HHS Public Access

Author manuscript

J Periodontal Res. Author manuscript; available in PMC 2020 October 01.

Published in final edited form as:

J Periodontal Res. 2019 October ; 54(5): 525–532. doi:10.1111/jre.12656.

Loss of alveolar bone density in post-menopausal, osteopenic women is associated with circulating levels of gelatinases

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Abstract

Objective: To determine whether circulating levels of two matrix metalloproteinases, MMP-2 and MMP-9, are associated with loss of alveolar bone density (ABD) or height (ABH), or with progression of periodontitis (relative clinical attachment level [RCAL]), among postmenopausal women with local and systemic bone loss.

Background: This study was planned as part of a two-year randomized, double-blind, placebo-controlled, clinical trial examining efficacy/safety of subantimicrobial dose doxycycline (20 mg bid) in postmenopausal osteopenic women. This study examines whether serum levels of gelatinases are associated with local changes in the periodontium.

Methods: A sample of 113 women received periodontal maintenance for moderate to advanced chronic periodontitis and consented to analysis of stored serum biomarkers. Posterior vertical bite-wings were taken, and serum collected, at baseline, one and two years. ABD was determined by computer-assisted densitometric image analysis (CADIA), ABH by the Hausmann et al. method [(1)], and RCAL by Florida Probe (every 6 months). MMPs were measured densitometrically on gelatin zymograms using denatured type I collagen as substrate and purified MMP-2 (72kDa) and MMP-9 (92kDa) as standards. Evidence of worsening in the periodontium at a tooth site was defined as a change from baseline of, for ABD, at least 14 densitometric units (for subcrestal locations) or 17 units (for crestal locations); of at least 0.4 mm for ABH; and of at least 1.5 mm for RCAL. Logistic regression models, while accounting for clustering, compared the odds of worsening in ABD, ABH or RCAL, after two years of observation, between groups defined by baseline and concurrent levels of serum gelatinases.

Results: Changes in ABH and RCAL were not associated with circulating levels of MMP-2 or MMP-9. However, elevated odds of ABD loss over 24 months were associated, among smokers, with both baseline and concurrent levels of MMP-9 in the middle and highest tertile, and with concurrent levels of MMP-2 in the middle (but not the highest) tertile. Elevated odds of ABD loss were also associated, among women within five years of menopause, with baseline levels of MMP-2 in the highest tertile.

Conclusion: Among post-menopausal osteopenic women, loss of ABD was associated, in smokers, with elevated circulating levels of MMP-9 and MMP-2. In those within five years of menopause, ABD loss was associated with elevated circulating levels of MMP-2.

Keywords

Collagen; Matrix metalloproteinases (MMPs); Periodontal disease/periodontitis

Introduction

Our group has proposed a “two-hit model” ([2]) that hypothesizes how two processes co-induce periodontitis. A local “hit” is initiated by periodontal pathogens in the subgingival biofilm. An additional “hit” involves exposure to high levels of systemic (circulating) mediators of inflammation and collagenolysis, including matrix metalloproteinases (MMPs). Elevated levels of these biomarkers are observed in the serum of individuals with systemic diseases including osteoporosis ([2]) and cardiovascular disease [3, 4]. We hypothesized that high serum levels of systemic MMPs can exacerbate the local inflammatory response in the periodontium.

We previously described the design of a double-blind, placebo-controlled randomized clinical trial [5, 6]) that, in accordance with CONSORT guidelines, examined the effects of subantimicrobial dose doxycycline (SDD) on changes in soft and hard tissue outcomes among postmenopausal osteopenic women with moderate to advanced chronic periodontitis.

This trial showed, in subgroup analyses, that SDD treatment improved radiologic measures, alveolar bone density (ABH) and alveolar bone height (ABH). For example, SDD reduced ABD loss in non-smokers over two years and reduced the odds of more progressive ABH loss in women who were beyond five years of menopause [5]. Based on intent-to-treat analyses, SDD also reduced progressive clinical attachment loss [6], reduced gingival crevicular fluid collagenase activity and the odds of elevated MMP-8 [7] and reduced the serum inflammatory biomarkers hs-CRP and MMP-9 [8]. Adverse event experiences did not differ significantly between SDD and placebo groups [5].

