Effects of Aromatase Inhibitors on the Periodontium among Postmenopausal Women with Breast Cancer

By

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Dedication

I dedicate this work and give special thanks to my family. Josh, you have been my strength. Maxwell and Alex, you are the reason I never gave up. Dan and Erin, you have been my biggest cheerleaders.

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Chapter I

Introduction

1.1 Problem Statement

Breast Cancer (BCA) is one of the most prevalent cancers affecting women. Postmenopausal women have an increased risk for developing breast cancer. Approximately 232,670 women living in the United States will be diagnosed with breast cancer in 2014.[1] Fortunately there is a 90% 5-year survival rate for women diagnosed with breast cancer.[1] In 2010, aromatase inhibitors (AI), anastrozole, letrozole, and exemestane were recommended to be included in the treatment of postmenopausal women with hormone receptor positive (HR+) breast cancer.[2] Although studies showed that AI's yield a higher survival rate than tamoxifen, and decrease the recurrence rate of the cancer, this class of drugs severely deplete the body of estrogen causing a greater than 10% side effect of osteoporosis and a 1-10% risk of bone fracture.[3]

To date the most common metabolic disorder in postmenopausal women is osteoporosis.[4] Postmenopausal osteoporosis is brought on by an inadequate amount of estrogen along with other risk factors associated with bone loss.[4] Estrogen deficiency is a dominant causative factor in postmenopausal osteoporosis.[5] Other risks for postmenopausal osteoporosis include lifestyle practices and nutrition.[4] Along with osteoporosis, postmenopausal women experience changes in their mouths.

Periodontitis is an inflammatory disease characterized by the loss of alveolar bone and clinical attachment loss of the soft tissues.[6] Although plaque is the primary

pathogen that initiates the inflammatory process, the progression of the disease is dependent on the role of systemic factors and the host response to bacterial mechanisms.[7,8] Osteoporotic changes caused by estrogen depletion may be a provoking component in periodontal disease. [8]

The depletion of estrogen in postmenopausal women plays a role in skeletal and alveolar bone loss.[9] Since the oral structures of mice are similar to humans, experimental models have been conducted to study the effects of estrogen on periodontal disease. [7] Skeletal responses of ovariectomy-induced osteopenia in mice parallel those of post-menopausal women.[7] A 2012 experimental study conducted by Kobayashi, et al. concluded that the ovariectomy of mice significantly increased alveolar bone loss. The study suggests that osteoporosis due to estrogen depletion, increases alveolar bone loss.[10]

Studies have shown a relationship between systemic and alveolar bone loss of the mandible, resulting in tooth loss.[4] Makker et al. concluded tooth loss in postmenopausal women may be an indicator of the onset of systemic osteoporosis.[4]

Post-menopausal women showed an association between mandibular bone mineral density and the bone mineral density of hip, neck, spine, hormones, and markers of bone resorption.[4] The analysis concluded a positive correlation between menopause, number of teeth present and mandibular bone mineral density.[4] Research has shown that low skeletal bone mineral density is correlated to alveolar bone loss and clinical attachment level loss placing postmenopausal women with osteoporosis at a greater risk for periodontal disease.[6,11]

Serum biochemical markers can be used to measure the rate of bone resorption and bone formation. The biomarker osteocalcin is a noncollagenous calcium binding protein synthesized mainly by osteoblasts.[12] Osteocalcin is present in gingival crevicular fluid (GCF) and reflects alveolar bone loss.[12] Payne et al. examined the relationship between serum biomarkers and bone mineral density in post-menopausal women with periodontitis and systemic osteopenia.[13] The study showed a positive relationship between serum bone biomarkers and alveolar bone loss.[13] More importantly, this study showed osteocalcin as a sensitive biomarker for alveolar bone loss.[13]

Due to the advancements in cancer medicine such as early detection and treatment, more and more women are surviving BCA.[14] AI's have become the gold standard of treatment for post-menopausal women with BCA.[15] Given the relationship between estrogen depletion, osteoporosis, and periodontitis, we are seeking to see which effects AI's have on alveolar bone loss.

1.2 Goal Statement

The goal of this investigation is to determine changes in the periodontium through the use of clinical parameters, salivary bone biomarkers, and the supplemental use of bisphosphonates, vitamin D, and calcium within postmenopausal women on AI.

1.3 Specific Aims

Specific Aim 1: To examine the changes in the periodontium as measured through the clinical periodontal parameters of clinical attachment levels, probing depths, bleeding on probing, and linear radiographic measurements in

postmenopausal breast cancer survivors on adjuvant aromatase inhibitor therapy as compared to those postmenopausal women not on adjuvant aromatase inhibitor therapy. *Hypothesis: Postmenopausal breast cancer survivors who are on adjuvant aromatase inhibitors will exhibit an increase in clinical attachment levels, probing depths, bleeding on probing, and a radiographic decrease in bone height as compared to those postmenopausal women who are not receiving adjuvant aromatase inhibitor treatment.*

Specific Aim 2: To determine if postmenopausal breast cancer survivors on adjuvant aromatase inhibitors exhibit an increase in alveolar bone loss as measured through the salivary biomarker osteocalcin as compared to postmenopausal women not on adjuvant aromatase inhibitors. *Hypothesis: Postmenopausal breast cancer survivors on adjuvant aromatase inhibitors will exhibit a higher level of osteocalcin as compared to those postmenopausal women not on adjuvant aromatase inhibitors.*

Specific Aim 3: To determine whether patient demographics and the use of bisphosphonates, vitamin D, and calcium have a differential impact on alveolar bone loss among postmenopausal breast cancer survivors on adjuvant aromatase inhibitors and postmenopausal women not on aromatase inhibitors. *Hypothesis: When controlling for demographics, we expect to see a difference in the effects of supplemental bisphosphonate, vitamin D, and calcium use between postmenopausal breast cancer survivors on aromatase inhibitors and postmenopausal women not on aromatase inhibitors.*

1.4 Significance:

AIs are widely used to treat breast cancer in postmenopausal women. These drugs profoundly deplete circulating estrogens which in turn may be associated with a loss of bone mineral density and an increased risk for osteoporosis and fracture. These conditions are associated with an increased risk for periodontal disease. At present, little is known about the oral side effects of AI. This is the first study to assess the impact of AI on the periodontium. It will aid in the understanding of oral care prior to starting aromatase inhibitor therapy by dental and medical professionals. The results of this study may have an impact on reimbursement policies for dental treatment prior to as well as during AI treatment.

1.5 Thesis Overview

Chapter II, the Review of the Literature, begins with an overview of the prevalence of breast cancer in the United States and adjuvant endocrine therapy. This is followed by a review of estrogen deficiency and how periodontal disease and osteoporosis are linked. Chapter II concludes with a review of salivary biomarkers and whole saliva sampling. Chapter III presents the materials and methods for the overall study and discusses the materials and methods of this analysis. The author presents the results in chapter IV, and provides the reader with a comprehensive discussion and conclusion in Chapters V and VI.

Chapter II

Review of the Literature

2.1 Prevalence of Breast Cancer in the United States

BCA is the most common cancer effecting women and the second leading cause of cancer death in women.[1] The American Cancer Society estimates that 232,000 women living in the United States will annually be diagnosed with breast cancer.[1] Although incidence of breast cancer is high, the prevalence of death is low. There is a 90% 5-year survival rate for women diagnosed with breast cancer. The etiology of BCA is unknown, but established risk factors for breast cancer include family history of breast cancer, obesity, an increase in age, dense breast tissue, alcohol consumption, and exogenous hormones.[16]

2.2 Adjuvant Endocrine Therapy

The main hormone involved in the development and growth of BCA tumors is estrogen.[17,18] Data have shown that adjuvant endocrine therapy is an effective treatment against tumor recurrence among women with HR+ breast cancer.[19,20] Anti-estrogen therapy is part of adjuvant endocrine care. It reduces recurrence of breast cancer and increases patient survival. Selective estrogen receptive modulators (SERM) such as tamoxifen, suppress the growth of hormone receptor positive breast tumors by binding the estrogen receptor.[18] In contrast, AIs impede the enzyme responsible for the synthesis of estrogens from androgenic substrates, causing a distinct suppression of

plasma estrogen levels in postmenopausal women.[18] Tamoxifen has previously been regarded as the gold standard of breast cancer treatment. AI's are now recommended to be a component of adjuvant endocrine therapy of postmenopausal women with early stage HR+ BCA because they further reduce the risk of disease recurrence.[17]

Data generated through Phase III randomized controlled clinical trials of postmenopausal women with hormone receptor positive breast cancer demonstrate that in comparison to tamoxifen alone, the use of an AI as a primary therapy or in sequential therapy with tamoxifen improves the length of disease free survival.[2] Hence, the AIs are a commonly prescribed medication for this population. AIs prevent conversion of androgens to estrogens and do not block ovarian estrogen production, t they are not indicated for premenopausal women.[18] In postmenopausal women, AIs cause relatively rapid decreases in circulating estrogen.[18] The toxicities of the AIs include the risk of accelerating bone loss and the development of osteoporosis and fractures, as well as a musculoskeletal syndrome characterized by bone and joint symptoms of pain and stiffness. The etiology and management of this musculoskeletal syndrome remains undefined and is undergoing investigation.[2] The estrogen deprivation associated with the aromatase inhibitors conceivably could affect the oral health of patients on these medications. There is currently limited research on the relationship of AIs and how the depletion of estrogen may impact the patient's oral health.

2.3 Estrogen Deficiency

Menopause

Estrogens are steroid compounds produced primarily by the ovaries that are important for normal development and functioning of female sexual development as well

as playing a crucial role in the skeletal growth and bone homeostasis of women. After the cessation of menstrual cycles and the onset of menopause, the primary source of circulating estrogen is derived from the conversion of androgens to estrogen in peripheral tissues.[23] Secondary estrogen sources are produced in small amounts in the liver, adrenal glands, fat cells and the breasts.[23] This secondary source of estrogen production is important for postmenopausal women but the estrogen produced is not at the same high levels as in a premenopausal woman. This lower level of circulating estrogen in menopause is associated a higher rate of bone resorption which may exceed that of bone deposition, leading to a net loss of bone mass and the risk of osteoporosis.

In addition to the changes to bone metabolism, menopausal symptoms may be present and include hot flashes, irritability, and vaginal atrophy.[23] In women with a history of breast cancer, use of exogenous estrogens to treat these symptoms is generally considered contra-indicated.

