Short Communication

Prediagnostic Serum Selenium Concentration and the Risk of Recurrent Colorectal Adenoma: A Nested Case-Control Study[1]

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Abstract

Several studies have suggested that selenium may help to prevent colorectal neoplasia. To investigate the relation between prediagnostic serum selenium concentrations and colorectal adenomas, we conducted a nested case-control study using data from a large, multicenter, adenoma prevention trial. Cases comprised a total of 276 patients who developed a colorectal adenoma between the year 1 and year 4 follow-up exam. Controls were 276 patients who did not develop an adenoma during this time interval, matched to case subjects on age, sex, and clinical center. Total and bound selenium concentrations were measured from baseline or year 1 serum samples using instrumental neutron activation analysis. We estimated the odds ratios of colorectal adenoma in relation to serum selenium concentrations adjusting for age, clinical center, and sex. Compared with the lowest quintile, the odds ratio for the highest quintile was 0.76 (95% confidence interval, 0.44–1.30) for total selenium and 0.60 (95% confidence interval, 0.34–1.05) for bound selenium, and there was no apparent trend in risk (P for trend = 0.50 for total selenium and P for trend = 0.20 for bound selenium). Thus, our findings do not indicate a clear association between serum selenium concentrations and adenoma recurrence.

Introduction

Selenium is an essential trace element hypothesized to play a preventive role in certain types of malignancies (1, 2). Several epidemiological studies have indicated an inverse association between measures of selenium and colorectal neoplasms, including colorectal adenomas, which are benign precursors of colorectal cancer (3–8). The strongest evidence that selenium may protect against colorectal cancer comes from secondary analyses of a randomized trial designed to test the effect of selenium supplements on the risk of skin cancer. In that trial, the incidence of colorectal cancer was 61% lower in the selenium-supplemented group than in those assigned to the placebo group (9). However, the results from epidemiological studies are not consistent (10–12), and the true nature of the association remains controversial. To clarify the relation between serum selenium and colorectal neoplasia, we conducted a nested case-control study of colorectal adenomas using data from a clinical trial of adenoma prevention (13).

Materials and Methods

This report is based on participants in a placebo-controlled, randomized trial of the effect of supplementation with vitamins C, E, and β-carotene on the recurrence of colorectal adenomas (13). Briefly, study participants were recruited at six clinical centers in the United States; each participant had a history of at least one colorectal adenoma excised within the 3 months before study entry and had no remaining polyps in the entire large bowel after complete colonoscopic examination. At enrollment, participants completed a questionnaire covering basic demographic characteristics, medical history, and usual diet (assessed using a food frequency questionnaire). Study colonoscopies were scheduled on two occasions: approximately 1 year and 4 years after the qualifying examination.

For these analyses, we considered a "case" to be any subject who developed at least one adenoma after the first (year 1) study colonoscopy and up to and including the second (year 4) study colonoscopy. Of the 751 participants who completed the trial, 278 developed an adenoma by year 4 of the study. We chose an equal number of controls from among patients who (*a*) completed the year 4 study colonoscopy and (*b*) had not developed an adenoma after the first study colonoscopy. We frequency-matched controls to the cases on sex, age (decade), and study center. Of these subjects, 276 cases and 276 controls had an available blood sample for analysis.

Blood samples were drawn on study participants at baseline, year 1, year 2, and at study completion (year 4) using 7-ml mineral-free, no additive, Vacutainer brand tubes. After collection, specimens were kept upright and protected from sunlight. Coordinators were instructed to centrifuge the specimens within 2 h of collection. Serum was transferred into 3.5-ml polypropylene tubes using disposable polyethylene transfer pipettes. Tubes were then placed upright in cardboard storage boxes and immediately frozen at −35°C. Once frozen, specimens were shipped to Dartmouth Medical School, where specimens were stored at −75°C.

