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Selenium and Risk of Bladder Cancer: A Population-Based Case-Control Study

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Abstract

Emerging evidence indicates a potential role of selenium in the prevention of several types of cancer, including bladder cancer. We investigated the association between toenail selenium concentrations and bladder cancer risk in a population-based case-control study in New Hampshire. We analyzed data from 857 incidence cases diagnosed between July 1, 1994 and June 30, 2001 and 1,191 general population controls. Newly diagnosed cases of bladder cancer were identified from the New Hampshire State Cancer Registry, which operates a rapid reporting system. Controls were selected from population lists (driver's license and Medicare enrollment). We used logistic regression analyses to generate odds ratios (OR) and 95% confidence intervals (95% CI), controlling for age, sex, and pack-years of smoking and conducted separate analyses according to the intensity of p53 immunohistochemical staining of the tumor. Overall, toenail selenium concentrations were not significantly related to bladder cancer [OR Q4 versus Q1, 0.90 (95% CI, 0.68–1.19); $P_{\text{trend}} = 0.15$]. However, within specific subgroups there were inverse associations, i.e., among moderate smokers [OR, 0.61 (95% CI, 0.39–0.96); $P_{\text{trend}} = 0.004$], women [OR, 0.66 (95% CI, 0.40–1.10); $P_{\text{trend}} = 0.11$], and those with p53-positive cancers [OR Q4 versus Q1, 0.57 (95% CI, 0.34–0.94); $P_{\text{trend}} = 0.01$]. Our results indicate that selenium is not inversely related to risk of bladder cancer overall; however, they raise the possibility that selenium may be preventive in certain molecular phenotypes of tumors (e.g., p53 positive) or within certain subsets of a population (e.g., women or moderate smokers).

Bladder cancer is the fourth most common cancer among men and the twelfth most common among women in the United States (1). More than 67,000 people will be diagnosed with bladder cancer this year and more than 13,000 deaths are expected (1). Cigarette smoking is the most common risk factor, accounting for possibly one half of all diagnoses in men and one quarter in women (2). Other risk factors include chemical exposures, male gender, infection, and possibly diet (2,3). One potential preventative dietary constituent is selenium (3).

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Selenium is an essential trace element commonly found in grains, nuts, and meats (3). Higher levels of selenium have been associated with a lower risk of many types of neoplasia, including prostate, lung, colorectal, and possibly bladder, although the data are inconsistent (3,4). Two studies to date involving >100 bladder cancer cases and controls reported inverse associations between toenail (5) or serum (6) selenium levels. In one of these, a nested case-control study, the inverse association was restricted to women (5). In a cohort study conducted in the Netherlands, higher toenail selenium levels were related to a significantly reduced risk of invasive bladder cancers (7). A small case-control study reported significantly lower serum selenium levels among patients with grade 2 and grade 3 transitional cell bladder cancers, but not grade 1 carcinomas, compared with healthy controls (8). In two of the above-mentioned studies, the inverse association between selenium level and bladder cancer was confined to former smokers (5,7), whereas in the α -Tocopherol β -Carotene Cancer Prevention Study, a nested case-control study, there was no association between toenail selenium levels and bladder cancer incidence among male smokers (9). Thus, it is possible that the selenium association to bladder cancer may be modified by gender, smoking status, or tumor severity (i.e., grade or degree of invasiveness).

Selenium is thought to inhibit carcinogenesis through several different mechanisms, including reduction of oxidative stress and inflammation, enhancement of immune response, induction of apoptosis, cell cycle arrest, and transactivation of DNA repair genes such as *p48XPE* or *Gadd45a* (4,10). Selenium may be involved directly in the DNA damage response through the transactivation of *p53* (4,10). One current hypothesis is that there are different molecular pathways by which bladder cancer evolves and one of the major pathways involves alterations in the *p53* gene (11). We investigated the association of toenail selenium concentration with bladder cancer incidence in a large population-based case-control study from New Hampshire. Additionally, we explored the effects of selenium status by gender, smoking, and tumor classification according to the presence or absence of *p53* alterations.

Materials and Methods

A detailed description of the study design appears in earlier reports (12). Briefly, cases were identified by the New Hampshire State Department of Health and Human Services' Cancer Registry and included New Hampshire residents, ages 25 to 74 y, first diagnosed with bladder cancer from July 1, 1994 through December 31, 2001. Under state mandate, the registry operates a rapid reporting system that requires an initial report of cancer within 45 d of diagnosis and a definitive report within 180 d. To be eligible for the study, we required that subjects have a listed telephone number and speak English. Before contacting potential participants, we requested physician consent.

