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Original Contribution

Is the Inverse Association Between Selenium and Bladder Cancer Due to Confounding by Smoking?

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Selenium has been linked to a reduced risk of bladder cancer in some studies. Smoking, a well-established risk factor for bladder cancer, has been associated with lower selenium levels in the body. We investigated the selenium-bladder cancer association in subjects from Maine, New Hampshire, and Vermont in the New England Bladder Cancer Case-Control Study. At interview (2001–2005), participants provided information on a variety of factors, including a comprehensive smoking history, and submitted toenail samples, from which we measured selenium levels. We estimated odds ratios and 95% confidence intervals among 1,058 cases and 1,271 controls using logistic regression. After controlling for smoking, we saw no evidence of an association between selenium levels and bladder cancer (for fourth quartile vs. first quartile, odds ratio (OR) = 0.98, 95% confidence interval (CI): 0.77, 1.25). When results were restricted to regular smokers, there appeared to be an inverse association (OR = 0.76, 95% CI: 0.58, 0.99); however, when pack-years of smoking were considered, this association was attenuated (OR = 0.91, 95% CI: 0.68, 1.20), indicating potential confounding by smoking. Despite some reports of an inverse association between selenium and bladder cancer overall, our results, combined with an in-depth evaluation of other studies, suggested that confounding from smoking intensity or duration could explain this association. Our study highlights the need to carefully evaluate the confounding association of smoking in the selenium-bladder cancer association.

bladder cancer; case-control study; selenium; smoking

Abbreviations: CI, confidence interval; CMS, Centers for Medicare and Medicaid Services; DMV, Department of Motor Vehicles; OR, odds ratio; SD, standard deviation; SE, standard error.

In the United States, an estimated 73,510 incident cases of urinary bladder cancer were diagnosed in 2012 (55,600 among men and 17,910 among women) (1). Cigarette smoking is a well-established risk factor for bladder cancer. Recent estimates of the population attributable risk for smoking and bladder cancer are 50% in men and 52% in women (2). Selenium, an essential trace metal present in a wide variety of foods, has been investigated as a possible chemopreventive agent for multiple types of cancer, possibly acting through selenoproteins (3, 4). Despite this, at high levels it has also been implicated as being associated with a number of adverse health outcomes, including certain types of cancer, such as prostate cancer (5, 6). For bladder cancer, some (but not all) observational studies have reported inverse associations with selenium, as measured in blood serum or toenails. A recent meta-analysis of 7 studies with measured selenium levels, and a Cochrane review that included 6 of these studies, suggested an overall inverse association (7, 8). Two studies have reported differences by sex, with a suggestion of stronger associations among women than among men (9, 10).

Smoking, however, is an independent predictor of selenium levels, with lower levels seen among smokers, even after controlling for dietary sources (11–14). While the mechanisms of how smoking impacts selenium levels are not known, this association indicates that careful adjustment for

smoking is needed when evaluating the selenium-bladder cancer association. In the population-based case-control New England Bladder Cancer Study, we evaluated the association between toenail selenium levels and bladder cancer with thorough consideration of potential confounding from smoking.

