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Evaluation of Non-Surgical Periodontal Treatment on Salivary Biomarkers in Patients Undergoing Bisphosphonate Therapy

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Research Article

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ABSTRACT

Objectives: Similar mechanisms and risk factors are thought to contribute to the progression of both osteoporosis and periodontitis. Bisphosphonates (BPs) exhibit anti-inflammatory effects and inhibit the release of proinflammatory cytokines, making them a potential adjunctive therapy in periodontal treatment. This study aimed to evaluate the impact of medical treatment for osteoporosis with BPs and non-surgical periodontal therapy on salivary levels of interleukin-1 beta (IL-1β), interleukin-17 (IL-17), and 8-Hydroxy-deoxyguanosine (8-OHdG), as well as on clinical outcomes.

Materials and Methods: Three groups of 75 participants were assigned based on their systemic and periodontal health. Group 1 included 25 postmenopausal women with both osteoporosis and periodontitis. Group 2 consisted of 25 systemically healthy women with periodontitis, while Group 3 comprised 25 women who were both systemically and periodontally healthy. Patients who used systemic alendronate once weekly (70 mg) for at least 3 months were included. Baseline and 1- and 3-month after periodontal therapy plaque index (PI), gingival index (GI), probing depth(PD), bleeding on probing(BOP), and clinical attachment levels (CAL) were assessed. The enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of IL-1β, IL-17, and 8-OHdG in salivary samples.

Results: The clinical parameters, including GI, PD, and CAL, were significantly higher in osteoporotic patients ($p < 0.05$). After periodontal therapy, Groups 1 and 2 demonstrated significant reductions in clinical parameters (PI, PD, BOP, and CAL) at the 3-month follow-up compared to baseline values ($p < 0.05$). In periodontitis groups, the mean IL-1β, IL-17, and 8-OHdG levels were found to be significantly higher ($p<0.001$). 1- and 3-month comparisons revealed significant reductions in IL-1β, IL-17, and 8- OHdG than the baseline values in periodontitis groups ($p<0.05$). In the patients with osteoporosis (group 1), baseline 8-OHdG levels were significantly greater ($p<0.001$). No significant differences regarding clinical measurements and biomarkers between the periodontitis groups were found following the initial phase of periodontal therapy.

Conclusions: Osteoporosis patients diagnosed with periodontitis and taking BPs exhibited elevated levels of the oxidative stress biomarker 8-OHdG compared to other patients. Salivary biomarkers and periodontal parameters responded similarly to initial periodontal therapy in patients receiving BPs. In the short term, taking oral BP medication did not significantly impact clinical parameters or salivary biomarkers.

Keywords: Bisphosphonates; 8-Hydroxydeoxyguanosine; Interleukin-1β; Interleukin-17; Non-surgical periodontal treatment

Bifosfonat Tedavisi Gören Hastalarda Cerrahi Olmayan Periodontal Tedavinin Tükürük Biyobelirteçleri Üzerine Değerlendirilmesi

Araştırma Makalesi

Süreç

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ÖZ

Amaç: Osteoporoz ve periodontitisin benzer risk faktörleri ve mekanizmalara sahip oldukları bilinmektedir. Bifosfonatlar pro-inflamatuar sitokinlerin salınımını inhibe eden ilaçlardır ve cerrahi olmayan periodontal tedavinin klinik sonuçlarını iyileştirmek amacıyla tedaviye ek olarak kullanılabilirler. Bu çalışmada osteoporoz tedavisi amacıyla bifosfonat reçete edilen bireylerde bifosfonatların cerrahi olmayan periodontal tedavinin klinik parametreler ve tükürük interlökin-1 beta (IL-1β) , interlökin-17 (IL-17) ve 8-Hydroxy-deoxyguanosine (8-OHdG) seviyeleri üzerine etkilerini incelemek amaçlanmıştır.

Gereç ve Yöntemler: Periodontitis ve osteoporoz görülen 25 (grup 1), sistemik açıdan sağlıklı periodontitis görülen 25 (grup 2) ve hem sistemik hem de periodontal açıdan sağlıklı 25 birey (grup 3) çalışmaya dâhil edildi. 1.gruptaki hastalar osteoporoz nedeniyle en az 3 aydır haftada bir kez alendronat (70mg) kullanan hastalardan oluştu. Klinik parametreler; plak indeksi (PI), gingival indeks (GI), sondlamada kanama indeksi (SKI), cep derinliği (CD), klinik ataçman seviyesi (KAS) ve tükürük örnekleri başlangıçta, tedavi sonrası 1. ve 3. aylarda değerlendirildi.

