

BRIEF COMMUNICATION

Prospective Study of Serum Vitamin E Levels and Esophageal and Gastric Cancers

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Participants in the General Population Trial, a randomized nutrition intervention trial in Linxian, China, who received a combination of selenium, β -carotene, and vitamin E supplements, had statistically significantly lower cancer mortality rates than those who did not receive the supplements. In the current study, we used a case-cohort design to examine the association between pre-trial serum vitamin E levels and the risks of developing esophageal and gastric cancers during the trial. We measured serum α - and γ -tocopherol and cholesterol levels in 1072 case patients with incident esophageal squamous cell carcinoma (ESCC), gastric cardia cancer (GCC), or gastric noncardia cancer (GNCC) and in 1053 control subjects. The relative risks for comparisons of the highest to the lowest quartiles of serum α -tocopherol were 0.63 (95% confidence interval [CI] = 0.44 to 0.91) for ESCC, 0.84 (95% CI = 0.55 to 1.26) for GCC, and 2.05 (95% CI = 0.89 to 4.75) for GNCC. Serum γ -tocopherol level was not associated with the incidence of any of these cancers. Our findings provide support for the role of α -tocopherol in the etiology of upper gastrointestinal cancers. [J Natl Cancer Inst 2003;95:1414-6]

Vitamin E, a group of eight substances found in nature that exhibit the biologic activity of α -tocopherol, is an essential nutrient that functions as the

body's primary fat-soluble antioxidant. Vitamin E has a number of known or presumed functions in addition to being an antioxidant that may play a role in carcinogenesis (1). Results of chemical carcinogenesis studies in rodents suggest that vitamin E inhibits development of cancer at numerous sites (2), whereas results of prospective cohort studies (3-10) and randomized intervention trials (11-13) in humans have also provided some evidence that vitamin E plays a role in the prevention of upper gastrointestinal cancers. In this study, we examined the relationship between pre-diagnostic serum levels of vitamin E (specifically α - and γ -tocopherol) and the subsequent development of esophageal squamous cell carcinoma (ESCC), gastric cardia cancer (GCC; i.e., gastric cancers located in the first 3 cm of the stomach at the gastroesophageal junction), and gastric noncardia cancer (GNCC; i.e., gastric cancers located in the remainder of the stomach) among participants in the General Population Trial in Linxian, China. We previously reported that study participants who received a combination of selenium, β -carotene, and vitamin E supplements had lower cancer mortality rates than participants who did not receive such supplements (11). Herein we evaluated these observational data in an attempt to further understand the role of tocopherols in upper gastrointestinal cancers.

The design (14) and results (11) of the General Population Trial have been previously described in detail. Participants included 29 584 men and women aged 40-69 years from Linxian, an area of China with extraordinarily high esophageal and gastric cardia cancer rates whose residents have numerous well-documented vitamin and mineral deficiencies. All participants provided written informed consent, and the trials were conducted in accordance with the U.S. Department of Health and Human Services Office for Protection from Research Risks guidelines. In the spring of 1985, each participant was interviewed, given a brief physical examination, and had blood drawn. The intervention began in March 1986 and continued through May 1991. The stratified case-cohort design, subject selection, quality-control procedures, and statistical analytic methods used in the current study were the same as those used and described in a previous publication on pre-

diagnostic serum selenium levels and upper gastrointestinal cancer (15). In the current study, we evaluated all case patients identified during the intervention (i.e., 590 patients diagnosed with ESCC, 395 patients diagnosed with GCC, and 87 patients diagnosed with GNCC) and 1053 control subjects for whom adequate serum was available. We used a modified simultaneous isocratic high-performance liquid chromatography assay, which was performed in the Rutgers University laboratory of Dr. C. S. Yang and was based on a previously described method (16,17), to determine serum α - and γ -tocopherol concentrations. Serum cholesterol levels were measured by the National Health and Nutrition Examination Survey laboratory at the Centers for Disease Control and Prevention (Atlanta, GA) with the use of an Ektachem 250 Dry Chemistry Analyzer and a single-slide two-point enzymatic cholesterol test (Eastman Kodak, Rochester, NY).

