Salivary Trefoil Factor (TFF3) in Stage I–II Periodontitis: A Prospective Clinical Study

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Abstract

Objective  This article evaluates the salivary trefoil factor levels using enzyme-linked immunosorbent assay and clinical parameters in stage I to II periodontitis subjects.

Materials and Method  A total of 44 subjects with periodontitis and healthy periodontium were enrolled for the study as per the inclusion criteria. The subjects were selected and categorized as group A (control group) and group B (test group). Scaling was performed on healthy subjects at baseline and 1 month if necessary and scaling and root planing was performed for periodontitis subjects. Trefoil factor 3 (TFF3) levels was analyzed at first and post-nonsurgical periodontal therapy followed by clinical parameters, respectively.

Statistical Analysis  Inferential statistics were performed using independent t-test and repeated measures of analysis of variance (ANOVA) test. Independent t-test was used for the intergroup comparison of all the variables. Repeated measures of ANOVA test along with post hoc Bonferroni test was used for the intragroup comparison and the level of statistical significance was set at 0.001.

Results  Difference in TFF3 levels and clinical parameters was seen between groups A and B, which was statistically significant.

Conclusion  Within the constraints of the study, it can be stated that TFF3 is a relevant biomarker to determine the activity and association of periodontal and systemic diseases, gastrointestinal disorders, and inflammatory bowel diseases.

Keywords
- trefoil factor
- periodontitis
- biomarker
- ELISA
- TFF3
- TFF1
- TFF2
- SRP
- NSPT

Introduction

The human immune-inflammatory response to microbial dental plaque is the key determinant of the initiation, progression, and severity of disease. The immune cells activation triggers the release of enzymes, which leads to the breakdown of bone and connective tissue.¹ A substance that can be measured and objectively studied as an indication of healthy biologic processes, harmful biologic processes, or pharmacological reactions to therapeutic treatments is known as a biomarker, also known as a biologic marker. Antimicrobial peptides have a role in both the innate and adaptive host responses, making them an essential part for understanding the progression of periodontal well-being.²

TFF3, or intestinal trefoil factors, are members of a mini-family of mucin-associated proteins. The term relates to one or more 38- or 39-amino acid domains where six cysteine residues form three disulfide connections to generate a...
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The operator, patient, and assistant all adhered to the coronavirus disease 2019 standard operating protocols. Along with regular sanitization, other precautions including hand hygiene and respiratory etiquette were taken. The subjects were selected based on age, probing depth (PD), and bleeding scores. On the basis of the 2017 Classification of Periodontal and Peri-Implant Diseases and based on the severity and extent of a patient’s disease, the participants on the basis of bleeding on probing scores were selected and categorized as group A (control group) having less than 10% BOP and group B (test group) with more than 10% bleeding on probing (BOP).

Oral hygiene measures were given at baseline and reinforced at all time intervals. Scaling was performed on healthy subjects at baseline and 1 month if necessary. As scaling and root planing (SRP) is the nonsurgical periodontal therapy to reduce/eliminate bacterial load and to remove endotoxin from the infected cementum, SRP was performed for periodontitis subjects who were eligible for study as per the inclusion criteria and unstimulated saliva samples for the estimation of salivary TFF3 was collected at baseline and 3 months to evaluate the effect of therapy on TFF3 concentration (►Fig. 2).

The following clinical parameters were noted at baseline, 1 month, and 3 months intervals:

1. Modified gingival index (MGI) (Lobene et al)\(^6\)
2. Plaque index (PI) (Turesky-Gilmore-Glickman modification of the Quigley and Hein)\(^9\)
3. Sulcus bleeding index (SBI; Mühlemann and Son)\(^10\)
4. PD (using UNC-15 probe)
5. Interdental clinical attachment loss (CAL) (using UNC-15 probe)

**TFF3 Estimation**

Unstimulated saliva was taken from all the subjects at initial visit and third visit, coded in Eppendorf tubes, and the biochemical analysis was done where the analyst was blinded toward the groups. The samples were stored at -80°C until analysis was done. The biochemical analysis was done after adding 97 µL of distilled water and the final volume of saliva obtained after dilution was 100 µL. The analysis was done with enzyme-linked immunosorbent assay (ELISA) reader of BeneSphera using the ElabScience kit using the sandwich-ELISA principal.\(^12\)