Bulgular: Başlangıçta GI, CD ve KAS değerlerinin osteoporozlu hastalarda, sistemik olarak sağlıklı bireylerle karşılaştırıldığında istatistiksel açıdan anlamlı düzeyde yüksek olduğu bulgulandı ($p<0.05$). 1. ve 2. grupta klinik parametreler PI, CD, SKI ve KAS periodontal tedavi sonrası 3. ayda başlangıca göre anlamlı düzeyde azaldı ($p<0.05$). Ortalama IL-1β, IL-17 and 8-OHdG seviyeleri 1. ve 2. grupta sağlıklı gruba göre anlamlı ölçüde yüksekti ($p<0.001$). Her iki periodontitisli grupta 1. ve 3. ay değerlendirmelerinde IL-1β, IL-17 ve 8- OHdG düzeyleri başlangıca göre anlamlı olarak azaldı ($p<0.05$). 1. grupta 8-OHdG seviyeleri 2.gruba göre anlamlı düzeyde yüksekti. Periodontal tedavi sonrası bifosfonat kullanan periodontitisli hastalarla ilaç kullanmayanlar arasında klinik parametreler ve biyobelirteç seviyeleri arasında anlamlı farklılık görülmedi.

Sonuçlar: Periodontitis görülen ve bifosfonat kullanan osteoporoz hastalarında, diğer hastalara kıyasla oksidatif stres biyobelirteci 8-OHdG düzeyleri yüksek bulunmuştur. Bifosfonat tedavisi gören hastalar periodontal parametreler ve tükürük biyobelirteçleri açısından cerrahi olmayan periodontal tedaviden, bifosfonat kullanmayan grupla benzer şekilde etkilenmektedir. Oral bifosfonatların kullanımının kısa vadede klinik parametreler ve tükürük biyobelirteçleri üzerine anlamlı bir etkisi olmamıştır.

Anahtar Kelimeler: Bifosfonatlar; 8-hidroksideoksiguanozin; İnterlökin-1beta; İnterlökin-17; Cerrahi Olmayan Periodontal Tedavi.

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Introduction

Periodontitis, known as a biofilm associated inflammatory disease that affects the tooth-supporting structures, causes attachment loss and alveolar bone loss.[1,2] Osteoporosis is defined by decreased bone mass and a degradation of bone architecture. Every bone in the body, including the jaw bone, is impacted by osteoporosis.[3] It has been suggested that periodontal pathogenic bacteria cause an increase in inflammatory cytokines.[4] Similar mechanisms and risk factors are considered to involve bone destruction in osteoporosis and periodontitis. Osteoporosis and periodontitis are both bone diseases that are strongly linked to aging and inflammation.[5]

The goal of periodontal therapy is to reduce the bacterial load, rebalance the composition of the oral microbiota, and eliminate inflammation. It includes oral hygiene motivation, biofilm control, scaling and root planing (SRP), and subgingival debridement to remove calculus and bacterial biofilm from tooth surfaces. The main aim of this therapeutic strategy has been shown to reduce long-term tooth loss and typically yields positive clinical outcomes, such as decreases in plaque index (PI), bleeding on probing (BOP), probing depth (PD), and clinical attachment level (CAL).[6,7] Indeed, non-surgical periodontal therapy has been demonstrated to eradicate acute periodontal inflammation and reduce markers in saliva and gingival crevicular fluid (GCF).[8-11]

Bisphosphonates (BPs) frequently prescribed to patients with osteoporosis, are potent inhibitors of osteoclastic activity affecting osteoclast-induced bone resorption.[12] Due to their impact on bone turnover and capacity to inhibit proinflammatory cytokines associated with periodontal tissue, BPs are now used along with SRP.[13,14] On the other hand, bisphosphonate therapy can alter local healing responses and may cause the development of osteonecrosis of the jaw bone (ONJ). Some studies have shown oxidative damage due to BPs in oral epithelium.[15] It is widely known that BPs should be used with caution since one of their undesirable side effects is ONJ, and the duration of BPs therapy is related to an increased risk of ONJ.[12,16-18]

The pro-inflammatory cytokine interleukin (IL)-1β has shown to be elevated in systemically healthy individuals with periodontitis. In addition to the clinical evidence supporting the association between IL-1β and periodontitis, elevated IL-1β triggers a cascade of

inflammatory responses, leading to bone resorption.[19] IL-1β is a key cytokine commonly measured in gingival tissues of individuals with periodontal disease. Elevated IL1-β levels have also been observed in osteoporotic patients receiving BPs.[11,20] Following SRP treatment, recent studies have demonstrated a decrease in IL-1β in saliva or GCF.[21,22]

IL-17 has been shown to increase bone resorption and exacerbate inflammation by stimulating the release of osteoclastic mediators and pro-inflammatory cytokines from gingival cells.[23] Studies indicate that IL-17A levels in serum, saliva, and GCF are elevated in individuals with periodontitis and correlate with clinical parameters such as BOP, PD, and CAL.[24,25] Higher GCF IL-17A levels have been detected in individuals with both osteoporosis and peridontitis compared to those with osteoporosis alone but without periodontitis.[26] In systemically healthy patients with periodontitis, non-surgical periodontal treatment has been shown to effectively reduce plasma IL-17 levels.[27]

Due to 8-Hydroxy-deoxyguanosine (8-OHdG) being known as a marker most representative of oxidative DNA damage, its salivary levels in oral diseases and periodontitis were extensively investigated. There is a correlation between 8-OHdG and periodontopathogenic bacteria. Consequently, this link demonstrates the value of 8-OHdG as a marker for evaluating the state of periodontal health and the effectiveness of periodontal therapy.[8,28] Salivary 8-OHdG levels were observed to be elevated in individuals with periodontal disease and to have significantly decreased following initial periodontal therapy.[29-31]

It has been suggested that BPs induce cellular lipid peroxidation and tissue damage, and osteoporotic patients are more prone to lipid peroxidation.[32,33] As people age, the accumulation of oxidative stress increases the progression of osteoporosis and periodontitis. Therefore, treatment for osteoporosis could help achieving promising outcomes in the management of periodontitis.[5]

The objective was to assess the impact of medical treatment for osteoporosis by BPs and non-surgical periodontal therapy on salivary levels of IL-1β, IL-17, and 8-OHdG, as well as the clinical outcomes.