Our earlier reports on the trial described the effects of host-modulation therapy (subantimicrobial-dose doxycycline, SDD) in these post-menopausal women on local biomarkers of inflammation, collagenolysis and bone resorption in the GCF [7] and on systemic biomarkers in the serum [8–10].

The present study explores our hypothesis that high levels of circulating MMP-2 and MMP-9 is associated with loss of ABH and of ABD, and with progression of clinical

periodontitis, as measured by relative clinical attachment loss (RCAL). The current analysis results from a supplement that was embedded in the main trial [5]. These additional, pre-planned analyses permitted us to explore associations in these women between changes over two years in ABH, ABD, and RCAL, and baseline and concurrent levels of matrix metalloproteinases MMP-2 and MMP-9.

Methods

Participants

The randomized clinical trial [5, 6] studied 128 postmenopausal osteopenic women. Women included in the study were 45−70 years of age at the time of telephone screening; had been postmenopausal for at least 6 months and not receiving hormone replacement therapy; and had evidence of systemic osteopenia defined by T-scores of −1.0 to −2.5 (inclusive) on DEXA scans of bone mineral density at the lumbar spine or femoral neck. Participants had at least 9 posterior teeth and were undergoing periodontal maintenance in connection with a history of generalized moderate to advanced periodontitis. Women were considered to have moderate to advanced periodontitis if they had at least two sites with probing depths of 5 mm or greater together with bleeding on probing, clinical attachment level loss of 5 mm or more, and radiographic evidence of alveolar bone height loss.

Patients were randomly assigned to a twice-daily subantimicrobial dose (20 mg tablets) of doxycycline (SDD; n=64) or placebo (n=64) for two years as an adjunct to periodontal maintenance every three to four months.

Among the 128 women who were randomized to treatment, 117 completed at least one annual follow-up visit; 113 (51 assigned to receive SDD and 62 assigned to placebo) also consented to the supplementary analysis of serum collagenolytic biomarkers on which this study reports. Baseline, one-year and two-year serum samples were assayed in 2008 by one investigator (Dr. Hsi-Ming Lee). Details on the consenting process, which was approved by the Institutional Review Boards of the University of Nebraska Medical Center and Stony Brook University, are published [8] as are other aspects of the original trial and its supplement. [5,6,7,9,10,11]

Changes in clinical and radiographic outcomes

Disease progression after two years of follow up was distinguished from improvement, or from lack of change, based on RCAL and ABH. We similarly distinguished among worsening, improvement or lack of change in ABD. To interpret measurements for each of these outcomes, we employed the standard deviation of replicate measurements that we calculated in reliability studies performed as part of the original trial.[5] We used a quantity equal to two times a measurement's standard deviation to define thresholds that distinguished between "worsening" or "disease progression" and "no change," and between "no change" and "improvement."

Relative clinical attachment level (RCAL) was measured every 6 months over the study's two years, as described previously [6]. Measurements were obtained at posterior interproximal sites using the Florida Disk Probe (Florida Probe Corporation, Gainesville,

FL). Using a quantity that was two times the standard deviation of replicate measures observed in reliability studies performed as part of our clinical trial[6], we defined increases from baseline of 1.5 mm or more as “disease progression;” absolute changes from baseline of less than 1.5 mm as “no change;” and decreases from baseline of 1.5 mm or more as “improvement.”

Alveolar bone density (ABD) was measured from posterior vertical bite-wings[5] using CADIA (computer-assisted densitometric image analysis), at crestal and subcrestal locations at posterior interproximal sites, at baseline, one year and two years. In reliability studies performed as part of the original trial[5], the standard deviation of replicate CADIA measures for ABD was 8.6 units for crestal locations and 7.0 units for subcrestal locations. Accordingly, we established thresholds of 17 units for crestal measurements and 14 units for subcrestal measurements to define worsening or improvement. We categorized decreases in ABD greater than the appropriate threshold as “worsening,” absolute changes less than the threshold as “no change,” and increases larger than the threshold as “improvement.” As reported previously[5], we created a sensitive measure of change by categorizing site-level change using the crestal or subcrestal measurement at the site that demonstrated the greater absolute change since baseline.

