Original Article

Estimation of gingival crevicular fluid matrix metalloproteinase-3 levels in chronic periodontitis before and after scaling and root planing: A clinicobiochemical study

ABSTRACT

Context: Matrix metalloproteinases (MMPs) have widely been demonstrated in inflamed periodontal tissues and oral fluids. MMP3 is one of the MMPs which is effective in the degradation of numerous extracellular matrix substrates. It also participates in the proteolytic activation cascades of latent pro-MMP1, -8, and -9 which mediate collagenosis.

Aims: This study aimed to estimate the gingival crevicular fluid (GCF) MMP3 levels in chronic periodontitis before and after scaling and root planing (SRP).

Settings and Design: A total of 60 subjects aged 25–55 years are randomly selected from the outpatient department of periodontology of our institute and categorized into two groups of 30 each; Group I - periodontally healthy and Group II - generalized chronic periodontitis.

Subjects and Methods: Clinical parameters such as plaque index, gingival index, probing depth, and clinical attachment loss were recorded in both groups. GCF was collected only once in Group I but twice in Group II. After the baseline records, Group II received SRP treatment followed by re-recording of clinical parameters and GCF sample collection 6 weeks posttreatment. GCF samples were analyzed for MMP3 molecule by enzyme-linked immunosorbent assay.

Statistical Analysis Used: Data were analyzed by Student's *t*-test and Pearson's correlation coefficient.

Results: All the clinical parameters showed improvements after the treatment procedure ($P < 0.05$). Baseline GCF MMP3 values in the test group were significantly higher than in controls ($P < 0.05$), and all the parameters decreased significantly after treatment ($P < 0.05$). Furthermore, the correlation between individual clinical parameters and biochemical parameter was positive but statistically insignificant ($P > 0.01$).

Conclusions: Within the confines of this study, GCF MMP3 was increased in Group II subjects, suggesting its role in chronic periodontitis and the possibility of it being used as an early diagnostic biomarker.

Keywords: Biomarkers, chronic periodontitis, gingival crevicular fluid, matrix metalloproteinases

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INTRODUCTION

Periodontitis is an inflammatory infectious disease in which the inflammation is initiated by bacteria, but the tissue breakdown events that lead to the clinical signs of

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the disease result from the host immuno-inflammatory response that develops to combat the challenge presented by the subgingival biofilm.[1] Proinflammatory mediators and destructive enzymes that orchestrate the host response play a crucial role in periodontal pathogenesis.[2] One such group of enzymes, matrix metalloproteinases (MMPs), is a group of zinc- and calcium-dependent endopeptidases capable of degrading almost all constituents of the extracellular matrix and the basement membrane. In addition, MMPs can cleave other molecules including cytokines, growth factor precursors, and cell adhesion molecules.[3] Due to this broad substrate specificity, MMPs may be regarded as a group of multifunctional enzymes involved in physiological processes, such as embryogenesis, normal tissue remodeling, wound healing, and angiogenesis.[4] However, evidence suggests that these MMPs are also involved in pathological conditions, such as rheumatoid arthritis, atherosclerosis, cancer, and periodontal diseases.[5] Stromelysin-1 (MMP3) has been shown to be necessary for the activation of pro-MMP8 and pro-MMP9 which are the major proteinases of periodontal tissue destruction.[6] Studies have shown that cells which produce collagenase are unable to degrade type I collagen unless MMP3 is present.[7] In the present study, the focus is to evaluate levels of MMP3 in the gingival crevicular fluid (GCF) as an early novel diagnostic biomarker in chronic periodontitis and also to determine the effect of scaling and root planing (SRP) on MMP3 levels in chronic periodontitis to evaluate its role as a prognostic biomarker as well.

