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Periodontal Pathogens and Risk of Incident Cancer in Postmenopausal Females: The Buffalo OsteoPerio Study

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Abstract

Background—Extraoral translocation of oral bacteria may contribute to associations between periodontal disease and cancer. The associations among the presence of three orange-complex periodontal pathogens (*Fusobacterium nucleatum*, *Prevotella intermedia*, and *Campylobacter rectus*), two red-complex periodontal pathogens (*Porphyromonas gingivalis* and *Tannerella forsythia*), and cancer risk were investigated.

Methods—A total of 1,252 postmenopausal females enrolled in the Buffalo Osteoporosis and Periodontal Disease Study were followed prospectively. Baseline subgingival plaque samples were assessed for the presence of periodontal pathogens using indirect immunofluorescence. Incident cancer cases were adjudicated by staff physicians via review of medical records. Cox proportional hazards regression was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations of periodontal pathogens with total cancer and site-specific cancer risk in unadjusted and multivariable-adjusted models.

Results—Neither the presence of individual pathogens nor the presence of any red-complex pathogens was associated with total cancer or site-specific cancers. Borderline associations were seen among the presence of any orange-complex pathogens (*F. nucleatum*, *P. intermedia*, and *C. rectus*), total cancer risk (HR = 1.35, 95% CI = 1.00 to 1.84), and lung cancer risk (HR = 3.02, 95% CI = 0.98 to 9.29).

Conclusions—No associations were found between the presence of individual subgingival pathogens and cancer risk. However, there were suggestions of borderline positive associations of the presence of any orange-complex pathogens with total cancer and lung cancer risk. The study is limited by the small number of cancer cases and the assessment of only five oral bacteria. Additional research is needed to understand the possi ble role of periodontal disease in carcinogenesis.

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The authors report no conflicts of interest related to this study.

Keywords

Bacterial infections; dental plaque; epidemiology; neoplasms; periodontal diseases; postmenopause

Periodontal disease, a common disease in the elderly, is characterized by chronic polymicrobial infection and inflammation of gum tissue.[1] Microbial colonization of the periodontal pockets is a necessary causal factor for periodontal disease development.[2]

Periodontal disease has been found to be associated with increased risk of certain cancers.[3–8] One underlying mechanism of this positive relationship may involve translocation of oral bacteria to extraoral body sites through the weakened periodontal pocket epithelium and the subsequent modulation of host cell environment and function that predisposes to increased risk of systemic disease. *Fuso-bacterium*, a common oral bacterium, has been found to be overrepresented in colorectal tumors and to play a role in carcinogenesis.[9–11] However, little is known about whether *Fusobacterium* and other oral pathogens detected in subgingival dental plaques are associated with cancer risk.

There is evidence that certain bacterial species are key players in the pathogenesis of periodontal disease.[12] Socransky et al.[13] developed a system categorizing 40 subgingival taxa into color-coded complexes based on their role in periodontal pathogenesis. In this study, cancer incidence in relation to the presence of five subgingival periodontal pathogens, including three orange-complex pathogens (*Fusobacterium nucleatum*, *Prevotella intermedia*, and *Campylobacter rectus*) and two red-complex pathogens (*Porphyromonas gingivalis* and *Tannerella forsythia*), were assessed. In the Socransky taxonomy, the three orange-complex pathogens were associated moderately with periodontal disease, and the two red-complex pathogens were associated strongly with periodontal disease. The aim of this study is to investigate the longitudinal associations between the presence of these subgingival pathogens and cancer risk in an ancillary study of the Women's Health Initiative Observational Study (WHI-OS),[6] the Buffalo Osteoporosis and Periodontal Disease (OsteoPerio) Study.

MATERIALS AND METHODS

Study Population

The OsteoPerio Study was designed to assess associations among osteoporosis, oral bone loss, and periodontal disease, and the eligible participants consisted of postmenopausal females who had participated in the WHI-OS[6] through the Buffalo Clinical Center from 1997 to 2001. The females needed six teeth to be able to undergo radiographic and probing assessment related to periodontal disease and oral bone loss. In addition, systemic bone loss and osteoporosis via dual-energy x-ray absorptiometry were assessed. Thus, females who had both hips replaced were limited in their ability to be evaluated for osteoporosis. Other bone disease and serious ongoing illnesses (cancer therapy) were also considered to be factors that would influence both systemic and oral bone measurements. A detailed description of the study design has been published previously.[14,15] In brief, among 2,249 females invited by mail to join the OsteoPerio study, 341 were not eligible (162 had less than

