Effect of Scaling and Root Planing Based on MMP-8 mRNA Expression and Clinical Parameters in Periodontitis Patients

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Abstract

Matrix metalloproteinase-8 (MMP-8) is an important host factor involved in the pathogenesis of periodontal diseases, which is the main collagenase detected in gingival tissues. Collagenases destruct periodontal tissue and result in pocket depth (PD), clinical attachment loss (CAL) and bleeding papilla for slight provocation.

This study aims to evaluate the correlation between *MMP-8* mRNA expression and clinical parameters in periodontitis patient after scaling and root planning (SRP).

GCF from periodontitis patients and healthy subject were sampled and assessed for the *MMP-8* mRNA expression by quantitative polymerase chain reaction (q-PCR). The clinical parameters which were PD, papillary bleeding index (PBI), and CAL were assessed 1 month before and after SRP and they were further analyzed for their correlation to MMP-8 mRNA expression. Wilcoxon test and paired t-test were used to compare the mRNA transcription level, PD, CAL and PBI before and after SRP. Furthermore, the relationship between the clinical parameters and the *MMP-8* mRNA expression was also evaluated by Spearman test.

A significant reduction in the clinical parameters was noted after SRP than before it, although the difference of *MMP-8 mRNA* expression was not significant. This result indicated a weak positive correlation between the reduction in *MMP-8* expression and altered clinical parameters. SRP resulted in the improvement of clinical conditions of periodontal tissues, but not *MMP-8 mRNA* expression.

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Introduction

Periodontitis is an outcome of interaction between bacterial biofilms and host immune response.[1] *Tannerella forsythia*, *Pophyromonas gingivalis,* and *Treponema denticola* are some of the most common bacteria detected in patients with chronic periodontitis.[2]

Virulence factors produced by these bacteria enable them to colonize tooth surfaces and periodontal tissues as well as avoid host immune surveillance.[3] One of the important host factor involved in periodontal disease is matrix metalloproteinases (MMPs).[4] MMP-8 is the main collagenolytic MMP that is detected in gingival tissues and oral fluids.[1]

Matrix metalloproteinase-8 is known as neutrophil collagenase or collagenase 2. MMP-8 has the unique ability to break down the type I and III collagen which is critical for periodontal destruction. The predominance of MMP-8 in GCF correlated with increased number of polymorphonuclear neutrophils (PMNs) recruited as a part of the inflammatory response, which suggests that neutrophil hyperresponsiveness may contribute to tissue destruction in periodontal diseases.[4]

The treatment of periodontal disease consists of a few phases, such as the nonsurgical phase, surgical phase, restorative phase, and maintenance phase. Nonsurgical periodontal treatment aims to control the periodontal inflammation by reducing the perio-pathogenic micro-organisms and their products and by giving the host the chance to heal. It results in a reduction in bleeding on probing and

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probing depth and a gain in clinical attachment.[5] Scaling and root planning (SRP) are a part of the nonsurgical phase.[6] Past literatures have reported contradictive results with regard to MMP-8. For instance, Oender et al. reported significant reduction in the MMP-8 levels from patients with rheumatoid arthritis and periodontitis, healthy patients with periodontitis, and patients with rheumatoid arthritis and gingivitis.[7] A similar result was also reported by Kinane et al., who noted reduction in the MMP-8 levels (ng/30s) following an initial therapy at 6-8 weeks, although the reduction was not significant as compared to the baseline value.[8] However, a different result was reported by Wahyukundari et al., who claimed that the MMP-8 levels increased after SRP as compared to that at the initial examination, and the difference was significant.[9] These authors did not exclude smoking from their research, while some past studies demonstrated that smoking could influence the MMP-8 and MMP-9 levels.[9,10] In the present research, we excluded the smoking factor; therefore, the aim of the study was to analyze the effect of SRP on the mRNA expression of *MMP-8* and correlation with clinical parameters in patients with periodontitis.

Materials and methods

Patient selection

The study protocol was approved by the research committee of the Faculty of Dentistry Universitas Indonesia. Informed consent was obtained from the patients with regard to their participation. Six untreated chronic adult periodontitis patients and a periodontally healthy subject (age: 30-55 years) were recruited from the clinics of periodontics. Each participant (4 men) had 4-6-mm pocket depth with ≥2-mm loss of attachment and bleeding on probing. None of the participants had caries or filling in their proximal site, were pregnant, used mouth rinse, consumed antibiotics and contraception in the past 3 months, had received any periodontal treatment in the past 6 months, had diabetes mellitus or other systemic diseases, or were smokers. The participants completed their SRP. The mean time between sampling was 4 weeks.