Materials and Methods

Study Design

The study was conducted after receiving ethical approval from the Ethical Board of Gazi University Faculty of Dentistry, Ankara, Turkey (Protocol number 2018/26120). The clinical trial is registered at ClinicalTrials.gov as NCT04299477. Each subject provided written informed consent. A total of 75 female patients, 50 postmenopausal individuals with clinical and radiographic diagnoses of periodontitis, were accepted to this study.

According to the consensus report published by Papapanou *et al*. (2018) if an individual case of periodontitis shows an interdental clinical attachment loss of 3 to 4 mm, maximum PD ≤ 5 mm, has mostly horizontal bone loss, radiographic bone loss coronal 1/3 (15% to 33%), it is identified as Stage II (moderate periodontitis).[1] The individuals in this study complied with the definition of periodontitis Stage II. The ratio of radiographic bone loss (%) to age was used to determine the grade after panoramic radiographs were used for confirmation. Grade B status was assigned to those patients with periodontitis whose bone loss (%)/age ratio values were between 0.25 and 1.

Based on World Health Organization guidelines[34], patients who were diagnosed with osteoporosis (bone mineral density that is 2.5 standard deviations or lower than the average value for young, healthy women a t-score of < - 2.5 SD) and were received oral bisphosphonate (alendronate) once weekly (70mg) for at least three months took part in the present study.[35] Although the risk of ONJ in the oral usage of BPs is lower than in individuals receiving intravenous (IV)[36], due to the potential risk, osteoporotic women who needed BPs and currently prescribed BPs were included in our study. Participants were excluded from the study if they were smokers, had taken other medications such as antibiotics, immunosuppressants, calcium channel blockers within the last sixth months, systemic conditions that could interfere with normal healing processes (such as diabetes). The research also did not include patients who had periodontal therapy in the three months prior.

Periodontal Parameters

The subjects (75) were split into three groups (n=25 each) based on their periodontal status. Patients in group 1 had osteoporosis and periodontitis and received BPs, while patients in group 2 were systemically healthy with periodontitis. The patients in group 3 were periodontally and systemically healthy.

Periodontal examinations were performed at baseline using a Williams probe (Hu-Friedy, Chicago, IL, USA) calibrated in millimeters. PI[37], gingival index (GI)[38], PD, BOP, and CAL scores were recorded. Participants with Stage II periodontitis received a quadrant non-surgical periodontal therapy protocol, which comprises SRP of teeth in one quadrant of the mouth at a time, with four different sessions over two weeks.[39,40] A periodontist (B.K) performed SRP procedures using hand scalers and curettes until the root surface was deemed clean and smooth. Local anesthesia was administered as needed. Antibiotics and mouth rinses were not recommended during or after the therapy. Following the periodontal treatment, re-evaluations were conducted at 1-month and 3-month follow-up visits. Patients were instructed to use interdental brushes once daily and to perform oral hygiene twice a day. During recall appointments, each patient received guidance on maintaining proper oral hygiene. However, no supra- or subgingival instrumentation was performed. In Group 3, SRP was not performed.

In order to determine intra-examiner reproducibility for each site the percentage of the sites examined on 10% of the sample for PD and CAL was calculated. 90% accuracy was determined by the mean difference of the scores. For PD and CAL, the intraexaminer kappa scores were 0.84 and 0.85, respectively.[41]

Saliva Sampling

Unstimulated saliva samples for biomarker assessment were taken prior to clinical measurements. The participants included in this study were told to avoid eating or drinking 1 hour before the saliva samples were taken. Samples of whole saliva were collected into polypropylene tubes over a 5-minute period in the morning and then stored at -80°C until subsequent examination via enzyme-linked immunosorbent assay (ELISA).[42]

Salivary Analyses

Following centrifuging at 3000 rpm for 10 minutes, the saliva samples were assessed for IL-1β, IL-17, and 8-OHdG. With a few slight modifications, ELISA kits (BT Lab, Cat. No. E0143Hu, China) were used in accordance with the manufacturer's instructions. Saliva samples were introduced into the wells of the microplate. Following this, 100 µL of reagent A, prepared in advance, was added to each well, and the plate was incubated at 37°C for 60 minutes. The plate was then washed three times with 400 µL of wash solution. Subsequently, 100 µL of the pre-prepared B-reactive agent was added to the wells, and the plate was incubated for an additional 30 minutes at 37°C. The plate underwent another round of washing, consisting of five washes with the wash solution. Afterward, 50 µL of substrate solution was added to each well, and the plate was incubated at 37°C for 15 to 20 minutes, until the solution developed a blue color. Finally, 50 µL of stop solution was introduced into each well, causing the color of the solution to transition to yellow. After the process finished, the optical densities (OD450) of the samples were immediately measured spectrophotometrically at 450 nm using an ELISA reader (BioTek, United Kingdom). The minimum detection thresholds for IL-1β, IL-17 and 8-OHdG were 4.69 pg/mL, 2 pg/ml, and 0.5 ng/ml respectively.