Osteoporosis

A common metabolic disorder in postmenopausal women is osteoporosis.[4] Osteoporosis is a systemic skeletal condition characterized by low bone mass and the deterioration of bone microstructure which leads to loss of bones strength and hence an increased susceptibility to fractures.[4] Approximately 40 percent of women over the age of 50 will experience a bone fracture related to postmenopausal osteoporosis during their lifetime.[5] Estrogen deficiency is a dominant risk factor for osteoporosis in postmenopausal women causing increased skeletal resorption and relatively decreased bone formation.[5]

In a low estrogenic state, osteoclasts may resorb bone at a rate that is uncoupled from sufficient bone formation by osteoblasts.[24] Bone loss ensues and ultimately, with the loss of bone mass, the individual becomes at increased risk for bone fractures in the bones of the hip, wrist and spine.[24]

A review by Weitzmann et al. discusses how postmenopausal osteoporosis should be viewed as a product of an inflammatory disease bearing similarities of an organ-limited autoimmune disorder, initiated by estrogen deficiency, and brought on by chronic mild decreases in T cell tolerance.[25] When estrogen deficiency provokes bone loss, an intricate interaction of hormones and cytokines converge to disrupt the process of bone remodeling.[25] Estrogen deficiency leads to an overall increase in interleukin seven (IL-7) production in target organs such as bone, thymus, and spleen, in part through decreases in transforming growth factor betta (TGF-β) and increased insulin growth factor one (IGF-1) production initiating T cell activation.[25] The activated T cells release interferon gamma (IFN-γ), which increases antigen presentation by dendritic cells and macrophages by upregulating major histocompatibility complex class II expression through the transcription factor class II major histocompatibility complex transactivator (CIITA).[25]

T cell activation and osteoclastogenesis is magnified by estrogen deficiency through down regulation of antioxidant pathways, which ultimately leads to an increase in Reactive Oxygen Species (ROS).[25] This stimulates antigen presentation and the production of tumor necrosis factor (TNF) by mature osteoclasts. Antigen presentation is distinctly enriched by the combined effect of IFN-γ and ROS, intensifying T cell activation and promoting release of the osteoclastogenic factors receptor activator

nuclear factor kappa B ligand (RANKL) and TNF.[25] Through the interleukin-one (IL-1) upregulation, stromal cell and osteoblast RANKL and macrophage colony stimulating factor production are further stimulated by TNF forcing osteoclast formation.[25] Direct repressive effects of osteoblasts cause TNF and IL-7 to further intensify bone loss by diminishing bone formation.[25]

Increasing evidence supports the association between osteoporosis and periodontal disease.[16] For every 1% per year decrease in whole-body bone mineral density, there is a more than four times increased risk of tooth loss in postmenopausal women.[26] These and similar statistics have caused the American Academy of Periodontology to consider osteoporosis as a risk factor for periodontal disease.[26]

2.4 Periodontal Disease

Periodontitis is a destructive inflammatory disease characterized by the loss of alveolar bone and clinical attachment loss of the soft tissues.[6,24] The precursor to periodontal disease is gingivitis. Gingivitis occurs when harmful bacteria accumulate in mass and thickness to form a film called plaque. When plaque adheres and remains on the tooth surface, especially at the gum line, the gingiva becomes inflamed and easily bleeds especially when brushing. At this point, any damage done is limited to the gingival tissues and is reversible through improved oral hygiene.[26] Although plaque is the primary factor that initiates the inflammatory process, the progression of periodontal disease is dependent on the role of systemic factors and the host response to bacterial mechanisms.[7,8] Periodontitis occurs when the inflammatory process is irreversible. In periodontitis, there is a turnover of alveolar bone with an increase of bone resorption and

decrease of bone growth resulting in alveolar bone loss, increase in probing depths, and clinical attachment loss[27] Symptoms such as tooth mobility, tooth loss, and abscesses appear late in the disease process.

2.5 Osteoporosis and Periodontitis

Osteoporotic changes have been seen in the oral cavity as a loss of alveolar bone, causing it to be a provoking component in periodontal disease.[8] The relationship between periodontal disease and osteoporosis was first addressed in 1990 by Kribbs et al.[28,29] They compared the mandibular bone mass of 85 osteoporotic women and 27 women without osteoporosis. Kribbs et al. reported a lower mandibular bone mass and density in the osteoporotic group, however no differences in clinical periodontal measurements were found between the two groups [Odds ratio (OR): 2.7 (95% CI: 1.1–6.5)].[28,29] Since then many studies have reported a positive relationship between osteoporosis and periodontal disease.[28,30-33]

To measure bone mineral density (BMD), Jeffcoat et al. used a dual energy x-ray absorptiometer (DXA) to measure the hip and quantitative digital radiography to measure mandibular bone in 158 postmenopausal women.[28,30] They found a significant positive correlation between mandibular basal bone and hipbone mineral density (OR: 5.23, $r=0.74$, $P<0.01$).[28,30] Tezal et al. also used DXA to measure skeletal systemic BMD and concluded that the mean alveolar bone level significantly correlated positively with systemic BMD($r= -0.20$ to -0.27) as well as finding a positive correlation between clinical attachment levels and BMD (OR: 2.89, $r= 0.10$ to 0.17).[28,31]

In 1995,Taguchi et al. studied 64 women age 50 to 70 years. The characteristics of osteoporosis studied were thoracic spine fracture, and periodontal characteristics studied included the number of teeth present, mandibular cortical width and alveolar bone resorption.[28,32] Their results showed a significant positive correlation with mean alveolar bone level and systemic BMD (Z=18.68-0.29).[28,32] Then in 2004, Taguchi et al. investigated the effects of estrogen use on tooth retention, oral bone height, and oral bone porosity in 264 postmenopausal women.[33] Multiple regression analysis showed that the duration of estrogen use was significantly associated with number of total ($p = 0.019$) and posterior ($p = 0.007$) teeth remaining, independent of age and oral bone height suggesting that estrogen may be a promoting factor in tooth retention by strengthening the periodontal attachment surrounding the teeth, but not increasing oral bone height and not decreasing oral bone porosity. [33]

A more recent study by Makker et al. has also shown a positive relationship between systemic and alveolar bone loss of the mandible, resulting in tooth loss.[34] In 2012, they concluded tooth loss in postmenopausal women may be an indicator of the onset of systemic osteoporosis.[34]

In contrast various studies have also shown no relationship between osteoporosis and periodontitis. In 1994, Von Wowern et al. used dual photon absorptiometry on 52 women with a history of osteoporotic fracture to measure mandibular bone mineral content.[28,35] They concluded that the osteoporotic women did not have a decrease of bone content in their jaw bones [OR: 1.00 (95% CI: 0.98–1.02)].[28,35] Lundstrom et al. found no statistically significant differences in gingival bleeding, probing pocket depths, gingival recession, or the marginal bone level between 15 women with osteoporosis and

41 women with normal BMD [OR: 1.3 (95% CI: 0.98–1.02)].[28,36] When comparing the clinical parameters of periodontitis and alveolar bone height with BMD of the lumbar and metacarpal bone, Elders et al. also did not find any statistically significant differences in gingival bleeding, probing pocket depths, gingival recession and marginal bone level of the subjects with low BMD compared to subjects with high BMD [OR: 1.46 (95% CI: 0.97–2.21)][28,37]

Skeletal and alveolar bone loss is accelerated by the depletion of estrogen in postmenopausal women.[9] When a depletion of estrogen causes bone resorption and remodeling, tooth support is negatively affected causing an increase in tooth mobility and tooth loss.[33] A longitudinal study was conducted by Jacobs et al. in 1996 assessing lumbar spine bone mineral density of 69 women receiving hormone replacement therapy, up to 5 years with dual photon absorptiometry of the lumbar spine.[28,38] They concluded that estrogen replacement therapy had a positive effect on the bone mass of the mandible and the lumbar spine.[28,38]

Out of 58 menopausal periodontal maintenance patients that Payne studied, 41 had normal bone mineral density and 17 were osteoporotic.[28,39] The osteoporotic estrogen-deficient women showed a greater alveolar bone loss, crestal and subcrestal density loss [OR: 1.73 (95% CI: 1.23–2.43)].[28,39] Fifty nine women with periodontitis and 16 non-periodontitis women, all within 5 years of menopause, were subjects in a study conducted by Reinhardt et al. that assessed bleeding on probing and clinical attachment levels.[28,5] The osteoporotic periodontitis patients with estrogen deficiency exhibited a greater amount of bleeding on probing and a greater rate of ≥ 2.0 mm clinical attachment level loss (3.8% versus 1.2%, 2 P<0.1) than estrogen sufficient subjects.[28,5]

Experimental mice models have also been conducted in order to study the effects of estrogen on periodontal disease.[7] Skeletal responses of ovariectomy-induced osteopenia in mice parallel those of post-menopausal women.[7] A 2012 experimental study on mice conducted by Kobayashi, et al. concluded that the ovariectomy of mice significantly increased alveolar bone loss, suggesting that osteoporosis due to estrogen depletion increases alveolar bone loss.[10] Studies done by Duarte, et al. have also discovered a direct relationship between periodontitis and estrogen deficiency.[8]

In summary, many of the above studies have been cross sectional in design using a small number of subjects. Future longitudinal studies will help strengthen the preliminary data to help better understand the relationship between postmenopausal osteoporosis, estrogen deficiency, and periodontitis.[30] Until then, estrogen depletion, low systemic bone mineral density and osteoporosis should be viewed as risk factors in periodontal disease.

2.6 Bisphosphonates and Periodontal Health

Bisphosphonates are widely used in the treatment of osteoporosis because of their ability to hinder bone resorption facilitated by a decreased function of osteoclasts thus improving bone density.[40] In diseases such as BCA, bisphosphonates help treat bone pain, improve quality of life, and can postpone skeletal events.[41] Studies have shown positive effects of bisphosphonate use in the treatment of periodontal disease.[42-44] Palomo et al. conducted a cross-sectional study to investigate the periodontal status of 60 age-matched postmenopausal women with mild to moderate osteoporosis.[42] The experimental group was undergoing systemic risedronate, a bisphosphonate therapy, for 3 months, while the

control group never used bisphosphonates.[42] Those on the risendronate therapy had an overall healthier periodontal status than control subjects exhibiting significant differences ($p<0.05$) in periodontal probing depth (2.6 vs 2.9 mm), gingival index (0.37 vs 0.71), plaque score (56.2 vs 77.0), attachment loss (2.8 vs 3.2 mm), and alveolar bone level (3.1 and 4.0).[42]

Palomo et al. then conducted a longitudinal study investigating the periodontal status of 28 white postmenopausal women with low bone density using bisphosphonate therapy for at least 2 years compared with that of a matching group not using bisphosphonate therapy.[43] Similar to the cross-sectional study results, women on bisphosphonates demonstrated statistically higher plaque score, lower probing depth, and lesser clinical attachment loss compared with the controls.[43] Although bisphosphonate users exhibited lower incidence of bleeding on probing, and lower alveolar bone height, the differences were not statistically significant.[43]

2.7 Calcium and Vitamin D Effects on Periodontal Health

Peak skeletal bone mass as it increases from infancy to early adulthood is influenced by hormonal factors, genetics, diet, exercise, and medications.[44] After that period of time, bone mass starts to decrease.[44] The degree of this increase and loss of bone mass is heavily relied on heredity and the availability of calcium.[44] Vitamin D promotes the absorption of calcium in the intestine while stimulating osteoblasts to support and preserve normal bone growth.[45] Both calcium and vitamin D are pivotal in the process of bone mineralization and the preclusion of osteoporosis.[45] 1a,25-dihydroxyvitamin, is vitamin D's biologically active form and possesses anti-inflammatory effects by inhibiting

the production of cytokine and stimulating monocytes and macrophages to conceal peptides with strong antibiotic activity.[45] Low levels of vitamin D cause the body to be vulnerable to infectious diseases and inflammatory conditions such as periodontitis.[45,46]

Miley et al. carried out a cross-sectional study of the effects of vitamin D and calcium supplementation on chronic periodontitis on 51 subjects.[47] 23 subjects were taking vitamin D (>or=400 IU/day) and calcium (>or=1,000 mg/day) supplementation. Although both groups improved in periodontal health with periodontal maintenance, the supplementation group had smaller probing depths, less bleeding on probing, lower gingival index values, fewer furcation involvements, less attachment loss, and less alveolar crest height loss. The differences between groups approached significance at (p=0.08).[47]

A study by Garcia et al. also found improvements in periodontal status when both vitamin D and calcium supplemental groups and control groups received regular periodontal maintenance.[45] When collectively looking at clinical attachment loss, bleeding on probing, gingival index, plaque index, and furcation, the differences between patients who did and did not take supplementation as part of their normal diets was modest (baseline (P= 0.061); 6 months (P= 0.049); and 12months (P= 0.114)).[45] There was no statistical difference found in radiographic measurements of alveolar crestal height between groups.[45]

It would appear that vitamin D and calcium supplementation is associated with improved periodontal health, however further studies are needed to solidify this hypothesis.[44-47]

2.8 Salivary Biomarker; Osteocalcin

Menopause and its low associated estrogen state is related to an increase in bone turnover that is complemented by an increase in bone formation and resorption, thus increasing serum bone formation and resorption biomarkers.[13] The biomarker osteocalcin is a noncollagenous calcium binding protein synthesized in mature human bone mainly by osteoblasts.[12,48]

