Prospective Study of Serum Micronutrients and Ovarian Cancer

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Background: Antioxidant micronutrients, such as α-tocopherol (vitamin E), the carotenoids, and selenium, may protect against the development of cancer by preventing free radical damage at the cellular level. Purpose: A nested case-control study was conducted among donors to a serum bank to examine the association between levels of serum micronutrients and/or cholesterol and the development of ovarian cancer. Methods: In 1974, sera were collected from 20,305 residents of Washington County, MD, over a 4-month period and stored at -70 °C. Serum micronutrient concentrations of women who developed ovarian cancer (case subjects, n = 35) were compared with those of women who remained free of cancer and who were matched to case subjects on age and menopausal status (control subjects, n = 67). Serum levels of retinol (vitamin A), α- and β-carotene (the major provitamin A), lycopene (a carotenoid), and α- and γ-tocopherol were measured using high-performance liquid chromatography. Serum selenium was measured by neutron activation analysis. Cholesterol was measured by enzymatic assay. The data were categorized into thirds and conditional logistic regression analyses were performed to determine the association between prediagnostic serum cholesterol and micronutrient levels and the development of ovarian cancer; matched odds ratios (ORs) were determined from these regression analyses. Results: Higher serum α-tocopherol levels were associated with an increased risk of ovarian cancer (P for trend = .04); however, this association diminished after adjustment for cholesterol. Women with higher serum cholesterol levels had an increased risk of ovarian cancer compared with women in the lowest third of cholesterol levels (OR = 3.2; 95% confidence interval = 0.9-11.3). The association between serum cholesterol levels and the risk of ovarian cancer was examined, stratifying by micronutrient level. The general pattern observed was an increased risk of ovarian cancer associated with cholesterol levels greater than 200 mg/dL, regardless of the micronutrient level. Serum selenium was associated with a decreased risk of ovarian cancer only among case participants diagnosed 4 or more years after blood collections (P for trend = .02). Concentrations of carotenoids and retinol were not associated with the development of ovarian cancer. Conclusions: Selenium may have a protective role against the development of ovarian cancer. Higher serum cholesterol levels were associated with an increased risk of developing ovarian cancer; an association that persisted regardless of serum micronutrient level. Implications: Given the small size of this study and the inconsistency of results among the few prospective studies of ovarian cancer conducted to test these associations, replications of these findings are highly desirable. [J Natl Cancer Inst 1996; 88:32-7]

Cellular damage by free radicals generated either by exogenous factors or in the process of normal oxidative metabolism has been postulated to be involved in the pathogenesis of cancer (1-3). Fortunately, such damage can be minimized by a variety of antioxidants that have been shown to quench free radicals (4-6). Potentially protective antioxidants include micronutrients such as the carotenoids, α-tocopherol, and selenium. Because free radicals have a very short half-life, effective protection must occur at the cellular level. The closest approximations to exposures occurring at the cellular level currently available for population-based studies are serum (or plasma) concentrations.

Few studies have prospectively examined the association between serum components and the subsequent risk of ovarian cancer. One prospective study (7) conducted in Finland found no association between prediagnostic α-tocopherol levels and the risk of gynecologic cancers on the basis of findings for 16 case subjects and 29 control subjects. A study of serum selenium levels and the subsequent risk of cancer was also conducted within the same cohort in Finland (8). Eighty-six cases of gynecologic cancers (International Classification of Diseases, 7th revision, Nos. 171-176) were grouped together; the number of cases of ovarian cancer was not stated. No association was observed between selenium levels and subsequent gynecologic cancers (8). In addition, a prospective study of serum cholesterol levels and the risk of cancer found no association between these levels and the development of ovarian cancer (9).

Case-control studies to evaluate possible associations between serum nutrient levels and ovarian cancer (10-12) have observed lower serum levels of vitamin A (10) and selenium (11,12) among case subjects compared with control subjects but no dif-

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See "Notes" section following "References."
ferences in the levels of total carotenoids and vitamin E (13). A reduced risk of ovarian cancer has been associated with high dietary intake of β-carotene (14) and carotene-rich foods, such as carrots and green vegetables (15-17). Most case-control studies of dietary fat intake and ovarian cancer have shown a small risk associated with increased intake of fat, particularly saturated fats (18,19).

