

RESEARCH ARTICLE

## Comparison of matrix metalloproteinase-13 and tissue inhibitor of metalloproteinase-1 levels and alveolar bone density in chronic periodontitis before and after scaling and root planning

Aini Hariyani Nasution\*✉, Lidya Irani Nainggolan\*\*, Widiyanto Meydhyono\*\*\*

\*Department of Periodontic, Faculty of Dentistry, Universitas Sumatera Utara, Medan, North Sumatra, Indonesia

\*\*Department of Dental Radiology, Faculty of Dentistry, Universitas Sumatera Utara, Medan, North Sumatra, Indonesia

\*\*\*Periodontics Specialty Program, Faculty of Dentistry, Universitas Sumatera Utara, Medan, North Sumatra, Indonesia

\*JI Alumni No 2, Universitas Sumatera Utara, Medan, North Sumatra, Indonesia; ✉ correspondence: [aini@usu.ac.id](mailto:aini@usu.ac.id)

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### ABSTRACT

Periodontitis is typically associated with disorders characterized by compromised tooth-supporting tissue. Damage to periodontal tissue is caused by an imbalance between matrix metalloproteinases and their inhibitors. Decreased tissue inhibitor and elevated matrix metalloproteinase levels result in collagen connective tissue and bone degradation. Several studies have shown that high levels of matrix metalloproteinase-13 (MMP-13) and low levels of tissue inhibitor of metalloproteinase-1 (TIMP-1) are also found in gingival crevicular fluid and saliva of patients with periodontitis. The purpose of this study was to determine the comparison of MMP-13 levels, TIMP-1 levels of saliva and bone density in patients with chronic periodontitis before and after scaling and root planning (SRP). The study samples were selected from patients who came for treatment at the Periodontics Installation of Universitas Sumatera Utara. A total of 16 patients were selected ( $n = 16$ ) with a diagnosis of chronic periodontitis. The result showed that salivary MMP-13 levels in chronic periodontitis patients before SRP were higher than salivary MMP-13 levels after SRP and the difference was statistically significant ( $p < 0.05$ ). It was also revealed that salivary TIMP-1 levels and alveolar bone density in chronic periodontitis patients before SRP were lower than that after SRP and the difference was statistically significant ( $p < 0.05$ ). There was a positive correlation between clinical parameters and salivary MMP-13 levels in patients with chronic periodontitis before and after SRP, but it was not statistically significant ( $p > 0.05$ ). There was a negative correlation between clinical parameters and salivary TIMP-1 levels in patients with chronic periodontitis before and after SRP, but it was not statistically significant ( $p > 0.05$ ).

**Keywords:** alveolar bone density; chronic periodontitis; MMP-13; saliva; TIMP-1

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### INTRODUCTION

Periodontitis is generally associated with conditions where the tooth supporting tissue is damaged.<sup>1</sup> Upregulation and Downregulation of matrix metalloproteinases is caused by some periodontal pathogens and by inflammatory cytokines.<sup>2</sup> In periodontal disease, the synthesis and secretion of matrix metalloproteinase (MMP) is impaired and there is an increase in neutrophil levels.<sup>3</sup> MMP is present in normal circumstances and is in the process of healing. Increased MMP levels will result in degradation of the protein matrix in periodontitis. Periodontal damage is the result of an imbalance between matrix metalloproteinase and its inhibitors. Decreased tissue inhibitor and increased matrix metalloproteinase level leads to degradation of collagen connective tissue and bone.<sup>4</sup>

MMP-13 was first discovered in breast cancer. MMP is involved in inflammatory diseases associated with bone resorption and destruction, such as rheumatoid arthritis and osteoarthritis. Studies have shown that high level of MMP-13 was found in the saliva of periodontitis patients.<sup>5</sup> On the other hand, the main function of tissue inhibitor of matrix metalloproteinase (TIMP) is to inhibit MMP. TIMP is also involved in transportation and stabilization of MMP. The imbalance between MMP and TIMP is responsible for the process of periodontal disease, which begins with damage to the extracellular matrix and ultimately to the alveolar bone.<sup>6</sup> TIMP-1 is known as an inhibitor of matrix metalloproteinase.<sup>7</sup>