It has been suggested that osteocalcin plays a role in bone resorption and deposition.[14] While being a specific marker of osteoblast function, osteocalcin in the plasma of postmenopausal women has also been known to be the best marker for spontaneous bone loss.[14] When resorption and deposition are coupled, serum osteocalcin is a marker of bone turnover.[14] When resorption and deposition is uncoupled, serum osteocalcin is a marker for bone formation.[14]

The mineralization of bone takes place due to the high attraction osteocalcin has for calcium.[48] Because of this, it exhibits a compact calcium dependent α helical conformation, in which the γ-carboxyglutamic acid (Gla) residues binds and promote absorption to hydroxyapatite in bone matrix.[48] In osteoporotic women, the formation of hydroxyapatite crystals is decreased through a deficiency of calcium and phosphorus. When the rate of bone mineralization is decreased, it allows free osteocalcin to be available for the circulation in the blood.[48] This explains the increase concentration of osteocalcin in serum levels of osteoporotic postmenopausal women.[48]

Hary Kumar et al. measured forearm, spinal, and femoral bone mineral density using dual-energy x-ray absorptiometry and markers of bone formation (serum osteocalcin and bone-specific alkaline phosphatase), serum osteocalcin and bone-specific alkaline phosphatase and bone resorption in 82 postmenopausal women with

untreated osteoporosis.[49] Serum osteocalcin was significantly different among the 3 study groups (4.1 +/- 2.7, 4.5 +/- 3.1, and 6.7 +/- 5.6 ng/mL, respectively; P = .0349) and had a significant negative correlation with BMD (r2 = -0.0779; P = .0168) concluding the significant correlation of osteocalcin to the bone mineral density in postmenopausal women with osteoporosis.[49]

Payne et al. examined the relationship between serum biomarkers and bone mineral density in 128 post-menopausal women with periodontitis and systemic osteopenia.[13] The study showed a positive relationship between serum bone biomarkers and alveolar bone loss.[13] Two-year changes in a serum bone biomarker were significantly associated with systemic bone mineral density loss at the lumbar spine (osteocalcin, bone-turnover biomarker, p = 0.0002) and femoral neck (osteocalcin p = 0.0025).[13] Two-year changes in serum osteocalcin and serum pyridinoline-crosslink fragment of type I collagen (ICTP; bone-resorption biomarker) were also significantly associated with alveolar bone density loss ($p < 0.0001$) and alveolar bone height loss (p = 0.0008).[13] More importantly, this study showed osteocalcin as a sensitive biomarker for alveolar bone loss. [13]

Makker et al. also concluded a strong positive statistical correlation between osteocalcin, systemic bone mineral density, and mandibular bone mineral density demonstrating osteocalcin levels of postmenopausal women are strong predictors of mandibular bone mineral density.[34]

2.9 Whole Saliva Sampling

Osteocalcin has been detected in whole saliva samples and GCF.[50,51] Using whole saliva collection methods to measure the biomarker osteocalcin is a fast and

convenient way to obtain samples. Whole saliva samples are potentially an overall representation of all periodontal sites providing an overall picture of a subject's periodontal disease status.[50,51]

Despite the advantages of whole saliva collection, the technique does have its limitations such as; the possible destruction of immunodeterminants necessary for immunoassay by the inhibitors or enzymes in saliva, the elevation of proteases in the saliva of periodontal subjects, which can ultimately decrease levels of protein biomarkers, subject oral hygiene, level of xerostomia, and variations in salivary flow rate.[50,52-54] A great amount of information regarding bone turnover specifically in the peridontium can be obtained through gingival crevicular fluid (GCF) samples, but this method can be time consuming and mirror gingival inflammation for the tested site only.[14,50]

Whole saliva methods have been used in studies to determine salivary biomarkers responsible for alveolar bone loss in postmenopausal women. A study by Scannapieco et al. provided preliminary evidence that several salivary biomarkers measured at baseline may serve to predict future alveolar bone loss.[55]

McGehee et al. used whole saliva samples to determine whether salivary concentrations of osteocalcin were statistically correlated with BMD. Results suggested that salivary osteocalcin concentrations could be used to predict both osteopenia and osteoporosis in human participants, as they positively correlated with calcaneal T scores.[56]

Conclusions and Recommendations:

Due to the advancements in cancer medicine such as early detection and treatment, more and more women are surviving breast cancer.[15] Aromatase inhibitors are becoming the gold standard of treatment for post-menopausal women with breast cancer, especially because their ability to lower the risk of tumor recurrence.[18] However, the use of AIs are associated with estrogen depletion and increased incidence of osteoporosis. Given the relationship between estrogen depletion, osteoporosis, and periodontitis, it is uncertain as to what the oral side effects of aromatase inhibitors are. Therefore this study aimed to determine the effects of aromatase inhibitors on the periodontium. Knowledge regarding the impact of AIs on periodontal health will aid in the appropriate oral and overall health care of these patients and help create a better standard of care for future patients. This knowledge will lead to an improved risk assessment of oral and overall health care of these patients and ultimately may lead to a better standard of care for future patients. Finally, this study will also provide a basis for further research on the oral effects of aromatase inhibitors.

Chapter III

Materials and Methods

3.1 Data Source

Data for this analysis was extracted from a 2009 18 month prospective cohort pilot study examining the oral health of 58 post-menopausal women (29 AI treatment group subjects and 29 control group subjects). This study was undertaken following IRB approval at the University of Michigan. Clinical dental data, standardized radiographs, oral fluid specimens, survey data on demographics, and perceived oral health were collected from all participants. Diagnosis date, cancer treatments, co-morbid conditions, and medication use was obtained from the patient's medical chart. The subjects did not undergo or receive any type of treatments.

Patient Recruitment

The recruitment of AI treatment group subjects was done by the University of Michigan Breast Care Center (UMBCC) Oncology Team. Control subjects were recruited through flyers approved by the institutional review board (IRB) posted within the University Campus and greater Ann Arbor Area, University of Michigan's Engage Website, the Clinical trials.gov website, the MCHOR website as well as at the University of Michigan Breast Imaging clinic (UMBI) for mammograms. The study consisted of 29 AI treatment group subjects and 29 control group subjects. The number of participants

for the study was determined through a power analysis. This sample size was chosen to meet recruiting feasibility limits. Based upon longitudinal pilot data of CAL in non-cancer patients, the sample size allowed for at least 80% power (with a Type I error rate of 5%) to detect a 10-point difference (i.e .20 vs. .10) in the 18 month change in percentage of sites with 3mm or more of CAL between the two groups of subjects (AI therapy vs. control). A single sample size that meets the power requirements for all hypotheses listed was calculated, but it was acknowledged, that statistical analysis may have been restricted by the sample size to a limited number of predictor variables for certain statistical models.

Eligibility

The following eligibility criteria were laid out for the 2009 prospective study.
Inclusion Criteria:

Postmenopausal as defined by National Comprehensive Cancer Network (any of the following)

- Prior bilateral oophorectomy
- Age equal to or greater than 60 years of age
- Age less than 60 and amenorrheic for 12 or more months in the absence of chemotherapy, tamoxifen, toremifen or ovarian suppression and FSH and estradiol in the postmenopausal range.
- If taking tamoxifen or toremifen and age less than 60y, then FSH and plasma estradiol level in postmenopausal ranges.
- Informed consent- Individuals capable of consenting and self-administering the survey instrument.

- Dentate- At least 15 teeth present.

AI users:

- Diagnosis of BCa- Histologic confirmed diagnosis of BCa: Stage 0, I, II, or III with no evidence of metastatic disease.
- Treatment- AI as clinically indicated (AI may be anastrozole, exemestane or letrozole). Subjects may have had prior tamoxifen or raloxifene. Subjects may have had chemotherapy and/or radiation therapy.

Controls:

- No Diagnosis of cancer- Patients must not have a diagnosis of any cancer.

Exclusion Criteria:

Medical history:

- Metastatic BCa (AI treated group: fully resected locally recurrent disease is permitted if the patient has been rendered without evidence of disease).
- Significant psychiatric illness/social situations that would preclude completion of questionnaire.

Medications:

- Chronic medications known to affect the periodontal status (calcium antagonist, anti-convulsives, immunosuppresives (> prednisone 7.5mg daily). NSAIDS and bisphosphonates are permitted.

Data Collection Procedures

Data were collected using both questionnaire and clinical examinations.

Questionnaire: Participants responded to self-report questionnaires at baseline, 6, 12 and 18 months. The questionnaire was completed with study personnel at the time of oral examination. The questionnaire included questions concerning the respondents' (a) demographic background such as their age, ethnicity/race, marital status, number of children, education, and income, (b) self-reported oral health such as the presence or absence of oral problems, self-perception of oral health, pain, dry mouth, and tooth loss, (c) dental care utilization such as their dental insurance status, the frequency of dental visits, reasons for not utilizing dental care services, dental consultations prior to breast cancer treatment, information given to patients concerning oral side effects of cancer therapy and oral hygiene self-care, (d) psychological factors such as depression and stress, and (e) lifestyle factors, namely use of tobacco and alcohol. [see appendix A for questionnaire].

Confidentiality was assured by assigning each survey a unique identification number. Only the PI, the research team, and statistician had access to the database.

Chart review: Patient charts were obtained and reviewed to extract information regarding cancer diagnosis, diagnosis date, cancer treatments, all other medical conditions, and a current medication list. [see appendix B for chart data abstraction sheet]

Clinical Data Collection Procedures

Examination Procedures: All dental examinations were performed at the Michigan Center for Oral Health Research (MCOHR) located at Domino's Farms. Dental examinations were done at baseline, 6, 12, and 18 months.

The examiner was a registered dental hygienist blinded to the cohort's group status. After the completion of inclusion and exclusion criteria, The examiner completed a full mouth standard comprehensive periodontal examination, including periodontal pocket depth, gingival recession, CAL, bleeding upon probing, plaque scores, missing teeth and supragingivial (above the gum line) plaque assessment on all teeth in each subject. Probing depths were measured with a University of North Carolina periodontal probe. Probing depth was measured on 6 sites per tooth. The loss of clinical attachment was defined as the distance in mm from the cemento-enamel junction to the base of the periodontal pocket. Probing depth was the distance from the free gingival margin to the base of the sulcus/pocket that could be probed. Distance was recorded to the next lowest millimeter. [see appendix C for the periodontal report form and clinical case report form.]

Standardized Radiographs: To measure changes in alveolar bone over time, 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. These x-rays are associated with 2 milli Severts of radiation which is a minimal amount of exposure for the patient and clinically not significant. All films were taken using an extended geometry method.[57,39] This technique assures that consistent projection geometry was achieved for each follow-up examination.

Whole Saliva Collection: To determine whether AI therapy increases bone remodeling biomarkers, osteoprotegerin (OPG) and osteocalcin was examined in the saliva of AI and non AI users at baseline, 12 and 18 months. Unstimulated whole saliva was collected from each subject as previously described by Mandel.[58] The procedures were stopped once a total of 2ml was collected or 15 minutes had elapsed. The sample was placed on

ice, aliquoted, and supplemented with two proteinase inhibitors (Aprotinin (1mg/ml) at a dilution of 1:100 and Phenylmethylsulphonyl fluoride (PMSF) (100 mM in MeOH) at a dilution of 1:200) and stored at -80 C.

Examiner Training and Calibration Session:

Prior to the start of the study, all study examiners attended a calibration session to ensure accuracy of data collection for all clinical parameters, which should increase intra and inter-rater reliability.

Human Subjects

Protection of Human Subjects: IRB approval was attained at the University of Michigan. The study utilized an informed consent procedure. Medical risks resulting from this research were extremely low. The study collected standard clinical dental data, clinically collected oral saliva specimens at relevant times and gathered materials from patient medical records.

The risk of breach of confidentiality of personal health information was present and was considered to be low due to safeguards implemented with the removal of patient identifiers and the use of secure databases, restricted access. Investigators and staff were well trained in the conduct of clinical research.

