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Periodontal Health in Breast Cancer Patients on Aromatase Inhibitors vs. Postmenopausal Controls: A longitudinal Analysis

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Abstract

Aim: This study was conducted to determine periodontal changes in postmenopausal breast cancer (BCa) survivors using aromatase inhibitors (AI) as compared to postmenopausal women without BCa.
Methods: An 18 month prospective examination of periodontal health in postmenopausal women (29 receiving AI therapy; 29 women without BCa) was conducted at University of Michigan. Comprehensive periodontal examinations including alveolar bone height (ABH) were conducted at baseline, 6, 12 and 18 months. Bisphosphonate, vitamin D, and calcium supplementation was collected via chart review. Linear mixed models were utilized to investigate the relationship between AIs and periodontal measures.
Results: AI users had significantly, deeper probing depths, more dental plaque and clinical attachment loss as compared to controls at the 6, 12, and18 month study visits ($P < 0.05$). ABH loss was seen over time within AI group. The linear mixed model showed a significant effect of time as well as an interaction between aromatase inhibitor use and calcium supplement status. AI users taking calcium experienced less ABH loss over the study than AI users not taking calcium ($P = .005$).
Conclusion: Aromatase inhibitor therapy has a negative impact on the periodontal health of postmenopausal BCa patients. Calcium supplementation appears to mitigate ABH loss in women on AIs.

Clinical Relevance

Scientific rational for the study: Aromatase inhibitor (AI) use is associated with severe and rapid depletion of estrogen and is known to accelerate osteoporosis within breast cancer patients. Estrogen deficiency has been shown to impact periodontal health. The effect of AIs on periodontal health is understudied.

Principal findings: Periodontal measures including clinical attachment loss, periodontal probing along with inflammatory salivary biomarkers increased over 18 months of AI use in early stage breast cancer patients compared to postmenopausal controls.

Practical implications: This study indicates that breast cancer patients should be regularly monitored for periodontal health changes during and after anti-estrogen therapy.

Introduction

Breast cancer (BCa) is the most common cancer affecting US women. In 2016, an estimated 232,670 women will be diagnosed with new cases of invasive BCa (American Cancer Society 2013). Although incidence of BCa is high, the 5 year survival rate is nearly 90% (Anderson et al. 2002). The large number of women affected by BCa, and the high survival rate makes cancer treatment toxicities a public health concern.

The majority of BCas are diagnosed in postmenopausal women. Approximately 75% of these cancers express the estrogen receptor/progesterone receptor and are considered hormone receptor positive (HR+) (Anderson et al. 2002). Adjuvant endocrine therapy (whereby estrogen signaling is disrupted) reduces the risk of BCa recurrence. Aromatase inhibitors (AIs) are the preferred agent in

postmenopausal women with HR+ BCa (Burstein et al. 2010). Side effects of AIs result from rapid decreases in circulating estrogen (Eastell et al. 2008).

The depletion of estrogen in postmenopausal women has been associated with skeletal and alveolar bone loss (Ramesh et al. 2011). Moreover, a relationship between skeletal and mandibular bone mineral density (BMD), hormones, and markers of bone resorption has been demonstrated (Makker et al. 2012). Low skeletal BMD correlates with alveolar bone loss and clinical attachment loss placing postmenopausal women with osteoporosis at a greater risk for periodontal disease (Vishwanath et al. 2011, Sultan et al. 2011).

Bone mass is influenced by multiple factors, including heredity and the availability of calcium (Hildebolt 2005). Vitamin D and calcium supplementation have been associated with improved periodontal health; however, previous studies show conflicting results (Hildebolt 2005, Garcia et al. 2011, Miley et al. 2009). Both calcium and vitamin D are pivotal in the process of bone mineralization and the prevention of osteoporosis. Furthermore, low levels of vitamin D increase vulnerability to infectious and inflammatory diseases such as periodontitis (Garcia et al. 2011).

Multiple studies have shown a positive relationship between biomarkers detected in the saliva and the different stages (inflammation, collagen degradation and bone turnover) in periodontal disease status (Ramseier et al. 2009, Miller et al. 2006). Interleukin (IL) IL-α, IL-1b, IL-6, and tumor necrosis factor α (TNF-a) have been associated with periodontal probing depths and bleeding on probing (Salminen et al. 2014, Ozçaka et al. 2011). Alveolar bone loss is associated with matrix metalloproteinase (MMP)-8, MMP-9, osteocalcin (OCN), osteoprotegerin (OPG) and stromal cell-derived factor 1 (SDF-1 or CXCL12) (Salminen et al. 2014, Havens et al. 2008). In postmenopausal women low osteocalcin levels and IL-6 have been associated with periodontal changes (Bullon et al. 2007, Strekus et al. 1997). Currently, little is known about the oral side effects of AI. The objective of this 18 month investigation was to determine changes in the periodontium in postmenopausal BCa patients undergoing AI treatment through clinical parameters, salivary bone biomarkers, and radiographic examinations. In addition, we examined the impact of bisphosphonate, vitamin D, and calcium supplementation on alveolar bone height (ABH).

