Periodontal Health in Women With Early-Stage Postmenopausal Breast Cancer Newly on Aromatase Inhibitors: A Pilot Study

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Background: Aromatase inhibitor (AI) use results in low estrogen levels, which in turn affect bone mineral density (BMD). Periodontitis, alveolar bone loss, and tooth loss are associated with low BMD. The goal of this study is to assess the prevalence of periodontitis and perceived oral health and evaluate salivary biomarkers in postmenopausal women who are survivors of early-stage (I to IIIA) breast cancer (BCa) and receive adjuvant AI therapy.

Methods: Participants included 58 postmenopausal women: 29 with BCa on AIs and 29 controls without BCa diagnoses. Baseline periodontal status was assessed with: 1) periodontal probing depth (PD); 2) bleeding on probing (BOP); and 3) attachment loss (AL). Demographic and dental utilization information was gathered by questionnaire. Linear regression modeling was used to analyze the outcomes.

Results: No differences were found in mean PD or number of teeth. The AI group had significantly more sites with BOP (27.8 versus 16.7; $P = 0.02$), higher worst-site AL (5.2 versus 4.0 mm; $P < 0.01$), and more sites with dental calculus (18.2 versus 6.4; $P < 0.001$) than controls. Linear regression adjusted for income, tobacco use, dental insurance, and previous radiation and chemotherapy exposure demonstrated that AI use increased AL by >2 mm (95% confidence interval, 0.46 to 3.92). Median salivary osteocalcin and tumor necrosis factor-α levels were significantly higher in the AI group than the control group.

Conclusion: This first investigation of the periodontal status of women initiating adjuvant AI therapy identifies this population as having an increased risk for periodontitis. *J Periodontol 2015;86:906-916.*

KEY WORDS

Aromatase inhibitors; biological markers; breast neoplasms; periodontal attachment loss; postmenopause; women's health.

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In 2013, ≈230,000 women were diagnosed with breast cancer (BCa) in the United States.[1] With an increase in early detection and improved therapies, more of these women have become survivors.[2] Nearly 75% of all BCa occurs in postmenopausal (PM) women, and 66% to 80% are hormone receptor positive and therefore amenable to hormone adjuvant therapy.[3] Tamoxifen, a synthetic selective estrogen modulator for estrogen receptor–positive (ER^+) BCa, was the drug of choice until the recent emergence of the aromatase inhibitors (AIs).[4] Because of their superior efficacy in reducing tumor recurrence[5] and their general tolerability, third-generation AIs (anastrozole, exemestane, and letrozole) are recommended as a component of the care plan in PM women with hormone receptor–positive early-stage BCa. AIs inhibit the conversion of androgen to estrogen in peripheral tissues, leading to a marked reduction in circulating estrogen. This pharmacology-induced drop in circulating estrogen levels is associated with negative effects on bone health. Loss of bone mineral density (BMD) and increased risk of fragility fracture are well-documented toxicities of adjuvant AI therapy.[6-9]

The density of the bones in the oral cavity is one aspect of systemic BMD and correlates with a risk for osteoporosis[10,11]

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and hip fracture in older women.[12] Currently, the potential impact of AI therapy on the periodontal health of BCa survivors is unknown. With an increasing use of AI in adjuvant therapy and potentially in the preventive setting as well,[13] the oral toxicities of AI use need to be better understood.

Periodontal diseases, alveolar bone loss, and tooth loss are associated with the low estrogen states of menopause and osteoporosis.[14,15] Although site-specific variations are known, osteoporosis is a systemic condition resulting in the loss of bone mass and microarchitecture. In general, patients diagnosed with osteopenia or osteoporosis have reduced jaw bone mass,[16] and changes in dental radiographs are correlated with hip fractures in PM women.[12] Proinflammatory biomarkers detected in the saliva have been associated with periodontitis, including interleukin (IL)-6, IL-1, IL-8, tumor necrosis factor (TNF)-α, matrix metalloproteinase (MMP)-8, and MMP-9.[17-19]

The effect of AIs on oral health is a neglected topic, particularly in light of the known systemic effects of AIs on bone remodeling among PM women, leading to a net bone loss. Evidence supports the role of low skeletal BMD and osteoporosis as risk indicators for reduced alveolar crestal height and attachment loss (AL).[20-24] Because low levels of circulating estrogen are an important risk factor for the development of osteoporosis, the role of AIs as possible risk factors for oral conditions among PM women needs to be evaluated. The objectives of this study are to explore: 1) the prevalence of periodontitis; 2) perceptions of oral health; and 3) salivary biomarkers in PM women initiating adjuvant AI therapy and control participants.