Saliva contains biomarkers specific to periodontitis and thus qualitative changes in

the composition of these biomarkers can be diagnosed. Experimental evidence suggests that the most important pathway involving MMP as active collagenase and gelatinase is found not only in gingival sulcus fluid but also in saliva and biopsy specimens in inflammatory periodontal tissue.<sup>8</sup> Saliva can be a useful diagnostic tool in periodontal disease, in which they consist of non-invasive procedures and are able to provide condition of oral cavity.<sup>3</sup>

Dental radiography is a supportive examination that is often used for diagnosis and treatment plan management. One of the frequently-used techniques of dental radiography is periapical radiography. Trabecular bone can be visualized on periapical radiography. Hard tissue damage can be seen from changes in trabecular density as an indicator of bone density reduction. The image processing on periapical radiographs by means of computerization using filters on ImageJ software can obtain fractal frequency images.<sup>9,10,11,12</sup>

Hernandez et al., who studied the levels of MMP-13 and TIMP-1 in the gingival crevicular fluid of patients with chronic periodontitis, demonstrated an increase in MMP-13 expression and a decrease in TIMP-1 levels.<sup>2</sup> This study aimed to determine the comparison of salivary MMP-13 levels and TIMP-1 levels and alveolar bone density in patients with chronic periodontitis patients before and after scaling and root planning (SRP).

## MATERIALS AND METHODS

This study was an observational analytic study, which was designed as a cross-sectional study and was performed in 2019. The research samples were selected by means of purposive sampling from patients who came for treatment at the Periodontics Installation of the Dental and Oral Hospital of Universitas Sumatera Utara (USU). The sampling selected 16 patients who were diagnosed with chronic periodontitis. Patients were selected based on the following inclusion criteria: patients had at least 15 teeth, had not performed periodontal treatment in the last 3 months, aged 25 - 55 years, were willing to undergo an examination

and sign an informed consent. Subjects of chronic periodontitis were patients who had lost of clinical attachment of  $\geq 3$  mm. The exclusion criteria were patients with a history of systemic disease, smoking, alcoholics, patients taking vitamins, antibiotics and anti-inflammatory drugs in the past month, using mouthwash regularly, having pregnancy and lactation.

Saliva was collected by draining method by instructing subjects to collect saliva on the floor of the mouth as much as  $\pm 2$  ml and spitting it on the saliva pot. Before the samples were taken, the patients were asked to rinse their mouth with water. Saliva samples were then put into a cooling box and then taken to the Integrated Laboratory in the Faculty of Medicine USU to be stored in a freezer ( $-80$  °C) before the examination was carried out.

Saliva samples were transported at a cold temperature to the Integrated Laboratory of the Faculty of Medicine, USU. Saliva were stored in the freezer ( $-80$  °C) because they were not examined immediately. The saliva was transferred to the 10  $\mu$ L eppendorf tube using a micropipette. After the number of samples was sufficient, saliva was examined.

Reagent preparation was carried out by diluting 30 mL of wash buffer concentrate into a 750 mL wash buffer solution using distilled water. Preparation of the standard solution was preceded by standard liquid centrifugation at 10,000 mg for 1 minute before 1 ml of the reference standard and a sample diluent was added. The dilution of the reference standard to various concentrations was determined. Subsequently, Biotinylated Detection Ab (1 : 100) and Concentrated HRP Conjugate (1 : 100) were diluted.

The examination procedure was started by adding 100  $\mu$ L standard solution or sample to each well and incubated at 37 °C for 90 minutes. The liquid was removed from each well without washing, then a 100  $\mu$ L Biotinylated Ab Detection working solution was added to each well and covered with a sealer plate. The liquid was stirred slowly and then incubated at 37 °C for 1 hour. The liquid was sucked and emptied from each well and 350  $\mu$ L of washing buffer was added to each well.

