Levels of Zinc, Selenium, Calcium, and Iron in Benign Breast Tissue and Risk of Subsequent Breast Cancer

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Abstract

Previous studies that have assessed breast cancer in relation to zinc, selenium, calcium, and iron have yielded inconsistent results but have not measured breast tissue levels. In a case-control study involving 252 matched pairs nested in a cohort of 9,315 women with benign breast disease, we investigated these associations by directly measuring elemental levels in breast tissue using X-ray fluorescence spectroscopy. Quintile analyses revealed positive associations of breast cancer, of borderline statistical significance, with zinc [highest versus lowest quintile: odds ratio (OR), 1.37; 95% confidence limit (95% CL), 0.91, 2.05; P_{trend} = 0.04], iron (highest versus lowest quintile: OR, 1.58; 95% CL, 1.02, 2.44; P_{trend} = 0.07), and calcium (highest versus lowest quintile: OR, 1.46; 95% CL, 0.98, 2.17; P_{trend} = 0.14), but little association with selenium (highest versus lowest quintile: OR, 1.10; 95% CL, 0.72, 1.68; P_{trend} = 0.76). The associations were weakened by mutual adjustment. Furthermore, after stratification by menopausal status, the positive association between iron and breast cancer was confined to postmenopausal women (highest versus lowest quintile: OR, 2.77; 95% CL, 1.25, 6.13; P_{trend} = 0.008), whereas the associations for zinc, calcium, and selenium did not differ by menopausal stratum. In conclusion, our data raise the possibility that relatively high levels of zinc, iron, and calcium in benign breast tissue may be associated with a modest increase in risk of subsequent breast cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(8):1682–5)

Introduction

Deficiency of zinc, selenium, and calcium may contribute to mammary carcinogenesis due to the roles of these elements in regulating cell proliferation, differentiation, and apoptosis (1-3). Additionally, zinc and selenium have immune-enhancing and antioxidant effects (2, 4, 5). In contrast, excessive intake of iron may predispose to mammary tumorigenesis due to the fact that free iron works as a catalyst for the generation of reactive oxygen species and the suppression of host defense cells (6). However, there have been few epidemiologic studies assessing breast cancer risk in relation to zinc (7-14) and iron intake (9, 12, 14-16), and studies of selenium (8, 13, 17-34) and calcium (12, 14, 15, 35-43) have been inconclusive. In these studies, exposure was assessed either by using food frequency questionnaires or by measuring elemental concentrations in blood, toenails, or hair. Such measurements are generally subject to various limitations. For example, bioavailability of these elements is not taken into consideration in food frequency questionnaires, and blood levels of zinc and calcium are maintained homeostatically, which make them weak markers of their status in humans (44). Most importantly, these measurements do not necessarily reflect levels of these elements in mammary tissue. In the study reported here, we used X-ray fluorescence spectroscopy to directly measure levels of zinc, selenium, calcium, and iron in benign breast tissue and related them to risk of subsequent breast cancer.

Materials and Methods

Study Population. We conducted a case-control study of breast cancer nested within a cohort of 9,315 women who received a histopathologic diagnosis of benign breast disease between 1970 and 1994 at Kaiser Permanente Northwest. Women who were diagnosed with breast cancer before, or within 1 year of, their benign breast biopsy were excluded from the cohort. Incident breast cancer cases among this cohort were ascertained by linking the cohort records to the Kaiser Permanente Northwest Tumor Registry. The ascertainment was supplemented by examination of inpatient discharge logs, referrals to radiation oncologists, and surveillance of radiology reports marked as particularly suspicious for cancer. Controls were individually matched to their corresponding case on age, age at diagnosis of benign breast disease, and duration of Kaiser Permanente membership and were randomly selected from women in the corresponding stratum who were alive but had not developed breast cancer by the date of diagnosis of the corresponding case. A total of 252 matched case-control pairs were included.