METHODS

Study design

We conducted a population-based case-control study in Maine, New Hampshire, and Vermont; the study has been described in detail elsewhere (15). Cases included individuals aged 30–79 years who were diagnosed with carcinoma of the urinary bladder between 2001 and 2004 in Maine and Vermont, or 2002 and 2004 in New Hampshire. Case subjects were ascertained through hospital pathology departments, hospital cancer registries, and state cancer registries. Of eligible patients who did not participate, 50% refused, 22% were deceased, 12% were too ill, 5.5% did not speak English fluently, 5% had a physician who refused, and 5% could not be located (percentages do not total 100% because of rounding). The study's expert pathologist carried out a blind review of the initial diagnostic slides to confirm diagnosis, histological classification, and tumor stage and grade. Based on the expert pathology review, 20 patients who did not have cancer and 22 who did not have urothelial carcinomas were excluded. We selected controls randomly from Department of Motor Vehicles (DMV) records in each state for those aged 30–64 years and from beneficiary records of the Centers for Medicare and Medicaid Services (CMS) for those aged 65–79 years. Controls were frequency-matched to cases by state, sex, and age at diagnosis within 5 years and interviewed between 2002 and 2005. Of the 1,878 eligible cases identified, 1,213 were interviewed (65%). A total of 1,418 controls participated (65% of those contacted from both DMV and CMS). Of the control subjects who did not participate, 70% of DMV and 65% of CMS control subjects refused, 24% of DMV and 11% of CMS control subjects could not be located, 3% of DMV and 10% of CMS control subjects did not speak English fluently, 1% of DMV and 7% of CMS control subjects were too ill to participate, and 1% of DMV and 7% of CMS control subjects were deceased. To evaluate potential bias in the selection of the controls, we limited results to cases with a driver's license (under age 65 years) or a Medicare card, and we found the results to be unchanged.

All participants completed an in-person interview at home and were asked to provide toenail clipping samples. In total, 1,124 cases (92.7%) and 1,348 controls (95.1%) provided samples. The median time from diagnosis to toenail collection in the cases was 5.9 months. The interview elicited information on a variety of factors, including a comprehensive smoking history. Using information from the interview, we defined "never smokers" as participants who reported smoking fewer than 100 cigarettes over their lifetime, and "occasional smokers" were defined as participants who reported smoking more than 100 cigarettes over their lifetime but never smoked cigarettes regularly (i.e., at least 1 cigarette per day for at least 6 months). Finally, "regular smokers" were defined as those who smoked at least 1 cigarette per day for at least 6 months. Regular smokers were further categorized as "former smokers" (i.e., those who quit smoking 1 year or more before the diagnosis date for case patients or before the selection date for control subjects) or "current smokers" (i.e., those who were still smoking regularly at the time of their interview or had quit within 1 year of the reference date). Participants also provided information on the duration of smoking (years) and number of cigarettes smoked per day.

Laboratory analyses

Once collected, the toenails were stored at room temperature until analysis. After the toenails were cleaned to remove external contaminants, selenium was quantified at the Trace Element Analysis Core at Dartmouth College (Dartmouth College, Hanover, New Hampshire), using inductively coupled plasma mass spectrometry. Samples were acid-digested with Optima HNO_3 (Fisher Scientific, St. Louis, Missouri) at 105°C, followed by the addition of hydrogen peroxide, further heating, and then dilution with deionized water. All sample preparation steps were recorded gravimetrically. Selenium (mass-to-charge ratio = 78) was analyzed by inductively coupled plasma mass spectrometry (Agilent7500cx; Agilent Technologies, Santa Clara, California) in reaction mode using hydrogen as the reaction gas. For quality control, each digestion batch included 1 standard reference material sample (GBW 07601, human hair, Institute of Geophysical and Geochemical Exploration, Langfang, Hebei Province, People's Republic of China, and National Institute for Environmental Studies no. 13 human hair, National Institute for Environmental Studies, Ibakari, Japan), a digestion blank, and 1 fortified blank for every 20 samples. Analysis quality control included initial and continuing calibration verification, analytical sample duplicates, and spikes. A 2-L volume of composited digested toenails was prepared and used as a laboratory control sample and an aliquot was tested at the beginning and end of each batch of toenail analysis for the duration of the study. The average measured selenium value for no. 13 was 1.66 (standard deviation (SD), 0.064) μg/g ($n = 102$) compared with a certified value of 1.79 (SD, 0.17) μg/g. For GBW 07601, the average measured selenium value was 0.557 (SD, 0.051) μg/g ($n = 54$) compared with a certified value of 0.60 (SD, 0.03) μg/g. The relative standard deviation of the laboratory control sample across all digestion batches was 3%, while the average within-batch relative percent difference was 2.3% (SD, 3). The average relative percent difference of analytical duplicate analyses was 4.6% (SD, 1.9) ($n = 147$), and the average percent recovery of the fortified blank was 99% (SD, 4) ($n = 80$). Overall, results were available for 1,058 cases (94.1% of those who provide samples) and 1,271 controls (94.3% of controls who provided samples).