The procedures for protecting against potential risks, including risks of privacy and confidentiality included removing identifiers from the study specimens, securing data in password protected electronic database Velos systems with limited access and security software and working within organized institutions with staff well trained in the protection of human subjects. There was a very high likelihood that these procedures would minimize risk. Events were not expected to occur often and would be handled on an

individual basis. Should a breach have occurred, it would be investigated and the situation corrected. In the unlikely chance that a breach of confidentiality occurred, the IRB would be notified and the investigators would respond to the guidance provided by the IRB. The privacy of all participants was protected under HIPPA provisions.

The same procedures for protecting against potential risks of privacy, confidentiality, and breach of confidentiality were carried out for the secondary analysis.

Serious Adverse Event (SAE) Reporting: No Data Safety Monitoring Board was proposed for the primary correlative study as it was not applicable. Although SAEs were not anticipated to occur within this study of oral exam and questionnaires, should any SAE have occurred, it would be reported to the IRB as soon as possible but no later than 7 days from coming to the attention of the investigator. The Study Team reviewed the protocol progress weekly with attention to accrual rate and safety issues.

Informed Consent Procedures: All patients participating in this study were required to sign a statement of informed consent prior to participation in the study that included; the nature and purpose of the proposed study and the possible benefits to the patient, the length of the treatment and follow up required, risks or discomforts involved, alternatives to proposed study, name of the investigator(s) responsible for the study, right of the patient to accept or refuse treatment and to withdraw from participation in this study at any time, and a statement that the patient's confidentiality would be maintained. The informed consent document was reviewed with the patient prior to obtaining consent, and

a signed copy of the consent was provided to the patient, filed in a medical record and provided to the MCOHR Office.

Subject registration: After completing screening and informed consenting, the patient was registered thorough the MCOHR office. Each patient was assigned a clinical study registration number which served to code their specimens and clinical data.

3.2 Materials and Methods for Secondary Data Analysis

Using the described prospective cohort study above we tested the following hypotheses; *Hypothesis1: Postmenopausal breast cancer survivors who are on adjuvant aromatase inhibitors will exhibit an increase in clinical attachment level, probing depths, bleeding on probing, and a radiographic decrease in bone height as compared to those postmenopausal women who are not receiving adjuvant aromatase inhibitor treatment. Hypothesis 2: Postmenopausal breast cancer survivors on adjuvant aromatase inhibitors will exhibit a higher level of osteocalcin as compared to those postmenopausal women not on adjuvant aromatase inhibitors. Hypothesis3: When controlling for demographics, we expect to see a difference in the effects supplemental bisphosphonate, vitamin D, and calcium use between postmenopausal breast cancer survivors on aromatase inhibitors and postmenopausal women not on aromatase inhibitors.* The implementation of this study and the results of the secondary analysis will provide insights into alveolar bone loss as a side effect of adjuvant AI in postmenopausal women with breast cancer.

Alveolar Bone Loss: To determine the changes of the periodontium as measured through the clinical periodontal parameters of CAL, PD, BOP, and linear radiographic measurements in postmenopausal breast cancer survivors on adjuvant aromatase inhibitor therapy compared to those postmenopausal women who did not receive adjuvant aromatase inhibitor therapy, a mean of CAL, PD, and BOP from the overall study was analyzed to determine the changes of alveolar bone loss. Utilizing the standardized radiographs taken from the previously described cohort study, the presence or absence of alveolar bone loss over time was determined using the *Image J* software program. Radiographs were transferred from the MCOHR facility to the University of Michigan Dental School by downloading all patient radiographs on a secure electronic server. Linear measurements between the cemento-enamel junction 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 were analyzed by a single calibrated examiner. All measurements were calibrated by the measurement of the inserted step-wedge. 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 used was millimeters. Alveolar bone loss was defined as a 0.4mm or greater reduction in bone height.

Salivary Biomarkers: In order to determine whether breast cancer survivors on aromatase inhibitors exhibit an increase in alveolar bone loss as measured through the

salivary biomarker osteocalcin, whole saliva was analyzed. The previously collected frozen samples were thawed at room temperature. Saliva OPG and osteocalcin levels were quantified in cell-free supernatants by ELISA (Osteoprotegerin; ALPCO Diagnostics, Salem, N.H., Osteocalcin ELISA; BTI, Stoughton MA).[59] Total protein levels were used to normalize the values (Sigma, St. Louis MO). Protein levels were compared to the clinical data including CAL, pocket depths, and radiographic data. All laboratory analyses were performed by a laboratory technician at the Russell Taichman laboratory in the University of Michigan, School of Dentistry.

Demographics, Bisphosphonates and Supplementation: To determine whether patient demographics and the use of bisphosphonates, vitamin D, and calcium have a differential impact on alveolar bone loss among postmenopausal breast cancer survivors on aromatase inhibitors as compared to postmenopausal women not on aromatase inhibitors: Data regarding subject age, ethnicity, marital status, education, smoking status, dental utilization, and dental insurance status was extracted from the survey questionnaire. Data regarding use of bisphosphonates, vitamin D and calcium supplementation was extracted from the survey questionnaire and medical charts to examine if the interaction of aromatase inhibitors in the presence of these supplements caused an effect on alveolar bone loss.

Age was collected as a continuous variable. AI status, marital status, dental insurance status, alcohol consumption, diabetes status, and current tobacco use were collected as categorical variables. Subjects answered yes or no for each question. Ethnicity was defined as white or non-white.

Education was collected as a categorical variable. The variable was defined as "some college or more" and "high school or less". Income was collected as a categorical variable. The variable was defined as "no income- $19,999", "$20,000-$39,999", "$40,000-$59,000", $60,000-$74,999", and "over "$75,000". Employment status was collected as a categorical variable. The variable was defined as "working full-time", "working part-time", "not actively employed". Bisphosphonate, calcium, and vitamin D supplementation were recorded as categorical values. Each subject answered a yes-1 or no-0 based on their supplement use at baseline.

Statistical Analysis Plan

Statistical analyses were done taking into account the study design and number of subject participants. Univariate statistics were calculated for continuous variables and frequency tables were generated for categorical variables. Independent sample t tests were done to investigate the differences of alveolar bone height between groups at each time point, the effects of bisphosphonate, calcium, and vitamin D supplementation on alveolar bone height between groups at each time point, as well as mean CAL, PD, and BOP. Paired t tests were done to investigate intragroup changes for bone height, CAL, PD, and BOB. Linear Pearson Correlation Coefficients were used to look at the correlation between salivary osteocalcin and bone height between groups. Linear mixed models were constructed to investigate bone height as a function of time, AI use, calcium, vitamin D and bisphosphonate status, along with an interaction between AI and calcium status. Data analysis was performed using the statistical package IBM SPSS.

Chapter IV

Results

Figure 4.1 provides an overview of the study participant recruitment and enrollment. Potential participants assessed for eligibility were n=142. Of the 142 eligible participants, 26 were excluded because they did not meet inclusion criteria and 45 declined to participate. A total of 63 study participants were enrolled, 29 control subjects, and 34 AI treatment subjects. Prior to baseline examination, 5 AI treatment subjects withdrew from the study n=29. After the baseline visit, 1 AI treatment subject withdrew and after the 6 month visit, 3 AI treatment subjects withdrew resulting in 25 study participants. There were no withdraws among the control subjects.

Among the 58 study participants, 29 were taking aromatase inhibitors and 29 were not. The age range of study participants was 44-75 years old. and the mean age of study participants was 61.66 years old. The majority of the study participants were Caucasian 89% (n=52) and 67.2% (n=39) were married. Approximately 66% of women (n=38) reported having some college education or more, while 33% (n=19) reported having a high school or less than high school education. Of the 58 study participants, 60.3% (n=35) reported drinking alcohol, 3.4% (n=2) were current tobacco users, and 8.6% (n=5) had a health history of diabetes. Slightly over half the study participants, 51.7% (n=30) were not actively working, 19% (n=11) working part time, and 29.3% (n=17) were full time workers. An income of over $75,000 was reported by 36.2 (n=21)

participants, 37.9% (n=22) reported an income of $20,000-$75,000, while 22.4% (n=13) reported an income of $19,000 or less. Forty-four women (75.9%) reported having dental insurance. At baseline, 27.6% (n=16) study participants were taking bisphosphonates, 53.4 (n=31) taking calcium supplements, and 58.6% (n=34) taking vitamin D. Of the 58 enrolled participants, 53 participants completed the study. Of the 53 participants, 24 were taking aromatase inhibitors and 29 were not.

Table 1 provides demographic characteristics of study participants broken out by AI status at baseline. The statistics for participants on AI and not on AI (controls) were similar for all categories. The average age for both groups was 61+ years. Both groups were comprised of nearly 90% (n=26) Caucasians. Income, education levels, and dental insurance status were also similar for both groups.

A descriptive summary of linear alveolar bone height by AI status is provided in Table 2. An increasing value for alveolar bone height from each time point indicates a greater loss of alveolar bone and the subject's periodontal status is worsening. The average difference in bone height from baseline to 18 months for participants on aromatase inhibitors was 0.32mm ± SD 0.36, indicating a decrease of about 0.32mm over the course of the study. Similarly, the average difference in bone height from baseline to 18 months for participants not on aromatase inhibitors was a decrease of 0.25mm ± SD 0.22. The difference in bone height from baseline to 18 months was not statistically different comparing those on AI and those not on AI (t(df)= 0.80(48), p=0.42). No statistically significant differences were found between the groups for average bone height at baseline, 12, or 18 months. When looking within the groups, the difference in bone height from baseline to 18 months was significantly different for those on AI (t(df)=

4.081(20), p=0.001, as well as the difference in the 12 to 18 month time point (t(df)= 3.504(20), p=0.002 (Table 3). Those not on AI showed a statistical difference in bone height between each time point (Table 4).

A descriptive summary of the average clinical attachment level (CAL) broken out by AI status is provided in Table 5. The mean CAL at baseline for participants not on AI was 1.42 ± SD 0.39. Similarly, the mean CAL for these participants at 18 months was 1.45 ± SD 0.20. The mean CAL of study participants on AI at baseline was 1.51 ± SD 0.75, and 1.84 ± SD 0.40 at 18 months. Comparing those on AI therapy and those not on AI therapy showed no statistical difference at baseline (t(df)=0.58(56) p=0.56). However there was a statistically significant difference between those on AI and those not on AI at 6 (t(df)= 3.23(54) p<0.001), 12 (t(df)= 3.43(50) p<0.001), and 18 months (t(df)= 4.53(50) p<0.001). Tables 6 and 7 are a summary of the average CAL over time within groups. In general, a statistical difference in CAL was also seen when looking over time within the groups. There was a statistical difference in CAL for those on AI between baseline and 6 months (t(df)= 2.990(26), p=0.006), baseline and 12 months (t(df)= 4.489(22), p<0.001), and baseline and 18 months (t(df)= 5.705(22), p<0.001). For those not on AI, the only statistical difference in CAL was at the baseline to 6 month time point (t(df)= 2.627(28), p= 0.014).

A descriptive summary of the average probing depth measurement (PD) broken out by AI status is provided in Table 8. The mean PD at baseline for participants not on AI was 2.00 $\pm$ SD 0.29. Similarly, the mean PD for these participants at 18 months was 2.00 $\pm$ SD 0.19. The mean PD for study participants on AIs at baseline was 2.00 $\pm$ SD 0.27 and 2.26 $\pm$ SD 0.30 at 18 months. When comparing PD for those on AI and not on

AIs there were statistical differences at 6 months (t(df)= 4.49(54) p< 0.001), 12 months (t(df)= 3.10(50) p=0.01), and 18 months (t(df)= 3.72(50) p<0.001). When looking at differences between baseline and 18 month time points for those on AI and not on AIs, a significant difference was also found (t(df)= -3.96(50), p<0.001). Participants on AIs had higher PD measures at each time point. No significant differences between the groups were found for average PD at baseline. Intragroup analysis showed a statistical difference in PD over time for those on AI between baseline and 12 months (t(df)= 3.355(22), p=0.003), as well as baseline and 18 months (t(df)= 4.756(22), p<0.001) (Tables 9 and 10).

