) and 95% confidence intervals (95% CI) for the association between squa-

mous dysplasia and sex-specific quartiles of serum 25(OH)D concentration.

Results: Two-hundred and thirty of 720 subjects (32%) had squamous dysplasia. Subjects with dysplasia had significantly higher median serum 25(OH)D concentrations than subjects without dysplasia, 36.5 and 31.5 nmol/L, respectively (Wilcoxon two-sample test, $P = 0.0004$). In multivariate-adjusted models, subjects in the highest compared with the lowest quartiles were at a significantly increased risk of squamous dysplasia (RR, 1.86; 95% CI, 1.35-2.62). Increased risks were similar when examined in men and women separately: men (RR, 1.74; 95% CI, 1.08-2.93); women (RR, 1.96; 95% CI, 1.28-3.18).

Conclusions: Higher serum 25(OH)D concentrations were associated with significantly increased risk of squamous dysplasia. No obvious source of measured or unmeasured confounding explains this finding. (Cancer Epidemiol Biomarkers Prev 2007;16(9):1889-93)

Introduction

Esophageal cancer is the 8th most common incident cancer worldwide and is the 6th most common cause of cancer death (1). Esophageal cancer incidence has an uneven geographic distribution, with high and nearby low incidence areas co-occurring in several parts of Asia and Africa. Within low risk areas, such as the U.S., there is also a distinct geographic pattern of incidence. The geographic distribution of esophageal cancer in the U.S. approximates the geographic distribution of solar radiation, with lower solar radiation areas having higher rates of esophageal cancer (2, 3). Lower exposure to type-B UV radiation leads to lower production of vitamin

D, and this led to the hypothesis that higher risk of esophageal cancer is due to low vitamin D status. Furthermore, one study reported that an index of variables related to higher predicted vitamin D status was associated with significantly reduced risk of esophageal cancer (4).

Linxian, People's Republic of China is a semiarid mountainous area in the center of the country (36 degrees North latitude) with a mainly rural population. This population has very high rates of esophageal squamous cell carcinoma (ESCC) and gastric cardia adenocarcinoma, with age-standardized rates for both men and women of $\sim 100/100,000/y$ for the two tumors combined (5). Residents of this area have a limited diet, and a nutritional deficiencies are common (6). Prospective epidemiologic studies in Linxian have shown increased risk of ESCC or gastric cardia adenocarcinoma in subjects with lower concentrations of serum selenium (7), serum α -tocopherol (8), and tissue zinc (9). For some other nutrients, such as β -carotene, β -cryptoxanthin, and retinol (10), deficiencies were common but were not associated with increased risk of these cancers. We recently reported that higher vitamin D status, as measured by serum 25-hydroxyvitamin D [25(OH)D], was associated with an increased risk of ESCC in a prospective study in this population (11).

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Esophageal squamous dysplasia is the precursor lesion for ESCC and is associated with significantly increased risk of ESCC (12). We previously showed that, in general, the risk factors for esophageal squamous dysplasia are similar to those for ESCC in the Linxian population (13). In the current study, we examine the association between serum 25(OH)D and the risk of esophageal squamous dysplasia in a cross-sectional study of apparently healthy Linxian residents.

Materials and Methods

Cohort Population and Subject Recruitment. In April 2002, we conducted a screening study of 724 apparently healthy adults ages 40 to 65 in Yaocun commune, Linxian, Henan Province, People's Republic of China. The recruitment of this cohort and the subjects' characteristics have previously been described in detail (13). This study was approved by the Institutional Review Boards of the Cancer Institute, Chinese Academy of Medical Sciences (Beijing, People's Republic of China), and the U.S. National Cancer Institute (Bethesda, MD). All subjects provided written informed consent.

Data and Biological Sample Collection. Subjects were administered a structured questionnaire that included questions about personal characteristics, habits, and living conditions. Subjects also received a brief physical exam which included measurement of height and weight. Fasting blood was collected by venipuncture and the separated serum was frozen at -70°C for future use.