To prospectively evaluate the association between serum micronutrient levels and the risk of developing ovarian cancer, a nested case-control study was conducted among donors to a serum bank. The Washington County Serum Bank was established in 1974 to study possible precursors of cancer. The serum specimens have been used for a considerable number of nested case-control studies examining the association between factors such as micronutrients and the subsequent development of cancer (20). Serum constituents included in the assays were several carotenoids (β-carotene, α-carotene, lycopene, lutien, and cryptoxanthin), α- and γ-tocopherol, selenium, retinol, and cholesterol.

Subjects and Methods

Study Population

In 1974, a blood collection campaign was conducted over a 4-month period in Washington County, MD, to establish a serum bank. After signing an informed consent, 20,305 county residents, representing about one third of the county population, donated a blood specimen. Compared with the county population identified in a private census in 1975, participants were more likely to be nonsmokers, women, and better educated. Adults over the age of 18 years who participated, the highest participation rate was among those aged 45-54 years (37.2%). At the time of blood collection, a brief questionnaire was administered to all participants to obtain basic demographic information, smoking history, time from last menstrual period, and information on all medications, including exogenous hormones, taken during the 48 hours prior to blood sampling. The study was approved by the Committee on Human Research, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

Forty-four incident ovarian cancer case subjects, diagnosed from 1975 through 1989, were identified by linking the serum bank records with the Washington County cancer registry maintained by the Training Center for Public Health Research. The mortality from ovarian cancer for this area has been similar to that for the United States as a whole for the period 1950 through 1980, suggesting relatively complete case subject ascertainment (21). The ratio of the observed number of ovarian cancer case subjects to expected is 1.17, based on race- and age-specific rates from the Surveillance, Epidemiology, and End Results (SEER) Registries for the years 1981 through 1985 (21). Ovarian cancer was histologically confirmed and case subjects had no record of any other cancer. Information on cell type was obtained through the tumor registry; 88% of the ovarian cancers were of epithelial origin.

For each case subject, two control subjects were selected who, as far as could be ascertained from mortality and cancer registry records, were alive and free from known cancer (with the possible exception of melanoma skin cancer and in situ cervical cancer) when the case subject was diagnosed. Control subjects were matched to case subjects on race (all were white), age within 1 year, time of day blood was collected, hours since last meal, and menopausal status. Premenopausal women were matched on the number of days from the beginning of the last menstrual period (within 1 day), and postmenopausal women were matched on the year from the last menstrual period. One case subject had insufficient serum available for assays, three case subjects could not be matched to control subjects, and five case-control sets were excluded because of reported hormone use at the time of blood donation. Case subjects reporting hormone use were excluded because serum from the same population had been used in another study to examine the association between endogenous hormone levels and ovarian cancer and oral contraceptive use has been reported to alter α-tocopherol levels and high-density lipoprotein (HDL) fraction (22). Two case subjects could each be matched to only one control subject; thus, 35 case subjects and 67 control subjects were available for analysis.

Laboratory Assays

Serum samples were stored at ~70°C until assayed for this study in 1990. A review of nested case-control studies has shown α-tocopherol, β-carotene, and retinol to be stable for at least 15 years when stored at ~70°C or colder (23). A recently published storage study (24) has demonstrated stable concentrations of tocopherols, carotenoids, and retinol in plasma stored at ~70°C for 4 years. Serum from case and control subjects were arranged in sets of three. Five sets also included an aliquot of pooled reference serum for quality control. All specimens from a given set were analyzed in duplicate in the same way and by the same laboratory personnel who were blinded to the case-control status. Serum levels of retinol, α- and β-carotene, cryptoxanthin, lutien, lycopene, and α- and γ-tocopherol were measured with the use of high-performance liquid chromatography (25); serum selenium levels were measured by neutron activation analysis (26); and cholesterol was measured by enzymatic procedures (Boehringer Mannheim Diagnostics Cat. No. 70412, Indianapolis, IN). Coefficients of variation based on pooled reference sera were 16.7% for α-carotene, 10.8% for cryptoxanthin, and less than 6% for all other nutrient assays.

