The Association Between Periodontal Disease and Breast Cancer in a Prospective Cohort Study

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ABSTRACT

Periodontal disease may be associated with increased breast cancer risk, but studies have not considered invasive breast cancer and ductal carcinoma *in situ* (DCIS) separately in the same population. We assessed the relationship between periodontal disease and breast cancer in a large prospective cohort study. The Sister Study followed women without prior breast cancer ages 35 to 74 years from 2003 to 2017 ($N = 49{,}968$). Baseline periodontal disease was self-reported, and incident breast cancer was ascertained over a mean follow-up of 9.3 years. We estimated hazard ratios (HR) and 95% confidence intervals (CI) using Cox proportional hazards regression, adjusting for multiple potential confounders, including smoking status. Heterogeneity in risk for invasive breast cancer versus DCIS was also estimated. About 22% of participants reported a history of periodontal disease at baseline. A total of 3,339 incident breast cancers (2,607 invasive breast cancer, 732 DCIS) were identified. There was no clear association between periodontal disease and overall breast cancer risk (HR = 1.02; 95% CI, 0.94–1.11). However, we observed a nonstatistically significant suggestive increased risk of invasive breast cancer (HR = 1.07; 95% CI, 0.97–1.17) and decreased risk of DCIS (HR = 0.86; 95% CI, 0.72–1.04) associated with periodontal disease, with evidence for heterogeneity in the risk associations (relative HR for invasive breast cancer versus DCIS = 1.24; 95% CI, 1.01–1.52). A case-only analysis for etiologic heterogeneity confirmed this difference. We observed no clear association between periodontal disease and overall breast cancer risk. The heterogeneity in risk associations for invasive breast cancer versus DCIS warrants further exploration.

Introduction

Characterized by chronic infection and inflammation (1), the prevalence of periodontal disease increases with age. Approximately 34% of U.S. female adults 30 years or older were affected by periodontal disease during 2009 to 2014 (2). Accumulating evidence suggests that periodontal disease is associated with increased risk of several cancers, especially oral cancer, lung cancer, and gastrointestinal cancer, with a hypothesized role of immune-inflammatory mechanisms that may be common to both periodontal disease and cancers (3–8).

Inflammation has also been implicated in the etiology of breast cancer, which is thought to be driven by immune system cells and associated inflammatory mediators in the tumor microenvironment (9). Studies also indicated that both breast tumor tissue and normal adjacent breast tissue from patients with breast cancer have different bacterial profiles from healthy controls (10, 11). Investigators have hypothesized that periodontal pathogens may directly influence breast carcinogenesis, citing evidence for the presence of oral bacterial species in breast tissues (12), whereas others have suggested that periodontal disease may influence and/or reflect systemic inflammatory processes that promote breast carcinogenesis (9, 13). However, no consensus has been reached for the relationship between periodontal disease and the risk of breast cancer, the most common cancer diagnosed among American women (14). Some studies have observed an increased risk of breast cancer among women with periodontal disease (6, 15, 16), whereas others have detected no relationship between periodontal disease and breast cancer risk (4, 17, 18). Two recent meta-analyses of studies reported that periodontal disease may increase breast cancer risk by about 1.2-fold (19, 20); however, notable limitations of prior studies included small sample sizes and inadequate adjustment for potential confounding factors. In prior studies, the sample sizes ranged from 151 (8) to 80,280 (6), with the Women's Health Initiative Observational Study (WHI OS; ref. 15) having the most breast cancer cases ($N = 2{,}124$), and all other studies each having fewer than 550 breast cancer cases. Larger prospective studies with robust multivariable adjustment, including controlling for the potential confounding effects of smoking, are needed to further examine the association between periodontal disease and breast cancer risk.

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Studies of periodontal disease appear to have only considered risk of overall breast cancer or invasive breast cancer, with no prior study having assessed the risk for invasive breast cancer and ductal carcinoma *in situ* (DCIS) separately. Although DCIS is considered to be a nonobligate precursor of invasive breast cancer (21), invasive breast cancers with or without history of DCIS have shown differences in tumor characteristics and protein expression, suggesting potential differences in etiologic risk factors (22). Furthermore, any observed differences in risk of invasive breast cancer versus DCIS associated with periodontal disease may point to important differences in behaviors and method of detection, because DCIS is largely identified through screening mammography (23).