Statistical methods

We used unconditional logistic regression to calculate odds ratios and 95% confidence intervals for toenail selenium levels. Selenium levels were categorized into quartiles based on the distribution among the controls, and odds ratios were calculated using the lowest quartile as the referent. We

adjusted for age (<55, 55–64, 65–74, and ≥75 years), sex, ethnicity (Hispanic or non-Hispanic), and state of residence (Maine, New Hampshire, or Vermont). We used information on smoking history to evaluate the association of confounding by smoking in a variety of ways, including adjustment for pack-years overall, and stratification by smoking status with further adjustment for pack-years. We also investigated differences by sex, tumor invasiveness (TNM classification <T2 vs. ≥T2), and potential confounding by other reported risk factors for bladder cancer, including employment in a high-risk occupation, and intake of arsenic and disinfection by-products in drinking water. Although the percentage of pipe and cigar users was low overall and less than 10% in the nonsmokers, in sensitivity analyses, we explored whether ever using pipes and cigars impacted risk estimates by excluding ever users of these 2 products from the nonsmoking category. We also evaluated whether the time between diagnosis and toenail collection in the cases influenced the results. Likelihood ratio tests were used to formally assess differences between strata. Tests for trend used the midpoint value of each exposure category treated as a continuous variable in regression models. All tests were 2-sided. Statistical analyses were conducted using SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Overall, men represented 76.5% of the 1,058 cases and 73.6% of the 1,271 controls with measured selenium values (Table 1). Cases were more likely than controls to be regular smokers ($P < 0.001$), and among those who were regular smokers, to have smoked more cigarettes per day ($P < 0.001$). The mean value of selenium was 0.94 μg/g (standard error (SE), 0.01) among cases and 0.94 μg/g (SE, 0.009) among controls. Among controls, the mean value was 0.96 μg/g (SE, 0.20) among nonsmokers, 0.95 μg/g (SE, 0.40) among former smokers, and 0.86 μg/g (SE, 0.24) among current smokers. In addition, among regular smokers with fewer than 20 pack-years, the mean value was 0.95 μg/g (SE, 0.23) as compared with 0.91 μg/g (SE, 0.32) for those with more than 60 pack-years.

In analyses adjusted for age, sex, state, and ethnicity, there appeared to be a reduced risk of bladder cancer with increasing selenium levels (for top quartile, odds ratio (OR) = 0.70, 95% confidence interval (CI): 0.56, 0.89; P for trend = 0.03) (Table 2). However, when pack-years were added to the model, the association was no longer apparent (for top quartile, OR = 0.98, 95% CI: 0.77, 1.25; P for trend = 1.0). We also observed no statistical interaction between smoking status and selenium concentration ($P = 0.4$).

We further explored the impact of smoking on the association between selenium and bladder cancer by examining results separately by smoking status. Among never smokers, there was no association between increasing selenium levels and bladder cancer (Table 2). Among regular smokers, with no additional adjustment for pack-years, there appeared to be an inverse association between selenium levels and bladder cancer (in highest quartile, OR = 0.76, 95% CI: 0.58, 0.99; P for trend = 0.03), similar to the overall results prior to adjustment for smoking. When we controlled for pack-years among the regular smokers, this association was no longer apparent (in highest quartile, OR = 0.91, 95% CI: 0.68, 1.20; P for trend = 0.4). We also examined associations among former and current smokers, with similar results for both groups. For former smokers, the highest odds ratio in the highest quartile was 0.95 (95% CI: 0.69, 1.32; P for trend = 0.8), and for current smokers it was 0.86 (95% CI: 0.47, 1.57; P for trend = 0.57).

Because of reports of sex differences in the association between selenium and bladder cancer risk in some studies, we examined associations separately in men and women. After adjustment for smoking, there was no association in either sex (Table 3).

