Original Research Paper

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Periodontics

COMPARATIVE EVALUATION OF PEPTIDYLARGININE DEIMINASE 2 LEVELS IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER SCALING AND ROOT PLANING AND FLAP SURGERY- A CLINICO BIOCHEMICAL STUDY

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ABSTRACT **BACKGROUND & OBJECTIVE**: The hunt for biomarkers of chronic periodontitis has progressed significantly over the years. Saliva, plaque, and gingival crevicular fluid have all been found to include biomarkers. Studies have found that people with periodontitis had higher levels of peptidylarginine deiminase 2 (PAD2) expression than those without periodontitis. Therefore peptidyl arginine deiminases can be used as biomarker for evaluation of periodontal disease. This study is aimed at comparing levels of peptidyl arginine deiminase 2 (PAD2) in the gingival crevicular fluid of chronic periodontitis patients before and after scaling and rootplaning and after flap surgery. **METHOD:** A total number of 20 subjects were included in the study. All patients received full mouth SRP followed by flap surgery for persisiting pockets . Clinical parameters including plaque index, gingival index, probing pocket depth, clinical attachment level , sulcus bleeding index were recorded and GCF was collected at baseline, 2 weeks after SRP, 1 month after flap surgery for assessment of PAD-2 levels. **RESULTS:** Mean PAD2 concentrations were significantly reduced, at two weeks after scaling and rootplaning and at 1 month after flap surgery ($p<0.05$) when compared to baseline PAD2 values. There was a statistically significant reduction in probing pocket depth and gain in clinical attachment level following non-surgical and surgical therapy when compared to baseline ($p<0.05$). **CONCLUSION:** Results indicate that PAD2 may play a role in the pathogenesis and diagnosis of periodontal disease and could be considered as disease predictive biomarker.

KEYWORDS : Periodontitis, Biomarker, peptidylarginine deiminase, Gingival crevicular fluid, chronic periodontitis

INTRODUCTION

Periodontal diseases are a set of infectious/inflammatory diseases of the tooth-supporting tissues that are caused mainly by pathogenic microorganisms in the microbial dental plaque.

A reliable assessment of periodontal inflammation is necessary for an accurate diagnosis and treatment decision. Clinical parameters such as probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP) recordings, and radiographic findings are currently used to diagnose periodontitis. PD and CAL measurements, as well as bone levels in radiographic films, provide information about periodontal tissue loss in the past, but they do not reveal the current state of disease activity or forecast future periodontal disease progression. An ideal periodontal diagnostic tool should be able to detect the presence and severity of periodontal disease, as well as anticipate the clinical course of periodontal disease and the current state of disease activity. The development of novel diagnostic tools that can detect the presence of active disease, predict future disease progression, and evaluate the response to periodontal therapy is needed to improve clinical management of periodontal patients. As a result, finding the best diagnostic method for periodontal disease is still an ongoing research topic. The diagnostic potential of sulcular fluid has been recognised for more than six decades, and researchers have been debating it since the 1950s, but it has yet to gain widespread acceptance to be used as a clinically significant tool. Periodontitis has been related to a biofilm containing a consortium of oral pathogens, including Porphyromonas gingivalis, a Gram negative anaerobe. P.gingivalis produces PPAD, a peptidylarginine deiminase (PAD) that converts peptidylarginine residues to citrulline and is distinctive among prokaryotes in this regard. Anti-citrullinated protein antibodies are antibodies that are generated against citrullinated proteins (ACPAs). Periodontitis has been suggested as a possible activator of ACPA formation. Periodontitis was linked to ACPA seropositivity in RA patients, according to studies supporting this notion. Another study found that citrullinated proteins produced in periodontal tissue samples were highly comparable to synovial tissue samples from people with RA, leading to the conclusion that periodontitis may be responsible for triggering an ACPA response that selectively targets citrullinated proteins. Patients with periodontitis have higher levels of PAD2 expression in gingival connective tissue than those with healthy gums. As a result, peptidylarginine deiminases can be used as a biomarker for periodontal disease evaluation. Therefore this study is aimed at comparing levels of PAD2 in gingival crevicular fluid of chronic periodontitis patients with before and after scaling and rootplaning alone and as an adjunct to scaling and rootplaning and flap surgery.