MATERIALS AND METHODS

This study was reviewed and approved by the Institutional Board at the University of Michigan before patients were enrolled and is registered with National Institutes of Health ClinicalTrials.gov (identifier no. NCT01272570). This paper conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational studies.[25]

Participants

Data were collected from 29 women with ER+ BCa who had been on AI therapy for 2 to 11 months and 29 PM women without BCa not using AI therapy. This sample size was chosen for feasibility, rather than to statistically power a specific hypothesis for the baseline data. Nonetheless, based on longitudinal pilot data of AL in patients without cancer, 58 patients would supply ≥80% power (with a Type I error rate of 5%) to detect a 10-point difference (i.e., 0.20 versus 0.10) in the 18-month change in percentage of sites with ≥3 mm AL between the two groups of participants (AI therapy versus control).

All 58 women (aged 44 to 75 years; mean age: 61 years) provided informed consent before participation. PM women with and without BCa diagnoses and having ≥15 teeth[26] (based on a previously published report) were eligible to participate in the study. Participants were recruited from April 2009 to September 2010. Menopausal status was determined using National Comprehensive Cancer Network criteria.[27] PM women with a histopathology-confirmed diagnosis of early-stage (I to IIIA) BCa who were newly on any adjuvant AI therapy (within 1 to 11 months of start) were recruited from the Breast Medical Oncology Clinic of the University of Michigan Comprehensive Cancer Center. AI prescriptions (which included anastrozole, exemestane, or letrozole) were provided by each patient's oncologist as clinically indicated. Participants may have had a history of tamoxifen use, chemotherapy, and/or radiation therapy. Women were excluded if they received a diagnosis of metastatic BCa.

The control group consisted of PM women without BCa diagnoses (or any other cancer other than thyroid or basal cell) and not on AI therapy. This group was chosen because women with ER+ BCa on tamoxifen may not be appropriate controls. Tamoxifen use is associated with an increase in BMD and thus may preclude the ability to examine how menopause and the loss of estrogen have an impact on alveolar bone changes.[28] The women were recruited from the University of Michigan Breast Imaging clinic at the time of routine mammography. Additional exclusion criteria for both groups included: 1) uncontrolled diabetes (glycated hemoglobin >7.2) as determined from self-reported screening within the last 2 months; and 2) the use of medications that affect periodontal status (calcium antagonists, anticonvulsives, and immunosuppressives such as prednisone >7.5 mg daily). Nonsteroidal anti-inflammatory drugs (occasional use only) and bisphosphonate usage were allowed.

Examination Procedures

All dental examinations were performed at the Michigan Center for Oral Health Research. Two trained and calibrated dental examiners (Karen Essell and Alaina Robinson from the University of Michigan), who were masked to the patient's status, completed a full-mouth comprehensive periodontal examination, excluding third molars, using a mouth mirror and periodontal probe. Study measurements included probing depth (PD), gingival recession (GR), AL, bleeding on probing (BOP), plaque scores, missing teeth, and presence of calculus on all teeth for each participant. PD was measured from the gingival margin to the base of the gingival sulcus/pocket with a 0.5-mm-diameter

calibrated probe.¶ Supragingival plaque was coded as 0 (absent) or 1 (present); supragingival calculus was defined as supragingival calcified deposits on tooth crowns and roots and was measured as 0 (absent) or 1 (present). PD was measured on six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual). All measurements were rounded to the lowest whole millimeter. AL was calculated using the same sites by first measuring the distance from the cemento-enamel junction (CEJ) to the gingival margin, then subtracting this distance from the PD. Values for interproximal sites were used to calculate the worst PD and AL sites. Periodontitis was defined as AL ≥3 mm. The presence of gingival bleeding was determined while obtaining the PD measurements. Gingival bleeding was coded as 0 (absent) or 1 (present) and was noted 10 seconds after removal of the periodontal probe.