Study outline

Baseline measurement, including that of papillary bleeding index, pocket depth, and clinical attachment loss, were recorded at the first visit for each patient. Gingival crevicular fluid (GCF) samples were collected after the first examination. Subsequently, SRP was performed on untreated chronic periodontitis patients. The periodontitis group was then reassessed 1 month after SRP; the post-treatment clinical measurements were recorded and the GCF samples were collected again from the same sites.

Clinical measurement, sampling, and laboratory technique

Pocket depths of 4-6-mm were selected, which was one pocket for one tooth. In the healthy group, the sulcus of the same site with the periodontitis group was selected. At each site, the papillary bleeding index and clinical attachment level were recorded. The pocket depth and clinical attachment level were measured with UNC-15 probe. Papillary bleeding index was measured by sweeping the margin gingiva in the buccal and palatal/lingual regions. Score 0 = no bleeding, 1 = a single bleeding point, 2 = several isolated bleeding points or a single fine line of blood, 3 = the interdental triangle fills with blood, and 4 = profuse bleeding.[11] GCF was collected by inserting a #20 paper point into the sulcus for 30 s. The expression of *MMP-8* mRNA was analyzed by quantitative polymerase chain reaction (q-PCR).

Analysis of MMP-8

The MMP-8 activity was analyzed by measurement of its mRNA expression by qPCR. RNA extraction was performed from the GCF sample by using guanidium thicyanite (TriPure Isolation Reagent). The extracted RNA was further reversed to cDNA by reverse transcriptase (ReverTra Ace® qPCR RT Master Mix with gDNA remover). All procedures were conducted in accordance with the company's guidelines. The primers used to amplify *MMP-8* mRNA were forward 5'-GCT GCT TAT GAA GAT TTT GAC AGA G-3' and reverse 5'-ACA GCC ACA TTT GAT TTT GCT TCA G-3'.[12] DNA or cDNA (3 μL) was amplified for the target genes in 7-μL reaction mixture containing 0.25-μL sense and antisense primers, 5-μL sybr (SensiFast™ SYBR® Hi-ROX Kit), and 1-μL nuclease-free water. *MMP-8* detection was performed with an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation (95°C for 30-35 s),

annealing (60°C for 35-60 s), and extension (72°C for 30-60 s).[12] Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers (forward 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse 5'-GAAGATGGTGATGGGATTTC-3') were used to detect the housekeeping gene. GAPDH detection was performed with an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation (94°C for 30 s), annealing (55°C for 30 s), and extension (72°C for 1 min).[13]

Statistical analysis

Wilcoxon test was used to analyze the mRNA expression of *MMP-8* and the clinical attachment loss before and after SRP because the distribution of data was not normal. Paired *t*-test was used to analyze the pocket depth and papillary bleeding index before and after SRP. Spearman test was used to analyze the correlation between the mRNA expression of *MMP-8* and the clinical parameters because the distribution of data was not normal.

Results

The healthy subject was not compared for clinical parameters (pocket depth, papillary bleeding index, and clinical attachment loss) because SRP was not performed to him. However, the data of fold gene expression of MMP-8 of healthy subject was used to determined relative quantification of MMP-8 mRNA expression in periodontitis subject.

The mean values used for papillary bleeding index and pocket depth because the distribution of data was normal. The median values used for clinical attachment loss and fold gene expression of MMP-8 because the distribution of data was not normal.

The results of clinical parameters (pocket depth, papillary bleeding index, and clinical attachment loss) and the fold *MMP-8* expression before and after SRP are given in Figure 1.

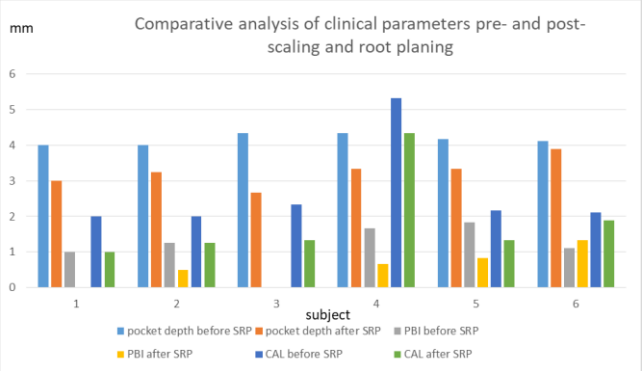

Figure 1. Comparison of clinical parameters before and after scaling and root planning.

Comparison of pocket depth before and after scaling and root planing

There was reduction of pocket depth (light blue and orange bars). Differences in the pocket depth before and after SRP were analyzed by paired *t*-test. A significant reduction in the pocket depth before and after SRP was noted (p=0.005).

Comparison of papillary bleeding index before and after scaling and root planing

There was reduction of papillary bleeding index (grey and yellow bars), except in subject 6. The absence bars in subject 1 and 3 showed no bleeding. Differences in the papillary bleeding index before and after SRP were analyzed by paired *t*-test. A significant reduction in the papillary bleeding index before and after SRP was noted (p=0.048).

