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EFFICACY OF SALIVARY TREFOIL FACTOR 3 AS BIOMARKER IN CHRONIC PERIODONTITIS PATIENTS.

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ABSTRACT

Background: Biomarkers are the molecules that help in evaluating the status of disease and the prognosis of treatment. One such molecule that gained popularity as a biomarker among chronic periodontitis patients in recent days is Trefoil Factor 3(TFF3). TFF3 was evaluated in saliva, but its clinical significance and correlation with periodontal pathogens among periodontitis patients have never been investigated.

Material & Methods: Saliva and supra, infra gingival plaque samples were collected from 30 periodontitis and 30 non-periodontitis individuals. Enzyme-linked immunosorbent assay was used to estimate the salivary levels of TFF3. The polymerase chain reaction was advocated for analyzing periodontal pathogens from plaque samples.

Results: Reduced salivary TFF3 levels were observed in chronic periodontitis patients, with a negative correlation between several periodontal pathogens and TFF3 levels.

Conclusion: Our study results suggest a negative correlation between inflammatory mediators and salivary TFF3 levels. Thus, the estimation of TFF3 can be considered as a potential biomarker in evaluating disease states among periodontitis patients.

INTRODUCTION

Periodontitis is a chronic disease of the oral cavity that represents a bacteria-induced inflammatory condition affecting supporting structures of dentition. The main causative bacterial species reported are Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Tannerella forsythia. These virulent bacteria produce several potentially harmful factors that induce host inflammatory mediators and are responsible for connective tissue breakdown and alveolar bone resorption.[1,2]

Saliva and Gingival tissues contain a large number of peptides that are responsible for initiating host defense and also for maintaining periodontal tissue integrity. Evaluation of these peptides helps in monitoring the chronic disease state and are termed Biomarkers.[3]

The science of biomarkers in dentistry dates back to the late 19th century and is slowly seeming to become extremely important for diagnosis, and monitoring treatment outcomes. Amidst the various biomarkers, there is a group of low-weight soluble proteins i.e., Trefoil factors (TFFs) which have been discovered in saliva, and recently their role in periodontal tissues is also brought to light.[4]

Mammalian TFFs were categorized based on their chemical structure into 3 types and were named TFF1, TFF2, and TFF3.[5] Among the 3 types TFF3 was isolated also from the oral cavity in the year 1999. It is said to be secreted from sub-mandibular and sublingual salivary glands along with mucin. Literature also shows the expression of TFF3 from the parotid gland duct.[6]

This small enigmatic family of TFFs has a multitude of functions that help in maintaining the homeostasis of the oral mucosa. They are Restitution, Regeneration, Anti-Apoptosis, Wound Repair, Neo-vascularization, and Mucin interaction.[7] These are also known for their ability to reduce pro-inflammatory mediators such as COX2 and inducible Nitric Oxide Synthase.[8]

To date, studies discussing about TFF 3 and its relation to periodontal tissues are scarce. Thus, this study intends to detect and quantify the levels of TFF3 in unstimulated saliva of healthy and chronic periodontitis patients.

MATERIALS AND METHODS

The present Clinic-Biochemical study was conducted among 60 systemically healthy patients, that includes 30 subjects with chronic periodontitis and 30 periodontally sound subjects. All the study subjects were selected from those attending the Department of Periodontics and Implantology from January 2020 to April 2020, CKS Theja dental college, Tirupati.

This research project was undertaken after getting approval from the institutional ethical board (CKS/PERIO/19-20/003). All the study subjects were explained about the need for the study in their local vernacular language and written informed consent was obtained for the same. All the study subjects were allocated into two groups that are Group 1 and Group 2, the control group and the periodontitis group respectively.

Inclusion criteria for the periodontal group:

· Patients with > 10 remaining teeth.

· Systemically healthy patients with chronic periodontitis.
· Patients who had not received any periodontal therapy in the past 6 months.
· Patients who had not taken anti-inflammatory agents, antibiotics, or immunosuppressants in the past 6 months.

