

Serum selenium and the risk of cervical cancer among women in the United States

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Abstract

Objective: To explore the relationship between serum selenium and cervical cancer.

Methods: We conducted a case–control study of cervical cancer in five areas around Birmingham, AL; Chicago, IL; Denver, CO; Miami, FL; and Philadelphia, PA. Community controls were selected by random-digit dialing and were matched to invasive cervical cancer cases by age, race/ethnicity, and telephone exchange. Serum selenium was determined by neutron activation analysis. Logistic regression analysis controlling for known risk factors of cervical cancer, including human papillomavirus (HPV) type-16 measured serologically, was performed on 227 invasive cases, 127 *in-situ* cases, and 526 controls.

Results: Values of serum selenium ranged from 67.5 to 185.0 ng/ml. Adjusted odds ratios for invasive cervical cancer by quintile were: 1.0 (highest selenium), 1.1, 1.0, 0.8, and 1.0 (lowest selenium), p for trend = 0.82. Similar patterns were observed for Stage I invasive, and Stages II–IV invasive cases, suggesting severity of disease did not influence the null results. Although no associations were seen among current or never smokers, a protective effect of selenium was suggested among former smokers. Effect modification was not evident for other variables examined.

Conclusions: This study does not support a relationship between serum selenium and invasive cervical cancer at typical serum selenium levels in the US.

Introduction

Selenium is an essential trace element for growth and development in humans [1]. Selenium is present in humans in at least 11 selenoproteins [2]. Severe selenium deficiency has been associated with cardiomyopathies,

including Keshan disease. The relationship of selenium to cancer prevention is actively under study.

Studies indicate chemopreventive effects of specific forms of dietary selenium in excess of nutritional requirements in a variety of animal models, including for example chemical induction in rat mammary glands [3] and chemical induction in mouse uterine cervix [4]. There is a wide variety of naturally occurring selenium-containing active chemical compounds. Although there is incomplete understanding of their precise biochemical actions, it is currently thought that selenium's roles in

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cancer prevention are through antioxidant enzymes and anticarcinogenic metabolites [5].

In regions of China where selenium deficiency is endemic, selenium supplementation was associated with reduced liver cancer incidence both in a general population and among hepatitis surface-antigen carriers [6]; selenium taken with β -carotene and vitamin E was associated with reduced stomach cancer mortality [7]. However, no protective effects were seen in patients with esophageal dysplasia [8]. In India a trial of vitamin A, riboflavin, zinc, and selenium in former smokers found a reduction in oral lesions in the treatment group [9]. The Nutritional Prevention of Cancer with Selenium Trial, a randomized, placebo-controlled trial conducted in the US, found no effect of daily supplementation with 200 μ g of selenium as selenized yeast on recurrent skin cancer, but did find a protective effect in the treatment group for lung, colorectal, and prostate cancers [10, 11].

The relationship of selenium to cervical cancer risk has not been studied extensively. While about 90% of cervical cancers throughout the world are attributed to infection with human papillomaviruses (HPV) [12], not all women exposed to HPV contract cancer. Thus, it is important to study potential co-factors. While animal models for HPV exposure of cervical tissue have been developed [13], no studies have used these models to examine the effect of selenium. Of the five human observational studies of *in-situ* or invasive cervical cancer and selenium identified, four found no relationship [14–17], and one found a statistically significant inverse relationship [18]. However, these studies were limited in size and/or in assessment and control of other known risk factors for cervical cancer.

During 1982–1983 a large case–control study of invasive cervical cancer with community controls was conducted at five sites in the US. The study was designed to explore relationships between various behavioral and nutritional factors and cervical cancer. This article presents results of analyses on serum selenium and invasive and *in-situ* cervical cancer.

