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COVER PAGE

Peri-implant Phenotype, Calprotectin and Mmp-8 Levels in Cases Diagnosed With Peri-implant Disease

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STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Aım of Research: In this study, it was aimed to investigate the effect of peri-implant soft tissue phenotype on the degree of peri-implant disease and its contribution to the results of non-surgical mechanical treatment, accompanied by clinical and biochemical parameters. The research hypothesis is that better clinical outcomes may be achieved in individuals with thicker soft tissue phenotype.

Scientific Basis and Validity of Medical Research:

Over the last two decades, dental implants have been used to replace missing teeth; It has become a widely accepted and applied therapeutic method to support fixed and removable prostheses (1). A dental implant is generally defined as a root form made of titanium that is placed in place of a natural tooth (2).

The soft tissues surrounding dental implants are called peri-implant tissues (peri-implant mucosa). Clinically healthy peri-implant mucosa is pink in color and firm in consistency. However, inflammatory gum diseases can develop around implants, just like natural teeth (3). These diseases are defined as peri-implant diseases. However, a consensus report was published by the European Federation of Periodontology (EFP) and the American Academy of Periodontology (AAP) at the World Workshop on Periodontology (WWP) in November 2017, providing a clear definition with clear clinical distinctions for peri-implant pathologies. It has been determined for both clinical applications and epidemiological studies. According to this consensus report, peri-implant diseases are divided into 4 classes. These conditions are defined as peri-implant health, peri-implant mucositis, peri-implantitis and the presence of peri-implant soft and hard tissue loss (4). Clinically, peri-implant health is characterized by the absence of signs of inflammation and bleeding observed on probing. Peri-implant health can exist around implants with normal or reduced bone support. It is not correct to classify health based on probing depth. Peri-implant mucositis is characterized by bleeding on probing and clinical signs of inflammation. Peri-implantitis is characterized by clinical inflammation occurring in the peri-implant mucosa and subsequent progressive loss of supporting bone. There is radiographic bone loss. Peri-implant soft and hard tissue loss; It is defined as the recession of the mucosa occurring in the implant or peri-implant tissue after the loss of the natural tooth, without evaluating the naturally occurring deficiencies, bone loss in the implant body and the grooves becoming clinically visible (5). Today, the methods used to diagnose peri-implant diseases are plastic probes and dental x-rays, but they may not provide accurate results for a full diagnosis. Bleeding on probing has been a measure of periodontal

inflammation for years, and this is an objective diagnostic finding for the diagnosis of peri-implant diseases (6). Despite successful osseointegration and function over the years, dental implants are susceptible to inflammatory peri-implant diseases that affect the supporting tissues and lead to partial or even total loss of osseointegration (7).

Bacterial pathogens (i.e., periodontal pathogens with a similar composition of the oral microbiota that cause chronic periodontitis) represent the primary etiological factor for peri-implant diseases. Additionally, an imbalance of individual local inflammatory response and host-parasite interaction is also implicated for the onset of inflammatory peri-implant diseases. Various risk factors (such as poor oral hygiene, smoking, uncontrolled diabetes, genetic characteristics, alcohol consumption and implant surface) play a role in peri-implant tissue destruction. Insufficiency of keratinized mucosa has also been investigated as a possible risk factor for periimplant diseases (8). Phenotype refers to a dimension that may change over time depending on environmental factors and clinical intervention, and may be site-specific. Periodontal phenotype (3-dimensional image of the gingiva) is determined by gingival phenotype (gingival thickness, keratinized tissue width) and bone morphotype (buccal bone thickness) (9). Peri-implant phenotype can be defined as the dimensional, morphological and topographic features that characterize the clinical condition of the tissues surrounding and supporting osseointegrated implants. The peri-implant phenotype encompasses a soft tissue component, including the osseous component characterized by peri-implant keratinized tissue width, mucosal thickness and supracrestal tissue height, and peri-implant bone thickness. This definition applies not only to buccal/facial regions, but also to lingual/palatal peri-implant regions. Like the periodontal phenotype, the peri-implant phenotype is site-specific and may change over time in response to environmental factors (10). Peri-implant mucosa thickness is the horizontal dimension of peri-implant keratinized or non-keratinized soft tissue. Peri-implant supracrestal tissue height is the vertical dimension of the soft tissue surrounding a dental implant from the mucosal margin to the crestal bone. Unlike keratinized mucosa width and mucosal thickness, this component of the peri-implant soft tissue phenotype courses circularly around an implant, including proximal regions. Peri-implant bone thickness is the horizontal dimension of the bone tissue supporting the osseotegrated implant. The optimal dimensions of all components of the phenotype to maintain peri-implant tissue health is a controversial issue. However, current studies show that keratinized mucosa, mucosal thickness and periimplant bone thickness are less than 2 mm; confirmed that marginal inflammation is greater in areas where the supracrestal tissue height is less than 3 mm (10). Biomarkers in peri-implant crevice fluid also provide promising results in terms of diagnostic