We analyzed serum samples obtained from cases and controls at enrollment (n = 546) or at the first study colonoscopy (n = 6) using instrumental neutron activation analysis (14) at the University of Missouri-Columbia Research Reactor Center. We used two measures of prediagnostic selenium con-

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centrations. The first method analyzed for total selenium, regardless of chemical form, from a 50-μl serum sample. Using this approach, the coefficient of variability was 5.4% in our 20 masked quality control samples. The second method used a membrane dialysis of the serum against dionized water, which removes electrolytes and retains the protein-bound selenium. This approach had a coefficient of variability of 4.0%. In the context of quantifying selenium by neutron activation analysis, there is a substantial analytical (signal to background) improvement in analyzing the dialyzed sample. In theory, however, non-protein-bound forms of selenium such as free selenomethionine or selenocysteine would be removed from this process. Nonetheless, the two measures were strongly correlated (correlation, $r = 0.88$; $P = 0.001$), largely due to the fact that all but trace amounts of selenium (including selenomethionine and selenocysteine) are protein bound.

Sera specimens used for our study had been stored for an average of 12 years and thus may have experienced water loss that could bias the analysis. To assess this, we measured sodium concentrations in all samples. Sodium content of human blood is under homeostatic control in a relatively narrow range; consequently, the sodium concentration measured in archived specimens will reflect sample concentration (or dilution), if either has occurred. In our study samples, the total selenium concentration was essentially independent of the sodium concentration with an adjusted R^2 of 0.015.

We used multiple logistic regression analysis to compute ORs[3] and 95% CIs to assess the association between at least one adenoma and serum selenium concentration while adjusting for age, sex, and study center location (15). Serum selenium concentrations were modeled both as a continuous variable and in quintiles (based on the control distribution). We also examined the potential confounding or modifying effects of fiber and red meat intake (the main dietary correlates of selenium intake), smoking status, body mass index, treatment assignment, number of polyps at study entry exam, and family history of colon cancer.

One year after randomization, subjects underwent a "cleaning" colonoscopy to remove any polyps that may have been missed at the baseline examination. Therefore, subjects who had an adenoma at the first, but not the second, study exam were eligible as controls. To assess the suitability of these subjects as controls, we performed a subgroup analysis classifying participants who had at least one adenoma after randomization (at the year 1 or subsequent examination) as cases and classifying those who did not develop an adenoma at any time during the follow-up period (either at year 1 or subsequently) as controls using logistic regression. Additionally, we examined the effect of adenoma size (adenomas < 0.5 cm or at least one adenoma ≥ 0.5 cm), number (single or multiple), and location (right colon, left colon, or both) using ANOVA among cases only. We defined the right colon as the cecum, ascending, and transverse colon and the left colon as the more distal portions of the bowel.

Results

Baseline characteristics for the 552 subjects (276 cases and 276 controls) are shown in Table 1. The mean age of the study participants was 61.5 years (SD = 8.0), and 71.8% were men. Cases and controls were comparable with respect to age, sex, and study center by the matched design. For the "non-matched" covariates, cases tended to have more baseline polyps. Cases and controls were similar with respect to smoking status, body mass index, family history of colon cancer, and dietary intake of grain fiber and servings of red meat.

Table 1 Baseline characteristics of colorectal adenoma cases and controls

Characteristic	Controls ($n = 276$)	Cases ($n = 276$)	P^a
Sex [no. (%)]			1.00
Male	226 (82)	226 (82)	
Female	50 (18)	50 (18)	
Age [mean (SD)] (yrs)	61.8 (8.1)	61.3 (7.9)	0.44
Smoking status [no. (%)]			0.19
Never	99 (36)	83 (31)	
Former/present	174 (63)	185 (69)	
BMI (kg/m^2) [no. (%)][b]			0.32
<25	87 (32)	88 (32)	
25–29.9	140 (51)	126 (46)	
30+	48 (17)	61 (22)	
No. of polyps at baseline [no. (%)]			0.001
1	132 (48)	90 (33)	
2–3	66 (24)	56 (21)	
4+	77 (28)	125 (46)	
Family history of colorectal cancer [no. (%)]			0.99
Yes	53 (20)	54 (20)	
No	215 (80)	219 (80)	
Fiber from grains per day [mean (SD)] (g)	5.64 (4.0)	5.21 (3.7)	0.21
Servings of red meat/week [mean (SD)]	3.72 (3.39)	3.74 (2.70)	0.95

[a] P represents the difference between cases and controls that was determined using a t test (continuous variables) or χ^2 test (for discrete variables).
[b] BMI, body mass index.