Materials and methods

Study design

The study sample was drawn from five US areas reporting to the Comprehensive Cancer Patient Data System centered around Birmingham, Alabama; Chicago, Illinois; Denver, Colorado; Miami, Florida; and Philadelphia, Pennsylvania. Eligible invasive cases were all residents of these communities aged 20–74 years who were diagnosed with histologically confirmed primary

invasive cancer of the uterine cervix during the period 1 April 1982–31 December 1983, at any of 24 participating hospitals. Eligible *in-situ* cases were newly diagnosed with histologically confirmed *in situ* cancer of the uterine cervix during the period 15 April 1982–31 August 1983 (Birmingham, Miami, and Philadelphia) or 15 April 1982–31 December 1983 (Chicago and Denver). Controls from the same communities were selected by random-digit dialing. Up to two controls were individually matched to each invasive case on age (± 5 years), race/ethnicity (white, Latino, African-American), and telephone exchange (first six digits). Controls were not matched to *in-situ* cases. About 26% of the selected controls reported a hysterectomy and were replaced with others from the control pool. Cases and controls with a history of cancer of other female genital organs were excluded.

Of the cases and controls eligible for the study, interviews were conducted with 481 invasive cases (73%), 293 *in-situ* cases (76%), and 801 controls (72%). After examination of staging data, one case previously considered invasive was reclassified to *in-situ*. Blood was obtained from 545 controls (68% of those interviewed), and at least six months after treatment from 245 invasive cases (51%) and 211 *in-situ* cases (72%). Reasons for non-participation in the interview and blood phases of the study are given in Brinton *et al.* [19], Jones *et al.* [20], and Weinstein *et al.* [21]. Subjects who participated in the blood draw were more often white, from particular study sites, and of higher socioeconomic status than were those who only completed the interview [21].

Insufficient serum was available to assay selenium levels for 11 invasive cases, four *in-situ* cases, and 16 controls. We excluded from this analysis subjects whose race/ethnicity was other than white, African-American, or Latino (seven invasive cases, three *in-situ* cases, and two controls) because their numbers in the population were too small to allow matching on race/ethnicity. One control who reported on interview possible cervical cancer was also excluded from this analysis.

Relatively few *in-situ* cervical cancer cases were identified in all study sites except Denver, because most cases of *in-situ* cancer are treated at local community hospitals rather than referred to regional medical centers, which provided most of the cases for this study. The Denver site, however, ascertained all *in-situ* cases because all hospitals in the defined geographical area were included. Because of the representativeness of the *in-situ* case series from Denver, analyses on *in-situ* cancer presented here are limited to Denver cases and controls. See Ziegler *et al.* [22] for further details.

Table 1 gives characteristics of the study participants included in these analyses: 227 invasive cases, 127 *in-situ*

Table 1. Characteristics of invasive and *in-situ* cases and controls

Demographic characteristic	Invasive cases (n = 227)		<i>In-situ</i> cases ^a (n = 127)		Controls (n = 526)	
	No.	Percentage	No.	Percentage	No.	Percentage
Age at diagnosis (years)						
<35	7	21	52	41	136	26
35–44	67	29	42	33	158	30
45–54	58	26	20	16	109	21
55+	55	24	13	10	123	23
Race/ethnicity						
White	155	68	120	94	358	68
African-American	49	22	1	1	130	25
Latino	23	10	6	5	38	7
Study site						
Birmingham	50	22	–	–	101	19
Chicago	38	17	–	–	116	22
Denver	63	28	127	100	129	25
Miami	32	14	–	–	72	14
Philadelphia	44	19	–	–	108	20

^a Only *in-situ* cases from Denver were included in the analysis.

cases, and 526 controls. The distribution of cases was comparable to that of the controls on age, ethnicity, and study site. However, cases who donated blood appeared to be of a lower socioeconomic status than controls, based on their report of less education ($p=0.001$) and lower income ($p=0.001$) [23]. Controls in Denver ($n=129$) served as controls for the Denver invasive cases and *in-situ* cases. *In-situ* cases in Denver were systematically oversampled from higher age strata, in order to reduce the age discrepancy anticipated between *in-situ* cases and controls matched on age to invasive cases. However, this procedure could not fully eliminate the discrepancy: *in-situ* cases continued to be younger and likely differed in other respects from the invasive controls.

