Evaluation of the GCF periostin, IL-17A and IL-17E levels of smoking and non-smoking periodontitis patients received periodontal treatment: short-term follow-up study

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Study Protocol and Statistical Analysis Plan

Before the study, One Way ANOVA test with a significance level of 5% was used for the sample size calculation. Based on this analysis, the sample size was determined as at least 11 people in each group and a total of 44 subjects to detect a difference between groups. This cross-sectional study was approved by Ondokuz Mayıs University Clinical Research Ethics Committee (OMUKAEK) Samsun, Turkey (protocol number; 2019/324).

Fourty four individuals, 22 males and 22 females who were older than 18 were enrolled in the study. The study groups were selected according to the following criteria from individuals who applied to the Ondokuz Mayıs University Faculty of Dentistry Periodontology Department Clinic for treatment or control, accepted to participate in the study, were systemically healthy, did not take any medication in the previous 6 months, and did not receive periodontal treatment in the previous 6 months:

Group 1: (SP) Stage 3 Grade C patients (n:11)

Group 2: (P) Stage 3 Grade A patients (n:11)

Group 3: (SH) Periodontally healthy smokers (n:11)

Group 4: (H) Non-smokers with periodontally healthy (n:11)

Individuals were included as smokers if they had smoked ≥10 cigarettes/day for ≥5 years, whereas individuals were included as nonsmokers if they had no previous history of smoking. The periodontitis stage III patients were evaluated clinically and radiographically, and were selected to have teeth with CAL ≥5 mm in at least 30% of them. Exclusion criterias were as follows; patients under the age of 18, pregnant or lactating women, patients who have a history of with systemic disease or cancer or chemotherapy and radiotherapy or ongoing drug therapy who might be affect the clinical characteristics of periodontitis, patients who smoke less than 10 cigarettes/day or who quit smoking.

In order to standardize the study, all radiological and clinical examinations were performed by a single investigator. In clinical examination, Plaque Index (PI), Gingival Index (GI), bleeding on probing (BOP), probing depth (PD) and clinical attachment loss (CAL) scores were measured and recorded from 6 regions of each tooth (buccomesial, midbuccal,

buccodistal, linguo/palatomesial, midingual/palatal, linguo/palatodistal regions) with using using millimetric (mm) calibrated University of North Carolina (UNC) (Hu-Friedy, Chicago, Illinois, USA). And up-to-date panoramic radiographs were taken from all patients on the day of the examination to determine periodontal status. After the clinical and radiographic evaluations, the participants were asked to come for GCF sample and treatment 1 day after the clinical evaluations, since bleeding may occur during probing.

From the patients in the SP and P groups, GCF samples were obtained from the 5 deepest pockets before the treatment, on the 15th and 30th days after the treatment. GCF samples were obtained taken from 5 randomly selected teeth with GI=0 PI=0 and SPD ≤3 from healthy individuals in the SH and H groups.

GCF samples were acquired by intrasulcular technique from selected pockets. Before sampling, the sites were isolated with cotton rolls, saliva and supragingival plaque were removed by cotton pellets, if present. Standardized filter paper strips (Periopaper® Oraflow Inc. New York, USA) were placed in the pocket for 30 seconds. Strips contaminated with blood or saliva were discarded. The absorbed GCF volume was measured with a precalibrated Periotron 8000 instrument (PeriotronR 8000, Pro Flow Inc., Amityville, NY, USA). Then, the strips were placed in sterile 0.5 ml Eppendorf (Eppendorf AG, Hamburg, Germany) tubes. Eppendorf tubes were stored at -80°C in Ondokuz Mayıs University Faculty of Medicine Department of Biochemistry until ELISA analysis. GCF samples were converted to an actual volume (μL) by reference to a standard curve.

Treatment and Follow-up Protocol

Patients in the SH and H groups were advised to come for routine controls by not applying any periodontal treatment. However for patients in the SP and P groups, non-surgical periodontal treatment was applied as full mouth debridement protocol in a single session, after the GCF samples were collected from selected pockets.

The periodontitis patients were called again on the 15th and 30th days after the treatment. First of all, the GCF sampling process was repeated from the teeth whose GCF sample was collected before. Then, clinical examination parameters (PI, GI, BOP, PD and CAL) values were measured again and recorded.

Determination of GCF Periostin, IL-17A, IL-17E Amount

GCF Periostin, IL-17A, IL-17E levels were analyzed by the Sandwich ELISA method with the commercially available Human Periostin ELISA kit (Bioassay Technology Laboratory, Cat No. E3226Hu, Zhejiang, China), Human IL-17A ELISA kit (Bioassay Technology Laboratory, Cat No. E0047Hu, Zhejiang, China), Human Interleukin-25 ELISA kit (Bioassay

Technology Laboratory, Cat No. E0054Hu, Zhejiang, China) in Ondokuz Mayıs University Faculty of Medicine, Department of Medical Biochemistry Research Laboratory. The study data were converted to pg/mL_{30sec} and statistical analyzes were made.

Statistical analysis

SPSS for Windows Ver. for statistical analysis and graphics. 22.0 (SPSS Inc., Chicago, ILL, USA) and MS-Excel 2016 programs were used. Statistical significance level was accepted as $p<0.05$.

The normal distribution of the data in our study was evaluated with the Shapiro-Wilks test. The mean±standard deviation was used for the descriptive statistics for the normally distributed variables, and the median (min-max) value for the non-normally distributed variables. While examining the distribution of the gender variable in the groups, the result of the Chi-Square test was evaluated.