Statistical Analysis

SPSS statistical software package program (Version 23.0 for Windows; Armonk, NY: IBM Corp.) was used to analyze the data. A sample size calculation was performed for the Repeated Measures ANOVA and a power of test approximately 80.57%. The result of the power analysis suggested that the number of patients was required to be at least 66 in total, with at least 22 in each group. Descriptive statistics were presented as mean ± standard deviation for continuous variables. The assumption of normalcy was examined using analytical (Kolmogorov-Smirnov/Shapiro–Wilk Test) and visual methods (histogram and plots). The Levene test was used to ensure homogenity of variances. The difference between the three groups was calculated using One-way ANOVA was utilized to determine differences among the three groups, followed by Tukey' post hoc test for binary comparisons. Independent samples t-test was employed to assess differences between the two groups. During the observation periods, repeated measurements were conducted using ANOVA (Repeated Measures ANOVA) to test intra-group differences. Pearson correlation coefficient was calculated to evaluate the relationship between the levels of biomarkers and clinical measurements. The significance value of the analyses was taken $p<0.05$.

Results

Clinical Findings

The study was completed with 25 women with osteoporosis and periodontitis (group 1, mean age: 57.04 ±7.54 years), 25 systemically healthy women with periodontitis (group 2, mean age: 49.72±6.90 years), and 25 periodontally and systemically healthy women (group 3, 39.60±8.90 years). No dropouts or unintended harms or effects occurred to the subjects. The mean age of the study groups were comparable (Table 1).

Table 1. Demographic data in Group 1 (osteoporosis with periodontitis), Group 2 (systemically healthy periodontitis) and Group 3 (systemically periodontally healthy) group at baseline.

	Age (years) (mean±SD)
Group 1 (n=25)	57.04 ±7.54
Group 2 (n=25)	49.72±6.90
Group 3 (n=25)	39.60±8.90

SD – standard deviation; n – number. [62]

Table 2. Clinical findings variables (mean±SD) of Group 1 (osteoporosis and periodontitis), Group 2 (systemically healthy with periodontitis) and Group 3 (periodontally and systemically healthy) at baseline (T0), 1 month after the non-surgical periodontal therapy (T1) and 3 months (T3) after the non-surgical periodontal therapy, incl. statistics.

	Group 1	Group 2	Group 3	p‡	p§
	(n=25)	(n=25)	(n=25)		
PI T0	0.726±0.633 [a]	0.528±0.342 [a]	0.072±0.036[1,2]	<0.001#	0.177
PI T1	0.326±0.343 [b]	0.157±0.161			0.013‖
PI T3	0.474±0.470 [c]	0.138±0.145 [c]			0.01
GI T0	0.173±0.119 [a,3]	0.332±0.202 [a,3]	0.008±0.028[1,2]	<0.001#	0.000‖
GI T1	0.101±0.101	0.089±0.079			0,671
GI T3	0.155±0.129	0.088±0.066 [c]			0,618
PD T0	5.214±0.774 [a,3]	4.262±0.822 [a,3]	1.826±0.166[1,2]	<0.001#	0.000‖
PD T1	2.564±0.391	2.518±0.398			0,671
PD T3	2.544±0.365 [c]	2.482±0.477 [c]			0,618
BOP 0	33.963±20.737 [a]	25.177±17.001 [a]	0.025±0.009 [1,2]	<0.001#	0.108
BOP 1	15.247±8.803	10.972±9.428			0.104
BOP 3	17.858±13.639 [c]	9.901±7.843 [c]			0.105
CAL 0	4.412±0.918 [a,3]	3.195±0.627 [a,3]	0.00±0.00		<0.001#
CAL 1	2.775±0.839	0.850±0.706			<0.001#
CAL 3	3.027±0.985 [c]	0.837±1.044 [c]			<0.001#

PD, probing depth (mm); CAL, clinical attachment level (mm); BOP, bleeding on probing (%); PI, plaque index (%); GI, Gingival index (%) Comparisons the difference among three groups assessed by One-way ANOVA test. p§: Independent Samples t Test, p‡: (ANOVA), ‖p<0.05; ¶p<0.01; #p<0.001.

1,2,3: Intergroup comparisons are shown in numbers. a,b,c: Intragroup comparisons are shown in lowercase. There is a statistically significant difference between the times indicated by different letters. [62]

The intra and intergroup comparisons with regard to the periodontal clinical parameters are displayed in Table 2. At the baseline, periodontitis groups had significantly higher PI values than the healthy group (p<0.001). Group 1 presented significantly higher mean PI values compared to Group 2 at the 1-month (p<0.05) and the 3-month visits (p<0.05), respectively (Table 2).