Data collection procedures

Informed consent was obtained from all participants in the study. A questionnaire requesting detailed information concerning demographic characteristics, sexual behavior, reproductive and menstrual history, use of contraceptives and female hormones, personal and family medical history, smoking, and diet was administered to each subject in her home by a trained interviewer. The relationships of dietary variables to *in-situ* and invasive cancer have been presented previously [22, 24].

For all participating invasive and *in-situ* cases, telephone questionnaires were periodically administered in order to ascertain when each woman had completed all treatment. Blood was drawn from participants with

invasive and *in-situ* disease at least six months after completion of treatment (days after treatment: 10th, 50th, 90th percentiles = 190, 342, and 669 days, respectively). Approximately 40 ml of blood were drawn from each patient into four vacutainer tubes, including three 15 ml serum separator (tiger-top) tubes. Samples were allowed to clot for 40 min at room temperature, and were then centrifuged to separate the serum. Serum samples were stored frozen at -70°C from the time of blood draw (March 1983–October 1985) until laboratory analysis.

Determination of serum selenium

Samples were prepared in batches of 20 subjects. Cases and their matched controls were included in the same batch. Two blinded quality control samples, both at either a low or a normal selenium concentration, were included in each batch.

Duplicate samples of 0.5 ml each were prepared and analyzed for selenium at the University of Missouri–Columbia Research Reactor Center by instrumental neutron activation analysis (INAA) using the Se-77m isotope [25], methodology used in other previous studies [14, 16, 17, 26]. This technique detects serum selenium that is bound to protein or to some other high molecular weight species.

The coefficients of variation (CV) between duplicates for serum selenium were nearly always under 10%, averaging 2.8% across all 977 samples analyzed. In each batch of samples, quality control procedures included analyses of National Institute of Standards and Technology Standard Reference Material #1577 Bovine

Liver and a human sera standard prepared at the University of Missouri laboratories by combining sera from two donors. For the latter, a set of serum standards was created by taking aliquots of four different quantities of human sera (3 ml, 2.5 ml, 1.5 ml, and 0.75 ml) and normalizing them to 3 ml each by adding saline solution. In addition, blinded quality control samples were monitored by NCI based on rules by Westgard *et al.* [27], and all batches were found to be within the acceptable range. For these quality control samples, the CV for the low serum values (mean = 83.6 ng/ml; SD = 5.4) was 4.7% and for normal serum values (mean = 95.8 ng/ml; SD = 7.3) was 3.4%. The mean serum sodium concentration observed among the samples (excluding the quality control samples) was 3201 $\mu\text{g/ml}$, within the expected range for normal sera, indicating no significant evaporation of the samples during long-term storage.

Human papillomavirus (HPV) type-16

The test for HPV type-16 serum antibodies used a well-characterized HPV-16 virus-like particle, enzyme-linked immunosorbent assay (ELISA) [28]. Samples were tested in duplicate. Prior to being averaged, the optical density (OD) readings were adjusted according to results of three control samples run in triplicate in each batch, to control for between-day and between-batch variability. An average OD < 0.904 was classified as seronegative; an average OD > 1.017 was classified as seropositive. Intermediate values (3.6% of the subjects tested) were considered indeterminate [28].

Analytical procedures

The odds ratio (OR) was used to estimate the relative risk of experiencing cervical cancer at different quantiles of the serum selenium distribution. Quantiles (given in tables) were defined based on the frequency distribution of the controls. Logistic regression was performed to obtain maximum-likelihood estimates of the ORs and corresponding 95% confidence intervals (CI), while adjusting for potential confounders. Tests for trend were obtained by assigning to each quantile level of serum selenium the median value of the controls, and treating this as a continuous variable in the logistic model.

