

Prolonged Suppressive Effects of Periodontitis on Salivary TFF3 Production

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Eur J Dent 2019;13:193–198

Abstract

Objective As a follow-up to our previous study that demonstrated decreased salivary trefoil factor family 3 (TFF3) peptide levels in chronic periodontitis patients, this current study aimed to observe the effects of nonsurgical periodontal treatment on salivary TFF3 peptides in patients with periodontal diseases.

Materials and Methods Eighty-seven volunteers that comprised of 30 individuals with healthy periodontium, 31 with gingivitis, and 26 with chronic periodontitis were considered for the study. Prior to periodontal treatment, a general periodontal examination was performed along with collection of saliva samples from each volunteer. Nonsurgical periodontal treatments were provided to patients with gingivitis and periodontitis. Two weeks post-treatment, saliva samples were recollected, and the periodontal status was re-evaluated. Salivary TFF3 concentrations were measured by enzyme-linked immunosorbent assay.

Statistical Analysis Mann–Whitney U test was used when the investigated data were not normally distributed. Chi-squared test was used when dealing with categorical data. Kruskal–Wallis test with post-hoc corrections was used to compare data among the three investigated groups. Two-tailed $p < 0.05$ was considered as statistically significant.

Results Prior to the periodontal treatment, salivary TFF3 concentrations in patients with gingivitis and periodontitis were significantly lower than those with healthy periodontium. Two weeks post-treatment, increased levels of salivary TFF3 were observed in patients with gingivitis, whereas the concentrations decreased in patients with chronic periodontitis.

Conclusion This study demonstrated the effects of periodontal disease on the production of salivary TFF3 peptides. Interestingly, nonsurgical periodontal treatment also affected the recovery of salivary TFF3 peptides but varied in their outcomes between gingivitis and periodontitis patients.

Keywords

- ▶ periodontal diseases
- ▶ saliva
- ▶ salivary glands
- ▶ trefoil factor



Introduction

Trefoil factor family (TFF) peptides are composed of TFF1, TFF2, and TFF3. Members of TFF peptides share a common molecular structure known as a trefoil domain.¹ It was reported that an interaction between a free cysteine residue in the C-terminal of this TFF peptide and other proteins would alter biological properties and activities of the TFF molecules.² The distribution and localization of TFF peptides vary according to organs, tissues, and body fluids.^{3,4} These peptides have several biological functions including cell migration⁵ and wound healing.⁶ Results from animal studies have shown that TFF3 modulated inflammation by interfering the production and secretion of inflammatory cytokines, such as interleukin-1beta (IL-1 β), IL-6, and IL-8.^{7,8} Recombinant human TFF3 peptides also inhibit the production of toll-like receptor 4, nuclear factor kappa B (NF- κ B), and tumor necrosis factor alpha (TNF- α) in epithelia of colitis mice.⁹ In contrast, it was demonstrated that NF- κ B signaling pathway was related with the downregulation of TFF expression.¹⁰ The TFF peptides have been intensively investigated in the gastrointestinal tract and are considered to exert their functions in maintenance and protection of mucosal tissues.¹¹ However, information on TFF peptides in the oral cavity is limited.

TFF peptides are identified in different oral tissues including salivary glands,¹² oral mucosa,¹³ gingiva,¹⁴ and saliva.⁴ They are mainly produced from the salivary glands¹⁵ with some addition from the parotid duct and oral mucosal epithelia.¹⁶ Among salivary TFF peptides, TFF3 is the most prominent, followed by TFF1 and TFF2.⁴ The previous report explained that TFF3 was a modifying factor involved in oral keratinocytes signaling pathways, such as cell survival, cell proliferation, and cell migration.¹⁷ Therefore, presence of TFF peptides in saliva may be crucial for the protection of oral mucosal against tissue damage. Previously, our cross-sectional study demonstrated reduced salivary TFF3 peptides in chronic periodontitis (CP) subjects, and the levels of TFF3 negatively correlated with the severity of periodontitis.¹⁴ Additionally, our *in vitro* study has revealed that TFF peptides could be digested by major proteolytic enzymes produced by periodontogenic bacteria.¹⁸ Taking these findings into account, periodontal inflammation mediated by periodontopathic bacteria may be a downregulating factor in the production of salivary TFF3 peptides in patients with CP. We hypothesized that reduction of periodontal inflammation by nonsurgical periodontal treatment would elevate the production of salivary TFF3 peptides. The present study was aimed to verify our hypothesis by examining salivary TFF3 peptide levels in gingivitis and periodontitis subjects prior to and following completion of nonsurgical periodontal treatment.