Given the limited and conflicting evidence on the association between periodontal disease and breast cancer risk, we aimed to assess the relationship between periodontal disease and breast cancer, separately for risk of DCIS and invasive breast cancer, controlling for potential confounding factors, in a large, prospective cohort study.

Materials and Methods

Study population

The Sister Study is a nationwide prospective cohort study, which was designed to study environmental and genetic risk factors for breast cancer (24). Briefly, 50,884 women ages 35 to 74, who had a sister diagnosed with breast cancer, but were without prior breast cancer themselves, were enrolled during 2003 to 2009. At baseline, characteristics of participants, including sociodemographic characteristics and reproductive information, were obtained via a computer-assisted telephone interview. Participants were contacted annually to complete a short questionnaire or a detailed follow-up questionnaire. The data presented in this study were obtained from Sister Study Data Release 7.0, which includes follow-up through September 15, 2017. The Sister Study was approved by the institutional review boards of the National Institute of Environmental Health Sciences and the Copernicus Group. All participants provided written informed consent.

Analytic population

Women diagnosed with breast cancer prior to completing baseline activities or with unknown timing of breast cancer diagnosis relative to baseline ($N = 80$) were excluded. Women who reported never having a menstrual period were also excluded ($N = 7$). For participants with missing menopausal status or age at menopause ($N = 360$), menopausal status and age at menopause were imputed using age 55 as the presumed age at menopause (24). Those who were missing values for other key variables were excluded from our analyses as follows: missing baseline periodontal disease history ($N = 76$), missing potential confounding factors ($N = 753$). After exclusions, a total of 49,968 women were included in this analysis.

Periodontal disease and breast cancer ascertainment

At baseline, participants were asked, "have you ever been told you had periodontal or gum disease?" They also were asked if they had been to a dentist within the 12 months prior to baseline.

Breast cancer diagnosis was self-reported during follow-up through 2017, and for 82.3% of the reported breast cancers medical records were acquired, which all confirmed the self-report (Sister Study Data Release 7.0, https://sisterstudy.niehs.nih.gov/English/images/SIS-BrCa-Inv-DCIS-DR7-508.pdf; ref. 25). Self-report was used when the medical record was not available given the high agreement between self-reported diagnosis and the medical records (24). The International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) was used to define DCIS and invasive breast cancer. Reports of patients' first DCIS or invasive breast cancer, regardless of possible prior lobular carcinoma *in situ* (LCIS) diagnosis, were used in our analyses.

Assessment of potential confounders

Factors that were associated with both periodontal disease and breast cancer risk were identified as potential confounders. We obtained baseline information for the following factors: age, education level (high school or less, some college or associate degree, bachelor's degree, master's or doctoral degree), race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic and other), smoking status (never, former, current), metabolic equivalent (MET)-hours physical activity per week that summed over self-reported sports/exercise and daily activities (≤27.06, >27.06 and ≤44.37, >44.37 and ≤67.21, >67.21), body mass index (BMI) was estimated using measured weight and height from examiner data, regular antibiotic use (never or unknown, former, current regular use, defined as ≥3 times per week for 3 months in a row or longer), menopausal hormone therapy (MHT) ever use [never, estrogen combined with progestin therapy (with or without ever use of estrogen-only therapy history), estrogen-only therapy, other therapy], tamoxifen use (never, ever) and age at first birth (nulliparity, <20, 20 to <30, ≥30). Menopausal status and age at menopause were assessed at baseline and updated on the basis of subsequent follow-up questionnaires.

Statistical analysis

Multivariable stepwise logistic regression and Cox proportional hazards regression were used to identify potential confounding factors associated with periodontal disease and breast cancer risk, respectively, and variables with an alpha level of 0.10 were retained in the model, in addition to *a priori* knowledge of potential confounders (race/ethnicity, BMI × menopausal status interaction).