six teeth, two had a history of bone disease, 16 had had bilateral hip replacement, 109 were diagnosed with cancer during the past 10 years, and 52 had other serious illnesses); 115 could not be reached; 370 were not interested or cancelled appointments and were not able to be rescheduled before the study ended; 52 were deceased; and 12 were ineligible because of recent exposure to dental radiographs or radioluminescent dye test, resulting in 1,359 eligible participants. Of these individuals, 107 were further excluded because of inadequate dental x-rays (n = 16), incomplete study questionnaires (n = 6), or missing oral bacteria data (n = 85), yielding an analytic sample of 1,252 postmenopausal females (aged: 53.2 to 83.1 years; mean age: 66.6 ± 7.0 years). The OsteoPerio Study was approved by the Health Sciences Institutional Review Board at the University at Buffalo. Written informed consent was obtained from participants for all aspects of the WHI-OS and the OsteoPerio Study.

Measurement of Periodontal Pathogens

Subgingival plaque samples were taken by placing fine paper points§ or absorbent points‖ in the gingival pockets of up to 12 prespecified teeth (six maxillary and six mandibular teeth) for 10 seconds. Index teeth (teeth #3, #5, #7, #9, #12, #14, #19, #21, #23, #25, #28, and #30) were usually sampled. Alternative teeth (teeth #2, #4, #8, #10, #13, #15, #18, #20, #24, #26, #29, and #31) were used if the corresponding index tooth was missing. No tooth was sampled when both the index tooth and its alternative were missing. Paper points containing subgingival plaque were placed directly into 4 mL lactated Ringer solution, and the samples for maxillary and mandibular teeth were pooled and vortexed for dispersion of microorganisms.

Indirect immunofluorescence microscopy was used to assess subgingival plaque samples for the presence of select established periodontal pathogens: *F. nucleatum*, *P. intermedia*, *C. rectus*, *P. gingivalis*, and *T. forsythia*. From the formalin-preserved subgingival plaque samples, a 10-μL suspension was smeared and heat fixed on a glass slide and then reacted with the same amount of species-specific antibodies. Slides were washed and incubated with fluorescein-conjugated immunoglobin G and washed again. The stained smears were assessed within a 2-hour interval with a microscope¶ equipped for phase-contrast illumination and incident light fluorescence. For each bacterial species, the emitted fluorescence was graded on a scale of 1+ to 4+, with grade 3+ and 4+ recorded as positive reactions. Plaque smears were considered positive if strong fluorescence was detected in at least five bacterial cells, with well-defined cell outlines and dark or lightly fluorescing centers, and constituted >1% of the total cell counts determined by phase-contrast microscopy. These procedures have been described previously in detail.[16–18]

Ascertainment of Cancer End Points

Participants were followed prospectively for incident cancer diagnosis from the beginning of the study (1997 to 2001) through September 2013. Incident cancer cases, excluding non-melanoma skin cancers, were identified from annual health updates and were adjudicated by trained physicians via review of medical records. Death certificates were used to identify

§Johnson & Johnson, East Windsor, NJ.
‖no. 504, Henry Schein, Melville, NY.
¶Standard 14 Fluorescent, Carl Zeiss MicroImaging, Thornwood, NY.

incident cancers only when no other medical records (i.e., pathology reports, admission history and physical, operative notes, and discharge summaries) were available; only 7% of cancer cases in the OsteoPerio Study were adjudicated using death certificates. A detailed description of cancer ascertainment and adjudication methods has been published previously.[19] Time-to-event was defined as the interval between dates of enrollment and diagnosis of the first primary cancer at any location (for analysis of total cancer risk) or diagnosis of the first primary cancer at a specific site (for analysis of site-specific cancer risk).

Assessment of Other Variables

Data on demographics, anthropometrics, lifestyle, oral health, and medical history were collected from self-administered questionnaires and a physical examination during the study visit. Participants were also asked to bring in all medications and supplements taken within the past month, and this information was documented. Participants' height and weight were measured using a calibrated clinical stadiometer and scale and used to calculate body mass index (BMI). BMI was further categorized into underweight (<18.5 kg/m^2), normal (18.5 to <25.0 kg/m^2), overweight (25.0 to <30.0 kg/m^2), and obese (≥30.0 kg/m^2) according to World Health Organization criteria.[20] Other participant information collected included the following: 1) age at study visit; 2) smoking status (never, former, current); 3) lifetime pack-years of smoking; 4) total recreational physical activity (metabolic equivalent task-hours per week [MET hour/week]); 5) total energy intake (kilocalories/day); 6) fruit and vegetables intake (servings/day); 7) red meat intake (servings/day); 8) alcohol consumption over the past year (ounces/ day); 9) race (white, other); 10) highest education level (high school, college, post-college); 11) post-menopausal combined hormone use (never, former, current); 12) history of diabetes (yes, no); 13) secondhand smoke exposure (yes, no); 14) frequency of toothbrushing (less than twice per day, at least twice per day); 15) frequency of tooth flossing (every day, not every day); 16) frequency of dental visits (no more than one time per year, more than one time per year); 17) use of calcium (yes, no); 18) use of vitamin D (yes, no); 19) current regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) (yes, no); 20) antibiotic use during the month before the study visit (yes, no); 21) family history of cancer (yes, no); 22) age at menopause; 23) age at menarche (9 to 11, 12 to 13, ≥14 years); and 24) parity (never pregnant or abortions/miscarriages only, one to two, three to four, at least five).