Statistical Analysis

We hypothesized that a dose-response relationship should exist between serum micronutrient levels and the risk of ovarian cancer. To assess for the presence of a trend in risk with increasing exposure levels, we categorized the data into thirds, using the distributions of the control group to determine the tertile cutoff points. Two indicator variables were coded to account for the median and high thirds, and both variables were entered in a conditional logistic regression equation. The matched odds ratios (ORs) from this regression analysis were inspected for the presence of a trend; if the data were compatible with a trend, they were assessed by entering a single three-level variable in a regression equation with the exposure score for each third corresponding to the control subjects’ median micronutrient level for that third (27). A statistically significant trend was judged to be present if the P value for the Likelihood Ratio Test comparing the model with this three-level variable to the null model was less than or equal to 0.05.

In specific instances, the data were assessed for the presence of statistical interaction. Serologic variables were first dichotomized on the basis of whether the study subjects’ serum levels were at or above versus below the median control value. The dichotomous variables for two serum analyses were both entered in a conditional logistic regression equation. The test for interaction was the P value from the Likelihood Ratio Test when an interaction term was added to the model.

The results for micronutrients are presented without adjustment and, when appropriate, adjusted for cholesterol. The association between cholesterol levels and ovarian cancer was examined after adjusting for other micronutrients. The regression adjustment approach (28) was used to account for the collinearity between cholesterol and tocopherol. This approach involves taking the residual from the regression of cholesterol on tocopherol to be the portion of an individual’s tocopherol level that is independent of cholesterol. Subsequent analyses are then based on this residual (with the mean tocopherol added back); the micronutrients were log transformed for this procedure. The independent and combined effects of cholesterol with each micronutrient were also assessed by stratified analyses. Micronutrients were stratified at the median value among the control subjects. Cholesterol levels were stratified at a level of 200 mg/dL. All P values resulted from two-tailed tests.

Results

Characteristics of case and control subjects are presented in Table 1. Because of matching, case and control subjects were similar with respect to age, month of blood collection, menstrual status, and hours since last meal prior to blood donation. Case subjects were more likely than control subjects to have ever smoked (P = .06) but were similar to control subjects in marital status (data not shown) and years of education. Twenty-three percent of the case subjects reported taking vitamins within 48 hours of blood collection compared with 16% of control subjects.
The median levels of the serum micronutrients and percent differences for case and control subjects are presented in Table 2. Case subjects had slightly lower levels of selenium and total carotenoids but had higher levels of cholesterol and α-tocopherol, the principal circulating form of vitamin E.

The risks of ovarian cancer according to the prediagnostic concentrations of serum constituents are presented in Table 2. Increased levels of α-tocopherol were associated with an increased risk of ovarian cancer in the crude analyses. Women with α-tocopherol levels in the highest third were almost four times more likely to develop ovarian cancer than women with levels in the lowest third (95% confidence interval [CI] = 1.1-14.0; \( P \) for trend = .04). The risk of ovarian cancer also increased with increasing concentrations of cholesterol (\( P \) for trend = .10). High serum selenium concentrations were associated with a decreased risk of ovarian cancer, but the trend was not statistically significant (\( P \) for trend = .33). The risk of ovarian cancer tended to increase with increasing concentrations of α-carotene and lycopene but decreased with increasing values of cryptoxanthin. None of these trends was statistically significant. Levels of total carotenoids were not associated with the development of ovarian cancer. Adjustment for smoking did not alter the associations. Current vitamin use was not associated with the risk of developing ovarian cancer. Neither adjustment for vitamin use nor parity status altered the observed associations; therefore, only the unadjusted ORs are presented.

α-Tocopherol is transported in the serum by lipoproteins, mainly HDL and low-density lipoprotein (29). Cholesterol levels were correlated with α-tocopherol levels among control subjects (\( r = .46 \)), and higher levels of cholesterol and α-tocopherol were associated with an increased risk of developing ovarian cancer. The independent influences of cholesterol and α-tocopherol were each adjusted for the influence of the other. Adjusting α-tocopherol for cholesterol attenuated the association for α-tocopherol (Table 2). The association between high serum cholesterol levels and ovarian cancer was reduced when adjustment was made for α-tocopherol, but a dose–response effect was still evident (Table 2). A statistically significant trend in risk was observed when cholesterol was adjusted for lycopene, selenium, and retinol (\( P = .003, .05, \) and .01, respectively).

The associations between nutrients and ovarian cancer were also examined in a stratified analysis; high levels of cholesterol were based on a cut point of 200 mg, and high levels of other nutrients were defined by the median level among the control subjects (Table 3). High cholesterol levels were consistently associated with an increased risk of ovarian cancer independent of serum micronutrient levels.