We estimated hazard ratios (HR) and 95% confidence intervals (CI) for the association between periodontal disease and breast cancer risk using multivariable Cox proportional hazards regression, with age as the primary time scale, adjusting for age at baseline, education, race/ethnicity, smoking status,

physical activity, BMI, regular antibiotic use, MHT use, tamoxifen use, age at first birth, menopausal status at baseline. A BMI × menopausal status at baseline interaction term was also included in multivariable models since the association between BMI and breast cancer changes after menopause (26). A joint Cox proportional hazards regression model was used to simultaneously estimate the HR for invasive breast cancer and DCIS, and the heterogeneity in risk for invasive breast cancer versus DCIS was estimated (27). This approach uses distinct baseline hazards for the subtypes, treating them as competing outcomes. Because DCIS and invasive breast cancer are not necessarily independent events, case-only logistic regression analysis that adjusted for age at diagnosis and menopausal status at diagnosis was also performed to test for etiologic heterogeneity in risk for invasive breast cancer versus DCIS (28).

We additionally conducted stratified analyses by menopausal status because alterations in endogenous sex hormone levels with menopause are thought to be related to periodontal disease as well as breast cancer (29, 30). When estimating the associations stratified by menopausal status, menopausal status was considered as a time-varying factor, so that the same woman could contribute person-time, for example, to both the premenopause and the postmenopause strata. Given that there is considerable heterogeneity in risk factor associations by tumor estrogen receptor (ER) status (31, 32), analyses evaluating risk by ER status were also conducted. Smoking is one of the major modifiable risk factors for periodontal disease, and may be weakly associated with risk for breast cancer (33–35). Smoking status may also be associated with other health behaviors, like cancer screening (36). Thus, we stratified analyses by smoking status at baseline.

The proportional hazards assumption was tested using an age times exposure interaction term, and the assumption did not appear to be violated. In multivariable models we additionally tested the interaction of periodontal disease with both menopausal status and smoking status with respect to breast cancer risk. A sensitivity analysis was conducted, restricting to women who reported both having seen the dentist and having had a mammogram within the 12 months prior to baseline (N = 35,362, 71%). We also conducted sensitivity analyses further adjusting for alcohol drinking status, recency of mammogram, age at menarche, and parity. All analyses were performed in SAS 9.4 software (SAS Institute Inc.).

Results

The mean age at enrollment was 56 years (SD = 9), and 22% of participants reported having ever been diagnosed with periodontal disease (**Table 1**). Women with periodontal disease tended to be older than those without (mean ages 58 and 55 years, respectively). Women with periodontal disease were more likely to be postmenopausal and slightly more likely to have seen a dentist or had a mammogram within the 12 months prior to baseline. Women with periodontal disease were more likely to be heavier, smokers, and MHT users, and were less likely to be non-Hispanic white, physically active, and to have ever used tamoxifen (**Table 1**). Multivariable associations between baseline participant characteristics and periodontal disease are shown in Supplementary Table S1.

A total of 3,339 incident breast cancer cases, including 2,607 invasive breast cancer and 732 DCIS, were identified during a mean (SD) follow-up of 9.25 (2.41) years. In the age-adjusted model, there was little evidence of association between periodontal disease and overall breast cancer risk (HR = 1.04; 95% CI, 0.96–1.13; **Table 2**). The multivariable-adjusted estimate was similar (HR = 1.02; 95% CI, 0.94–1.11). In multivariable analyses, there was heterogeneity in the association of periodontal disease with risk of invasive breast cancer versus DCIS (relative HR = 1.24; P-het = 0.04), such that periodontal disease was associated with a small nonstatistically significant increased risk for invasive breast cancer (HR = 1.07; 95% CI, 0.97–1.17) and decreased risk for DCIS (HR = 0.86; 95% CI, 0.72–1.04; **Table 2**). In case-only analysis, the estimated odds ratio (OR) for risk of invasive breast cancer versus DCIS (OR = 1.24; 95% CI, 1.01–1.52) was the same as the relative HR from the joint model (Supplementary Table S2).