Data Collection. For each study subject, one block of formalin-fixed, paraffin-embedded benign breast tissue was retrieved from the Kaiser Permanente Northwest Department of Pathology warehouse. A single 5-μm-thick section was cut from each tissue block by microtome and dry mounted onto Ultralene XRF films (SPEX CertiPrep, Metuchen, NJ). To avoid contamination, tissue sections were not stained. The tissue concentration of elements was measured by X-ray fluorescence (45) on beamline 2-ID-E of the Advanced Photon Source (Argonne National Laboratory, Argonne, IL). Briefly, 14.1-keV X-rays were used to illuminate a 0.5-mm-diameter area on each mounted sample. The area was chosen such that there were few ripples or folds, tissue-like structures were visible, and the measured spectral signature showed the presence of sulfur. Sulfur was detected in the breast tissue but not in the paraffin-only regions. Fluorescence spectra were acquired for 300 s, intensities were determined by fitting the fluorescence peaks with modified Gaussians, and elemental content was quantified by comparison to NIST standards NBS 1832 and NBS 1833. To minimize the influence of variation in the tissue density as well as to account for areas only partially containing tissue or with some folding over, we normalized

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Table 1. Baseline characteristics of the cases and controls

	n (%)	
	Cases (*N* = 252)	Controls (*N* = 252)
Age at menarche (y)		
<12	51 (20.3)	49 (19.4)
12	52 (20.6)	56 (22.2)
13	64 (25.4)	73 (29.0)
14+	62 (24.6)	55 (21.8)
Missing	23 (9.1)	19 (7.6)
Parity		
0	36 (14.3)	30 (11.9)
1-2	111 (44.1)	113 (44.8)
3-4	82 (32.5)	90 (35.7)
5+	19 (7.5)	15 (6.0)
Missing	4 (1.6)	4 (1.6)
Age at first live birth (y)		
Nulliparous	36 (14.3)	30 (11.9)
30+	25 (9.9)	19 (7.5)
25-<30	46 (18.3)	52 (20.6)
20-<25	93 (36.9)	106 (42.1)
<20	32 (12.7)	26 (10.3)
Missing	20 (7.9)	19 (7.5)
History of bilateral oophorectomy		
Yes	31 (12.3)	33 (13.1)
No	195 (77.4)	187 (74.2)
Missing	26 (10.3)	32 (12.7)
Family history of breast cancer		
Yes	43 (17.1)	34 (13.5)
No	195 (77.4)	209 (83.0)
Missing	14 (5.5)	9 (3.5)
Body mass index (kg/m^2)		
<25	119 (47.2)	119 (47.2)
25-<30	73 (29.0)	71 (28.2)
30+	46 (18.2)	48 (19.0)
Missing	14 (5.6)	14 (5.6)
Ever smoked cigarettes		
Yes	92 (36.5)	102 (40.5)
No	78 (31.0)	82 (32.5)
Missing	82 (32.5)	68 (27.0)
Menopausal status		
Premenopausal	110 (43.7)	96 (38.1)
Postmenopausal	125 (49.6)	138 (54.8)
Missing	17 (6.7)	18 (7.1)
Ever used oral contraceptives		
Reported	54 (21.4)	64 (25.4)
No/not reported*	198 (78.6)	188 (74.6)
Ever used postmenopausal hormones		
Reported	113 (44.8)	125 (49.6)
No/not reported*	139 (55.2)	127 (50.4)
Presence of proliferative changes in benign breast tissue		
Yes	172 (68.2)	150 (59.5)
No	80 (31.8)	102 (40.5)

*The medical records of 2 cases and 3 controls reported never use of oral contraceptives, whereas the records of the remaining subjects did not indicate ever use; for use of postmenopausal hormones, the records of 7 cases and 5 controls indicated never use.

the measured elemental concentrations by the sulfur content [(element concentration / sulfur content) × 100]. Element concentration data were obtained for 251 cases and 249 controls, among which there were 248 matched pairs. Data on well-documented risk factors for breast cancer were obtained by abstracting data from the Kaiser Permanente Northwest medical records. In addition, histologic sections were reviewed and classified using standard criteria (46).

Statistical Methods. The Wilcoxon rank-sum test was used to compare the concentrations of the elements in cases and controls. Associations of breast cancer with these elements were evaluated using conditional logistic regression. In multivariate models, we controlled for age at menarche, parity, age at first live birth, history of bilateral oophorectomy, family history of breast cancer, body mass index, smoking status, menopausal status, oral contraceptive use, postmenopausal hormone use, and presence of proliferative changes in the benign breast tissue. For tests of trend across successive levels of categorical variables, we assigned the categories their ordinal number and then fitted the resulting variable as continuous variables in the regression models. *P* values were two sided.

Results

The distribution of the study subjects by baseline characteristics and outcome is shown in Table 1. Higher proportions of cases than controls had a family history of breast cancer, had proliferative changes in their breast tissue, and were premenopausal; a smaller proportion had ever smoked cigarettes.