Table 1. Distribution of Selected Factors Among Cases and Controls in the New England Bladder Cancer Study, 2001–2004

Characteristic	Cases ($n = 1,058$)		Controls ($n = 1,271$)	
	No.	%	No.	%
Age, years				
<55	166	15.7	231	18.2
55–64	285	26.9	299	23.6
65–74	387	36.6	488	38.4
≥75	220	20.8	253	19.9
Sex				
Male	809	76.5	936	73.6
Female	249	23.5	335	26.4
Smoking status				
Never smoker	163	15.4	423	33.3
Occasional smoker	20	1.9	37	2.9
Regular smoker[a]	875	82.7	811	63.8
Pack-years of smoking (among regular smokers)				
<20	188	21.5	314	38.7
20–<40	261	29.8	226	27.9
40–<50	138	15.8	82	10.1
50–<60	90	10.3	52	6.4
≥60	191	21.8	127	15.7
Unknown	7	<0.1	10	1.2
Hispanic ethnicity				
No	1,039	98.2	1,248	98.2
Yes	19	1.8	21	1.7
Unknown			2	0.1
State				
Maine	519	49.1	660	51.9
Vermont	180	17.0	221	17.4
New Hampshire	359	33.9	390	30.7

[a] Regular smokers were defined as those who smoked at least 1 cigarette per day for at least 6 months. Regular smokers were further categorized as "former smokers" (i.e., those who quit smoking 1 year or more before the diagnosis date for case patients or before the selection date for control subjects) or "current smokers" (i.e., those who were still smoking regularly at the time of their interview or had quit within 1 year of the reference date).

Table 2. Odds Ratios for the Association Between Toenail Selenium and Bladder Cancer, by Smoking Status, in the New England Bladder Cancer Study, 2001–2004

Selenium Concentration, μg/g	Overall					Never Smokers				Regular Smokers				
	No. of Cases	No. of Controls	OR[a]	95% CI	OR[b]	95% CI	No. of Cases	No. of Controls	OR[a]	95% CI	No. of Cases	No. of Controls	OR[a]	95% CI	OR[c]	95% CI
≤0.81	315	340	1.00	Referent	1.00	Referent	76	28	1.00	Referent	309	232	1.00	Referent	1.00	Referent
>0.81–0.89	312	246	0.74	0.59, 0.93	0.89	0.70, 1.13	105	38	0.89	0.70, 1.13	203	196	0.78	0.60, 1.01	0.84	0.64, 1.10
>0.89–1.00	319	229	0.67	0.53, 0.85	0.87	0.69, 1.11	108	42	0.87	0.69, 1.11	179	199	0.67	0.52, 0.88	0.74	0.57, 0.98
>1.00	325	243	0.70	0.56, 0.89	0.98	0.77, 1.25	134	55	0.98	0.77, 1.25	184	184	0.76	0.58, 0.99	0.91	0.68, 1.20
P for trend[d]				0.03		1.0				1.0				0.03		0.4

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Estimates were adjusted for age, sex, state, and ethnicity.

[b] Estimates were adjusted for age, sex, ethnicity, state, and smoking status.

[c] Estimates were adjusted for age, sex, ethnicity, state, and pack-years of smoking.

[d] Tests for linear trend were performed by including the median of each quartile of the toenail trace element concentration as a continuous variable in the logistic regression model.

We evaluated the impact of other known or suspected bladder cancer risk factors on the overall associations and observed no impact on the odds ratio for selenium after adjustment for exposures such as arsenic or disinfection byproducts in drinking water or employment in a high-risk occupation. We also conducted several sensitivity analyses, including creating categories based on the distribution in the cases rather than the controls, excluding ever users of pipes and cigars among the nonsmokers, and stratifying analyses by time between diagnosis and toenail collection, and there was no change in risk estimates. We examined differences by muscle invasiveness and saw no clear trends in our study, although reduced odds ratios were observed in higher quartiles of selenium (Appendix Table 1).