Alveolar bone height (ABH) was measured from the same radiographs and at the same posterior interproximal sites used to measure ABD. ABH was determined using a method described by Hausmann and colleagues[1]. Linear measurements between the fixed reference point (cemento-enamel junction or restoration margin) and the alveolar crest were made at baseline, one year and two years. Reliability studies performed as part of the original trial[5] found the standard deviation of replicate measures of ABH to be 0.2 mm. Accordingly, increases of 0.4 mm or more in ABH compared to baseline values signified alveolar bone loss and “disease progression.” Absolute changes since baseline of less than 0.4 mm signified “no change,” and decreases since baseline of 0.4 mm or more were regarded as “improvement”.[5]

Measures of gelatinase biomarkers in serum

Pro- and active forms of MMP-2 and MMP-9 were analyzed from assays performed on serum samples collected and frozen at −80°C at the time of the original clinical trial, as previously described [4, 8]. This study reports on samples that were obtained at baseline and at two years, concurrently with the assessment of disease progression. Samples were assayed by means of gelatin zymography, and gelatinase levels were expressed as densitometric units. Our previous research found the 92-kDa pro form of MMP-9 to be the dominant circulating type IV collagenase.[8] Consequently, this study focuses on that gelatinase and on the 72kDa pro form of MMP-2.

Statistical analysis

This study reports on analyses that were embedded in the original randomized clinical trial (5) and planned at the time the trial was conducted. Pre-planned analyses of changes in inflammatory mediators and lipid profiles are reported elsewhere.[8] Logistic regression models, estimated using generalized estimating equations (GEE), assessed the association

between the odds of disease progression over 24 months and baseline and concurrent levels of serum gelatinases. These models estimated the ratio of the odds of disease progression or of worsening of outcomes, compared to the odds of improvement or of “no change.” To limit the number of comparisons and the probability of detecting and reporting spurious associations, this manuscript assessed associations only over 24 months, and tested hypotheses against an alpha of 0.01. To avoid ignoring potentially important subgroup differences, we assessed tests of effect modification (statistical interaction) at an alpha of 0.10. When effect modification was detected, subgroup-specific hypotheses were assessed for significance against an alpha of 0.01. P values reported in tables relate to tests of hypotheses that the true odds ratios are equal to one. While we defined significance at α=0.01, we accompany estimates of odds ratios with 95% confidence intervals (CI).

Separate models defined disease progression with respect to each of the study’s three outcome measures: ABD, ABH and RCAL. Within each of the three models, separate analyses considered, as a primary independent variable, the serum gelatinases MMP-2 or MMP-9. To create groups of roughly equal size and to preserve statistical power, we categorized measurements of MMP-2 and MMP-9 levels, at baseline and at 24 months, in tertiles. Statistical models were estimated using GEE, which permitted us to treat each individual as an independent cluster, and to adjust for correlation among each individual’s site-level measurements.

To account for the potential influence of other explanatory factors, each statistical model was initially constructed with interaction terms to detect whether an association between disease progression and the circulating levels of gelatinases differed in subgroups defined by randomized treatment (SDD versus placebo), smoking status (smoker versus nonsmoker) or postmenopausal status (within five years of menopause versus more than five years from menopause). When evidence of interaction was detected, stratified models were reported. When interactions were nonsignificant, the interaction terms were eliminated and the models were refit. We also assessed whether estimated associations differed meaningfully if we adjusted for treatment, smoking or postmenopausal status. We verified that adjustment for these covariates did not affect model estimates and, therefore, we report unadjusted estimates.

Results

Table 1 summarizes the demographic characteristics of the 113 participants for whose samples MMP-2 and MMP-9 were assayed using gelatin zymography. Table 2 reports, by tertiles, circulating levels of the two gelatinases.