Statistical Analyses

Presence of individual periodontal pathogens, of any orange-complex pathogen (*F. nucleatum*, *P. intermedia*, or *C. rectus*), of any red-complex pathogen (*P. gingivalis* or *T. forsythia*), and of any periodontal pathogen (*P. gingivalis*, *T. forsythia*, *F. nucleatum*, *P. intermedia*, or *C. rectus*) by total cancer or site-specific cancer status were assessed using χ^2 tests or Fisher exact tests. Comparisons of personal characteristics of study participants by the presence of any periodontal pathogens were performed using independent *t* tests or Wilcoxon rank-sum tests for continuous variables or χ^2 tests for categorical variables.

Cox proportional hazard regression was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations of periodontal pathogens with total cancer and site-specific cancer risk in unadjusted models and multivariable-adjusted models. Potential

confounders were chosen on the basis of biologic plausibility, previous literature, and their associations with periodontal pathogen infection, total cancer, and common site-specific cancers (i.e., breast cancer, colorectal cancer, and lung cancer) in the current study. Using the change-in-estimate method, the potential confounding effects of age, BMI, smoking, secondhand smoking, education, family history of cancer, combined hormone use, and frequency of tooth flossing on the associations among the presence of periodontal pathogens, total cancer, and site-specific cancers were assessed. For colorectal cancer, the effects of alcohol consumption, physical activity, NSAID use, dietary variables (i.e., total energy intake, red meat intake, fruits and vegetables intake, calcium intake, and vitamin D intake), and history of diabetes were additionally assessed. For breast cancer, the effects of alcohol consumption, NSAID use, age at menopause, age at menarche, and parity were additionally assessed. Because age is a recognized risk factor for many different types of cancer, it was included in all multivariable-adjusted models. If the addition of any variables listed above, one at a time, changed the age-adjusted HR estimates for total cancer or any of the site-specific cancers by >10%, the variable was included in all multivariable-adjusted models. Sensitivity analyses were performed by reanalyzing the data after exclusion of those reporting antibiotic use during the month before the study visit, because it may influence oral microbiota composition. A two-sided α value of 0.05 was used to evaluate statistical significance. A statistical software package# was used for all statistical analyses.

RESULTS

During a mean ± SD follow-up of 11.8 ± 3.8 years, 171 confirmed incident cancer cases occurred among 1,252 postmenopausal females, including 67 invasive breast cancers, 17 colorectal cancers, and 17 lung cancers. *P. intermedia* was the most prevalent periodontal pathogen (43.2% of the females were positive), followed by *T. forsythia* (37.8%), *C. rectus* (17.3%), *P. gingivalis* (14.9%), and *F. nucleatum* (14.0%). There were 775 females (61.9%) who carried at least one of the five subgingival pathogens. Frequencies of the presence of an individual pathogen, any orange-complex pathogen, any red-complex pathogen, and any periodontal pathogen by incidence of total cancer and incidence of the three most common site-specific cancers in females are shown in Table 1.

Distribution of demographics and other personal characteristics of study participants by the presence of any periodontal pathogens are shown in Table 2. Females who harbored any of the periodontal pathogens tend to be older ($P = 0.02$), former/current smokers ($P = 0.03$), heavy smokers measured by pack-years ($P = 0.03$), and to have higher BMI ($P < 0.01$), although the absolute differences in these measures were relatively small. Females who did not carry any of the assessed periodontal pathogens were more likely to have used combined postmenopausal hormone therapy ($P < 0.01$) and to have flossed their teeth daily ($P < 0.01$).

Associations of periodontal pathogen infection with total cancer and site-specific cancers are shown in Table 3. When analyzed singly, individual pathogens were not associated statistically with total cancer or site-specific cancers. The presence of any periodontal pathogen was associated with increased lung cancer risk in the unadjusted model (HR =

#SAS v.9.3 software, SAS Institute, Cary, NC.

