The Effects of Scaling and Root Planing on Mrna Expression of Matrix Metalloproteinase-1 and Clinical Parameters

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Abstract

The etiology of periodontal disease is multifactorial; however, studies show gram-negative microorganisms on subgingival plaque biofilms and host response, play important roles in initiation and progression of periodontitis. Matrix Metalloproteinase (MMP-1) is one of the pathogenic enzymes responsible for collagen and other protein degradation in periodontitis. The purpose of this research is to assess the effects of scaling and root planing on MMP-1 gene expression and how these processes correlate with other selected clinical parameters. We used quantitative real time PCR (qPCR) to compare the transcription level of MMP-1, before and after treatment of patients with periodontitis (n=6). Clinical data (pocket depth, bleeding index, and clinical attachment loss) were collected. There was no significant difference on mRNA expression of MMP-1 before and after scaling and root planing ($p \geq 0.05$). The transcription level of MMP-1 and pocket depth before treatment were significantly correlated ($p < 0.05$); however, other variables had no significant correlation. As initial therapy, scaling and root planing effectively reduces pocket depth in patients with chronic periodontitis and pocket depths of 4–6 mm. However, MMP-1 expression could not be used as a clinical biomarker for evaluating scaling and root planing. Therefore, further studies with larger sample sizes are necessary.

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Introduction

Periodontitis is a progressive disease affecting tooth-supporting tissues, such as gingiva, cementum, the periodontal ligament, and alveolar bone.[1] Periodontitis is a common disease, affecting 5–20% of the population worldwide. Periodontitis is a global health problem that can cause tooth loss and increase the risk of other systemic diseases, such as cardiovascular disease and stroke, diabetes, low birth weight babies, arthritis, and lung inflammation. Periodontitis should be diagnosed as early as possible and be treated as soon as possible to keep the teeth, periodontium, or peri-implant tissue healthy, comfortable, aesthetically pleasing, and functional.[2]

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Periodontal disease is a disease with multifactorial etiology; however, studies show gram-negative microorganisms on subgingival plaque biofilms, as well as host response, play an important role in initiation and progression of periodontitis. Matrix metalloproteinase (MMPs) are calcium-dependent, calcium-containing endopeptidase, and they are responsible for the degradation of the extracellular matrix. Human MMPs are divided into several subgroups, such as collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, -11, and -12), matrilysins (MMP-7 and -26), membrane type MMPs (MMP-14, -15, -16, -17, -24, and -25) and other MMPs (MMP-18, -19, -20, -21, -23, -27, and -28).[3] MMPs participate in physiological (bone remodeling, wound healing, angiogenesis, and apoptosis) and pathological processes (heart disease, arthritis, cancer and periodontal disease). In physiological processes, there is a balance between MMPs and their inhibitors, called *tissue inhibitors of metalloproteinase* (TIMPs). In patients with periodontal disease, MMP expression and activation are influenced by virulent bacterium

and cytokines. MMP levels that increase and exceed TIMPs will disrupt the balance between MMPs and TIMPs. This imbalance will cause enzyme activation that degrades the extracellular matrix and alveolar bone.[4,5]

MMP-1 or interstitial collagenase, plays an important role in the destruction of connective tissue during inflammatory conditions.[6] Kubota et al. showed an increase in MMP-1 mRNA expression in the gingival tissue of patients with periodontitis, compared to healthy patients.[7] We sought to analyze clinical improvements in periodontal tissue, pocket depth, clinical attachment, gingival bleeding index, and MMP-1 mRNA expression, before and 1 month after scaling and root planing in patients with chronic periodontitis with absolute pockets 4–6 mm in depth. The quantitative real time polymerase chain reaction (qPCR) method was used to determine MMP-1 mRNA expression. The qPCR method was chosen because it is a fast, accurate, and sensitive means of quantifying gene expression.

