Cancer Risk in Relation to Serum Copper Levels

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ABSTRACT

A nested, matched case-control study was conducted to assess the relationship between serum levels of copper and the subsequent risk of cancer. One hundred thirty-three cases of cancer were identified during 1974–1984 among 5000 members of a northwest Washington State employee cohort from whom serum specimens had been previously obtained and stored. Two hundred forty-one controls were selected at random from the cohort and were matched to the cases on the basis of age, sex, race, and date of blood draw. Serum copper levels were measured by atomic absorption spectrometry. Risk of a subsequent diagnosis of cancer was positively associated with serum copper levels, but only among those cases diagnosed within 4 years of the time the serum specimens were collected. Among cases diagnosed more than 4 years after specimen collection, there was no consistent association between serum copper levels and risk. Adjustment for age, sex, race, occupational status, cigarette smoking, family history of cancer, alcohol consumption, and, among females, use of exogenous hormones had no appreciable effect on these relationships. The findings suggest that the presence of cancer may increase serum copper levels several years prior to its diagnosis. They are less supportive of the hypothesis that serum copper levels affect cancer risk.

INTRODUCTION

A number of studies have found that in comparison with control populations the serum copper levels in cancer cases are elevated (1). Serum copper levels have been found to be higher in patients with late stage disease than among those with less severe disease, to be reduced following cancer treatment, and to increase prior to relapse (1). However, evidence from other research suggests that dietary intake of copper and serum copper levels may affect subsequent risk of cancer. In laboratory experiments, increased intake of copper has been found to reduce the occurrence of cancer in test animals (2), but population correlation studies have found the incidence of cancer to decrease with serum copper levels in blood donors (3). In addition, case-control studies have found increased risk of a subsequent diagnosis of cancer among individuals with elevated serum copper levels (4, 5) and among those with low serum copper levels (5).

To further study the relationship between serum copper levels and the subsequent risk of cancer, we conducted a case-control study of cancer among members of a cohort from whom blood samples had been obtained and frozen 0 to 10 years prior to diagnosis.

MATERIALS AND METHODS

As part of an investigation of serum lipids and cardiovascular diseases, during 1972–1974 the Northwest Lipid Research Center collected fasting blood specimens, demographics, and information on factors which affect lipid levels, including use of exogenous hormones, from 5000 Pacific Northwest Bell employees in Seattle, WA (6). Demographic characteristics of the Northwest Lipid Research Center study participants closely resembled those of residents of the Seattle-Everett Standard Metropolitan Statistical Area. Twenty-four % of the participants were managerial, 44% were clerical and sales, and 32% were craft employees.

To identify persons who subsequently developed disease, names and birth dates of those employees were matched against the records of the Cancer Surveillance System. The Cancer Surveillance System, a participant in the Surveillance Epidemiology and End Results program of the National Cancer Institute, has obtained diagnostic information on all new cancer cases in 13 counties of northwest Washington since January 1974.

One hundred forty-four cases were identified, 75% of the 192 anticipated given the age and sex distribution of the study cohort and the number of years of follow-up. The difference between the expected and identified numbers of cases was most likely due to migration of cohort members out of the 13-county registry area, although some of the reduction in the observed cancer incidence may be due to a "healthy worker effect" (7) in this employed population. Of those 144 eligible for the study, 11 cases could not be included because serum samples had not been retained in the storage freezers. Those specimens had been used previously in other studies, primarily in replications of lipid measurements. The remaining 133 constituted the case group.

Controls were selected from the same cohort and were matched on the basis of age (5-year groups), sex, race (white/nonwhite), year of blood draw, and season of blood draw (January–March, April–June, July–September, October–December). A ratio of 2 controls per case was desired, but for 25 cases only a single control was eligible.

This study population is described in Table 1. There were minor differences in the age and sex distributions of the expected case group and those for the study population. Forty-eight % of the study population was male, and 97% was white. Almost one-half of the participants were in their 50s when they participated in the Northwest Lipid Research Center blood collection during 1972–1974. There were few cases of any specific site, the largest numbers being 25 gastrointestinal tract (19%), 19 breast (14%), 12 cervix (8%), and 11 prostate (8%).