MMP-9

Multivariable logistic regression models detected evidence that associations between circulating levels of MMP-9, at baseline and at two years, and change in ABD differed between smokers and non-smokers (tests of interaction $p<0.04$ for each; Table 3). Table 3 illustrates how smokers with concurrent or baseline levels of circulating MMP-9 in either the second or third tertiles had higher odds of ABD loss over two years than those whose levels of circulating MMP-9 were in the lowest tertile. Among smokers, the odds of ABD loss over

24 months were 11.78 times higher (95%CI: 4.13, 33.64; p<0.0001) among those whose concurrent levels of circulating MMP-9 were in the highest tertile compared to smokers in the lowest tertile. Among smokers whose concurrent levels of circulating MMP-9 were in the second tertile, the odds of ABD loss over two years were 6.07 times higher (95%CI: 2.05, 18.00; p=0.0011)

The odds of ABD loss among smokers was 5.86 times higher (95%CI: 2.24, 15.58; p=0.0003) for those whose baseline levels of MMP-9 were in the highest tertile compared to smokers in the lowest tertile. Among smokers whose baseline levels of MMP-9 were in the second tertile, the odds of ABD loss were 10.17 times higher (95%CI: 4.94, 20.95; p<0.0001).

Among non-smokers, no associations were detected between circulating levels of MMP-9 and change in ABD. Across all participants, MMP-9 levels in serum were unassociated with changes in ABH or RCAL.

MMP-2

Multivariable logistic regression models detected evidence that associations between levels of circulating MMP-2 and change in ABD differed between smokers and non-smokers, and between those within and beyond five years of menopause (test of interaction p<0.09; Table 4).

Among smokers, those with concurrent levels of MMP-2 in the middle tertile had higher odds of decreased ABD over 24 months (OR: 2.99; 95% CI: 1.43, 6.23; p=0.0035) than those whose levels were in the lowest tertile. No association was observed in non-smokers.

Among women within five years of menopause, the odds of ABD loss were 4.54 times higher (95% CI: 2.04, 10.10; p=0.0002) among those whose baseline MMP-2 levels were in the highest tertile than among those whose baseline MMP-2 levels were in the lowest tertile. No association was detected among women who were more than five years after menopause.

Neither baseline nor concurrent MMP-2 levels were associated with disease progression over two years as defined by measurement of ABH or RCAL.

Discussion

We hypothesized that circulating levels of MMP-2 and MMP-9 were associated with periodontitis progression in postmenopausal women with chronic periodontitis. Differences in levels of these gelatinases were unassociated with disease progression defined by changes in ABH or RCAL. However, we found significant associations, in certain subgroups, between levels of MMP-9 and MMP-2, measured in serum, and ABD loss.

Higher circulating levels of MMP-9, at both baseline and at 24 months, were consistently and strongly associated with ABD loss among women who smoked. No association was detected among non-smokers. Elevated MMP-2 levels over 24 months were significantly associated with ABD loss in smokers, and elevated baseline levels of MMP-2 also were associated with ABD loss among women within five years of menopause. However,

associations between elevated levels of circulating MMP-2 and ABD loss were smaller in magnitude than corresponding associations between elevated MMP-9 levels and ABD loss.

The absence of associations between circulating MMP-2 and MMP-9 with change in either ABH or RCAL may have been due to the small percentage of tooth sites at which change in these measures surpassed thresholds that signified either improvement or worsening following two years of study[5, 6]. Across all 113 patients, ABH had improved after two years at 1.5% of tooth sites and had worsened at 8.2%. RCAL had improved after two years at 4.5% of tooth sites and had worsened at 2.8%. The relative stability of ABH and RCAL reflects the relatively minimal progression of periodontitis and systemic bone loss that characterized the patients enrolled in the clinical trial. Because the trial excluded women receiving hormone replacement therapy, bisphosphonates or other medications that would have influenced bone remodeling, the cohort did not include patients with severe systemic disease such as osteoporosis. Moreover, the cohort's relative periodontal stability may relate to the treatment the participants received. Women in both groups received periodontal maintenance, at no cost, every three to four months throughout the two-year clinical trial. Participants complied with periodontal maintenance; over 90% of women in both the control and SDD groups completed at least one periodontal maintenance visit between each of the semi-annual study visits. In addition to these periodontal maintenance visits, participants attended a baseline visit and four semi-annual study visits, each of which included periodontal measurements that likely heightened their awareness of their periodontal status and interest in their periodontal health.