In analyses stratified by menopausal status, we observed similar patterns of association for premenopausal and postmenopausal person-time. Multivariable HRs for overall breast cancer, invasive breast cancer and DCIS were 1.02 (95% CI, 0.80–1.29), 1.05 (95% CI, 0.80–1.38), and 0.90 (95% CI, 0.55–1.49) among premenopausal women, and 1.02 (95% CI, 0.93–1.11), 1.00 (95% CI, 0.55–1.80), and 0.85 (95% CI, 0.70–1.04) among postmenopausal women, respectively. Statistical heterogeneity in the association of periodontal disease with invasive breast cancer versus DCIS was most apparent in the larger group of postmenopausal women (relative HR = 1.25; 95% CI, 1.00–1.55; **Table 3**), and the case-only analysis demonstrated similar heterogeneity in results (OR = 1.25; 95% CI, 1.01–1.56). Consistent with the patterns of association that we observed for overall breast cancer risk, statistically significant heterogeneity in the risk associations for invasive breast cancer versus DCIS was also observed for ER-positive breast cancer (relative HR = 1.35; 95% CI, 1.06–1.72; **Table 4**); the case-only analysis showed similar results (OR = 1.35; 95% CI, 1.05–1.73). In analyses stratified by smoking status, the estimated HRs was strongest among former smokers, with almost statistically significant heterogeneity in risk associations for invasive disease versus DCIS observed among former smokers (relative HR = 1.34; 95% CI, 0.98–1.84; **Table 5**); in case-only analysis, the estimated OR was 1.35 (95% CI, 0.98–1.85) in former smokers. We did not detect multiplicative interactions between periodontal disease and menopausal status or smoking status with respect to breast cancer risk by either the Cox model or the case-only analyses (all P values for interaction > 0.2).

In sensitivity analyses restricted to participants who reported both having seen a dentist and having had a mammogram within the 12 months before study enrollment, and in analyses further adjusting for additional potential confounding factors,

Table 1. Baseline characteristics of participants in the Sister Study, United States, 2003–2009.[a]

	All participants ($n = 49{,}968$)			Women with history of periodontal disease ($n = 10{,}830$)			Women without history of periodontal disease ($n = 39{,}138$)		
	Mean (SD)	No. of women	Frequency (%)	Mean (SD)	No. of women	Frequency (%)	Mean (SD)	No. of women	Frequency (%)
Baseline age (years)	55.6 (9.0)			57.9 (8.3)			54.9 (9.0)		
Follow-up time (years)	9.3 (2.4)			9.2 (2.5)			9.3 (2.4)		
BMI[b] (kg/m^2)	27.8 (6.3)			28.3 (6.4)			27.7 (6.2)		
Education									
High school or less		7,643	15.3		1,680	15.5		5963	15.2
Some college or associate degree		16,881	33.8		3,780	34.9		13,101	33.5
Bachelor's degree		13,494	27.0		2,687	24.8		10,807	27.6
Master's or doctoral degree		11,950	23.9		2,683	24.8		9,267	23.7
Race/ethnicity									
Non-Hispanic White		41,852	83.8		8,700	80.3		33,152	84.7
Non-Hispanic Black		4,371	8.8		1,298	12.0		3,073	7.9
Hispanic		2,444	4.9		527	4.9		1,917	4.9
Other		1,301	2.6		305	2.8		996	2.5
Smoking status									
Never smoker		28,057	56.2		4,685	43.3		23,372	59.7
Former smoker		17,817	35.7		4,786	44.2		13,031	33.3
Current smoker		4,094	8.2		1,359	12.6		2,735	7.0
Physical activity (MET-hours per week)									
≤27.06 (Q1)		12,489	25.0		2,972	27.4		9,517	24.3
27.06–44.37 (Q2)		12,499	25.0		2,709	25.0		9,790	25.0
44.38–67.21 (Q3)		12,488	25.0		2,639	24.4		9,849	25.2
>67.21 (Q4)		12,492	25.0		2,510	23.2		9,982	25.5
Had been to a dentist 12 months prior to baseline									
No		7,164	14.3		1,472	13.6		5,692	14.6
Yes		42,796	85.7		9,356	86.4		33,440	85.5
Had a mammogram 12 months prior to baseline									
No		9,186	18.6		7,097	18.4		2,089	19.5
Yes		40,218	81.4		31,572	81.7		8,646	80.5
Menopause status at baseline									
Premenopausal		16,887	33.8		2,509	23.2		14,378	36.7
Postmenopausal		33,081	66.2		8,321	76.8		24,760	63.3
Age at first birth									
Nulliparity		9,055	18.1		2,023	18.7		7,032	18.0
<20		6,629	13.3		1,586	14.6		5,043	12.9
20–29		26,979	54.0		5,802	53.6		21,177	54.1
≥30		7,305	14.6		1,419	13.1		5,886	15.0
Take antibiotics regularly									
Never user or missing		43,621	87.3		9,565	88.3		34,056	87.0
Former user		5,159	10.3		987	9.1		4,172	10.7
Current user		1,188	2.4		278	2.6		910	2.3
MHT use									
Never user		27,429	54.9		5,222	48.2		22,207	56.7
EPT user (with/without ET)		11,303	22.6		2,966	27.4		8,337	21.3
ET user		9,889	19.8		2,364	21.8		7,525	19.2
Other user		1,347	2.7		278	2.6		1,069	2.7
Tamoxifen use									
Never user		48,951	98.0		10,634	98.2		38,317	97.9
Ever user		1,017	2.0		196	1.8		821	2.1