A descriptive summary of average bleeding on probing (BOP) broken out by AI status is provided in Table 11. The mean BOP at baseline for participants not on AI was 0.107 $\pm$ SD 0.080. The mean BOP for these participants at 18 months was 0.148 $\pm$ SD 0.132. For those on AI, mean BOP at baseline was 0.176 $\pm$ SD 0.141and 0.198 $\pm$ SD 0.121at 18 months. When comparing BOP for those on AIs and not on AIs, there were statistical differences at baseline (t(df)= 2.288(44.250), p= 0.027, 12 months (t(df)= 2.734(50), p=0.009, and when looking at the difference between baseline and 18 months (t(df)= 3.247(39.237), p=0.002. Interestingly, within groups, BOP was statistically significant over time at each time point for those not on AIs and statistically significant at baseline to 12 months for those on AIs (t(df)= 2.351(22), p= 0.028 (Tables 12 and 13).

Table 14 illustrates the effects of vitamin D use on alveolar bone height for those on AI while Table 15 illustrates its effects on alveolar bone height for those not on AI. The average difference in bone height from baseline to 18 months for participants on AI and vitamin D was 0.31 $\pm$ SD 0.32, indicating an increase of about 0.31mm over the course

of the study. Similarly, the average difference in bone height from baseline to 18 months for participants on vitamin D and not on AI was an increase of 0.24 ± SD 0.20. The difference in bone height from baseline to 18 months was not statistically different for those on AI and vitamin D (t(df)= 0.65(16), p=0.52). For those on AI, the average bone height at baseline, 12 months, and 18 months did not differ significantly between those on vitamin D and those not on vitamin D. Further, for those not on AI, average bone height at baseline, 12 months, and 18 months did not differ significantly between those on vitamin D and those not on vitamin D.

Table 16 illustrates the effects of calcium use on alveolar bone height for those on AI while Table 8 illustrates its effects on alveolar bone height for those not on AI. The average difference in bone height from baseline to 18 months for participants on AI and calcium was 0.26 ± SD 0.34, indicating a decrease of about 0.26mm over the course of the study. The average difference in bone height from baseline to 18 months for participants on calcium and not on AI was a decrease of 0.21 ± SD 0.12. For those on AI, the average bone height at baseline (t(df)= -2.56(17), p=0.02), 12 months (t(df)= -2.44(16), p=0.03), and 18 months (t(df)= -2.13(16), p=0.05) differed significantly between those on calcium and those not on calcium. The difference in bone height from baseline to 18 months was not statistically different for those on AI and calcium (t(df)= -0.27(16), p=0.78). For those not on AI, average bone height at baseline, 12 months, and 18 months did not differ significantly between those taking calcium and those not taking calcium (Table 17).

Table 18 illustrates the effects of bisphosphonate use on alveolar bone height for those on AI while Table 10 illustrates its effects on alveolar bone height for those not on

AI. The average difference in bone height from baseline to 18 months for participants on AI and bisphosphonates was 0.08 ± SD.27, indicating a decrease of about 0.08mm over the course of the study. The average difference in bone height from baseline to 18 months for participants on bisphosphonates and not on AI was a decrease of 0.20 ± SD 0.14. For those on AI, the difference in bone height from baseline to 18 months was significantly different for those on bisphosphonates compared to those not on bisphosphonates ($t(df)= -2.12(16)$, $p=0.05$). We did not see significant differences for those on AI at baseline, 12, and 18 months for those on bisphosphonates compared to those not on bisphosphonates. For those not on AI, average bone height at baseline, 12 months, and 18 months did not differ significantly between those on bisphosphonates and those not on bisphosphonates.

A linear mixed model (Table 20-24) was constructed to investigate bone height as a function of time, AI, calcium, vitamin D and bisphosphonate status, along with an interaction between AI and calcium statuses. Linear mixed models account for the dependence in the data due to repeated measures per study participant. When controlling for the other variables in the model, a significant effect of time was found along with a significant AI status by calcium use interaction. Those on AI and calcium have a significantly lower bone height value (Est. Mean=2.50, SE=0.13) than those on AI but not on calcium (Est. Mean=3.32, SE=0.23) ($p=0.005$) but no significant difference was found between those on calcium and those not for those not on AI. Bone height decreased significantly over time when controlling for the other covariates in the model.

Table 25 illustrates salivary osteocalcin levels for those on AI and controls. There was no statistical difference in salivary osteocalcin levels between the two groups at

baseline t(df)=-.31(54) p=0.76, 12 months t(df)= 1.36(44) p= 0.18, and 18 months t(df)= -0.57(45) p=0.23. When looking at the correlation between salivary osteocalcin and bone height, there was no significant relationship at baseline, 12, and 18 months using Pearson Correlation Coefficients (Tables 26,27).

Chapter V

Discussion

The aims of this study were to examine the changes of the periodontium as measured through the clinical periodontal parameters in postmenopausal breast cancer survivors on adjuvant AI as compared to those not on AI. Secondary aims of this study were to determine if these women exhibit a decline in the periodontium as measured through the salivary biomarker osteocalcin, and whether patient demographics and the use of bisphosphonates, vitamin D, and calcium supplements have a differential impact on alveolar bone loss. This investigation suggests that adjuvant AI therapy does have an impact on the oral health of postmenopausal women on AIs. Furthermore, in postmenopausal women on AIs, calcium supplementation decreases alveolar bone loss. The results also suggest that clinical attachment levels, BOP and PD increase over time in postmenopausal women on AI's.

Periodontitis is an inflammatory disease characterized by the loss of alveolar bone and clinical attachment loss of the soft tissues while osteoporosis is characterized by bone loss leading to structural bone transformation.[4,6] Osteoporotic changes have been seen in the oral cavity as a loss of alveolar bone, causing it to be a provoking component in periodontal disease.[8]

Previous investigations have illustrated the advantageous effects of hormone replacement therapy on clinical attachment levels.[60,5] A longitudinal study by Reinhardt studied 59 osteoporotic postmenopausal women, and found that estrogen supplementation was associated with less clinical attachment loss.[5] Our group of postmenopausal women taking AI showed significant differences compared to postmenopausal women not taking AI for average CAL and PD at 6,12, and 18 months, and also when looking at the difference in CAL and PD from baseline to 18 months. When looking within groups, we also saw significant differences over time for CAL and PD for those women on AI. This suggests AI causes a negative effect on CAL and PD that increases with time. In a cross-sectional study, Aspalli et al. concluded a definite relationship between osteoporosis and periodontitis based on PD and CAL.[61] As the BMD decreases, the PD and CAL increases.[61] Shen et al. also suggests osteoporosis is a risk factor for periodontitis after finding an increase in attachment loss in osteoporotic subjects.[62] Using the NHANES III data, Ronderos et al. in a found that women with high calculus index and low BMD had significantly more CAL than women with a similar calculus index and normal BMD.[63]

Studies have shown that postmenopausal women on bisphosphonates have improved periodontal disease and bone turnover including those with osteoporosis.[42,43,65] Postmenopausal subjects on bisphosphonates demonstrated a significantly less plaque accumulation, less gingival inflammation, lower probing depths, less periodontal attachment loss, and greater alveolar bone levels, suggesting that bisphosphonate therapy may play a beneficial role in periodontal status.[42] When investigating the effects of bisphosphonate supplementation on alveolar bone loss, we saw no significant

differences for those postmenopausal women taking AI and bisphosphonates at baseline, 12, and18 months as compared to those taking AIs and not taking bisphosphonates. We did, however see an overall statistical significance in those on AIs and bisphosphonates when looking at the difference in bone height from baseline to 18 months t(df)= -2.12(16) p= 0.05. For postmenopausal women not on AI, average bone height at baseline, 12 months, and 18 months did not differ significantly between those on bisphosphonates and those not on bisphosphonates. For postmenopausal women not on AI, average bone height at baseline, 12 months, and 18 months did not differ significantly between those on bisphosphonates and those not on bisphosphonates. As the sample size for this pilot study was small, it may have impacted our ability to detect a significant difference in bisphosphonate use. Further longitudinal studies with larger sample size should be done to gain a better understanding of the relationship between bisphosphonate use and alveolar bone loss among AI users.

Although sustaining ideal levels of calcium and vitamin D is essential for maintaining bone in postmenopausal women, there are limited studies documenting their role in periodontal health.[66,67] Based on data from the 3rd National Health and Nutrition Examination Survey (NHANES III), Dietrich et al. found a significant correlation between vitamin D levels and clinical attachment loss in both men and women over the age of 50.[67,68] Those on vitamin D had lower clinical attachment loss than those not on vitamin D. Dietrich et al. also determined an association between low vitamin D serum levels and an increase of bleeding on probing establishing the positive anti-inflammatory role vitamin D plays in gingival inflammation and periodontitis.[67,68] This investigation did not find the supplementation of vitamin D to have a significant effect in bone height in either

the AI or control groups. In a 7 year prospective study of 62 year old men, 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.[66,67] Evidence suggests increased levels of calcium are positively correlated with a reduced prevalence of clinical attachment loss and a lower risk of tooth loss.[66] Utilizing the NHANES III data, Nishida et al. found subjects with inadequate calcium levels exhibited more severe periodontal disease.[67,69] This study showed significantly lower bone height values for postmenopausal women taking AI and calcium supplements compared to those taking AI but not taking calcium. AI users supplementing with calcium exhibited less bone loss at baseline and each subsequent time point than those AI users not taking calcium; however, there was no significant difference between these groups in bone height change from baseline to 18 months. One explanation for a non-significant finding is the loss to follow up that occurred within the AI group impacting 18 month measures (loss to follow up n=4). This loss of participants may have affected the ability to detect a difference in bone height. Millen et al., conducted one of the largest prospective study on the effects of vitamin D on the advancement of periodontal disease in postmenopausal women.[70] The authors did not note an association between vitamin D status and alveolar bone height as well as CAL, PD, and gingival bleeding.[70] The cohort of postmenopausal women in the Millen study did not have a high prevalence for periodontal risk factors such as smoking, as in our study.[70] As there is conflicting evidence available, there is a need to conduct randomized controlled clinical trials to determine the effects of both vitamin D and calcium supplementation on alveolar bone loss. Evidence from studies has linked an association between calcium use and an increased risk for cardiovascular events

among patients undergoing chemotherapy.[71,72] Advice about supplement use, such as calcium, needs to be individualized and come from a credible source, and it is best communicated by the physician.

This study did have some limitations. First, subjects initially had minimal periodontal attachment loss and changes in both groups for PD and CAL, though statistically significant, were of small magnitude. Thus, the effect size was relatively small. Although we saw statistically significant changes in linear radiographic bone height over time when looking within groups for both women on AI and not on AI, the investigation, as originally hypothesized did not show a difference in alveolar bone height between the two groups of postmenopausal women. When looking at table 2, if one subtracts the baseline mean alveolar bone height from the 18 month alveolar bone height, the difference (2.86-2.67=0.19mm) does not agree with the reported difference of 0.32mm because the computed mean difference was based on sample sizes for those on AI that varied slightly over time because of the withdrawal of AI users due to drug toxicity. In addition, we may not have seen any changes in bone height because the accuracy of the linear measurements may be have been limited by the ununiformed technical quality of the x-rays and unintentional differences in x-ray techniques. Although a beam guiding/ positioning device, bite registration, and step wedge were used, the film placement varied and both vertical and horizontal images were taken. If radiograph placement had been uniform, subtraction radiography may have been possible. Digital subtraction radiography is a technique used to determine both qualitative yes/no results and quantitative results expressed in absolute units, mg of bone, or relative units in the changes that occur between two or more images taken at different points in time.[73]

Subtraction radiography has been suggested to be a more diagnostically accurate way to identify alveolar bone change compared to conventional x-ray techniques.[74] Perhaps the use of a vertical posterior bitewing compared to a posterior periapical may have made the linear measurements more accurate. Overall, both the control and AI subjects presented with mild disease to a fairly healthy periodontium. It is difficult to determine in such a small and healthy population whether or not AI is affecting alveolar bone height, especially since we have not provided any type of oral health treatment for these women.