Materials and methods

The Ethical Committee of the Faculty of Dentistry at Universitas Indonesia approved this study (No: 17/Ethical Approval/FKGUI/III/2018). Written informed consent was obtained from each study patient. This study is a clinical and microbiological experimental study, and the subjects were patients who came to the teaching hospital Fakultas Kedokteran Gigi Universitas Indonesia (FKG UI) and were diagnosed with chronic periodontitis. The periodontal statuses of all the included subjects were assessed according to the methods for classifying periodontal disease established by the American Academy of Periodontology. We included males and females, age 35–55 years, diagnosed with chronic periodontitis (Pocket depths of 4–6 mm, loss of attachment ≥4 mm, and bleeding on probing). The exclusion criteria were pregnancy, smoking, use of antibiotics or mouthwash within the last 3 months, periodontal therapy in the past 6 months, or the presence of systemic diseases such as diabetes mellitus, osteoporosis, and immune deficiency syndrome.

Two dentists performed all the clinical examinations. The examiners were blinded to patient assignment to the various interventions. All the patients underwent full mouth examination of periodontal status, including pocket depth, loss of attachment, and papilla bleeding index at six sites (buccal-mesial, mid-buccal, buccal-distal, lingual-mesial, mid-lingual, and lingual-distal). Sampling of gingival crevicular fluid was performed to determine MMP-1 mRNA expression. Gingival Crevicular Fluid (GCF) was taken from 6 patients with chronic periodontitis and from 1 healthy control subject. The patients were considered healthy if they exhibited probing depths <3 mm and had no clinical attachment loss. GCF sampling was taken twice: at the first examination (before scaling and root planing) and 1 month after scaling and root planing. Sharp sickles and ultrasonic instruments were used to perform scaling and root planing. GCF was sampled by gently inserting #20 sterile paper point into each periodontal pocket with pocket depth of 4–6 mm and the selected site was the site that has the deepest pocket. After 20 seconds, three paper points from the sampled tooth were placed immediately in a microcentrifuge tube containing 1 ml of sterile TE buffer. All the samples were stored at -80^{O}C immediately after collection.

Laboratory examination to determine mRNA expression of MMP-1 using qPCR was conducted in the Oral Biology Laboratory of FKG UI. All the samples were run in duplicate in MicroAmp™ Fast Optical 48-well plates in Applied Biosystems StepOne Plus (Applied Biosystems, Foster City, CA, USA). The protocol was as follows: 95^{o}C for 10 minutes, followed by 40 cycles of 95^{o}C for 30 s, 60^{o}C for 30 s, and 72^{o}C for 1 minute. Following amplification, melting curve analysis was performed to verify the authenticity of the amplified products by their specific melting temperatures (Applied Biosystems). PCR amplification was carried out in 10-µl reactions containing 3 µl of cDNA, 0.5 µl of each specific primer (1 µl), 5 µl of SensiFAST SYBR® Hi-ROX Kit, and 1 µl of nuclease free water. Primer sequences of MMP-1 (Forward 5'-GAAGGTGATGAAGCAGCCCAGATGT-3' and reverse 5'-CAGTTGTGGCCAGAAAACAGAAATGAAA-3') were as described by Guan et al., 2009.[8] The sequence for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the mRNA expression of MMP-1. Primer sequences of GAPDH (Forward 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse 5'-GAAGATGGTGATGGGATTTC-3')[9] was used,

and the qPCR protocol consisted of 95°C for 10 minutes, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C, where ΔΔCt = ΔCt (treated sample) – ΔCt (control/healthy person). SPSS 22.0 was used to analyze the differences in expression of mRNA MMP-1 between the 2 groups (before and after treatment) using Wilcoxon test ($p<0.05$).

We used normality testing to check the data distributions and selected either parametric or non-parametric tests based on the results. To assess the efficacy of scaling and root planing for changing the clinical parameters and microbiology, before and 1 month after treatment, we used paired *t*-tests or the Wilcoxon test.

Results

Four male and 2 female patients with an average age of 42.67 ± 8.33 years were recruited for this study. Table 1 presents the demographic data.

We used the Shapiro-Wilk test of normality because each patient group had fewer than 50 members (Table 2). Pocket depth after scaling and the bleeding index before scaling were normally distributed; however, the other data were non-normally distributed, so a comparative analysis was done using the Wilcoxon (non-parametric) test.

