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Effect of bisphosphonate as an adjunct treatment for chronic periodontitis on gingival crevicuar fluid levels of nuclear factor-κB ligand (RANKL) and osteoprotegerin in postmenopausal osteoporosis

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Abstract: Osteoporosis and periodontal disease are linked by an altered receptor activator of nuclear factor κB ligand and osteoprotegerin ratio (RANKL/OPG), and medical treatment with bisphosphonate (BP) may help control these molecules. The effect of BP on clinical findings and gingival crevicular fluid (GCF) values of RANKL and OPG using enzyme-linked immunosorbent assays was evaluated in postmenopausal women; 13 patients with both chronic periodontitis and osteoporosis (group A), 12 systemically healthy patients with chronic periodontitis (group B), 12 periodontally healthy patients with osteoporosis (group C), and 10 systemically and periodontally healthy individuals (group D). Recordings were repeated at the end of months 1, 6, and 12 in groups A, B, and C. At the baseline, groups A and B exhibited the lowest OPG values ($P < 0.05$). After periodontal treatment, OPG values were markedly increased at the end of 6th month in group A and 12th month in group B ($P < 0.008$). There was no significant difference in GCF RANKL values among groups ($P > 0.05$) or during the observation period ($P > 0.008$). The use of BP may be effective in preventing periodontal breakdown by controlling the levels of these markers in osteoporosis as an adjunct to periodontal treatment.

Keywords: bisphosphonate; non-surgical periodontal treatment; osteoporosis; RANKL; OPG; periodontitis.

Introduction

Receptor activator of nuclear factor κB ligand (RANKL), its cellular receptor; receptor activator of nuclear factor κB (RANK), and its decoy receptor; osteoprotegerin (OPG) play critical roles in osteoclastogenesis (1). RANKL, a member of the tumor necrosis factor (TNF) ligand superfamily is expressed by osteoblasts, fibroblasts, and activated T- and B-cells (2). RANKL binds to RANK on the surface of pre-osteoclasts and leads to the differentiation and fusion of osteoclast precursor cells, and stimulates the activity of the mature osteoclast (3). The actions of RANKL are counteracted by OPG, a soluble neutralizing decoy receptor that is also a member of TNF receptor superfamily. OPG binds to RANKL and blocks its interaction with RANK (4). Bone resorption and formation is regulated by the concentration of RANKL and RANK on the osteoclast surface (5) and the presence of OPG (4). Thus, the balance between the RANKL and OPG plays an important role in

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induction and inhibition of osteoclast formation. Under some inflammatory conditions, the balance of RANK/RANKL/OPG is altered and bone resorption occurs, such as that observed in periodontitis (6) and osteoporosis (7). Increased levels of RANKL and decreased levels of OPG have been observed in osteoporosis (8) and periodontal disease (9,10).

Osteoporosis is defined as a disorder of decreased bone mass, commonly seen in postmenopausal women, and is associated estrogen deficiency (11). Previous studies have reported a correlation between osteoporosis and periodontitis with older age and cytokines production with respect to enhanced osteoclast-mediated resorption of skeletal and alveolar bone (12). Systemic factors in postmenopausal osteoporosis may combine with periodontitis and enhance alveolar bone loss. Predisposition to periodontal diseases and alveolar bone loss was reported in postmenopausal osteoporosis (13), thus osteopenia/osteoporosis is now accepted as a risk factor for periodontal disease (14,15).

Bisphosphonate (BP), the most commonly prescribed agent for treatment of osteoporosis, is a potent inhibitor of osteoclastic activity that affects osteoblast-induced osteoclastic bone resorption (16,17). BP inhibits bone resorption by induction of bone resorptive factors and inhibition of osteoclast-mediated bone resorption by induction of osteoclastic apoptosis (18). BP affects the RANKL/OPG system by increasing OPG production and decreasing RANKL production (19,20). BP was shown to increase alveolar bone mineral density in postmenopausal women with osteoporosis (21) and reported to be beneficial to the periodontium and non-surgical periodontal treatment (22).

It is hypothesized that osteoporosis and periodontal disease may be linked with elevations in RANKL and altered RANKL/OPG ratio. Hence, medical treatment of osteoporosis with BP may help to control these molecules, which are central to alveolar bone resorption. Therefore, the aim of the present study was to investigate the effect of BP as an adjunct to periodontal treatment in patients with osteoporosis and chronic periodontitis by comparing the clinical findings with the RANKL and OPG changes in gingival crevicular fluid within a 12-month follow-up.