DISCUSSION

Selenium was first reported to be inversely associated with cancer at multiple sites by ecologic studies focused on geographical variability in selenium levels (16). Because of the suggestion of selenium's chemopreventive properties, intervention trials have been conducted for a variety of cancer types. A recent review and meta-analysis of these randomized controlled trials failed to demonstrate a beneficial effect for any cancer type, including bladder cancer (8). Analytical studies with information on either dietary selenium or selenium concentrations measured in serum or toenails have supported the hypothesis that selenium is associated with reduced risk of bladder cancer. In a meta-analysis of 7 published studies, the overall meta–odds ratio for selenium and bladder cancer was reported to be 0.6 (7). However, there was heterogeneity across studies in the approach used to evaluate potential confounding from smoking.

Several studies have indicated that smoking is an independent predictor of selenium levels (11–14). While some researchers have suggested that the differences might be partially explained by dietary differences between smokers and nonsmokers (14, 17), others have demonstrated that smoking has been shown to be an independent predictor of selenium levels (11). It is not currently known why smoking impacts selenium levels. One hypothesis is that the oxidative stress associated with cigarette smoking (18) might deplete selenium stores and therefore lower selenium levels within the body. This is supported by observations in our study and in previously published studies of lower selenium levels in current smokers (but not former smokers) as compared with nonsmokers (11). Other investigators have postulated that cadmium, another trace element found in cigarettes, might cause selenium to be secreted at a higher rate (19). Regardless of the mechanism, given the strong association between smoking and bladder cancer, it is necessary to thoroughly control for confounding by smoking when evaluating the association between selenium and bladder cancer.

In our study, prior to controlling for confounding by smoking, an inverse association between toenail selenium levels and bladder cancer was present. After controlling for smoking, however, we observed no overall association between toenail selenium and bladder cancer. Among nonsmokers, no association between selenium levels and bladder cancer was detected. Among regular smokers, before controlling for

Table 3. Odds Ratios for the Association Between Toenail Selenium and Bladder Cancer, by Sex, in the New England Bladder Cancer Study, 2001–2004

Selenium Concentration, μg/g	Men				Women			
	No. of Controls	No. of Cases	OR	95% CI[a]	No. of Controls	No. of Cases	OR	95% CI[a]
≤0.81	249	283	1.00	Referent	66	57	1.00	Referent
>0.81–0.89	225	182	0.81	0.62, 1.06	87	64	1.10	0.65, 1.86
>0.89–1.00	242	170	0.73	0.56, 0.96	77	59	1.26	0.74, 2.15
>1.00	220	174	0.89	0.68, 1.18	105	69	1.18	0.7, 1.99
P for trend[b]			0.4				0.5	
P for interaction				0.5				

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Estimates were adjusted for age, state, ethnicity, and pack-years of smoking.

[b] Tests for linear trend were performed by including the median value from each quartile of the toenail trace element concentration as a continuous variable in the logistic regression model.

smoking, we observed an inverse association with selenium and bladder cancer. However, when we further controlled for pack-years, the results were attenuated. In our study, pack-years and toenail selenium levels were negatively correlated among regular smokers in the controls, although this correlation was not statistically significant. Previous studies have suggested differences by sex or tumor invasiveness. We observed inverse odds ratios among invasive tumors as found by Zeegers et al. (20), but after we controlled for smoking, we saw no evidence of trending or any differences by sex.