Controls were shared with a study of nonmelanoma skin cancer covering a diagnostic span from July 1, 1993 to March 30, 2000 (12), along with additional controls frequency matched to the bladder cancer cases on age and gender. Controls <65 y of age were selected using population lists obtained from the New Hampshire Department of Transportation. Controls ≥ 65 y of age were chosen from data files provided by the Centers for Medicare & Medicaid Services of New Hampshire residents.

Before recruitment, we sent a letter to potential cases and controls explaining the general purpose of the study and that they would be contacted by telephone. Subjects who agreed to participate in the study underwent a detailed personal interview, usually at their home. The interviewer asked sociodemographic questions, including level of education, occupational history, detailed information about the use of tobacco products, and medical history before the reference date. Additionally, subjects were asked to provide toenail samples, which were analyzed for selenium using an established protocol and an instrumental neutron activation

analysis at the University of Missouri Research Reactor Center (Columbia, MO; ref. 13). Before analysis, nail samples were carefully washed to remove external contamination. Each batch of analyses included quality control samples composed of matrix-matched samples with known content and analytic blanks along with study samples and standards. The between-assay coefficient of variability for matrix-matched samples is ~8%. All samples were labeled with an identification number that did not disclose the case-control status of the study participants. All procedures and study materials were approved by the Committee for the Protection of Human Subjects at Dartmouth College.

In addition to a histopathology re-review of the tumors, we evaluated immunohistochemical staining for p53 protein using previously described methods (14). The intensity of nuclear staining was graded on a semiquantitative scale (0–3) using the dominant pattern within the tumor. p53 positivity (i.e., strong intensity) was defined as 3+.

Statistical analysis

We computed odds ratios (OR) and 95% confidence intervals (95% CI) for the association between toenail selenium concentration and risk of all bladder cancer, transitional cell bladder cancer, and invasive and noninvasive bladder cancers. Generation of the quartiles was based on the control distribution. In each regression analysis, we controlled for age (linear), sex, and pack-years of smoking. We also examined sex, pack-years, and smoking status (never, former, current) as potential effect modifiers of selenium status by using product interaction terms in the regression models. We modeled the interaction between selenium and pack-years both as continuous and in three groups (never, moderate, and heavy). The cutoff point for “moderate” and “heavy” was the median of pack-years (excluding never smokers). Statistical significance was assessed using the likelihood ratio test and Wald statistics. Tests of trend were done for each regression analysis using the appropriate orthogonal linear contrasts. In addition, we examined the association between quartiles of selenium and p53 staining (positive versus negative) using logistic regression analyses, controlling for age and pack-years of smoking. Alterations in p53 also are associated with advanced tumor stage and grade, and for this reason, we conducted a case-only analysis classifying tumors based on p53 staining (positive versus negative), adjusting for tumor stage and grade in addition to age, sex, and pack-years.

Results

We interviewed a total of 857 bladder cancer cases and 1,191 controls (85% of cases, 70% of controls participated) and obtained toenail selenium levels for 1,875 of the 2,048 (92%) eligible subjects, including 767 of the 857 (89.5%) cases and 1,108 of the 1,191 (93%) controls. More than 90% of the toenail samples were collected at the time of interview (>95% within 12 months of interview) and the majority of cases were interviewed within 14 months of diagnosis.

Demographic characteristics of the cases and controls are shown in Table 1. There was a higher portion of males and smokers in the case group compared with controls. Cases also tended to be slightly older than controls.