Serum aliquots had been stored in sealed copper-free Wheaton serum bottles with rubber stoppers and frozen at approximately −20 °C. Specimens were delivered frozen to the nutrition laboratory at the Department of Laboratory Medicine, University of Washington, where the aliquots were thawed and thoroughly mixed and the serum copper levels were measured using atomic absorption spectrometry (8). Triad Comprehensive Custom Unassayed Chemistry Control Sera (Beckman Instruments, Inc., Brea, CA) at normal and elevated levels were routinely assayed at each run. The coefficient of variation for quality control specimens was 3.7%.

During 1984–1985, information on known risk factors for major cancer sites, including cigarette smoking, alcohol consumption, and family history of cancer, was collected by interview. Permission to contact cases or their next of kin was obtained from the case physicians. Cases, controls, and next of kin were written and then telephoned for a 10- to 15-min interview. In those instances in which the case was deceased and an interview with a next of kin was obtained, interviews with next of kin were sought for the matched controls. Overall, information was obtained on 101 (76%) cases and 210 (87%) controls. Thirty-two (34%) of case interviews were with next of kin, and 58 (30%) of control interviews were with next of kin.

To assess the relationship between serum nutrient levels and subsequent case status, two different statistical techniques were used, both...
of which retained the case-control matching and thus adjusted for age, sex, race, and year and season of blood draw. The serum copper level of the cases and controls were compared using a paired t test (9). The nutrient level of each case was compared to that of his or her control or with the mean of the two controls. In addition, risk of subsequent cancer as a function of serum copper levels was estimated using a conditional logistic regression (10) procedure which treated each case and its control(s) as a separate stratum. For this analysis, subjects were categorized into quartile groups which were defined by the serum copper concentrations in the control group (Table 2). Additional analyses were conducted to determine if the addition of occupational status, cigarette smoking, family history of cancer, alcohol consumption, and, for females, use of exogenous hormones to the regression equation altered the relationship between serum copper levels and cancer risk.

RESULTS

The serum copper level in the control group was 115 ± 36 (SD) μg/dl, whereas the case group mean was 123 ± 37 μg/dl. Adjusted for age, sex, race, and year and season of blood draw, P for the case-control difference was 0.07.

Table 2 presents relative risk estimates of cancer, all sites, by quartile level of serum copper level, and by level of exposure to other factors which may potentially affect both cancer risk and serum copper levels, Seattle, WA, 1972–1984.

use of exogenous estrogens. Risk varies somewhat with the level of exposure to these factors but all risks are consistent with there being no true association.

Further analyses (not shown) of the relationship between serum copper levels and cancer risk adjusted for family history of cancer, cigarette smoking, alcohol consumption and, for females, use of exogenous hormones was completed. The adjusted relative risk estimates were not appreciably different from the unadjusted risk estimates. However, these analyses were conducted on a sample which excluded subjects for whom the 1984–1985 interview data were missing. Although the adjustment for potential confounders did not materially affect the relative risk estimates, the exclusion of subjects from the analysis did so. Therefore, additional analysis was conducted using the larger sample, and adjustments were made for matching variables available for all study subjects and for occupational status which was available for all but one control (Table 3).

To address the concern that preclinical disease might have affected nutrient levels in the subjects with cancer, cases were subdivided into five 2-year groups defined by the time between the blood sample collection and the cancer diagnosis. The risk of cancer by quartile level of serum copper was estimated for each of the five groups of cases and matched controls (Table
CANCER RISK IN RELATION TO SERUM COPPER LEVELS

Table 3  Risk of cancer, all sites, by quartiles of serum copper by time between blood draw and diagnosis, Seattle, WA, 1972–1984

<table>
<thead>
<tr>
<th>Time between blood draw and diagnosis (mo)</th>
<th>No. of cases/controls</th>
<th>Relative risk* by serum copper quartile</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–24</td>
<td>25/46</td>
<td>1.0</td>
<td>2.6</td>
<td>7.6</td>
<td>32.9</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>25–48</td>
<td>27/46</td>
<td>1.0</td>
<td>2.2</td>
<td>3.1</td>
<td>6.6</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>49–72</td>
<td>25/45</td>
<td>1.0</td>
<td>0.8</td>
<td>1.2</td>
<td>0.9</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>73–96</td>
<td>27/52</td>
<td>1.0</td>
<td>1.2</td>
<td>4.4</td>
<td>2.5</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>97–129</td>
<td>29/51</td>
<td>1.0</td>
<td>0.4</td>
<td>0.3</td>
<td>1.7</td>
<td>0.46</td>
<td></td>
</tr>
</tbody>
</table>

* Conditional logistic regression estimates, adjusted for age, sex, race, year of blood draw, season of blood draw, and occupational status.