Our results agree for the most part with those of other studies that have controlled for smoking in a comprehensive manner. Of the 7 studies that have evaluated selenium levels and bladder cancer (see Web Table 1, available at http://aje.oxfordjournals.org/), 2 had a very limited number of cases ($n = 29$ and $n = 35$) with which to evaluate associations. Investigators of both of these studies reported statistically nonsignificant inverse associations after controlling for current cigarettes per day (21) and smoking status (22). The remaining 5 studies were able to control for smoking in various ways. In a cohort study of Finnish men who smoked, there was no association between selenium levels and bladder cancer after additional control for smoking by dose and duration (relative risk in the highest tertile = 0.9, 95% CI: 0.46, 1.76; *P* for trend = 0.79) (23). In a nested case-control study carried out in the United States, Michaud et al. (10) reported an inverse association among women, and a nonsignificant increased risk among men with higher selenium levels. Their results were nearly identical regardless of whether they controlled for smoking status (never, former, current) or additionally controlled for pack-years and current heavy smoking for both men and women. However, a potentially important difference in study design between this study by Michaud et al. and other studies was that cases and controls were matched on smoking status. The percentage of women who were reported to be current smokers was nearly 50%, in contrast to the men in the study, 16% of whom were current smokers, which might also contribute to the differing results for men and women. In a case-control study that controlled for pack-years, Wallace et al. (9) reported no association with toenail selenium levels (OR = 0.9, 95% CI: 0.68, 1.19). Among never smokers in that study, there was no statistically significant association for any category of exposure and no trend (*P* for trend = 0.12). In the remaining 2 studies, there was some evidence of an inverse association between selenium levels and bladder cancer in the primary analyses. In the study by Zeegers et al., the overall odds ratio for the highest level of exposure was 0.67 (95% CI: 0.47, 0.96; *P* for trend < 0.01) after controlling for cigarettes per day and years of smoking (20). However, when the results were stratified by smoking status, similar to the results of our study, there was no evidence of an association in never smokers, where the odds ratio for the highest category was 1.36 (95% CI: 0.5, 3.69; *P* for trend = 0.62), or among current smokers after control for years of smoking and cigarettes per day, where the odds ratio in the highest quintile was 1.13 (95% CI: 0.56, 2.26; *P* for trend = 0.62). The inverse association was observed only among the former smokers. However, we saw no such association among former smokers in our study. In the final study, Kellen et al. (24) reported an inverse association after controlling for current smoking status, cigarettes per day, and duration of smoking in a case-control study of 178 cases and 362 controls. Findings from these 7 studies were summarized in a recent meta-analysis, in which Amaral et al. (7) reported an overall meta–odds ratio of 0.61 (95% CI: 0.42, 0.87). However, when the results were restricted to the 2 studies with specific information on never smokers there was no association (OR = 0.85; 95% CI: 0.54, 1.34), which was reflected in both of the studies individually (9, 20). This is consistent with the results of our study. Amaral et al. noted that smoking was a potential source of considerable heterogeneity. In 1 other small study of transitional cell carcinoma, Yalçin et al. (25) reported lower selenium levels in cases ($n = 68$) than in controls ($n = 23$), but the authors did not report selenium levels or adjust for any potentially confounding factors, making interpretation difficult.

Evidence of lack of an association between selenium and bladder cancer further comes from other sources. First, in an analysis from the Selenium and Vitamin E Cancer Prevention Trial (SELECT), a randomized controlled trial designed to

evaluate the effect of selenium and vitamin E supplementation, there were no differences in bladder cancer risk between men in the placebo group and those receiving selenium supplementation alone (hazard ratio = 1.13, 95% CI: 0.70, 1.84, $P = 0.52$) or with vitamin E (hazard ratio = 1.05, 95% CI: 0.63, 1.70, $P = 0.86$) (26). Other observational studies also provided some evidence of no association between selenium and bladder cancer risk, including an analysis from the Vitamins and Lifestyle (VITAL) Study, which found no association between selenium supplementation and subsequent bladder cancer risk (27). Two other studies evaluated all urinary tract cancers and found no association between selenium levels and kidney and bladder cancer combined (28, 29).

In our study, results did not vary by sex, based on 249 female cases, 335 female controls, 809 male cases, and 936 male controls. Two previous studies reported sex-specific estimates. Given the small number of women in other studies of bladder cancer, most were unable to evaluate sex differences. In addition to the study by Michaud et al. (10) described previously, which showed evidence of association among women but not men, there was a population-based case-control study that evaluated sex differences (9). With 206 female cases, there was evidence of interaction with sex (P for interaction = 0.06), with the suggestion of an inverse association reported among the women but not the men (9), although with limited statistical power.