When measuring osteocalcin levels, the investigation also did not show a difference in alveolar bone height between the two groups of postmenopausal women. Many women who were taking AIs were unable to give a saliva sample. We had 1 missing sample at baseline, 10 at 12 months, and 11 at 18 months. It was difficult to determine whether the dry mouth was a side effect of their AI therapy or other medications that might have a side effect of xerostomia such as antidepressants, antihistamines, and medications that that treat high blood pressure and heart conditions. The number of missing saliva samples coupled with the loss of AI users at the 12 and 18 month time point may have affected our ability to find a correlation between the salivary biomarker osteocalcin and bone height. Although whole saliva samples are an overall representation of all periodontal sites providing an overall picture of a subject's periodontal disease status, GCF samples provide a great amount of information regarding bone turnover specifically in the peridontium, and may have been easier to collect, especially in women who could not produce whole saliva samples.[50,51,14]

As this was a pilot study the sample size was small. Beginning trends in bone loss may have been seen, but may not have had enough power to detect a difference or perform more advanced statistical analysis.

However, this study had some notable strengths including the comprehensive periodontal examinations and detailed demographic and cancer information. Although the sample size was small, there were power calculations for the primary endpoint.

This study is among the first to investigate the oral effects of AI on postmenopausal women with breast cancer. The results from this study indicate those on AI experience greater increases in CAL, PD, and BOP. Future longitudinal studies lasting longer than 18 months, with a larger number of subject participants, may give evidence and demonstrate an even greater negative effect on the periodontium while on AI treatment.

Chapter VI

Conclusions

The objective of this investigation was to determine changes in the periodontium through the use of clinical parameters, salivary bone biomarkers, and the supplemental use of bisphosphonates, vitamin D, and calcium within postmenopausal women with breast cancer on aromatase inhibitors. The results from this investigation indicate that prolonged use of AI may cause an increase in CAL loss, PD, and BOP. AI use supplemented with bisphosphonates and calcium, may cause an increase in alveolar bone loss.

New guidelines on adjuvant hormonal therapy have been developed by The American Society of Clinical Oncology (ASCO) recommending all women diagnosed with hormone-receptor-positive breast cancer be offered the option of taking hormonal therapy for 10 years.[75] Postmenopausal women who have originally received 5 years of tamoxifen, should be offered the choice of continuing tamoxifen for up to 5 more years or switching to an aromatase inhibitor for 10 years total adjuvant endocrine therapy.[75] The Knowledge about the prolonged use of AI will lead to an improved risk assessment of oral and overall health care of these patients and ultimately may lead to a better standard of care for future patients. This study will also provide a basis for further research on the oral effects of AI.

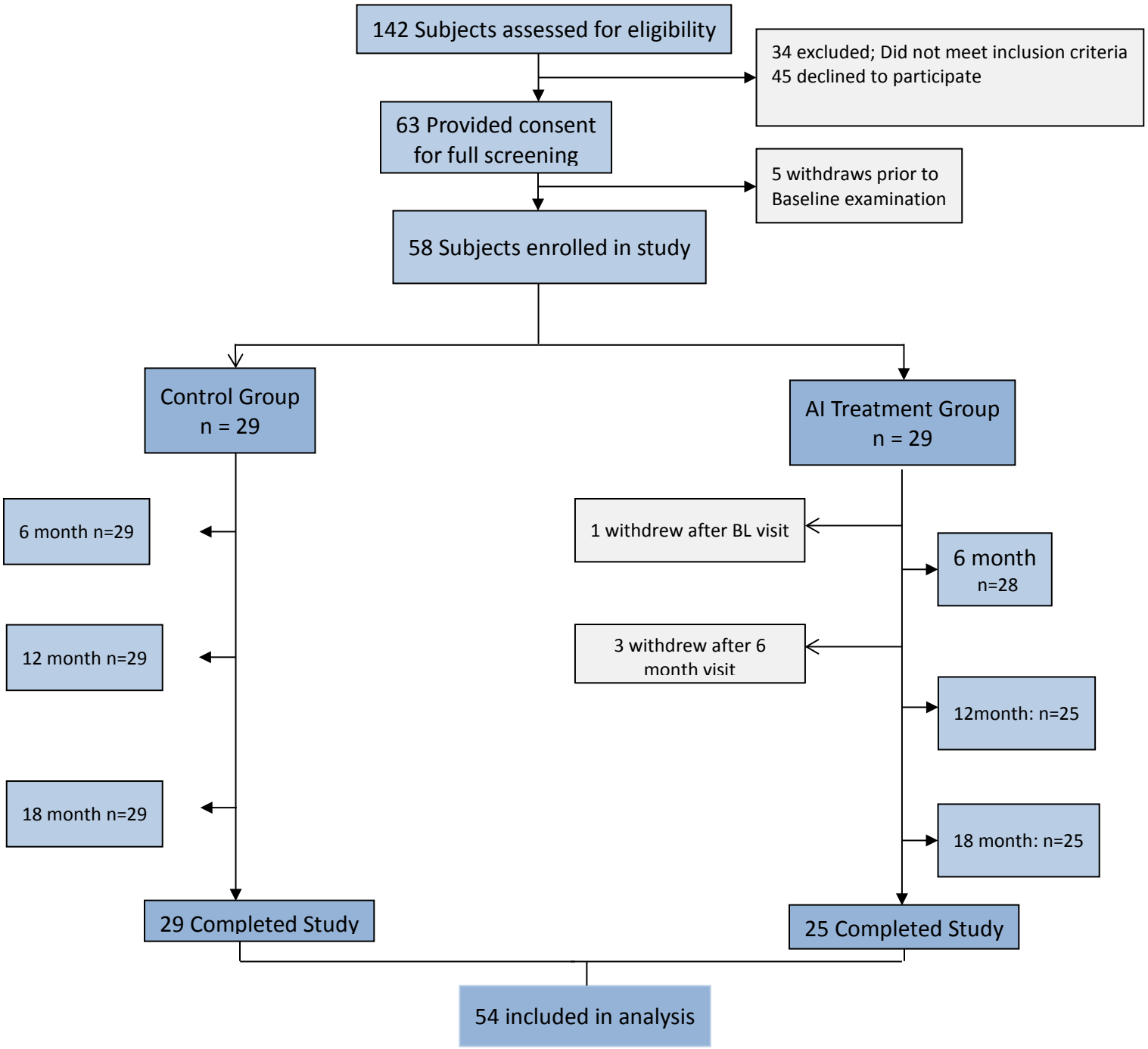
Figures


Progression of Gum Disease
Healthy Teeth and Gums
Gingivitis
Early Periodontitis
Moderate Periodontitis
Advanced Periodontitis
Pink gums
No bleeding gums with flossing and brushing
Fresh breath
Red swollen gums
Bleeding gums with flossing and brushing
Possible bad breath or taste
No bone loss
No tooth mobility
Red swollen gums
Bleeding gums flossing and brushing
Persistent bad breath or taste
Slight bone loss
Possible tooth mobility
Red swollen gums
Bleeding gums flossing and brushing
Persistent bad breath or taste
Moderate bone loss
Tooth mobility and root exposure
Red swollen gums
Bleeding gums flossing and brushing
Persistent bad breath or taste
Severe bone loss
Severe tooth mobility and root exposure
Possible tooth loss

Figure 1; Images of Gingivitis and Periodontal Disease.

http://dentistatrajkot.com/Treatments/Gum-Problems/1474

Figure 2: Study Subject Participation Consort Chart



Tables

Table 1.
Demographic Characteristics of Study Patients on AI and Controls[+]

	On AI N=29		Control N=29		
Analysis variable	Obs	%	Obs	%	P Value
Age		61.7 (7.6)		61. 6 (5.4)	0.92
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 diploma	6	20.7	5	17.9	
More than high school	20	68.8	18	64.3	0.82
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	
Not Married	8	27.6	11	37.9	0.36
Has Dental Insurance					
Yes	21	72.4	23	79.3	
No	8	27.6	6	20.7	0.76
Last Dental visit					
Within last 6 months	25	89.3	27	93.1	
More than 6 months	3	10.6	2	6.90	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	
No	18	62.1	24	82.8	0.07

Calcium Supplement					
Yes	20	71.4	11	45.8	
No	8	28.6	13	54.2	0.42
Vitamin D Supplement					
Yes	19	65.5	15	51.7	
No	10	34.5	13	44.8	0.52

[+]Descriptive statistics

Table 2.
Descriptive Summary of Mean Alveolar Bone Height Among AI users and controls at Baseline,12, and 18 months[+]

Time of Measurement	On AI Mean (SD) (N=24)[*]	Control Mean (SD) (N=29)	t(df)	p value
Mean bone height Baseline[*]	2.67(.63)	2.68(.45)	-0.09(51)	0.92
Mean bone height 12 months[*]	2.73(.52)	2.85(.55)	-0.75(50)	0.46
Mean bone height 18 months[*]	2.86(.55)	2.94(.49)	-.50(48)	0.61
Mean difference in average bone height from baseline to 18 months[*]	0.32 (.36)	0.25(.22)	0.80(48)	0.42

*Maximum sample size across measures. Sample sizes vary slightly from measure to measure. Counts and percentages shown are calculated among non-missing. N= 23 at 12months, N= 21 at 18 months for those on AI.
[+]Descriptive statistics, Independent samples-t test

Table 3.
Descriptive Summary of Mean Difference in Alveolar Bone Height Among AI users at Baseline,12, and 18 months[+]

Time of Measurement	Mean(SD) (N=24)[+]	t(df)	p value
Baseline-12months	0.115(0.360)	1.536(22)	0.139
Baseline-18 months	0.327	4.081(20)	0.001
12-18 months	0.198(0.260)	3.504(20)	0.002

[+]Paired t test.
[+] Maximum sample size across measures. Sample sizes vary slightly from measure to measure. N= 23 at 12months, N= 21 at 18 months.

Table 4.
Descriptive Summary of Mean Difference in Alveolar Bone Height Among Controls at Baseline,12, and 18 months[+]

Time of Measurement	Mean(SD) (N=29)	t(df)	p value
Baseline-12months	0.169(0.233)	3.892(28)	0.001
Baseline-18 months	0.259(0.228)	6.105(28)	0.000
12-18 months	0.090(0.217)	2.230(28)	0.034

[+]Paired t test

Table 5.
Mean Clinical Attachment Level Among AI Users and Controls at Baseline, 6, 12,and 18 months*

Time of Measurement	On AI Mean (SD) (N=29)+	Control Mean (SD) (N=29)	t(df)	p value
Baseline Mean CAL	1.51(.75)	1.42(.39)	0.58(56)	.56
6 month Mean CAL	1.73(.74)	1.24(.29)	3.23(54)	0.001
12 month Mean CAL	1.72(.36)	1.40(.29)	3.43(50)	0.001
18 month Mean CAL	1.84(.40)	1.45(.20)	4.53(50)	0.001
Mean difference in CAL from baseline to 18 months	0.46(.38)	0.03(.33)	-4.31(50)	0.001

+Descriptive statistics, independent sample T Test
+ Maximum sample size across measures. Sample sizes vary slightly from measure to measure. N=29 at baseline, 27 at 6 months, N=23 at 12,18 months for those on AI

Table 6.
Mean Difference in Clinical Attachment Level Among AI Users at Baseline, 6, 12,and 18 months[+]

Time of Measurement	Mean(SD) (N=29)[+]	t(df)	p value
Baseline-6months	0.201(0.349)	2.990(26)	0.006
Baseline-12 months	0.335(0.358)	4.489(22)	0.000
Baseline-18 months	0.457(0.384)	5.705(22)	0.000

[+]Paired t test
[+] Maximum sample size across measures. Sample sizes vary slightly from measure to measure. N=29 at baseline, N=23 at 12,18 months.