**Results**

**Clinical Parameters**

The mean PI change for group B was 0.52 ± 0.27, 0.20 ± 0.24, and 0.72 ± 0.37 and in group A was 0.02 ± 0.01, 0.01 ± 0.00, and 0.02 ± 0.00 from baseline to first subsequent visit, first to third month, and baseline to third month, respectively. Mean MGI changed significantly from baseline to 1 month, and from 1 to 3 months for both groups A and B. Mean changes in SBI in group A were 0.0009 ± 0.009, 0.011 ± 0.009, and 0.125 ± 0.004 and in group B were 0.53 ± 0.26, 0.74 ± 0.28, 0.26, 0.74 ± 0.28, and 0.26.
and 0.21 ± 0.14 from baseline to 1 month, baseline to 3 months, and between 1 and 3 months, respectively. This comparison concluded significant changes in SBI scores for group A and B (►Table 1). The mean change in probing depth (PD) for group A was 0.00 ± 0.00, 0.00 ± 0.00, and 0.00 ± 0.00 and in group B was 0.36 ± 0.49, 1.27 ± 0.45, and 0.90 ± 0.42 from baseline to first month, baseline to third month, and from first to third month, respectively, during the comparison between the two groups. The mean CAL scores in group A were 0.00 ± 0.00, 0.00 ± 0.00, and 0.00 ± 0.00 and in group B were 0.31 ± 0.47, 1.13 ± 0.46, and 0.81 ± 0.50 from baseline to first month, baseline to third month, and from first to third month, respectively. The mean change in clinical attachment level from baseline to first month, from first to third month, and from baseline to third month in group B was significantly significant (►Table 2).

**Biochemical Parameters**

The mean increase in TFF3 in group B was higher than in group A. In both groups, the mean change of TFF 3 recorded posttreatment was 0.07 ± 0.12 and 0.39 ± 0.30, respectively. Statistically significant results were obtained for the mean

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**Fig. 2** Flowchart.

**TFF3 - Trefoil factor**

49 PATIENTS

5 Dropouts

44 PATIENTS

**GROUP A** (Periodontally Healthy Patients- Control Group)

22 Patients

Saliva collection (Salivary TFF3 estimation) and *Clinical parameters at baseline.

Scaling (Baseline) and Oral hygiene instructions given

*Clinical parameters at 1 month and Oral Hygiene Instructions reinforced

*Clinical parameters, Saliva collection and TFF3 estimation at 3 months

**GROUP B** (Stage I-II periodontitis patients- Test Group)

22 Patients

Saliva collection (Salivary TFF3 estimation) and *Clinical parameters at baseline.

Scaling and Root Planing (Baseline) and Oral Hygiene instructions given

***Fig. 2 Flowchart.***
### Table 1  Mean changes in PI, MGI, and SBI at intervals

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Change from baseline to 1 mo</th>
<th>Change from 1 to 3 mo</th>
<th>Change from baseline to 3 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Plaque index (PI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy group (group A)</td>
<td>0.0067</td>
<td>0.00835</td>
<td>-0.0076</td>
</tr>
<tr>
<td>Periodontitis group (group B)</td>
<td>0.5277</td>
<td>0.27951</td>
<td>0.2005</td>
</tr>
<tr>
<td>p-Value of intergroup comparison between group A and B</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group A</td>
<td>0.003*S</td>
<td>&lt; 0.001*S</td>
<td>0.003*S</td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group B</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
</tr>
<tr>
<td>Modified gingival index (MGI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy group (group A)</td>
<td>0.0204</td>
<td>0.00918</td>
<td>0.0113</td>
</tr>
<tr>
<td>Periodontitis group (group B)</td>
<td>0.3805</td>
<td>0.19815</td>
<td>0.2550</td>
</tr>
<tr>
<td>p-Value of intergroup comparison between group A and B</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group A</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group B</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
</tr>
<tr>
<td>Sulcus bleeding index (SBI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy group (group A)</td>
<td>-0.0009</td>
<td>0.00949</td>
<td>0.0125</td>
</tr>
<tr>
<td>Periodontitis group (group B)</td>
<td>0.5327</td>
<td>0.26114</td>
<td>0.2164</td>
</tr>
<tr>
<td>p-Value of intergroup comparison between group A and B</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group A</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group B</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
</tr>
</tbody>
</table>

Abbreviations: MGI, modified gingival index; PI, plaque index; SBI, sulcus bleeding index; SD, standard deviation; S, significant.

### Table 2  Mean changes of probing depth and clinical attachment loss

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probing depth (PD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy group (group A)</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Periodontitis group (group B)</td>
<td>0.3636</td>
<td>0.49237</td>
<td>0.9091</td>
<td>0.42640</td>
<td>1.2727</td>
<td>0.45584</td>
</tr>
<tr>
<td>p-Value of intergroup comparison between group A and B</td>
<td>0.002*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group A</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group B</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical attachment loss (CAL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy group (group A)</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Periodontitis group (group B)</td>
<td>0.3182</td>
<td>0.47673</td>
<td>0.8182</td>
<td>0.50108</td>
<td>1.1364</td>
<td>0.46756</td>
</tr>
<tr>
<td>p-Value of intergroup comparison between group A and B</td>
<td>0.005, S</td>
<td>&lt; 0.001, S</td>
<td>&lt; 0.001, S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group A</td>
<td>&lt; 0.001, S</td>
<td>&lt; 0.001, S</td>
<td>&lt; 0.001, S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Value of intragroup comparison among group B</td>
<td>0.019*NS</td>
<td>&lt; 0.001*S</td>
<td>&lt; 0.001*S</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NS, nonsignificant; S, significant; SD, standard deviation.  
*p < 0.001.  
*p > 0.001.
Discussion