The Wilcoxon test was used to analyze the differences in pocket depth before and after scaling and root planing. Table 3 shows that patients with chronic periodontitis had significantly deeper pocket depths before scaling and root planing, compared to after scaling and root planing ($p<0.05$).

	Chronic periodontitis
Demographic characteristics	
Age (years)	42.67±8.33
Sex	
Male	4 (66.67%)
Female	2 (33.33%)
Clinical Parameters	
PD	4.08±0.27

Table 1. Demographic Data. PD=pocket depth

Variables	p-value
Pocket depth before scaling	0.000
Pocket depth after scaling	0.184
Bleeding index before scaling	0.089
Bleeding index after scaling	0.025
CAL before scaling	0.000
CAL after scaling	0.000
Fold Gene Expression of MMP-1 before scaling	0.007
Fold Gene Expression of MMP-1 after scaling	0.000

Table 2. Normality Test Results. *Shapiro-Wilk* test*;* CAL, clinical attachment level; *p<0.05 normal data distribution.

Pocket depth	Mean (SD)	p-value
Before SRP	4.36 (0.804)	0.027
After SRP	3.22 (0.431)	

Table 3. Comparative Analysis of Pocket Depth in Patients with Chronic Periodontitis Before and After Scaling and Root Planing.

The Wilcoxon test was used to analyze the differences in pocket depth before and after scaling and root planing. Table 3 shows that patients with chronic periodontitis had significantly deeper pocket depths before scaling and root planing, compared to after scaling and root planning ($p<0.05$).

The Wilcoxon test was used to analyze the differences in bleeding index before and after scaling and root planing. Table 4 showed higher bleeding index in patients with chronic periodontitis before scaling and root planing compared to after scaling and root planing; however, the difference was not significant ($p\geq0.05$).

The Wilcoxon test was used to analyze the differences in clinical attachment loss before and after scaling and root planing. Our results indicated significantly higher clinical attachment loss in patients with chronic periodontitis before scaling and root planing, compared to after scaling and root planing (Table 5) ($p < 0.05$).

The Wilcoxon test was used to analyze the differences in fold gene expression of MMP-1 before and after scaling and root planing. There was higher fold gene expression of MMP-1 in patients with chronic periodontitis before scaling and root planing, compared to after scaling and root planing; however, the difference did not rise to the level of statistical significance ($p \geq 0.05$).

Based on diagram 1, fold gene expression of MMP-1 in patients with chronic periodontitis before treatment was 0.6x compared to the control group. After treatment, fold gene expression of MMP-1 was 0.1x compared to the control group. The diagram showed there was downregulation of MMP-1 fold gene expression after treatment.

The Spearman test was used to analyze the correlations between fold gene expression of MMP-1 and pocket depth, between fold gene expression of MMP-1 and bleeding index, and between fold gene expression of MMP-1 and clinical attachment loss. Table 7 shows that the fold gene expression of MMP-1 and pocket depth, before scaling and root planing, had a significant negative correlation ($p < 0.05$) and very strong relationship ($r = -0.880$); meanwhile, bleeding index and clinical attachment loss had no statistically significant correlation with fold gene expression of MMP-1 ($p \geq 0.05$).

Bleeding index	Mean (SD)	p-value
Before SRP	1.40 (0.781)	0.066
After SRP	0.54 (0.777)	

Table 4. Comparative Analysis of Bleeding Index in Patients with Chronic Periodontitis Before and After Scaling and Root Planing. Wilcoxon test; SRP, scaling and root planing; *p<0.05 indicates a significant difference.

CAL	Mean (SD)	p-value
Before SRP	4.50 (1.225)	0.020
After SRP	3.33 (0.816)	

Table 5. Comparative Analysis of Clinical Attachment Loss in Patients with Chronic Periodontitis Before and After Scaling and Root Planing.

Fold gene expression of MMP-1	Mean (SD)	p-value
Before SRP	0.64 (0.904)	0.249
After SRP	0.11 (0.190)	

Table 6. Comparative Analysis of Fold Gene Expression of MMP-1 in Patients with Chronic Periodontitis Before and After Scaling and Root Planing.