Inclusion criteria for the control group:
· Patients with > 10 remaining teeth.
· Patients with probing pocket depth ≤3mm.

Exclusion Criteria
· Patients with uncontrolled diabetes, anticoagulant therapy, immunosuppressive therapy, and other systemic diseases were excluded from the study.
· Patients with aggressive periodontitis.
· Patients who have received any surgical or non-surgical or antibiotic therapy, 6 months before the start of the study.
· Pregnancy females and lactating mothers.
· Patients who were smokers/alcoholics.

Periodontal examination for all the study subjects was performed at baseline using a UNC-15 probe and it included: detailed dental and medical history, periodontal pocket depth[9], clinical attachment level[9], and bleeding on probing[10]. Errors during the recording of clinical parameters were maintained at a minimum by constant intraexaminer comparisons.

***Collection of Saliva*:**
All the study subjects refrained from eating and drinking 1 hour before sample collection. Patients were instructed to thoroughly rinse the oral cavity with water and then, not swallow the saliva, subsequently allowing it to pool sublingually. Participants then spit the saliva into a container which should be cold after collection (4° c) and freeze (-20° to -70° c) as soon as possible.[11]

Evaluation of TFF3:
Trefoil factor 3 assays were done by using an enzyme-linked immunosorbent assay kit (ELISA) (Sun Red Biotechnology Company) [Figure 1]. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay. Recombinant human TFF3 was advocated in the calibration of salivary supernatants that are vortexed and diluted with assay buffer. Before the assay further dilutions were performed to check if the dilutions were too low or too high to estimate. Recombinant human TFF3 peptide was diluted in assay buffer to the concentrations of 0.0003–0.183 nmol/dl.[4]

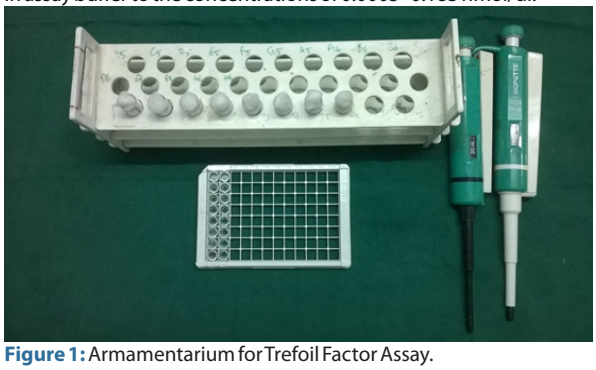


Figure 1: Armamentarium for Trefoil Factor Assay.

Microbial Analysis:
For the detection of periodontal pathogens (P.gingivalis, A.actinomycetemcomitans, T.forsythia) in the subgingival plaque sample [Figure 2], a conventional Polymerase chain reaction was used. The selected tooth was dried with sterile cotton swabs, and the supragingival plaque was removed manually. The subgingival plaque sample was collected by using a sterile curette.

The collected sample was transferred to a vial of transport media containing Tris HCL EDTA buffer (TE buffer). The vial was stored at 4° Cand PCR analysis of the collected sample was done within 48hours. Identification by PCR method involved DNA isolation, polymerase chain reaction, gel electrophoresis, and observation under an ultraviolet transilluminator.[4,12]