Radiographic Measures

Standardized periapical digital radiographs were taken in the posterior dentition of all participants using a parallel technique. The radiographs were standardized with the use of bite registration material and an aluminum step wedge of known density[29] with the same settings (63 kV, 8 mA, 0.1 second).# An average of the distance measured in pixels was used to establish the distance of the step wedge. Linear bone measurements were taken between the CEJ or on the apical border of a restoration on the mesial and distal surfaces of the first molars for the determination of alveolar bone height. A higher value for alveolar bone height indicates greater bone loss and worse periodontitis. The radiographs were analyzed by a trained examiner (Iwonka Eagle, University of Michigan) with the use of a computer software measurement tool.[30]

Examiner Training and Calibration for Clinical Measurements

The dental examiners were calibrated before the study. Examiners demonstrated ≥94% of PD measurements within 1 mm of each other with a 95% confidence interval (CI) of 0.84 to 0.95 and ≥85% of AL measurements within 1 mm of each other with a 95% CI of 0.72 to 0.93. Examiners were masked to the cancer history of the patient.

Saliva Biomarkers

Unstimulated whole saliva was collected from all study participants via passive drooling into a sterile plastic tube as previously described by Mandel and Wotman.[31] Saliva collection was stopped once a total of 2 mL was collected or 15 minutes had elapsed, whichever occurred first. The sample was immediately placed on ice, aliquoted, supplemented with proteinase inhibitors aprotinin and phenylmethylsulfonyl fluoride, and stored at –80°C.[18] Saliva samples were analyzed for IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-17, IL-18, TNF-α, C-reactive protein, MMP-8, MMP-9, osteocalcin, osteoprotegerin, vascular endothelial growth factor (VEGF), TNF-α–related activation-induced cytokine, and stromal cell–derived factor 1 (SDF-1 or CXCL12). Protein biomarker levels were determined through a custom human array-based multiplex sandwich enzyme-linked immunosorbent assay system** as previously reported.[19]

Questionnaire

A survey was used to collect demographic information such as age (years), ethnicity/race (white, other), education (less than high school, high school, more than high school), income, oral health–related behaviors and dental care utilization, and prior periodontal therapy or scaling and root planing. To measure the participants' perceptions of oral health, four questions were included regarding the perception of the health of their teeth and gums, the importance of oral health, and their perception of mouth dryness.[32] The patient responses to the two questions "How would you describe the health of your teeth?" and "How would you describe the health of your gums?" were given on 5-point rating scales ranging from 1 (poor) to 5 (excellent). Respondents were asked to rate the importance of their dental health on 5-point rating scales ranging from 1 (not at all important) to 5 (very important). Finally, respondents were asked to rate the dryness of their mouth. The 5-point scale ranged from 1 (very little saliva) to 5 (perfect amount of saliva). The average response to these items was used as an assessment of their oral health perceptions. The questionnaire was pretested with 10 patient volunteers from the University of Michigan Comprehensive Cancer Center. Feedback concerning the clarity of some questions was used to finalize the survey. Cancer-related data such as the diagnosis, time since cancer diagnosis, cancer treatments, medical conditions, and medication use were obtained from the patient's medical chart.

Statistical Analyses

All site-specific measures were averaged within each participant before being analyzed, and all biomarker measures were examined for normality before being analyzed. Between-group differences in demographic and behavioral characteristics were assessed with a χ^2 test of association (categorical) or Wilcoxon rank sum test (continuous). Between-group differences in clinical measures were assessed with either a χ^2 test of association (categorical) or two-sample t test (continuous). Between-group differences in biomarker levels were assessed with the Wilcoxon rank sum test.

¶ UNC probe, Hu-Friedy, Chicago, IL.
# PLANMECA Intra DC, Helsinki, Finland.
** Quantibody Human Cytokine Custom Array, RayBiotech, Norcross, GA.