In terms of GI values, at baseline, a significantly higher score was seen in systemically healthy individuals with periodontitis compared to osteoporotic women (group 1) and healthy women (group 3) (p<0.001). Mean GI values at three months of group 1 were higher than those of group 2 (p<0.05).

At the 3-month follow-up, the intragroup comparison showed that group 1 had significantly lower mean PI values than baseline while group 2 had significantly lower mean PI and GI values than baseline (p<0.05).

The mean PD, BOP, and CAL scores were significantly higher for periodontitis groups than group 3 at baseline (p<0.001). The mean CAL was significantly higher for group 1 than group 2 (p<0.001). In contrast, no significant differences were seen between the groups at the first and third-month follow-up visits for the mean PD and BOP values.

For the intragroup comparison (Table 2), significant reductions were noticed for all groups three months after periodontal treatment compared to the mean PD, BOP, and CAL baseline (p<0.05).

Biochemical Findings

The biochemical findings at all time-points are displayed in Table 3.

Mean salivary IL-1β levels were significantly higher in periodontitis groups than in group 3 at baseline. At one month following periodontal therapy, IL-1β levels in both groups 1 and 2 significantly decreased. At three months, IL-1β levels in group 1 significantly reduced in comparison to baseline (p<0.001), whereas IL-1β levels in group 2 did not change significantly from baseline to three months. For group 1, statistically higher IL-1β levels were observed in comparison with group 2 (p<0.05) at baseline and 1-month follow-up (p<0.05).

Baseline salivary IL-17 levels were significantly higher in both periodontitis subjects (p<0.001). IL-17 levels in group 1 at 1 and 3 months were significantly lower than baseline. In group 2, a significant decrease in IL-17 levels was observed after one month; however, this decrease was not statistically significant at 3 months. In the intergroup comparison, the mean IL-17 levels at baseline (p<0.05) and the 1-month re-examination visit (p<0.05) were statistically higher in group 1 than in group 2.

At baseline, significantly higher 8-OHdG levels were seen in both periodontitis groups than in healthy individuals (p<0.001), and for group 1, 8-OHdG levels were significantly higher than group 2 (p<0.05). When compared to baseline both periodontitis groups exhibited a significant decrease in 8-OHdG levels at the 1- and 3-month follow-up (p<0.05).

1- and 3-month evaluations revealed significant reductions in IL-1β, IL-17, and 8- OHdG compared to baseline in group 1 and group 2 (p<0.05).

Table 3. Biomarkers of Group 1 (osteoporosis and periodontitis), Group 2 (systemically healthy with periodontitis) and Group 3 (periodontally and systemically healthy) at baseline (T0), 1 month after the non-surgical periodontal therapy (T1) and 3 months (T3) after the non-surgical periodontal therapy, incl. statistics.

	Group 1	Group 2	Group 3	p‡	p§
	(n=25)	(n=25)	(n=25)		
IL1β 0	3741.80±1118.668Aa	2872.60±1499.146Ba	742.80±276.888C	<0.001#	0.024‖
IL1β 1	2806.40±740.625Ab	1890.80±1059.224Bb			0.001‖
IL1β 3	2952.44±1076.875Abc	2359.40±1288.334Aa			0.084
IL17 0	211.056±97.787Aa	151.776±98.581Ba	67.632±54.981C	<0.001#	0.038‖
IL17 1	145.624±88.640Ab	76.512±79.009Bb			0.049‖
IL17 3	149.176±108.562Aab	141.648±122.205Aa			0.819
OHdG 0	74.523±22.996Aa	52.348±35.027Ba	74.523±22.996C	<0.001#	0.011‖
OHdG 1	46.60±25.696Ab	39.468±27.072Ab			0.344
OHdG 3	57.868±26.914Ab	46.148±29.105Aab			0.146

A,B,C: Intergroup comparisons are shown in upper case. a,b,c: Intragroup comparisons are shown in lowercase. There is a statistically significant difference between the times indicated by different letters.[62]

Table 4. The correlations of salivary IL-1β, IL-17 and 8-OHdG levels with clinical parameters at baseline (T0), 1 month after the non-surgical periodontal therapy (T1) and 3 months (T3) after the non-surgical periodontal therapy in group 1 are shown in table 4