We previously hypothesized[9] that ABD loss may precede ABH loss. However, we have since examined the data from this group of women and failed to detect this hypothesized sequence in periodontitis progression,[11] possibly because the post-menopausal women in this study exhibited minimal local or systemic bone loss over the two-year study[5]. The current study's finding that ABD loss was more common than periodontitis progression (measured by either ABH or RCAL) suggests that change in ABD may be a more sensitive diagnostic measure in patients undergoing periodontal maintenance.

Research has established [12–14] that both bone formation and resorption rates increase dramatically in the years immediately following menopause; however, the rate of bone resorption exceeds the rate of formation, resulting in high-turnover bone loss. The effects we found among women within five years of menopause, where significant associations were observed between ABD loss and levels of circulating MMP-2 in the highest tertile, is consistent with these participants experiencing a period of active bone turnover and remodeling that favored excessive bone resorption. We detected no associations among women more than five years from menopause, in whom less active bone remodeling results in low turnover bone loss.

The associations found in this study between circulating gelatinolytic MMPs (particularly MMP-9 and, to a lesser extent, MMP-2) with ABD loss in certain subgroups of post-menopausal women may reflect two issues discussed below:

1. MMP-9 is a diagnostic marker of inflammation. Activated macrophages and other inflammatory cells are sources of MMPs (including MMP-9) in the vasculature.[15] Blankenburg et al.[3] found that MMP-9 levels are associated, in patients with coronary artery disease, with cardiac events and cardiac-induced death. MMP-9 is a major constituent of acute inflammatory cells, and is stored in the specific granules of polymorphonuclear (PMN) leukocytes. MMP-9 also mediates the degradation of collagen and other connective tissue constituents as well as bone resorption in various diseases. In fact, both acute and chronic inflammatory cells either release this gelatinase on degranulation (PMN leukocytes) or secrete it into the extracellular matrix (macrophages). MMP-9 helps mediate tissue breakdown via its secretion by osteoclasts (and other bone cells) when the organic matrix of bone is being degraded as part of the bone resorption process.

Smoking induces vascular inflammation. Sivaraman and colleagues[15] found both circulating MMP-2 and MMP-9 to be elevated, compared to controls, in patients with acute myocardial infarction (AMI). These authors also observed a significant elevation in MMP-9 in AMI patients who were smokers compared to those who were non-smokers. However, they observed no significant difference, between smokers and non-smokers with AMI, in levels of MMP-2. Also consistent with the current study, circulating MMP-9 has been shown to be elevated in people with chronic periodontitis who are also smokers[16].

It is of note that serum MMP-2, which is neither a marker of inflammation nor a constituent of PMN leukocytes, was less strongly associated with ABD loss in the current study. Also consistent with the current study, numerous studies of different clinical conditions[4, 8] [17] [18] have found MMP-2 to be a less sensitive biomarker than MMP-9.

2. A number of studies have found that MMP-9 in biologic fluids, such as blood, urine, synovial fluid, and gingival crevicular fluid, is a diagnostic marker of local and systemic diseases including cardiovascular diseases[3, 4, 19, 20]; pulmonary disease[18, 21]; rheumatoid arthritis and osteoarthritis[22–24]; ophthalmologic diseases such as cataracts and glaucoma[25, 26]; sepsis and mortality[27]; and periodontitis[28].

MMP-9 is now known to have multiple biological effects beyond its originally recognized ability to degrade denatured collagen fragments (gelatin) and basement membrane (type IV) collagen. These effects include activation of chemokines and cell surface receptors and modulation of several inflammatory pathways[20].

This study’s results support proposed links among local changes in the periodontium and two circulating gelatinases that are elevated with systemic disease. With respect to the two-hit hypothesis[2], relatively high levels of MMP-9 circulating in the serum may serve as the second hit that initiates an amplifying inflammatory/collagenolytic cascade and results in the elevated odds of ABD loss that we observed.

Further research can address certain limitations of this study. While we found circulating levels of two serum gelatinases to be associated with ABD loss, we characterized levels of these gelatinases in tertiles, in order to preserve statistical power. Consequently, we cannot yet propose threshold values that clinicians can regard as prognostic markers for changes in alveolar bone density. Moreover, the study’s design cannot illuminate change over time in

circulating levels of MMP-9 and MMP-2, but instead focuses on cross sectional comparisons using measurements of the gelatinases at baseline and at two years. Finally, like all approaches that rely on measurements in the circulation, it cannot verify whether circulating levels of MMP-9 and MMP-2 reliably represent local exposures or conditions in the periodontium.