Observed differences between studies might be partially due to differing selenium levels. In our study, the levels of selenium in toenails were more similar to values reported in studies conducted in the United States and Finland (9, 10, 23) than to the study in the Netherlands, which were lower than the values reported here (20). Other studies reported selenium levels in serum (21, 22, 24), so it was not possible to directly compare levels with those observed in our study. However, in most of the United States, selenium intake is generally high compared with other parts of the world, including parts of Europe (30), where many of the other studies were conducted. It has been suggested that any beneficial health effects of selenium might be most pronounced in populations in which there is a selenium deficiency (31), which does not appear to have been the case in our study.

Another possibility is that selenium might be related to certain subgroups of tumors based on their molecular phenotype. This was observed in the study by Wallace et al. (9), in which an inverse association was observed among persons with bladder tumors with p53 alterations. In the study by Zeegers et al. (20), a stronger inverse association was observed among invasive tumors than among noninvasive tumors, and our study was consistent with this, but only weakly so.

To our knowledge, our investigation represents the largest study of selenium body burden and bladder cancer to date, with 1,058 cases and 1,271 controls. This, combined with our comprehensive information on smoking, allowed us to fully evaluate whether smoking impacted the association between selenium and bladder cancer. Another strength of our study was the large number of female cases, considerably more than any other study published to date, which allowed us to evaluate possible sex differences with statistical precision.

Selenium levels were based on 1-time measurements in the toenails collected post-diagnosis, which is a limitation. Toenail selenium levels are estimated to represent approximately 12 months of exposure (14), and levels in toenails have been shown to reflect selenium levels when selenium supplementation is given, suggesting that it is a reasonable biomarker of exposure (32). Additionally, a 1-time measurement of selenium has been shown to be correlated with intake and long-term exposure, particularly for the ranking of individuals (33, 34). However, we were unable to account for the species of selenium in this analysis; other studies have suggested that different selenium species might exert different biological properties and differentially influence health outcomes (35).

In summary, in this large population-based case-control study, with adequate control for smoking, we concluded that toenail selenium levels were not associated with bladder cancer risk. Although the exact mechanism is unknown, there is mounting evidence that cigarette smoking reduces selenium levels in the body. In conclusion, to evaluate whether selenium might influence bladder cancer risk over a range of dietary selenium levels, it is important to fully consider the impact of smoking on such associations. Despite reports of inverse associations between selenium and bladder cancer, most studies, including this one, that have been able to control for smoking intensity or duration in their evaluation have shown no such association.

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(Appendix follows)

Appendix Table 1. Odds Ratios for the Association Between Toenail Selenium Concentration and Bladder Cancer, by Tumor Invasiveness, in the New England Bladder Cancer Study, 2001–2004

Selenium Concentration, μg/g	Tumor Invasiveness							
	Superficial (TNM <T2)				Invasive (TNM ≥T2)			
	No. of Controls	No. of Cases	OR[a]	95% CI	No. of Controls	No. of Cases	OR[a]	95% CI
≤0.81	315	278	1.00	Referent	315	61	1.00	Referent
>0.81–0.89	312	219	0.96	0.74, 1.2	312	27	0.59	0.36, 0.98
>0.89–1.00	319	198	0.90	0.7, 1.2	319	31	0.75	0.46, 1.22
>1.00	325	215	1.0	0.8, 1.3	325	27	0.68	0.40, 1.12
P for trend[b]			0.80				0.19	

Abbreviations: CI, confidence interval; OR, odds ratio; TNM, tumor, node, metastasis scale.

[a] Estimates were adjusted for age, state, ethnicity, and pack-years of smoking.

[b] Tests for linear trend were carried out by including the median value from each quartile of toenail trace element concentration as a continuous variable in the logistic regression model.