	Group 1	IL-1β T0	IL-1β T1	IL-1β T3	IL-17 T0	IL-17 T1	IL-17 T3	8-OHdG T0	8-OHdG T1	8-OHdG T3
PI T0	Pearson correlation coefficient	.288	.053	-.091	.160	-.114	-.204	-.044	-.070	-.369
	p-value	.162	.802	.667	.446	.588	.328	.835	.741	.070
PI T1	Pearson correlation coefficient	.452	.283	.010	.368	.047	.023	.098	-.054	-.235
	p-value	.323	.171	.961	.070	.823	.912	.640	.799	.258
PI T3	Pearson correlation coefficient	.278	.172	.036	.171	-.076	-.042	.012	.062	-.230
	p-value	.179	.411	.864	.415	.716	.844	.956	.769	.269
GI T0	Pearson correlation coefficient	.659**	.303	-.082	.577**	.450	-.089	.168	.049	-.301
	p-value	.000	.141	.697	.003	.224	.671	.422	.815	.144
GI T1	Pearson correlation coefficient	.249	.226	-.030	.396	.028	-.067	-.048	.028	-.189
	p-value	.230	.276	.886	.050	.893	.749	.819	.893	.364
GI T3	Pearson correlation coefficient	.446*	.311	-.154	.453	.208	-.206	.006	-.090	-.326
	p-value	.025	.130	.463	.323	.318	.323	.978	.668	.111
PD T0	Pearson correlation coefficient	.128	.101	.155	-.139	-.034	-.007	.097	-.004	-.132
	p-value	.543	.630	.460	.509	.873	.974	.646	.985	.528
PD T1	Pearson correlation coefficient	.057	.027	-.271	.436	.279	-.175	-.064	.055	-.110
	p-value	.788	.898	.190	.129	.177	.403	.761	.792	.599
PD T3	Pearson correlation coefficient	-.030	-.073	-.176	.338	.072	-.083	-.049	.182	.023
	p-value	.888	.727	.400	.099	.733	.693	.818	.384	.912

BOP T0	Pearson correlation coefficient	.150	.263	-.150	.331	-.016	.073	.122	-.022	.092
	p-value	.473	.203	.474	.106	.938	.729	.561	.917	.661
BOP T1	Pearson correlation coefficient	.056	-.026	-.092	.103	-.159	.004	-.141	.074	-.045
	p-value	.789	.903	.662	.625	.447	.985	.501	.726	.829
BOP T3	Pearson Correlation	.084	.144	-.251	.237	-.187	-.146	-.057	.082	-.094
	p-value	.688	.494	.226	.254	.372	.486	.785	.695	.654
CAL T0	Pearson Correlation	.204	.151	-.208	.067	-.064	-.019	-.042	.047	-.233
	Sig. (2-tailed)	.351	.492	.342	.760	.773	.930	.848	.831	.285
CAL T1	Pearson correlation coefficient	.163	.008	-.289	.167	-.165	-.093	-.064	-.024	-.210
	p-value	.459	.971	.181	.446	.452	.672	.771	.915	.336
CAL T3	Pearson correlation coefficient	.077	.040	-.229	.083	-.145	-.077	-.045	.009	-.208
	p-value	.728	.857	.294	.705	.510	.726	.837	.968	.341

PD. probing depth (mm); CAL. clinical attachment level (mm); BOP. bleeding on probing (%); PI. plaque index (%); GI. Gingival index (%) Spearman's Rank Correlation Test; *p<0.05; **p<0.01[62]

Table 5. The correlations of salivary IL-1β. IL-17 and 8-OHdG levels with clinical parameters at baseline (T0). 1 month after the non-surgical periodontal therapy (T1) and 3 months (T3) after the non-surgical periodontal therapy in group 2 are shown in table 5

	Group 2	IL-1β T0	IL-1β T1	IL-1β T3	IL-17 T0	IL-17 T1	IL-17 T3	8-OHdG T0	8-OHdG T1	8-OHdG T3
PI T0	Pearson correlation coefficient	.252	.215	.040	.198	.160	.243	.061	.001	.021
	p-value	.225	.303	.848	.343	.445	.241	.773	.997	.919
PI T1	Pearson correlation coefficient	-.229	-.184	-.339	.133	.096	-.260	-.080	-.086	-.250
	p-value	.271	.378	.097	.525	.649	.210	.702	.683	.227
PI T3	Pearson correlation coefficient	-.231	-.158	-.196	.247	.187	-.136	-.036	-.093	-.172
	p-value	.266	.451	.348	.234	.371	.518	.866	.658	.411
GI T0	Pearson correlation coefficient	.148	.093	.290	.249	.168	.191	.120	.082	.124
	p-value	.479	.657	.159	.229	.421	.359	.567	.698	.554
GI T1	Pearson correlation coefficient	-.479*	-.435*	-.384	-.276	-.325	-.485	-.288	-.230	-.228
	p-value	.015	.030	.058	.182	.113	.314	.162	.268	.274
GI T3	Pearson correlation coefficient	-.369	-.363	-.265	-.074	-.084	-.352	-.085	-.063	-.004
	p-value	.070	.074	.201	.725	.689	.084	.686	.765	.986
PD T0	Pearson correlation coefficient	-.070	-.196	.134	-.215	-.202	-.208	-.165	-.092	-.026
	p-value	.741	.347	.523	.303	.333	.318	.431	.661	.902

