Evaluation of periostin levels in gingival crevicular fluid and peri-implant sulcus fluid in patients with periodontal and peri-implanter disease: A cross-sectional study

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Abstract
Aim: Periodontal and peri-implanter diseases are chronic, infectious and inflammatory diseases that manifest as a result of the relationship between their primary etiologic factor, i.e. microbial dental plaque, and the host defense system. Periostin is a protein that has been shown to play a functional role in wound repair, cardiovascular diseases, bone and tooth regeneration and tooth morphogenesis. In this study, the aim was to understand the role of periostin in periodontal (Gingivitis and Periodontitis) and peri-implant (Peri-implant mucositis and Peri-implantitis) disease.

Material and Methods: Forty-two subjects were enrolled in this cross-sectional study. In the clinical measurements, PPD, GI and PI values were measured from the implants and natural teeth. Peri-implant sulcus and gingival crevicular fluids were collected from the patients using paper strips. Periostin levels were measured using ELISA. All parameter was statistically tested by using third party software (SPSS v24.)

Results: In terms of PPD, there was a statistically significant difference between the gingivitis-periodontitis, gingivitis-peri-implantitis, peri-implant mucositis-periodontitis and peri-implant mucositis-peri-implantitis groups. There was a statistically significant difference between the gingivitis-periodontitis, gingivitis-peri-implantitis and peri-implant mucositis-peri-implantitis groups in terms of GCF/PISF volume. Although there was no statistically significant difference between the groups in terms of the amount of GCF/PISF periostin, periostin values were higher in peri-implant mucositis and gingivitis samples as compared to the peri-implantitis and periodontitis groups.

Conclusion: Clinical parameters are a valid diagnostic tool for peri-implanter disease; there is a need for multicenter studies to understand the inflammatory basis of peri-implanter diseases that are biochemically prospective.

Keywords: Periodontitis; periostin; peri-implantitis; peri-implanter mucositis, gingivitis.

INTRODUCTION
Periodontal diseases are chronic, infectious and inflammatory diseases that manifest as a result of the relationship between their primary etiologic factor, i.e. microbial dental plaque, and the host defense system. (1) Tooth loss can be frequently encountered when the supporting tissues around the tooth are affected because of periodontal diseases. While periodontal diseases and loss of teeth due to tooth decay can be eliminated using conventional fixed and removable dentures, dental implant treatment can also be provided to treat the same conditions in order to enhance the comfort and quality of life of the patients (2,3). Although dental implants have been used as a reliable treatment option for many years, re-treatment can be necessary due to the diseases that develop in peri-implant tissues. These diseases consist of peri-implantitis and peri-implant mucositis. Peri-implant mucositis is an initial lesion similar to gingivitis in terms of its clinical manifestations, whereby it displays the cardinal signs of inflammation such as redness, swelling and pain (4). Peri-implant mucositis is reversible through patient care and periodontal treatment (5). Peri-implantitis describes a clinical presentation in which the inflammatory lesion in peri-implant mucosa has spread to the peri-implant bone. Therefore, the diagnosis of peri-implantitis requires is based on the presence of bleeding.
on probing and also on the evaluation of the marginal bone loss using radiography (6).

Periodontal and peri-implant diseases are similar in many ways in terms of the pathogenesis of the disease due to both being inflammatory in nature and sharing site-specific characteristics (7). Although the primary etiologic factor for peri-implant and periodontal diseases is microbial dental plaque, the immune response of the host to the existing microbial dental plaque affects the severity and course of the disease (8,9). Thus, the importance of the local immune response has been underlined in relevant studies (10)(10,11). Local immune responses can be clinically monitored with parameters such as gingival index and bleeding on probing (BOP) (12,13). There are also studies showing that the immune response can be monitored biochemically from the gingival crevicular fluid (GCF), peri-implant sulcus fluid (PISF), saliva and serum(14,15). There are studies indicating that GCF and PISF can be more suitable materials for periodontal studies since they are site-specific and sensitive(16). Cytokines, growth factors and such materials in GCF and PISF have been addressed in previous studies in order to evaluate the development and course of periodontal disease (17,18).