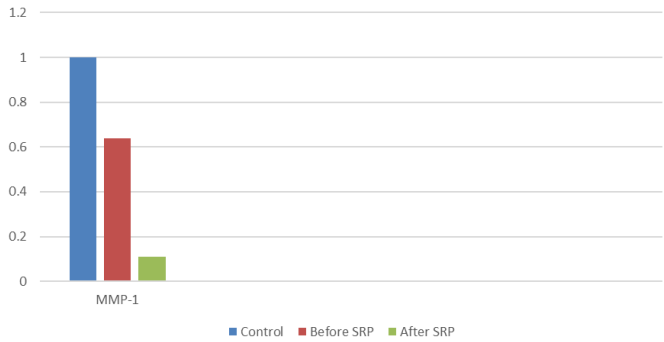

Diagram 1. Fold Gene Expression of MMP-1 in Patients with Chronic Periodontitis Before and After Scaling and Root Planing.

	r	p-value
Pocket depth – MMP-1 before	-0.880	0.021
Pocket depth – MMP-1 after	-0.319	0.538
Bleeding index – MMP-1 before	-0.464	0.354
Bleeding index – MMP-1 after	-0.030	0.954
CAL – MMP-1 before	-0.393	0.441
CAL – MMP-1 after	-0.655	0.158

Table 7. Correlation Analysis of Pocket Depth, Bleeding Index, and Clinical Attachment Loss with Fold Gene Expression of MMP-1 in Patients with Chronic Periodontitis Before and After Scaling and Root Planing. *Spearman* test; CAL, clinical attachment loss; *p<0.05 indicates a significant correlation.

Discussion

We treated patients with chronic periodontitis with scaling and root planing (non-surgical periodontal therapy) and oral hygiene instruction. Our data showed that pocket depth was significantly improved after nonsurgical periodontal treatment; however, the bleeding index was not significantly reduced.

After treatment, the pocket depth was reduced from 4.36 to 3.22, and the clinical attachment loss was reduced from 4.50 to 3.33, which were statistically significant changes ($p < 0.005$). These results are similar to those found by Ghodpage et al. and Pozo et al.[10,11] Additionally, while the bleeding index reduced, the reductions did not rise to the level of statistical significance ($p \geq 0.005$). This result is similar to Pozo et al., but differs from Ghodpage et al., who found that all clinical

parameters, including papillary bleeding index, were significantly reduced 6 weeks after scaling and root planning.[10,11] This could be because nonsurgical treatments, such as scaling and root planing, often reduce inflammation, but do not eliminate inflammation completely;[12] therefore, the bleeding index was not significantly reduced.

When we analyzed MMP-1 gene expression in GCF in patients with chronic periodontitis, we found that after treatment, the transcription level of MMP-1 mRNA was reduced from 0.64 to an after-treatment level of 0.11; however, this down regulation was not statistically significant. A similar result was reported previously by Mouzakiti et al.[13]

We further evaluated the possible correlation between RNA expression of the targeted gene and select clinical parameters (pocket depth, bleeding index, and clinical attachment loss). We found a significantly strong and negative correlation between fold gene expression of MMP-1 and pocket depth before scaling and root planing ($p < 0.05$). Meanwhile, the correlations between fold gene expression of MMP-1 and other clinical parameters (bleeding index and clinical loss attachment) were not significant. Additional studies, using a similar methodology and larger sample size, should be performed to confirm this result. This is because different methods of GCF sampling and laboratory analysis techniques, and variations in follow up time, may influence the MMP-1 gene expression and clinical parameter results. These factors may also affect the observed correlations between clinical and microbiological parameters.

Conclusions

Scaling and root planing are effective initial therapies for reducing pocket depth in patients with chronic periodontitis and pocket depths of 4–6 mm. Second, MMP-1 gene expression cannot be used as biomarker for periodontal disease progression. Further studies with larger sample sizes are needed to clarify the involvement of MMP-1 in the healing processes of periodontitis.

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Declaration of Interest

The authors report no conflict of interest.

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