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ORIGINAL ARTICLE

Effects of alendronate and hormone replacement therapy, alone and in combination, on saliva, periodontal conditions and gingival crevicular fluid matrix metalloproteinase-8 levels in women with osteoporosis

S Eviö[1], L Tarkkila[2], T Sorsa[2,3], J Furuholm[2], MJ Välimäki[4], O Ylikorkala[1], A Tiitinen[1], JH Meurman[2,3]

[1]*Department of Obstetrics and Gynecology, University of Helsinki;* [2]*Helsinki University Central Hospital, and Institute of Dentistry, University of Helsinki;* [3]*Department of Oral and Maxillofacial Diseases, University of Helsinki;* [4]*Division of Endocrinology, Department of Medicine, University of Helsinki, Helsinki, Finland*

OBJECTIVE: To compare the effects of hormone replacement therapy (HRT), alendronate and their combination on oral health of elderly postmenopausal women with osteoporosis.

MATERIALS AND METHODS: Sixty patients, aged 65–80 years (mean 71 years), with a T-score of bone mineral density of −2.5 s.d. or less at either the lumbar spine or the femoral neck, were randomized to receive 2 mg of estradiol plus 1 mg norethisterone acetate (HRT) ($n = 20$), 10 mg of alendronate ($n = 18$), or their combination ($n = 22$) for 2 years. Periodontal and oral status and mouth symptoms were recorded, and salivary analyses made at the beginning and at the end of the study. Gingival crevicular fluid (GCF) matrix metalloproteinase (MMP-8) levels were determined to address destructive events in periodontal tissue.

RESULTS: Resting salivary flow rate decreased by 19% ($P < 0.05$), and GCF MMP-8 tended to increase in the alendronate group. None of the regimens affected subjective feelings of dry or burning mouth. There were no significant changes in dental or periodontal status, stimulated flow rate or composition of saliva during the study.

CONCLUSIONS: Alendronate decreased resting salivary flow rate but otherwise HRT or alendronate separately or in combination had no effect on oral health in elderly women with osteoporosis.

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Keywords: alendronate; hormone replacement therapy; osteoporosis; dental health; periodontitis; matrix metalloproteinase

Correspondence: Sirpa Eviö, Department of Obstetrics and Gynecology, Helsinki University Central Hospital, PB 140, 00029 Helsinki, Finland. Tel: +358 9 4711, Fax: +358 9 47174801, E-mail: sirpa.evio@hus.fi
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Introduction

A chronic imbalance in bone-remodeling process results in osteoporosis, which is characterized by reduced skeletal mass and micro-architectural deterioration. Postmenopausal estrogen deficiency results in increased bone resorption and a reduction in bone mass. Of 65–70-year-old women, 30% have osteoporosis (Consensus Development Conference, 1993). Women with osteoporosis are at increased risk of attachment loss of teeth, which risk may be attenuated by the use of estrogen replacement therapy (Grodstein *et al*, 1998; Payne *et al*, 1999; Ronderos *et al*, 2000). In addition to estrogen bisphosphonates also inhibit bone resorption. Women with severe osteoporosis or those who have failed to respond optimally to estrogen or bisphosphonate alone might benefit from an additive effect when they combine these two antiresorptive agents which have different mechanisms of action (Lindsay *et al*, 1999; Bone *et al*, 2000; Tiras *et al*, 2000).

Deficiency of estrogen after menopause causes oral health problems (Grodstein *et al*, 1998). Buccal mucosa and salivary gland tissue contain estrogen receptors and might be estrogen responsive tissue (Leimola-Virtanen *et al*, 2000), and hormone replacement therapy (HRT) has been reported to ameliorate dry mouth feelings (Laine and Leimola-Virtanen, 1996; Leimola-Virtanen *et al*, 1997; Friedlander, 2002; Eliasson *et al*, 2003).