Periostin is a cellular matrix protein that consists of 835 amino acids, weighs 90 kDa and is included in the fasciclin family (19,20). Periostin production is induced by TGF-BETA. Periostin affects the mechanical properties of collagen cross-linking and connective tissue. In addition to extracellular matrix proteins, it also interacts with integrins, i.e. cell membrane proteins. TGF-BETA affects osteoblast replenishment and attachment by inducing periostin production (21). It has been shown that periostin plays a functional role in wound repair, cardiovascular diseases, bone and tooth regeneration and tooth morphogenesis (20,22,23). In order to investigate the effects of periostin on human PDL (hPDL) cells under inflammatory conditions, hPDL cells were treated with different concentrations of periostin using LPS of porphyromonas gingivalis and TNF-α. The results showed that periostin played a key role in periodontal integrity and was associated with important cellular events, such as cell proliferation, migration and survival signaling pathway activation, when exposed to inflammatory mediators and bacterial virulence factors. (24)

In this cross-sectional study, the aim was to evaluate the periostin level and its relationship with the clinical parameters in periodontal (Gingivitis and Periodontitis) and peri-implant (Peri-implant mucositis and Peri-implantitis) disease.

**MATERIAL and METHODS**

**Study Population**

The minimum number of subjects in each group that was required to evaluate a change of 1.4±1.5 (Effect size=7.6-9) units in periostin levels as significant between the groups with periodontal and peri-implant disease was determined to be 19 (α =0.05, 1- β =0.80).

Fourty-two subjects, i.e. 21 females and 21 males, were enrolled in this cross-sectional study. Approval for the study was obtained from the Gaziantep University Ethics Committee on 26/09/2018. All participants were informed about the study before their consent was obtained.

**Inclusion and exclusion criteria**

Patients who were older than 18, did not have any systemic disease, had not taken antibiotics or anti-inflammatory drugs within the previous 6 months, or received dental treatment within the previous 6 months were included in the study.

**Clinical examination**

GI and PI values were measured from four zones of the implants and natural teeth, and PPD and CAL values were measured from six zones of the natural teeth and four zones of the implants. In the clinical measurements, a Williams probe was used for natural teeth, and a Plastic probe for the implants.

**GCF and PISF sampling and analysis**

GCF/PISF samples were collected before conducting the clinical measurements. The deepest pockets were preferred from radiographic images when selecting the teeth/implants. During sample collection, the tooth/implant was isolated using dental cotton rolls, dried gently using air spray and then paper strips were inserted into the pocket until there was pressure. After waiting for 60 seconds, the paper strips were placed in a Periotron 8000 device that had previously been calibrated to measure the GCF/PISF volume. The measurements were recorded. The samples were stored at -80 degrees until the day of analysis. The periostin level in the GCF/PISF samples was measured in accordance with the manufacturer’s instructions and a research(25) using the *Cloud-Clone Corp. ELISA (Enzyme Linked Immunosorbent Assay) method.

**Statistical analysis**

In the statistical evaluation of the data, normal distribution of numerical data was tested by the Shapiro Wilk test. The Mann Whitney U Test was used to compare variables that did not have a normal distribution in the two groups. ANOVA and LSD multiple comparison tests were used to compare the numerical data with normal distribution between the 4 groups, whereas Kruskal Wallis and All pairwise tests were used for the comparison of data with non-normal distribution between the 4 groups. Correlation between categoric variables and correlation between numerical variables were tested with the Chi-Square test and Spearman’s Rank correlation coefficient, respectively. Descriptive statistics were provided with mean±std. deviation for numerical variables and with numbers (%) for categorical variables. SPSS 22.0 package software was used in the analyses. P<0.05 was considered statistically significant.
RESULTS

Eighty samples collected from 42 patients, i.e. 21 males and 21 females, aged between 22-75 were used in the current study (P; n=20 G; n=20 PI n=20 PM n=20). One sample from the peri-implant mucositis group was excluded from the study since it was not read biochemically. The mean age ± standard deviation value of the participants was 54.92±12.79.

Statistical evaluation showed that there was no statistically significant difference between the groups in terms of the sample PI and sample GI parameters (p>0.05) (Table 1). Evaluation of the PPD parameters of the sampled teeth and implants showed that there was a statistically significant difference between the gingivitis-periodontitis, gingivitis-peri-implantitis, peri-implant mucositis-periodontitis and peri-implant mucositis-peri-implantitis groups (p<0.05).

There was a statistically significant difference between the gingivitis-periodontitis, gingivitis-peri-implantitis and peri-implant mucositis-peri-implantitis groups in terms of GCF/PISF periostin volume (p<0.05). On the other hand, the amount of periostin in GCF/PISF did not exhibit a statistically significant difference between the groups (p>0.05). (Table 2)

DISCUSSION

Peri-implant diseases are one of the common complications of dental implant treatment (26). They are similar to periodontal diseases in terms of their inflammatory nature (8). There are indices and biomarkers that enable the clinical and biochemical follow-up of both disease groups such IL-1b (27,28). Periostin is a matricellular molecule in the fasciclin family. Periostin, which is especially synthesized by fibrous connective tissue such as the periosteum and periodontal ligament, plays a key role in tissue integrity and maturation, wound repair and periodontal ligament integrity (20). Periostin is a novel biomarker which has been recognized for nearly a quarter of a century. Many different studies have focused on the potential role of periostin in inflammation. It has been included in many in vitro and in vivo studies in dentistry (29) (29,30). Aral et al. (31), Akman et al. (32), Balli et al. (25), and Padial-Molina et al. (33). have also conducted studies similar to the results in this study. According to our knowledge, this is the first study to evaluate the periostin level in patient with peri-implanter mucositis.