SUBJECTS AND METHODS

A gender-matched, clinicobiochemical, single-blinded study was conducted with 60 participants in the age group of 25–55 years selected from the outpatient department of periodontics and oral implantology of our institute. The study was explained in detail to the participants and informed consent was taken from participants. Ethical clearance was obtained before the commencement of the study. The subjects were divided into Group I - control (healthy) and Group II - test group (chronic periodontitis). Inclusion criteria for Group II were patients with generalized chronic periodontitis having a minimum of 25 teeth present in the dentition, clinical attachment loss (CAL) evident and/or probing depth (PD) of more than 4 mm, and radiographic evidence of bone loss. Patients who received periodontal therapy or antibiotic treatment in the last 6 months, with known systemic disease or disorders, and on long-term medications, pregnant women, lactating mothers, postmenopausal women, and smokers were excluded from the study. Clinical parameters recorded were plaque index (PI) (Silness P and Loe H, 1964), gingival index (GI) (Loe H and Silness J, 1963), PD, and CAL. The periodontal examination was carried out with a mouth mirror and a Williams graduated periodontal probe after taking a GCF sample. With the help of disposable micropipettes, 3 µl GCF (by capillary principle) was collected from the upper anterior sextants of all 60 patients by placing the tip of the pipette extracervicular (unstimulated) for 5–20 min after site isolation. GCF was collected only once in Group I and twice in Group II, at baseline and 6 weeks after SRP. GCF contaminated with blood or saliva was discarded. SRP was performed once a week for 2 weeks with autoclaved ultrasonic and hand instruments. Mechanical periodontal therapy was not accompanied by any medications such as antibiotics and analgesics. GCF samples were sent for laboratory analysis by the ELISA kit (RayBio Human MMP3 ELISA Kit Protocol Cat#: ELH-MMP3-001) for MMP3 in vials containing phosphate-buffered saline on the same day. Samples were stored at −80°C until analyzed with an ELISA reader, according to the instruction manual in the kit.

Statistical analysis

Descriptive statistics such as mean, standard deviation (SD), and percentage were used. Intra- and inter-group comparison was done using Student's *t*-test. A comparison of qualitative data was done using Chi-square test. Pearson's correlation coefficient was used to find the relationship between biochemical parameters and clinical parameters. A $P < 0.05$ was considered statistically significant. Data analysis was done using the software Minitab v14.0 Statistical Software (2004). [Computer software]. (State College, PA: Minitab, Inc.).

RESULTS

The mean age in Group I and Group II was 37.7 years (SD ± 5.98) and 39 years (SD ± 5.49). Both the groups consisted of 15 males and 15 females [Table 1].

Clinical parameters

The mean PI at baseline in Group I was 0.20 (SD ± 0.06), whereas in Group II, it showed a higher mean value of 1.64 (SD ± 0.12). The mean PI decreased at 6 weeks after SRP giving a value of 1.29 (SD ± 0.08). Similarly, the mean GI at baseline in Group I was 0.23 (SD ± 0.07), and Group II showed a higher mean value of 1.81 (SD ± 0.11). The mean GI was decreased at 6 weeks after SRP giving a value of 1.35 (SD ± 0.14). The mean PD in Group I was 1.91 (SD ± 0.47), whereas Group II showed a higher mean value of 4.19, with SD being 0.68 at baseline. The mean PD decreased 6 weeks after treatment giving a value of 2.71 (SD ± 0.47). CAL in Group I was 0.00 (SD ± 0), whereas in Group II, it showed a higher mean value of 1.49 (SD ± 0.62) at baseline. The mean CAL decreased

Table 1: Mean age and sex data in healthy group

	Healthy group	Test group
Age (years)	37.7±5.98	39±5.49
Male/female	15/15	15/15

6 weeks after treatment giving a value of 0.86 (SD ± 0.36). Comparison between both groups at baseline in all clinical parameters showed a highly significant difference with $P < 0.0001$. A similar statistical difference was seen in their values when compared before and after treatment in the test group. When compared between control and posttreatment in the test group, $P < 0.0001$ indicated a high statistically significant difference [Tables 2-4].