Osteoclasts resorb bone by secreting acid and proteolytic enzymes into an extracellular resorption lacuna. Two major groups of proteinases, matrix metalloproteinases (MMPs) and cysteine proteinases, play the greatest role in degradation of the organic matrix, which is mainly composed of type 1 collagen (Parikka *et al*, 2001). Estrogen reduces the depth of resorption pits by disturbing the organic bone matrix degradation activity of mature osteoclasts and decreases cysteine proteinases

(Parikka *et al*, 2001) and MMPs (Liao and Luo, 2001). Bisphosphonates can inhibit the catalytic activities of MMPs *in vitro*, and this might be one potential mechanism behind down-regulation of bone resorption (Teronen *et al*, 1999). Matrix metalloproteinases represent the family of tissue-degradative host proteinases, and they are not only involved in pathologic tissue destruction but also in tissue remodeling associated with tooth development and wound healing (Birkedal-Hansen, 1993; Salo *et al*, 1994; Pirilä *et al*, 2001). Type I and III collagens produced by periodontal ligament and gingival fibroblasts are the predominant extracellular matrix components of the periodontium (Birkedal-Hansen, 1993). The initial split of interstitial collagens is a key feature of progressive and active periodontitis lesions, and this cleavage is carried out by host cell-derived interstitial collagenolytic MMPs (Birkedal-Hansen, 1993; Sorsa *et al*, 1998).

Collagenase activities in gingival crevicular fluid (GCF) are elevated in periodontitis compared with the periodontally healthy gingival tissue and GCF (Ingman *et al*, 1994, 1996; Nomura *et al*, 1998). Active forms of MMPs are associated with the active phase of periodontitis (Nomura *et al*, 1998; Sorsa *et al*, 1998), and periodontal treatment is followed by a reduction of increased GCF MMPs (Golub *et al*, 1997; Chen *et al*, 2000). Assessment of MMP levels or collagenase levels in GCF reflects the degree of pathological periodontal collagen catabolism and may be of diagnostic value (Golub *et al*, 1997; Chen *et al*, 2000; Mäntylä *et al*, 2003). The major collagenase species detected in inflamed human periodontium are collagenase-2 or MMP-8 (Golub *et al*, 1995; Sorsa *et al*, 1998, 1999; Mäntylä *et al*, 2003). Recently it has been shown that MMP-8 in addition to its destructive action (Sorsa *et al*, 2004) also exerts anti-inflammatory or defensive characteristics (Owen *et al*, 2004).

The present study was designed to compare the efficacy of HRT, the bisphosphonate alendronate and their combination in treatment of osteoporosis and associated conditions in postmenopausal women. The study design was a randomized longitudinal study with a 2-year follow up. This paper reports findings on oral health parameters with special emphasis on periodontal disease, saliva, and GCF MMP-8 levels. The hypothesis was that these three treatments of osteoporosis might differently affect the oral symptoms and clinical, salivary and GCF parameters.

Materials and methods

Study population

The original study population comprised 90 female patients with diagnosed osteoporosis (Eviö *et al*, 2004). The patients were 65–80 years old (mean age 71 years) and their T-score of bone mineral density (BMD) was −2.5 s.d. or less at either the lumbar spine or at the femoral neck. The women were randomized (by computer program) to one of three treatment regimens: continuous combined HRT [2 mg estradiol plus 1 mg norethisterone acetate orally; Kliogest®; Novo Nordisk, Copenhagen, Denmark; $n = 30$], alendronate (10 mg Fosamax®; Merck & Co. Inc., NJ, USA; $n = 30$), or HRT plus alendronate ($n = 30$). The principle of double dummy technique was followed so that each preparation was similar in appearance. The women were instructed to take alendronate or its placebo in the morning, at least 30 min before the first meal of the day, with a glass of water, and to remain upright for at least 30 min after dosing. HRT or its placebo was taken in the evening. At baseline, dietary calcium intake was assessed by using a questionnaire. In addition to the study medication, the participants were instructed to take calcium supplementation (500–1000 mg day^{-1}) and vitamin D (400 IU day^{-1}) during the fall and winter months (October to April), but they were not provided by us. Compliance in use of the study medication was assessed by counting the unused tablets. The medical status of the subjects was assessed at baseline and 6, 12, 18 and 24 months.