We considered three models: a “design-adjusted” model, an “adjusted without HPV-16” model, and a “fully adjusted” model. The first adjusted for design variables alone (age at diagnosis, race/ethnicity, study center). The second adjusted for the design variables as well as potential confounding variables identified in previous analyses of these data [19, 22, 24] but not HPV-

16 serologic status. These variables were included if their addition to the (otherwise) fully adjusted model changed the OR for any stratum by at least 0.1. This second group of potential confounders included in the fully adjusted model includes: number of sexual partners, age at first intercourse, years of oral contraceptive use, history of nonspecific genital infection, years since last Pap smear, number of abnormal Pap smears, and cigarette smoking status and intensity. The third model adjusted for all the variables in the second model and HPV-16 serologic status. Effect modification was evaluated for selected variables by testing the statistical significance of added interaction terms in the fully adjusted model and by stratified analyses.

For many cases and controls who participated in the blood phase of the study, their individually matched controls and cases did not participate in this phase. Thus, conditional analyses requiring individually matched case-controls were based on much smaller numbers and were less stable than unconditional analyses. The results presented are those from the unmatched (*i.e.* unconditional) analyses. However, for the principal findings of the study, results of conditional analyses confirmed results of the unconditional analyses.

Results

The distribution of serum selenium varied across the sites in the study. Serum selenium levels were highest in Denver and Chicago, sites closest to the high selenium belt in South Dakota, lower in Miami and Philadelphia, areas of low selenium soil concentration, and lowest in Birmingham (Figure 1). While Alabama is listed as an adequate selenium area, bordering Georgia is a low selenium area.

HPV exposure is believed to be responsible for over 90% of all invasive cervical cancers [29]. The HPV-16

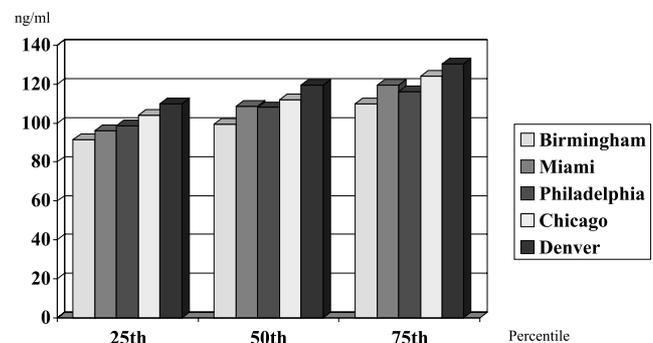


Fig. 1. 25th, 50th, and 75th percentile serum selenium (ng/ml) of controls by study center.

ELISA test used in our study had some limitations: it may be less sensitive than DNA hybridization assays, which require cervical tissue scrapings; seropositivity decreases in some women after surgical treatment for cervical cancer; and it tested for only one of the oncogenic HPV strains. However, only serum was available for HPV testing in this study. Despite the limitations, the design-adjusted risk estimate of invasive cervical cancer for seropositive HPV-16 exposure was 3.5 (95% CI = 2.35–5.09).

The design-adjusted, adjusted without HPV, and fully adjusted risk estimates of invasive cervical cancer, by quintile of serum selenium, are shown in Table 2. None of the risk estimates for any of the models was significantly different from 1.0; no trend in risk estimates across quintiles was apparent. Risk estimates varied only slightly among the three models presented, indicating minimal confounding of risk estimates from the broad array of risk factors included.

To examine the role of selenium among women who had been exposed to HPV, we examined the association of selenium with cervical cancer risk using only the controls with positive HPV-16 serologic tests. All cases were assumed to have been exposed to oncogenic HPV at one time, even though HPV-16 status was positive only in 33%. Again, no relationship between serum selenium and invasive cervical cancer was found; the fully adjusted odds ratios for the highest to lowest serum quartiles were 1.0, 1.1, 1.1, and 1.1.

In order to examine the possibility of a non-linear relationship between serum selenium and invasive cervical cancer, we estimated the adjusted risks by decile of serum selenium. Again, neither the individual risk estimates nor the test for trend was statistically signifi-

cant, nor was there any apparent curvilinear relationship or threshold effect evident in any of the three models evaluated. The fully adjusted odds ratios for the highest to lowest serum deciles were 1.0, 0.8, 0.8, 1.1, 0.6, 1.2, 0.4, 1.1, 1.1, and 0.7.