The presence of occult disease at the time of blood collection may affect serum nutrient levels. Therefore, additional analyses were conducted limiting the study population to the 27 case subjects diagnosed 4 or more years after blood donation (Table 4). The protective association with selenium became more pronounced; for selenium, those in the highest third were five times less likely to develop ovarian cancer (OR = 0.23; 95% CI = 0.1-0.9) compared with those in the lowest (\( P \) for trend = .02). The association between higher serum cholesterol levels and the risk of ovarian cancer persisted but was attenuated on adjustment of cholesterol for α-tocopherol.

Information on parity could be established for 21 case subjects and 39 control subjects. Within this subset, the results were similar for parous and nulliparous women.

### Discussion

- Selenium, α-tocopherol, and the carotenoids were hypothesized to be protective against ovarian cancer because of their antioxidant function. Only selenium was associated with a decreased risk of ovarian cancer in this study. The temporal nature of the association, the dose–response trend, and biological plausibility of the association suggest that the association with selenium is not a chance finding. Blood levels were prediagnostically, and the observed association persisted and became stronger after exclusion of women diagnosed within 3 years of blood collection. A statistically significant dose–response trend was ob-
served for selenium and the risk of ovarian cancer that was most prominent among case subjects diagnosed 4 years or more after blood collection. Selenium is presumed to function as a protective agent against cancer through multiple mechanisms, including the function of selenium-dependent glutathione peroxidase, and has been shown to have a cancer-preventive effect in animal models (30). Our findings are consistent with results of case-control studies that showed lower selenium levels in patients with ovarian cancer at the time of diagnosis than in control subjects (10–12). Selenium has been used as an imaging agent and thus may be sequestered by tumors, accounting for lower levels among case patients than control subjects in these studies (31). Because our study examined prediagnostic levels and the findings were stronger among later-diagnosed case patients, tumor sequestration is unlikely to explain our findings. Our findings, however, do not agree with the results of a prospective study of toenail selenium levels and subsequent cancer among a cohort of U.S. registered nurses; that study (32) found no association with all cancers or in the subgroup of women who developed ovarian cancer. Toenail selenium levels measured 2 years apart have been shown to have a correlation of .48 (33), and toenail levels are highly correlated with serum selenium levels (r = .89) (34). Exposure in the Nurses’ Health Study as well as in our study was based on a single measurement of micronutrient status. The use of a single measurement of exposure is similar to the situation in studies of the association between blood pressure and cardiovascular disease (35,36). Single blood pressure measurements have been consistently associated with cardiovascular disease despite marked fluctuations in blood pressure (35,36). Because of the conflicting results in these prospective studies, the findings should be replicated in other cohorts.

Contrary to our expectations, higher α-tocopherol levels were associated with a significantly increased risk of developing ovarian cancer. α-Tocopherol levels are closely correlated with cholesterol levels because lipoproteins, particularly HDL, are

### Table 3. Risk of developing ovarian cancer by stratified serum micronutrient and cholesterol levels, Washington County, MD, 1974*

<table>
<thead>
<tr>
<th>Micronutrient, units</th>
<th>Median</th>
<th>Matched odds ratio by thirds (95% confidence interval [CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
</tr>
<tr>
<td>Retinol, µg/dL</td>
<td>57.3</td>
<td>56.2</td>
</tr>
<tr>
<td>Total carotenoids, µg/dL</td>
<td>99.0</td>
<td>107.7</td>
</tr>
<tr>
<td>α-Carotene, µg/dL</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>β-Carotene, µg/dL</td>
<td>16.5</td>
<td>18.2</td>
</tr>
<tr>
<td>Lutein, µg/dL</td>
<td>22.0</td>
<td>19.1</td>
</tr>
<tr>
<td>Lycopene, µg/dL</td>
<td>28.6</td>
<td>30.9</td>
</tr>
<tr>
<td>α-Tocopherol, mg/dL</td>
<td>1.17</td>
<td>0.96</td>
</tr>
<tr>
<td>γ-Tocopherol, mg/dL</td>
<td>0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>Selenium, µg/dL</td>
<td>10.6</td>
<td>11.1</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>230</td>
<td>198</td>
</tr>
</tbody>
</table>

*Percentile cutoff points: retinol (µg/dL) 48.10, 59.90; total carotenoids (µg/dL) 89.60, 118.30; α-carotene (µg/dL) 0.90, 3.07; β-carotene (µg/dL) 13.60, 23.20; cryptoxanthin (µg/dL) 7.07, 13.37; lutein (µg/dL) 16.00, 23.30; lycopene (µg/dL) 21.90, 35.20; α-tocopherol (mg/dL) 0.89, 1.15; γ-tocopherol (mg/dL) 0.18, 0.25; selenium (µg/dL) 10.50, 11.60; and cholesterol (mg/dL) 184.66, 230.67.