Methods

This study was approved by The University of Michigan Institutional Review Board and conducted from April 2009 to September 2013. Written informed consent prior to enrollment of all participants

was required. The authors have followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) strategies outlined for the reporting of observational studies (Von Elm 2007).

Observations were conducted over an 18 month period in a prospective cohort design of 29 postmenopausal BCa patients treated with AIs and 29 postmenopausal controls. A detailed description of the study protocol and baseline findings was previously reported (Taichman et al. 2015). In brief, postmenopausal women with and without BCa diagnosis having at least 15 teeth were recruited from the University of Michigan Hospital, Ann Arbor, Michigan. BCa patients had histologically confirmed diagnosis of BCa: Stage 0, I, II, or III with no evidence of metastatic disease and were undergoing treatment with an AI (AI could be anastrozole, exemestane or letrozole). The comparison group was postmenopausal women without a BCa diagnosis. This comparison group was chosen to investigate how AI therapy might disrupt oral health from the "healthy normal" of the general population. The control group was recruited at the hospital's Breast Imaging Clinic. Women using chronic medications that were known to affect the periodontal status or having conditions such as uncontrolled diabetes (A1c, >7.2) were excluded. Both groups used oral bisphosphonates of similar potency. The patient recruitment and follow-up flow diagram is shown in Figure 1.

Clinical and Radiographic Measures

Examination Procedures: Dental examinations were conducted at baseline, 6, 12, and 18 months by two trained and calibrated examiners blinded to the cohort's group status. A comprehensive periodontal examination, including periodontal pocket depth (PD), clinical attachment levels (CAL), bleeding upon probing (BOP), and dental plaque was conducted on all teeth for each subject. Clinical measures were taken using a University of North Carolina periodontal probe (Hu-Friedy, Chicago IL) on 6 sites per tooth. PD was the distance from the free gingival margin to the base of the sulcus/pocket that could be probed. The loss of CAL was defined as the distance in mm from the cemento-enamel junction (CEJ) to the base of the periodontal pocket. Distance was recorded to the next lowest millimeter. BOP and dental plaque were recorded as present (score of 1) or absent (score of 0).

In order to calibrate and set the measurement scale, vertical measurements of the inserted step wedge were taken from 5 separate radiographs.

Standardized Radiographs: Two periapical radiographs, each positioned to visualize the premolar area in the mouth, were taken using F-speed #2 size intra-oral film at baseline 12, and 18 months. All films were taken using the method described by Zaki and colleagues (Zaki, et al 2015).

Utilizing the standardized radiographs, the presence or absence of alveolar bone loss over time was determined using the *Image J* software program. Linear measurements between the CEJ or restoration margin, and the alveolar crest of first molars were made on baseline, 12 and 18 months radiographs. Two separate linear measurements were taken at all time points, and the average of both measurements was recorded. All radiographs measurements were performed by a single calibrated examiner (I.E.). In order to set the measurement scale, vertical measurements of the step wedge were taken from 5 separate radiographs. The average of the measurements determined the distance in pixels value. The measured known distance of the step wedge was 5.0mm and the pixel aspect ratio was 1.0. Unit of length was recorded in millimeters.

Saliva Collection: To determine whether AI therapy increases bone remodeling biomarkers, IL-1α, IL-1 β, IL-6, IL-8, IL-10, IL-17, IL-18, as well as TNF- α , CRP , MMP-8, MMP-9, OCN, OPG, VEGF, SDF-1 or CXCL12 were examined in the saliva of AI and non AI users at baseline, 12 and 18 months. Whole saliva (unstimulated) was collected from all participants at baseline, 12 months and 18 months through passive drooling into a sterile plastic tube. (Mandel et al 1976) Saliva collection was conducted until either 2ml was collected or up to 15 minutes. Protein biomarker levels in the saliva samples were analyzed using a custom human array-based multiplex sandwich ELISA system as previously reported (Quantibody Human Cytokine Custom Array, RayBiotech, Norcross, GA.).