We estimated relative risks (RRs) and 95% confidence intervals (CIs) by using the case-cohort estimator for the stratified Cox proportional hazards model (18-21). (The data conformed to proportional hazards assumptions.) We estimated relative risks by using measures of serum α - and γ -tocopherol on three scales: continuous, quartile, and ordinal. All reported relative risks were adjusted for sex, age, serum cholesterol level, body mass index, smoking status, and alcohol status. Treatment group assignment did not affect relative risk estimates and therefore was not included

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in the models. We also estimated the relative risks for baseline serum α -tocopherol levels separately for those randomly assigned to the selenium/ β -carotene/vitamin E supplement group and those who did not receive this supplement, and found that there was no interaction between pre-trial serum levels and treatment group assignment (data not shown).

Our analyses considered two different endpoints: all incident cancers, both fatal and nonfatal, and the more restricted endpoint of fatal cancers. Because the results of analyses that used the two endpoints were similar, we present only the data for the incident cancer endpoint. In addition to the site-specific relative risk estimates, we estimated the relative risk of the combined endpoint of ESCC and GCC. This combined category was the primary cancer endpoint of the General Population Trial. In this and two other analyses of pre-trial nutrient levels (15,22), we found that the exposure-cancer associations were similar at these two sites.

Table 1 shows the number of subjects studied according to case-control status as well as median age and serum levels of α - and γ -tocopherol and cholesterol at the start of the trial. For comparison, the median serum α -tocopherol level in the latest (i.e., the third) National Health and Nutrition Examination Survey in the United States was 969 $\mu\text{g/dL}$ (23), which is 17% higher than the median value reported here from the General Population Trial cohort in Linxian. Neither the geometric mean (data not shown) nor the median values for α - or γ -tocopherol differed statistically significantly between case patients and control subjects. Table 2 shows the rela-

tive risks and 95% confidence intervals for regression models of the associations between cancer risk and serum α - and γ -tocopherol levels, which were considered to be continuous variables (where all values were standardized so that a change of one unit corresponds to a change of approximately 25% of the distribution) as well as by quartiles (15). As shown, each standardized unit increase in serum α -tocopherol was associated with a 10% decreased risk of ESCC incidence ($P = .016$), a 2% decreased risk of GCC ($P = .644$), a 19% increased risk of GNCC ($P = .047$), and a 7% decreased risk of the combined ESCC and GCC endpoint ($P = .049$). Compared with subjects in the lowest quartile of serum α -tocopherol, those in the highest quartile of serum α -tocopherol had a 37% lower risk of ESCC ($P = .015$), a 16% lower risk of GCC risk ($P = .40$), a 105% higher risk of GNCC ($P = .093$), and a 29% lower risk of ESCC and GCC combined ($P = .030$). Serum γ -tocopherol levels were not associated with cancer risk at any of the cancer sites studied in any of the models evaluated. The relative risks for α -tocopherol were unchanged when we included both α - and γ -tocopherol in the same model, and no differences in the relative risks were seen when we excluded cases that occurred in the first 2 years of follow-up or between subgroups defined by sex, smoking status, alcohol status, or intervention assignment (data not shown).

Several other prospective studies of serum vitamin E levels and upper gastrointestinal cancers have been reported, but most are serial updates from the same few cohorts, and all are based on small numbers of case patients. The largest studies to date have included

only 28 esophageal cancers (6) and 70 gastric cancers (5). By contrast, our results are based on more than 1000 cancers at these sites identified among a unique population with marginal serum levels of vitamin E and for which both observational and intervention data are available. We found that high α -tocopherol levels were associated with a reduced risk of ESCC (statistically significant), a reduced risk of GCC (not statistically significant), and an increased risk of GNCC (of marginal statistical significance). Our previous analyses of serum selenium levels in this same cohort showed that, compared with the lowest quartile, the highest quartile of selenium was strongly associated with a reduced risk of ESCC (RR = 0.56, 95% CI = 0.44 to 0.71) and GCC (RR = 0.47, 95% CI = 0.33 to 0.65) but not with the risk of GNCC (RR = 1.07, 95% CI = 0.55 to 2.08) (15). By contrast, we found no association between serum β -carotene levels and cancer risk at any of the three cancer sites studied here (22). The relative risk and 95% confidence interval estimates for selenium, α -tocopherol, and β -carotene were unchanged when they were adjusted for each other (data not shown).