The mean concentrations of total selenium were similar for cases and controls: 131.5 μg/liter (SD = 19.7 μg/liter) for cases and 130.3 μg/liter (SD = 17.8 μg/liter) for controls, compared with the lowest quintile, the highest quintile of total serum selenium was associated with a modest reduction in risk of adenoma recurrence (OR, 0.76; 95% CI, 0.44–1.30), but there was no apparent trend in risk (Table 2). When we analyzed total serum selenium as a continuous variable, the adjusted OR associated with a 25 μg/liter increase in serum selenium was 0.91 (95% CI, 0.73–1.16).

The mean concentrations of selenium using the dialyzed method (bound method) did not differ among cases and controls. Values were 133.1 μg/liter (SD = 19.6 μg/liter) among controls and 130.9 μg/liter (SD = 16.8 μg/liter) among cases. The adjusted OR for the highest *versus* lowest quintile of bound selenium was 0.60 [95% CI, 0.34–1.05 (Table 2)]. As a continuous variable, the adjusted OR associated with a 25 μg/liter increase in serum selenium was 0.86 (95% CI, 0.67–1.09).

We did not detect any appreciable confounding or effect modification by number of adenomas at study entry, smoking status, body mass index, family history of colon cancer, dietary intake of grain fiber, servings of red meat, or treatment assignment (data not shown). When we defined cases as those with at least one adenoma after randomization and controls as subjects with no adenomas after randomization, the OR for the highest *versus* lowest quintile was 0.57 (95% CI, 0.32–1.02) for total selenium and 0.52 (95% CI, 0.29–0.94) for bound selenium.

We did not find that mean selenium concentrations markedly differed by adenoma size, location, or number of adenomas. The mean total selenium concentration was 129.6 μg/liter

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval.

Table 2 Total and bound serum selenium levels in relation to the subsequent adenoma occurrence

A. Total selenium

	Quintiles				
	1	2	3	4	5
Se total (μg/liter)	≤116	117–125	126–133	134–147	>147
No. of controls	55	59	52	55	55
No. of cases	65	54	47	62	48
OR (95% CI)[a]	1.00 (reference)	0.78 (0.46–1.31)	0.77 (0.45–1.34)	0.97 (0.57–1.63)	0.76 (0.44–1.30)
P for trend = 0.50[b]					

B. Bound selenium

	Quintiles				
	1	2	3	4	5
Se bound (μg/liter)	≤119	119–127	128–133	134–147	>147
No. of controls	57	60	49	55	55
No. of cases	70	56	43	67	40
OR[a] (95% CI)	1.00 (reference)	0.77 (0.46–1.28)	0.72 (0.41–1.25)	1.01 (0.60–1.69)	0.60 (0.34–1.05)
P for trend = 0.20[b]					

[a] Adjusted for age, sex, and study center.
[b] Based on modeling selenium concentrations as a continuous variable.

(SD = 20 μg/liter) among subjects with at least one adenoma ≥ 0.5 cm (n = 101) and 130.6 μg/liter (SD = 16.5 μg/liter) among subjects with adenomas < 0.5 cm (n = 175; P = 0.27). The mean total selenium concentrations were 131.4 μg/liter (SD = 18.1 μg/liter) among cases with right-sided adenomas (n = 116), 130.1 μg/liter (SD = 16.1 μg/liter) among those with left-sided adenomas (n = 106), and 128.2 μg/liter (SD = 20.4 μg/liter) among those with both right- and left-sided adenomas (n = 54; P = 0.54). Mean selenium levels were slightly higher among cases with single adenomas (n = 164; mean = 132.2 μg/liter; SD = 17.7 μg/liter) than those with multiple adenomas (n = 112; mean = 128.8 μg/liter; SD = 17.9 μg/liter); however, this difference was not statistically significant (P = 0.17). The results were similar for bound serum selenium in each of these analyses.