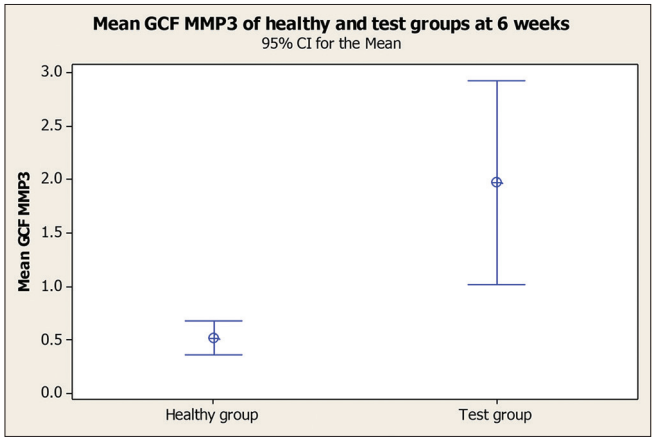
Gingival crevicular fluid matrix metalloproteinase 3 levels

The GCF MMP3 values in Group I was 0.52 (SD ± 0.4), whereas Group II showed a higher mean value of 6.31 (SD ± 6.71) at baseline. The mean GCF MMP3 decreased at 6 weeks after treatment (SRP) with a value of 1.97 (SD ± 2.54). When compared in both groups at baseline, a highly statistically significant difference was found in the values ($P < 0.0001$). A similar statistical difference was seen in values when compared before and after treatment in the test group ($P < 0.0002$). In comparison between the control and posttreatment test group, the difference was statistically significant ($P < 0.003$) [Tables 5-7 and Graphs 1, 2].

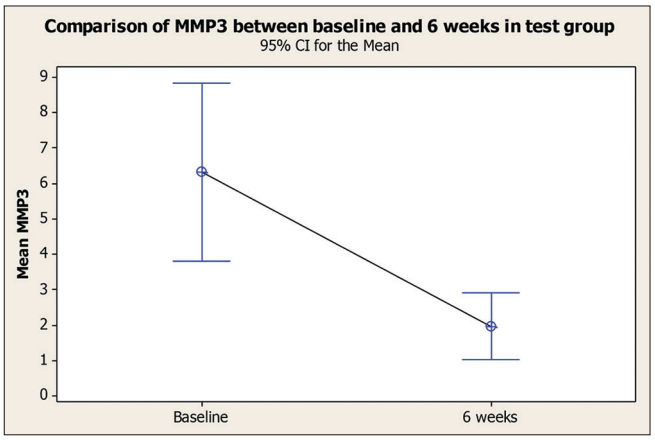
The correlation between clinical parameters with a biochemical parameter was done by Pearson's correlation coefficient. The level of significance was calculated as $P < 0.01$. All the clinical parameters showed a positive correlation with the biochemical parameter at baseline and 6 weeks posttreatment (determined by *r* value). However, the *P* value obtained as per the calculations was $P > 0.01$, which indicated that the difference was not statistically significant even after a positive relation.

DISCUSSION

Pathogenic organisms might mediate tissue degradation in periodontal diseases through the ability of the cell wall antigens to stimulate cytokine production. These would then induce MMP synthesis by resident gingival cells and initiate the degradative events. MMP3 is effective in degrading proteoglycans and fibronectin, which must be removed first in order for the collagenase to have access to the collagen substrate. Reynolds *et al*. have concluded that the host cell production of MMPs may contribute to tissue remodeling in periodontal disease.[8] Kubota *et al*., 1996 have shown



Graph 1: Mean GCF MMP3 of healthy and test group at 6 weeks. GCF MMP3: Gingival crevicular fluid matrix metalloproteinases 3, CI: Confidence interval



Graph 2: Comparison of GCF MMP3 between baseline and 6 weeks in test group. GCF MMP3: Gingival crevicular fluid matrix metalloproteinases 3, CI: Confidence interval

Table 2: Comparison of clinical parameters between healthy and test group (mean±standard deviation) at baseline

Clinical parameters	Healthy group	Test group	Mean difference	95% CI difference	*t*	*P*
PI	0.20±0.06	1.64±0.12	1.44	1.38-1.49	56.47	<0.0001
GI	0.23±0.07	1.81±0.11	1.58	1.53-1.63	65.48	<0.0001
PD	1.91±0.47	4.19±0.68	2.28	1.97-2.58	15.01	<0.0001
CAL	0±0	1.49±0.62	1.49	1.26-1.73	13.11	<0.0001