Breast tissue levels of zinc, calcium, selenium, and iron were slightly higher in the cases than in the controls, and the case-control differences for zinc and iron were statistically significant (Table 2). Quintile analyses revealed positive associations of breast cancer, of borderline statistical significance, with zinc [highest versus lowest quintile: odds ratio (OR), 1.37; 95% confidence limit (95% CL), 0.91, 2.05; P_{trend} = 0.04], iron (highest versus lowest quintile: OR, 1.58; 95% CL, 1.02, 2.44; P_{trend} = 0.07), and calcium (highest versus lowest quintile: OR, 1.46; 95% CL, 0.98, 2.17; P_{trend} = 0.14), but little association with selenium (highest versus lowest quintile: OR, 1.10; 95% CL, 0.72, 1.68; P_{trend} = 0.76; Table 3). Due to the fact that proliferative changes in the benign breast tissue might be an intermediate variable rather than a confounder, we also analyzed the data without adjusting for it in multivariate models. The results were similar to those yielded from models with adjustment for proliferative changes. Tissue levels of these elements were moderately strongly correlated (ranging from 0.10 for iron and calcium to 0.42 for iron and zinc). After mutual adjustment, the point estimates for zinc (highest versus lowest quintile: OR, 1.13; 95% CL, 0.66, 1.94; P_{trend} = 0.38) and iron (highest versus lowest quintile: OR, 1.45; 95% CL, 0.83, 2.56; P_{trend} = 0.28) were closer to unity, whereas that for calcium (highest versus lowest quintile: OR, 1.49; 95% CL, 0.92, 2.42; P_{trend} = 0.32) was largely unchanged; all confidence intervals were wider.

Stratified analyses showed little association of breast cancer with zinc, calcium, and selenium within strata defined by menopausal status and by the presence of proliferative changes in the benign breast tissue (data not shown). For iron, a positive association with breast cancer was observed among postmenopausal women (highest versus lowest quintile: OR, 2.77; 95% CL, 1.25, 6.13; P_{trend} = 0.008) but not among premenopausal women. Moreover, the association with iron among postmenopausal women remained positive (highest versus lowest quintile: OR, 3.68; 95% CL, 1.38, 9.82; P_{trend} = 0.02) after mutual adjustment for the other elements. Risk did not vary by the presence or absence of proliferative changes in the breast tissue (data not shown).

Table 2. Tissue levels of zinc, selenium, calcium, and iron in cases and controls

Element	[Tissue concentration (ng/cm^2) / sulfur content (ng/cm^2)] × 100		
	Median (interquartile range)		*P**
	Cases (*n* = 251)	Controls (*n* = 249)	
Zinc	0.91 (1.04)	0.81 (0.83)	0.01
Selenium	0.031 (0.027)	0.027 (0.023)	0.94
Calcium	8.33 (18)	7.48 (12)	0.17
Iron	2.38 (6.13)	2.12 (4.36)	0.04

**P* values were derived from the Wilcoxon rank-sum test with normal approximation.

Table 3. ORs and 95% CLs for associations between levels of zinc, selenium, calcium, and iron in breast tissue and subsequent breast cancer risk

Element	Quintiles					P_{trend}
	1	2	3	4	5	
Zinc						
Model 1*	1.0	+0.85 (0.53, 1.37)	1.15 (0.76, 1.73)	1.18 (0.78, 1.79)	1.37 (0.91, 2.05)	0.04
Model 2†	1.0	0.85 (0.53, 1.37)	1.16 (0.77, 1.75)	1.18 (0.78, 1.78)	1.32 (0.89, 1.98)	0.06
Selenium						
Model 1*	1.0	1.23 (0.84, 1.82)	0.92 (0.60, 1.40)	0.95 (0.61, 1.46)	1.10 (0.72, 1.68)	0.76
Model 2†	1.0	1.21 (0.82, 1.79)	0.94 (0.62, 1.43)	0.97 (0.63, 1.49)	1.06 (0.70, 1.62)	0.72
Calcium						
Model 1*	1.0	1.17 (0.77, 1.77)	1.04 (0.67, 1.63)	1.01 (0.65, 1.57)	1.46 (0.98, 2.17)	0.14
Model 2†	1.0	1.17 (0.77, 1.77)	1.03 (0.66, 1.61)	1.01 (0.65, 1.57)	1.44 (0.96, 2.14)	0.15
Iron						
Model 1*	1.0	1.41 (0.91, 2.18)	1.24 (0.78, 1.99)	1.45 (0.93, 2.27)	1.58 (1.02, 2.44)	0.07
Model 2†	1.0	1.42 (0.92, 2.20)	1.28 (0.80, 2.04)	1.45 (0.93, 2.28)	1.56 (1.01, 2.41)	0.08