* Categories defined in Table 2.

* From x² trend.

Table 4  Mean serum copper levels of cases and matched controls by time between blood draw and diagnosis, Seattle, WA, 1972–1984

<table>
<thead>
<tr>
<th>Time between blood draw and diagnosis (mo)</th>
<th>No. of cases/controls</th>
<th>Mean serum copper (mg/dl)</th>
<th>Mean difference*</th>
<th></th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>01–24</td>
<td>25/46</td>
<td>22 ± 9</td>
<td>0.26</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>25–48</td>
<td>27/47</td>
<td>22 ± 8</td>
<td>0.07</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>49–72</td>
<td>25/45</td>
<td>22 ± 6</td>
<td>0.26</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>73–96</td>
<td>27/52</td>
<td>22 ± 8</td>
<td>0.26</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>97–109</td>
<td>29/51</td>
<td>22 ± 6</td>
<td>0.26</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>133/241</td>
<td>22 ± 3</td>
<td>0.26</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

* Based on paired t test, adjusted for age, sex, race, year of blood draw, and season of blood draw.

3). Within 2 years of blood draw, a new diagnosis of cancer was approximately 33 times more common among persons in the highest quartile than among those in the lowest quartile of serum copper (P = 0.001). In the high quartile group, this value declined to 6.6 during the period 2–4 years following blood draw (P = 0.06). Beyond 4 years after blood draw, relative risk estimates for those in the middle and higher quartile groups varied from 0.3 to 4.4, risk did not increase systematically with serum copper levels, and the associations were consistent with there being no true relationship between the copper levels and cancer risk.

This same issue also was approached by calculating the difference between serum copper levels of each case and his/her matched control, separating the cases according to the time interval between blood draw and diagnosis. Table 4 presents the results of those comparisons. The largest case-control difference (22 mg/dl) was present within 2 years of blood draw. The observed difference declined to 6 mg/dl in the period 3 to 4 years after blood draw. Among those cases diagnosed more than 4 years after blood draw, differences from their controls were smaller still and variable in direction.

DISCUSSION

Inherent in this type of study are limitations which must be acknowledged. The means of identifying cases of cancer among members of the original study population led to a potential underascertainment of approximately 25%; 144 cases were identified, rather than the 192 cases anticipated. Furthermore, 6% of the identified cases could not be studied for lack of a serum sample. This resulted in a study case group that was similar to the expected group but was younger and had a somewhat higher proportion of females than males (Table 1). The demographic and cancer site distribution of the cases for whom specimens were available was quite similar to that of identified cases for whom specimens were unavailable (Table 1). Although the study group was somewhat different from the expected case group, no obvious bias could be identified. However, if the serum copper levels of unidentified cases and of cases for whom specimens were not available were substantially different from the study cases, a bias could have resulted.

Due to the small number of cancer cases of any particular site, site-specific analyses could not be performed within the groups defined by time between blood draw and diagnosis. Therefore, the study could not assess the relationship between serum copper levels and subsequent risk of a given type of cancer.

In the first published report on the relationship between prediagnostic serum copper levels and subsequent cancer risk, Haines et al. (4) presented data from a study of 28 cases identified within 7 years of participation in the Northwick Park Heart Study and 84 controls selected from that cohort matched to the cases on the basis of age, sex, specimen storage time, and smoking status. The serum copper level among cases (87.64 µg/dl) was somewhat higher than the control group mean (82.61 µg/dl). Cases identified within 2 years of blood draw were excluded from the study, but authors did not report results from additional analysis on the effect of time between blood draw and diagnosis on the difference in copper levels.

In a more recent study, Kok et al. (5) examined the relationship between serum copper and zinc levels and risk of death from cancer and from cardiovascular disease. One hundred cases who died of cancer 1–9 years after participation in a cardiovascular disease study were identified from a Netherlands cohort of 10,532 subjects. Two controls per case were selected from among the cohort and matched by sex, age, and cigarette smoking status. Compared with subjects whose serum copper levels were 105–143 µg/dl (the middle 3 quartiles), subjects with lower and higher levels were more likely to die of cancer, all sites. The relative risks were 1.8 and 3.7, respectively.

Analyses were adjusted for serum cholesterol, blood pressure, body mass index, serum selenium, serum vitamin A, serum vitamin E, education, and week of blood collection. Exclusion of cases who died within 4 years of blood draw resulted in only minor changes in risk estimates. Further evaluation of the effect of time between blood draw and diagnosis on risk was not reported.

