Investigation of the Effects of Bisphosphonate on the Gingival Crevicular Fluid Levels of Sclerostin and the DKK-1 in Individuals with Postmenopausal Osteoporosis with Periodontal Changes

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Study Protocol

Individuals with chronic periodontitis and osteoporosis between 51 and 66 years (mean age: 57.15 ± 4.93) (group A, n=12), 52 to 66 years (mean age: 57.41 ± 4.94) with systemic healthy chronic periodontitis (group B, n=10), between the ages of 50-63 (mean age: 55.64 ± 3.79) periodontally healthy osteoporotic individuals (group C, n=11) and 52-64 years (mean age: 56.55 ± 4.06) periodontal and systemically healthy individuals (group D, n=10) were included in study. 43 postmenopausal women in total were included in the study. This study involved postmenopausal women admitted to the Ondokuz Mayıs University Faculty of Medicine, Department of Endocrinology and Metabolism between 2016 and 2018. The study protocol was approved by Ethical Board of Ondokuz Mayis University School of Medicine (OMU KAEK 2016/100) 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.

Bisphosphonates were started in the patients who were diagnosed with osteoporosis. Individuals smokes, who had received antibiotic treatment in the last 3 months and/or periodontal treatment in the last 6 months were excluded from the study. Following medical

record retrieving, patients were leaded to Ondokuz Mayıs University Faculty of Dentistry Periodontology Department for periodontal examination, receipt of clinical records and gingival crevicular fluid (GCF) samples. Following the receipt of medical records; standard periodontal recordings including plaque index (PI) (1), gingival index (GI) (2), bleeding on probing index (BOP), pocket depth measurement (PD), clinical attachment loss measurement (CAL), dental radiographs were received from each patient. Clinical measurements were taken from the region under each tooth (mesio-vestibule, mid-vestibule, disto-vestibule, mesio-lingual, mid-lingual, disto- lingual) using Williams probe. In the first clinical and radiographic evaluation, groups with postmenopausal osteoporosis and healthy groups were divided into 2 subgroups as chronic periodontitis and healthy control groups. The diagnosis of chronic periodontitis was made clinically and radiographically according to the criteria determined by the "1999 Periodontology Workshop" (3). Periodontitis patients consisted of patients with 4 or more probes with bleeding index (BOP) positive, 5 mm or more deeper pocket depth (PD) and ≥6 mm attachment loss (CAL) and radiographically determined alveolar bone loss. Individuals with <3 mm PD and no radiographically determined alveolar bone loss were preferred to form groups with healthy periodontium. In the groups with chronic periodontitis gingival

crevicular fluid (GCF) was collected with the help of periopaper (Periopaper®; Oraflow, New York, NY, USA) from the regions with the deepest pocket depth with periodontal defect, and in healthy groups it was collected by taking the right mandibular molar region as reference. Each time, the volume value of the collected GCF was determined by the Periotron 8000 device. GCF collected paper strips were placed in eppendorf tubes and stored at -80 ° C until biochemical analysis was performed (NUAIRE Ultra-Low Freezer Model no: Nu-6420E). Following the initial sample collection and recording of patient data (medical, periodontal and radiographic), patients were placed under phase 1 periodontal treatment (scaling and root planing), and oral hygiene habits of the patients were corrected and followed up for 1 year. In this period, periodontal treatments, controls and sample collections of the patients in Group A and Group B who completed phase 1 treatment were repeated at the end of 6th month and at the end of the 12th month after the end of the treatment, their clinical changes were recorded. The clinical data and GCF SOST and DKK-1 levels of osteoporosis groups (Group A and C) and non-osteoporotic chronic periodontitis group (Group B) with bisphosphonate were reevaluated in the determined times.

Biochemical Analysis

SOST and DKK-1 levels in gingival crevicular fluid were determined by ELISA (Enzyme-linked immunosorbent assay) kits (SunRed Human DKK1 ELISA Kit, Cat.No:201-12-063, and SunRedHuman SOST ELISA Kit, Cat No: 201-12 -5418) The SOST and DKK1 assay ratios according to the manufacturer's instructions are 0.2-60 ng / mL 0.5-78 ng / mL, respectively. Sensitivity is 0.412 ng / mL for DKK-1 and 0.17 ng / mL for SOST. Color intensity is measured at 450 nm and the results were calculated using standard curves for each kit. GCF SOST and DKK-1 concentrations were obtained by dividing total amount of SOST and DKK1 values by GCF volume (microliter). Concentration values will be presented in ng / mL.

Statistical Analysis

Statistical analysis was performed using SPSS (version 21, SPSS Inc. Chicago, IL, USA) program. The Shapiro-Wilk test was used to show if the data were consistent with the normal distribution. Comparisons of biochemical data between groups were performed with non-parametric Kruskal-Wallis test and $p < 0.05$ was considered as statistically significant. In determining the

difference group, p value was divided by the number of comparison and p <0.016 was accepted as significance level (Mann- Whitney U test with Bonferroni correction). Friedman test was used to determine the time difference in groups and p<0.05 was considered as statistically significant.

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2. Loe H, Silness J. Periodontal disease in pregnancy. I. prevalence and severity. Acta Odontol Scand 21:533-51, 1963.
3. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 4,1:1-6, 1999.