After 1-2 minutes the liquid was sucked back in and the back of the well was tapped dry.

Each well was added with 100 µL HRP conjugate working solution. Sealer plate was closed again and incubated for 30 minutes at 37 °C. Each well was sucked again and the washing process was repeated 5 times. After that, 90 µL of Reagent Substrate was added to each well, covered again with a plate sealer and incubated for 15 minutes at 37 °C. The plates were protected from light with aluminum foil. Afterwards, each well was added with a 50 µL stop solution before the optical density (OD) value of each well was determined using a microplate reader with a wavelength of 450 nm. The procedure of scaling and root planing was carried out in patients with chronic periodontitis and then observations were made at the first month.

After the research subjects signed their consent, a periapical X-ray was taken at the Dental Radiology Unit of USU Hospital before scaling and 1 month after scaling. Furthermore, periapical X-ray photographs were collected from the research subjects followed by an image processing to calculate fractal dimension values using imageJ software. Finally, the region of interest was determined in the distal mesial region until quantitative fractal dimension values were obtained.

## RESULTS

This study was conducted to see differences in salivary MMP-13 levels and TIMP-1 levels and alveolar bone density in chronic periodontitis patients before and after scaling and root planing and the relationship of MMP-13 TIMP-1 with clinical parameters of periodontal disease. Subject groups were selected based on inclusion and exclusion criteria. The subjects of chronic periodontitis patients were 16 patients who came for treatment at the Periodontics Installation of the Dental and Oral Hospital of Universitas Sumatera Utara.g

Table 1 shows that the average age of the chronic periodontitis group was  $49.06 \pm 7,353$  years. Gender differences of all the chronic periodontitis

patients who served as the study subjects were almost evenly distributed. All subjects brushed their teeth regularly every day and almost all of them brushed their teeth with a frequency of more than 2 times a day. Most of the subjects of chronic periodontitis had never been treated with scaling. Table 2 shows that pocket depth, attachment loss, gingival index and papillary bleeding index in the chronic periodontitis of the group before SRP were significantly higher ( $p < 0.05$ ) than in the chronic periodontitis of the group after SRP. Wilcoxon test results showed that salivary MMP-13 levels in the chronic periodontitis of the group before SRP were significantly higher than in the chronic periodontitis of the group after SRP ( $p < 0.05$ ). (Table 3)

Furthermore, Wilcoxon test also revealed that salivary TIMP-1 levels in the chronic periodontitis of the group before SRP were significantly lower than in the chronic periodontitis of the group after SRP ( $p < 0.05$ ) (Table 4). Alveolar bone density in the chronic periodontitis of the group before SRP was lower than in the chronic periodontitis of the group after SRP and the difference was statistically significant ( $p < 0.05$ ). (Table 5)

Table 6 shows that patients of chronic periodontitis in the group before and after SRP indicated a positive correlation between salivary MMP-13 with clinical parameters although it was

**Table 1.** Characteristics of research subjects

Characteristics	Chronic periodontitis (n = 16)
Age	
Mean ± SD	(49.06 ± 7.353)
Sex [n (%)]	
Male	7 (43.8)
Female	9 (56.3)
Frequency of tooth brushing [n (%)]	
0 – 1	5 (31.3)
≥ 2	11 (68.8)
Scaling experience [n (%)]	
No	12 (75.0)
Yes	4 (25.0)

**Table 2.** Independent t-test results on pocket depth, attachment loss, and papillary bleeding index and mann-whitney u test results on the gingival index between chronic periodontitis subjects before and after SRP