The mean toenail selenium concentration was lower among cases than controls ($P < 0.001$). For cases, the mean selenium level was 0.86 $\mu\text{g/g}$ (± 0.19), and for controls, 0.88 $\mu\text{g/g}$ (± 0.18). After controlling for age, sex, and pack-years of smoking, the ORs among those in the third and fourth quartiles were only slightly below 1 and not statistically significantly ($P_{\text{trend}} = 0.15$). For women, the OR for those in the highest versus lowest quartile was 0.66 (95% CI, 0.40–1.10; $P_{\text{trend}} = 0.11$; Table 2), and among men the OR was 0.97 (95% CI, 0.70–1.36; $P_{\text{trend}} = 0.35$; gender-selenium $P_{\text{interaction}} = 0.06$). For never smokers, the OR among those in the highest versus lowest was 0.78 (95% CI, 0.44–1.37; $P_{\text{trend}} = 0.12$); among moderate smokers

the OR was 0.61 (95% CI, 0.39–0.96; $P_{\text{trend}} = 0.004$); and among heavy smokers the OR was 1.44 (95% CI, 0.91–2.27; $P_{\text{trend}} = 0.09$). The interaction between pack-years and selenium was statistically significant ($P = 0.01$). Results did not markedly differ when the data were restricted to transitional cell bladder cancers or when the data were similarly restricted to invasive and noninvasive tumors (data not shown). For tumors with strong intensity p53 staining, there was evidence of a dose-response relation across the quartiles among both men and women combined ($P_{\text{trend}} = 0.01$). Selenium was unrelated to cancers with p53 low intensity stained tumors. The case-only analysis of p53 status adjusted for tumor stage and grade yielded similar results to the main immunohistochemical analysis.

Discussion

In this population-based case-control study, we found that higher toenail selenium levels were not significantly associated with a lower risk of bladder cancer. However, we found evidence of an inverse association among women, moderate smokers, and those with p53 intensely stained tumors.

Several epidemiologic studies have examined the association between selenium and the risk of bladder cancer (5-7,9,15), and a number of these have observed inverse associations (5-7, 15). However, a number of these were relatively small, with only four having >100 cases (5-7,9). One nested case-control study had >100 women cases (5) and, interestingly, also observed a reduced OR in the highest versus lowest quartile of toenail selenium among women (OR, 0.36; 95% CI, 0.14–0.91) but not men (OR, 1.17; 95% CI, 0.66–2.07; ref. 5). Our findings also suggest the possibility of a differential association of selenium with the risk of bladder cancer among men and women, although the interaction term was not statistically significant ($P = 0.06$). Future research with a larger population of women may help to clarify the association between selenium, gender, and risk of bladder cancer.

Modification of effects by cigarette smoking status has also been observed. Two previous studies found stronger inverse associations between selenium and bladder cancer among past smokers than for never or current smokers, one in women only (5) and the other for both sexes (7). In the α -Tocopherol β -Carotene Cancer Prevention Study trial conducted among male smokers, no association was found between selenium and bladder cancer (9). In our study, we observed a strong inverse relationship between selenium and bladder cancer among those reporting low to moderate levels of smoking, and a similar non-significant trend was observed among nonsmokers. Additionally, we observed an increased risk among heavy smokers in relation to selenium concentrations. In theory, heavy smoking could potentially overwhelm any beneficial effects of selenium on bladder cancer, or worse, act as a promoter of the neoplastic process, analogous to the findings for β -carotene among smokers for lung cancer (16,17) or for colorectal adenomas (18).

Experimental data indicate potential anticarcinogenic effects of selenium, yet the exact molecular mechanism(s) remains unknown. Selenium is associated with several preventive processes such as reduction of DNA damage, oxidative stress, and inflammation, as well as immune enhancement (4). Alternatively, there is accumulating evidence that selenium or selenium metabolites may activate genes or modify proteins associated with the p53 pathway, which is frequently altered in human cancers (4,10,19), including a subset of bladder cancers (11,14,20). Selenomethionine has been shown to mediate p53-induced cell cycle arrest and apoptosis in colon cancer cells (21), whereas selenite seems to enhance superoxide production and apoptosis in human prostate cancer via the p53 pathway (22). As Luis et al. (11) describe, p53 alterations (i.e., abnormal staining or mutation) seem to play a central role in bladder carcinomas progressing from carcinoma *in situ*/dysplasia to high-grade invasive tumors, but not in the pathway progressing from hyperplasia to low-grade, noninvasive papillary tumors.

Albeit speculative, one possibility is that selenium plays a role in the molecular pathogenesis of p53-altered bladder cancers.

One of the limitations of our study is that we did not have the subjects' prediagnostic toenail selenium levels. This could have affected the results if the disease process changed the selenium levels. However, this possible source of bias is minimized by the use of toenail samples, which likely reflect exposures several months before the date of collection, and by our population-based design that included a large fraction of noninvasive tumors (i.e., less patient morbidity). In conclusion, our results indicate that a higher intake of selenium is not associated with a lower risk of bladder cancer overall. Our findings and others suggest the possibility of selenium affecting subsets of individuals (e.g., women or moderate smokers) or types of tumors (e.g., such as tumors evolving through the p53 pathway).