Materials and Methods

The study groups were selected from postmenopausal patients referred to the Department of Endocrinology, School of Medicine, Ondokuz Mayis University, Samsun, Turkey, between 2014 and 2015. The study protocol was approved by Ethical Board of Ondokuz Mayis University School of Medicine (OMÜ KAEK 2012/138) and was carried out in accordance with the ethical standards established in the 1964 Declaration of Helsinki, as revised in 2008. Written informed consent was obtained from each subject before enrollment in the study. This study is registered at ClinicalTrials.gov under ID. NCT02808988.

According to the study design, a total of 47 postmenopausal women were enrolled in this study: 13 women with osteoporosis and chronic periodontitis (CP) (group A, mean age: 57.15 ± 4.93 years); 12 systemically healthy women with CP (group B, mean age: 57.41 ± 4.94 years); 12 periodontally healthy women with osteoporosis (group C, mean age: 55.64 ± 3.79 years); and 10 periodontally and systemically healthy women (group D, mean age: 56.55 ± 4.06 years).

Subject exclusion criteria

The subject exclusion criteria were i) any known systemic disease other than osteoporosis that can affect periodontal status; ii) smoking; iii) antibiotic therapy within the last 3 months; and iv) surgical or non-surgical periodontal treatment within the last 6 months.

Subject inclusion criteria

The subject inclusion criteria were i) postmenopause, defined as absence of menstruation for at least 12 months; ii) diagnosis of osteoporosis in accordance with World Health Organization criteria (23) and defined as a T score of less than −2.5 at L1-L4, the femoral neck, or total femur (bone mineral density was measured by dual energy X-ray absorptiometry (Hologic QDR, Bedford, MA, USA) from L1-L4 and/or femur (g/cm^2); iii) patients with CP; and iv) subjects demonstrating good periodontal health and willingness to undergo regular examinations for 1 year.

Periodontal disease definition and sample site selection

Patients were directed to Department of Periodontology, Faculty of Dentistry, Ondokuz Mayis University for detailed periodontal examinations, clinical findings, and collection of gingival crevicular fluid (GCF) for sampling of markers. The clinical diagnosis was based on plaque (PI) (24) and gingival (GI) indices (25), bleeding on probing (BOP), probing depth (PD), clinical attachment loss (CAL), and full-mouth radiographs. Clinical measurements were performed at six sites per tooth (mesio-vestibular, mid-vestibular, disto-vestibular, mesio-lingual, mid-lingual, and disto-lingual) using a Williams probe (Hu-Friedy, Chicago, IL, USA) calibrated in millimeters. GCF sampling and clinical

examinations were carried out by the same investigator who was blinded to the study. Following the clinical and radiographic examinations, the BP groups and healthy subjects were divided into two groups as CP and clinically healthy controls. CP was diagnosed according to 1999 classification of periodontal disease via clinical and radiographic criteria (26). CP was identified as a minimum of six teeth exhibiting CAL and a PD of 5 mm or greater, positive for BOP in multiple regions and bone loss affecting more than 30% of the existing teeth. The clinically healthy control groups were selected on the basis of no radiographic bone loss or CAL and PD ≤ 3 mm. Sample sites were selected from the deepest pockets of single rooted teeth in different quadrants.

Flow of the study plan

Study groups A and B received periodontal phase 1 therapy, which consisted of scaling and root planing with an ultrasonic scaler (Woodpecker UDS-A; Guilin Woodpecker Medical Instrument Co., Ltd., Guangxi, China) and hand instruments (Hu-Friedy) under local anesthesia by the same investigator after baseline recordings and GCF sampling. The treatment was planned as once per week for 4 weeks and repeated in 6th and 12th months. groups A and C received BP therapy after baseline examinations and prior to periodontal phase 1 therapy using Aclasta (Novartis, Stockholm, Sweden), a once-yearly intravenous infusion of 5 mg of zoledronic acid in 100 mL ready to infuse solution. Acute phase reactions, including fever, myalgia, arthralgia, and flu-like symptoms, were noted in 10% of the patients. Postdose symptoms were reduced by paracetamol administration shortly after zoledronic acid infusion. No serious adverse effects of the drug (osteonecrosis of the jaw, renal impairment, hypocalcaemia, seconder hyperparathyroidism, and gastrointestinal, eye, cardiac, renal, and urinary disorders) (27) occurred in any patient.

Clinical recordings and GCF sampling were performed at baseline and repeated at 1, 6, and 12 months after periodontal phase 1 therapy.