The study also included a dental examination. Of all the subjects, 60 women (66%) were willing to participate at baseline (Table 1), while only 40 subjects (44%) came to the dental examination 2 years later. The main reason for refusal was the lack of interest, and the drop-outs were evenly distributed in all the study groups.

Ethical consideration

The study protocol was approved by the Ethical Committee of the Helsinki University Central Hospital and informed written consent was obtained from all subjects before the study.

Clinical dental recordings and questionnaire

Dental, periodontal and intra- and extraoral status were recorded in the beginning and at the end of the study. Because of saliva and GCF tests (below) the subjects were told not to eat or smoke 2 h prior to the examination. Panoramic tomography of the jaws (OPTG) was taken before the clinical examinations. A structured questionnaire was given to all the subjects prior to the examinations. The questionnaire comprised multiple-choice questions on smoking, self-assessed general and dental health, and an enquiry regarding the last visit to a dentist. Subjective concepts of periodontal health of the women were investigated by means of multiple-choice questions on gingival bleeding during tooth brushing.

Table 1 Baseline characteristics of the study groups

	HRT group	*Alendronate group*	*Alendronate + HRT group*
n	20	18	22
Age, mean ± s.d.	72 ± 4	70 ± 4	70 ± 3
(range in years)	(67–78)	(66–79)	(65–80)
Current smokers, *n* (%)	4 (20)	3 (18)	–
Former smokers, *n* (%)	2 (10)	3 (18)	4 (21)
Non-smokers, *n* (%)	14 (70)	11 (65)	15 (79)
Long-term disease, *n* (%)	11 (58)	8 (44)	9 (45)
Regular medication, *n* (%)	14 (70)	10 (56)	16 (73)

HRT, hormone replacement therapy.

All clinical dental examinations were carried out by the same examiner (L.T.). Dental status was recorded using the WHO criteria (World Health Organization, 1987). Because not all women remembered their history of dental treatment, especially reasons for tooth extractions, the third molars were included in recording the numbers of decayed teeth (DT), filled teeth (FT) and the total number of teeth. However, in calculating the WHO DMFT-index the third molars were not included (M = missing teeth). The OPTG X-ray films with written statement from an oral radiologist were available at the clinical examination. The number of periapical radiolucencies, endodontically treated teeth, and furcation lesions of multi-rooted teeth were counted from the X-ray films.

Periodontal health status and the need for treatment were recorded using the WHO Community Index of Periodontal Treatment Need (CPI) (Ainamo *et al*, 1982). All surfaces of the teeth were examined. Mouth sextants were scored on the basis of the worst finding in the respective sextant. Periodontal probing depths were measured to the nearest mm from the gingival margin to the bottom of the periodontal pocket at four surfaces of each tooth with a WHO periodontal ball-point probe (tip diameter 0.5 mm). As the CPI indicates periodontal treatment needs, no recordings of plaque or gingival recessions were included. The number of >6 mm periodontal pockets was recorded separately.

Gingival crevicular fluid sampling and analysis for MMP-8
Gingival crevicular fluid samples for MMP-8 levels were collected from periodontal pockets of two teeth of each patient ($n = 34$). Exclusion criteria for MMP-8 sampling were gingival bleeding or edentulous state. Before taking the sample for MMP-8 supra-gingival plaque was removed and the sampling sites were isolated with cotton rolls and dried gently to avoid saliva contamination. Samples of GCF were taken with a filter-paper sampling strip. The strip was placed into the gingival crevice for 30 s. It was then placed in a test tube and frozen at $-20°C$ until analyzed (Sorsa *et al*, 1999; Mäntylä *et al*, 2003).