Table 7.
Mean Difference in Clinical Attachment Level Among Controls at Baseline, 6, 12,and 18 months[+]

Time of Measurement	Mean(SD) (N=29)	t(df)	p value
Baseline-6months	0.177(0.362)	2.627(28)	0.014
Baseline-12 months	0.015(0.357)	0.225(28)	0.823
Baseline-18 months	0.032(0.326)	0.531(28)	0.600

[+]Paired t test

Table 8.
Mean Probing Depths Among AI Users and Controls at Baseline, 6, 12, and 18 Months*

Time of Measurement	On AI Mean (SD) (N=29)+	Control Mean (SD) (N=29)	t(df)	P value
Baseline Mean PD	2.00(.27)	2.00(.29)	0.05(56)	0.96
6 month Mean PD	2.09(.31)	1.76(.24)	4.49(54)	0.001
12 month Mean PD	2.16(.29)	1.95(.20)	3.10(50)	0.001
18 month Mean PD	2.26(.30)	2.00(.19)	3.72(50)	0.001
Mean difference in PD from baseline to 18 months	0.28(.28)	0.01(.22)	-3.96(50)	0.001

+Descriptive statistics, independent sample T Test
+ Maximum sample size across measures. Sample sizes vary slightly from measure to measure. N=29 at baseline, N= 27 at 6 months, N=23 at 12,18 months.

Table 9.
Mean Difference in Probing Depths Among AI Users at Baseline, 6, 12, and 18 Months*

Time of Measurement	Mean(SD) (N=29)+	t(df)	p value
Baseline-6months	0.093(0.262)	1.856(26)	0.075
Baseline-12 months	0.186(0.266)	3.355(22)	0.003
Baseline-18 months	0.284(0.286)	4.756(22)	0.000

+Paired t test

+ Maximum sample size across measures. Sample sizes vary slightly from measure to measure. N=29 at baseline, N= 27 at 6 months, N=23 at 12,18 months..

Table 10.
Mean Difference in Probing Depths Among Controls at Baseline, 6, 12, and 18 Months*

Time of Measurement	Mean(SD) (N=29)	t(df)	p value
Baseline-6months	0.234(0.260)	4.952(28)	0.000
Baseline-12 months	0.050(0.226)	1.197(28)	0.242
Baseline-18 months	0.245(0.186)	7.097(28)	0.878

+Paired t test

Table 11.
Mean BOP for those on AI and Controls at Baseline, 6, 12, and 18 Months*

Time of Measurement	On AI Mean (SD) (n=29)+	Control Mean (SD) (n=29)	t(df)	Pvalue
Baseline Mean BOP	0.176(.141)	0.107(.080)	2.288(44.250)	0.027
6 month Mean BOP	0.191(.139)	0.175(.155)	0.420(53.920)	0.676
12 month Mean BOP	0.267(.147)	0.166(.022)	2.734(50)	0.009
18 month Mean BOP	0.198(.121)	0.254(.169)	1.393(49.589)	0.170
Mean Difference in Average BOP from Baseline to 18 months	0.002(0.180)	0.148(0.132)	3.247(39.237)	0.002

+Descriptive statistics, independent sample T Test
+ Maximum sample size across measures. Sample sizes vary slightly from measure to measure. N=29 at baseline, N= 27 at 6 months, N=23 at 12, 18 months.

Table 12.
Mean Difference in Bleeding on Probing Among AI Users at Baseline, 6, 12, and 18 Months[+]

Time of Measurement	Mean(SD) (N=29)[+]	t(df)	p value
Baseline-6months	0.010(0.153)	0.342(26)	0.735
Baseline-12 months	0.071(0.145)	2.351(22)	0.028
Baseline-18 months	0.002(0.180)	0.053(22)	0.958

[+]Paired t test

[+] Maximum sample size across measures. Sample sizes vary slightly from measure to measure. N=29 at baseline, N= 27 at 6 months, N=23 at 12, 18 months.

Table 13.
Mean Difference in Bleeding on Probing Among Controls at Baseline, 6, 12, and 18 Months[+]

Time of Measurement	Mean(SD) (N=29)	t(df)	p value
Baseline-6months	0.068(0.140)	2.617(28)	0.014
Baseline-12 months	0.059(0.101)	3.171(28)	0.004
Baseline-18 months	0.148(0.132)	6.008(28)	0.000

[+]Paired t test

Table 14.
Effect of Vitamin D Use on Bone Height Among AI Users at Baseline, 12, and 18 Months[+]

Time of Measurement	On vit D Mean (SD) (N=16)[*]	not on vit D Mean (SD) (N=8)[+]	t(df)	P value
Mean bone height Baseline	2.52(.58)	2.85(.68)	-1.11(17)	0.28
Mean bone height 12 months	2.63(.44)	2.67(.61)	-0.17(16)	0.86
Mean bone height 18 months	2.84(.50)	2.90(.78)	-0.19(16)	0.85
Mean difference in bone height from baseline to 18 months	0.31(.32)	0.19(.45)	0.65(16)	0.52

[+]Descriptive statistics, independent sample T Test
*N=14 at 18 months for AI users on vit D
[+]N=7 at 18 months for AI users not on vit D

Table 15.
Effect of Vitamin D Use on Bone Height Among Controls at Baseline, 12, and 18 Months[+]

Time of Measurement	On vit D Mean (SD) (N=15)	not on vit D Mean (SD) (N=13)	t(df)	P value
Mean bone height Baseline	2.79(.480)	2.55(.43)	1.33(26)	0.20
Mean bone height 12 months	2.97(.639)	2.69(.44)	1.31(26)	0.20
Mean bone height 18 months	3.03(.543)	2.84(.45)	1.01(26)	0.32
Mean difference in bone height from baseline to 18 months	0.24(.20)	0.28(.26)	-0.43(26)	0.67

[+]Descriptive statistics, independent sample T Test. 1 missing

Table 16.
Effect of Calcium Use on Bone Height Among AI Users at Baseline, 12, and 18 Months[+]

Time of Measurement	On calcium Mean (SD) (N=17)[*]	Not on calcium Mean (SD) (N=7)[+]	t(df)	P value
Mean bone height Baseline	2.45(.58)	3.18(.37)	-2.56(17)	0.02
Mean bone height 12 months	2.51(.46)	3.11(.26)	-2.44(16)	0.03
Mean bone height 18 months	2.71(.54)	3.36(.50)	-2.13(16)	0.05
Mean difference in bone height from baseline to 18 months	0.26(.34)	0.31(.47)	-0.27(16)	0.78

*N=16 at 18 months for AI users on calcium
[+]N=6 at 12months, N= 5 for AI users not on calcium
[+]Descriptive statistics, independent sample T Test

Table 17.
Effect of Calcium Use on Bone Height Among Controls at Baseline, 12, and 18 Months[+]

Time of Measurement	On calcium Mean (SD) (N=11)	Not on calcium Mean (SD) (N=13)	t(df)	P value
Mean bone height Baseline	2.70(.55)	2.72(.41)	-0.09(22)	0.92
Mean bone height 12 months	2.91(.72)	2.89(.46)	0.10(22)	0.92
Mean bone height 18 months	2.91(.57)	3.01(.50)	-0.42(22)	0.67
Mean difference in bone height from baseline to 18 months	0.21(.12)	0.28(.20)	-1.04(22)	0.31

[+]Descriptive statistics, independent sample T Test

Table 18.
Effect of Bisphosphonate Use on Bone Height Among AI Users at Baseline, 12, and 18 Months[+]

Time of Measurement	On bisphosphonate Mean (SD) (N=10)[*]	Not on bisphosphonate Mean (SD) (N=14)[#]	t(df)	P value
Mean bone height Baseline	2.82(.33)	2.51(.76)	1.05(17)	0.31
Mean bone height 12 months	2.73(.40)	2.58(.56)	0.64(16)	0.53
Mean bone height 18 months	2.91(.25)	2.81(.77)	.32(16)	0.75
Mean difference in bone height from baseline to 18 months	0.08(.27)	0.42(.37)	-2.12(16)	0.05

[*]N=9 at 18 months for AI users on bisphosphonates
[#]N=13 at 12 and 18 months for AI users not on bisphosphonates
[+]Descriptive statistics, independent sample T Test

Table 19.
Effect of Bisphosphonate Use on Bone Height Among Controls at Baseline, 12, and 18 Months[+]

Time of Measurement	On bisphosphonates Mean (SD) (N=5)	Not on bisphosphonates Mean (SD) (N=24)	t(df)	P value
Mean bone height Baseline	2.79(.38)	2.66(.47)	0.60(27)	0.56
Mean bone height 12 months	3.00(.65)	2.82(.54)	0.64(27)	0.52
Mean bone height 18 months	3.00(.37)	2.93(.52)	0.28(27)	0.78
Mean difference in bone height from baseline to 18 months	0.20(.14)	0.27(.24)	-0.59(27)	0.56

[+]Descriptive statistics, independent sample T Test

Table 20.
Estimates of Fixed Effects[a]

Variable	Estimate	Std. Error	Sig.
Intercept	2.42	0.17	<.001
Baseline	Ref	Ref	Ref
12 Months	0.13	0.04	0.001
18 Months	0.27	0.04	<.001
No Vitamin D at Baseline	-0.15	0.17	0.37
Vitamin D At Baseline	Ref	Ref	Ref
No Calcium at Baseline	0.81	0.27	0.005
Calcium at Baseline	Ref	Ref	Ref
No Bisphosphonate Use at Baseline	0.04	0.17	0.81
Bisphosphonate Use at Baseline	Ref	Ref	Ref
Not on AI	0.27	0.20	0.19
On AI	Ref	Ref	Ref
Not on Calcium and Not on Baseline Interaction	-0.70	0.34	0.04

[a] Dependent Variable: Average Bone Height

Table 21.
Estimates of Covariance Parameters[a]

Covariance Parameters	Estimate	Std. Error
Residual	0.03	0.005
Intercept	0.24	0.060

[a] Dependent Variable: Average Bone Height

Table 22.
Pairwise Comparisons for Calcium Use within AI Status[a]

Pairwise Comparisons: Calcium	Mean	Std. Error	Sig.
On AI: Yes Calcium	2.50	0.13	0.005
On AI: No Calcium	3.32	0.23	
Not on AI: Yes Calcium	2.78	0.18	0.648
Not on AI: No Calcium	2.89	0.15	

[a] Dependent Variable: Average Bone Height

Table 23.
Estimates[a]

Estimated Means for Time	Mean	Std. Error
Baseline	2.73	0.09
Wave 2: 12 Months	2.87	0.09
Wave 3: 18 Months	3.01	0.09

[a] Dependent Variable: Average Bone Height

Table 24.
Pairwise Comparisons[a]

Pairwise Comparisons: Time		Mean Difference	Std. Error	Sig.
Baseline	12 Months	-0.13*	0.04	.003
	18 Months	-0.27*	0.04	<.001
12 Months	18 Months	-0.13*	0.04	.003

[a] Dependent Variable: Average Bone Height

Table 25.
Osteocalcin Level at Baseline, 12 Months, and 18 Months for Those on AI and Controls[+]

Time of Measurement	On AI Mean (SD) (N=28)	Control Mean (SD) (N=28)	t(df)	P value
Osteocalcin at Baseline[*]	182.30(287.17)	150.16(455.46)	-0.31(54)	0.75
Osteocalcin at 12 months[*]	278.11(349.80)	463.50(513.80)	1.36(44)	0.18
Osteocalcin at 18 months[*]	262.26(534.61)	184.39(388.67)	-0.57(45)	0.57

*Maximum sample size across measures. Sample sizes vary slightly from measure to measure. Counts and percentages shown are calculated among non-missing. At 12 months on AI n=19, control n=27. At 18 months on AI n=18, control n=29.
[+]Independent sample t test

Table 26.
Correlation of Osteocalcin on Bone Height Among Those on AI at Baseline, 12 Months, and 18 Months+

Time of Measurement	Bone Height r(Sig)
Osteocalcin at Baseline	0.28(.19)
Osteocalcin at 12 months	0.16(.50)
Osteocalcin at 18 months	0.22(.37)

+Pearson Correlation

Table 27.
Correlation of Osteocalcin on Bone Height Among Controls at Baseline, 12 Months, and 18 Months+

Time of Measurement	Bone Height r(Sig)
Osteocalcin at Baseline	-0.21(.27)
Osteocalcin at 12 months	0.09(.63)
Osteocalcin at 18 months	0.14(.46)

+Pearson Correlation

Appendix

Appendix A

Oral health and oral health-related quality of life in early stage breast cancer survivors: The role of aromatase inhibitors

ID Number:_____

Thank you very much for filling out this questionnaire. We want to assure you that all your answers are strictly confidential. Your name will not appear on any material connected with the data. Please, be honest and try to answer all questions.