Questionnaire: Self-report questionnaires were used to collect data on sociocultural-demographics, dental care utilization, history of periodontal cleanings, dental insurance status, behavioral factors (smoking, home care frequency) and the use of bisphosphonates, vitamin D, and calcium (Yes/No) prior to the oral examination at each study visit. Patient charts were reviewed to extract information regarding cancer diagnosis, diagnosis date, cancer treatments, all other medical conditions, and verify the medication list including vitamin D, calcium, and bisphosphonate use.

Statistical Analyses: Data analysis was performed using the statistical package IBM SPSS version 21 (IBM SPSS,2012). Between-group differences in demographic, behavioral characteristic and periodontal measures were assessed at each time point for using either an X^2 test of association for categorical measures or an independent sample t-test for continuous measures. Paired t-tests were used to investigate within group changes over time for ABH, CAL, PD, and BOP. Bonferroni adjustments were applied as appropriate. Linear mixed models were used to investigate the impact of AI use over time along with calcium, vitamin D and bisphosphonate status on periodontal outcomes. This

modelling approach allowed for incomplete data due to loss to follow up (without any imputation of missing values) and provided valid estimates of treatment differences under the assumption that such data are missing at random. Potential confounders included dental insurance status, a history of periodontal cleanings and previous chemotherapy treatment. A backward-step selection was used to determine the final model. Interactions between AI and calcium status were tested. As AI severely diminishes estrogen, negatively impacting bone, BCa patients using AIs are more likely to be prescribed bisphosphonates therefore we kept bisphosphonates in the models in an effort to control for this potential confounder. The mean salivary biomarker levels were assessed for both between group differences (Wilcoxon Rank Sum test) and intragroup differences over time (Wilcoxon Rank Signed test).

Results

There were 142 potential participants assessed for eligibility in the study. Of these, 58 participants met study eligibility criteria and were enrolled; 29 control participants, and 29 AI treatment participants. After the baseline visit, one AI treatment participant withdrew due to AI toxicity. After the 6 month visit, 3 AI treatment participants withdrew due to AI toxicities thus resulting in 25 AI study participants at the 18 month study visit. There were no withdrawals among the control participants. **Table 1** provides summary baseline characteristics of study participants according to AI status which have been previously reported in full (Taichman et al. 2015). There were no significant differences between the groups.

An overview of the clinical data for both groups is shown in **Table 2**. Significant differences in clinical parameters were noted between the groups at each study visit as well as within study groups over time. Significant increases in the mean PD between the baseline and 18 month follow-up visit were observed in the AI group as well as between the AI and control group at the last study visit. When mean CAL was considered, statistically greater attachment loss was observed over time in the AI group as well as between both groups at all study visits ($P < 0.001$). Significant differences in BOP were found between the groups from baseline to 18 month visits with a greater increase seen in the control group than the AI group ($P = 0.002$). Plaque scores between groups were significantly different at baseline, 6, and 12 month visits. No within-group differences were seen between the baseline and 18 month visits.

An increasing value for ABH from each time point indicates a greater loss of alveolar bone (Table 2). No differences were identified between AI users and controls for ABH at baseline, 12, or 18 month visits. However within groups, ABH loss was seen from baseline to 18 months for women on AIs (0.32mm ± SD 0.36, P=0.001), as well as controls, (0.19mm ± SD 0.22 P=0.002).

Table 3 shows ABH by bisphosphonate, vitamin D and calcium use. Focusing on AI users, significant ABH loss was seen during the 18 month observation period for those women who were not using bisphosphonates compared to those who did (p=0.04). Likewise ABH changes were seen in AI users when examining calcium supplementation. The average ABH among AI users differed significantly at each exam visit between women on calcium supplementation verses women who were not on calcium supplementation. AI users not using calcium demonstrated a significant average ABH loss over the study period (baseline to 18 months). ABH did not differ among AI users by vitamin D use or over time. Bisphosphonate, vitamin D and calcium usage had no significant impact on average ABH in the control group.

Linear mixed models were fit using restricted maximum likelihood estimation to account for the dependence in the data due to repeated measures per study participant. The results of these models are presented in Table 4. When controlling for time, AI use, history of periodontal cleanings, dental insurance, calcium supplementation, vitamin D and bisphosphate use in the model, PD and CAL increased, and ABH significantly decreased over time. Having had history of periodontal cleanings was significantly correlated with both the CAL and ABH models, but dental insurance was not. A significant effect of time was found along with a significant AI status by calcium use interaction when examining ABH and controlling for the other variables in the model. Those receiving AIs and calcium had significantly less ABH loss (Mean=2.50, SE=0.13) than those on AI but not taking calcium (Mean=3.32, SE=0.23) (P=0.005) but no impact was observed among controls.