GCF sampling

GCF was collected from the two deepest site of single rooted teeth in different quadrants with commercially available paper strips (Periopaper; Oraflow Inc., Smithtown, NY, USA) by the same investigator. The selected sites were isolated with cotton rolls and cleared of supragingival plaque. Then, the crevicular site was gently dried with an air syringe. The paper strips were gently placed into the periodontal pocket until mild resistance was felt and not more than 1 mm, and left in place for 30 s. Strips contaminated with blood or debris were discarded. The GCF volume of each strip was measured with a calibrated GCF meter (Periotron 8000; Oraflow Inc., Hewlett, NY, USA) and then the readings were converted to an actual volume (μL) by reference to a standard curve. All samples were pooled into an Eppendorf tube to make one sample and stored at −80°C (NuAire Ultra-Low Freezer Model no. Nu-6420E; NuAire, Caerphilly, UK) before laboratory analysis.

The fluid was eluted by centrifugation with portions of buffers to completely extract the sample from the paper. Each strip was placed in a sterile Eppendorf tube and incubated in 200 μL of phosphate buffer solution (50 mM; pH 7.2) with albumin (2 g/dL) for 30 min. Thereafter, each tube was centrifuged at 15,000 g and 4°C for 10 min. After removal of the strips used centrifugal filtration from the eppendorf tube, samples were separated for biochemical analysis. Results after measurements for all samples were multiplied by a dilution factor of 200 μL/GCF volume of each patient (28).

RANKL and OPG analysis in GCF

RANKL and OPG in the samples were determined using commercially available ELISA kits in accordance with the manufacturer's instructions (Total sRANKL ELISA kit: Cat. No.: RD193004200R, Lot No: E15-062, and Osteoprotegerin ELISA kit: Cat. No.: RD194003200, Lot No: E15-079; BioVendor GmbH, Heidelberg, Germany). These kits are based on sandwich enzyme immunoassays that used monoclonal anti-human OPG antibody for quantitative measurement of RANKL and OPG in human serum, plasma, and tissue fluids. Absorbance of the colored compound at the end of the reaction at 450 nm is proportional to the concentrations of RANKL and OPG. Intra-assay coefficients of variation for RANKL and OPG of these kits are 11.51% and 3.8%, respectively. Expected values of RANKL and OPG in human serum are 339.34 ± 42.30 pmol/L and 4.1 ± 2.3 pmol/L, respectively. All samples were measured twice.

Statistical analysis

A sample size of 20 patients in each group (20 patients with osteoporosis and 20 healthy controls) allowed 80% power with $\alpha = 0.05$ (5% probability). Values of the maximum difference between means and standard deviation were 15.27 and 14.01, respectively (Minitab, version 15). To avoid any possible dropouts, 25 patients were included in the osteoporosis groups (groups A and C). Statistical analysis was performed using SPSS for Windows (version 21, SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to investigate

whether the data were normally distributed. One-way analysis of variance (ANOVA) and the post-hoc Tukey test were performed to identify differences between groups. A P value of <0.05 was considered statistically significant. Repeated measures ANOVA was performed to compare intragroup differences throughout the observation periods with a P value of <0.05 considered as statistically significant. The degree of significance was analyzed using the paired samples t-test with Bonferroni correction. A P value of <0.008 (P value/6) was used for comparisons. Pearson correlation analysis was performed to investigate possible relationships between the biochemical and clinical parameters. The level of significance was $P < 0.05$.

Results

At the baseline

There was no statistically significant difference in mean patient age among the groups ($P = 0.74$, $P > 0.05$). All clinical parameters (PI, GI, PD, and CAL) were higher in groups A and B than in groups C and D ($P < 0.05$). GCF volume was lowest in group D, as compared with the other groups ($P < 0.05$). Group D (healthy control group) had the highest OPG value ($P < 0.05$), while there were no significant differences in baseline RANKL values among groups ($P > 0.05$) (Table 1).

Intergroup analyses of the test groups (groups A, B, and C)

At the baseline, clinical parameters were lowest in group C ($P < 0.05$). At the 1st month, there was a statistically significant difference in mean PI between the periodontitis groups and group C ($P < 0.05$). At the end of 6th month, PI was more reduced in group B than the other periodontitis group of subjects with osteoporosis (group A) and there was a difference between group A and the other test groups ($P < 0.05$). But no difference was observed at the end of the 12th month within the groups ($P > 0.05$). There was no significant difference in GI among groups at the 1st, 6th, and 12th months ($P > 0.05$), and the GI indices of both periodontitis groups were reduced. There was a significant difference in mean PD and CAL at the 1st, 6th, and 12th months between the periodontitis groups (groups A and B) and the non-periodontitis osteoporosis group (group C; $P < 0.05$). There was no significant difference in mean PD and CAL between the periodontitis groups (groups A and B; $P > 0.05$). There was a decrease in GCF volumes of both periodontitis groups (groups A and B) at the end of periodontal therapy, although this difference was not statistically different from that of the periodontally healthy osteoporosis group (group C; $P > 0.05$).