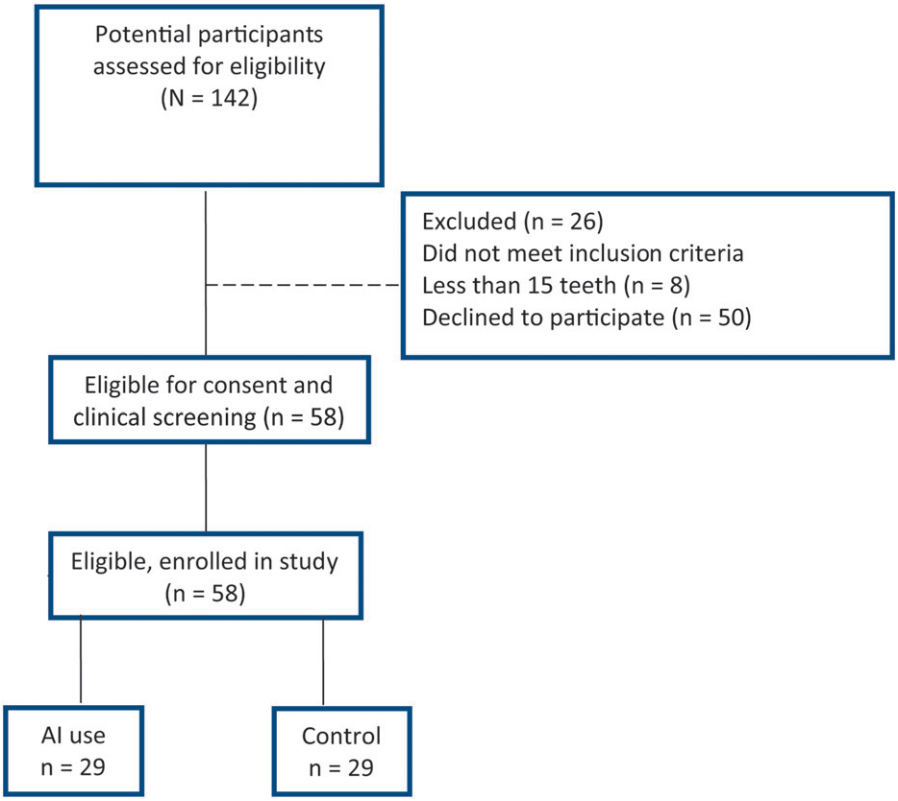

Figure 1.
Schematic of enrollment.

Between-group differences in self-perceived oral health measures were assessed with an independent sample *t* test. Between-group differences in maximum AL and percentage of sites with BOP were further assessed using multiple linear regression to adjust for possible confounders using a manual backward selection technique. A full model with all candidate confounders was fitted; then, the predictor with the lowest *P* value was removed, and the model was refitted. The next least significant predictor was removed, and so forth, until all predictors in the model were statistically significant or clinically relevant. Variables explored were AI duration, local factors such as dental plaque and calculus, oral health behaviors (brushing and flossing), past periodontal treatment (deep cleanings), sociodemographic factors, and bisphosphonate use. As predictors were found to be not informative, they were dropped from the final model. Although chemotherapy and radiation exposure did not show statistical significance, because they may have a negative impact on a patient's periodontal health they were retained as possible confounders in the linear regression models. Data analyses were performed using a statistical analysis software package.†† Statistical significance is defined as a *P* value <0.05.

RESULTS

The study met its target accrual of 58 PM woman, 29 with BCa on AI and 29 controls (Fig. 1). Descriptive characteristics for the sample stratified by AI status are presented in Table 1. The majority of participants were white and married. The mean age for both groups was 61 years. Characteristics were similar in the AI versus control group regarding education, income level, dental visits, and dental insurance status. Bisphosphonate use was reported in 38% of the AI users compared to 17% of the controls ($P = 0.11$). Oral health behaviors and lifestyle behaviors such as tobacco and alcohol use and toothbrushing behaviors were not statistically different between study arms. Of note, 20% to 28% of all participants did not have dental insurance. The two groups did not differ in regard to having received periodontal treatment or periodontal cleanings ($P = 0.44$; data not tabulated).

The mean age at BCa diagnosis was 59.3 years (SD, 7.1; age range, 42 to 73 years). A total of 51.7% had been diagnosed with Stage I and 31% with Stage II ER^+ BCa. The time since diagnosis was 1.3 years (SD, 6.1 months; range, 8 months to 1.7 years). Adjuvant cancer treatments included chemotherapy (37.9%) and radiation therapy (86.9%), and 17.2% had received tamoxifen before treatment with an AI. The distribution of AI medications were as follows: 20 women indicated anastrozole use, two indicated exemestane use, and seven indicated letrozole use. The median time of AI duration was 5.7 months (SD, 3.1 months; range, 2 to 11 months) (data not tabulated).

A comparison of periodontal measures in AI users and controls is shown in Table 2. Compared with the control group, participants receiving AI therapy had a significantly higher mean number of gingival bleeding sites (27.8 versus 16.7; $P < 0.02$), higher mean worst-site AL (5.2 versus 4.0 mm; $P < 0.01$), and approximately three times the number of sites with dental calculus and dental plaque. No difference between the two groups was found concerning the mean PD and the number of teeth present. The percentage of women

†† STATA Statistics and Data Analysis, v.11, STATA Corp., College Station, TX.