and prognostic values. A biomarker is a parameter that is objectively measured and evaluated as an indicator of normal biological, pathogenic processes or responses to therapeutic intervention. The imbalance between bacterial load and host response at the soft tissue-implant interface triggers an inflammatory process. Cytokines such as tumor necrosis factor alpha, Interleukin-1-beta, and Interleukin-6 are produced by dendritic cells, connective tissue fibroblasts, macrophages, and neutrophils, as well as a number of enzymes, such as matrix metalloproteinases, by neutrophils, fibroblasts, and osteoclasts, which promote the synthesis of connective tissue collagen. and causes deterioration of alveolar bone. Such cytokines and enzymes can be detected in peri-implant groove fluid by various tests and allow objective evaluation in determining prognosis (11). Nowadays, periodontal and transgingival probing measurement techniques are frequently used to determine gingival thickness. In our study, peri-implant mucosa phenotype will be measured with a newly developed probe (Hu-Friedy Colorvue Biotip Probe®) with a colored tip that will be placed in the gingival sulcus, and the relationship between peri-implant disease and peri-implant phenotype will be investigated. Additionally, since the method to be applied is different, whether it is a reliable method will be compared with the literature and the conventional method (transgingival probing). In our study, connective tissue graft, one of the soft tissue augmentation methods, will be applied to the areas where thin biotypes are detected as a result of our analysis methods. Recent studies (12) have emphasized that soft tissue augmentation procedures applied in areas with peri-implant disease can provide significant reductions in bleeding on probing and gingival index values, and in addition, there may be an increase in marginal bone levels. In the light of all this information, the aim of our study is to investigate the effect of peri-implant soft tissue phenotype on the degree of peri-implant disease and its contribution to the results of non-surgical mechanical treatment, accompanied by clinical and biochemical parameters.

Study Protocol, Methods and Procedures to be Applied:

Patient Selection and Study Protocol

The patients to be included in the research will be selected among 50 volunteer individuals aged between 18-65 who applied or were referred to Van Yüzüncü Yıl University Faculty of Dentistry Department of Periodontics with complaints of inflammation in the peri-implant area and were diagnosed with peri-implantitis disease. People who have systemic diseases, smokers, have used antibiotics or received any other medical treatment within 6 months, and are pregnant or breastfeeding will be excluded from the study. Additionally, individuals who

met the definition of peri-implant health and individuals who required surgical treatment of peri-implantitis were excluded from the study.
The peri-implant soft tissue phenotype of all participants who are detected to have peri-implant disease and will be included in the study will be measured, necessary clinical and radiological examinations will be performed, necessary samples will be collected and conventional mechanical treatments will be applied. The data of all participants will be collected again in the 6th month following the treatment.

Patient Data Records, Peri-implant Disease Treatment

The details of the application and the research to be conducted will be explained to the patients who will participate in the study, and an informed consent form will be signed. Patients diagnosed with peri-implant disease will be asked for routine examinations before treatment, intraoral photographs will be taken, and clinical and radiological examinations will be performed in detail. Detailed anamnesis of patients who meet the selection criteria will be taken and clinical and radiological examinations will be performed. Patients with caries on their teeth will be directed to Restorative Dentistry Department and restorations of existing caries will be made at the relevant clinic. Before starting their treatment, patients who meet the selection criteria will be given detailed information about periodontal and peri-implant diseases, microbial dental plaque that causes these diseases, and methods of preventing microbial dental plaque. Individuals will be taught how to brush their teeth, how to use dental floss and/or how to use an interdental brush.

Peri-implant crevice fluid (PIOS) samples will be collected from all individuals before proceeding with mechanical treatment of diseased implant sites. The areas will be isolated from saliva with cotton pellets, dried with light air, and paper strips (PerioPaper®) will be kept in the trough for 30 seconds (13). Paper strips obtained from each implant will be placed in Eppendorf tubes containing isotonic Ph:7.4 phosphate buffer solution and stored at -40 ° C until analyzed in the laboratory department of our clinic. Routine periodontal parameters such as plaque index (PI) (14), gingival index (GI) (14), bleeding on probing and probing pocket depth scores of the patients whose PIOS samples are collected will be recorded with a Williams-type plastic probe. Following these procedures, the patients' peri-implant phenotype will be evaluated. In the evaluation, the traditional transgingival probing technique and a newly developed technique will be used simultaneously and their correlations with each other

will be examined. A digital caliper with a sensitivity of 0.01 mm will be used to measure transgingival probing values to determine peri-implant mucosa thickness. Measurements will be made from two points: apical of the free gingival sulcus and coronal of the mucogingival junction. After the peri-implant mucosa thickness measurement points are determined with a marker pen, Xylocaine® spray (Vemcaine 10%, lidocaine) or, if necessary, local anesthetic (Maxicaine, lidocaine hydrochloride) will be applied to prevent the patient from feeling pain. Measurements will be made from the marked points in a direction perpendicular to the peri-implant mucosa, using a 10-gauge endodontic spreader (G-Star Medical Co., Ltd., Guangdong, China) with a silicone stopper until its contact with the alveolar bone is felt. Since excessive force will cause the spreader to exceed the soft tissue and advance in the alveolar bone, care should be taken to apply only light forces that can be limited to the soft tissue. After all measurements are repeated twice by the same researcher at 10-minute intervals, the amount of peri-implant mucosa thickness in each region will be determined by taking the average of the two measurements.