Abbreviations: ET, estrogen therapy; EPT, estrogen plus progestin therapy; Q, quartile.

[a]Missing data: had been to a dentist 12 months prior to baseline, 2 women with periodontal disease, 6 women without periodontal disease; mammogram 12 months prior to baseline, 469 women with periodontal disease, 95 women without periodontal disease.

[b]BMI calculated as weight (kg)/height (m)^2.

Table 2. Association of periodontal disease with risk of breast cancer, Sister Study, 2003–2017 ($N = 49{,}968$).

	IBC and DCIS combined		IBC only		DCIS only		Heterogeneity[c]	
	No. of cases	HR (95% CI)	No. of cases	HR (95% CI)	No. of cases	HR (95% CI)	Relative HR (95% CI)	*P*-het
Model 1[a]	3,339	1.04 (0.96–1.13)	2,607	1.09 (0.99–1.19)	732	0.88 (0.73–1.05)	1.24 (1.01–1.52)	0.04
Model 2[b]	3,339	1.02 (0.94–1.11)	2,607	1.07 (0.97–1.17)	732	0.86 (0.72–1.04)	1.24 (1.01–1.52)	0.04

Abbreviation: IBC, invasive breast cancer.
[a]Model 1 adjusted for age at baseline.
[b]Model 2 adjusted for age at baseline, education level, race/ethnicity, smoking status, BMI, menopausal status at baseline, BMI × menopause, antibiotics regular use, menopausal hormone therapy use, tamoxifen use, physical activity, age at first birth.
[c]Heterogeneity in the associations of periodontal disease with risk of IBC versus DCIS.

findings were consistent with those observed among the overall cohort (Supplementary Tables S3 and S4).

Discussion

In this large, prospective cohort study, we did not observe a clear association between self-reported periodontal disease and overall breast cancer risk. However, we observed suggestive heterogeneity in risk for invasive breast cancer versus DCIS, with periodontal disease being associated with a nonstatistically significant higher incidence of invasive disease and lower incidence of DCIS. In addition, these findings persisted under adjustment for multiple potential confounders and in sensitivity analyses restricted to those with recent dental exams and mammograms. Overall, in one of the largest studies to date to examine the association between periodontal disease and breast cancer risk, our findings suggest that periodontal disease is not strongly associated with breast cancer risk. However, the observed heterogeneity in periodontal disease risk associations for invasive breast cancer versus DCIS warrants further exploration.