Analysis of the inflammatory and bone turnover markers in saliva demonstrated significant differences between the experimental and control groups as well as within-group differences over the study period. Between-group differences were demonstrated at baseline for TNFα, osteocalcin, IL-8, OPG, MCP1 with the AI group exhibiting higher levels (Table 5). The two groups also differed significantly at the at 18 month visit regarding levels of salivary TNFα, Il-6, IL-8, and OPG , with the

AI group exhibiting higher levels than the control group participants. The salivary IL-1b levels in the AI group were significantly decreased from the baseline to the 18 month follow-up. In contrast, the salivary levels of IL-6, (P=0.02), IL18 (P= 0.03) and osteocalcin (P = 0.02) in the AI group were significantly increased at 18 months when compared to the baseline visit.

Discussion

AIs have become a standard intervention for postmenopausal women with early stage HR+ BCa because they reduce the risk of disease recurrence (Burstein et al. 2010). Therefore, tens of thousands of women use these drugs. Thus, understanding the impact of AIs on periodontal health is important to BCa survivors. Postmenopausal women with a history of BCa taking AIs demonstrated significant differences in average PD and CAL and at the 6, 12, and 18 month visits compared to postmenopausal controls. Furthermore, for AI users, the mean PD and CAL increased significantly from the baseline to 18 month visit. These clinical changes were accompanied by small, but statistically significant alterations in the radiographic bone levels of AI users over the same period. Linear regression mixed models demonstrated that time was significantly associated with all periodontal outcomes in both adjusted and unadjusted models. These findings suggest that women who use AIs may be at greater risk for adverse periodontal outcomes.

Estrogen has been shown to play a critical role in the maintenance of oral tissues (Grossi 1998). Hormone replacement therapy has a positive impact on clinical attachment levels (Reinhardt et al. 1999). Furthermore, estrogen status has been reported to influence alveolar bone density changes among postmenopausal women (Payne et al. 1997). AI associated skeletal bone loss is estimated between 3.9% to 4.1% loss within the first 2 years of treatment (Eastell et al. 2008). Thus it is reasonable to hypothesize that the loss of estrogen signaling related to AI would lead to greater risk for periodontal disease and significant alveolar bone loss use in postmenopausal BCa patients. This longitudinal analysis demonstrates continued impacts on clinical periodontal measures while patients are exposed to AIs (Taichman et al. 2015).

Our studies sought to explore and validate the association between salivary bone turnover markers and AI use. While direct comparisons to other investigations exploring salivary biomarkers in BCa patients using AIs is not possible, the biomarker concentrations reported in this study are similar to those reported in investigations on postmenopausal women (Rahnama et al. 2013, Strekus et al. 1997).

In this study several of the biomarkers of bone turnover demonstrated significant changes between groups and over time. OPG, a rank ligand inhibitor, has been shown to reduce inflammatory and neoplastic bone remodeling (Lerner 2006). OPG levels in AI users were higher than controls at each time point examined. Over time OPG levels decreased in the AI group but these changes did not reach a level of significance. Consistent with previous studies, OPG levels decrease over time in postmenopausal women not using HRT (Rahnama, et al. 2013). The cytokines IL-1, IL-6 and TNFα are well established mediators of osteoclastic resorption in periodontitis (Okada & Murakami 1998). Matching the OPG results differences in osteocalcin, TNFα, MCP1, and IL18 were observed between the groups, along with a significant increase in TNFα, IL6, and osteocalcin from baseline to 18 months. These observations are in keeping with the known activity of estrogen which has been shown to inhibit the expression of bone-resorbing cytokines IL-1, TNFa, and IL-6 in postmenopausal women (Payne et al. 1997, Kwan 2004). Our results suggest that AI users, who are in a severely estrogen-deficient state, may be at higher risk for the production of elevated pro-inflammatory cytokines, and ultimately enhanced destruction of the periodontium.

Postmenopausal women using bisphosphonates have been shown to have less plaque accumulation, less gingival inflammation, lower PD, less CAL and alveolar bone loss, suggesting bisphosphonates play a beneficial role in periodontal status (Palomo et al. 2005, Palomo et al. 2011, Rocha 2004). AI users on bisphosphonates in this study demonstrated less bone loss over the 18 month relative to AI users not on bisphosphonates in our unadjusted analysis ($p=0.05$). However, bisphosphonate use was not a significant factor relative to the periodontal outcomes in the linear mixed models. One possible reason for these observations is that bisphosphonate use was defined as use/no use rather than by duration or type of drug. Another reason may be the small number of AI users taking bisphosphonates. To determine the benefits of bisphosphonates on periodontal health, an effective dose related to the periodontium (Palomo et al. 2007) as well as a more robust sample size is needed .