Endoscopy with Lugol's iodine staining and biopsy was done as previously described (14). For higher grades of dysplasia and early cancer, the use of Lugol's iodine staining has a sensitivity of between 91% and 100%, and a specificity of between 40% and 95% (reviewed in ref. 14). These figures are somewhat lower for mild dysplasia. The biopsies were fixed in 95% ethanol, embedded in paraffin, cut in 5- μm sections, and stained with H&E. The biopsy slides were read independently by two experienced pathologists without knowledge of the patient's history or the visual endoscopic findings, and discrepant results were adjudicated by joint review. The histologic criteria were based on previous descriptions (15). All 724 subjects had at least one technically sufficient squamous biopsy.

Laboratory Analysis. We measured serum 25-hydroxyvitamin D concentrations using OCTEIA 25-hydroxyvitamin D enzyme immunoassay (IDS, Inc.). The International Vitamin D Quality Assessment Scheme³ concluded that this specific assay is accurate and reliable (16). Measurements were carried out in the nutrition laboratory of the Cancer Institute, Chinese Academy of Medical Sciences. Laboratory personnel were blind to the identity of all samples. The coefficient of variation for a pooled serum sample used to assess assay reproducibility was 17%. We successfully measured serum 25(OH)D concentrations for 722 of the 724 subjects.

Statistical Analysis. All statistical analyses were carried out using SAS 9.1 (SAS Institute). Throughout

the article, all P values we report are from two-sided tests and $\alpha < 0.05$ is considered statistically significant. We graphically examined the shape of the serum 25(OH)D distributions using histograms and we found that the distribution was skewed, but log-transformation improved normality. We categorized each subject by his or her worst squamous diagnosis, tabulated subjects by the presence or absence of squamous dysplasia, and examined the frequency or distribution of different factors potentially associated with serum 25(OH)D concentration. Differences in the distribution of 25(OH)D by groups were tested with nonparametric Wilcoxon two-sample tests. We also tested potential predictors of serum 25(OH)D concentration using the log-transformed data in a multiple linear regression model. All analyses were carried out on 720 of the 722 subjects with complete data on potentially confounding covariates.

Because the prevalence of dysplasia was high in the cohort (32%) and because this prevalence approximates the prevalence in the underlying source population, we used generalized linear models with a binomial distribution and a log-link to estimate the association between serum 25(OH)D concentration and risk of esophageal squamous dysplasia. These models allow us to estimate prevalence relative risks (RR), which are not prone to overestimating the magnitude of the association that occurs with odds ratios. We fit two models with serum 25(OH)D concentrations represented as sex-specific quartiles based on the distribution in the nondysplastics: (a) crude models; and (b) multivariate models adjusted for age, sex, height, weight, and tooth loss category. We selected variables for the final model either *a priori* or because they changed the β by $\sim 10\%$ or more. Our previous prospective analysis suggested that there was an interaction between 25-hydroxyvitamin D and sex in the risk for ESCC; therefore, we tested whether the association between serum 25(OH)D concentration and squamous dysplasia differed by sex, using a linear variable to maximize power.

Results

Table 1 presents the distribution of serum 25-hydroxyvitamin D concentrations by subject characteristics. Subject characteristics by dysplasia status have previously been published (13). Thirty-two percent of the cohort was histologically diagnosed with any grade of squamous dysplasia in their esophagus.

The overall median serum 25(OH)D concentration was 33.1 nmol/L and the medians for men and women were 47.0 and 28.5, respectively (Table 1). Subjects with squamous dysplasia had significantly higher median serum 25(OH)D concentrations than those without, 36.5 and 31.5, respectively (Wilcoxon two-sample test, $P = 0.0004$).

We examined several different potential predictors of serum 25(OH)D concentration using multiple linear regression, including variables previously shown to be associated with esophageal squamous dysplasia or of interest *a priori* (Table 1). We used multivariate linear regression to examine predictors of serum concentration using log-transformed 25(OH)D. The total r^2 for the model was 23% with the strongest predictors being sex

³ <http://www.deqas.org/>

Table 1. Serum 25(OH) vitamin D concentration (nmol/L) geometric means and selected quantiles overall and by sex in the Cytology Sampling Study 2 cohort

