Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2019; 19:e5210
DOI:http://doi.org/10.4034/PBOCI.2019.191.134
ISSN 1519-0501

ORIGINAL ARTICLE

Scaling and Root Planing Effect to mRNA Expression of Matrix Metalloproteinase-9 and Periodontal Clinical Parameters on Chronic Periodontitis

Mikha Sundjojo[1], Robert Lessang[2], Yuniarti Soeroso[3], Boy Muchlis Bachtiar[4]

[1]Periodontology Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. 0000-0002-5184-8883
[2]Department of Periodontology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. 0000-0001-6997-5736
[3]Department of Periodontology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. 0000-0003-4660-3045
[4]Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. 0000-0002-4027-778X

Author to whom correspondence should be addressed: Robert Lessang, Department of Periodontology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. Phone: +62 213911502. E-mail: robertlessang@gmail.com.

Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 12 March 2019 / Accepted: 16 August 2019 / Published: 09 September 2019

Abstract

Objective: To evaluate the relationship between the mRNA transcription level of Matrix Metalloproteinase-9 (MMP-9) and the selected clinical periodontal healing at one month of scaling and root planing. **Material and Methods:** A total of six chronic periodontitis patients and one periodontally healthy subject were recruited. The gingival crevicular fluid was collected from all subjects, and the expression level of MMP-9 mRNA was measured by quantitative real-time PCR. Pocket depth, papilla bleeding index, and clinical attachment loss were measured on day 1 at baseline and day 30. Scaling and root planing was performed on day 1. Data were analyzed using SPSS 22.0 software. **Results:** In comparison to the control, periodontal clinical parameters in the treatment group were significantly reduced after scaling and root planing. MMP-9 mRNA expression did not show a significant change after the 30th day. A weak correlation was noted between the MMP-9 mRNA transcription level and the changed PBI measurement. **Conclusion:** Scaling and root planing is clinically effective for chronic periodontitis with a 4-6 mm pocket, whereas the expression of MMP-9 mRNA was not altered. Further studies with a more extended observation period are needed to confirm or reject the present findings.

Keywords: Matrix Metalloproteinase 9; Dental Scaling; Root Planing; Periodontal Pocket.

Introduction

Periodontal diseases have a high prevalence, affecting 20-50% of the global population, and it is one of the major causes of tooth loss in adults [1]. Interaction of bacteria plaque with host response triggers inflammation, which leads to periodontal attachment loss and bone destruction [3,4].

The tissue breakdown events that lead to the clinical signs of disease are a result of the host inflammatory response to the bacterial plaque [5]. Matrix metalloproteinases (MMPs) are involved in the physiological degradation of extracellular matrix proteins and basement membranes. They are considered mediators of tissue destruction and are strongly correlated with the occurrence of periodontal diseases. MMP-9, also known as gelatinase B, degrades type-IV collagen and regulates basement membrane remodeling. MMP-9 is expressed by polymorphonuclear leukocytes, macrophages, and epithelial cells, which reflect periodontal disease severity, progression, and treatment response [3,4,6]. MMP-9 level increases in the gingival crevicular fluid (GCF) of sites with active periodontal diseases [7,8].

Non-surgical therapy is known to successfully arrest periodontitis progression in shallow to moderate pockets. The goal of non-surgical treatment is to eliminate or reduce pathogens (shifting microbial flora) to create a favorable environment, which will stabilize periodontal conditions achieved by scaling and root planing (SRP), dental health education, and patient motivation [9].

We hypothesized that the mRNA expression of MMP-9 in GCF decreases following SRP performed on CP patients with a pocket depth (PD) of 4-6 mm. Therefore, the present study aimed to assess the effect of scaling and root planing on the mRNA expression of MMP-9 in gingival crevicular fluid over one month as well as to evaluate its correlation with the clinical parameters of the healing progress of the periodontal tissues.

Material and Methods

Subject Selection

The study was conducted from March 2018 to June 2018 at the Periodontic Clinic Faculty of Dentistry, Universitas Indonesia. The study included six chronic periodontitis patients and one healthy subject. The inclusion criteria were chronic periodontitis patients of age 35-55 years with 4 to 6 mm pockets, loss of clinical attachment ≥2 mm, bleeding on probing, and exclusion of the third molar. The exclusion criteria were patients with systemic diseases, smokers, patients who had undergone a periodontal treatment in the past six months, pregnant or breastfeeding patients, patients using contraceptives, and patients who have taken antibiotics in the last three months.

The healthy periodontal subject exhibited a probing depth of <3 mm, no bleeding on probing, and no clinical attachment loss. The subjects signed an informed consent form after receiving information about this study. Data collected during the first visit were followed by SRP using an ultrasonic instrument and collected again after one month.