Materials and Methods

Study Population and Clinical Examination

This prospective study was performed at the Dental Hospital, Khon Kaen University, Thailand during 2014 to 2016. All procedures were approved by Khon Kaen University Ethics committee (HE551372). Eighty-seven systemically healthy

volunteers including 26 CP patients, 31 dental plaque induced gingival diseased (GD) patients, and 30 clinically periodontally healthy (PH) individuals participated in this study. Written informed consents were obtained from all participants. All subjects were nonsmokers who had at least 15 remaining teeth. A general periodontal examination periodontal parameters including bleeding on probing (BOP), probing depth (PD), and clinical attachment level (CAL) on six sites of each remaining tooth, except third molar, were measured and recorded by one periodontist. Diagnosis of periodontal diseases was based on the 1999 International Workshop for classification of Periodontal Diseases and Conditions.¹⁹ Two weeks following completion of the conventional periodontal treatment, a second full-mouth general periodontal examination was performed and the parameters were recorded.

Saliva Collection

Saliva collection was performed as previously described.¹⁴ Each volunteer was asked to refrain from eating or drinking for at least 1 hour prior to saliva collection. After rinsing the mouth thoroughly with water, unstimulated whole saliva sample (3–5 mL) was obtained in a 50 mL centrifuge tube. The first saliva collection was done in the morning during 9 to 11 am, followed by the first periodontal examination for each volunteer. Two weeks post-treatment, second saliva collection and periodontal examination were performed with the GD and CP groups. Saliva samples were immediately placed in an ice container, followed by centrifugation at 10,000 \times g at 4°C for 10 minutes. Saliva samples were stored at –80°C until further measurements. Total protein concentrations from each saliva sample were determined employing a commercially available protein assay kit (Qubit Protein Assay kit; Thermo Fisher Scientific, Invitrogen, United States) to test whether any differences in salivary TFF3 concentrations could be referred to the differences in the total salivary protein concentrations.

Measurement of Salivary TFF3 Concentrations

For the quantification of salivary TFF3 concentrations, we used our generated monoclonal antibody (mAb) clones 286 and 116 for a modified sandwich enzyme-linked immunosorbent assay (ELISA) technique.²⁰ To develop the sensitivity, fluorescein isothiocyanate (FITC) and anti-FITC identification methods were used in this sandwich ELISA technique. An antihuman TFF3 mAb clone 286 was used as the plate-coating antibody to capture the peptides in saliva samples. The antihuman TFF3 mAb clone 116 labeled with FITC was applied to detect the captured TFF3 peptides. Horseradish peroxidase (HRP)-conjugated sheep anti-FITC antibodies along with TMB substrate were used as colored markers. The color intensity was measured using a microplate reader at 450 nm. A standard curve revealing salivary TFF3 between 0.5 and 32 ng/mL was observed. All details of salivary TFF3 concentrations were normalized by total salivary protein concentrations and reported as TFF3 concentrations (ng)/salivary protein concentration (mg).

Nonsurgical Periodontal Treatments

Nonsurgical mechanical treatments were provided for gingivitis and periodontitis patients in the periodontal clinic,

Faculty of Dentistry, Khon Kaen University. The treatment included plaque control by providing oral hygiene instructions and mechanical debridement by full-mouth scaling and root planing.²¹⁻²³ Periodontal treatments were performed in single or multiple visits, utilizing manual curettes (Hu-Friedy, Chicago, Illinois, United States) and ultrasonic instruments (P5 Newtron, Acteon, France). Two weeks following completion of nonsurgical periodontal treatment, the periodontal health was re-examined by the same periodontist.

Statistical Analysis

IBM SPSS software version 19.0 was used for the statistical analyses. Student's *t*-test was used to evaluate normally distributed data between the two groups, whereas Mann-Whitney U test was used when the investigated data were not normally distributed. Chi-squared test was used when

dealing with categorical data. Kruskal-Wallis test with post-hoc corrections was used to compare data among the three investigated groups. Spearman's rank correlation was used to evaluate correlations between salivary TFF3 peptides levels and periodontal parameters. Two-tailed *p* < 0.05 was considered as statistically significant.

Results

Demographic and Clinical Characteristics of the Study Population

Demographic and clinical characteristics of three investigated groups were as shown in ►Tables 1 and 2. The mean age of PH individuals and GD patients was similar. In contrast, the mean age of CP patients was significantly higher than those in the PH and GD groups. Females were predominant in all the three groups. Prior to the nonsurgical periodontal

Table 1 Demographic data of the study population

	Investigated groups			
	Healthy	Gingivitis	Periodontitis	<i>p</i> -Value
Number	30	31	26	
Age in years (mean ± SD)	32.19 ± 1.61	32.13 ± 1.81	44.04 ± 2.74	0.001 ^a
Male	5	5	9	
Female	25	26	14	0.085 ^b

Abbreviations: ANOVA, analysis of variance; SD, standard deviation.