PD: Probing depth, CAL: Clinical attachment loss, GI: Gingival index, PI: Plaque index, CI: Confidence interval

Table 3: Comparison of clinical parameters between healthy and test group (mean±standard deviation) at 6 weeks

Clinical parameters	Healthy group	Test group	Mean difference	95% CI difference	*t*	*P*
PI	0.20±0.06	1.29±0.08	1.08	1.04-1.12	54.42	<0.0001
GI	0.23±0.07	1.35±0.14	1.12	1.06-1.17	38.55	<0.0001
PD	1.91±0.47	2.71±0.47	0.79	0.55-1.05	6.52	<0.0001
CAL	0±0	0.86±0.36	0.86	0.73-1.00	13.11	<0.0001

PD: Probing depth, CAL: Clinical attachment loss, GI: Gingival index, PI: Plaque index, CI: Confidence interval

Table 4: Comparison of clinical parameters between baseline and 6 weeks in test group (mean±standard deviation)

Clinical parameters	Baseline	6 weeks	Mean difference	95% CI difference	*t*	*P*
PI	0.20±0.06	1.29±0.09	1.084	1.04-1.12	54.42	<0.0001
GI	0.23±0.07	1.35±0.14	1.12	1.06-1.18	38.55	<0.0001
PD	4.19±0.68	2.71±0.47	1.48	1.33-1.63	20.009	<0.0001
CAL	1.49±0.62	0.87±0.36	0.63	0.51-0.75	10.58	<0.0001

PD: Probing depth, CAL: Clinical attachment loss, GI: Gingival index, PI: Plaque index, CI: Confidence interval

Table 5: Comparison of biochemical parameters between healthy and test group (mean±standard deviation) at baseline

Biochemical parameter	Healthy group	Test group	Mean difference	95% CI difference	*t*	*P*
GCF MMP3	0.52±0.4	6.31±6.71	5.79	3.33-8.25	4.72	<0.0001

GCF: Gingival crevicular fluid, MMP: Matrix metalloproteinases, CI: Confidence interval

Table 6: Comparison of biochemical parameters between healthy and test group (mean±standard deviation) at 6 weeks

Biochemical parameter	Healthy group	Test group	Mean difference	95% CI difference	*t*	*P*
GCF MMP3	0.52±0.4	1.97±2.54	1.45	0.51-2.39	3.08	<0.003

GCF: Gingival crevicular fluid, MMP: Matrix metalloproteinases, CI: Confidence interval

Table 7: Comparison of biochemical parameters between baseline and 6 weeks in test group (mean±standard deviation)

Biochemical parameter	Baseline	6 weeks	Mean difference	95% CI difference	*t*	*P*
GCF MMP3	6.31±6.71	1.97±2.54	4.34	2.26-6.42	4.26	<0.0002

GCF: Gingival crevicular fluid, MMP: Matrix metalloproteinases, CI: Confidence interval

that MMP3 or stromelysin-1 is a broad-spectrum MMP and a pivotal activator of latent MMPs.[9] Studies have also demonstrated that MMP3 could activate procollagenases including MMP1, -8, and -9.[9-12] Therefore, keeping in mind the important role of MMP3 in the pathogenesis of the disease at the initial stage, the levels of MMP3 were evaluated in the GCF to evaluate its role as a diagnostic and prognostic marker.

The present study was a case–control prospective study that included 60 subjects categorized equally into two groups that were age and gender matched. In this study, all the clinical parameters and GCF MMP3 levels were found to be significantly higher in the periodontitis subjects as compared to the controls. Similar findings were also noted by Haerian *et al*., 1995 in who observed that GCF MMP3 and Tissue inhibitors of metalloproteinases (TIMP) levels can differentiate between healthy and diseased sites.[13] Beklen *et al*. in an *in vitro* study showed that fibroblast synthesis of MMP3 was stimulated by tumor necrosis factor alpha. They also suggested that the largest difference in health and disease is seen in the MMP3 levels rather than MMP8 and -9, demonstrating the possible role of MMP3 to activate MMP8 and -9.[14]