At baseline, GCF values of OPG were lower in groups A and B than group C ($P < 0.05$), although the difference was not significant between the periodontitis groups ($P > 0.05$). After the end of phase I periodontal therapy, there were no differences in OPG values among the groups ($P > 0.05$), although the OPG values of the test groups increased (groups A-C). The recordings were close to that of the control group (group D).

There were no significant differences in baseline RANKL values among groups ($P > 0.05$) throughout the observation periods. However, there was a slight reduction in the periodontitis group (group B), but this change was not statistically significant ($P > 0.05$; Table 1).

Intragroup analyses

Group A

As expected, all clinical parameters decreased from baseline ($P < 0.008$). There was no significant difference in PI between the 6th and 12th months' ($P > 0.008$), but these values were less than at the end of the 1st month ($P < 0.008$). There were no significant differences in mean GI, PD, and CAL between 1st, 6th, and 12th months ($P > 0.008$). There was a statistically significance difference between GCF volume at baseline and at the end of the 1st and 12th months ($P < 0.008$), but not at the end of 6th months ($P > 0.008$). No difference was observed in RANKL values within the sampling period ($P > 0.008$) (Table 1). However, baseline OPG values were lower than at the end of 6th and 12th months ($P < 0.008$). There was a statistically significant difference between the 1st and the 12th months and those between the values of 6th and 12th months. There was an apparent increase in the OPG values at the end of the study ($P < 0.008$) (Table 1).

Group B

There were significant differences between the baseline values of some clinical parameters and the values at the end of the 1st, 6th, and 12th months ($P < 0.008$). The mean PI at the end of the 1st month was greater than that at the end of 6th and 12th months ($P < 0.008$). No differences were observed in GI, PD and CAL values between the 1st, 6th, and 12th months ($P > 0.008$). However, there was more gain in the mean at the end of the 12th month when compared with that at the end of the 1st month ($P < 0.008$). The decrease in GCF volume was only statistically significant between the baseline and at the end of the 12th month ($P < 0.008$). There was no statistical difference in RANKL values throughout the observation period (Table 1). Although a decrease was observed at the end of 1st month, this change was not significant (P

Table 1 Clinical parameters of GCF RANKL and OPG values of the sampling areas during the observation periods (mean ± standard deviation)

Parameters	Group A (n = 13)	Group B (n = 12)	Group C (n = 12)	P value	Group D (n = 10)
PI					
Baseline	2.92 ± 0.27[a]	2.12 ± 1.08[a]	1.42 ± 0.75**,[a]	0.000	0.55 ± 0.52*
1st month	0.76 ± 0.67[e]	0.79 ± 0.58	0.08 ± 0.28**	0.004	
6th month	0.19 ± 0.32**	0.00 ± 0.00	0.00 ± 0.00	0.02	
12th month	0.15 ± 0.37	0.00 ± 0.00	0.00 ± 0.00	0.15	
GI					
Baseline	1.96 ± 0.32[a]	1.58 ± 0.66[a]	0.57 ± 0.51**,[b]	0.00	0.00 ± 0.00*
1st month	0.15 ± 0.37	0.25 ± 0.45	0.00 ± 0.00	0.20	
6th month	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	
12th month	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	
PD (mm)					
Baseline	4.69 ± 0.63[a]	5.00 ± 1.70[a]	1.71 ± 0.46**	0.00	1.33 ± 0.50*
1st month	3.61 ± 0.86	4.08 ± 1.37	1.58 ± 0.51**	0.00	
6th month	3.15 ± 0.68	3.75 ± 1.28	1.50 ± 0.67**	0.00	
12th month	3.00 ± 0.81	3.50 ± 1.38	1.58 ± 0.66**	0.00	
CAL (mm)					
Baseline	6.76 ± 1.11[a]	6.41 ± 1.88[a]	1.96 ± 0.57**	0.00	1.77 ± 0.26*
1st month	5.69 ± 1.03	5.45 ± 1.49[g]	1.95 ± 0.68**	0.00	
6th month	5.50 ± 1.06	5.08 ± 1.22	1.87 ± 0.85**	0.00	
12th month	5.19 ± 1.28	4.79 ± 1.28	1.87 ± 0.85**	0.00	
GCF volume (μL)					
Baseline	0.67 ± 0.25[c]	0.53 ± 0.23[h]	0.25 ± 0.40**	0.02	0.19 ± 0.04*
1st month	0.34 ± 0.13	0.41 ± 0.27	0.24 ± 0.24	0.04	
6th month	0.39 ± 0.25	0.35 ± 0.20	0.21 ± 0.24	0.21	
12th month	0.27 ± 0.14	0.31 ± 0.14	0.28 ± 0.15	0.19	
GCF RANKL (pmol/L)					
Baseline	29.91 ± 19.29	28.66 ± 13.42	32.57 ± 9.67	0.84	23.02 ± 7.73
1st month	31.59 ± 21.99	24.08 ± 16.87	30.14 ± 14.96	0.61	
6th month	33.95 ± 21.57	25.64 ± 19.57	29.91 ± 17.81	0.61	
12th month	35.33 ± 19.26	25.93 ± 20.80	36.69 ± 19.51	0.41	
GCF OPG (pmol/L)					
Baseline	0.55 ± 0.49[d]	0.71 ± 0.54[h]	1.46 ± 1.51**,[d]	0.04	2.89 ± 1.53*
1st month	1.12 ± 0.74	1.95 ± 1.20	2.09 ± 1.42[e]	0.08	
6th month	1.82 ± 1.04	3.02 ± 2.70	2.99 ± 1.73	0.20	
12th month	4.32 ± 3.45[f]	3.07 ± 1.86	3.76 ± 2.15	0.51	