In the General Population Trial itself, the participants were randomly assigned to receive a combination of 50 μg of selenium, 15 mg of β -carotene, and 30 mg of α -tocopherol or no such supplements. Although reductions in cancer mortality and incidence were observed in the supplemented group, the simultaneous administration of three supplements prevented assessment of potentially differential effects of selenium versus β -carotene versus α -tocopherol. Our observational analyses of the association between cancer risks and pre-trial serum levels of these compounds do not overcome this non-separability problem. However, our findings suggest that α -tocopherol and selenium are more likely than β -carotene to be involved in the reduced cancer rates observed with supplementation during the trial. Assuming that α -tocopherol is an inhibitor of esophageal and gastric cardia cancers, we have no ready explanation for our finding that higher blood levels of α -tocopherol were associated with an elevated relative risk of GNCC, particularly when considering that trial subjects who took the supplements had lower risks for each type of stomach cancer

Table 1. Median age and serum levels at entry for case patients with incident site-specific upper gastrointestinal cancer and control subjects in the General Population Trial from Linxian, China

Characteristic	Control subjects (n = 1053)	Incident cancer case patients		
		Esophageal (n = 590)	Gastric cardia (n = 395)	Gastric noncardia (n = 87)
Age, y	57	57	58	60
Serum α -tocopherol, $\mu\text{g/dL}$ †	804.1*	786.0	786.1	804.5
Serum γ -tocopherol, $\mu\text{g/dL}$ †	57.7*	60.1	57.4	57.2
Serum cholesterol, mg/dL †	147*	148	142	144

*Difference between case patients and control subjects was not statistically significant (all $P \geq .25$).

†Adjusted by sampling weights so that these values approximate the distribution of those in the entire General Population Trial, and not just the analyzed group (15).

Table 2. Relative risks (RRs) and 95% confidence intervals (CIs) for changes in serum α - and γ -tocopherol concentrations, as continuous variables and as quartiles (range, $\mu\text{g/dL}$), by incident cancer site in the General Population Trial, Linxian, China*

Cancer site	RR (95% CI)	P	1		2		3		4		$P_{\text{trend}}^{\dagger}$
			(referent)	RR	95% CI	RR	95% CI	RR	95% CI		
α-tocopherol/140 $\mu\text{g/dL}^{\ddagger}$			(≤ 676)		(677–804)		(805–955)		(>955)		
Esophagus	0.90 (0.83 to 0.98)	.016	1.00	0.79	0.59 to 1.06	0.66	0.48 to 0.91	0.63	0.44 to 0.91	.008	
Gastric cardia	0.98 (0.89 to 1.07)	.644	1.00	0.79	0.57 to 1.10	0.70	0.48 to 1.00	0.84	0.55 to 1.26	.290	
Gastric noncardia	1.19 (1.00 to 1.41)	.047	1.00	1.32	0.69 to 2.53	2.21	1.13 to 4.32	2.05	0.89 to 4.75	.030	
Esophagus + gastric cardia	0.93 (0.87 to 1.00)	.049	1.00	0.79	0.61 to 1.01	0.67	0.51 to 0.89	0.71	0.52 to 0.97	.016	
γ-tocopherol/15.4 $\mu\text{g/dL}^{\ddagger}$			(≤ 44)		(45–57)		(58–75)		(>75)		
Esophagus	1.01 (0.95 to 1.08)	.654	1.00	1.37	1.01 to 1.87	1.36	0.99 to 1.87	1.26	0.89 to 1.77	.291	
Gastric cardia	0.98 (0.91 to 1.06)	.675	1.00	0.87	0.62 to 1.22	0.81	0.57 to 1.15	0.89	0.61 to 1.28	.476	
Gastric noncardia	0.97 (0.82 to 1.15)	.749	1.00	0.75	0.40 to 1.43	1.17	0.63 to 2.15	0.95	0.46 to 1.93	.789	
Esophagus + gastric cardia	1.00 (0.95 to 1.06)	.934	1.00	1.13	0.87 to 1.47	1.09	0.84 to 1.43	1.08	0.81 to 1.43	.723	

*RRs, 95% CIs, and two-sided P values (score tests) come from regression models stratified on sex and age, with additional adjustment by separate continuous age variables for each stratum and variables for cholesterol, smoking status, alcohol use, and body mass index.

\dagger The P_{trend} is from the score test for the addition of a variable where an individual's value is the quartile in which their serum value fell compared to a model with no variable for α - or γ -tocopherol.

\ddagger The RRs and 95% CIs for the continuous measures were standardized to the average size of the two central quartiles. The standardization unit is $(Q3 - Q1)/2$ where $Q3 - Q1$ is the difference between the serum values of the third and first quartile. Therefore, these RRs are associated with a 25% increase in serum concentration relative to the population distribution.

individually. Taken together with the findings of the General Population Trial, the results reported here provide support for a role of α -tocopherol in the etiology and prevention of upper gastrointestinal cancers.

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NOTE

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