Like bisphosphonate use, vitamin D supplementation did not have a significant impact on ABH in either group. Our results are similar to Millen et al., who conducted a large prospective study on the effects of vitamin D and the progression of periodontal disease in postmenopausal women who found no association between vitamin D status and ABH as well as CAL, PD, and gingival bleeding (Millen et al. 2014). Likewise, Krall showed no link between vitamin D and alveolar bone loss; however the authors did find a 30% higher loss of alveolar bone among those men with low calcium levels (Krall

2001). However, other clinical studies have demonstrated a positive impact of vitamin D on periodontal health (Garcia et al. 2011).

Increased levels of calcium are positively correlated with a reduced prevalence of clinical attachment loss and a lower risk of tooth loss (Krall 2001). In this study, AI users supplementing with calcium exhibited less alveolar bone loss at baseline and at each subsequent time point compared to AI users not taking calcium; however, there was no significant difference between these groups in ABH change from baseline to 18 months. One possible explanation for these findings is the loss of four patients which may have impacted our ability to detect a difference in ABH among AI users at the18 month examination. As there is conflicting evidence regarding the impact of vitamin D and calcium supplementation on alveolar bone loss, a randomized controlled clinical trial is needed.

Participants at baseline were socio-economically homogenous with low disease levels reporting high dental care utilization. The changes in both groups for PD and CAL, though statistically significant, were of small magnitude perhaps a reflection of high dental utilization. There is still debate as to the clinical significance of very small changes in clinical parameters as the population level may not reflect individual changes. Nevertheless, for some individuals the changes can be significant both in terms of the clinical measures but also in terms of the relatively short duration of this investigation (18 months). These findings expand our understanding of estrogen deficiency and periodontal changes. Future studies examining more socio-economically diverse populations for longer periods should be considered. Although significant within-group changes in linear radiographic ABH occurred over time for both the AI and non- AI groups, no differences in ABH were seen between the groups. The computed mean difference of ABH was based on sample sizes for those on AI that varied slightly over time because of the withdrawal of AI users. Furthermore, baseline ABH measures were taken after the start of AI initiation limiting the ability to determine loss within the first few months of use. Finally, as BCa patients may have additional exposures or behaviors related to having a cancer diagnosis which could impact their oral health, future studies should consider a control group consisting of women with BCa but not treated with AIs. Despite these limitations, this study provides a unique contribution to understanding the impact of AI use on periodontal health in BCa survivors. Notable strengths for this investigation included a comprehensive periodontal which when coupled with the salivary cytokine levels suggests that cytokine levels in saliva mirror bone and tissue loss identified in clinical and radiographic analyses.

Conclusions

This study is the first to investigate the oral effects of AI on postmenopausal women with breast cancer within the first 18 months of use. AI users experienced greater increases in CAL, PD, and ABH loss. Interestingly, AI use supplemented with calcium, may help mitigate AIs impact on alveolar bone loss. Increased knowledge about the oral effects of the prolonged use of AI will lead to an improved risk assessment of oral and overall health care of BCa survivors.

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Figure Legend:

Figure 1: Study Schematic

Table 1: Baseline Characteristics of Study Patients on AI and Controls[+]

	On AI N=29	Control N=29	P Value
Characteristic	**%**	**%**	
Age, mean (SD), year	61.7±7.6	61.6 ±5.4	0.92
Race/ethnicity			
White	26(89.7)	26(89.6)	
Non White	3(10.3)	3(10.4)	0.92
Education			
Less than high school	3(10.5)	5(17.8)	
High school	6(20.7)	5(17.9)	
More than high school	20(68.8)	18(64.3)	0.70
Income			
No income to $19,999	5(17.9)	8(28.6)	
$20,000-$39,999	3(10.7)	5(17.9)	
$40,000-$59,999	3(10.7)	3(10.7)	
$60,000-$74,999	6(21.4)	2 (7.1)	
Over $75,000	11(39.3)	10(35.7)	0.22
Marital Status			
Married	21(72.4)	18(62.1)	0.29
Has Dental Insurance			
Yes	21(72.4)	23(79.3)	0.76
Last Dental visit			
Within last 6 months	25(89.3)	27(93.1)	0.67
Smoking Status			
Current	1(3.4)	1 (3.4)	
Past	16(55.0)	10(34.4)	
Never	12(41.6)	18(62.2)	0.11