One way ANOVA (post-hoc Tukey test) was used for intergroup analysis; *P < 0.05 (difference between group D and the other groups), **P < 0.05 (difference between group A, group B and group C). The paired samples t-test and Bonferroni correction was used for intragroup analysis (P < 0.008); [a]Baseline was different from the other periods, [b]Baseline was different from 1st and 6th months' values, [c]Baseline was different from 1st and 12th months' values, [d]Baseline was different from 6th and 12th months' values, [e]Value at 1st month was different from those at 6th and 12th months', [f]12th month was different from 1st and 6th months', [g]Difference between the 1st and 12th months, [h]Difference between the baseline and 12th months. GCF, gingival crevicular fluid; PI, plaque index; GI, gingival index; PD, probing depth; CAL, clinical attachment loss; RANKL, Receptor activator of NF-κB ligand; OPG, osteoprotegerin.

≥ 0.008). There were significant differences in OPG from baseline to 12th month (P < 0.008) (Table 1).

Group C

There were statistically significant differences in PI from the baseline to the end of 1st, 6th, and 12th month scores (P < 0.008). Baseline GI scores were significantly different from scores at the end of the 1st and 6th month scorings (P < 0.008), but not from the 12th months' (P > 0.008). There were no statistical differences between the mean PD, CAL, and GCF volume (P > 0.008).

No differences were observed in RANKL values during the observation period (P > 0.008) (Table 1). Baseline OPG values were lower than the 6th and 12th month values (P < 0.008). The values for 6th and 12th months were also higher than those of the 1st month (P < 0.008) (Table 1).

Inter- and intra-group differences and mean values of

Table 2 Correlations between the clinical and biochemical parameters at the observation periods

Parameters	Baseline		1st month		6th month		12th month	
	RANKL	OPG	RANKL	OPG	RANKL	OPG	RANKL	OPG
Group A								
PD (mm)	−0.267	0.006	0.033	0.174	0.570*	0.186	−0.172	0.046
CAL (mm)	−0.243	0.128	−0.049	0.361	0.151	0.346	0.011	0.212
GCF volume (μL)	−0.320	−0.128	0.072	−0.178	−0.317	−0.268	0.041	−0.444
OPG	0.103	–	0.606*	–	−0.180	–	−0.025	–
Group B								
PD (mm)	−0.202	−0.327	−0.347	−0.549	−0.334	−0.174	−0.336	−0.549
CAL (mm)	−0.150	−0.451	−0.242	0.604*	−0.230	−0.209	−0.172	−0.459
GCF volume (μL)	0.382	−0.415	−0.475	−0.229	0.512	0.037	0.471	−0.232
OPG	−0.456	–	0.565	–	0.124	–	0.270	–
Group C								
PD (mm)	−0.124	0.662*	0.457	−0.289	0.195	0.137	0.672*	0.139
CAL (mm)	0.092	−0.461	0.188	−0.004	0.365	0.072	0.528	0.011
GCF volume (μL)	−0.174	−0.151	−0.214	0.803*	−0.056	0.039	0.649*	−0.392
OPG	0.589	–	0.041	–	0.137	–	0.371	–
Group D								
PD (mm)	−0.463	−0.341						
CAL (mm)	−0.007	0.007						
GCF volume (μL)	−0.383	−0.543						
OPG	0.027	–						

Pearson correlation analysis was used. PD, probing depth; CAL, clinical attachment loss; GCF, gingival crevicular fluid; OPG, osteoprotegerin (pmol/L); RANKL, Receptor activator of NF-κB ligand (pmol/L); group A, BP chronic periodontitis group; group B, healthy chronic periodontitis group; group C, BP control group.*Significant at $P < 0.05$.

the parameters during the observation periods are shown in Table 1.