4.67, 95% CI = 1.07 to 20.43). The estimate was attenuated and no longer statistically significant after adjusting for age and smoking status (HR = 3.97, 95% CI = 0.90 to 17.45). The presence of any red-complex pathogens was not statistically associated with total cancer risk or site-specific cancer risk in unadjusted or multivariable-adjusted models. The presence of any orange-complex pathogens was significantly associated with increased total cancer risk (HR = 1.39, 95% CI = 1.02 to 1.88) and lung cancer risk (HR = 3.18, 95% CI = 1.04 to 9.77) in the unadjusted models. After adjusting for age at visit and smoking status, borderline positive associations were seen among the presence of any orange-complex pathogens, total cancer risk (HR = 1.35, 95% CI = 1.00 to 1.84) and lung cancer risk (HR = 3.02, 95% CI = 0.98 to 9.29). The presence of any orange-complex pathogens was not associated with invasive breast cancer or colorectal cancer. In a sensitivity analysis excluding participants reporting recent antibiotic use (n = 183) (data not shown), associations among the presence of any orange-complex pathogens, total cancer risk (HR = 1.46, 95% CI = 1.05 to 2.04) and lung cancer risk (HR = 3.28, 95% CI = 0.91 to 11.77) became more apparent. Interestingly, the association between *P. intermedia* and total cancer risk also became stronger and statistically significant (HR = 1.45, 95% CI = 1.04 to 2.00).

DISCUSSION

There are several epidemiologic studies that suggest a positive association between periodontal disease and cancer risk. One possible mechanism involves translocation of periodontal pathogens to extraoral body sites, in which they promote carcinogenesis.[21] No consistent associations among the presence of individual periodontal pathogens, total cancer risk, and site-specific cancer risk were found. However, borderline positive associations were found among the presence of any orange-complex pathogens, total cancer risk (multivariable-adjusted HR = 1.35, 95% CI = 1.00 to 1.84) and lung cancer risk (multivariable-adjusted HR = 3.02, 95% CI = 0.98 to 9.29).

Few studies have examined the presence of specific periodontal pathogens and cancer risk.[22,23] In a case-control study, certain bacterial species were increased in the saliva of 10 pancreatic cancer patients compared with that of 10 healthy controls.[22] However, the bacteria identified in this case-control study did not target the periodontal pathogens investigated by the authors, and it was limited by both its small sample size and retrospective design. In another case-control study, nested in a large European cohort and investigating the association between pancreatic cancer and antibodies to 25 oral bacteria, there was a two-fold higher risk of pancreatic cancer among individuals with higher levels of antibodies against *P. gingivalis*.[23] However, no information on use of medications (e.g., antibiotics) that may affect the presence, abundance, or diversity of oral bacteria was available. There is no association of oral *P. gingivalis* with total cancer or common site-specific cancers in the present study, and the association between *P. gingivalis* and pancreatic cancer attributable to the low number of cases could not be assessed. Nonetheless, the study provides additional insight into a possible link between a certain group of periodontal bacteria and cancer risk. Notably, although no significant associations between individual bacteria and cancer were observed, the estimates for the three orange-complex bacteria and cancer risk were in the same direction. Because the prevalence of each individual bacterium was low, the standard errors were large and *P* values did not reach statistical significance. Assessing the group-

level effect of these three bacteria (as the orange complex) yielded more statistical power and may have allowed detection of a modest, borderline positive association.

The mechanisms through which periodontal bacteria may be involved in carcinogenesis have yet to be elucidated. It has been shown that oral bacteria could enter the systemic circulation through weakened periodontium, facilitated by activities such as mastication, toothbrushing, and scaling.[24] Subsequent bacteremia then permits the seeding of extraoral body sites. Additionally, aspiration and ingestion of oral bacteria could lead to colonization of the lungs[25] and gastrointestinal tract.[26] The migration of periodontal pathogens to these new niches may promote carcinogenesis. Persistence of oral bacterial infection initiates a signaling cascade in immune cells and epithelial cells that activate systemic inflammatory processes known to promote carcinogenesis.[27,28] Certain oral bacterial species could also exert direct genotoxic effects or engage in molecular events that perturb host cellular survival and proliferation pathways.[29,30] In accordance with this view, *F. nucleatum* has been detected in colorectal tumors and was shown to have direct tumorigenic effects on colonic epithelial cells.[10,11] Similarly, oral bacteria have been identified in breast tissues, and breast cancer has been associated with microbial dysbiosis.[31,32] Moreover, oral bacteria could facilitate formation of established carcinogens, including nitrosamines and acetaldehyde.[33,34] The possible associations between certain periodontal pathogens and cancer risk could further be ascribed to common lifestyle risk factors or shared host risk factors, such as genetic variation and age-related changes in immune function.[35–37]