^aKruskal-Wallis one-way ANOVA.

^bChi-squared test.

Table 2 Clinical parameters and salivary characteristic data of the investigated groups prior to and 2 weeks following nonsurgical periodontal treatment

Clinical parameters		Investigated groups			
		Healthy (<i>n</i> = 30)	Gingivitis (<i>n</i> = 31)	Periodontitis (<i>n</i> = 26)	<i>p</i> -Values
PD (mm; mean ± SD)	Before treatment	1.89 ± 0.19	1.94 ± 0.16	2.64 ± 0.59	<0.001 ^a
	After treatment	–	1.86 ± 0.14	1.98 ± 0.25	<0.001 ^c
CAL (mm; mean ± SD)	Before treatment	0.52 ± 1.77	0.55 ± 0.12	2.35 ± 1.07	<0.001 ^a
	After treatment	–	0.54 ± 0.14	2.09 ± 0.92	0.358 ^d
BOP (mean ± SD)	Before treatment	15.42 ± 3.57	38.28 ± 9.98	51.7 ± 15.29	<0.001 ^a
	After treatment	–	23.02 ± 6.93	30.47 ± 12.65	0.015 ^d
Salivary characteristics					
Total salivary protein concentrations (mg/mL; mean ± SD)	Before treatment	0.59 ± 0.28	0.57 ± 0.31	0.73 ± 0.76	0.775 ^a
	After treatment	–	0.49 ± 0.16	0.75 ± 0.29	<0.001 ^c
pH	Before treatment	6.99 ± 0.24	6.98 ± 0.29	6.79 ± 0.4	0.083 ^a
	After treatment	–	6.89 ± 0.3	6.76 ± 0.36	0.153 ^d
Normalized salivary TFF3 concentrations (ng/mg protein)	Before treatment	228.46 ± 149.0	93.05 ± 83.33	156.73 ± 147.23	<0.001 ^a
	After treatment	–	252.73 ± 171.17	40.74 ± 41.77	<0.001 ^d

Abbreviations: ANOVA, analysis of variance; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level; TFF3, trefoil factor family 3; SD, standard deviation.

^aKruskal-Wallis one-way ANOVA.

^bChi-squared test.

^cMann-Whitney U test.

^dStudent's *t*-test.

treatment, the total salivary protein concentrations were not significantly different among the three groups. Following treatment, salivary protein concentrations in the GD group were significantly different than those in the CP group ($p < 0.001$). In regard to the periodontal parameters prior to the treatments, the mean BOP value in the PH group was significantly lower than the GD and CP groups ($p < 0.001$). PD and CAL measures from the CP group were significantly greater than those from PH and GD groups ($p < 0.001$). Improvement in periodontal health status at 2 weeks following the treatments was observed in GD and CP groups.

Levels of Salivary TFF3 Peptides in GD and CP Groups before and after Nonsurgical Periodontal Treatments

The median, mean, upper, and lower quartiles values of normalized salivary TFF3 concentrations were as shown in ►Fig. 1. The trend of alternative salivary TFF3 in the GD and CP groups following nonsurgical periodontal treatment was as shown in ►Fig. 2. Prior to the periodontal treatment, mean normalized salivary TFF3 concentrations in PH, GD, and CP groups were 228.46, 93.05, and 156.73 ng/mg, respectively. Normalized salivary TFF3 concentrations in the PH group were significantly higher than the GD and CP groups