Correlations with OPG and RANKL

At the baseline, OPG was negatively correlated with PD in group C ($P < 0.05$). However, no correlations in the other parameters were observed among the groups. At the end of the 1st month, OPG was negatively correlated with RANKL in group A ($P < 0.05$). Negative correlation was investigated between the OPG value and the mean CAL of the sampling sites in group B ($P < 0.05$), which showed that OPG was positively correlated with GCF volume of the sampling sites in group C. RANKL was positively correlated with PD of the sampling sites at the end of the 6th months in group A. A negative correlation was observed between RANKL and GCF volume, and mean PD was positively correlated with RANKL of the sampling sites at the end of the 12th month in group C. Correlations between the clinical and biochemical parameters at the observation periods are summarized in Table 2.

Discussion

The purpose of this study was to evaluate the effect of BP together with non-surgical periodontal treatment on periodontal parameters and levels of GCF, RANKL, and OPG in postmenopausal women with osteoporosis and chronic periodontitis. The effect of periodontal treatment together with BP therapy on clinical parameters in osteoporosis has not yet been clarified. Based on the pharmacological action of BP as an adjunct to periodontal treatment, it was hypothesized that an increase in OPG and decrease in RANKL levels will result in improved clinical outcomes.

The systemically healthy subjects with periodontitis (group B) exhibited lower OPG levels in GCF when compared with periodontally healthy subjects (group D), in accordance with the results of previous studies (9,29-31). RANKL levels in GCF were not statistically different from the periodontally healthy groups, contrarily to some reports (9,29,31), although in accordance with the results of a recent study, which demonstrated no significiant difference in GCF concentrations of sRANKL and OPG (32).

The strongest finding of the present study was the long-term (1 year) results of BP and periodontal phase I therapy, while all previous studies about the effect of periodontal treatment on GCF levels of only OPG and RANKL values included short-term results. GCF concentration of OPG was lower in chronic periodontitis, but increased after periodontal phase I therapy, although not significantly (31). The RANKL/OPG ratio was also reported unchanged at 2, 3, and 4 months after periodontal therapy (33,34). In accordance with these

studies, the RANKL value was not altered in any of the treatment periods in the healthy periodontitis group (group B). While OPG concentration was not changed at the end of 1st and 6th months after periodontal treatment, an increase was observed at the end of 12th month in the healthy periodontitis group. This result may have resulted from periodontal treatment reflecting the positive results on GCF concentration of OPG over a longer period. Also, the discrepancies with the existing studies might be caused by methodological variations or intra-individual differences of the study populations.

In the present study, the clinical parameters of PI, GI, PD, and CAL decreased in the osteoporosis and chronic periodontitis groups using BP (group A) following by phase I therapy at the end of the 1st month and did not tend to increase during the other observation periods. According to a study by Lane et al. (22), BP therapy improved the clinical outcome of non-surgical periodontal therapy in patients with chronic periodontitis at the end of one year and in a latest study of Bhavsar et al. (35), BP therapy as an adjunct to periodontal phase I therapy was found to have beneficial clinical effects (decrease in PI and GI, gain in CAL, and reduction in PD) and an increased alveolar bone density at the end of 12 months. Our results are in agreement with the clinical improvement observed in the BP-treated periodontitis group. At the same time, both periodontitis groups (groups A and B) in the present study revealed clinical improvement within time.

Serum levels of RANKL have been shown to decrease and the level of OPG was shown to increase after 3 and 6 months in patients with postmenopausal osteoporosis receiving BP treatment (36). This result shows that BP may play a role in decreasing bone breakdown via decreasing serum RANKL concentrations. Also, a 6-month follow-up of postmenopausal women with osteoporosis who started BP therapy at the beginning of the study revealed that serum OPG and RANKL levels remained unchanged (37). There exists some data on the interplay between RANKL-OPG and osteoporosis in periodontitis under long-term BP therapy in GCF samples. In a recent study, BP did not change the concentration of RANKL and OPG and the ratio in GCF of patients with periodontal disease and postmenopausal osteoporosis (38) which included only chronic periodontitis and osteoporosis/osteopenia groups with or without BP therapy. In contrast, the control group (group D), which consisted of systemically healthy post-menopausal women, showed a marked significance in OPG levels. Furthermore, the present study is the first to reveal GCF alterations of RANKL and OPG after periodontal treatment, and correlations with the clinical parameters. One of the most impressive findings of this study was the initiation of BP treatment increased OPG earlier in the chronic periodontitis group with osteoporosis (group A) than that in the systemically healthy chronic periodontitis group (group B), although both groups received periodontal phase I therapy for one year. At the same time, the similar early increase in the other osteoporosis group without periodontitis (group C) may be concluded as a sign of the potentially beneficial effect of BP on bone biomarkers during periodontal phase I therapy.