Bisphosphonate Use			
Yes	11(37.9)	5((17.2)	0.07
Calcium Supplement			
Yes	20(68.9)	11(37.9)	0.42
Vitamin D Supplement			
Yes	19(65.5)	15(51.7)	0.52
Cancer Characteristics			
Age at BCA diagnosis-yrs	59.3 ± 7.1	(42-73)	
Time since BCA diagnosis-mos	15.1 ± 6.1	(8 -19)	
AI duration - mos	5.7 ± 3.1	**(**2-11)	
Tumor stage at diagnosis			
Stage I	15(51.7)		
Stage II	9(31.0)		
Stage III	5(17.3)		
BCA treatment			
Prior Adjuvant chemotherapy	11(37.9)		
Prior radiation	26(86.9)		
Prior Tamoxifen use	5(17.2)		

Table 2: Clinical and Radiographic Measures of AI Users and Non AI Users

Index	Group	Baseline (mean± SE)	6 months (mean± SE)	12 months (mean± SE)	18 months (mean± SE)	Δ (mean± SE)
PD(mm)	Control	2.00 ± 0.29	1.73 ± 0.24	1.94 ± 0.20	2.01 ± 0.19	0.01 ± 0.22
Mean	AI user	2.00 ± 0.27	2.12 ± 0.31	2.23 ± 0.29	2.35 ± 0.30	0.35 ± 0.28 [a,b]
CAL (mm)	Control	1.42 ± 0.39	1.42 ± 0.29	1.40 ± 0.29	1.45 ± 0.20	0.03 ± 0.22
	AI user	1.51 ± 0.75	1.73 ± 0.74	1.72 ± 0.36	1.96 ± 0.40	0.45 ± 0.38 [a,b]
BOP	Control	0.11 ± 0.80	0.17 ± 0.15	0.17 ± 0.02	0.25± 0.12	0.14± 0.13[a,b]
	AI user	0.18 ± 0.14 [a]	0.19 ± 0.13	0.26 ± 0.14	0.19 ± 0.16	0.02 ± 0.36
Alveolar Bone	Control	2.65 ± 0.63	N/D	2.73 ± 0.52	2.86 ± 0.55[a]	0.19 ± 0.22 [b]
Height	AI user	2.69 ± 0.45		2.85 ± 0.55	2.94 ± 0.49[a]	0.32 ± 0.36[b]
Plaque	Control	0.10 ± 0.11	0.19 ± 0.17	0.30 ± 0.19	0.46 ± 0.26	0.36 ± 0.14[b]
	AI user	0.36 ± 0.23[a]	0.44 ± 0.28[a]	0.52 ± 0.27	0.59 ± 0.25	0.24 ± 0.37

Values are means ± SE

Δ, Change using a paired t-test

PD, probing depth; CAL, clinical attachment level; BOP, Bleeding on Probing; PI, Plaque Index; RBL, radiographic bone level; SE, standard error.

[a] Significant difference between AI users and controls ($P < 0.05$).

[b] Significant difference within group over time ($P < 0.05$).

Table 3: Alveolar bone measures of calcium, bisphosphonate and Vitamin D users

Index	Group	Baseline (mean± SE)	12 months (mean± SE)	18 months (mean± SE)	Δ (mean± SE)
AI user					
Calcium	**No Calcium**	3.11 ± 0.37	3.18 ± 0.37	3.36 ± 0.50	0.31 ± 0.47[c]
	Calcium	2.45 ± 0.58 [a]	2.51 ± 0.46 [a]	2.71 ± 0.54[a]	0.26 ± 0.34
Bisphosphonate	**No use**	2.51 ± 0.76	2.58 ± 0.56	2.81 ± 0.77[b]	0.42 ± 0.37[c]
	Bisphosphonate	2.82 ± 0.33	2.73 ± 0.40	2.91 ± 0.25	0.09 ± 0.27
Vitamin D	**No Vitamin D**	2.85 ± 0.68	2.67 ± 0.61	2.90 ± 0.78	0.19 ± 0.45
	Vitamin D	2.52 ± 0.58	2.63 ± 0.44	2.84 ± 0.50	0.31 ± 0.32
Control					
Calcium use	**No Calcium**	2.72 ± 0.41	2.89 ± 0.46	3.01 ± 0.50	0.28 ± 0.20
	Calcium	2.70 ± 0.55	2.91 ± 0.72	2.91 ± 0.57	0.21 ± 0.12
Bisphosphonate	**No use**	2.66 ± 0.47	2.82 ± 0.54	2.93 ± 0.52	0.27 ± 0.24
	Bisphosphonate	2.79 ± 0.38	3.00 ± 0.65	3.00 ± 0.37	0.20± 0.14
Vitamin D	**No Vitamin D**	2.55 ± 0.43	2.69 ± 0.44	2.84 ± 0.45	0.28 ± 0.26
	Vitamin D	2.79 ± 0.48	2.97± 0.64	3.03 ± 0.54	0.24 ± 0.24

Values are means ±SE

[a] Significant differences among AI users on calcium and no calcium supplements

[b]Significant differences among AI users on bisphosphonates and no bisphosphonates ($P < 0.05$).