Table 1.

Demographic and Behavioral Characteristics [n (%)] of 58 PM Women Stratified by AI Status

Variable	No AI Use (n = 29)	AI Use (n = 29)	*P*
Mean age in years (SE)	61.6 (5.4)	61.7 (7.6)	0.92*
Ethnicity			
White	26 (89.7)	26 (89.7)	
Non-white	3 (10.3)	3 (10.3)	0.92†
Education			
Less than high school	5 (17.8)	3 (10.5)	
High school diploma	5 (17.9)	6 (20.7)	
More than high school	18 (64.3)	20 (68.8)	0.70†
Income			
≤$19,999	8 (28.6)	5 (17.9)	
$20,000 to $39,999	5 (17.9)	3 (10.7)	
$40,000 to $59,999	3 (10.7)	3 (10.7)	
$60,000 to $74,999	2 (7.1)	6 (21.4)	
>$75,000	10 (35.7)	11 (39.3)	0.22†
Marital status			
Married	18 (62.1)	21 (72.4)	
Not married	11 (37.9)	8 (27.6)	0.29†
Dental insurance			
Yes	23 (79.3)	21 (72.4)	
No	6 (20.7)	8 (27.6)	0.76†
Last dental visit			
Within 6 months	27 (93.1)	25 (89.3)	
>6 months	2 (6.90)	3 (10.6)	0.67†
Smoking status			
Current	1 (3.4)	1 (3.4)	
Past	10 (34.4)	16 (55.0)	
Never	18 (62.2)	12 (41.6)	0.11†
Bisphosphonate use			
Yes	5 (17.2)	11 (37.9)	
No	24 (82.8)	18 (62.1)	0.07†
Diabetes			
Yes	1 (3.4)	4 (13.8)	
No	28 (96.6)	25 (86.2)	0.16†
Frequency of brushing			
Every day	10 (34.5)	12 (41.4)	
More than once a day	19 (65.5)	17 (58.6)	0.58†
Frequency of flossing			
Every day	14 (48.3)	12 (41.5)	
Nearly every day	12 (41.4)	11 (37.9)	
Occasionally	3 (10.3)	6 (20.6)	0.20†
Alcohol use			
Yes	19 (65.52)	16 (57.1)	
No	10 (34.48)	12 (42.9)	0.51†

* Two-sample *t* test.
† χ^2 test of association.

Table 2.

Periodontal Measures Among Study Participants by AI Use [mean ± SD or n (%)]

Characteristic	No AI Use (n = 29)	AI Use (n = 29)	*P*
Number of teeth	26.6 ± 1.6	26.1 ± 2.3	0.39*
Number of plaque/biofilm sites	16.3 ± 6.6	55.4 ± 3.4	0.03*
Number of calculus sites	6.4 ± 1.7	18.2 ± 3.0	0.001*
Number of gingival bleeding sites	16.7 ± 12.3	27.8 ± 23.4	0.02*
PD (mm)	2.0 ± 0.29	2.0 ± 0.27	0.95*
Worst-site PD (mm)	4.2 ± 1.4	4.6 ± 0.75	0.21*
AL (mm)	1.4 ± 0.39	1.5 ± 0.75	0.56*
Worst-site AL (mm)†	4.0 ± 1.0	5.2 ± 2.3	0.01*
GR	0.28 ± 0.44	0.36 ± 0.67	0.06*
Radiographic bone (mean bone height)	2.69 ± 0.46	2.65 ± 0.63	0.06*
Women with GR			0.35‡
0 to 1 mm	8 (27.6)	2 (6.9)	
2 mm	7 (24.1)	11 (37.9)	
3 mm	11(37.9)	10 (34.5)	
4 mm	3 (10.3)	6 (20.7)	
Women with periodontitis§			0.03‡
AL 3 mm (mild)	8 (27.5)	5 (17.2)	
AL ≥4 mm but <6 mm (moderate)	17 (58.3)	14 (48.3)	
AL ≥6 mm (severe)	2 (6.9)	9 (31.0)	

* Two-sample *t* test.
† Calculated using interproximal values.
‡ χ^2 test of association.
§ One patient in each group for this measure did not have periodontitis (n = 28).

Table 3.