Our decisively null findings for the association between periodontal disease and overall breast cancer risk are consistent with several previous studies that incorporated detailed exposure assessment. In the Atherosclerosis Risk in Communities (ARIC) cohort study, which had ascertained clinical records of periodontal disease, increasing severity of periodontal disease was associated with overall cancer risk but not specifically with breast cancer (4). In the Buffalo OsteoPerio Study, a prospective study where oral alveolar crestal bone height was measured, as a biomarker, to examine the association between periodontal disease and invasive breast cancer risk, no association was detected, but the number of breast cancer cases was small ($N = 9$; ref. 18). In contrast, a retrospective cohort study within the Taiwan National Health Insurance Program, where participants with chronic periodontitis were ascertained on the basis of medical insurance claims, found increased rates of overall cancer, including higher rates of breast cancer specifically, with a hazard ratio of 1.23 (95% CI, 1.11–1.36); however, the cancer cases in this study were sourced from an administrative database, with the authors noting possible inaccuracy in diagnosis confirmation and potential survival bias (6). Results from the WHI OS, which identified 2,124 postmenopausal invasive breast cancer cases after a mean follow-up of 6.7 years, found that periodontal disease was related to a borderline increased risk of invasive breast cancer among postmenopausal women, with an estimated HR of 1.11 (95% CI, 1.00–1.23; ref. 15). Differences in participants (both pre- and postmenopausal women or postmenopausal women only), methods of exposure ascertainment (self-reported with or without clinical dental measurements), severity of periodontal disease (not specified, mild, moderate or severe), as well as adjustment

Table 3. Association of periodontal disease with risk of breast cancer by menopausal status, Sister Study, 2003–2017.

	IBC and DCIS combined		IBC only		DCIS only		Heterogeneity[d]	
	No. of cases[c]	HR (95% CI)	No. of cases	HR (95% CI)	No. of cases	HR (95% CI)	Relative HR (95% CI)	*P*-het
Premenopausal women								
Model 1[a]	594	0.98 (0.77–1.24)	451	1.01 (0.77–1.32)	143	0.87 (0.53–1.43)	1.17 (0.67–2.05)	0.59
Model 2[b]	594	1.02 (0.80–1.29)	451	1.05 (0.80–1.38)	143	0.90 (0.55–1.49)	1.17 (0.67–2.05)	0.59
Postmenopausal women								
Model 1	2,745	1.05 (0.96–1.15)	2,156	1.03 (0.57–1.86)	589	0.88 (0.73–1.07)	1.25 (1.00–1.55)	0.046
Model 2	2,745	1.02 (0.93–1.11)	2,156	1.00 (0.55–1.80)	589	0.85 (0.70–1.04)	1.25 (1.00–1.55)	0.046

Abbreviation: IBC, invasive breast cancer.
[a]Model 1 adjusted for age at baseline.
[b]Model 2 adjusted for age at baseline, education level, race/ethnicity, smoking status, BMI, menopausal status as a time-varying variable, antibiotics regular use, menopausal hormone therapy use, tamoxifen use, physical activity, age at first birth.
[c]Number of cases based on the menopausal status at diagnosis within 91,970 premenopausal person-years, and 370,246 postmenopausal person-years.
[d]Heterogeneity in the associations of periodontal disease with risk of IBC versus DCIS.

Table 4. Association of periodontal disease with risk of breast cancer by estrogen receptor status, Sister Study, 2003–2017.

	IBC and DCIS combined		IBC only		DCIS only		Heterogeneity[c]	
	No. of cases	HR (95% CI)	No. of cases	HR (95% CI)	No. of cases	HR (95% CI)	Relative HR (95% CI)	*P*-het
ER-positive								
Model 1[a]	2461	1.03 (0.94–1.13)	1937	1.09 (0.99–1.22)	524	0.80 (0.64–1.00)	1.35 (1.06–1.73)	0.02
Model 2[b]	2461	1.02 (0.93–1.23)	1937	1.09 (0.98–1.21)	524	0.80 (0.64–1.00)	1.35 (1.06–1.72)	0.02
ER-negative								
Model 1	436	0.95 (0.76–1.20)	334	0.94 (0.72–1.23)	102	0.99 (0.62–1.58)	0.95 (0.55–1.63)	0.84
Model 2	436	0.95 (0.75–1.20)	334	0.94 (0.71–1.23)	102	0.98 (0.61–1.58)	0.95 (0.55–1.63)	0.84

Abbreviation: IBC, invasive breast cancer

[a]Model 1 adjusted for age at baseline.

[b]Model 2 adjusted for age at baseline, education level, race/ethnicity, BMI, menopausal status at baseline, BMI × menopause, antibiotics regular use, menopausal hormone therapy use, tamoxifen use, physical activity, age at first birth.