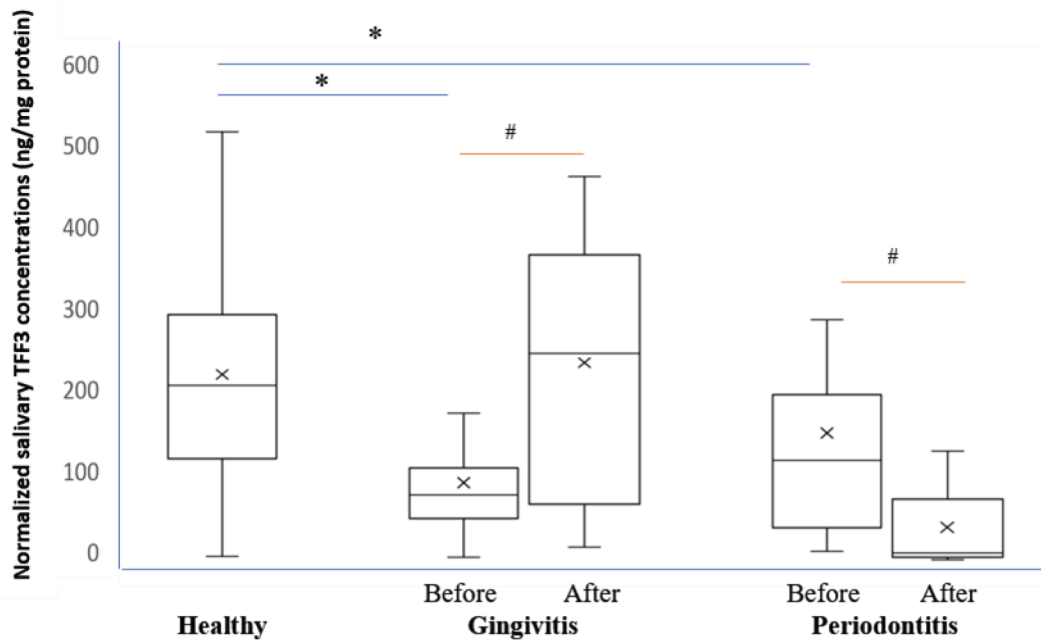


Fig. 1 Normalized salivary Trefoil factor family 3 (TFF3) concentrations (ng/mg) among the three investigated groups including clinically periodontal healthy (healthy, $n = 30$); gingival diseased (gingivitis, $n = 31$); and chronic periodontitis (periodontitis, $n = 26$) individuals prior to and 2 weeks following nonsurgical periodontal treatment. Middle line indicates median, cross indicates mean, box indicates the upper and lower quartiles, and whiskers indicate minimum and maximum values. The asterisk represents the p -value < 0.05 (Mann-Whitney U test). The sharp represents the p -value < 0.001 (Wilcoxon signed-rank test).

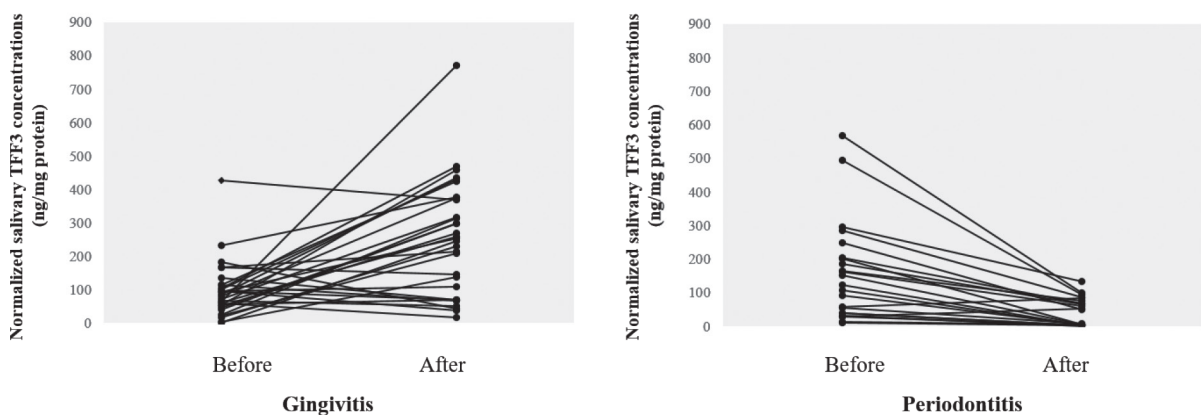


Fig. 2 A trend of alternative salivary trefoil factor family 3 (TFF3) in the gingival disease (gingivitis, $n = 31$) and chronic periodontitis (periodontitis, $n = 26$) patients prior to and 2 weeks following nonsurgical periodontal treatment.

($p < 0.05$). Two weeks following completion of periodontal treatment in the GD group, there was a significant increase in the salivary TFF3 (252.73 ng/mg) ($p < 0.001$). The level of salivary TFF3 peptides in the GD group after receiving the treatment was comparable with those in the PH group. On the other hand, salivary TFF3 peptides in the CP group after receiving the treatment significantly decreased as compared with those before the treatment ($p < 0.001$).

Correlations between Salivary TFF3 Concentrations and Periodontal Parameters

Spearman's rank correlation analysis was performed on all the subjects with periodontal diseases. Prior to the periodontal treatment, significantly negative correlations were observed between salivary TFF3 levels and BOP (correlation coefficient = -0.379, $p = 0.005$). On the other hand, no correlation was observed between salivary TFF3 levels and other periodontal parameters. Likewise, following periodontal treatment, no correlation was demonstrated between salivary TFF3 levels and periodontal parameters.