	N	Geometric mean	Quartiles		
			25th	50th	75th
Overall	720	34.13	24.1	33.1	48.2
Height*					
<Median	350	34.40	23.9	33.8	49.0
≥Median	370	33.87	24.3	32.8	47.4
Weight*					
<Median	353	33.71	23.4	32.8	47.1
≥Median	367	34.53	25.0	33.6	49.4
People in household					
<Median	359	34.28	24.4	33.9	49.5
≥Median	361	34.00	24.0	32.3	47.1
Household income					
<Median	326	34.94	24.1	33.4	48.3
≥Median	394	33.33	24.1	33.0	48.0
Tooth loss					
0-3 teeth lost	164	36.68	25.7	33.7	52.1
4-6 teeth lost	138	34.46	24.4	34.0	50.5
7-11 teeth lost	161	34.39	24.4	35.0	47.5
12-31 teeth lost	133	32.10	23.6	30.5	44.0
Edentulous	124	32.44	22.9	32.4	43.7
Men	304	44.28	33.2	47.0	59.6
Age (<55)	138	42.08	32.6	44.3	56.9
Age (≥55)	166	46.19	33.3	47.6	64.0
Ever smokers	192	45.09	33.1	45.0	59.0
Never smokers	112	43.81	33.9	47.3	62.2
Women	416	28.21	21.6	28.5	36.5
Age (<55)	226	28.76	22.2	29.3	35.9
Age (≥55)	190	27.57	21.2	28.1	36.5
Esophageal squamous dysplasia					
No	490	32.68	23.0	31.5	47.1
Yes	230	37.39	27.0	36.5	50.9

*The cohort was divided using sex-specific median splits.

(male, $\beta = 0.52$, $P \leq 0.0001$); height (+1 cm, $\beta = -0.0077$, $P = 0.018$); weight (+1 kg, $\beta = 0.0037$, $P = 0.078$); and the fourth category of tooth loss, 12 to 31 teeth lost ($\beta = -0.086$, $P = 0.082$). Smoking tobacco and drinking alcohol were not significantly associated with serum concentration and neither was number of people in the household nor household income.

Table 2 presents the relative risk of any dysplasia conferred by higher serum 25(OH)D concentration estimated using crude or multivariate-adjusted regres-

sion models. The risk of any dysplasia increased monotonically and significantly by quartile of serum 25(OH)D concentration. Using sex-specific quartiles and the lowest quartile as the referent group, subjects in the highest quartile of serum 25(OH)D had a RR of 1.86; 95% confidence intervals (95% CI) of 1.35 to 2.62 for esophageal squamous dysplasia. Despite the large difference in the distribution of serum 25(OH)D concentration in men and women, the association with risk of dysplasia was similar for the two sexes when analyzed

Table 2. Crude and adjusted associations between serum 25(OH)D concentration and risk of squamous dysplasia in the Cytology Sampling Study 2 cohort

	25(OH)D Quartiles*				$P_{\text{trend}}^{\dagger}$
	Q1 (ref)	Q2, RR (95% CI)	Q3, RR (95% CI)	Q4, RR (95% CI)	
Overall					
Crude	1.0	1.37 (0.96-1.99)	1.42 (1.00-2.06)	1.82 (1.32-2.57)	0.0003
Multivariate [‡]	1.0	1.43 (1.00-2.06)	1.47 (1.04-2.12)	1.86 (1.35-2.62)	0.0002
Men					
Crude	1.0	1.38 (0.84-2.36)	1.41 (0.86-2.41)	1.57 (0.98-2.64)	0.0848
Multivariate [§]	1.0	1.52 (0.92-2.59)	1.51 (0.92-2.58)	1.74 (1.08-2.93)	0.0373
Women					
Crude	1.0	1.36 (0.82-2.30)	1.43 (0.87-2.41)	2.04 (1.33-3.31)	0.0010
Multivariate [§]	1.0	1.37 (0.84-2.32)	1.45 (0.89-2.43)	1.96 (1.28-3.18)	0.0021

*25(OH)D quartiles defined for men and women separately using the distribution in the subjects without dysplasia.

[†] P for trend comes from a model in which quartiles were entered as ordinal variables.

[‡]Adjusted for age, height, and weight as continuous variables, sex, and tooth loss category.

[§]Adjusted for age, height, and weight as continuous variables, and tooth loss category.

separately: men (RR, 1.74; 95% CI, 1.08-2.93); women (RR, 1.96; 95% CI, 1.28-3.18). There was no evidence of a statistically significant difference (i.e., interaction) in the risk conferred by sex (P for interaction = 0.36).