[c]Heterogeneity in the associations of periodontal disease with risk of IBC versus DCIS.

factors (differences in potential confounding factors) may explain part of the discrepancies observed across these observational studies. In the two recent meta-analyses (19, 20), modest positive associations between periodontal disease and breast cancer risk were observed, with estimated RRs of 1.18 (95% CI, 1.11–1.26) and 1.22 (95% CI, 1.06–1.40), and the WHI OS contributing the most to the overall RRs of any prior study (weights of 25.6% and 52.9%, respectively).

This is the first analysis to consider the association of periodontal disease with breast cancer risk separately for invasive breast cancer and DCIS, and a number of factors could contribute to our observed differences in associations for the invasive versus *in situ* lesions. First, prior work has suggested etiologic heterogeneity for DCIS as compared with invasive breast cancer for several breast cancer risk factors (22, 37). Although biologic differences in the oral microbiota of women diagnosed with DCIS versus invasive disease have not been previously explored, numerous taxa have been found to be significantly enriched in healthy oral samples as compared with those from breast cancer cases studied prior to any systemic therapy, and these taxonomic differences corresponded to functional differences (38). Further investigation is needed to evaluate whether periodontal disease or the oral microbiota differentially affect DCIS and invasive breast cancer. Alternatively, the suggestive association of increased risk of invasive breast cancer associated with periodontal disease could reflect confounding by surveillance behavior if women who tend to have periodontal disease (and a history of poor dental hygiene) also tend to get diagnosed at a later stage of tumor progression. In such a scenario, some of the tumors that would have been DCIS are not found until they are invasive, creating an artifactual "protection" against DCIS. We therefore conducted sensitivity analyses restricted to the participants who had reported

Table 5. Association of periodontal disease with risk of breast cancer by smoking status, Sister Study, 2003–2017.

		IBC and DCIS combined		IBC only		DCIS only		Heterogeneity[c]	
	No. of participants	No. of cases	HR (95% CI)	No. of cases	HR (95% CI)	No. of cases	HR (95% CI)	Relative HR (95% CI)	*P*-het
Never smoker									
Model 1[a]	28,057	1,794	1.02 (0.90–1.15)	1,383	1.06 (0.92–1.21)	411	0.90 (0.69–1.18)	1.17 (0.87–1.58)	0.31
Model 2[b]	28,057	1,794	1.01 (0.89–1.15)	1,383	1.05 (0.91–1.20)	411	0.90 (0.68–1.17)	1.17 (0.87–1.58)	0.31
Ever smoker									
Model 1	21,911	1,545	1.03 (0.92–1.15)	1,224	1.08 (0.96–1.22)	321	0.86 (0.66–1.10)	1.26 (0.95–1.67)	0.10
Model 2	21,911	1,545	1.03 (0.92–1.15)	1,224	1.07 (0.95–1.21)	321	0.85 (0.66–1.10)	1.26 (0.95–1.67)	0.11
Former smokers									
Model 1	17,817	1,302	1.05 (0.93–1.18)	1,030	1.11 (0.97–1.27)	272	0.82 (0.62–1.09)	1.34 (0.98–1.84)	0.07
Model 2	17,817	1,302	1.03 (0.91–1.17)	1,030	1.09 (0.95–1.25)	272	0.82 (0.61–1.08)	1.34 (0.98–1.84)	0.07
Current smokers									
Model 1	4,094	243	0.99 (0.76–1.30)	194	0.98 (0.73–1.32)	49	1.06 (0.58–1.91)	0.93 (0.48–1.80)	0.82
Model 2	4,094	243	0.98 (0.75–1.28)	194	0.96 (0.71–1.30)	49	1.04 (0.57–1.88)	0.93 (0.48–1.80)	0.82

Abbreviation: IBC, invasive breast cancer.

[a]Model 1 adjusted for age at baseline.

[b]Model 2 adjusted for age at baseline, education level, race/ethnicity, BMI, menopausal status at baseline, BMI × menopause, antibiotics regular use, menopausal hormone therapy use, tamoxifen use, physical activity, age at first birth.