We explored the possibility of effect modification by demographic characteristics and selected risk factors, including smoking. There was no evidence for effect modification by study center, race/ethnicity, age at first intercourse, and number of sexual partners.

A potential effect modification emerged for smoking (Table 3). For both never smokers and current smokers, the lack of relationship between serum selenium and cervical cancer persisted. However, for former smokers there was a suggestion of an elevated risk in the lowest serum selenium quartile (OR = 4.6; 95% CI = 0.89–23.60); the test for trend was marginally statistically significant ($p = 0.054$). We further examined this possible relationship in former smokers for light vs heavy cigarette smoking (data not shown). Although based on small numbers, the risk among former smokers in this sample did not appear to differ by smoking intensity.

We explored the associations of serum selenium with cervical cancer risk in three subgroups that differed by severity of disease: *in-situ* cases (Denver site only), Stage I invasive cases, and Stages II–IV invasive cases. For *in-situ* cases (Table 4), after adjustment for the design variables, estimated risks increased with decreasing serum selenium ($p = 0.009$), and the lowest level of serum selenium was associated with an OR of 2.7 (CI = 1.17–6.34). When confounders (including HPV-16 status) were added to the model, the excess risk observed at the lower selenium levels noticeably decreased; neither any of the individual risk estimates nor the overall trend

Table 2. Design-adjusted, adjusted without HPV-16, and fully adjusted odds ratios (OR) of invasive cervical cancer by quintile of serum selenium (ng/ml)

Selenium quintile	No.		Design-adjusted ^a OR	Adjusted without HPV-16 ^b OR	Fully adjusted ^c	
	Cases	Controls			OR	95% CI
Highest (>124.0 ng/ml)	45	104	1.00	1.00	1.00	–
4 (114–124.0 ng/ml)	46	107	0.98	1.02	1.06	0.59–1.90
3 (107–113.9 ng/ml)	44	104	0.99	0.99	1.00	0.55–1.82
2 (97.5–106.9 ng/ml)	40	102	0.94	0.88	0.81	0.43–1.50
Lowest (<97.5 ng/ml)	52	109	1.10	1.06	1.01	0.55–1.86
<i>p</i> for trend			0.77	0.96	0.82	

^a Adjusted for age at diagnosis, race/ethnicity, and study center.

^b Adjusted for age at diagnosis, race/ethnicity, study center, number of sexual partners, age at first intercourse, years of oral contraceptive use, history of nonspecific genital infection, smoking status and intensity, years since last Pap smear, and number of abnormal Pap smears. One control was dropped from the analysis due to missing data on smoking status.

^c Adjusted for age at diagnosis, race/ethnicity, study center, HPV-16 serologic status, number of sexual partners, age at first intercourse, years of oral contraceptive use, history of nonspecific genital infection, smoking status and intensity, years since last Pap smear, and number of abnormal Pap smears. Three cases and two controls were dropped from the analysis due to missing data on HPV or smoking status.

Table 3. Fully adjusted odds ratios (OR) of invasive cervical cancer by quartile of serum selenium (ng/ml) by smoking status

Selenium quartile ^b	Never smokers				Former smokers				Current smokers			
	No.		Fully adjusted ^a		No.		Fully adjusted ^a		No.		Fully adjusted ^a	
	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI
Highest	20	70	1.00	–	13	24	1.00	–	20	40	1.00	–
3	28	71	1.14	0.50–2.59	14	23	1.49	0.31–7.21	14	40	0.82	0.30–2.24
2	28	67	1.45	0.63–3.32	11	22	1.85	0.35–9.83	17	38	1.14	0.43–3.04
Lowest	20	69	0.68	0.27–1.74	21	23	4.60	0.89–23.60	19	39	0.75	0.27–2.07
<i>p</i> for trend			0.28				0.05				0.84	

^a Adjusted for age at diagnosis, race/ethnicity, study center, HPV-16 serologic status, number of sexual partners, age at first intercourse, years of oral contraceptive use, history of nonspecific genital infection, years since last Pap smear, and number of abnormal Pap smears. Three cases and two controls were dropped from the analysis due to missing data on HPV or smoking status.