Ryu *et al*., 2008 showed similar results saying that MMP3 and Membrane type- Matrix metalloproteinases 1 may be partly involved in the progression of periodontal inflammation associated with diabetes mellitus.[15] Contrasting results were shown by Offenbacker *et al*. in a stent-induced gingivitis experimental study, suggesting that there was a significant decrease in the multiple chemokines such as MMP1, -3, and -13 as opposed to the significant increase in interleukin 1 (IL1)α and IL1β.[16]

In the present study, a highly significant difference was found in the clinical parameters and GCF MMP3 levels in the test group before and after treatment. Individual clinical parameters and GCF MMP3 levels at baseline and 6 weeks after treatment in the test group showed positive but a weak correlation. This was in accordance with the study by Haerian *et al*., 1996 who demonstrated that MMP3 and TIMP levels were reduced after treatment.[17]

Similar results were noted by Tuter *et al*., 2005 saying that there was an improvement in the clinical parameters after phase I periodontal therapy accompanied by a reduction in the GCF MMP3 and increasing GCF TIMP levels.[18] The duration of 6 weeks in the present study was also in accordance with other studies.[9,18] However, in contrast to the present study, they found a significant correlation between the clinical parameters and GCF MMP3 and TIMP levels. This difference in results from the present may be attributed to the difference in the sampling procedure and the ELISA kit used by a different manufacturer.

Pourtaghi *et al*. evaluated the effect of different antibiotics on GCF MMP3 and TIMP levels and observed unchanged levels in SRP alone and SRP + metronidazole group, which was in contrast to the present study. They suggested a significant reduction in Gingival crevicular fluid stromelysin and TIMP values in the SRP + tetracycline fibers and SRP + minocycline

group. There was a gain in the clinical attachment in all groups after treatment as in the present study.[19] Adjunctive antibiotic treatment was not used in the present study to avoid bias. The different methods of sampling and laboratory techniques, as well as variations in results analysis, may have influenced the presence and extent of correlation between clinical and biochemical parameters.

Comparison of clinical parameters and GCF MMP3 between Group I and post-treatment values in Group II showed statistically significant reduction compared to the test group baseline values but were higher than the controls. This was in accordance with the study done by Tuter *et al*., who observed similar findings.[18]

By utilizing ELISA in the present study, we were able to detect only the total enzyme MMP3 in the GCF (latent and active form). It is possible that posttreatment levels of the active enzyme was low. Other methods that can be used for the detection of this enzyme include western blotting, zymography, and bead-based multiplexing analysis for several molecules together. In addition, the sample used in the study was GCF which was collected with microcapillary pipettes by the extracrevicular technique described by Brill *et al*.[20] and retrieved by insulin syringes. Other samples that could be used for the same purpose as in some studies are gingival biopsies specimens or saliva. Several methods can be used to collect the GCF such as paper strips, preweighed twisted threads, microcapillary tubes, microsyringes, and plastic strips.[21]

The GCF samples underwent the assay procedure as described by the ELISA kit manufacturer and were analyzed in an ELISA reader. These analyzers were self-monitored with computer-based programs. Therefore, the results obtained were accurate and reliable with reduced errors. Thus, all samples were assessed with a high degree of reproducibility. The clinical parameters were assessed by a single examiner to reduce interexaminer error and get more accurate readings. Furthermore, the sample collection was done between 10 a.m. and 12 p.m. with complete isolation with cotton rolls. This time was selected as the GCF flow rate was not affected much during this period of the day.[22]

The limitations of the present study were that the generalized chronic periodontitis group was not categorized into mild, moderate, and severe groups. Larger sample size may be required to find out a definite correlation between the clinical parameters and biochemical parameters (GCF MMP3).

CONCLUSIONS

Within the limitations of our study, we can conclude that GCF MMP3 may be used as a novel early diagnostic biomarker in chronic periodontitis cases. We recommend that further studies need to be conducted to assess the role of MMP3 as a prognostic marker.

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Conflicts of interest

There are no conflicts of interest.

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