PD T1	Pearson correlation coefficient	-.218	-.185	.026	-.117	-.144	-.192	-.171	-.098	.021
	p-value	.294	.376	.901	.578	.491	.357	.414	.643	.920
PD T3	Pearson correlation coefficient	-.305	-.246	-.031	-.146	-.168	-.195	-.215	-.137	-.039
	p-value	.139	.236	.884	.487	.422	.351	.302	.515	.853
BOP T0	Pearson correlation coefficient	.229	.378	.534	-.103	-.197	.411	.040	.106	.177
	p-value	.272	.063	.116	.625	.345	.341	.851	.615	.396
BOP T1	Pearson correlation coefficient	.029	.288	.328	.208	.159	.258	.243	.301	.251
	p-value	.892	.163	.109	.319	.448	.213	.242	.143	.225
BOP T3	Pearson correlation coefficient	-.076	.119	.059	.009	.005	-.066	.222	.144	.124
	p-value	.716	.570	.778	.967	.980	.754	.287	.492	.556
CAL T0	Pearson correlation coefficient	.066	.080	.080	.064	.092	.256	.343	.438	.515*
	p-value	.808	.767	.767	.815	.734	.339	.194	.089	.041
CAL T1	Pearson correlation coefficient	.165	.234	-.030	.368	.454	.198	.530*	.504*	.418
	p-value	.542	.384	.911	.160	.078	.462	.035	.047	.107
CAL T3	Pearson correlation coefficient	-.084	-.127	-.239	-.043	.056	-.128	.114	.143	.118
	p-value	.756	.639	.373	.873	.837	.636	.674	.597	.663

PD. probing depth (mm); CAL. clinical attachment level (mm); BOP. bleeding on probing (%); PI. plaque index (%); GI. Gingival index (%) Spearman's Rank Correlation Test; *p<0.05; **p<0.01[62]

Correlations

In group 1, there was a weak positive correlation between baseline IL-1β and IL-17 levels and GI (p<0.01) (Table 4). However, no correlations were found in the other parameters at baseline, while at 1-month, IL-1β was negatively correlated with the GI in group 2 (p < 0.05) (Table 5). For group 2, we found a weak positive correlation between 8-OHdG and CAL (p < 0.05).

Discussion

The mechanism between osteoporosis and periodontal diseases needs to be clearly understood. Various studies analyzing the relationship between osteoporosis and periodontal parameters including CAL and PD concluded that bone mineral density T-score is correlated with PD and CAL scores in osteoporotic postmenopausal subjects.[43,44] The findings of our study showed that, at baseline individuals diagnosed with osteoporosis exhibited higher PI, PD, BOP, and CAL scores in comparison to those in the group identified as systemically healthy. Despite the usage of BPs in individuals with osteoporosis. higher PD, BOP, and CAL scores have been found in group 1 before and after treatment. Therefore, our results could be interpreted as osteoporosis may worsen periodontal status. and using BPs systemically did not yield a significant improvement in clinical outcomes.

Rocha *et al*. examined the effects of alendronate on periodontal parameters six months after treatment in diabetic patients and post-menopausal women.[45,46] Their findings showed a significant decrease in gingival bleeding in patients who received alendronate. The study demonstrated clinical attachment gain in patients taking alendronate as well. Lane *et al*. stated that after six months, alendronate and risedronate did not significantly alter clinical outcomes when compared to conventional therapy along with placebo in systemically healthy individuals.[47] The authors reported that improved CAL, PD, and BOP in individuals who undergo bisphosphonate therapy were significant 12 months after treatment. In another study by Graziani *et al.* a powerful nitrogen-containing amino bisphosphonate neridronate exhibits no significant differences in CAL and PD.[39] According to the authors, the drug's molecular action may be the reason for the lack of effects three months after therapy began. The dosage used (12.5 mg/week) in their study was

insufficiated to be expected to have a significant impact on the osseous metabolism of the alveolar bone. Several studies[13,14,48] that used 1% alendronate gel locally revealed that PD and BOP decreased. and a more significant gain in clinical attachment has been found. except for one study.[49] At baseline, the authors found no difference in PD and CAL in smokers and nonsmokers. At the 3-month follow-up, the maximum decrease in PD and CAL gain was observed in the alendronate nonsmokers group. This could be explained by the immunological responses of smokers and nonsmokers being different. Our study found statistically significant improvements in all clinical measurements in both periodontitis patients at 1- and 3-month follow-ups. In parallel with Graziani *et al.*[39] and Lane *et al.*[47] there was no significant benefit of BPs in our study in addition to conventional periodontal treatment. Despite the absence of statistically significant differences between the groups, it is noteworthy that individuals in group 1 demonstrated a more pronounced reduction in both BOP and PD. The reason why no significant changes were observed could be related to the duration and the dosage of administered BPs. This led us to conclude that oral BPs may not have a significant effect on clinical parameters in the short term.

Several studies have shown that there was a change in cytokine levels in the inflammatory process of osteoporosis and periodontal diseases, and medical treatment of osteoporosis could prevent periodontal destruction by controlling the cytokines. hence alveolar bone resorption.[50,51] Given that saliva is easily accessible and non-invasive, and it contains a large number of indicators, it has been thoroughly investigated as a possible diagnostic tool.[52,53]

The way local tissues react to periodontal infection may also be affected by systemic variables affecting bone remodeling. It is known that people who have systemic bone loss produce IL-1 and IL-6, which may influence the oral tissues. It has been demonstrated that periodontal infection enhances local cytokine production. promoting local osteoclast activity, increasing bone resorption.[54] Several BPs. including disodium clodronate, etidronate, and tiludronate, have been shown to exhibit anti-inflammatory properties through inhibition of the synthesis of IL-1, IL-6, and tumor necrosis factor.[55] Research conducted by Bagan *et al.* revealed that there were no statistically significant variances in IL-1β values when compared to the patients who had received IV BPs and control patients not treated with BPs.[56] In the current study. the intergroup analysis between the periodontitis groups showed that IL-1β levels were significantly higher in osteoporotic patients with periodontitis than in the systemically healthy periodontitis group and healthy control group. In contrast to Bagan *et al.'s* findings, our investigation showed that patients receiving BPs had greater baseline levels of IL-1β.