^b Quartiles were defined for each stratum using the controls in that stratum. Cut-points are: never smokers: 99.50, 110.00, 121.00; former smokers: 99.75, 111.00, 122.50; current smokers: 98.00, 110.00, 121.50.

Table 4. Crude and adjusted odds ratios (OR) of *in-situ* cervical cancer by quintile of serum selenium (ng/ml): Denver cases and controls only

Selenium quintile	No.		Design-adjusted ^a		Fully adjusted ^b	
	Cases	Controls	OR	95% CI	OR	95% CI
Highest (>133.5 ng/ml)	14	26	1.00	–	1.00	–
4 (124.5–133.5 ng/ml)	18	25	1.28	0.51–3.20	0.92	0.29–2.95
3 (116.0–124.4 ng/ml)	23	27	1.52	0.63–3.67	1.09	0.35–3.35
2 (108.5–115.9 ng/ml)	28	26	1.91	0.80–4.55	2.12	0.68–6.57
Lowest (<108.5 ng/ml)	44	25	2.72	1.17–6.34	1.63	0.54–4.88
<i>p</i> for trend			0.01		0.16	

^a Adjusted for age at diagnosis and race/ethnicity.

^b Adjusted for age at diagnosis, race/ethnicity, HPV-16 serologic status, number of sexual partners, age at first intercourse, years of oral contraceptive use, history of nonspecific genital infection, smoking status and intensity, years since last Pap smear, and number of abnormal Pap smears.

Table 5. Fully adjusted odds ratios (OR) of invasive cervical cancer by quartile of serum selenium (ng/ml) for Stage I and Stage II–IV disease

Selenium quartile	Stage I				Stage II–IV			
	No.		Fully adjusted ^a		No.		Fully adjusted ^a	
	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI
Highest (>121.0 ng/ml)	28	133	1.00	–	11	133	1.00	–
3 (110.0–121.0 ng/ml)	31	137	0.94	0.49–1.80	15	137	1.02	0.36–2.90
2 (99.0–109.9 ng/ml)	41	128	1.23	0.65–2.31	7	128	0.47	0.14–1.53
Lowest (<99.0 ng/ml)	33	126	0.93	0.47–1.84	15	126	1.24	0.43–3.58
<i>p</i> for trend			0.78				0.36	

^a Adjusted for age at diagnosis, race/ethnicity, study center, HPV-16 serologic status, number of sexual partners, age at first intercourse, years of oral contraceptive use, history of nonspecific genital infection, smoking status and intensity, years since last Pap smear, and number of abnormal Pap smears. Forty-nine cases were dropped from the analysis because of missing data on stage or HPV; two controls were dropped because of missing data on HPV or smoking status.

(*p* = 0.16) was statistically significant (Table 4). Serum selenium level was unrelated to cervical cancer for both Stage I invasive disease and Stages II–IV invasive disease (Table 5).

A concern with the retrospective design is the possibility that the treatment may be affecting serum selenium levels. In our study we compared pre-treatment serum selenium values with post-treatment serum sele-

mium values for 20 invasive cases and found a non-significant ($p = 0.19$) increase in mean serum selenium levels of 5.4 ng/ml in the post-treatment samples.

Discussion

The role of selenium in cancer etiology is unclear. While many studies have found inverse associations between selenium and cancer, others have found that selenium may enhance certain cancers in humans [26, 30]. Selenium has antioxidant and antiproliferative properties [31, 32], and has been shown in some animal models to inhibit initiation and promotion [33]. Because selenium's actions are associated with many different selenoenzymes and metabolites, its chemopreventive effects may occur in both nutritionally deficient states and adequate states [5], and may even require pharmacologic levels comparable to the supplemental selenium provided in the Nutritional Prevention of Cancer with Selenium Trial [10].

