The Analysis of Matrix Metalloproteinase-8 in Gingival Crevicular Fluid and Periodontal Diseases

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Abstract

Background: Periodontal disease, also generally called periodontitis or gum disease, is a chronic infection-induced inflammatory disease that causes tooth loss if not properly treated, and is considered as a modifying factor in systemic health. Gingival crevicular fluid (GCF) matrix metalloproteinase-8 (MMP-8) is an inflammatory marker found in periodontal pathologic conditions. Gingivitis, a nondestructive type of periodontal disease, can progress to periodontitis if left untreated. Therefore, assessing the level of MMP-8 with comfortable methods and no tissue intervention can determine the progression of the periodontal disease for a better treatment. **Objective:** The purpose of the present study is to determine the relationship between MMP-8 in GCF and periodontal disease. **Setting and Design:** This is a cross-sectional study that took place in West Sumatra, Indonesia from June to December 2013. **Materials and Methods:** This study involves 60 respondents who are divided into three groups based on the periodontal disease index. The samples consist of 20 healthy individuals, 20 with mild gingivitis, and 20 periodontitis initial. GCF was collected from each group. MMP8 level in GCF was tested by using ELISA technique. **Statistical Analysis:** Data were analyzed with SPSS version 17 Software. ANOVA test was used to determine the differences in average levels of MMP-8. Bonferroni *post hoc* test was used to discover which spesific means differed. **Results:** The level of MMP-8 is significantly different between the healthy group and mild gingivitis group, between the healthy group with mild periodontitis group, and also between groups with mild gingivitis and mild periodontitis ($P < 0.05$). **Conclusion:** The findings of this study can be used by practitioners of dentistry to establish a proper diagnosis and appropriate treatment of periodontal disease by measuring the scale of MMP-8, to prevent or to minimize further complication in periodontitis patients.

Keywords: *Matrix metalloproteinase-8, periodontal disease, gingival crevicular fluid*

Introduction

Periodontitis (inflammation around the tooth) is a chronic infection-induced inflammatory disease that causes tooth loss and is considered a modifying factor in systemic health.[1] Periodontal disease is a disease of the periodontal tissues that results in attachment loss and destruction of alveolar bone. Several pathogens are associated with periodontitis. Porphyromonas gingivalis is one of the major pathogens in chronic periodontitis (CP). The natural history of periodontal disease, in some but not all patients, results in tooth loss. Periodontal disease, however, encompasses a wider spectrum of diseases than only periodontitis and the recognition of these diseases requires a diagnosis be made.[2] Periodontology nowadays is very different from what it was 20 years ago, and will most likely become as different over the next 20 years to come. Assessment of patients will be more specific, and the development of risk profiles will allow identification of individuals who require greater or lesser amounts of care. Finally, as patients continue care on a maintenance schedule, their status will be assessed by sensitive tests that will predict the onset of an active phase of disease so that appropriate interceptive treatment can be provided.[3] The "gold standard" for active periodontal disease is not available and inflammation due to gingivally confined lesions (gingivitis) and periodontal inflammation which results in attachment loss is a potential confounder of any test based on assessing the host response elements of the disease.[4]

Gingival crevicular fluid (GCF) can be found in the physiologic space (gingival sulcus), as well as in the pathological space (gingival pocket or periodontal pocket) between the

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gums and teeth. In the first case, it is a transudate and in the second case, it is an exudate. The constituents of GCF originate from serum, gingival tissues, and from both bacterial and host response cells present in the aforementioned spaces and the surrounding tissues. The collection and analysis of GCF are the noninvasive methods for the evaluation of host response in periodontal disease. These analyses mainly focus on inflammatory markers involved in periodontal disease progression.[5] Matrix metalloproteinases (MMPs) are a family of 24 proteases that act in physiological and pathological conditions. They can degrade almost all extracellular matrix constituents and regulate inflammatory processes. They are mainly inhibited by tissue inhibitors of metalloproteinases.[6] MMP-8 (collagenase 2) is a collagenolytic enzyme that can initiate the digestion of type I collagen, the most dominant interstitial collagen type in the periodontal tissues. Collagen degradation is considered as one of the key factors in the uncontrolled tissue destruction in periodontitis.[1] MMP-8 correlated positively with clinical signs of gingival/periodontal inflammation.[7]