Study Participants' Self-Perceived Oral Health, Level of Saliva, and Importance of Dental Health (mean ± SD)

Question	No AI Use (n = 29)	AI Use (n = 29)	*P**
How would you describe the health of your teeth?†	3.69 ± 0.96	3.14 ± 1.18	0.056
How would you describe the health of your gums?†	3.34 ± 1.04	2.97 ± 1.29	0.22
How much saliva do you have?‡	4.34 ± 0.97	4.03 ± 1.08	0.25
How important is your dental health?§	4.97 ± 0.18	4.72 ± 0.75	0.09

* Two sample *t* test.
† 1 = poor to 5 = excellent.
‡ 1 = very little saliva to 5 = perfect amount of saliva.
§ 1 = not at all important to 5 = very important.

Table 4.

Multiple Linear Regression Models for BOP and CAL in PM Women

	Mean Sites With BOP (%)				Worst-Site CAL (mm)			
Independent Variable	Coefficient (B)	SE	*P*	95% CI	Coefficient (B)	SE	*P*	95% CI
AI use								
No	Reference				Reference			
Yes	11.22	4.28	0.02	1.63 to 22.00	2.03	0.99	0.02	0.46 to 3.92
Income								
≤$19,000	Reference				Reference			
$20,000 to $75,000	−6.81	6.77	0.30	−20.58 to 6.77	−0.612	0.55	0.15	−1.73 to 0.51
>$75,000	−1.50	7.10	0.83	−15.67 to 12.71	−1.05	0.58	0.07	−2.22 to 0.15
Tobacco use								
No	Reference				Reference			
Yes	−5.08	0.44	0.41	−15.9 to 5.75	0.22	0.52	0.61	−0.66 to 1.11
Dental insurance								
No	Reference							
Yes	−5.14	6.31	0.36	−17.4 to 7.55	−1.10	0.53	0.09	−1.10 to 1.03
Radiation treatment								
No	Reference							
Yes	4.82	12.0	0.69	19.3 to 29.0	−0.88	1.11	0.43	−3.13 to 1.35
Chemotherapy								
No	Reference							
Yes	−11.61	8.68	0.18	−29.0 to 5.85	−1.09	0.71	0.13	−2.52 to 0.34

with AL ≥6 mm was significantly higher in the AI group compared with the control group (31.0% versus 6.9%; $P = 0.03$) (Table 2). Radiographic linear mean bone levels were not statistically different between the two groups (AI 2.65 ± 0.63 mm, control 2.69 ± 0.45 mm; $P = 0.057$).

Table 3 compares the self-reported oral health perceptions of the two groups. AI users had a lower perception of health of their teeth compared with controls, although it did not research statistical significance (on a scale of 1 [poor] to 5 [excellent]: AI 3.14, control 3.69; $P = 0.056$). The two groups did not differ in their perceptions of the health of their gums, the importance placed on dental health, or the amount of saliva in or dryness of their mouths.

Multivariate analyses describing the periodontal health of AI users and non-users are shown in Table 4. AI users had significantly higher worst-site AL values than non-users after adjusting for income, tobacco use, dental insurance status, and previous radiation and chemotherapy treatments. Furthermore, when examining BOP, a linear regression model demonstrated that AI use was significantly correlated with the presence of bleeding. On average, those women using an AI had 12 more sites of bleeding than those not using AIs after controlling for AI status, presence of dental insurance status, tobacco use, and income level.

Table 5 provides the data on the biomarker results. The two groups differed significantly in the level of salivary TNF-α, with the AI group exhibiting higher levels than the control group (median 9 [range 0 to 632] versus 2 [0 to 27] pg/mL; $P < 0.003$) as well as osteocalcin (182 [72 to 323] versus 121 [47 to 40] pg/mL; $P = 0.03$). Salivary levels of receptor activator of nuclear factor-κB ligand (RANKL) ($P = 0.058$) showed a trend toward significance. No other markers suggested differences between the study groups.

DISCUSSION

AIs are an important therapy in the management of ER^+ PM early-stage BCa. Because the use of AIs is often recommended for 5 years, and these women are being treated for a cure, it is important to assess the potential impact of these drugs on oral health. The present study is the first to specifically investigate the effects of AI treatments on oral health. The authors found that AI use is associated with an increased prevalence of periodontitis.

PM participants in this study had good periodontal health with regard to mean whole-mouth PD, AL, and radiographic bone height measures. However, women on adjuvant AI therapy for a median of 5.7 months

Table 5.