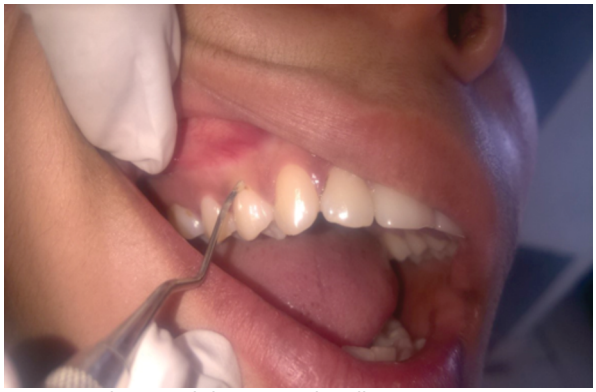


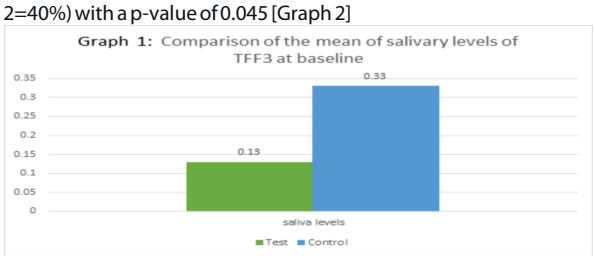
Figure 2: Sub Gingival Plaque Sample Collection.

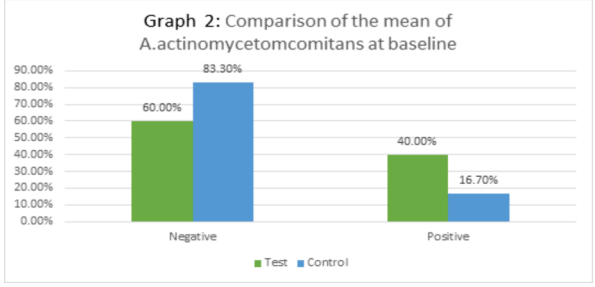
***Statistical Analysis*:**
All the analysis was done using SPSS version 18 software with a p-value of <0.05 was considered statistically significant. Independent sample T-test was advocated for the analysis of clinical parameters, periodontal pathogens, and salivary TFF3 levels between both groups.

RESULTS
The control group (Group 1) consisted of 30 subjects with ages ranging from 20-60 years, 30 Subjects of the periodontitis group (Group 2) were of 35-70 years. The mean probing depth of group 1 subjects was 3.64±0.70 and in group 2 it was found to be 4.09±0.78. On intergroup comparison of mean probing depth among both the groups statistically significant difference was noticed with a p-value of 0.022.

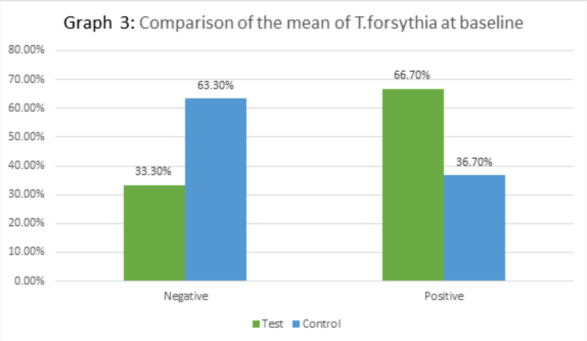
Means of clinical attachment level in group 1 and group 2 were 3.97±0.77 and 4.49±0.95 respectively. A statistically significant difference was noticed in comparison of mean clinical attachment level between both the study groups with a p-value of 0.024. Mean Bleeding on probing among group 1 subjects was 1.75±0.30 and 2.30±0.27 in group 2, with a statistically significant difference in the comparison (p<0.001).

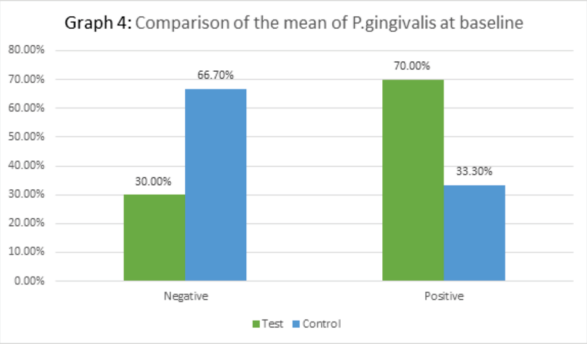
The mean values of salivary TFF3 at baseline in group 1 and group 2 subjects were 0.33±0.08 and 0.13±0.03 respectively with a statistically significant p-value of 0.001 on the intergroup comparison [Graph 1]. In the comparison of Aggregatibacter actinomycetemcomitans values, a significant difference was noticed among both the study groups (group1=16.7%; group 2=40%) with a p-value of 0.045 [Graph 2]





Estimation of periodontal pathogen Tannerella forsythia showed 36.7% in group 1 and 66.7% in the group with a significant difference (p-value 0.02) [Graph 3]. Porphromonas gingivalis was found to be 33.3% in group 1 and 70% in group 2 with a p-value of 0.004 which is significant [Graph 4].