Materials and Methods

This cross-sectional study was approved by the Ethical Committee of the Faculty of Medicine, Andalas University under the authorization number 247/KEP/FK/2013. The researchers are trained dentists who are competent in their respective fields. The sample sized was calculated by the formula:

$$n1 = n2 = n3 = 2\left[\frac{(z\alpha + z\beta)s}{\chi_1 - \chi_2}\right]^2$$

$$n1 = n2 = n3 = 2\left[\frac{(196 + 0842)507)}{(2350 - 1850)}\right]^2$$

$$n1 = n2 = n3 = 2\left[\frac{(14,207)}{(5)}\right]^2$$

$$n1 = n2 = n3 = 16,154$$

The study sample includes 20 patients with mild gingivitis, 20 with mild periodontitis, and 20 healthy others. Subject are patients who visit and seek for dental treatment in Rasidin Municipal Hospital from June to December 2013. They consist of 14 males and 46 females whose ages range from 17 to 35.

Periodontal disease index

Periodontal disease index was first used in periodontal tissue screening. Gums or periodontal tissue was inspected by using an instrument called a periodontal probe (©Osung). This tool was used to measure the depth of the sulcus gums (which are V-shaped gaps between the teeth and gums). The normal depth of gums sulcus ranges from 0 to 3 mm. Gingivitis or periodontitis can cause increased sulcus depth and form a pocket. The higher the degree of severity of the disease, the deeper the pocket formed. The periodontal probe can also be used in determining the severity of bleeding of the gums. In healthy individuals, there is no sign of inflammation. Mild gingivitis signs were reddish shiny gingiva with edema, bleeding on probing. If one of the two sides are measured, the gingival sulcus extends to 3 mm and the apical cementoenamel junction, then it is mild periodontitis.

Gingival crevicular fluid collection

The patients sat in the dental chair and the plaque in the area where GCF was extracted was cleaned up and dried. To equalize conditions and to minimize the involvement of oral bacteria, patients were instructed to rinse their mouths with a solution of 2% chlorhexidine. Then, lip retractor was attached and the area isolated with a cotton roll. Absorbent paper (©Gapadent) was inserted by using superficial intracrevicular technique and was left in place for 3 min. The absorbent paper was removed and put in 5 ml Eppendorf tubes (©Eppendorf) already containing phosphate buffered saline buffer solution. Specimens were labeled clearly. Samples which were taken to be analyzed by using ELISA were stored at −20°C.

ELISA

This study uses ELISA Kit, reagan/KIT MMP-8 RPN2619. Human Biotrak System by Amersham Biosciences, Reagents, samples and a number of wells (that which was not used was stored back in 40C temperatures) were prepared. A volume of 150 mL assay diluent was poured into each well. A 50 mL standard and the sample were poured into each well that has been set. The plate was closed with a plastic cover then was incubated for 2 h at room temperature in a 50–500 rpm shaker. All the fluid was discharged in the well and set aside some time after 400 µl wash buffer was added. The washing process was repeated as much as three times. A volume of 200 mL of MMP-8 Conjugate was added to each well and was incubated for 2 h at room temperature. A volume of 200 µl substrate solution was added to each well that was incubated for 30 min at room temperature. Then 50 mL stop solution was added to each well. The liquid changed color from blue to yellow. Elisa plate was read at a wavelength of 450 nm.

Statistical analysis

Data were analyzed by using SPSS version 17 (SPSS.inc, Chicago). MMP-8 were tabulated and verified statistically by using the Kolmogorov–Smirnov test to determine the normal distribution of data. The relationship between MMP-8 and periodontal disease index level was tested by using ANOVA.

Results

Samples were taken from patients who visited the hospital dental clinic in Rasidin Padang Municipality during the

year 2013. The study involves 60 patients consisting of 20 healthy individuals, 20 patients with mild gingivitis and 20 patients with mild periodontitis who meet the inclusion criteria of a consecutive sampling method.

The average age of female $\bar{\chi}$ ± standard deviation (SD) = 23.39 ± 4.39 is higher than male ± SD = 21.15 ± 31.18. This difference is statistically insignificant, which means that by age, men and women are equal [Table 2].

There are differences in the average levels of MMP-8 and the terms of PDI group, which is the highest in the mild periodontitis with mean = 40.16 ± 2.64 ng/dl. The above table also indicates that light gingivitis patients are likely to have elevated levels of MMP-8 5.2-fold compared to healthy condition patients, while the condition of mild periodontitis patients is 9.5-fold compared to healthy conditions [Table 1]. The following table shows the differences between groups in accordance with *post hoc* Bonferroni test [Table 3].