[c] Significant difference within group over time ($P < 0.05$) using a paired t-test

Table 4: Mean Periodontal Pocket Depth, Mean Clinical Attachment Loss, and Mean Alveolar Bone Height Loss (in mm) between 0 and 12 months and 12 and 18 months

	Periodontal Pocket			Attachment Loss			Alveolar bone height		
Variable	**Est.***	**Std. Error**	**P-Value**	**Est.**	**Std. Error**	**P-Value**	**Est**	**Std. Error**	**P-Value**
Intercept	1.9	0.21	<.001	1.4	0.23	<.001	2.42	0.17	<.001
Time									
Baseline	Ref			Ref			Ref		
6 Months	-0.08	0.03	0.02	-0.03	0.04	0.52	NA	NA	NA
12 Months	0.06	0.03	0.04	0.11	0.04	0.020	0.13	0.04	<0.001
18 Months	0.13	0.04	<0.001	0.17	0.04	<0.001	0.27	0.04	<0.001
AI use									
Not on AI	-0.23	0.08	0.008	-0.44	0.20	0.038	0.27	0.20	0.19
On AI	Ref			Ref			Ref		
History of periodontal cleanings									
No	0.21	0.07	0.05	0.35	0.12	0.04	0.86	0.29	0.052
Yes	Ref			Ref			Ref		
Bisphosphonate use at baseline (Y/N)									
No Bisphosphonate use	0.06	0.06	0.36	0.23	0.14	0.12	0.04	0.17	0.81
Bisphosphonate use	Ref			Ref			Ref		
Dental insurance									
No	0.10	0.07	0.15	-0.01	0.16	0.95	0.12	0.05	0.16
Yes	Ref			Ref			Ref		
Adjuvant chemotherapy									
No	0.11	0.08	0.20	0.29	0.18	0.13	-0.20	0.21	0.39

Yes	Ref			Ref			Ref		
Vitamin D supplement use at baseline									
No Vitamin D	-0.03	0.06	0.69	0.07	0.15	0.65	-0.15	0.17	0.37
Vitamin D	Ref			Ref			Ref		
Calcium supplement use at baseline									
No Calcium use	0.02	0.07	0.69	-0.05	0.20	0.81	0.81	0.27	0.005
Calcium use	Ref			Ref			Ref		
Not on Calcium and Not on AI Baseline Interaction	-0.17	0.12	0.17	-0.17	0.27	0.53	-0.70	0.34	0.04

Est* Estimates of Fixed Effects

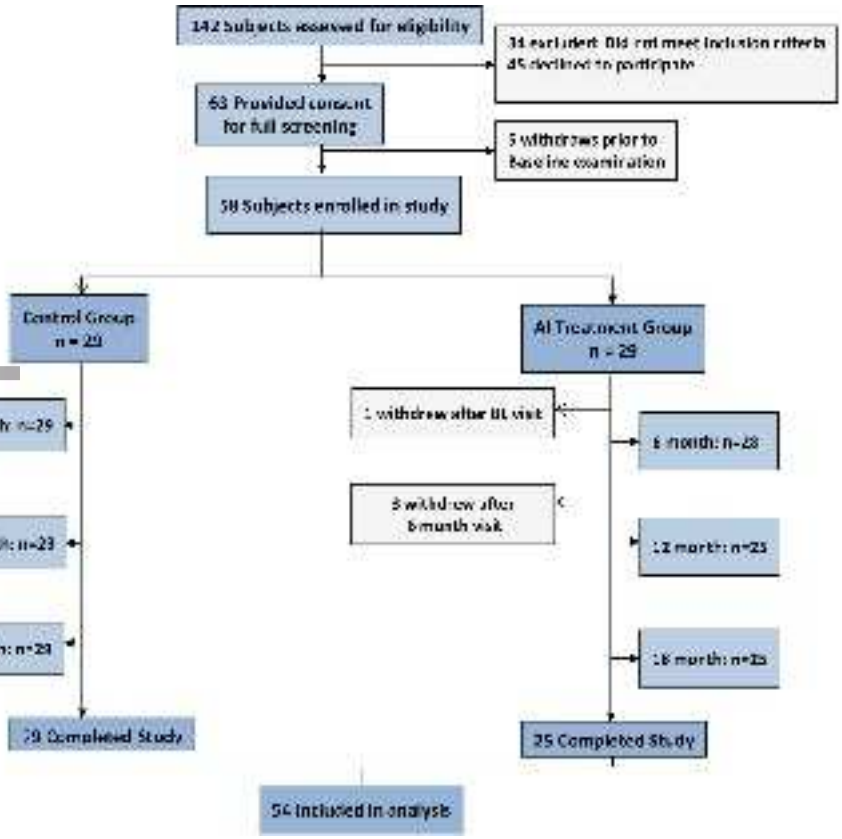
Table 5: Salivary Biomarkers (log10(pg/ml)