The MMP-8 levels in the GCF samples were determined by time-resolved immunofluoresence assay (IFMA). The monoclonal MMP-8 specific antibodies 8708 and 8706 were used as catching and tracer antibodies, respectively, and the tracer antibody was labeled using europium-chelate (Hanemaaijer *et al*, 1997; Liede *et al*, 1999; Chen *et al*, 2000; Mäntylä *et al*, 2003). The assay buffer contained 20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 5 mM $CaCl_2$, 50 mM $ZnCl_2$, 0.5% bovine serum albumin, 0.05% sodium azide and DTPA at 20 mg l^{-1} (Liede *et al*, 1999). Samples were diluted in assay buffer and incubated for 1 h in microplate wells after which the cells were washed, followed by incubation for 1 h with the tracer antibody. After 1 h the cells were washed again, enhancement solution was added to the wells, and after 5 min fluorescence was measured using a 1234 Delfia Research Fluorometer (Wallac, Turku, Finland) (Hanemaaijer *et al*, 1997; Liede *et al*, 1999; Sorsa *et al*, 1999). The specificities of monoclonal antibodies against MMP-8 corresponded to those of polyclonal MMP-8 antibodies and they did not detect other MMPs (Lauhio *et al*, 1994; Hanemaaijer *et al*, 1997; Liede *et al*, 1999; Sorsa *et al*, 1999). The results were reported as total MMP-8 (μg l^{-1}) in the sample.

Saliva sampling and analyses
Resting and stimulated flow rates of saliva were measured. Resting saliva was collected with the free flowing method for 3 min (Meurman and Rantonen, 1994; Närhi, 1994). For stimulated saliva, a 1-g piece of paraffin wax was given to the women to chew. The collection time for stimulated saliva was also 3 min. During this time the subject chewed the paraffin wax at a constant rate (about once a second). Saliva collected during the first 30 s, however, was discarded. Resting flow below 0.1 ml min^{-1} and stimulated flow below 0.7 ml min^{-1} were regarded as reduced salivary flow rates (Meurman and Rantonen, 1994; Närhi, 1994). Buffering capacity was assessed immediately after collecting saliva using the Dentobuff Strip® method (Orion Diagnostica Ltd., Espoo, Finland). According to the manufacturer, Dentobuff score 1 corresponds to buffering end-pH >6, score 2 to pH 4.5–5.5 and score 3 to end-pH <4.5. A pH value <4.5 (score 3) was recorded as low buffering capacity (Närhi *et al*, 1993). For analyzing the prevalence of oral yeasts, the Dentocult CA® dip slide method (Orion Diagnostica Ltd.) was used. For this, samples were taken with a sterile spatula from the tongue surface. After incubation for 5 days at 37°C the colonies were counted and classified into three groups according to the manufacturer: score 1 corresponds 0–20 colony-forming units (CFU) per ml, score 2 corresponds 21–50 CFU ml^{-1}, and score 3 to more than 50 CFU ml^{-1}.

Salivary total protein and albumin concentrations were determined by colorimetric analyses and salivary immunoglobulin concentrations (IgG, IgM, IgA) were analyzed by means of enzyme immunoassays according to validated methods in our laboratory (Meurman *et al*, 1998).

Statistical analyses
All results were analyzed statistically by using SPSS for Windows version 11.0.1 software (SPSS Inc., Chicago, IL, USA) to detect differences between and changes within the study groups. Because of small sample sizes only non-parametric analyses (Kruskal–Wallis test, χ^2 test, Wilcoxon test) were used. P-values <0.05 were considered statistically significant.

Results

The baseline characteristics of the subjects included are given in Table 1. No differences were seen between the three study groups in age, amount and nature of concomitant medication and the number of chronic diseases. The 20 women who discontinued the trial were distributed evenly in the three study groups (five in the HRT group, seven in the alendronate group and eight in the combination group).