The color-coded bacterial complex scheme suggested by Socransky et al.[13] is used widely to categorize periodontal microorganisms by their timing of colonization and strength of association with periodontal disease. Although this classification serves as a useful heuristic framework in the microbiology of periodontitis, its utility in the context of systemic disease is unclear. This may be in part attributable to the limited number of prospective epidemiologic studies that have the capacity of characterizing periodontal pathogens, as well as sufficient sample size and follow-up to examine their associations with incidence of clinical disease outcomes. A borderline positive association between the presence of orange-complex bacteria with total cancer risk was observed. Orange-complex bacteria have been detected in diverse extraoral sites related to systemic disease, including atherosclerotic lesions, placental and fetal tissues of patients with adverse pregnancy outcomes, synovial fluid from patients with rheumatoid arthritis, colorectal tumors, and abscesses of the brain, lung, liver, and spleen.[38] In particular, *F. nucleatum* is one of the most common species found in extraoral infections. Rubinstein et al.[39] reported that *F. nucleatum* may activate oncogenes *Myc* and *cyclin D1* and inflammatory responses that stimulate the growth of colorectal cancer cells. Kostic et al.[11] also showed that *F. nucleatum* may selectively attract tumor-infiltrating myeloid cells, thereby creating a proinflammatory microenvironment. No association of *F. nucleatum* with total cancer risk or colorectal cancer risk is observed in the present sample. However, it is not known whether the *F. nucleatum* found in colorectal tumors are of the same strains as those found in subgingival dental plaques. Moreover, the prevalence of *F. nucleatum* in this study is lower than that observed in previous reports.[40,41] This occurrence may be attributable to differences in the study populations. The study has a larger sample size and is community based, in contrast to some previous studies that were conducted in participants recruited from dental clinics or based on their periodontal disease

status. Furthermore, because the prevalence of periodontal disease is lower among females than among men,[42] it follows that the prevalence of *F. nucleatum*, a periodontal pathogen, could be lower in females compared with males as well.

With the advent of next-generation metagenomic sequencing technology, >700 bacterial species have been detected in the oral cavity.[43] Periodontal disease is characterized by polymicrobial infection, but only five pathogens are assessed in this study. Although these five bacterial species are well-established periodontal pathogens, the presence of other pathogens and commensals that may synergistically or antagonistically interact with these bacteria to influence cancer risk was not determined. Importantly, some individuals in the reference group defined as the absence of these specific bacteria/bacterial complexes may not be representative of a lower-risk group if those individuals had other, unmeasured oral bacteria that increased cancer risk. The current study used indirect immunofluorescence, which was not able to distinguish among the different bacterial subspecies or strains. Future epidemiologic research on the association between periodontal pathogens and chronic disease should characterize the presence, abundance, and diversity of subgingival microbial communities using high-throughput sequencing technology. However, it is noted that these studies would require even larger sample sizes and would need to correct for multiple comparisons.

The study data were also limited to the presence/absence of the bacterial species of interest; the association between the relative abundance of the periodontal pathogens and cancer risk was not investigated. It is possible that the quantity rather than the presence/absence of a bacterial species influences cancer risk. However, accurate measures of bacterial abundance depend on factors including sampling method, sites sampled, the number of samples taken per participant, sample preparation, the extent of contamination, and sequencing techniques. At the incipient stage of investigation pertaining to oral bacteria and their association with cancer risk, using a more reliable measure, such as what was used in the present study for determining presence/absence of bacteria, provides some evidence in a cost-effective manner. The hypothesized association between extraoral translocation of periodontal pathogens and cancer risk was not tested directly. Future studies should examine whether the same oral bacterial strains are found in the dental plaque and tumor tissue of the same participant. In this study, statistical power is limited by the small number of cancer cases. Although positive associations between the presence of orange-complex bacteria and lung cancer (HR = 3.02, 95% CI = 0.98 to 9.29) and total cancer (HR = 1.35, 95% CI = 1.00 to 1.84) was observed, these relationships did not reach statistical significance. The small number of cancer cases likely resulted in a lack of statistical power and wide CIs. Few cancer cases also hindered the exploration of the potential effect of modification by factors that may influence both cancer risk and oral bacterial composition, such as age, smoking, and BMI.[44,45] Although smoking in the multivariable-adjusted models was adjusted for, residual confounding by smoking could explain the pattern of observed associations. However, it should also be noted that, if smoking and periodontal disease acted synergistically to increase the risk of cancer development, controlling for smoking might have led to over-adjustment. Additionally, the generalizability of the results may be somewhat limited, because the study population is relatively well educated and had a high

frequency of healthy behaviors. Furthermore, the possibility of chance findings attributable to multiple comparisons in these analyses could not be eliminated.