Clinical Assessment

Periodontal clinical parameters consist of Pocket Depth (PD), Papilla Bleeding Index (PBI), and Clinical Attachment Loss (CAL). The parameters were measured on six sides of each tooth (i.e., mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) using a periodontal probe instrument UNC-15 (MND Co. Ltd, Seoul, Korea). PD is the distance (in millimeters) from the free gingival margin to the base of the pocket. PBI is measured by carefully inserting a periodontal probe into the margin of the gingival sulcus and scored as follows: 0 =no bleeding; 1 = bleeding in the form of a point; 2 = bleeding in the form of a line; 3 = bleeding in the form of a triangle; and 4 = widespread bleeding. CAL is the distance (in millimeters) from the border of the cemento-enamel junction to the base of the pocket.

GCF Collection and RNA Extraction

Teeth were isolated using a cotton roll, followed by the removal of the supragingival plaque to avoid GCF contamination. Then, the crevicular site was gently dried with air syringe, followed by inserting three paper points (number 20) into the deepest pocket site for 20 s. The paper points were further placed into a microcentrifuge tube containing 1 mL of sterile TE buffer in an icebox. All clinical samples were immediately transferred to a laboratory for in vitro processing. In this study, RNA extraction and transcription to cDNA were performed using a Toyobo kit. All procedures were performed in accordance with the instructions provided by the company. For RT-PCR reaction, the final volume was 10 µL per microtube, which contained 3 µL of cDNA, 0.5 µLeach of forward and reverse primers, 5 µL of the SensiFAST SYBR® Hi-ROX Kit, and 1 µL of nuclease-free water. The amplification cycling conditions were initial denaturation at 95°C for 10 min, followed by 40 cycles of annealing at 60°C for 60s and an extension at 72°C for 30 s [10]. All samples were run in duplicate in the MicroAmp™ Fast Optical 48-well Plates in Applied Biosystems StepOne Plus (Applied Biosystems Inc., Foster City, CA, USA). The primers used in the amplification phase were MMP-9 primers (forward 5′-GGG ACG CAG ACA TCG TCA TC-3′ and reverse 5′-TCG TCA TCG TCG AAA TGG GC-3′). The primers for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used to normalize the mRNA expression of MMP-9. The primer sequences of GAPDH consisted of forwarding primer 5′-GAAGGTGAAGGTCGGAGTC-3′ and reverse primer 5′-GAAGATGGTGATGGGATTTC-3′ [11], and the PCR cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles at 94°C for 30 s, at 55°C for 30 s, and at 72°C for 30 s. The formula used to calculate the difference in the expression of mRNA MMP-9 between the two groups (before and after treatment) was $2^{-\Delta\Delta Ct}$.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS Statistics Software, version 22 (IBM Corp., Armonk, NY, USA), where a p-value of <0.05 was considered statistically significant. A normality test was performed to examine the normality of each data. According to the results, we

selected either parametric tests or non-parametric tests to analyze the data to investigate the effectiveness of SRP for changing them RNA expression of MMP-9 and the clinical parameters before SRP and 1 month afterward. Wilcoxon test was used to analyze CAL and fold-gene expression of MMP-9. PD and PBI were analyzed using a paired t-test.

The correlation analysis of MMP-9 and periodontal clinical parameters from the same site of GCF collection was performed at baseline and 1 month after collecting the SRP data. In this study, MMP-9 showed an abnormal data distribution; therefore, analyses were performed with the Spearman test, considering $p<0.05$ to be statistically significant.

Ethical Aspects

This research was approved by the Ethical Committee of the Faculty of Dentistry, Universitas Indonesia (Protocol No. 090240218).

Results

A statistically significant difference was noted in the periodontal clinical parameters between baseline and at 1 month of SRP on the 4-6 mm pocket of CP patients. No statistically significant difference in MMP-9 was noted between baseline and at 1 month after SRP on the 4-6 mm pocket of CP patients. A paired t-test was used to analyze PD and PBI, and the Wilcoxon test was used to analyze CAL and MMP-9. The results of these comparative analyses are shown in Table 1. Correlation analysis was performed with the Spearman test (Table 2).

Table 1. Distribution of clinical parameters after one month of SRP.

Variables	Mean (SD)		Median (Minimum–Maximum)		p-value
	Baseline	One Month	Baseline	One Month	
PD	4.10 (0.10)	3.21 (0.33)			0.002
PBI	1.12 (0.62)	0.56 (0.48)			0.048
CAL			2.07 (2.00–5.25)	1.30 (1.00–4.25)	0.024
MMP-9			1.61 (0.51–99.73)	1.26 (0.19–2.89)	0.463

*$p<0.05$ indicate a significant difference.

A weak correlation in the positive direction was revealed on the alteration of MMP-9 with the alteration of PBI (Figure 1).