Periodontitis is characterized by change in bacterial poly- 
genicity leading to deterioration and destruction of the sup- 
porting structures of the teeth including the alveolar bone. The symptoms include gingival bleeding, pocket for- 
mation, interdental attachment loss, and resorption of bone. A new classification of periodontitis was proposed in 2017, based on the extent and severity of the disease. Periodontal health and gingival inflammation were the key points of discussion in this workshop.13

TFF3 is chosen out of the three trefoil factors as the salivary biomarker since TFF1 and TFF2 were shown to have no noticeable impact on the keratinocytes of the mouth. Also, its concentrations in gingival tissues did not significantly change in people with periodontally healthy gingiva.15 Abundance and the noninvasive method of collection of saliva constitute it as the chosen medium for evaluation of the biomarker TFF3.

The intragroup comparison of the clinical parameters for both groups showed significant changes from baseline to 1 month. These results are attributed to the impact of nonsurgical periodontal therapy (SRP) in group B and scaling in group A, which is regarded as the benchmark for the treatment of periodontitis for stage I and II periodontitis.14 The changes in these scores were significant in both groups from the initial level to third month and from 1 to 3 months.15 These outcomes were related to the efficient use of standardized oral hygiene instructions throughout the study time period after the baseline treatment.16

On intergroup comparisons, there was a statistically significant change obtained in clinical parameters. This occurs because of the reduction in proteolytic activity of microorganisms and reduction of oxidative stress after nonsurgical therapy which further leads to the reduction in periodontal inflammation. However, subjects with periodontitis continue to have a higher chance of recurrence and hence they do not become nonperiodontitis subjects unless they are kept on professional mechanical plaque removal regimen.17 On intergroup comparisons of PD and CAL, there was a statistically significant difference seen at all time intervals between the groups. This is because more subjects were included from stage II periodontitis according to the 2017 Classification of Periodontal and Peri-Implant Diseases.18

The intragroup comparison of the TFF3 scores for both groups showed significant changes from baseline to 3 months. Gingival tissues of chronic periodontitis subjects reported lower levels of TFF3 along with its expression compared with the healthy group.16

Intergroup comparison of mean TFF showed that at base- 
line the mean TFF remained high in group A compared with 
group B, whereas it was reversed after 3 months of SRP. 
Also, the mean change in TFF levels in group B continued to be higher than in group A.

This is because TFF3 is linked to periodontitis and after 
scaling also, nonperiodontitis state cannot be achieved accord- 
ing to the 2017 classification. The levels of TFF3 were shown to be negatively correlated with the other periodontal parameters. These results complemented the findings of the study done by Meesala et al15 and Keles Yucel et al2 where salivary TFF3 increased after nonsurgical periodontal treatment in periodontitis subjects. Therefore, it was suggested for periodontal degradation leading to inflammation and cellular damage, TFF3 might be upregulated to ensure epithelial restoration and formation of new periodontal tissues. The limitations of the study include separate analysis 
done for both the stages of periodontitis, that is, stage I and stage II. An estimation of biomarker, that is, TFF3, should 
be performed at 1 month and microbiological analysis could have been done along with the estimation of TFF3.

Conclusion

Within the constraints of the study, it can be stated that TFF3 is an important biomarker to determine the activity of periodontal disease. It is advised that the subjects should be kept in maintenance therapy at all intervals. An additional long-term longitudinal study with bigger sample size should be conducted to assess the importance of TFF3 levels in periodontitis subjects.

Future Prospective

TFF3 is a protein anti-inflammatory biomarker secreted from salivary glands, from gastric mucosa, and is associated with

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Table 3 Mean changes in salivary TFF3 concentration

<table>
<thead>
<tr>
<th>TFF3</th>
<th>Baseline (initial visit)</th>
<th>At 3 mo</th>
<th>From baseline (initial visit) to 3 mo</th>
<th>Baseline (initial visit) to 3 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Healthy group (group A)</td>
<td>0.55982</td>
<td>0.141077</td>
<td>0.63127</td>
<td>0.160105</td>
</tr>
<tr>
<td>Periodontitis group (group B)</td>
<td>0.33182</td>
<td>0.156982</td>
<td>0.72673</td>
<td>0.376094</td>
</tr>
<tr>
<td>p-Value</td>
<td>&lt; 0.001&quot;S</td>
<td>0.043&quot;NS</td>
<td>&lt; 0.001&quot;S</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; TFF3, trefoil factor 3; S, significant; NS, non-significant.
gastrointestinal diseases and inflammatory bowel disease. It would act as a systemic diseases indicator and a link between periodontal disease and systemic diseases.

**Funding**
None.

**Conflict of Interest**
None declared.

**References**
2. Keles Yucel ZP, Afacan B, Atmaca Ilhan H, Kose T, Emingil G. The trefoil factor family 1 (TFF-1) and 3 (TFF-3) are upregulated in the saliva, gingival crevicular fluid and serum of periodontitis patients. Oral Dis 2022;28(04):1240–1249