Strengths of the study include the well-characterized community-based cohort of postmenopausal females and the extended period of follow-up. All cancer cases were confirmed by trained physician adjudicators using medical records including pathology reports, reducing the likelihood of outcome misclassification. In addition, the OsteoPerio Study included standardized oral examinations, identification of specific periodontal pathogens, and comprehensive data on baseline participant characteristics. It is the first study to investigate the longitudinal association among measured periodontal pathogens, total cancer risk, and risks of common site-specific cancers. Last, the majority of previous studies on this topic focused on patients with periodontal disease. In comparison, results of this study are more generalizable to the general population and postmenopausal females.

CONCLUSIONS

In this cohort of postmenopausal females, no associations are observed among the presence of individual periodontal pathogens, total cancer risk, and site-specific cancer risk. There were suggestions of borderline positive associations of the presence of orange-complex bacteria with total cancer and lung cancer. These results are novel but should be interpreted with caution, because conclusions are limited by the small number of site-specific cancers and by the evaluation of only five bacterial species. More research using a larger cohort and a more comprehensive assessment of the presence and quantity of oral bacteria is needed to evaluate the association between periodontal bacteria and cancer.

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Table 1

Distribution of Periodontal Pathogens by Status of Total Cancer and Site-Specific Cancers (N = 1,252)

	Total Cancer (n = 171)			Invasive Breast Cancer (n = 67)			Colorectal Cancer (n = 17)			Lung Cancer (n = 17)		
Periodontal Pathogen Infection	**Yes, n (%)**	**No, n (%)**	***P*** *	**Yes, n (%)**	**No, n (%)**	***P*** *	**Yes, n (%)**	**No, n (%)**	***P*** *	**Yes, n (%)**	**No, n (%)**	***P*** *
P. gingivalis			0.90			0.16			0.16			0.30
Absent	146 (85.4)	919 (85.0)		61 (91.0)	1,004 (84.7)		12 (70.6)	1,053 (85.3)		13 (76.5)	1,052 (85.2)	
Present	25 (14.6)	162 (15.0)		6 (9.0)	181 (15.3)		5 (29.4)	182 (14.7)		4 (23.5)	183 (14.8)	
T. forsythia			0.15			0.42			0.22			0.07
Absent	98 (57.3)	681 (63.0)		39 (58.2)	740 (62.5)		13 (76.5)	766 (62.0)		7 (41.2)	772 (62.5)	
Present	73 (42.7)	400 (37.0)		28 (41.8)	445 (37.6)		4 (23.5)	469 (38.0)		10 (58.8)	463 (37.5)	
F. nucleatum			0.79			0.82			>0.99			0.28
Absent	146 (85.4)	931 (86.1)		57 (85.1)	1,020 (86.1)		15 (88.2)	1,062 (86.0)		13 (76.5)	1,064 (86.2)	
Present	25 (14.6)	150 (13.9)		10 (14.9)	165 (13.9)		2 (11.8)	173 (14.0)		4 (23.5)	171 (13.8)	
P. intermedia			0.09			0.31			0.19			0.87
Absent	87 (50.9)	624 (57.7)		34 (50.7)	677 (57.1)		7 (41.2)	704 (57.0)		10 (58.8)	701 (56.8)	
Present	84 (49.1)	457 (42.3)		33 (49.3)	508 (42.9)		10 (58.8)	531 (43.0)		7 (41.2)	534 (43.2)	
C. rectus			0.60			0.85			>0.99			0.52
Absent	1 39 (81.3)	897 (83.0)		56 (83.6)	980 (82.7)		14 (82.4)	1,022 (82.8)		13 (76.5)	1,023 (82.8)	
Present	32 (18.7)	184 (17.0)		11 (16.4)	205 (17.3)		3 (17.6)	213 (17.2)		4 (23.5)	212 (17.2)	
Any periodontal pathogens			0.17			0.69			0.21			**0.02**
Absent	57 (33.3)	420 (38.9)		24 (35.8)	453 (38.2)		4 (23.5)	473 (38.3)		2 (11.8)	475 (38.5)	
Present	114 (66.7)	661 (61.1)		43 (64.2)	732 (61.8)		13 (76.5)	762 (61.7)		15 (88.2)	760 (61.5)	
Any red-complex pathogens†			0.19			0.78			0.60			0.05
Absent	92 (53.8)	639 (59.1)		38 (56.7)	693 (58.5)		11 (64.7)	720 (58.3)		6 (35.3)	725 (58.7)	
Present	79 (46.2)	442 (40.9)		29 (43.3)	492 (41.5)		6 (35.3)	515 (41.7)		11 (64.7)	510 (41.3)	
Any orange-complex pathogens‡			**0.046**			0.21			0.10			**0.03**
Absent	72 (42.1)	544 (50.3)		28 (41.8)	588 (49.6)		5 (29.4)	611 (49.5)		4 (23.5)	612 (49.6)	
Present	99 (57.9)	537 (49.7)		39 (58.2)	597 (50.4)		12 (70.6)	624 (50.5)		13 (76.5)	623 (50.4)	