Table 2. Correlational analysis of the alteration of the fold gene expression of MMP-9 with the alteration of PD, PBI, and CAL.

Variables	R	p-value
Alteration of MMP-9 – PD	-0.030	0.954
Alteration of MMP-9 – PBI	0.116	0.827
Alteration of MMP-9 – CAL	-0.135	0.798

*$p<0.05$ indicate a significant difference

No correlation was noted in the negative direction on the alteration of MMP-9 with the alteration of PD and on the alteration of MMP-9 with the alteration of CAL.

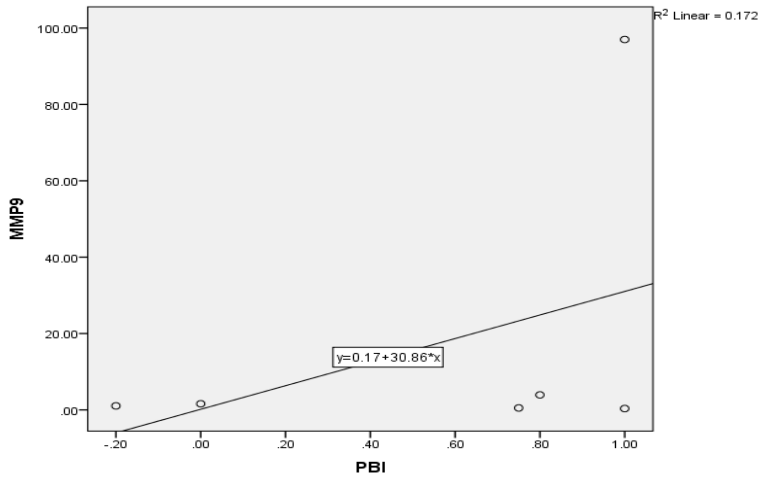

Figure 1. Correlational diagram between the Alteration of MMP-9 and alteration of PBI at 1 month after SRP.

Discussion

In this study, we found an improvement in the periodontal clinical parameters and reduced expression of MMP-9 in GCF after 1month of scaling and root planing in CP patients with 4-6 mm pockets. However, the reduced MMP-9 was not statistically significant. Alteration of MMP-9 expression showed no negative correlation with the alteration of PD and CAL, and a weak correlation in the positive direction was noted with the alteration of PBI.

Cumulatively, our results suggest that SRP succeeded in treating CP with 4-6 mm pockets, in accordance with the previous findings [12]. Thus, periodontal clinical parameters are essential in diagnosing periodontal tissue destruction, and PBI is more correlated with periodontal disease activity. To evaluate the activity of periodontal diseases or predict future prognosis, investigation of the inflammatory molecule level was considered [13].

MMPs contribute to both normal and pathological tissue remodeling. The physiological roles of MMPs include cell migration, tissue remodeling during organogenesis and growth, wound healing, angiogenesis, enamel formation, and antigen processing and presentation. These roles are strongly correlated to periodontal diseases since MMPs are the major players in collagen breakdown during periodontal tissue destruction. Gelatinases (MMP-2,-9) degrade the denaturated collagen gelatin [4].

The presence of MMP in oral fluids provides additional information about disease progression. Oral fluid samples were used to investigate MMP and their regulators as potential candidates in chair-side tests for monitoring periodontal and peri-implant diseases. For estimating the level of MMP, samples can be collected from the saliva, GCF, and tissues [5]. A previous study found that the analysis of the MMP-9group in the saliva before and after SRP was not a precise indicator of periodontal disease using the zymographic assay [6]. Several studies have suggested that MMP-9 in GCFhas a higher concentration following a severe inflammation [5]. Here, no statistically significant reduction of MMP-9 was noted at 1 month of SRP. Our findings are in line

with those of past studies that showed decreased MMP-9 levels in non-surgical therapy. Some authors reported decreased MMP-9 in GCF (over three months) and circulating blood after non-surgical treatment [7,14.], while others reported increased MMP-9 during a periodontal disease, which effectively reduced with non-surgical therapy [4].

MMPs as inflammatory molecules released from different cell types (e.g., macrophages, leukocytes, fibroblast, and other resident cells) in a lesion play a direct role in the degradation of both non-mineralized and mineralized tissues of the periodontium. MMP is produced at low levels or not at all in resting-state adult tissues [5]. A good body of evidence suggests that MMP-9is essential in bone resorption for osteoclast to access the resorption site, and it has been recently considered as important for osteoblast in bone development and formation [4]. The alteration of MMP-9 is weakly correlated in the positive direction with the alteration of PBI; this result may be attributed to ongoing inflammatory activities. Regarding the evidence of MMP's role in the periodontal destruction process, the study of MMP may provide information about whether, in the future, it will be important to use a host modulatory agent such as an MMP inhibitor in adjunctive therapy combined with conventional treatment.