Clinical Parameters	Before SRP	After SRP	p
Pocket depth	4.20 ± 0.926	3.56 ± 0.695	0.00*
Attachment loss	5.50 ± 0.932	4.75 ± 0.622	0.00*
Gingival index	2.06 (1.04 – 2.78)	0.23 (0.08 – 0.38)	0.00*
Papillary bleeding index	75.31 (61 - 90)	8.13 ± (7 - 9)	0.00*

Independent t-test (pocket depth, attachment loss)

\*significant p < 0.05

Wilcoxon Test (gingival index and papillary bleeding index)

\*significant p < 0.05

**Table 3.** Salivary MMP-13 levels in chronic periodontitis patients before and after SRP

Variable	Before SRP	After SRP	p
Salivary MMP-13 level (ng/ml)	0.85 (0.33 – 1.41)	0.46 (0.08 – 1.40)	0.005

Wilcoxon test

\*significant p < 0.05

**Table 4.** Salivary TIMP-1 levels in chronic periodontitis patients before and after SRP

Variable	Before SRP	After SRP	p
Salivary TIMP-1 level (ng/ml)	265.75 (100 - 458)	378.50 (114 - 497)	0.034*

Wilcoxon test

\*significant p < 0.05

**Table 5.** Alveolar bone density in chronic periodontitis patients before and after SRP

Variable	Before SRP	After SRP	p
Alveolar bone density	0.85 ± 0.11	0.97 ± 0.12	0.001*

Independent t-test

\*significant p < 0.05

not statistically significant (p < 0.05). Table 7 shows that in the group of chronic periodontitis patients before and after SRP, there was a negative correlation between salivary TIMP-1 with clinical parameters although it was not statistically significant (p < 0.05).

## DISCUSSION

In this study, MMP-13 levels in the group of patients with chronic periodontitis saliva before SRP were higher than the MMP-13 levels of patients with

the chronic periodontitis saliva after SRP and the difference was statistically significant. This result is in accordance with the research of Pawar, et al who examined the effects of phase 1 periodontal therapy on matrix metalloproteinase-3 and -13 levels in gingival crevicular fluids. Pawar found that MMP-3 and MMP-13 levels of gingival crevicular fluid was higher in the chronic periodontitis group before SRP than that in the chronic periodontitis group after SRP and the results were statistically significant.<sup>13</sup> Another similar study was conducted

**Table 6.** Correlation test between clinical parameters and salivary MMP-13 levels in chronic periodontitis patients before and after SRP

Clinical parameters	Salivary MMP-13 levels			
	Before SRP		After SRP	
	r	p	r	p
Pocket depth	0.150	0.578	0.046	0.867
Attachment loss	0.309	0.244	0.228	0.395
Gingival index	0.079	0.770	0.063	0.818
Papillary bleeding index	0.013	0.963	0.130	0.631

Spearman Test, 2-tailed before SRP: (MMP-13 → gingival index); after SRP: (MMP-13 → pocket depth, attachment loss, gingival index and papillary bleeding index)

\*significant p < 0.05

Pearson test, 2-tailed before SRP: (MMP-13 → pocket depth, attachment loss, and papillary bleeding index)

\*significant p < 0.05

**Table 7.** Correlation test between clinical parameters and salivary TIMP-1 levels in chronic periodontitis patients before and after SRP

Clinical Parameters	Salivary TIMP-1 levels			
	Before SRP		After SRP	
	r	p	r	p
Pocket depth	-0.524	0.037	-0.241	0.368
Attachment loss	-0.270	0.312	-0.097	0.721
Gingival index	-0.208	0.441	-0.012	0.965
Papillary bleeding index	-0.146	0.588	-0.019	0.945

Pearson test, 2-tailed before SRP: (TIMP-1 → pocket depth, attachment loss, and papillary bleeding index)

\*significant p < 0.05

Spearman Test, 2-tailed before SRP: (TIMP-1 → gingival index); after SRP: (TIMP-1 → pocket depth, attachment loss, gingival index and papillary bleeding index)