If the millimetric values obtained as a result of measurements are less than 1 mm, thin phenotype; If it is more than 1 mm, it will be classified as thick phenotype (15). The second gingival phenotype evaluation in individuals with peri-implant disease will be performed with the Hu-Friedy Colorvue Biotip Probe®, a newly developed probe with a colored tip that will be placed in the peri-implant pocket. The white probe will first be placed in the peri-implant pocket with a pressure of less than 25 N. If the color of the probe reflects from the gingival tissue, the phenotype will be recorded as thin. If the white color is not visible, the green probe will be placed in the pocket in the same way and if the color is reflected, the phenotype will be classified as medium thickness. If the Green tip is not visible through the gingival tissue, the Blue probe will be used and if the only color seen is Blue, the phenotype will be classified as thick. If blue is not visible, the peri-implant mucosa tissue will be recorded as too thick. Then, traditional mechanical debridement with titanium curettes will be applied to the areas with peri-implant disease without losing the effect of anesthesia. The area will be irrigated with physiological saline from time to time. Following the procedure, patients will be called for control at the 1st, 3rd and 6th months. Oral hygiene instructions will be repeated when necessary. At the 6th month, all clinical parameters examined will be measured again and PIOS samples will be collected again. 6th month following the operation; PIOS samples will be collected again from all operated patients and the clinical parameters examined will be recorded.

Immunohistochemical Analysis

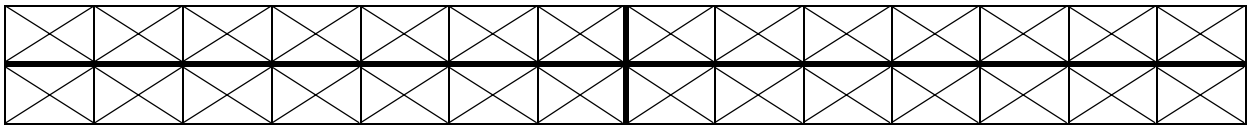
After the target number of patients is reached, the PICF samples collected will be sent to the laboratory for analysis using ELISA (Enzyme-linked immunosorbent assay) kits.

Statistical analysis

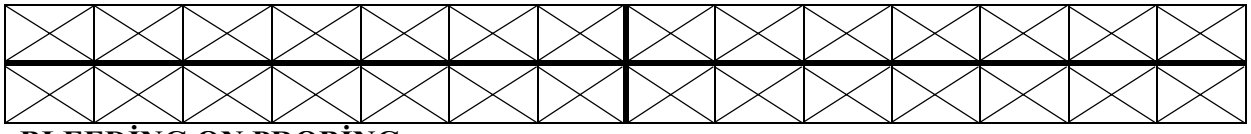
Descriptive statistics for the features emphasized will be expressed as Median, Average, Standard Deviation, Minimum and Maximum value. In terms of these characteristics, two-factor and one of the factors repeated-measures analysis of variance will be used to compare group averages and periods (0th month and 6th month). To determine the relationship between variables, Pearson or Spearman correlation coefficients will be calculated separately in the groups. In the calculations, the statistical significance level will be taken as 5% and the SPSS statistical package program will be used for the calculations.

Fıgure- 1. Clinical Index Form

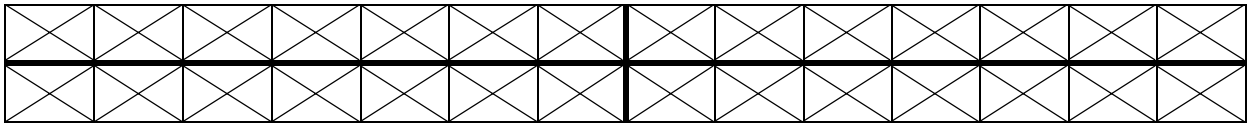
PLAQUE INDEX



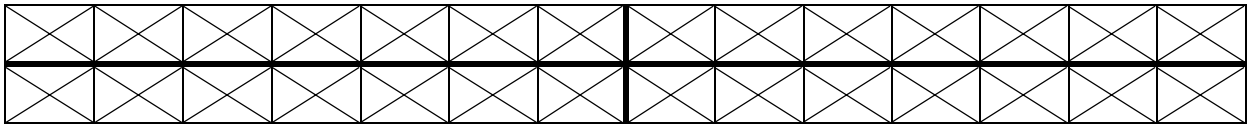
GINGIVAL INDEX



BLEEDİNG ON PROBİNG



POCKET DEPTH



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