OneWay ANOVA test was applied to the variables showing normal distribution in the comparison of the data between groups. In post-hoc analyses, homogeneously distributed data according to Levene variance homogeneity test were evaluated with Tukey test, and data that were not homogeneously distributed were evaluated with Tamhane T2 test. Variables that did not show normal distribution were evaluated with the Kruskal-Wallis test. Bonferroni corrected Mann-Whitney U test was used in order to determine the group that caused the difference in the variables with a significant difference as a result of the Kruskal-Wallis test.

Paired-Sample T Test and Repeated Measured Analysis of Variance were used for the variables showing normal distribution in the evaluation of the within-group changes according to time. Significant differences as a result of analysis of variance were examined with Bonferroni, one of the multiple comparison tests. Variables that did not show normal distribution were analyzed with the Friedman test. The Wilcoxon test was used to determine the group that caused the difference in the variables with a significant difference as a result of the Friedman test.

RESULTS

Demographic Findings

The distributions of gender and age of the groups were as follows: SP (seven male and four female subjects; mean age: 40,9 ± 9,28 years); P (three male and eight female subjects; mean age: 41 ± 12.30 years); SH (seven male and four female subjects; mean age: 30.27 ± 9.99 years); H (three male and eight female subjects; mean age: 26.36 ± 4.20 years).

The study groups were evaluated in terms of age and gender, and a significant difference was found between the groups in terms of mean age (χ^2=16.567; p=0.001). There was no

significant difference in terms of gender (χ^2=0.364; p=0.546). The mean age of the H group was lower than the mean age of the patients in the SP (Z=18,000; p=0.006) and P groups (Z=16.682; p=0.014). It was determined that the mean age of the individuals was similar (Z=3,682; p=1,000) between group H and group SH.

Clinical Parameters

Full Mouth Clinical Findings

When the pre-treatment (day 0) data were evaluated, a significant difference was found between the groups in all clinical parameters (PI, GI, BOP, PD and CAL). The measurements of SP and P groups were higher than the healthy groups ($p<0.05$).

When the results of the in-group treatment were evaluated, the data before the treatment (day 0), the 15th day and the 30th day after the treatment were compared and it was observed that there was a statistically significant decrease in all clinical parameters (PI, GI, BOP, PD, CAL) in both the SP group and the P group.

There was no statistically significant difference between the periodontitis groups (SP-P) and healthy groups (SH-H) on the 0th, 15th and 30th days in terms of smoking ($p>0.05$).

Sample Teeth Clinical Findings

GCF was taken from the 5 deepest pockets measured before the treatment from the patients in the SP and P groups of the study groups before the treatment, on the 15th and 30th days after the treatment. GCF was taken from 5 randomly selected teeth with GI=0 PI=0 and PD $\leq$3 from individuals in SH and H groups.

All clinical parameters (PI, GI, BOP, PD, CAL and GCF volume) of these selected teeth were analyzed statistically. There was a significant difference between the groups in all clinical parameters before treatment. The measurements of SP and P groups were higher than the healthy groups ($p<0.05$).

There was a significant difference between the groups in terms of BOP, PD, CAL and GCF volumes on the 15th and 30th days after treatment.

There was no statistically significant difference between the periodontitis groups (SP-P) and healthy groups (SH-H) on the 0th, 15th and 30th days in terms of smoking ($p>0.05$).

Biochemical Parameters

GCF Periostin levels

When the GCF Periostin data were examined, the difference between the groups was found to be statistically significant at the beginning ($p=0.003$). In terms of periodontal disease, the amount of GCF Periostin in the P group was found to be significantly lower than in the H group ($p=0.007$), while there was no statistically significant difference between the SP group and the SH group.

When evaluated in terms of smoking, it was observed that the GCF Periostin amount of the SP group was significantly higher than the P group ($p=0.021$). There was no significant difference between the SH and H groups ($p>0.05$).

A statistically significant difference was found between the groups on the 15th day ($p<0.001$). When examined in terms of periodontal disease in the post-hoc analysis, it was found that the amount of GCF Periostin in the P group was significantly lower than in the H group, as at the beginning ($p=0.002$). It was determined that there was no statistically significant difference between the SP group and the SH group. When evaluated in terms of smoking, it was observed that the GCF Periostin amount of the SP group was significantly higher than the P group ($p=0.003$).

However, on the 30th day, it was determined that there was no statistically significant difference between the groups.

In the in-group evaluation of the change with treatment on a time basis, it was found that the GCF Periostin level in the SP group decreased over time ($p<0.001$). In post-hoc analyzes, this decrease was observed to be significant between all times ($p=0.009$(0-15.days, respectively), $p=0.001$). (day 0-30) and $p=0.003$(day 15-30)). In the P group, there was no statistically significant difference in the evaluations of the 0th day, 15th day and 30th day within the group ($p>0.05$).

GCF IL-17A levels

In the analysis of GCF IL-17A values, no statistically significant difference was found between the groups in terms of both periodontal disease and smoking at any time ($p>0.05$).

In the evaluation of the change with non-surgical periodontal treatment according to time, no statistically significant difference was found in the GCF IL-17A levels of the SP group between the baseline, the 15th day and the 30th day ($p>0.05$). only in the P group, it was found that the GCF IL-17A level increased on the 30th day, with a statistically significant difference ($p<0.05$). Significant differences were found between day 0 and day 30, and day 15 and day 30 in post-hoc analyzes ($p=0.009$, $p=0.005$, respectively).

GCF IL-17E levels

In the analysis of GCF IL-17E levels, there was no statistically significant difference between the groups in terms of both periodontal disease and smoking at any time ($p>0.05$).

In the evaluation of the change with non-surgical periodontal treatment, there was no statistically significant change in GCF IL-17E levels at any time in both the SP group and the P group ($p>0.05$).