A comparable reduction was noted in group 1 compared to group 2 following the completion of non-surgical periodontal therapy. At baseline, IL-1β was positively correlated with the GI in group 1. For group 2, IL-1β levels correlated negatively with the GI at 1-month follow-up. Similar to the studies[20,57] our findings support the role of IL-1β in both periodontitis and osteoporosis mechanisms as a valuable indicator. However, bisphosphonate treatment along with periodontal therapy did not show a considerable impact in reducing IL-1β.

Yang *et al.* concluded a notable reduction in IL-17 levels in saliva following non-surgical treatment at both 1 and 3 months.[23] They also indicated that all clinical parameters and baseline salivary IL-17 levels had a positive correlation. The findings of our study were consistent with those of Yang *et al.* The significant decrease in IL-17 levels after treatment is a result of periodontal treatment, and BPs did not appear to have any noticeable impact on salivary IL-17 levels during the follow-up periods. Molnar *et al.* reported that increased levels of IL-17A are linked to postmenopausal osteoporosis and have a role in bone resorption.[58] Gumus *et al.* stated that although there were no significant differences in GCF IL-17 levels between osteoporotic patients with periodontitis and systemically healthy individuals, serum IL-17A concentrations were significantly elevated in the osteoporosis group.[26] In line with these studies. osteoporotic patients had significantly higher saliva concentrations of IL-17 in the current study. This may be because of the association of osteoporosis with increased salivary biomarker levels in periodontitis. At the 1-month follow-up, BPs did not show a noticeable benefit on IL-17 levels in saliva.

There is limited information available regarding the 8-OHdG levels in the saliva of individuals with osteoporosis. However, several studies have been conducted to analyze the level of 8-OHdG in individuals diagnosed with periodontitis.[8,59,60] Takane *et al*. revealed elevated levels of 8-OHdG in saliva among individuals with chronic periodontitis compared to periodontally healthy individuals. and observed a significant reduction in these levels following periodontal therapy.[8,9] Miricescu *et al*. showed that individuals diagnosed with chronic periodontitis exhibited markedly higher levels of 8-OHdG in saliva in comparison to healthy counterparts but could not detect the relationship with bone resorption biomarkers.[59] Yang *et al*. indicated a significant reduction in salivary 8-OHdG values following the initial phase of non-surgical therapy in periodontitis patients.[23] The study also found PD and CAL had significantly positive effects on salivary 8-OHdG levels. Sezer *et al*. reported that 8-OHdG levels in saliva were found to be significantly correlated with the sites with PD and CAL (≥ 3mm). In comparison to gingivitis and healthy groups. subjects with chronic periodontitis had greater levels of 8-OHdG.[28] In the present study, 8-OHdG values were significantly higher in the group with both periodontal disease and osteoporosis. This finding suggests that oxidative DNA damage may be associated with clinical attachment level in patients with osteoporosis. 8-OHdG and CAL were found to be positively correlated. which is consistent with the previously cited findings.

According to some studies biphosphonate treatment can produce oxidative stress in oral epithelium.[15] Patients who had received IV BP treatment showed higher levels of oxidative stress compared to the healthy controls.[61] The results of our study are in accordance with these findings just mentioned. To our knowledge. This study is the first to examine the effects of non-surgical periodontal therapy on 8-OHdG in patients taking BPs. Our findings highlight that BPs and osteoporosis may create additional oxidative stress in periodontal tissues. The decrease in 8-OHdG levels after periodontal treatment indicates that periodontal therapy positively affects the biochemical markers associated with periodontitis.

The decrease in 8-OHdG levels after periodontal treatment may be indicative of the positive effect of conventional periodontal treatment on clinical outcomes.

The current study has several limitations. Notably. the average age of the subjects with periodontitis was higher compared to the healthy group. and there was no control group of periodontally healthy individuals with osteoporosis. Additionally, the concentration of biochemical parameters is influenced by volume. and the absence of data on the total amount in our study can be considered a limitation.

Conclusions

Considering the limitations of the present study. our findings indicated that osteoporosis patients diagnosed with periodontitis and taking BPs exhibited elevated levels of the oxidative stress biomarker 8-OHdG compared to other patients. Patients on BPs therapy were affected similarly by the initial phase of periodontal therapy regarding periodontal parameters and salivary biomarkers. Although BPs tended to have better results in terms of PI, PD, and BOP, there was no significant effect. In the short term. there were no significant effects of oral BPs (70 mg per week) on clinical parameters or salivary biomarkers. Future research may be warranted to evaluate the additional use of BPs tends to favor better clinical outcomes.

Conflicts of Interest Statement

None

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