The role of selenium in cervical cancer etiology has not been extensively studied. In an ecological study in China, serum selenium was negatively but not significantly correlated with cervical cancer mortality rates [34]. Of the five observational studies published, two were case-control studies nested in cohorts, using prospectively collected blood [14, 17] and three were case-control studies with blood or dietary information collected after cancer diagnosis [15, 16, 18]. While two studies provided data on more than 100 *in-situ* cancers [15, 16], none provided data on more than 40 invasive cases. Four studies used serum or plasma selenium as the selenium measure [14, 16–18]; one used dietary selenium [15]. Of these five studies, only one [18] reported a statistically significant inverse association between serum selenium and cervical cancer. However, that study reported on a small case series (37 invasive cases) and did not ascertain or control for accepted risk factors in cervical cancer etiology. Our study provides data on a large, broadly representative, case series (227 invasives), utilizes neighborhood matched controls, and adjusts for potential confounding by known risk factors for cervical cancer, including HPV. We found generally no relationship between serum selenium and invasive cervical cancer, consistent with most of the cervical cancer findings published thus far.

Because those who participated in the blood draw phase of the overall study and who are analyzed here differed from those who participated only in the interview phase of the study, the possibility of participation bias arises. However, cases and controls who participated in the blood phase of the study did not

differ from each other in the study matching factors of age, race/ethnicity, or study site. In addition, risk estimates of invasive cancer for socioeconomic status, age at first intercourse, number of sexual partners, time since last Pap smear, and other cervical cancer risk factors were computed for the interviewed subjects and only those participating in the blood draw. Similar patterns of risk were seen for both groups, suggesting minimal participation bias [21].

Exposure to oncogenic HPV is a dominant risk factor for cervical cancer. We attempted to control for its potential confounding effect by including in our models HPV-16 serologic status as well as questionnaire-derived variables indicative of timing and extent of exposure. We compared risk estimates for cervical cancer by serum selenium levels using different models: without any HPV variables, with questionnaire-derived variables, and with in addition HPV-serologic status. In addition, we examined the relationship of selenium to cervical cancer restricting the analyses to only women who were likely to have a history of HPV infection. In all analyses the results showed consistently no relationship between serum selenium and invasive cervical cancer. Thus, while exposure to oncogenic HPV was imperfectly captured, there was no evidence that the imperfect measure was altering the selenium-cervical cancer relationship.

A potential limitation of this study is that serum was collected from cases after diagnosis of cervical cancer and subsequent treatment. In addition, the serum selenium measure is a short-term assessment and not an integrated measure of long-term selenium status. However, failure to measure selenium status accurately during the critical period in carcinogenesis might have hidden a modest association, but is unlikely to have blurred a strong relationship.

To our knowledge no longitudinal data have been presented which compare blood selenium values for the same individuals before onset of cancer, at diagnosis, and during and following treatment. However, information is available for shorter periods along this continuum. Some studies have found decreased serum selenium levels with increased overall tumor burden [35, 36]; it has been shown that certain tumors concentrate selenium [37]. Studies have also shown decreased selenium levels after radiation therapy [35, 38], surgery [39], and during long-term parenteral feeding [35, 40].

We considered the possibility that either treatment or stage of disease had an impact on our findings. Our comparison of pre-treatment and post-treatment serum selenium levels in a small number of invasive cases indicated a non-significant increase in serum selenium, suggesting at most a minimal effect of treatment. Comparison of risk estimates for subjects with less

advanced Stage 1 disease to subjects with more advanced cervical cancer (Stages II–IV) showed similar null relationships between serum selenium and cervical cancer for early and advanced invasive disease, indicating no significant disease effect. Finally, if a strong treatment or disease effect were present, we would expect to see an inverse relationship between serum selenium and cervical cancer, which we did not observe.