AIM & OBJECTIVE OF THE STUDY

To evaluate and compare the levels of clinical parameters and peptidylarginine deiminase 2 levels in chronic periodontitis patients before and after SRP and flap surgery.

MATERIALS AND METHODS

This Clinico-biochemical study was carried out on gingival crevicular fluid samples collected from 20 patients in an age group ranging from 30-60 years reporting to department of periodontics, Coorg Institute of Dental Sciences, Virajpet. The study was reviewed and approved by the Institutional Review Board of Coorg Institute of Dental Sciences. The nature and purpose of the study and the recall protocol was explained to the subject and a written consent was obtained before commencing the study.

A group of 20 patients are selected who are diagnosed with Chronic periodontitis with probing depth>5mm were selected. The demographic characteristics of the patients (age and sex), clinical parameters were recorded initially and GCF samples were collected from sites with deepest periodontal pockets at baseline visit to evaluate PAD2 levels. Then the patients were subsequently subjected for

scaling and root planing. , two weeks after Phase I therapy GCF samples were collected from the deepest pocket sites . After Phase I therapy, patients were evaluated for persisting pockets in at least three quadrants and flap surgery were performed. The patients were re-called for post surgical follow-up (1 month) after flap surgery and subsequent GCF samples were collected to further evaluate the PAD2 levels.

Inclusion Criteria:
1) Subjects aged 30-60 yrs.
2) Subjects with a minimum complement of 20 teeth.
3) Subjects with chronic periodontitis indicated for flap surgery with persisting pockets in at least three quadrants with probing depth>5mm

Exclusion Criteria:
1. Subjects with any systemic disease.
2. Subjects who have undergone any periodontal treatment in the last 6 months.
3.Subjects who have received antibiotics/anti-inflammatory drugs/steroids immunosuppressive chemotherapy within the last 6 months.
4. Pregnant or lactating women.
5. Subjects who are tobacco or alcohol users.

Periodontal status :
All the patients received a full mouth periodontal examination consisting of following indices:
1. Sulcus Bleeding Index (SBI) – (Muhlemann and Sons 1971)
2. Gingival Index (GI) – (Loe H and Silness J 1963)
3. Plaque Index (PI) – (Silness & Loe, 1964)
4. Probing Pocket depth (PPD) -
5. Clinical Attachment Level (CAL) -

PROCEDURE

Sample collection:
Micro-capillary pipettes of standardized length and diameter were used to collect GCF. After gently drying the sites with air syringe and cotton rolls, micropipettes were placed inside the pocket. A standardized volume of 2µl was collected. The collected sample was transferred into the storage medium and stored at a temperature of -70□C.

Method of collection of data
All the teeth were probed using a UNC-15 periodontal probe. Each tooth was probed at six sites from gingival margin to the base of the pocket (buccal, mesiobuccal, distobuccal, lingual, mesiolingual and distolingual) reading was taken nearest mm with 0.5 mm being round off to the lower number. The clinical attachment level was recorded from a fixed reference point (stent) to the base of the pocket. The probing pocket depth was recorded from the margin of the gingival to the base of the pocket. Total score of all teeth divided by the number of teeth examined gave the average clinical attachment loss and probing pocket depth for the patient per tooth. A full mouth periodontal examination was done at baseline and after 2 weeks and 1 month after treatment to evaluate the status of periodontium. The peptidylarginine deiminases 2 levels in GCF samples were measured using Enzyme linked immunoassay test.

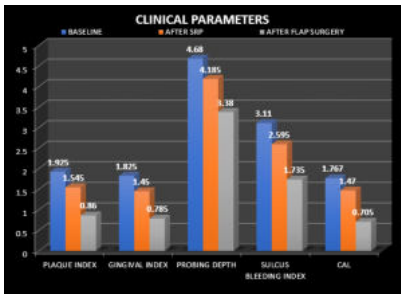
RESULTS
A total of 20 patients between age group ranging from 30-60 years were included. The demographic characteristics of the patient (age and sex), were recorded at baseline.