Table 2 Baseline and follow-up oro-dental status findings

	HRT group		*Alendronate group*		*Alendronate + HRT group*	
	Baseline	*Follow up*	*Baseline*	*Follow up*	*Baseline*	*Follow up*
n	20	15	18	11	22	14
No. of teeth, mean ± s.d.	19 ± 8	17 ± 9	16 ± 9	17 ± 10	17 ± 10	17 ± 10
Edentulous, *n* (%)	1 (5)	1 (7)	3 (17)	1 (9)	4 (18)	3 (21)
Prosthesis in upper jaw, *n* (%)	9 (45)	7 (47)	11 (61)	4 (36)	9 (41)	6 (43)
Prosthesis in lower jaw, *n* (%)	7 (35)	6 (40)	7 (39)	4 (36)	7 (32)	6 (43)
DMF index, mean ± s.d.	23 ± 3*	24 ± 4	23 ± 3	22 ± 3	23 ± 4	23 ± 4
DT, mean ± s.d.	0.8 ± 1.3	0.7 ± 1.0	0.4 ± 0.9	0.3 ± 0.5	0.5 ± 0.9	0.7 ± 1.3
FT, mean ± s.d.	13 ± 6	13 ± 7	11 ± 7	12 ± 7	12 ± 8	11 ± 9
No periodontitis, *n* (%)	1 (5)	2 (14)	1 (7)	1 (11)	1 (6)	–
Mild periodontitis, *n* (%)	9 (47)	3 (21)	4 (27)	1 (11)	3 (17)	–
Severe periodontitis, *n* (%)	9 (47)	9 (64)	10 (67)	7 (78)	14 (78)	11 (100)
No. of teeth with gingival pockets >6 mm, mean ± s.d.	1.5 ± 2.0*	2.1 ± 2.2	1.6 ± 1.6	2.8 ± 2.0	1.5 ± 1.9*	2.8 ± 1.9
Lesions of mouth mucosa, *n* (%)	15 (75)	12 (80)	12 (67)	6 (54)	13 (59)	9 (64)
Signs of TMJ dysfunction, *n* (%)	15 (75)	10 (67)	5 (28)	3 (72)	10 (45)	5 (36)

HRT, hormone replacement therapy; DMF, Diseased, missing, filled teeth; DT, diseased teeth; FT, filled teeth; TMJ, temporomandibular joint.
*$P < 0.05$.

Table 2 shows the oro-dental status of the subjects at baseline and at the end of the study. No significant differences were observed in any dental or oral health status parameters between the groups. The number of patients with severe periodontitis increased in all the study groups in terms of both the CPI values and in the number of deep periodontal pockets. The increase in the number of deep periodontal pockets between baseline and follow-up recordings was significant in the HRT and combination therapy groups. Periodontitis was highly prevalent in all three groups.

Table 3 shows the salivary flow rates, results from protein analyses and the GCF MMP-8 levels. In comparison with baseline values, the resting salivary flow rate decreased 19.4% in the alendronate group ($P < 0.05$). The number of women reporting subjective feelings of dry or burning mouth remained the same in each group, with no difference between the groups (Table 4). The levels of GCF MMP-8 increased in one of the periodontal pockets sampled (A2) in the alendronate group ($P < 0.05$), but in the other pocket (A1) the increase was not significant. In the HRT and combination groups no changes were detected in the concentrations of GCF MMP-8 or in salivary protein concentrations. No statistically significant changes or intergroup differences were observed in salivary yeast counts.

Discussion

The present study represent the first randomized trial in which the effects of different osteoporosis treatment regimens on oral health and on saliva have been investigated. Alendronate caused a decrease in resting salivary flow rate, but no effect was observed on any other oral health parameters. The finding on saliva is

Table 3 Baseline and follow-up salivary flow rates, buffering capacity, yeast counts and biochemical constituents

