

**Evaluation of Gingival Crevicular Fluid and Saliva SOST and TWEAK, Gingival  
Crevicular Fluid RANKL and OPG Levels in Smokers and Non-smokers with  
Periodontitis**

**14.09.2023**

## **Study Protocol**

### **Study Population**

Participants who applied to Gazi University Faculty of Dentistry Department of Periodontology for periodontal treatment or control, who were systemically healthy and who agreed to participate in the study were included in the study.

Participants who agreed to participate in the study were evaluated clinically and radiologically and diagnosed in accordance with the Classification of Periodontal and Peri-implant Diseases and Conditions 2017 Workshop criteria. Accordingly, the individuals who will take part in the study are; Systemically healthy, non-smoking periodontitis group (Group 1, number = 26), systemically healthy, smoker periodontitis group (Group 2, number = 26) and systemic and periodontal healthy group (Group 3, number = 26).

### **Clinical examination**

In order to evaluate the clinical conditions of the participants in the study; Plaque index, gingival index, percentage of bleeding on probing, pocket depth and clinical attachment loss were recorded for each tooth from four regions (mesiobuccal, buccal, distobuccal, lingual/palatinal) using the Williams probe. Clinical indices were measured and recorded by a single calibrated investigator. Stages and grades in the classification of periodontitis patients; Radiographic bone loss was determined according to the parameters of pocket depth, amount of attachment loss, number of teeth lost due to periodontitis, bone loss%/age and case phenotype.

### **GCF collection**

GCF samples were collected from all individuals participating in the study with paper strips. GCF samples were placed in 2 Eppendorf tubes with paper strips in fours. After the tubes were closed with parafilm, they were stored at -30°C until ELISA examination to determine cytokine levels.

### **Saliva collection**

Total saliva samples were collected from the individuals participating in the study by spitting the saliva accumulated at the bottom of the mouth into a test tube every 60 seconds for a period of 5 minutes. The collected saliva samples were stored at -30°C until ELISA examination to determine cytokine levels.

### **Laboratory analysis**

Gingival crevicular fluid and saliva samples were examined by the ELISA method in accordance with the manufacturer's instructions (Bioassay Technology Laboratory Human Sklerostin SOST BT-LAB ELISA Kit, Bioassay Technology Laboratory Human Osteoprotegerin OPG OPG BT-LAB Kit ELISA kit, Bioassay Technology Laboratory Human Tumour necrosis related weak inducer of apoptosis, TWEAK BT LAB ELISA kit, Human Receptor Activator of Nuclear Factor Kappa B Ligand RANKL BT-LAB ELISA kit) to determine biomarker levels.