Salivary Biomarkers Identified in AI Users and Controls

Biomarker (pg/mL) and Group	Median (range)	P*
C-reactive protein		0.13
No AI use	888 (12 to 10,513)	
AI use	1,481 (25 to 10,137)	
IL-1α		0.28
No AI use	214 (44 to 4,794)	
AI use	262 (44 to 3,561)	
IL-1β		0.89
No AI use	69 (0 to 1,224)	
AI use	151 (4 to 1,319)	
IL-6		0.90
No AI use	59 (0 to 239)	
AI use	42 (2 to 332)	
IL-8		0.71
No AI use	265 (56 to 1,253)	
AI use	335 (50 to 978)	
IL-10		0.82
No AI use	214 (0 to 552)	
AI use	220 (96 to 488)	
IL-17		0.48
No AI use	32 (0 to 103)	
AI use	25 (0 to 85)	
IL-18		0.48
No AI use	320 (35 to 12,038)	
AI use	463 (34 to 13,085)	
MCP-1		0.76
No AI use	1,421 (280 to 6,335)	
AI use	1,842 (428 to 4,736)	
MMP-8		0.98
No AI use	7,206 (3,875 to 9,840)	
AI use	7,076 (4,550 to 8,929)	
MMP-9		0.54
No AI use	38,883 (21,159 to 53,477)	
AI use	38,034 (13,646 to 52,003)	
Osteoprotegerin		0.67
No AI use	4,174 (896 to 32,616)	
AI use	4,848 (123 to 41,012)	
Osteocalcin		0.03†
No AI use	121 (47 to 405)	
AI use	182 (72 to 323)	
SDF-1α		0.59
No AI use	270 (23 to 15,415)	
AI use	508 (35 to 16,803)	

demonstrated significantly more localized AL compared with the women in the control group (worst-site AL 5.2 versus 4 mm; $P < 0.01$). The relationship between AI use and worst-site AL held after adjustment for dental insurance status, tobacco use, income, and previous radiation and chemotherapy exposure. It has been reported that whole-mouth mean values for periodontal measures may not reflect the level of disease at individual affected sites,[33] thus supporting the examination of the worst-site mean values. The present data showed that worst-site mean values for AL and PD were two- to threefold greater than whole-mouth measures, and nearly one-third of AI users had AL ≥6 mm.

As noted previously, AI use in PM patients with BCa demonstrates enhanced rates of skeletal bone loss[34] estimated between 2.6% to 5.3% within the first 6 to 12 months of use[7,35,36] compared with untreated PM women, whose rate is estimated to be 2%.[37] To the authors' knowledge, there has not been a report on the use of AIs and periodontal health with which to compare these findings. However, the reduction of endogenous estrogen after the cessation of menses and the resulting low estrogen state among PM women has been shown to play a role in the progression of oral bone loss and AL.[38-40] The differences in AL were supported by the results in salivary bone turnover markers. Significantly higher levels of TNF-α ($P = 0.003$) and osteocalcin ($P = 0.03$) were observed in the AI group compared with controls. Salivary levels of RANKL ($P = 0.06$) were higher in the AI group, but the difference did not reach statistical significance. TNF-α is an important cytokine involved in many inflammatory responses including bone metabolism in health and disease.[41] Likewise, osteocalcin and RANKL are bone-related proteins associated with osteoblast activity.[42] The higher biomarker values noted in those treated with an AI suggests that the osteoblastic/osteoclastic activities may be increased.[43] Given that osteoblast signaling is integral to osteoclastic activity, it is hypothesized that these cytokine results reflect enhanced bone and connective tissue turnover and breakdown.[44]

In a more general sense, osteoclast formation, locally in periodontitis and systemically in PM osteoporosis, shares many similar pathways for activation and function in pathogenic situations.[45] The salivary findings herein support previous investigations of serum bone turnover biomarkers in blood or urine, which noted increased TNF-α concentrations in both estrogen-deficient[46] PM women and women undergoing AI therapy.[6] In treatment-naïve PM women with BCa, AIs have been shown to increase the levels of serum osteocalcin, TNF-α, RANKL, and IL-6 markers by 10% to 35% in comparison with baseline PM levels.[47]

Table 5. (continued)

Salivary Biomarkers Identified in AI Users and Controls

Biomarker (pg/mL) and Group	Median (range)	*P**
TNF-α		0.003†
No AI use	2 (0 to 27)	
AI use	9 (0 to 632)	
RANKL		0.058
No AI use	1,110 (0 to 7,028)	
AI use	1,861 (0 to 5,194)	
VEGF		0.59
No AI use	3,410 (1,714 to 7,957)	
AI use	3,276 (539 to 7,683)	

RANKL = receptor activator of nuclear factor-κB ligand.
* Wilcoxon rank sum test used to test the median of means.
† Significant differences between groups.