*Adjusted for matching variables, age at menarche, parity, age at first live birth, history of bilateral oophorectomy, family history of breast cancer, body mass index, smoking status, menopausal status, oral contraceptive use, postmenopausal hormone use, and presence of proliferative changes in benign tissue.
† Adjusted for all of the variables in model 1 except for proliferative changes in benign breast tissue.

Discussion

To our knowledge, this is the first study that has prospectively assessed levels of zinc, selenium, calcium, and iron in breast tissue in relation to subsequent breast cancer risk. Among the strengths are the population-based study design and the evaluation of elemental concentration without knowledge of disease status. Our study, however, had several limitations. First, the study was restricted to women with benign breast disease, so that the study results cannot necessarily be extrapolated to women in general. Second, we did not elucidate the form of elements (e.g., valence state) or their binding status by using techniques such as micro-Xanes (x-ray absorption near edge structure) because of the significant amount of additional beamtime this would have required as well as the uncertainty about whether the chemical state of the elements of interest would have been preserved adequately during chemical fixation and paraffin embedding. Third, although we controlled for a panel of potential confounding factors in multivariate analyses, residual confounding cannot be excluded. Fourth, a moderate proportion of study subjects had missing values for at least one factor. Missing values were handled in several ways, including imputation by using the corresponding mean or median (for continuous variables), sensitivity analyses by including subjects with missing data into one category at a time (for categorical variables), and treating subjects with missing values as a separate category. The results of the analyses based on these approaches differed little from those presented here (data not shown). Fifth, because a significant proportion of the samples had selenium levels at or below the minimum detection level, we cannot rule out the possibility of an association between selenium and cancer risk at very low selenium levels.

In this study, there was some indication that relatively high levels of zinc and calcium in benign breast tissue were positively associated with risk of subsequent breast cancer. Although it is possible that this was a chance finding, it might suggest that benign breast tissue that accumulates relatively high concentrations of the essential elements zinc and calcium is predisposed to progress to breast cancer because an adequate supply of zinc and calcium is necessary to sustain the proliferation of breast tissue. Indeed, experimental studies have shown that zinc accumulates in *N*-methyl-*N*-nitrosourea–induced mammary tumors in rats and that low zinc intake can suppress *N*-methyl-*N*-nitrosourea–induced mammary tumorigenesis in rats (47, 48). However, whether low calcium intake can suppress chemical-induced mammary carcinogenesis has not been well documented.

Four hospital-based case-control studies have evaluated dietary iron intake in association with breast cancer risk, with two reporting inverse associations (15, 16) and the other two reporting null results (12, 14). In addition, a cohort study showed no association between toenail levels of iron and breast cancer risk (9). In contrast to these findings, our results indicated that a relatively high concentration of iron in benign breast tissue was positively associated with subsequent breast cancer risk. Due to the fact that we were unable to separate our measure of iron levels into free iron and iron in conjugation with enzymes, we are unable to differentiate between two possible explanations for this finding. One explanation is that high levels of free iron in benign breast tissue might increase breast cancer risk due to the catalytic effects of iron on mutagenic radicals and a suppressant effect on host immune function (6). The other explanation is that both proliferative benign breast tissue and breast cancer cells might demand high levels of iron to sustain their proliferation given that iron is required for ribonucleotide reductase, a key enzyme in DNA synthesis (49). Thus, benign breast tissue that accumulates a high concentration of iron might predispose to breast cancer. When examined by menopausal status, the association between iron and breast cancer risk was evident only in postmenopausal women. This might reflect the fact that iron tends to accumulate in intracellular complexes with increasing age, particularly in postmenopausal women, thereby increasing the likelihood of iron-induced oxidative damage (50).

In conclusion, our data do not support the hypothesis that levels of zinc, calcium, and selenium are associated with a decrease in breast cancer risk, and indeed, raise the possibility that zinc, calcium, and iron may be associated with a modest increase in risk of subsequent breast cancer among women with benign breast disease.

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