The present study had a number of strengths, including the follow-up of patients with osteoporosis using BP from the beginning to the end of the first year, both clinically and biochemically. However, the study also has a number of potential limitations, such as the cohort was limited to Caucasian postmenopausal women. Therefore, the results cannot be appropriated to other races. Factors, such as smoking, age, alcohol use, and diet, should be included in future studies and correlations between serum and GCF levels of biochemical markers in osteoporosis should be elucidated. There is also a need for further studies investigating and comparing the effect of different BP drugs in respect to dosage and application method. This study included patients with osteoporosis that were in need of BP therapy and any group including those patients without BP therapy was considered to be unethical. So, it was not possible to compare osteoporotic patients those receiving BP therapy and other non-users. Meanwhile, previous data may provide a basis for comparisons between osteoporosis and healthy subjects.

In conclusion, BP therapy as an adjunct to periodontal treatment in postmenopausal osteoporotic women with chronic periodontitis may have beneficial effects on GCF levels of OPG. Postmenopausal women should be monitored at regular periodontal visits and receive periodontal treatment to reduce the risk of further periodontal damage.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Rogers A, Eastell R (2005) Circulating osteoprotegerin and receptor activator for nuclear factor κB ligand: clinical utility in metabolic bone disease assessment. J Clin Endocrinol Metab 90, 6323-6331.

2. Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C et al. (1999) OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 397, 315-323.
3. Gori F, Hofbauer L, Dunstan C, Spelsberg T, Khosla S, Riggs B (2000) The expression of osteoprotegerin and RANK ligand and the support of osteoclast formation by stromal-osteoblast lineage cells is developmentally regulated. Endocrinology 141, 4768-4776.
4. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R et al. (1997) Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89, 309-319.
5. Boyle WJ, Simonet WS, Lacey DL (2003) Osteoclast differentiation and activation. Nature 423, 337-342.
6. Nagasawa T, Kiji M, Yashiro R, Hormdee D, Lu H, Kunze M et al. (2007) Roles of receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin in periodontal health and disease. Periodontol 2000 43, 65-84.
7. Lerner UH (2006) Inflammation-induced bone remodeling in periodontal disease and the influence of post-menopausal osteoporosis. J Dent Res 85, 596-607.
8. Lerner UH (2006) Bone remodeling in post-menopausal osteoporosis. J Dent Res 85, 584-595.
9. Mogi M, Otogoto J, Ota N, Togari A (2004) Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. J Dent Res 83, 166-169.
10. Cochran DL (2008) Inflammation and bone loss in periodontal disease. J Periodontol 79, 8 Suppl, 1569-1576.
11. Lane JM, Russell L, Khan SN (2000) Osteoporosis. Clin Orthop Relat Res 372, 139-150.
12. Wactawski-Wende J (2001) Periodontal diseases and osteoporosis: association and mechanisms. Ann Periodontol 6, 197-208.
13. Juluri R, Prashanth E, Gopalakrishnan D, Kathariya R, Devanoorkar A, Viswanathan V et al. (2015) Association of postmenopausal osteoporosis and periodontal disease: a double-blind case-control study. J Int Oral Health 7, 119-123.
14. Martínez-Maestre MÁ, González-Cejudo C, Machuca G, Torrejón R, Castelo-Branco C (2010) Periodontitis and osteoporosis: a systematic review. Climacteric 13, 523-529.
15. Genco RJ, Borgnakke WS (2013) Risk factors for periodontal disease. Periodontol 2000 62, 59-94.
16. Sato M, Grasser W, Endo N, Akins R, Simmons H, Thompson DD et al. (1991) Bisphosphonate action. Alendronate localization in rat bone and effects on osteoclast ultrastructure. J Clin Invest 88, 2095-2105.
17. Licata AA (2005) Discovery, clinical development, and therapeutic uses of bisphosphonates. Ann Pharmacother 39, 668-677.
18. Palomo L, Bissada NF, Liu J (2005) Periodontal assessment of postmenopausal women receiving risedronate. Menopause 12, 685-690.
19. Pan B, Farrugia AN, To LB, Findlay DM, Green J, Lynch K et al. (2004) The nitrogen-containing bisphosphonate, zoledronic acid, influences RANKL expression in human osteoblast-like cells by activating TNF-α converting enzyme (TACE). J Bone Miner Res 19, 147-154.
20. Dobnig H, Hofbauer LC, Viereck V, Obermayer-Pietsch B, Fahrleitner-Pammer A (2006) Changes in the RANK ligand/osteoprotegerin system are correlated to changes in bone mineral density in bisphosphonate-treated osteoporotic patients. Osteoporos Int 17, 693-703.
21. Reddy MS, Morgan SL (2013) Decreased bone mineral density and periodontal management. Periodontol 2000 61, 195-218.
22. Lane N, Armitage GC, Loomer P, Hsieh S, Majumdar S, Wang HY et al. (2005) Bisphosphonate therapy improves the outcome of conventional periodontal treatment: results of a 12-month, randomized, placebo-controlled study. J Periodontol 76, 1113-1122.
23. Kanis JA, Melton LJ III, Christiansen C, Johnston CC, Khaltaev N (1994) The diagnosis of osteoporosis. J Bone Miner Res 9, 1137-1141.
24. Silness J, Loe H (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 22, 121-135.
25. Loe H, Silness J (1963) Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 21, 533-551.
26. Armitage GC (1999) Development of a classification system for periodontal diseases and conditions. Ann Periodontol 4, 1-6.
27. Black DM, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA et al. (2007) Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. N Engl J Med 356, 1809-1822.
28. Uematsu S, Mogi M, Deguchi T (1996) Interleukin (IL)-1β, IL-6, tumor necrosis factor-α, epidermal growth factor, and β2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. J Dent Res 75, 562-567.
29. Bostanci N, Ilgenli T, Emingil G, Afacan B, Han B, Töz H et al. (2007) Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. J Clin Periodontol 34, 370-376.
30. Babür C, Özcan G, Çebi DU, Pervane B, Özdemir B, Yücel A et al. (2012) Gingival crevicular fluid levels of osteoprotegerin (OPG) in premenopausal and postmenopausal women with or without chronic periodontitis. J Dent 40, 364-371.
31. Baltacıoğlu E, Kehribar MA, Yuva P, Alver A, Atagün OS, Karabulut E et al. (2014) Total oxidant status and bone resorption biomarkers in serum and gingival crevicular fluid of patients with periodontitis. J Periodontol 85, 317-326.
32. Gümüş P, Buduneli E, Bıyıkoğlu B, Aksu K, Saraç F, Nile C et al. (2013) Gingival crevicular fluid, serum levels of receptor activator of nuclear factor-κB ligand, osteoprotegerin, and interleukin-17 in patients with rheumatoid arthritis and osteoporosis and with periodontal disease. J Periodontol 84, 1627-1637.
33. Bostanci N, Saygan B, Emingil G, Atilla G, Belibasakis GN (2011) Effect of periodontal treatment on receptor activator

of NF-κB ligand and osteoprotegerin levels and relative ratio in gingival crevicular fluid. J Clin Periodontol 38, 428-433.
34. Balli U, Aydogdu A, Dede FO, Turer CC, Guven B (2015) Gingival crevicular fluid levels of sclerostin, osteoprotegerin, and receptor activator of nuclear factor-κB ligand in periodontitis. J Periodontol 86, 1396-1404.
35. Bhavsar NV, Trivedi SR, Dulani K, Brahmbhatt N, Shah S, Chaudhri D (2016) Clinical and radiographic evaluation of effect of risedronate 5 mg as an adjunct to treatment of chronic periodontitis in postmenopausal women (12-month study). Osteoporos Int 27, 2611-2619.
36. Dundar U, Kavuncu V, Ciftci IH, Evcik D, Solak O, Cakir T (2009) The effect of risedronate treatment on serum cytokines in postmenopausal osteoporosis: a 6-month randomized and controlled study. J Bone Miner Metab 27, 464-470.
37. Anastasilakis AD, Goulis DG, Polyzos SA, Gerou S, Koukoulis G, Kita M et al. (2008) Serum osteoprotegerin and RANKL are not specifically altered in women with postmenopausal osteoporosis treated with teriparatide or risedronate: a randomized, controlled trial. Horm Metab Res 40, 281-285.
38. Verde ME, Bermejo D, Gruppi A, Grenón M (2015) Effect of bisphosphonates on the levels of RANKL and OPG in gingival crevicular fluid of patients with periodontal disease and post-menopausal osteoporosis. Acta Odontol Latinoam 28, 215-221.