STATISTICALANALYSIS
The data was collected, coded and fed in SPSS (IBM version 23) for statistical analysis. The descriptive statistics included mean and standard deviation.The inferential statistics included ANOVA followed by post hoc Tukeys test. The level of significance was set at 0.05 at 95% confidence interval.

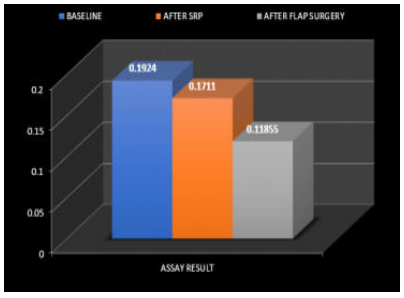
CLINICAL PARAMETERS
When overall analysis were compared all the clinical parameters showed a significant reduction in their values between baseline to 2 weeks after scaling and root planning and 1 month after flap surgery with p value <0.001. (**Graph 1**)

BIOCHEMICALANALYSIS
GCF samples subjected to ELISA assay showed statistically highly significant results with a f value 55.351. (Graph 2)

GRAPH 1 : COMPARISON OF CLINICAL PARAMETERS BETWEEN BASELINE, SRPAND FLAPSURGERY



GRAPH 2 : COMPARISON OF PADI 2 ASSAY VALUES BETWEEN BASELINE, SRPAND FLAPSURGERY



DISCUSSION
Periodontal disease has historically been diagnosed using a patient's clinical performance in BOP, PD, and CAL tests as well as radiographic evidence of alveolar bone loss. These methods are reliable, but are depend upon a clinician's professional experience.
Periodontitis has been linked to a biofilm that contains a consortium of oral pathogens that includes the Gram negative anaerobe Porphyromonas gingivalis. P. gingivalis expresses a peptidylarginine deiminase (PAD) known as PPAD, an enzyme that modifies peptidylarginine residues to citrulline and is unique in this regard amongst prokaryotes.

In the present study, patients with chronic periodontitis had elevated plasma PAD2 levels. In our study we have checked for PAD2 levels at baseline and after phase I therapy and 2 weeks after scaling and root planning, the PAD2 levels were checked again and patient had been evaluated for residual periodontal pockets in atleast 3 quadrants and flap surgery was done and further evaluation of PAD2 levels was checked 1 month after completion of the flap procedure. This is in agreement with studies done by Foulquier C and Damgaard et al who concluded that expression level of PAD-2 was correlated with the intensity of inflammation and disease activity[1] In a study conducted by Lappin DF et al , it has been demonstrated that untreated periodontitis patients had higher ACPA titres than healthy controls. Engström et al. reported that there was an increased expression of PAD2 in gingival connective tissue of patients with periodontitis compared to periodontal healthy group whereas similar levels of PAD2 was observed in the gingival epithelium of the two groups.

GCF was used for assessment of PAD2 levels because although GCF has been shown to originate from the serum in proximal blood vessels, it is generally considered to reflect the ongoing processes in the surrounding periodontal tissues, including inflammation, turnover of connective tissue and resorption of alveolar bone. Collection of GCF using micropipettes appears to be ideal as it provides an undiluted sample of 'native' GCF whose volume can be accurately assessed.

PAD2 could be detected in all the samples from sites with chronic periodontitis which was in accordance with a similar study done by Gozde Veyisoglu.

This study reported a decrease in GCF PAD 2 levels from baseline to 2 weeks after receiving scaling and rootplaning which is statistically significant .This was in accordance with the findings of Gozde Veyisoglu., where GCF PAD2 levels were higher in the chronic periodontitis group[2] and with Kardum et al, who stated that Scaling and root planing (SRP) is one of the most commonly utilized procedures for the treatment of periodontal diseases and has been used as the

"gold" standard therapy in comparison to other therapeutical procedures.