†Referent category.

‡P for trend = from likelihood ratio test when variable coded with exposure score added to model. N/A = does not apply because no trend.

§Adjusted matched odds ratio by thirds Low Middle High

<table>
<thead>
<tr>
<th>Micronutrient, units</th>
<th>Adjusted matched odds ratio by thirds (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low†</td>
</tr>
<tr>
<td>Retinol adjusted for cholesterol (95% CI)</td>
<td>1.0</td>
</tr>
<tr>
<td>Cholesterol adjusted for α-tocopherol (95% CI)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Stratified level: cholesterol, high = >200 mg/dL, and low = ≤200 mg/dL; micronutrients, high = at or above median and low = below median. 
†P_interaction = the likelihood ratio test when the interaction term was added to the model. 
‡Unmatched odds ratio, unable to maximize in matched analysis.
the principal carriers of α-tocopherol in the serum. Thus, it has been suggested, primarily in studies of cardiovascular disease, that lipid-adjusted α-tocopherol levels are the most relevant measure to control for potential confounding effects of cholesterol (29). In our study, prediagnostic cholesterol and α-tocopherol levels were correlated, and high serum cholesterol was associated with an increased risk of developing ovarian cancer. Thus, cholesterol may be distorting the association between α-tocopherol and ovarian cancer. However, since cholesterol-laden lipoproteins are the principal carriers of α-tocopherol in the serum, sorting out the role of cholesterol as a confounder, effect modifier, or as part of the causal chain is problematical. If cholesterol is part of the causal chain, lipid adjustment would not be appropriate. The observed association between elevated levels of α-tocopherol and ovarian cancer risk, however, was attenuated when adjusted for cholesterol levels. In a nested case-control study of 16 ovarian cancer case patients and 29 control subjects, Knekt (7) found a risk of developing cancer of 1.3 among women in the highest compared with the lowest quintile of vitamin E levels, but no dose-response trend was evident. A case-control study by Heinonen et al. (13) found vitamin E levels to be 12% higher, but not statistically different, among ovarian cancer case patients than in the noncancer control subjects. Vitamin E levels were found to be lower among case patients compared with family and household control subjects in a case-control study conducted in Poland (12).

The association between serum cholesterol level and the risk of ovarian cancer appears to be independent of serum micro-nutrient levels, including α-tocopherol levels. Our finding of an increased risk with serum cholesterol levels is consistent with a case-control study of an association between intake of saturated fat, including cholesterol from egg sources, and ovarian cancer (18). A lower risk of ovarian cancer associated with vegetable fiber intake was also observed (18). However, previous case-control studies have not found an increased risk associated with dietary fat (14), animal fat (19), or combined fat (16) intake. Our results also fail to agree with a large prospective study of serum cholesterol and cancer incidence that included 190 cases of ovarian cancer in the cohort (9). Members of that cohort had cholesterol levels drawn between 1964 and 1972 and were followed through 1980. No association was observed between cholesterol levels and subsequent ovarian cancer. Given these conflicting results, it would be helpful to examine this association in other prospective studies.

We did not observe a significant association between the levels of retinol or carotenoids and the risk of ovarian cancer, a finding inconsistent with the results of some case-control studies of dietary intake of β-carotene or carotenoid-rich foods and the risk of ovarian cancer (14-17). Although our results are not incompatible with a protective effect of β-carotene, it may be that nutrients present in these food sources, other than the carotenoids, may be the protective factor.

References


Notes

Editor’s note. SEER is a set of geographically defined, population-based central tumor registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Each registry annually submits its cases to the NCI on a computer tape. These computer tapes are then edited by the NCI and made available for analysis.

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Editor’s note. This article has been reviewed in the context of its potential for occupational health and safety (OHS) implications. The review was conducted by an independent expert in the field of OHS.

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