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Table 1

Characteristics among bladder cancer cases and controls

Characteristic	Cases (<i>n</i> = 857)	Controls (<i>n</i> = 1,191)
Sex, <i>n</i> (%)		
Male	651 (76)	729 (61.2)
Female	206 (24)	462 (38.8)
Age (SD), y	62.01 ± 9.5	61.0 ± 10.5
Smoking status, <i>n</i> (%)		
Never	147 (17.4)	399 (33.7)
Former	419 (49.7)	579 (48.9)
Current	277 (32.9)	205 (17.3)
Pack-years of smoking (±SD)	37.9 (35.6)	20.3 (27.4)
Never smoker (0 PY)	147 (18.0)	399 (35.7)
Moderate smoker* (<32 PY)	268 (32.7)	428 (38.3)
Heavy smoker* (>32 PY)	405 (49.4)	291 (26.0)
Pack-years of smoking by gender		
Male never smoker (0 PY)	91 (11.1)	196 (17.5)
Female never smoker (0 PY)	56 (6.8)	203 (18.2)
Male moderate smoker* (<32 PY)	202 (24.6)	261 (23.4)
Female moderate smoker* (<32 PY)	66 (8.1)	167 (15.0)
Male heavy smoker* (>32 PY)	333 (40.6)	228 (20.4)
Female heavy smoker* (>32 PY)	72 (8.8)	63 (5.6)
Family history of bladder cancer		
Yes, <i>n</i> (%)	39 (5.1)	14 (1.2)
Median selenium level (±SD)	0.86 (0.19)	0.88 (0.18)

Abbreviation: PY, pack-years.

* Moderate smoker defined as those below the median in pack-years and heavy as those above the median in pack-years. Median pack-year cutoff value was calculated using subjects that had >0 pack-years (i.e., excluding never smokers).

Table 2

The association between toenail selenium levels and risk of bladder cancer

	Q1 <0.77 µg/g	Q2 0.77–0.86 µg/g	Q3 0.87–0.95 µg/g	Q4 >0.95 µg/g	<i>P</i> _{trend} [*]	<i>P</i> _{interaction} [†]
All bladder cancers						
Controls	280	275	276	277		
Cases	245	217	138	167		
OR, [‡] overall	1.00	1.04 (0.79–1.35)	0.73 (0.55–0.98)	0.90 (0.68–1.19)	0.15	
OR, [§] men	1.00	1.27 (0.93–1.71)	0.83 (0.59–1.16)	0.97 (0.70–1.36)	0.35	
OR, [§] women	1.00	0.53 (0.30–0.93)	0.50 (0.29–0.84)	0.66 (0.40–1.10)	0.11	0.06
OR, never smoker (0 PY) [¶]	1.00	1.05 (0.59–1.88)	0.53 (0.29–1.00)	0.78 (0.44–1.37)	0.12	
OR, moderate smoker (<32 PY) [¶]	1.00	0.85 (0.56–1.31)	0.45 (0.28–0.72)	0.61 (0.39–0.96)	0.004	
OR, heavy smoker (>32 PY) [¶]	1.00	1.09 (0.73–1.64)	1.30 (0.83–2.01)	1.44 (0.91–2.27)	0.09	0.01
p53 immunohistochemical staining						
Negative cases	135	137	97	111		
Negative OR, [‡] overall	1.00	1.31 (0.83–1.50)	0.97 (0.63–1.20)	1.20 (0.86–1.65)	0.97	
Positive Cases	67	44	28	26		
Positive OR, [‡]	1.00	0.82 (0.53–1.27)	0.58 (0.36–0.95)	0.57 (0.34–0.94)	0.01	

^{*} *P*_{trend} determined using the appropriate orthogonal linear contrasts.[†] *P*_{interaction} determined using the likelihood ratio test with and without product interaction terms.[‡] OR adjusted for age, sex, and pack-years of smoking.[§] OR adjusted for age and pack-years of smoking.^{||} OR adjusted for age and sex.[¶] Moderate smoker defined as those below the median in pack-years and heavy as those above the median in pack-years.