Conclusion

Among post-menopausal osteopenic women, loss of ABD was associated, in smokers, with elevated circulating levels of MMP-9 and MMP-2. In those within five years of menopause, ABD loss was associated with elevated levels of MMP-2. Although serum MMP-2 and MMP-9 were associated with ABD loss, they were not associated with changes in ABH or RCAL. Future studies in patients with more active periodontitis are needed to determine whether ABD loss precedes ABH loss and to determine whether circulating MMPs are associated with periodontitis progression.

Acknowledgements

The clinical trial was supported by National Institute of Dental and Craniofacial Research Grants (NIDCR/NIH) R01DE012872 (JBP, PI and LMG, Co-PI) and R03DE019805 (JAS). Data analysis was also supported in part by a National Institute of General Medical Sciences grant 1U54GM104938 (Judith James, PI). Subantimicrobial dose doxycycline and placebo tablets were provided by CollaGenex Pharmaceuticals (Newtown, PA), since acquired by Galderma R&D (Fort Worth, Tx) and Dr. Golub has consulted for both. Dr. Golub is listed as an inventor on patents on the test medication in this trial, and those patents have been fully assigned to his institution, Stony Brook University, Stony Brook, NY.

Supported by NIDCR grants R01DE012872 and R03DE019805-01A1 and by NIGMS grant 1U54 GM104938. Clinical trial registered at ClinicalTrials.gov. Identifier: NCT00066027.

Abbreviations:

RCAL	relative clinical attachment level
ABH	alveolar bone height
ABD	alveolar bone density
MMP	matrix metalloproteinase

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Table 1.

Demographic characteristics (at baseline) of 113 participants who consented to this supplementary analysis

Age (years)	58.3 (5.8)
Ethnicity	
Hispanic or Latino	5 (4%)
Not Hispanic or Latino	108 (96%)
Race	
Asian	3 (3%)
African American	2 (1%)
White	108 (96%)
Years postmenopausal	
5 or fewer years	42 (37%)
More than 5 years	71 (63%)
Smoking status	
Former smoker or non-smoker	90 (80%)
Current smoker	23 (20%)

Data are reported as mean (standard deviation) for age and as counts (%) for categorical variables.

Table 2.

Circulating levels of MMP-2 and MMP-9, measured in densitometric scanning units on gelatin zymograms, at baseline and at 24 months, reported by tertiles.

	First tertile			Second tertile			Third tertile		
	n	**min**	**max**	**n**	**min**	**max**	**n**	**min**	**max**
MMP-2 (72 kDa)									
Baseline levels	37	122.48	190.88	38	191.27	219.33	38	220.71	284.23
Concurrent levels	37	89.91	186.21	38	187.27	215.58	38	216.41	297.31
MMP-9 (92 kDa)									
Baseline levels	37	185.96	315.52	38	316.18	364.78	38	365.29	462.93
Concurrent levels	37	184.65	309.38	38	310.09	367.40	38	368.77	464.00

Table 3.

Odds for worsening of clinical and radiographic outcomes (RCAL, ABH and ABD) over two years, in groups with circulating levels of MMP-9 in the second and third tertiles, compared to odds of worsening in group with circulating MMP-9 levels in lowest (first) tertile.

		Odds ratios (OR) and 95% CI (in parentheses) for worsening of clinical and radiographic outcomes over 24 months since baseline					
Comparison		**RCAL**		**ABH**		**ABD**	
						Smokers* **(n=23)**	**Non-smokers*** **(n=90)**
Concurrent Levels of MMP-9	2nd tertile vs 1st	OR= 0.79 (0.50, 1.27) p=0.34		OR=0.78 (0.48, 1.26) p=0.31		***OR=6.07 (2.05, 18.00) p=0.0011***	OR=1.09 (0.60, 1.96) p=0.78
	3rd tertile vs 1st	OR= 1.00 (0.64, 1.55) p=0.99		OR=0.91 (0.58, 1.42) p=0.68		***OR=11.78 (4.13,33.64) p<0.0001***	OR=1.71 (0.96, 3.01) p=0.066
Baseline levels of MMP-9	2nd tertile vs 1st	OR=1.24 (0.81, 1.92) p=0.32		OR=1.36 (0.88, 2.10) p=0.17		***OR=10.17 (4.94, 20.95) p<0.0001***	OR=1.59 (0.87, 2.88) p=0.13
	3rd tertile vs 1st	OR=0.91 (0.57, 1.44) p=0.68		OR=0.88 (0.55, 1.41) p=0.60		***OR=5.86 (2.24, 15.58) p=0.0003***	OR=1.15 (0.65, 2.05) p=0.63