Comparison of clinical attachment loss before and after scaling and root planing

There was reduction of clinical attachment loss (dark blue and green bars). Differences in the clinical attachment loss before and after SRP was analyzed by Wilcoxon test. A significant reduction the in the clinical attachment loss was noted before and after SRP (p=0.026)

Comparison of fold gene expression of MMP-8

Fold gene MMP-8 expression as showed in Figure 2 and 3 was reduced but not statistically significant. The fold gene expression of MMP-8 was reduced from 4218.59 to 11.8216. Differences in the fold *MMP-8* expression before and after SRP were analyzed by Wilcoxon test. No significant reduction was noted in the fold

MMP-8 expression before and after SRP (p=0.25) even the diagram showed a significant reduction. That result was caused by the distribution of data was not normal.

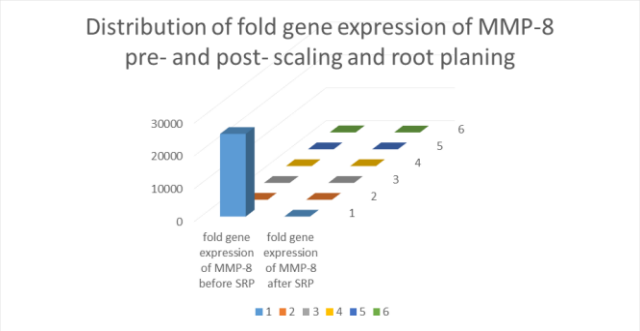

Figure 2. Distribution of fold gene expression of MMP-8 before and after SRP.

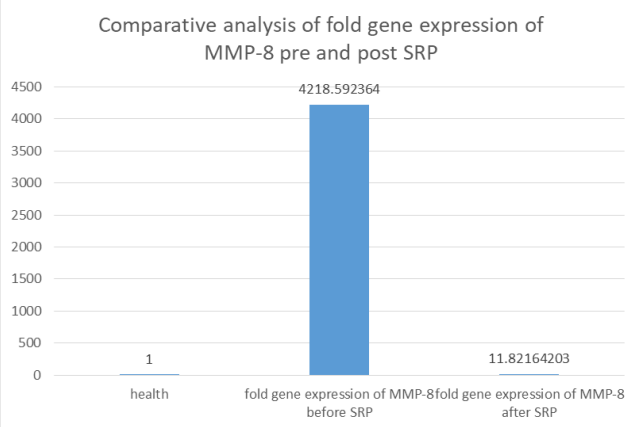

Figure 3. Comparative analysis of fold gene expression of MMP-8 before and after SRP.

Correlation of clinical parameters with mRNA expression of MMP-8

Correlation between the clinical parameters (i.e., alteration of pocket depth, papillary bleeding index, and clinical attachment loss) and the mRNA expression of *MMP-8* was analyzed by Spearman test. The results revealed a weak positive linear correlation between the alterations of fold *MMP-8* expression with the alterations in the pocket depth, papillary bleeding index, and clinical attachment loss (r = 0.232; r = 0,213; r = 0.213), which was not significantly correlation (p>0.05). Figure 4-6 shows correlation between alterations in the pocket depth, papillary bleeding index, and clinical attachment loss with the change in the fold *MMP-8* expression.

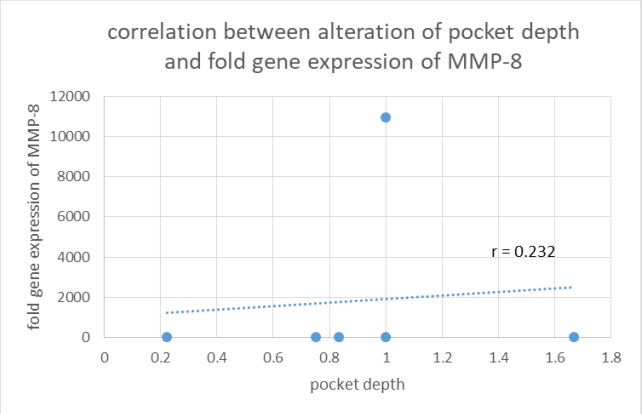

Figure 4. Correlation between alteration of pocket depth and fold gene expression of MMP-8.

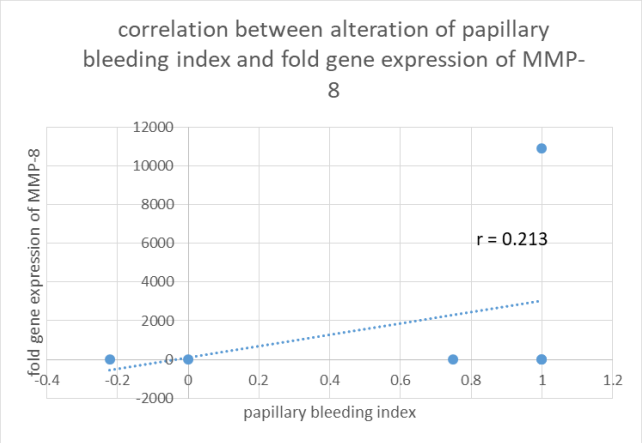

Figure 5. Correlation between alteration of papillary bleeding index and fold gene expression of MMP-8.