Table 1. Comparison of clinical parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>P</th>
<th>G</th>
<th>PI</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>1.488±0.732</td>
<td>1.039±0.567</td>
<td>1.066±0.506</td>
<td>1.000±0.459</td>
</tr>
<tr>
<td>GI</td>
<td>1.400±0.328</td>
<td>1.276±0.322</td>
<td>1.500±0.333</td>
<td>1.323±0.232</td>
</tr>
<tr>
<td>PPD(mm)</td>
<td>3.422±0.885*</td>
<td>2.119±0.595*</td>
<td>3.513±0.911*</td>
<td>2.213±0.586*</td>
</tr>
</tbody>
</table>

* Significant difference between groups (p < 0.05)

Table 2. Comparison of GCF/PISF volume and Periostin levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>P</th>
<th>G</th>
<th>PI</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCF/PISF volume(μl)</td>
<td>0.509±0.314*</td>
<td>0.166±0.116*</td>
<td>0.680±0.319*</td>
<td>0.321±0.220*</td>
</tr>
<tr>
<td>Periostin (ng/30sn)</td>
<td>19.705±3.98</td>
<td>19.26±7.57</td>
<td>18.76±5.43</td>
<td>20.09±6.45</td>
</tr>
</tbody>
</table>

* Significant difference between groups (p < 0.05)
data reported by Ballı et al. It is believed that this stems from the fact that Ballı et al. included a healthy subject group.

In the current study, periostin levels in GCF and PISF samples were measured using a Biotech device. Although there is no defined standard value for the GCF volume in healthy gums, it was reported that impaired gingival health and increased inflammation led to an increase in the amount of GCF (35).

Ballı et al. (25) found a statistically significant difference between all groups in terms of GCF volume and the amount of GCF periostin in their study, whereas Akman et al. (32) did not report any statistically significant difference in terms of GCF/PISF volume and the amount of GCF/PISF periostin. In another study by Aral et al. (31), it was found that the highest GCF periostin amount was observed in the aggressive periodontitis group, and the lowest in the non-periodontitis group. Padial-Molina et al. observed in their study that GCF periostin levels increased consistently with the recovery pattern following periodontal surgery compared to the disease and health states (34).

In this study, there was a statistically significant difference between all groups in terms of GCF/PISF periostin volume, similar to the findings of Ballı et al., whereas there was no statistically significant difference between any group in terms of the amount of GCF/PISF periostin, similar to the findings of Akman et al. (32) (25).

Studies report that the amount of GCF periostin decreases as the inflammation becomes more severe, and the decrease in the periostin level is described by two mechanisms. The first mechanism is based on the fact that the fight against bacteria in the environment could modulate periostin expression. The second mechanism associates the aforementioned decrease with decreased periodontal ligament cells, which are among the major producers of periostin, in the progression of the disease. (34)

Experimental periodontitis has been created in an animal study. This study also showed that periostin levels decreased as a response to the inflammatory process similar to the other studies (33).

According to the results of the study by Ballı et al., there was a statistically significant difference between all groups in terms of GCF periostin levels, whereas there was no statistically significant difference between the groups in terms of serum periostin levels. In the study, it was observed that GCF periostin levels were statistically decreased moving gradually from the healthy group towards the chronic periodontitis group. The investigators underlined the fact that GCF periostin levels decreased with inflammation. They hypothesized that the aforementioned decrease stems from the fact that periostin has a function in healthy tissue. Experimental periodontitis has been created in an animal study. This study showed that periostin levels decreased as a response to the inflammatory process, similar to the findings of Ballı et al. In this study, there was no statistically significant difference between the groups in terms of GCF periostin levels. It is believed that this inconsistency between the two studies stems from the fact that Ballı et al. had a healthy subject group (25).

Within the limitation of the previous cross-sectional study, although the difference was not statistically significant, periostin values were higher in peri-implant mucositis and gingivitis groups as compared to the peri-implantitis and periodontitis groups. It may concluded that, periostin level may be useful clinically for differential diagnosis of peri-implantitis and peri-implant mucositis, but it is required to conduct further multicenter studies with larger sample size to understand the potential role of periostin in periodontal and peri-implant inflammation.

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Competing interests: The authors declare that they have no competing interest.

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