	HRT group		*Alendronate group*		*Alendronate + HRT group*	
	Baseline	*Follow up*	*Baseline*	*Follow up*	*Baseline*	*Follow up*
n	20	15	18	11	22	14
Resting salivary flow rate (ml min^{-1}, mean ± s.d.)	0.54 ± 0.32	0.65 ± 0.43	0.72 ± 0.46*	0.58 ± 0.31	0.59 ± 0.36	0.70 ± 0.42
Stimulated salivary flow rate (ml min^{-1}, mean ± s.d.)	1.54 ± 0.82	1.96 ± 1.06	1.83 ± 0.90	1.71 ± 0.83	1.78 ± 0.71	2.23 ± 1.12
High buffering capacity, *n* (%)	14 (78)	8 (57)	16 (89)	8 (89)	19 (91)	10 (77)
Medium buffering capacity, *n* (%)	3 (17)	5 (36)	2 (11)	1 (11)	2 (10)	3 (23)
Low buffering capacity, *n* (%)	1 (6)	1 (7)	–	–	–	–
Positive yeast count, *n* (%)	14 (78)	10 (67)	11 (61)	4 (36)	16 (76)	11 (85)
MMP-8 1A ($\mu g\ ml^{-1}$), mean ± s.d.	299 ± 245	296 ± 286	169 ± 115	199 ± 101	212 ± 155	189 ± 189
MMP-8 A2 ($\mu g\ ml^{-1}$), mean ± s.d.	262 ± 205	426 ± 525	208 ± 157*	300 ± 185	136 ± 128	242 ± 213
Albumin ($\mu g\ ml^{-1}$), mean ± s.d.	277 ± 140	262 ± 134	250 ± 143	248 ± 201	252 ± 107	247 ± 144
Salivary total protein (mg ml^{-1}), mean ± s.d.	1.58 ± 0.33	1.55 ± 0.51	1.64 ± 0.35	1.56 ± 0.44	1.55 ± 0.36	1.40 ± 0.43
IgA ($\mu g\ ml^{-1}$), mean ± s.d.	34.1 ± 15.4	28.1 ± 15.1	27.3 ± 7.0	26.5 ± 10.8	28.0 ± 15.2	27.9 ± 33.9
IgG ($\mu g\ ml^{-1}$), mean ± s.d.	26.4 ± 19.3	22.2 ± 18.6	17.3 ± 16.0	13.8 ± 14.1	24.0 ± 14.1	21.7 ± 18.6
IgM ($\mu g\ ml^{-1}$), mean ± s.d.	1.99 ± 1.93	1.87 ± 2.65	1.26 ± 1.00	1.18 ± 1.44	1.10 ± 0.60	0.99 ± 1.74

HRT, hormone replacement therapy; MMP, matrix metalloproteinase; Ig, immunoglobulin.
*$P < 0.05$.

Table 4 Baseline and follow-up recordings of self-assessed oral health and oral health habits (yes/no)

	HRT group		*Alendronate group*		*Alendronate + HRT group*	
	Baseline	*Follow up*	*Baseline*	*Follow up*	*Baseline*	*Follow up*
n	20	15	18	11	22	14
Dry mouth, *n* (%)	5 (26)	2 (13)	2 (13)	–	7 (40)	3 (21)
Pain or burning sensation in mouth, *n* (%)	1 (6)	1 (7)	2 (11)	–	1 (5)	2 (15)
Inadequate mastication, *n* (%)	3 (17)	2 (27)	5 (28)	2 (20)	4 (19)	5 (36)
Pain in masticatory muscles, *n* (%)	–	–	1 (6)	–	1 (5)	2 (15)
Bruxism, *n* (%)	2 (11)	–	2 (11)	1 (9)	–	–
Visits dentist regularly, *n* (%)	18 (90)	14 (100)	16 (94)	9 (90)	17 (77)	11 (85)
Gingival bleeding when brushing, *n* (%)	8 (44)	5 (36)	6 (33)	4 (36)	11 (61)	5 (42)
Satisfactory self-assessed oral health, *n* (%)	11 (55)	8 (57)	5 (25)	5 (46)	13 (62)	9 (64)

HRT, hormone replacement therapy.

clinically important, because low resting salivary flow is detrimental to the teeth and oral mucosa.

In the main study (Eviö *et al*, 2004) increases of 9.1–11.2% in lumbar spine BMD at 2 years were similar in the three study groups ($P < 0.0001$). Only HRT increased femoral neck BMD statistically significantly ($P < 0.0001$), at both 1 (+4.9%) and 2 years (+5.8%; $P < 0.05$ *vs* the other groups). Total hip BMD increased similarly in all three study groups. The combination of HRT and alendronate did not offer an extra gain of bone mass in comparison with either treatment alone among elderly postmenopausal women with osteoporosis. In terms of BMD changes the single treatments were equally effective, but the reductions in markers of bone turnover were less with HRT than with alendronate (Eviö *et al*, 2004).