Caffesse et al., had stated that non-surgical treatment approach frequently results in insufficient root debridement especially at sites with deep pockets. Hence, all sites were subjected to SRP followed by open flap debridement. This was in accordance with the study done by Kim et al., Becker et al., and Silva et al., and explains the improvement in periodontal parameters from baseline to pre-surgical time intervals.

In the present study showed significant reduction after SRP and there was significant decrease in GCF PAD2 concentrations from baseline to 1 month post-surgery and these findings are in agreement with those of Veyisoğlu et al, and Engström et al. This could be due to the elimination of the microbial factors which causes a gradual decrease in the level of bacterial toxins followed by decline in the production of inflammatory mediators and stimulants as well.

There was a significant decrease in GCF PAD concentrations from 2 weeks after SRP to 1 month Post-surgery in this study and these findings are in agreement with those of Veyisoğlu et al and Engström et al. The effectiveness of treatment was based on reduction of gingival inflammation and bacterial deposits and improvements in probing depth and clinical attachment levels.

We have evaluated PAD2 at baseline , 2 weeks after scaling and rootplaning and 1 month after flap surgery and have seen that PAD2 levels had been reduced significantly after each procedure. The difference is highly statistically significant (P = 0.00). This was in accordance with the findings of Veyisoglu et al, and Engstrom et al. and may be considered supporting evidence showing the possible role of PAD2 in chronic periodontitis.

The present clinical trial shows that the nonsurgical periodontal therapy and periodontal flap surgery in patients with chronic periodontitis resulted in statistically significant improvement in clinical parameters such as plaque index, gingival index, sulcus bleeding index, probing pocket depth and as correlated from baseline to 2 weeks after SRP and 1 month after flap surgery. Except for clinical attachment level which the mean difference results show as nonsignificant from baseline to SRP. However the mean difference results shows highly significant from baseline to 1 month after flap surgery and from SRP to flap surgery, thus there is statistically improvement in clinical attachment level after surgical periodontal therapy.

The improvement in periodontal parameters following treatment in this study were in accordance with a systemic review done by Heitz-Mayfield et al., which established that in deep periodontal pockets (≥6 mm), surgical therapy resulted in more probing pocket depth reduction and more attachment gain than the non-surgical therapy.

So, this enzyme may also be used in determining the intensity of inflammation in periodontal pockets considering the correlation between PAD2 levels and disease activity in RA. This result may be considered supporting evidence showing the possible role of PAD2 in periodontal inflammation. Our results were in agreement with the study by Engström et al. in terms of findings about PAD2 levels in gingival connective tissue in their study.

To the best of our knowledge this is the first study reporting information on the GCF levels of changes in PAD2 molecules after periodontal flap surgery in chronic periodontitis patients.

There are certain limitations in our study, which include a smaller sample size and shorter follow up period , short time interval observations and absence of a control group. Further long term studies with larger study subjects are required to understand the impact of various treatment modalities like oral prophylaxis, SRP and conventional flap surgery on controlling the periodontal inflammation and relationship between the PAD2 molecules and chronic periodontitis. Since this was a prospective study with statistical comparison of select parameters over a set course of time, the requirement for a control group was not warranted and hence not included in the study design.

CONCLUSION

Within the limitations of the present study, the following conclusions can be made:-

1. Increased GCF levels of PAD2 were observed in chronic periodontitis patients.
2. PAD2 levels had been reduced significantly after SRP and flap surgery in chronic periodontitis patients along with improvement in clinical parameters.
3. The results of the present study provide site-specific information on significant changes in PAD2 levels and periodontal parameters as a result of periodontal disease and treatment.
4. Therefore, the present study indicates that PAD2 may play a role in the pathogenesis and diagnosis of periodontal disease.
5. GCF total amounts of this biomarker may have a potential to be used as diagnostic tools for periodontal disease.
6. Furthermore, their roles in pathogenesis of periodontal disease should be confirmed in additional studies.

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