Bold text indicates statistical significance.

*χ^2 test or Fisher exact test.

† Red-complex pathogens: *P. gingivalis* and *T. forsythia*

‡ Orange-complex pathogens: *C. rectus, F. nuleatum,* and *P. intermedia.*

Table 2

Baseline Characteristics by Presence of Any of the Five Subgingival Pathogens in the Buffalo OsteoPerio Study (N = 1,252)

Characteristics	No (n = 477)	Yes (n = 775)	*P*
Age at visit (years), mean ± SD	66.0 ± 7.2	67.0 ± 6.9	**0.02** *
BMI (kg/m^2), mean ± SD	26.2 ± 5.2	26.8 ± 5.1	0.06 *
Smoking (pack-years), mean ± SD	9.1 ± 17.5	10.9 ± 18.9	**0.03** *
Missing	6	24	
Physical activity (MET hour/week), mean ± SD	14.7 ± 13.9	14.3 ± 14.8	0.30 *
Missing	12	20	
Total energy intake (kcal/day), mean ± SD	1,569 ± 516	1,536 ± 550	0.30 *
Missing	17	27	
Alcohol consumption (ounces/day), mean ± SD	0.47 ± 0.70	0.43 ± 0.67	0.55 *
Missing	7	10	
Age category (years)			**0.03** †
<60	112 (23.5)	137 (17.7)	
60 to <70	215 (45.1)	358 (46.2)	
≥70	150 (31.5)	280 (36.1)	
BMI category (kg/m^2)			**<0.01** †
Underweight (<18.5)	7 (1.5)	10 (1.3)	
Normal (18.5 to <25.0)	237 (49.7)	302 (39.0)	
Overweight (25.0 to 29.9)	144 (30.2)	287 (37.0)	
Obese (≥30.0)	89 (18.7)	176 (22.7)	
Race			0.12 †
White	469 (98.3)	751 (96.9)	
Other	8 (1.7)	24 (3.1)	
Highest education			0.63 †
High school	95 (20.3)	164 (21.5)	
College	206 (43.9)	345 (45.3)	
Post-college	168 (35.8)	253 (33.2)	
Missing	8	13	
Smoking status			**0.03** †
Never	274 (57.4)	389 (50.3)	
Former	192 (40.3)	355 (45.9)	
Current	11 (2.3)	30 (3.9)	
Missing	0	1	
Combined hormone therapy			**<0.01** †
Never	143 (30.0)	269 (34.7)	
Former	78 (16.4)	172 (22.2)	

Characteristics	No (n = 477)	Yes (n = 775)	*P*
Current	256 (53.7)	334 (43.1)	
History of diabetes			0.37†
No	450 (94.3)	740 (95.5)	
Yes	27 (5.7)	35 (4.5)	
Secondhand smoke exposure			0.31†
No	24 (5.1)	30 (3.9)	
Yes	443 (94.9)	737 (96.1)	
Missing	10	8	
Frequency of toothbrushing			0.73†
<2 times/day	105 (22.0)	177 (22.8)	
≥2 times/day	372 (78.0)	598 (77.2)	
Frequency of tooth flossing			**<0.01** †
Not every day	245 (51.4)	458 (59.6)	
Every day	232 (48.6)	311 (40.4)	
Missing	0	6	
Frequency of dental visits			0.15†
≤1 time/year	100 (21.0)	190 (24.5)	
> 1 time/year	377 (79.0)	585 (75.5)	
Recent antibiotic use			0.30†
No	401 (84.1)	668 (86.2)	
Yes	76 (15.9)	107 (13.8)	

Data are presented as n (%) unless otherwise noted. Bold text indicates statistical significance. The five subgingival pathogens include *P. gingivalis*, *T. forsythia*, *F. nucleatum*, *P. intermedia*, and *C. rectus*.