\*significant p < 0.05

by Goncalves, et al on periodontal treatment for decreasing levels of MMP in localized aggressive periodontitis. Goncalves found that there was a decrease in MMP-1, MMP-8, MMP-9, MMP-12, and MMP-13 in the gingival sulcus fluid in patients with localized aggressive periodontitis after phase 1 treatment compared to healthy areas.<sup>14</sup>

This study points out that salivary MMP-13 levels are positively correlated with levels of clinical parameters (pocket depth, attachment level, gingival index and papilla bleeding index) in patients with chronic periodontitis and in healthy patients. This is consistent with the research by Gonzalves et al, which showed that MMP-13 levels were correlated with the average percentage of

pocket depth before and after phase 1 treatment. Accordingly, this study disclosed that MMP-1, MMP-8, MMP-9 levels, MMP-12 and MMP-13 were associated with the periodontal disease process and they were also shown to decrease the levels of MMP-8, MMP-9, and MMP-13 after phase 1 treatment.<sup>14</sup>

In this study, the levels of TIMP-1 saliva in the chronic periodontitis group before SRP were lower than the TIMP-1 levels in the chronic periodontitis group after SRP and the difference was statistically significant. These results are consistent with the study of Ghodpage, et al who found out that low salivary TIMP-1 levels in the chronic periodontitis group before SRP was 12.88,

much lower than those in the chronic periodontitis group after SRP with a value of 20.46 and this difference was statistically significant.<sup>15</sup> Other related study was conducted by Kumar, et al on the comparison of MMP-3 and TIMP-1 levels of gingival sulcus fluid in healthy, diseased, and after treatment patients. Kumar demonstrated that TIMP-1 levels in the gingival sulcus fluid of chronic periodontitis patients decreased with the course of the disease and TIMP-1 levels in periodontitis patients increased after scaling and root planning treatment.<sup>16</sup>

This study highlighted that salivary TIMP-1 levels were negatively correlated with levels of clinical parameters (pocket depth, attachment level, gingival index and papilla bleeding index) in patients with chronic periodontitis before SRP and in patients with chronic periodontitis after SRP. This is consistent with the research by Fenol, et al, which showed that the TIMP-1 level of the gingival crevicular fluid was negatively correlated with the level of attachment and gingival index in patients with chronic periodontitis before and after SRP, although this correlation was not statistically significant.<sup>4</sup>

In this study, alveolar bone density in the chronic periodontitis group before SRP were lower than that in the chronic periodontitis group after SRP and the difference was statistically significant. These results are consistent with a research conducted by Barros, et al who figured out that there was an increase in alveolar bone density 90 and 180 days after non-surgical treatment.<sup>17</sup> These results are also in accordance with research by Salim, et al, on the effects of scaling and root planning on alveolar bone density and on the number of *Porphyromonas gingivalis* and *Treponema denticule*. Salim pinpointed that there was an increase in bone density radiographically before and after scaling and root planning treatments.<sup>18</sup>

Saliva is one of the most studied fluids in the body to detect a disease. Saliva contains protein, mRNA, and DNA used as a biological marker for clinical applications. Saliva and blood serum contain the same protein and RNA. In the examination of periodontal disease, many investigators focus primarily on biomarkers of gingival fluid that

provide information on local disease status, but it is a difficult approach to use as a clinical application. Saliva has many advantages as a clinical tool, including the simplicity of its collection, ease of storage and delivery, cost effectiveness, ease of availability at large sample volume for analysis, and possibility for repeated sampling.<sup>19,20</sup>

## CONCLUSION

Salivary MMP-13 levels in chronic periodontitis patients before SRP were higher than salivary MMP-13 levels in chronic periodontitis patients after SRP, while salivary TIMP-1 levels and alveolar bone density in chronic periodontitis patients before SRP were lower than salivary TIMP-1 levels in chronic periodontitis patients after SRP. Saliva can be used as an early diagnostic tool for the development of periodontal disease.

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