Significant associations (p<0.01) between measures of RCAL, ABH and ABD, and serum levels of MMP-9, are noted in italics. All associations were examined for effect modification by treatment group, time since menopause, and smoking status. Because adjustment for these covariates did not affect model estimates, unadjusted odds ratios are presented.

* Multivariable models of alveolar bone density (ABD) detected effect modification between smoking status and both concurrent measures of MMP-9 levels (p=0.0329) and baseline measures of MMP-9 (p=0.0109). Therefore, analyses that relate ABD to measures of MMP-9 are stratified by smoking status.

Table 4.

Odds for worsening of clinical and radiographic outcomes (RCAL, ABH and ABD) over two years, in groups with circulating levels of MMP-2 in the second and third tertiles, compared to odds of worsening in group with circulating MMP-2 levels in lowest (first) tertile.

		Odds ratios (OR) and 95% CI (in parentheses) for worsening of clinical and radiographic outcomes over 24 months since baseline						
Comparison		**RCAL**		**ABH**			**ABD**	
				Placebo * (n=62)	**SDD * (n=51)**		**Smokers ** (n=23)**	**Non-Smokers ** (n=90)**
Concurrent Levels of MMP-2	2nd tertile vs 1st	OR=1.19 (0.62, 2.30) p=0.61		OR=0.73 (0.39, 1.37) p=0.32	OR=1.83 (0.83,4.04) p=0.13		**OR=2.99 (1.43, 6.23) p=0.0035**	OR=0.76 (0.42, 1.37) p=0.35
	3rd tertile vs 1st	OR=2.07 (1.14, 3.76) p=0.017		OR=1.77 (1.05, 2.99) p=0.032	OR=1.52 (0.72, 3.18) p=0.27		OR=2.62 (1.02, 6.73) p=0.046	OR=1.24 (0.73, 2.11) p=0.42
Baseline levels of MMP-2								
							Within five years of menopause * (n=42)**	**More than five years post-menopause *** (n=71)**
	2nd tertile vs 1st	OR=0.75 (0.37, 1.54) p=0.43		OR=0.90 (0.55, 1.48) p=0.67			OR=2.44 (1.04, 5.76) p=0.041	OR=0.94 (0.52, 1.71) p=0.85
	3rd tertile vs 1st	OR=1.48 (0.90, 2.42) p=0.12		OR=0.99 (0.64, 1.54) p=0.98			**OR=4.54 (2.04, 10.10) p=0.0002**	OR=0.78 (0.42, 1.44) p=0.42

Significant associations ($p<0.01$) between measures of disease progression (RCAL, ABH and ABD) and serum levels of MMP-2 are noted in italics. All associations were examined for effect modification by treatment group, time since menopause, and smoking status. Because adjustment for these covariates did not affect model estimates, unadjusted odds ratios are presented.

* Multivariable models of alveolar bone height (ABH) detected effect modification between treatment status and concurrent measures of MMP-2 (p=0.0782). Therefore, analyses that relate ABH to concurrent measures of MMP-2 are stratified by treatment status.

** Multivariable models of alveolar bone density (ABD) detected effect modification between smoking status and concurrent measures of MMP-2 (p=0.0886). Therefore, analyses that relate ABD to concurrent measures of MMP-2 are stratified by smoking status.

*** Multivariable models of alveolar bone density (ABD) detected effect modification between post-menopausal status and baseline measures of MMP-2 (p=0.0097). Therefore, analyses that relate ABD to baseline measures of MMP-2 are stratified by post-menopausal status.