While the data suggested a possible weak inverse relationship between serum selenium and *in-situ* cancer, we view this finding cautiously for several reasons. First, we would expect a strong inverse relationship in *in-situ* cases to lead to an inverse relationship in invasive cases, which we did not see. Second, risk estimates diminished dramatically as confounders were added to the model. While these estimates were still elevated at low selenium levels, they were not indicative of a trend and their confidence limits were wide (Table 4). A similar pattern of substantially attenuated risk estimates after controlling for confounding was observed in data from this study by Ziegler *et al.* [22] in an investigation of the role of dietary intake in *in-situ* cervical cancer. Finally, as noted earlier, Denver controls were selected to reflect the demographics of the invasive and not the *in-situ* cases. As a result there is a greater likelihood of bias and uncontrolled confounding in the analysis of these cases, even when the models incorporate the accepted covariates.

Many studies (reviewed in ref. 41, with commentary in ref. 42) have found associations between cigarette smoking and cervical cancer, independent of sexual behavior such as early age at first sexual intercourse and multiple sex partners. Some have found that the estimated relationships between cervical cancer and cigarette smoking increase with the amount and duration of cigarette smoking [41]. A significant association between smoking and cervical cancer was found in our study population [19].

Several mechanisms for a causal relationship of smoking to cervical cancer have been posited, for example, through its immunosuppressive effects, or as a co-carcinogen with HPV [42]. Cigarette smoking may lower serum levels of some antioxidants, including some carotenoids [43], vitamin C [44], and selenium. Cigarette smoking significantly lowered serum selenium in a subgroup nested in the Hypertension Detection Follow-Up Program [45]. In the Nurses' Health Study, current smokers had significantly lower toenail selenium levels compared to non-smokers; a significant dose-response was observed [46]. In our study, however, serum selenium levels in current smokers were not statistically significantly lower than serum selenium levels in never smokers.

We were interested in exploring the possibility that selenium might modify the effect of cigarette smoking on

cervical cancer. We found that serum selenium was marginally significantly protective for invasive cervical cancer among former smokers, but not current smokers. Clark *et al.* in their Nutritional Prevention of Cancer with Selenium Trial had a similar finding for total cancer incidence. Although not statistically significant, the effect of selenium treatment on incidence of total cancer was stronger among former smokers (RR = 0.68; 95% CI = 0.44–1.06) than among current smokers (RR = 0.82; 95% CI = 0.59–1.32) and never smokers (RR = 0.74; 95% CI = 0.38–1.42) (Safety and Monitoring Advisory Committee Technical Report, Clark LC, Combs Jr GF, and Turnbull, BW, 1994, unpublished). Nomura *et al.* found statistically significant trends of increased protection for prostate cancer with higher levels of serum selenium among current and former smokers but not among never smokers (47). These findings taken together suggest the possibility that selenium may be exerting some protection against the carcinogenic effects of smoking, but that this protection may be overwhelmed with years of smoking exposure. On the other hand, we had small numbers of smokers and former smokers, the confidence intervals were quite large, and we performed many statistical tests, so our finding may be spurious.

There is tremendous variation in blood selenium values throughout the world [48, 49]. For example, China has some of the lowest (16–21 ng/ml) and some of the highest (357–494 ng/ml) serum values; Europe generally falls on the lower end of the distribution (*e.g.* 63–94 ng/ml in Germany; 88–132 ng/ml in England), with the US somewhat higher (72–198 ng/ml). Blood values in our study were typical of those found in other US studies at that time [10, 48]. Blood values reported in the other observational cervical cancer studies range from a mean of 65/111 ng/ml (cases/controls) in Finland [18] to 125/125 ng/ml in Maryland [17]. The values of 162/162 ng/ml in Washington State reported by Coates *et al.* [14] are considered high and possibly biased. Our mean values were 111/111 ng/ml. A substantially higher level of serum selenium of 190 ng/ml was attained within the first year of supplementation in the intervention group of the Nutritional Prevention of Cancer with Selenium Trial [11]. It may be that, if selenium has a role in cervical cancer prevention, it is apparent only when a high blood value is achieved.

Conclusions

These data from a large retrospective study of cervical cancer do not support the existence of a relationship between serum selenium and cervical cancer in typical

serum selenium ranges in the US. The possibility of a protective effect of serum selenium on cervical cancer in populations with higher selenium exposure and among smokers or former smokers requires further exploration.

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