Discussion

In this study, there was no clear association between serum selenium levels and risk of recurrence of colorectal adenoma over the ensuing several years. Our results did not change, even after taking into account several potentially confounding variables. Furthermore, we did not find statistically significant associations between serum selenium levels and adenoma size, number, or location; however, we had limited power to detect such differences.

Several previous observational studies have found an inverse association between colorectal cancer and selenium levels (3–4, 8), although not consistently so (10–11). The most compelling evidence that selenium protects against colorectal neoplasia comes from a placebo-controlled randomized study of oral supplementation with 200 μg/day selenium in yeast taken for an average of 4.5 years (9). The study involved 1312 non-melanoma skin cancer patients from low selenium areas of the United States and was originally intended to test whether selenium would reduce the incidence of basal cell and squamous cell skin cancer; the occurrence of these cancers, however, was similar in the two groups. This trial found significantly reduced overall cancer mortality and incidence rates in selenium-supplemented patients. Specifically, colorectal cancers occurred 61% less often in the intervention group than in the placebo group.

To date, three studies (5–7) have reported inverse associations between selenium concentrations and adenoma formation, whereas two others have not (12, 16). In a cross-sectional study, Clark *et al.* (5) found an increased prevalence of one or more adenomas in relation to low serum selenium levels. They further reported an inverse relation between serum selenium and number of adenomas. Two other investigations (6, 7) also specifically found an inverse association between serum selenium and multiple adenoma occurrences. In contrast, our study and other studies (12, 16) did not detect such an association. Many studies, including ours, had limited power to detect small to moderate effects, and lack of statistical precision may account for some of the apparent discrepancies across studies.

Unlike previous investigations, we measured both total and protein-bound serum selenium. In our data, results were generally similar for the two measures. A limitation of our analysis is that we were unable to speciate the forms of selenium either bound or unbound, *i.e.,* selenomethionine or selenocysteine. Future investigations may want to consider the possible effect of the various forms of selenium on adenoma risk.

There are several advantages to our study, including a large, well-characterized, and carefully followed population. Using a nested design, we had the advantage of measuring serum selenium levels before diagnosis, contrary to most previous studies. However, our study also has limitations. First, all participants, both cases and controls, had to have had at least one neoplastic lesion at the baseline exam to be enrolled in the prevention trial. Thus, it is conceivable that the association between selenium and adenoma risk could differ between our group of patients (*i.e.,* those with a history of adenoma) and the general population. In theory, selenium could protect against the initial development of polyps but not their recurrence, or individuals who might experience protection from selenium would not be represented in our study group. Second, a pro-

portion of our control subjects actually had an adenoma during the first year study examination. It is possible that these represented newly developed adenomas rather than missed polyps on the baseline examination. If so, our results could be biased toward the null due to nondifferential misclassification. Indeed, our findings were more suggestive of a reduced risk when we examined all patients who had at least one adenoma after randomization compared with those who did not. Third, as mentioned, we had limited statistical power to detect small differences in risk.

Other factors could account for the absence of a consistent association between serum selenium and adenoma recurrence in our data. It may be that selenium is more effective when taken as a dietary supplement such as in the prevention trial conducted by Clark *et al.* (9). Alternatively, selenium may act primarily on the later stages of carcinogenesis. However, we did not detect an association with more advanced lesions (*e.g.*, large or villous adenomas), albeit with limited statistical power. Our results certainly do not exclude the possibility that selenium protects against colorectal neoplasia, but more conclusive evidence is needed before any dietary recommendations can be made.

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