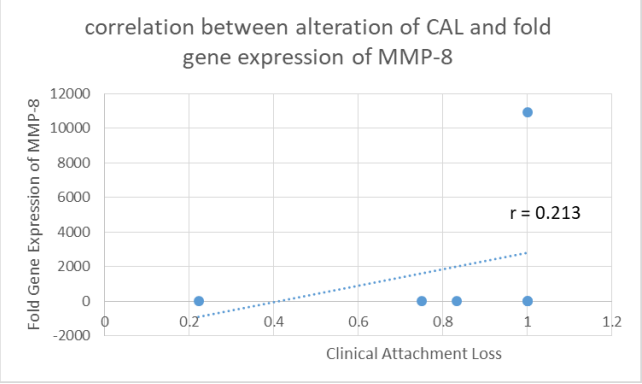

Figure 6. Correlation between alteration of papillary bleeding index and fold gene expression of MMP-8.

Discussion

The present study evaluated whether the efficiency of a scaling and root planing can be monitored by the expression of *MMP8* mRNA

and to analyze how the efficiency correlates with the clinical parameters or periodontal conditions in patients with chronic periodontitis. Scaling and root planing (SRP) are the first-line of treatment for chronic periodontitis and is accepted as the gold standard. They ensures the mechanical elimination of the source of periodontal pathogens and their byproducts.[14] SRP are included in nonsurgical therapy. Scaling is the process by which biofilm and calculus can be removed from both the supragingival and subgingival tooth surfaces. Root planing is the process by which residual embedded calculus and portions of cementum can be removed from the roots to produce a smooth, hard, and clean surface. The primary objective of SRP is to restore the gingival health by completely removing elements that provoke gingival inflammation (such as biofilms, calculus, and endotoxin) from the tooth surface.[6]

SRP therapy has been studied extensively to evaluate its effects on periodontal diseases. Suvan suggested that, in periodontitis patients, mechanical nonsurgical pocket therapy reduces inflammation and pocket depth and increases the clinical attachment level.[15] In the present study, the results revealed significant differences in the pocket depth, papillary bleeding index, and clinical attachment loss. The same results showed that the pocket depth reduction was significant.[14] The magnitude of pocket depth reduction is correlated with greater pocket depth before the treatment.[15] Studies ranging from 1 month to 2 years in length have demonstrated up to 80% reduction in bleeding on probing as well as a mean probing depth reduction of 2-3 mm. Other studies have demonstrated that the percent of periodontal pockets of depth ≥4 mm was reduced >50% and up to 80%.[6] A different result was reported by Konopka et al., who observed no significant changes in the mean clinical attachment loss values in chronic periodontitis patients during 4 weeks of periodontal therapy. However, SRP resulted in statistically significant decrease in the mean pocket depth on days 7 and 28 in comparison to that on day 0.[16]

In this report, the fold *MMP-8* expression revealed a reduction in the expression before and after treatment, albeit the difference was not significant. The same result was reported by Mouzakiti et al., who found that the mRNA expression of *MMP-8* was not significantly reduced after a nonsurgical treatment in comparison to that before. This result might be explained by the possible residual inflammation at the second sampling sites and the association of *MMP-8* with inflammation.[5] In the present study, a subject showed high papillary bleeding index even after nonsurgical therapy. A different result was reported by Konopka et al., who found that SRP resulted in a significant decrease in the amount of MMP-8 expression in the GCF.[16]

The correlation between alterations of MMP-8 expression with the clinical parameters revealed a weak positive linear relationship in the present study. This observation is in line with the report of Konopka et al., who showed that the levels of MMP-8 following SRP did not correlate with the change in clinical parameters.[16]

Limitations of this study include lack of sample size and healthy control. It could influence the result of the study.

Conclusions

Our observation indicated that 4 weeks after SRP, a significant improvement was noted in the resultant clinical parameters. However, the *MMP-8* mRNA expression did not significantly decrease after SRP. These findings may suggest SRP resulted in an improvement in the clinical condition of the periodontal disease, but not the MMP-8 mRNA expression. The correlation between alterations of MMP-8 expression and clinical parameters showed weak linear relationship. A study with larger sample size is needed.

Declaration of Interest

The authors report no conflict of interest and the study was funded by authors and Hibah PITTA Universitas Indonesia.

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