The findings of the present study are consistent with those of Haines et al. (4) in that prior to diagnosis cases had somewhat higher serum copper levels than did controls. As did Kok et al. (5), this study found that risk was elevated among those with higher copper levels. In this study population there was, however, no evidence of increased risk among those with serum copper levels below 105 µg/dl. Compared with subjects whose serum copper levels were 105–145 µg/dl, the risk in this low serum copper level group was 0.6.

The relationship between serum copper levels and cancer risk was not due to the potentially confounding effects of age, sex, race, occupational status, cigarette smoking, family history of cancer or, among females, use of exogenous hormones. Adjustment for these variables did not appreciably affect the relative risk estimates.

The major difference between the findings of this study and those of the earlier studies is that the positive relationship between cancer risk and serum copper levels was strong and systematic only among cases who were diagnosed within 4 years of blood draw. Among cases diagnosed more than 4 years after the blood samples were collected, the relationships were weaker and inconsistent. This suggests that the higher levels of serum copper are more likely due to the presence of undiagnosed cancer and are thus more likely to be markers for cancer than
to actually affect subsequent cancer risk. Since the period of time between exposure to most carcinogens and the diagnosis of cancers is generally more than several years (11), if the serum copper level were truly a risk factor for cancer, an association between serum copper levels would have been expected among the cases diagnosed several years after the blood samples had been drawn. Furthermore, serum copper levels have been found to be elevated in individuals with a variety of acute and chronic diseases including cystic fibrosis, myocardial infarction, rheumatoid arthritis, and cancers (12, 13). This elevation in copper levels has been found in patients with many different types of cancers (14–18). Serum copper levels have also been found to be positively associated with stage of disease, to decline in patients responding to treatment, and generally to increase in patients prior to relapse (18).

The reasons for this increase in serum copper levels among cancer patients are not known. It may result from increased liver production of copper-containing ceruloplasmin as an inflammatory response to the cancer or from a tumor-induced decrease in catabolism of the serum ceruloplasmin (13, 18). Unfortunately, although a high copper level may predate diagnosis and although serum copper levels may predict relapse in cancer patients (13), serum copper levels are not useful in cancer screening, even in relatively high risk populations. The point can be illustrated using serum copper and risk data from this study and cancer incidence rates from the northwest Washington State Cancer Surveillance System. Among the cases in this study who were diagnosed with cancer within 2 years of blood draw, 12 of 25 or 48% fell into the high quartile with serum copper levels of 125 μg/dl or more. Among controls only 9 of 46 (19.6%) fell into this group. If this serum copper level were used to indicate a positive test, the sensitivity would be 48%, and the specificity of the test would be 80.4%. Given annual incidence rates of 1,521/10^5 for males ages 60–69 years and 1,212/10^5 for females ages 60–69 years, from a screened population of 100,000 men and women of those ages, one would expect approximately 2,714 cancers to occur over a 2-year period. Applying the 48% sensitivity rate to the 2,714 who would develop cancer and the 80.4% specificity to the 97,286 who would not, a table can be constructed which allows estimation of the predictive value of a positive test in that population (Table 5). Only 1,303 of 20,766 (6.4%) of those who test positive would actually develop cancer during the following 2 years.

These findings suggest then that the presence of cancer may increase serum copper levels several years prior to its diagnosis. However, while an elevated serum copper level is a marker for cancer, the low predictive value of such an elevation limits its usefulness in cancer screening.

Acknowledgments

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Table 5 Hypothetical results of screening for undiagnosed cancer in a high risk population* of 100,000 men and women using serum copper levels, Seattle, WA, 1972–1984

<table>
<thead>
<tr>
<th>Serum copper screening level</th>
<th>Cancer</th>
<th>Not cancer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (&gt;125 μg/dl)</td>
<td>1,303</td>
<td>19,034</td>
<td>20,337</td>
</tr>
<tr>
<td>Negative (&lt;126 μg/dl)</td>
<td>1,411</td>
<td>78,252</td>
<td>79,663</td>
</tr>
<tr>
<td>Total</td>
<td>2,714</td>
<td>97,286</td>
<td>100,000</td>
</tr>
</tbody>
</table>

* Men and women, ages 60–69 years.

* Assumes an incidence rate of 1,367/10^5/year; thus prevalence of undiagnosed cancer is 2.7%.

References

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