Discussion

In this cross-sectional study of a population with low vitamin D status, we found that higher serum 25(OH)D concentration was associated with a significantly increased risk of esophageal squamous dysplasia in both men and women. Despite substantial differences in the serum 25(OH)D concentrations between men and women, the risk conferred by quartile was similar for each sex. The magnitude of the increased risk was similar to what we previously reported for risk of ESCC in a prospective study conducted in the same population (11), although in that study, the increased risk was limited to men.

We have now shown, in two independent data sets, that higher serum 25(OH)D concentration is associated with increased risk of esophageal neoplasia in this population. Two previous prospective studies have shown that men with higher vitamin D status are at an increased risk of pancreatic (17) or prostate (18) cancer. This is the first study to find that higher serum vitamin D is associated with increased risk in women, albeit not for cancer but for a preneoplastic lesion.

Confounding is always a potential explanation for associations reported in observational epidemiologic studies. It is possible that vitamin D could be correlated with intake of an environmental contaminant that co-occurs with a vitamin D food source. However, this seems unlikely because the typical diet in Linxian provides little vitamin D: fatty fish and liver are rarely consumed (6) and median egg intake is also low (19). A second possibility is that our result is due to some unmeasured confounder that is correlated with outdoor activity, which is the major contributor to vitamin D status other than constitutional differences between individuals. The majority of the Linxian population and the members of this cohort are subsistence farmers, however, and all spend large amounts of time outdoors. Finally, although there is some variation in socioeconomic status, and household income is associated with risk of esophageal squamous dysplasia (13), we saw no association between household income and serum 25(OH)D status.

The association between higher vitamin D status and increased risk of ESCC and its precursor lesion may be due to an interaction with another factor rather than simply due to vitamin D itself. For example, the population of Linxian relies on coal for both cooking and heating, and previous studies have shown that the people are heavily exposed to polycyclic aromatic hydrocarbons (20). A recent study showed that in the intestines of vitamin D-deficient rats, an injection of the active vitamin D metabolite 1,25(OH)D induced the expression of both phase I and phase II enzymes (21), which metabolize polycyclic aromatic hydrocarbons. Although phase II enzymes participate only in the detoxification of these pro-carcinogens, phase I enzymes create reactive intermediate which could be detoxified or could form adducts with DNA or protein and increase the risk of cancer. The balance of activation and detoxification in this system can depend on many factors,

including the amount and type of polycyclic aromatic hydrocarbons or other chemical exposure, genetic variation in the genes encoding the metabolizing enzymes, the availability of conjugating factors necessary for the phase II enzymes (e.g., glutathione), etc. If higher vitamin D status leads to more phase I enzyme activity in humans, this may have an adverse effect in a polycyclic aromatic hydrocarbon-exposed group such as the population of Linxian (22). Therefore, it is plausible that some other factors in this population may explain the unexpected direction of the observed association between vitamin D and esophageal squamous dysplasia.

Our study has several strengths and weaknesses. We studied the association between serum 25(OH)D concentration and the established preneoplastic lesion for ESCC in a large, asymptomatic group of subjects from a high-risk region for ESCC. We used the best marker for vitamin D status, serum 25(OH)D concentration. We used the gold standard diagnostic technique, endoscopy with Lugol's iodine staining and biopsy (14), to establish the presence or absence of esophageal squamous dysplasia. Our study is limited by the use of a preneoplastic lesion, which confers a significantly increased risk of ESCC (12) but is not itself cancer. Also, our study is cross-sectional, so it is possible that the higher level of serum 25(OH)D is somehow secondary to the dysplasia, although this seems unlikely. Our similar finding in a prospective study (11) from this same population suggests that reverse causation is an unlikely explanation for our findings.

In conclusion, we found that a higher serum 25(OH)D concentration was associated with an increased risk of esophageal squamous dysplasia, the precursor lesion for ESCC. This finding concurs with our previous prospective study which found that higher vitamin D status was associated with increased risk of incident ESCC in this same population. These unexpected findings suggest that further studies of the association of vitamin D and digestive tract cancers are needed before the effect of vitamin D in different populations can be elucidated.

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