Conclusion

Scaling and root planing is clinically effective for chronic periodontitis patients with 4-6-mm pockets, despite no statistically significant result of MMP-9 mRNA expression in the gingival crevicular fluid after 1 month of scaling and root planing. The weak correlation between MMP-9 and Papilla Bleeding Index may be attributed to ongoing inflammatory activities. Further studies with a longer observation period are needed to confirm or reject the above findings.

Authors' Contributions: MS performed the data collection, data analysis and interpretation, and wrote the manuscript. RL and BMB designed the study, performed data analysis, data interpretation and reviewed the manuscript. YS performed the data analysis, data interpretation, and reviewed the manuscript. All authors made significant contribution to this content and approved to be published.

Financial Support: Faculty of Dentistry, Universitas Indonesia and funds from HIBAH PITTA 2018, DRPM Universitas Indonesia (Grant No. 2179/UN.Rs.1/HKP.05.00/2018).

Conflict of Interest: The authors declare no conflicts of interest.

References

[1] Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci 2017; 11(2):72-80.

[2] Tadjoedin FM, Fitri AH, Kuswandani SO, Sulijaya B, Soeroso Y. The correlation between age and periodontal diseases. J Int Dent Med Res 2017; 10(2):327-32.

[3] Franco C, Patricia H, Timo S, Claudia B, Marcela H. Matrix metalloproteinases as regulators of periodontal inflammation. Int J Mol Sci 2017; 18(2):E440. https://doi.org/10.3390/ijms18020440

[4] Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand 2007; 65(1):1-13. https://doi.org/10.1080/00016350600963640

[5] Navjot KP, Nisha KJ, Sanjeela G, Shyam P. Role of matrixmetalloproteinases in periodontal disease - A review. Biomed J Sci Tech Res 2018; 2(1):2099-2104. https://doi.org/10.26717/BJSTR.2018.02.000632

[6] Gonçalves RP, Damante CA, Lima FLM, Imbronito AV, Nunes FD, Pustiglioni FE. Detection of MMP-2 and MMP-9 salivary levels in patients with chronic periodontitis before and after periodontal treatment. Rev Odonto Ciência 2009; 24(3):264-9.

[7] Marcaccini AM, Meschiari CA, Zuardi LR, de Sousa TS, Taba M Jr, Teofilo JM, et al. Gingival crevicular fluid levels of MMP-8, MMP-9, TIMP-2, and MPO decrease after periodontal therapy. J Clin Periodontol 2010; 37(2):180-90. https://doi.org/10.1111/j.1600-051x.2009.01512.x

[8] Hadi CA, Suhartono W, Sulijaya B, Sarwono AT, Yuniastuti M, Auerkari EI. Distribution of matrix metalloproteinase – 9 (Mmp-9) – 1562 C/T gene polymorphism in Indonesian males with periodontitis. J Int Dent Med Res 2017; 10:778-81.

[9] Aljateeli M, Koticha T, Bashutski J, Sugai JV, Braun TM, Giannobile WV, et al. Surgical periodontal therapy with and without initial scaling and root planing in the management of chronic periodontitis: A randomized clinical trial. J Clin Periodontol 2014; 41(7):693-700. https://doi.org/10.1111/jcpe.12259

[10] Mu X, Urso ML, Murray K, Fu F, Li Y. Relaxin regulates MMP expression and promotes satellite cell mobilization during muscle healing in both young and aged mice. Am J Pathol 2010; 177(5):2399-2410. https://doi.org/10.2353/ajpath.2010.091121

[11] Radonic A, Thulke S, Mackay IM, Landt O, Siegert W, Nitsche A. Guideline to reference gene selection for quantitative real-time PCR. Biochem Biophys Res Commun 2014; 313(4):856-62. https://doi.org/10.1016/j.bbrc.2003.11.177

[12] Meseli SE, Kuru B, Kuru L. Relationships between initial probing depth and changes in the clinical parameters following non-surgical periodontal treatment in chronic periodontitis. J Istanbul Univ Fac Dent 2017; 51(3):11-7. https://doi.org/10.17096/jiufd.40993

[13] Buduneli N, Kinane DF. Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. J Clin Periodontol 2011; 38(Suppl 11):85-105. https://doi.org/10.1111/j.1600-051X.2010.01670.x

[14] Marcaccini AM1, Novaes AB Jr, Meschiari CA, Souza SL, Palioto DB, Sorgi CA, et al. Circulating matrix metalloproteinase-8 (MMP-8) and MMP-9 are increased in chronic periodontal disease and decrease after non-surgical periodontal therapy. Clin Chim Acta 2009; 409(1-2):117-22. https://doi.org/10.1016/j.cca.2009.09.012