The above study results, clearly demonstrate that there was a negative co-relation between salivary TFF3 levels and periodontal pathogens. It is also clear that TFF3 levels reduce with an increase in periodontal clinical scores.

DISCUSSION

TFFs are small soluble proteins with a three-looped structure formed by interchain disulfide bonds, hence the name "Trefoil Factors". The first member of this family was discovered thirty years back and it was named a pancreatic spasmolytic peptide now termed as TFF2.[5] the second isolated member was TFF1 which was named a breast cancer-associated peptide.

TFF3 the last known member of the family was isolated from rat intestinal epithelial cells.[13] In the study conducted by Samson et al.,[4] authors noticed no significant relation between TFF1 and TFF2 with chronic periodontics except for TFF3. Hence in the present study salivary levels of TFF3 were considered for evaluation.

Normal TFFs are secreted from gastrointestinal epithelial cells along with mucin granules, that form a protective covering over intestinal mucosa.[14] Other than the gastrointestinal tract these peptides were also produced from respiratory epithelium, ocular mucus-producing cells, breast milk-producing cells, and salivary glands.[15]

The main function of TFFs is restitution, a process wherein epithelial cells migrate and seal the superficial wounds after injury.[16] Antiapoptotic property is the other main function that helps in preventing apoptosis of migrating epithelial cells, thus favoring repair.[17] Likewise, TFF3 promotes cell migration and survival of oral keratinocytes.[18]

TFF3 is the only known member found in saliva produced by all salivary glands. Assessment of the literature has shown a negative correlation between salivary levels of TFF3 and chronic periodontitis.[4] Hence present study was aimed at co-relating salivary TFF3 levels with that of periodontal pathogens and clinical periodontal parameters.

To the best of our knowledge, this study is the first of its kind to compare salivary levels of TFF3 with that of the expression of periodontal pathogens. The age of participants in the present study ranged from 35 -70 years among chronic periodontitis patients. This can be attributed to the prevalence of periodontitis among older patients.

The means of clinical parameters like bleeding on probing, periodontal pocket depth, and clinical attachment loss were greater in the periodontitis group than the control group. This was in accordance with the study conducted by Chaiyarit et al, where a statistically significant difference was noticed for change in clinical parameters between study and control groups.[19]

Salivary TFF3 levels in the periodontitis group were lower when compared to the test group with a statistically significant difference. This was in accordance with studies conducted by Chaiyarit et al,[19] in which they estimated all the 3 members of the Trefoil group. Of all the three estimated TFF3 showed statistically significant results than the rest two.

In the comparison of periodontal pathogens, there was a negative correlation between TFF3 and pathogens, with a statistically significant difference between the two.[20] The main limitations of the present study were the number of subjects and not considering TFF3 levels of gingival tissue. Clinical parameters were also estimated manually using the UNC-15 probe although it is a third-generation probe, would have been more appropriate for estimation.

Furthermore, investigations at the molecular level are essential to shed light on mechanisms in regulating TFF3 expression in oral tissues and the role of TFF3 in periodontal diseases.

CONCLUSION

Within the limitations of the study, it can be concluded that salivary TFF3 levels are lower in chronic periodontitis patients than in healthy subjects. There is also a negative correlation between the amount of P.gingivalis and T.forsythia and A. actinomycetemcomitans, clinical parameters, and Salivary TFF3. There appear to be multiple pathways for signaling and activation/expression of TFF3. How and which pathway will get activated is still not clear. Further studies are required that analyze the ligands associated with the activation of pathways that induce expression of TFF3 and their threshold levels required for activation/inhibition of TFF3.

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