Bisphosphonates, such as alendronate, have been shown to inhibit the catalytic activities of MMPs including MMP-8 *in vitro* (Teronen *et al*, 1999; Heikkilä *et al*, 2002). However, instead of measuring the catalytic activity at GCF MMP-8 we measured the MMP-8 immunoreactive protein levels in GCF. Alendronate did not down-regulate GCF MMP-8 level or expression *in vivo*, and no effect or slight tendency to increase GCF MMP-8 level was observed. Estrogen also has an effect on MMPs (Liao and Luo, 2001), and in this study there was no placebo group, because it was considered to be unethical in an osteoporosis study. Further, weaknesses of the study were the relatively small number of patients in each treatment group and the observational nature of the trial. The small number of patients at the follow-up examination, in particular, decreased the power of the study, but this could not be avoided as so many of the elderly women were not interested in participating in the follow-up dental examination. However, the drop-outs were evenly distributed in the three study groups.

Early detection of tissue destruction is desirable to prevent further irreversible loss of connective tissue attachment of teeth and adjacent alveolar bone (Wactawski-Wende *et al*, 1996; Tezal *et al*, 2000). Females with osteoporosis are at an increased risk of attachment loss of teeth, and this risk may be attenuated by the use of HRT (Paganini-Hill, 1995; Grodstein *et al*, 1998; Jeffcoat, 1998; Krall *et al*, 1998; Payne *et al*, 1999; Reinhardt *et al*, 1999; Ronderos *et al*, 2000; Hildebolt *et al*, 2004). Alveolar crest height and alveolar bone density is higher in postmenopausal women receiving HRT compared with placebo (Civitelli *et al*, 2002), and severe clinical attachment loss (11.9% *vs* 18.6%) and alveolar bone loss (20.3% *vs* 34%) is decreased in HRT-users compared with non-users (Grossi, 1998). Calcium and vitamin D supplementation also decrease alveolar bone loss (Krall *et al*, 2001; Hildebolt *et al*, 2004). In this regard, there is some evidence to show that bisphosphonates might also have a role as an adjunct therapy for periodontal disease (Jeffcoat, 1998; El-Shinnawi and El-Tantawy, 2003). For this purpose the ability of bisphosphonate or some other MMP-inhibitory drugs, such as low-dose doxycycline (Golub *et al*, 1997) to act as MMP inhibitor could be useful. However, this area of investigation requires more clinical human studies.

In previous studies climacteric symptoms despite HRT have been reported to be risk factors of burning mouth syndrome (Tarkkila *et al*, 2001), which is common complaint among elderly women (Wardrop *et al*, 1989; Hakeberg *et al*, 1997). In the present study, however, subjective sensations of a painful or dry mouth were similar in all three study groups throughout the observational period.

Salivary protein analyses can be used to measure serum-derived proteins in saliva as markers of the integrity of mouth mucosa and very small amounts of these proteins are usually detected in the saliva in healthy subjects (Laine *et al*, 1992; Pajukoski *et al*, 1997; Meurman *et al*, 1998). The present results showed that the treatments for osteoporosis did not affect mouth mucosa in this regard. However, it needs to be re-emphasized that a significant decrease in the mean resting salivary flow rate in the alendronate group was not desirable from the clinical point of view. Resting saliva is the moisturizing and lubricating component of the oral defense system and a decrease in its secretion is often reflected in symptoms of dry mouth and burning mouth and it also renders the patient liable to oral yeast infection (Pajukoski *et al*, 2001). In the present study, however, no difference was observed between the groups

in the numbers of patients with mucosal pathology or positive yeast counts, or among women reporting or not reporting symptoms of the mouth.

In conclusion, alendronate caused a decrease in resting salivary flow rate and did not decrease but instead tended to increase GCF MMP-8 levels. Depending on either destructive (Sorsa *et al*, 2004) or anti-inflammatory role (Owen *et al*, 2004) of MMP-8 in oral inflammation, this may even be useful. Otherwise, treatment with HRT and alendronate, alone or in combination, had no effect on oral health parameters in elderly women with osteoporosis.

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