Biomarker	Group	Baseline (mean± SE)	12 months (mean± SE)	18 months (mean± SE)
CRP	**No AI**	2.96 ± 1.2	2.73 ± 0.92	2.89 ± 0.95
	AI	3.15 ± 0.76	2.70 ± 0.76	2.71 ± 1.12
TNFa[a]	**No AI**	0.97 ± 0.96	1.63 ± 0.66	1.30 ± 0.70
	AI	1.60 ± 0.73[a]	1.53 ± 0.45	1.88 ± 1.14[a]
Osteocalcin[a,b]	**No AI**	2.24 ± 1.2	2.52 ± 0.47	2.36 ± 0.50
	AI	2.28 ± 0.62	2.19 ± 0.40	2.60 ± 0.40 [a,b]
IL-1a	**No AI**	2.31 ±0.51	2.57 ± 0.53	2.51 ± 0.51
	AI	2.41 ±0.52	2.34 ± 0.49	2.45 ± 0.64
IL-1b[b]	**No AI**	1.86 ± 0.66	1.78 ± 0.67	1.99 ± 0.68
	AI	2.18 ± 0.75	2.01 ± 0.61	1.63 ± 0.50[b]
IL-6[a,b]	**No AI**	1.66 ± 0.40	1.69 ± 0.61	1.45 ± 0.52
	AI	1.62 ±0.60	1.70 ± 0.27	1.83 ± 0.33[a,b]
IL-8[a]	**No AI**	2.29 ± 0.35	2.20± 0.30	2.13 ± 0.36
	AI	2.52 ± 0.37[a]	2.86 ± 0.61[a]	2.68 ± 0.59[a]
IL-10	**No AI**	0.09 ± 0.46	0.01 ± 0.46	0.30 ± 0.36
	AI	0.03 ± 0.55	0.04 ± 0.38	0.26 ± 0.46
IL-17	**No AI**	0.63 ± 0.42	0.91 ± 0.40	1.06 ± 0.42
	AI	1.04 ± 0.36	1.01 ± 0.29	1.13 ± 0.29
IL-18	**No AI**	2.41 ± 0.62	2.89 ± 0.67	2.77 ± 0.59
	AI	2.66 ± 0.63	2.86 ± 0.61	2.98 ± 0.59 [b]
MCP1[a]	**No AI**	3.01± 0.33	2.96 ± 0.29	2.99 ± 0.34
	AI	3.26±0.26[a]	3.06 ± 0.29	3.34 ± 0.32
MMP8	**No AI**	3.85 ± 0.10	3.86 ± 0.11	3.84 ± 0.10
	AI	3.84 ± 0.07	3.87 ± 0.10	3.87 ± 0.10
MMP9	**No AI**	5.11 ± 0.11	5.07± 0.12	5.05 ± 0.18
	AI	5.11 ± 0.14	5.05± 0.10	5.08 ± 0.22
OPG[a]	**No AI**	4.77 ± 0.53	4.65 ± 0.54	4.41 ± 0.50
	AI	5.00 ± 0.69[a]	4.77 ± 0.62 [a]	4.72 ± 0.78[a]

SDF-1	**No AI**	2.74 ± 0.73	2.80 ± 0.59	2.38 ± 1.06
	AI	2.77 ± 1.05	2.79 ± 0.59	2.71 ± 0.90
TRANCE	**No AI**	3.10 ± 0.52	3.36 ± 0.44	3.01 ± 0.53
	AI	3.28 ± 0.57	3.32 ± 0.52	3.40 ± 0.46
VEGF	**No AI**	3.52 ± 0.15	3.49 ± 0.16	3.47 ± 0.19
	AI	3.51 ± 0.21	3.47 ± 0.14	3.56 ± 0.21

a: Wilcoxon Ranksum Test used to test for significant difference between AI users and Non-users

b: Wilcoxon Signed Rank Test used to test for significant difference between baseline and 18 months



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