At present, the mechanism connecting AI use with loss of periodontal structures is unclear, as duration of AI use was not found to play a significant role. One mechanism that could play a role is change related to the oral microflora. Previous reports have linked changes in *Bacteroides* and other species associated with periodontal disease progression to changes in local or systemic estrogen and progesterone levels.[48] However, the direct link between oral microfloral changes and AI usage is limited to reports focused on mucosal lesions, which cannot be directly attributed to AI usage alone independent of chemotherapy.[49] A second mechanism that may explain the differences in AL and PD may be the effect of AIs on oral wound healing. The present findings demonstrate no changes in MMP-8 or MMP-9 levels in saliva, but do show significant changes in osteocalcin levels between the two study populations. These results suggest, but do not prove, that the tissue turnover phase of wound healing may not be altered in and of itself. Thus, the repair phase of wound healing may be different in AI users. Yet, here too, none of the three cytokines assayed that are associated with vascular remodeling (VEGF and SDF-1, but not IL-8) approached significance for being altered in the AI users over controls. Thus it remains unclear how tissue turnover differences in AI users versus controls could contribute to the clinical differences observed. A third mechanism that could account for these observations is that the immune response is altered in AI users. As estrogen has been shown to inhibit the expression of bone-resorbing cytokines such as IL-1, TNF-α, and IL-6, in AI users, who are in a severely estrogen-deficient state, higher amounts of these cytokines may be produced, leading to the enhanced progression of bone loss in AI users compared with non-users.[45] Clearly, further investigation is warranted to discern the mechanisms responsible for the periodontal impact of AI use.

Women on AIs demonstrated a trend toward lower perception of their oral health compared with PM controls ($P = 0.056$). These findings are in contrast to a population-based analysis of the National Health and Nutrition Examination Survey (NHANES) 1999 to 2004 data of PM women, which reported that women with a diagnosis of BCa had a significantly higher perception of their oral health than women without a BCa diagnosis.[50] One possible reason for the difference in results is that the NHANES analysis did not have complete data on anti-estrogen therapy exposure. Patients on AIs frequently report disturbed sleep, joint pain and arthralgia, and mood disturbances, which result in higher levels of psychologic distress, anxiety, and depression.[51] This increased psychologic anxiety in turn could affect these women's subjective oral health perceptions. The present observations in women on AIs may indicate an AI-specific change in oral health perceptions and will require further investigation.

Interestingly, there were no significant differences between AI users and controls regarding the question "How important is your dental health?" The authors were surprised by this finding, given the psychologic stress and anxiety that a diagnosis of cancer can bring to an individual. Yet, the number of sites demonstrating significant biofilms and calculus were significantly higher in the AI-user group. From these two sets of data, the authors conclude that although dental/oral health is of great importance to women on AIs, they may not be able to achieve optimal oral care. The barriers to achieving optimal oral care among AI users is being evaluated in ongoing clinical trials.

The strengths of this study include comprehensive whole-mouth periodontal examinations and biomarker data. Furthermore, an extensive collection of the participants' demographics, cancer characteristics and treatments, oral health behaviors, and lifestyle behaviors have been established. However, this study also has some limitations. First, the homogeneity of the study participants related to race/ethnicity and other sociodemographic characteristics requires careful interpretation and caution when generalizing to other groups. Second, these data consider only information of ≤12 months of use of AIs. Future research should consider the effects of long-term AI use on these women's periodontal health. Finally, this study is limited by its cross-sectional design, which prevents the determination of causality between AI use and periodontal parameters. An ongoing clinical study (NCT01693731) is being conducted to address some of these concerns.

CONCLUSIONS

Oral health has significant implications for overall systemic health; thus oral health is an important component of BCa survivorship care. The authors have shown for the first time that adjuvant AI use is associated with increased AL and gingival bleeding in PM early-stage ER^+ BCa survivors. Because long-term survival rates are high in patients with early-stage BCa who receive AIs, and treatment may continue for many years, the complications arising from therapy in this patient population can have long-term effects and may ultimately impact patients' quality of life. Additional prospective clinical studies with women on adjuvant AI are needed.

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