D1. How old are you? I am_____years old.

D2. What is your ethnic / racial background? _____

D3. How many years of schooling did you have starting with Kindergarten?_____Years

D4. What is your employment status?
_____Working full-time;
_____Working part-time
_____Not actively employed

Other(please specify)_____

D5. The monthly combined income of your family is best represented by:

_____No income
_____0-$19,999
_____between $20,000 and $39,999
_____between $40,000 and $59,999
_____between $60,000 and $74,999
_____over $75,000

D6. Do you have dental insurance? _____YES _____ NO
Is your insurance through the source of employment (yours or others) ____YES____NO

D7. Are you currently married or living as married? _____YES _____ NO

D8. How many children do you have? I have _____child/ children.

D8a. How many are at home?_____

D9. Have you ever smoked 100 cigarettes? _____YES _____ NO

D9a. Do you smoke now? _____YES _____ NO

D10. Do you drink alcohol? _____Never _____YES _____ NO

D10a.If Yes how many drinks a day_____

The following questions are concerned with your teeth and dental health:

D11. How would you describe the health of your teeth?

❑1	❑2	❑3	❑4	❑5
poor	fair	good	very good	excellent

D12.. How would you describe the health of your gums?

❑1	❑2	❑3	❑4	❑5
poor	fair	good	very good	excellent

If not excellent why not?_____

D13. How important is your dental health to you?

❑1	❑2	❑3	❑4	❑5
Not at all Important				Very Important

D14. Think about how dry your mouth usually feels. On a scale from 1 = "much too little saliva" to 5 = "perfect amount of saliva", how much saliva do you have?

❑1	❑2	❑3	❑4	❑5
Much too Little saliva				perfect amount of saliva

D115. When was the last time you visited a dental office?

❑1 Never	❑2	❑3	❑4
	3-6 months	7-12 months	Over 13 months

D6. How often do you brush your teeth?

❑1	❑2	❑3	❑4	❑5
never	rarely	nearly every day	every day	more than once a day

D17. How often do you floss your teeth?

❑1	❑2	❑3	❑4	❑5
never	rarely	nearly every day	every day	more than once a day

D18. How many of the following dental procedures did you have done in the past 12 months?

Tooth/teeth pulled _____ Fillings _____

Root canals _____ Crowns _____

Others_____

These next questions ask you about problems with your teeth or mouth. We would like to know if you have had these problems. Gum disease is a common problem with the mouth. People with gum disease might have swollen gums, receding gums, sore or infected gums, or loose teeth.

Do you think you might have gum disease?	❑Yes	❑ No	❑ Don't know
Have you noticed bleeding from your gums during toothbrushing in the last month?	❑Yes	❑ No	❑ Don't know
Have you noticed gingival swelling-do tissues look puffy in the last month?	❑Yes	❑ No	❑ Don't know
Have you ever had treatment for gum disease, such as scaling and root planing, also called "deep" cleaning?	❑Yes	❑ No	❑ Don't know
Have you ever had any teeth become loose on their own, without an injury?	❑Yes	❑ No	❑ Don't know
Have you ever been told by a dental professional that you lost bone around your teeth?	❑Yes	❑ No	❑ Don't know
During the past 3 months, have you noticed a tooth that doesn't look right?	❑Yes	❑ No	❑ Don't know
Do you think can see more of the roots of your teeth than in past?	❑Yes	❑ No	❑ Don't know
Have you had sore areas on your gums that have lasted more than a week within the last month?	❑Yes	❑ No	❑ Don't know
Have you had painful tingling in your gums or tongue within the last month?	❑Yes	❑ No	❑ Don't know
Do you currently have any teeth that are sensitive to hot, cold or sweets?	❑Yes	❑ No	❑ Don't know

The following questions are concerned with your visits to your dentist. Please check the appropriate box.

Did you visit your dentist after your cancer diagnosis but before the start of cancer treatment?	❑Yes	❑ No	❑ Don't know

I f no, why not?_____

If yes, at that dental visit did your dentist explain the need for regular dental examinations during treatment?	❑Yes	❑ No	❑ Don't know
Did you visit your dentist during your cancer treatment?	❑Yes	❑ No	❑ Don't know

If yes, how often_____

Appendix B

Oral health and oral health-related quality of life in early stage breast cancer survivors: The role of aromatase inhibitors

Data abstraction sheet

_____Patient Code (study number)

Patient Age at survey and age at cancer diagnosis

Patient race/ethnicity

Cancer Diagnosis

- Date
- Stage
- Treatment
  - Chemotherapy ❑Yes ❑ No
    - Drugs used
    - Start & stop dates
  - Radiation therapy ❑Yes ❑ No
    - Start & stop dates
  - Tamoxifen ❑Yes ❑ No
    (Start and stop dates)
  - AI therapy ❑Yes ❑ No
    Start and stop dates of any AI used

Comorbid conditions

Medications (chronic and PRN)

Tobacco exposure

Alcohol exposure

Dental practices
- Cleaning
- Procedure
- Personal oral hygiene behaviors
- Dental insurance plan

Appendix C

Oral Health and OHQoL in Early Stage Breast Cancer Survivors: The Role of AIs			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

Patient Screening Date: _____
(MON / DD / YY)

Patient Screening Date: _____
(MON / DD / YY)

Inclusion Criteria:	**Yes**	**No**
1. Is the patient postmenopausal as defined by NCCN?	☐	☐
a. Prior bilateral oophorectomy or: b. Age ≥ 60 years or: c. Age < 60 and amenorrheic for 12 or more months in the absence of chemotherapy, tamoxifen, toremifene or ovarian suppression with FSH and estradiol in the PM range		
2. Does the patient have a diagnosis of ER+ breast cancer? ☐	☐	
3. If patient had ER + breast cancer, has the patient been using an AI ☐ (such as Arimidex, Aromasin or Femara) for less than 1 year?	☐	
4. Does the patient have 15 teeth? ☐	☐	

Exclusion Criteria:

1. Has the patient received a diagnosis of metastatic breast cancer? ☐ ☐

2. Has the patient received a diagnosis of any type of cancer other than ☐ ☐
early stage breast cancer (Not incl history of thyroid or skin cancer)?

3. Has the patient been told they need antibiotics prior to dental treatment? ☐ ☐

4. Has the patient received long term use of medications known to affect periodontal status such as immunosuppresives? ☐ ☐

5. If the patient has diabetes, do they have a Hemoglobin AC1 level of >7.2%? ☐ ☐

6. Does the patient have any disease of the immune system or any medical conditions that may influence the outcome of the study (neurologic, psychiatric disorders, systemic infections)? ☐ ☐

Oral Health and OHQoL in Early Stage Breast Cancer Survivors: The Role of AIs			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

General Information

Date of Birth MON/DD/YY
/ /

Gender
M F

1. Does the patient use tobacco products? Yes No
   If yes, circle the products used:

   cigarettes cigars chewing tobacco other

   If yes: Quantity per day? _____

2. Has the patient used tobacco products in the past? Yes No

   If yes: Start Year: _____ Quit Year: _____ Quantity per day: _____

	Yes	**No**
3. Does the patient take bisphosphonates?	☐	☐

Start date: _____ Stop date: _____

Drugs used: _____

Oral Health and OHQoL in Early Stage Breast Cancer Survivors: The Role of AIs			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

ADVERSE EVENTS		
Have there been any adverse events? (If "Yes" complete AE form)	**YES**	**NO**

Informed Consent Form (ICF) Signed? ☐ **Yes** ☐ **No**

ICF Completion Documented? ☐ **Yes** ☐ **No**

Medical/Oral History

Were there any changes to the med/oral history? ☐ **Yes** ☐ **No**

If yes, comment below.

Quality of Life

Was quality of life assessment completed? ☐ **Yes** ☐ **No**

Oral Exam

Was an oral exam performed? ☐ **Yes** ☐ **No**

Please indicate any abnormalities below.

Caries Assessment

Were obvious caries noted? ☐ **Yes** ☐ **No**

If so, please indicate tooth number(s) below.

Radiography

Were standardized radiographs taken? ☐ **Yes** ☐ **No**

Areas **1** _____ Areas **2** _____

Clinical Periodontal Measures

Were clinical measures taken? ☐ **Yes** ☐ **No**

Oral fluid sampling

Was saliva collected? ☐ **Yes** ☐ **No**

Volume saliva collected? **_____ ml (max. 2.0ml) collected in _____ Mts. (max. 15 Mts.)**

Comments:

_____

_____

_____

_____

Examiner Signature: _____

Date: _____

Oral Health and OHQoL in Early Stage Breast Cancer Survivors: The Role of AIs			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

UPPER RIGHT

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
FGM																								
PD																								
CAL																								
BOP																								
Supragingival Plaque																								
Supragingival Calculus																								
Exudate																								
(Y = 1 / N = 0)	**1**			**2**			**3**			**4**			**5**			**6**			**7**			**8**		
FGM																								
PD																								
CAL																								
BOP																								
Supragingival Plaque																								
Supragingival Calculus																								
Exudate																								
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

Oral Health and OHQoL in Early Stage Breast Cancer Survivors: The Role of AIs			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

UPPER LEFT

m	b	d	m	b	d	m	b	d	m	b	d	m	b	d	m	b	d	m	b	d	m	b	d	BUCCAL
																								FGM
																								PD
																								CAL
																								BOP
																								Supragingival Plaque
																								Supragingival Calculus
																								Exudate
9			10			11			12			13			14			15			16			(Y = 1 / N = 0)
																								FGM
																								PD
																								CAL
																								BOP
																								Supragingival Plaque
																								Supragingival Calculus
																								Exudate
m	l	d	m	l	d	m	l	d	m	l	d	m	l	d	m	l	d	m	l	d	m	l	d	LINGUAL

Oral Health and OHQoL in Early Stage Breast Cancer Survivors: The Role of AIs			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

LOWER LEFT

m	b	d	m	b	d	m	b	d	m	b	d	m	b	d	m	b	d	m	b	d	m	b	d	BUCCAL
																								FGM
																								PD
																								CAL
																								BOP
																								Supragingival Plaque
																								Supragingival Calculus
																								Exudate
24			23			22			21			20			19			18			17			(Y = 1 / N = 0)
																								FGM
																								PD
																								CAL
																								BOP
																								Supragingival Plaque
																								Supragingival Calculus
																								Exudate
m	l	d	m	l	d	m	l	d	m	l	d	m	l	d	m	l	d	m	l	d	m	l	d	LINGUAL

University of Michigan	Date (MM / DD / YY) / /	Patient Number	Patient Initials

LOWER RIGHT

BUCCAL	d	b	m	d	b	m	d	b	m	d	b	m	d	b	m	d	b	m	d	b	m	d	b	m
FGM																								
PD																								
CAL																								
BOP																								
Supragingival Plaque																								
Supragingival Calculus																								
Exudate																								
(Y = 1 / N = 0)	32			31			30			29			28			27			26			25		
FGM																								
PD																								
CAL																								
BOP																								
Supragingival Plaque																								
Supragingival Calculus																								
Exudate																								
LINGUAL	d	l	m	d	l	m	d	l	m	d	l	m	d	l	m	d	l	m	d	l	m	d	l	m

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