[c]Heterogeneity in the associations of periodontal disease with risk of IBC versus DCIS.

having had both a dental appointment and a mammogram within the 12 months before baseline, and the findings were hardly altered. Although it is possible that unmeasured confounders may have influenced our findings, risk estimates were very similar for our two multivariable models as well as in sensitivity analyses, suggesting little evidence for confounding by the measured covariates.

The associations between periodontal disease and breast cancer for premenopausal and postmenopausal women were similar, consistent with the absence of an interaction between periodontal disease and menopausal status with respect to breast cancer risk. Among postmenopausal participants in the WHI OS, the highest HR for invasive breast cancer was observed among former smokers, especially for those who quit in the past 20 years (HR = 1.36; 95% CI, 1.05–1.77; ref. 15). The estimated association with invasive breast cancer was also most apparent among former smokers, although not statistically significant, in our study (HR = 1.09; 95% CI, 0.95–1.25). These results may reflect a potential role of former smoking exposure in breast cancer risk, and persisting effects on the oral microbiome (15, 39). There were too few current smokers in our analysis to disentangle the effects of ever versus former smoking. However, alternative explanations including differential screening behavior by smoking status may also contribute to the apparent heterogeneity in risk for invasive disease versus DCIS among former smokers. Indeed, in a prior study by Sanford and colleagues, compared with never smokers, former smokers were more likely to undergo mammographic screening, while current smokers were less likely to receive screening (36). In our study, we observed a similar trend for recent breast cancer screening. Although our observed findings remained robust to a series of adjustments by potential confounding factors, we cannot rule out the possibility of chance findings. Future studies with more extended follow-up periods may permit evaluation of the effects of latency on risk associated with changes in periodontal disease over the course of study follow up. On the other hand, more frequent screening *per se* would be expected to favor DCIS over invasive disease, and thus would not explain our findings.

Strengths of our study should be highlighted. First, this is a prospective cohort study with a large population size and long follow-up time. This population has been well-characterized, which enabled us to control for multiple potential confounders. Second, because all participants had a sister with breast cancer, the women in this study had a higher risk, on average, than that in the general population, allowing us to accrue a large number of breast cancer cases in a short amount of time. Third, women in this volunteer cohort are highly compliant, with more than 90% responding to their most recent follow-up request. Fourth, this is the first study to assess the risk association with periodontal disease separately for invasive breast cancer and DCIS, which suggests future avenues of research will need to allow for potential etiologic heterogeneity for these subtypes of breast cancer.

Limitations in our study should also be noted. First, self-reported periodontal disease history was used, and it may have been under-reported. Such misclassification may lead to the underestimation of the association between periodontal disease and breast cancer risk. However, self-reported periodontal disease has been shown to have acceptable validity for use in large epidemiologic studies (40). Second, we did not have data regarding the frequency of breast cancer screening during follow-up, and were unable to address whether periodontal disease risk associations varied by screening behavior. However, as participants in the Sister Study are at elevated risk and the majority reported having had a mammogram within the 12 months prior to baseline, presumably they continued to undergo breast cancer screening during follow-up (41, 42). Third, DCIS and invasive breast cancer are not necessarily independent events, suggesting that the assumptions of the competing risks approach we used may not apply. However, we also considered case-only analyses to test for etiologic heterogeneity, and found similar results.

In conclusion, our findings indicate no clear association between periodontal disease and breast cancer. Additional studies are warranted to explain potential heterogeneity in the risk for invasive breast cancer versus DCIS, given that we observed evidence of non-statistically significant elevated risk for invasive breast cancer and reduced risk for DCIS associated with history of periodontal disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

M. Jia: Formal analysis, writing-original draft, writing-review and editing. **Z. Wu:** Formal analysis, writing-review and editing. **E. Vogtmann:** Conceptualization, formal analysis, supervision, writing-original draft, writing-review and editing. **K.M. O'Brien:** Formal analysis, writing-original draft, writing-review and editing. **C.R. Weinberg:** Resources, formal analysis, writing-review and editing. **D.P. Sandler:** Resources, writing-original draft, writing-review and editing. **G.L. Gierach:** Conceptualization, formal analysis, supervision, writing-original draft, writing-review and editing.

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