Discussion

According to our observations, the levels of salivary TFF3 peptides in gingivitis and periodontitis patients prior to the periodontal treatment were lower than those in PH subjects. These results were in agreement with our previous study¹⁴ and suggested that decreased levels of salivary TFF3 peptides could be due to local and systemic effects of periodontal diseases. Regarding the local effects, our previous in vitro study demonstrated proteolytic activity of gingipains, one of the major proteolytic enzymes produced by *Porphyromonas gingivalis*, on digesting TFF peptides.¹⁸ These findings imply that periodontopathic enzymes may locally reduce TFF3 peptides in saliva. As TFF3 peptides are mainly produced by salivary glands, decreased levels of salivary TFF3 peptides may reflect the systemic impact of periodontal diseases on the functions of the salivary gland. Several investigations have demonstrated the association between periodontal diseases and biological functions of salivary glands.^{24,25} One study demonstrated hyposalivation in experimental periodontitis model rats.²⁴ Similarly, alterations of inflammatory microRNA in salivary glands of rats infected with periodontal bacteria have also been observed.²⁵ Our findings provide new implication that periodontal diseases may be a downregulating factor in the production of TFF3 from the salivary glands. Regarding the pathological effects of periodontal diseases on salivary gland dysfunctions, further studies to validate correlations between periodontal diseases and protein synthesis from salivary glands would be of importance.

The effects of nonsurgical periodontal treatments on salivary compositions were investigated in patients with periodontitis, and outcomes were varied.²⁶⁻²⁹ Some studies reported changes in salivary components,^{26,27,30} whereas others demonstrated no significant impact of nonsurgical periodontal treatment on salivary components.^{25,26} Nonsurgical

periodontal treatments have been observed to improve the levels of proinflammatory cytokines, including TNF- α and endothelial growth factor A, in serum and gingival crevicular fluid.³¹ In our results, nonsurgical periodontal treatment increased levels of salivary TFF3 peptides in gingivitis patients to the same levels as in PH individuals. Taking these findings into account, it could be possible that gingival inflammation could be a temporary downregulator of salivary TFF3 production, and the reduction in proinflammatory cytokines related to gingivitis by nonsurgical periodontal treatments would help to regain salivary TFF3 production. In contrast, the periodontal therapy had no effect on the recovery of salivary TFF3 peptides in periodontitis patients. These results are indicative of the suppressive effects of periodontitis on the functioning of salivary glands and reflect differential effects of nonsurgical periodontal treatment on the production of salivary TFF3 peptides in periodontitis patients as compared with those in gingivitis patients. However, it should be noted that the follow-up period in this study was 2 weeks following periodontal treatment. It is possible that at this time point the ongoing inflammatory response due to CP may still remain in effect and could interrupt the recovery of salivary glands' functions. Thus, an extended follow-up study should be conducted to re-examine the levels of salivary TFF3 peptides.

Previous studies demonstrated that TFF3 peptides had a protective effect on mucosal tissues by the downregulation of proinflammatory cytokines.⁷⁻⁹ It was reported that recombinant human TFF3 administered as a topical oral spray could reduce oral mucositis in colorectal cancer patients who received fluorouracil-based chemotherapy.³² Thus, the presence of salivary TFF3 peptides may be an important protective factor against oral mucosal tissue damage. However, it remains unclear whether salivary TFF3 plays any role in periodontal diseases. Our previous observations¹⁴ combined with the present results demonstrated inverse correlations between levels of salivary TFF3 peptides and periodontal parameters in patients with periodontal diseases. It would be of interest to further investigate whether administration of topical TFF3 peptides could help to reduce periodontal inflammation.

In conclusion, the present study demonstrated the impact of periodontal diseases on the production of salivary TFF3 peptides, and confirmed our previous findings of decreased levels of salivary TFF3 peptides in patients with periodontal diseases. Nonsurgical periodontal treatments had varied results on the recovery of salivary TFF3 peptides between gingivitis and periodontitis patients. The treatment elevated salivary TFF3 peptides in gingivitis patients but the treatment had no effect on the recovery of salivary TFF3 peptides in periodontitis patients. Our findings provide a new implication that periodontal diseases may be a downregulator of salivary glands in the context of TFF3 production.

Funding

This work was supported by the TRF Mid-Career Research Scholar (RSA5680010) and Khon Kaen University (KKU 600301).

Conflict of Interest

None declared.

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