* Independent *t* test or Wilcoxon rank-sum test.

† χ^2 test.

Table 3

Cox Regression Analyses for Associations Among Periodontal Pathogens and Total and Most Common Site-Specific Cancers in Females (N = 1,252)

Periodontal Pathogen Infection	Total Cancer (n = 171)		Invasive Breast Cancer (n = 67)		Colorectal Cancer (n = 17)		Lung Cancer (n = 17)	
	Unadjusted Model	Age and Smoking Adjusted Model*	Unadjusted Model	Age and Smoking Adjusted Model*	Unadjusted Model	Age and Smoking Adjusted Model*	Unadjusted Model	Age and Smoking Adjusted Model*
P. gingivalis								
Absent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Present	0.95 (0.62 to 1.46)	0.92 (0.60 to 1.40)	0.55 (0.24 to 1.27)	0.54 (0.24 to 1.26)	2.32 (0.82 to 6.60)	2.23 (0.78 to 6.35)	1.74 (0.57 to 5.34)	1.45 (0.47 to 4.45)
T. forsythia								
Absent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Present	1.28 (0.95 to 1.73)	1.23 (0.90 to 1.67)	1.23 (0.76 to 1.99)	1.22 (0.75 to 2.00)	0.51 (0.17 to 1.57)	0.46 (0.15 to 1.43)	2.39 (0.91 to 6.27)	1.86 (0.69 to 4.99)
F. nucleatum								
Absent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Present	1.07 (0.70 to 1.63)	1.05 (0.68 to 1.60)	1.10 (0.56 to 2.15)	1.06 (0.54 to 2.09)	0.84 (0.19 to 3.67)	0.78 (0.18 to 3.43)	1.93 (0.63 to 5.92)	2.27 (0.73 to 7.03)
P. intermedia								
Absent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Present	1.31 (0.97 to 1.77)	1.28 (0.95 to 1.73)	1.30 (0.80 to 2.10)	1.29 (0.80 to 2.09)	1.86 (0.71 to 4.88)	1.80 (0.68 to 4.74)	0.92 (0.35 to 2.41)	0.87 (0.33 to 2.29)
C. rectus								
Absent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Present	1.12 (0.77 to 1.65)	1.09 (0.74 to 1.60)	0.96 (0.50 to 1.82)	0.93 (0.49 to 1.79)	1.03 (0.30 to 3.58)	0.95 (0.27 to 3.32)	1.49 (0.49 to 4.56)	1.35 (0.44 to 4.15)
Any periodontal pathogens								
Absent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Present	1.28 (0.93 to 1.75)	1.23 (0.89 to 1.70)	1.13 (0.69 to 1.87)	1.12 (0.68 to 1.86)	2.02 (0.66 to 6.20)	1.90 (0.62 to 5.85)	**4.67 (1.07 to 20.43)**	3.97 (0.90 to 17.45)
Any red-complex pathogens†								
Absent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Present	1.24 (0.92 to 1.68)	1.20 (0.88 to 1.63)	1.10 (0.68 to 1.78)	1.10 (0.67 to 1.79)	0.77 (0.28 to 2.08)	0.71 (0.26 to 1.94)	2.59 (0.96 to 7.02)	2.00 (0.73 to 5.51)

Periodontal Pathogen Infection	Total Cancer (n = 171)		Invasive Breast Cancer (n = 67)		Colorectal Cancer (n = 17)		Lung Cancer (n = 17)	
	Unadjusted Model	Age and Smoking Adjusted Model*	Unadjusted Model	Age and Smoking Adjusted Model*	Unadjusted Model	Age and Smoking Adjusted Model*	Unadjusted Model	Age and Smoking Adjusted Model*
Any orange-complex pathogens‡								
Absent	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Present	**1.39 (1.02 to 1.88)**	1.35 (1.00 to 1.84)	1.39 (0.86 to 2.26)	1.38 (0.85 to 2.24)	2.34 (0.82 to 6.64)	2.24 (0.79 to 6.38)	**3.18 (1.04 to 9.77)**	3.02 (0.98 to 9.29)

Bold text indicates statistical significance.

*Models adjusted for age at visit and smoking status (never, former, current).

†Red-complex pathogens: *P. gingivalis* and *T. forsytrtia*.

‡Orange-complex pathogens: *C. rectus*, *F. nulealum*, and *P. intermedia*.