According to Table 4, there are significant differences of the average MMP-8 between the healthy group and mild gingivitis, healthy group and mild periodontitis, and between mild gingivitis and mild periodontitis ($P < 0.05$).

Discussion

MMP-8 is a promising biomarker candidate for oral fluid (GCF, peri-implant sulcular fluid and saliva) and mouthrinse chair-side/point-of-care diagnostics to predict, diagnose, and determine the progressive phases of episodic periodontitis and peri-implantitis, as well as to monitor the treatments and medications.[8] The results of the current study show that the mean MMP-8 concentrations are higher in mild periodontitis patients than it is in mild gingivitis and healthy individuals. The increase in levels seen among three groups is statistically significant ($P < 0.05$). This is in line with the study of Leppilahti *et al*. who identified the diagnostic accuracy of GCF biomarker candidate to discriminate periodontitis from the inflamed and healthy sites, and to compare the performance of MMP-8 immunoassays. MMP-8 is higher in periodontitis than it is in gingivitis and healthy sites ($P < 0.05$).[9] The study of Killi *et al*. (2002), on the other hand, shows that the percentage of MMP-8 enzyme correlates significantly with gingival and bleeding index ($P < 0.05$).[10] The study by Marcaccini which aimed at comparing the levels of MMP-8 in the GCF of CP patients and controls at baseline and 3 months after nonsurgical therapy shows that higher levels of MMP-8 are found in periodontitis patients compared with controls ($P < 0.001$), and these molecules decreased after 3 months periodontal therapy ($P < 0.03$). Rai *et al*. compared MMP-8 level in GCF in 15 healthy individuals, 18 individuals with gingivitis, and 20 individuals with periodontitis. They concluded that elevated MMP-8 levels were highly correlated to probing depth, clinical loss of attachment, bleeding on probing, and age in a manner consistent with the features of the periodontal disease.[11]

The presence of some collagen fibers such as types I, IV, and VI has already been observed in healthy and diseased gingival tissue.[12] Type 1 collagen is the major extracellular matrix component of gingiva.[13] It acquires a significant

Table 1: Periodontal disease index criteria

Score	Criteria
0	Negative. There is neither overt inflammation in the investing tissues nor loss of function due to destruction of supporting tissue
1	Mild gingivitis. There is an overt area of inflammation in the free gingiva which does not circumscribe the tooth
2	Gingivitis. Inflammation completely circumscribes the tooth, but there is no apparent break in the epithelial attchment
6	Gingivitis with pocket formation (mild periodontitis). The epithelial attachment has been broken and there is a pocket (not merely a deepened gingival crevice due to swelling in the free gingiva). There is no interference with normal masticatory function, the tooth is firm in its socket, and has not drifted
8	Advanced destruction with loss of masticatory function. The tooth may sound dull on percussion with a metallic instrument; may be depressible in its socket

Table 2: Average age of research subjects by sex

Age	Sex	*n*	Mean±SD	*P*
20- 35	Male	14	21,0±3,18	0.14
17 - 35	Female	46	23,39±4,39	

SD=Standard deviation

Table 3: Average difference of matrix metalloproteinase-8 (ng/dl) in gingival crevicular fluid and periodontal disease based on periodontal disease index

Enzyme	PDI	*F*	Mean±SD	*P*
MMP8	Healthy	20	4,1±4,41	0,001
	Mild gingivitis	20	21,69±3,94	
	Mild periodontitis	20	40,16±2,64	
	Total	60	22,02±15,25	

PDI=Periodontal disease index, SD=Standard deviation, MMP=Matrix metalloproteinase

Table 4: Results of *post hoc* Bonferroni test enzyme levels of matrix metalloproteinase-8 between periodontal disease index groups

	MMP-8 level		
Enzyme	Healthy	Mild gingivitis	Mild periodontitis
Healthy	-	0.00	0.00
Mild gingivitis	0.00	-	0.00
Mild periodontitis	0.00	0.00	-

MMP=Matrix metalloproteinase

role in disease progression, as collagen degradation is pointed as the main marker of periodontal disease progression. Collagen I represents the bulk component of the periodontal extracellular matrix. Accordingly, collagenase or collagenolytic MMPs (MMP-1, -8, -13, and -14) and gelatinase MMPs (MMP-2 and -9) play a pivotal role in the loss of periodontal support on the basis of their collagen-degrading properties.[8] MMP-8 is the most effective in hydrolyzing Type I collagen. MMP-8 share the unique ability to cleave Types I, II, and III collagen and probably serve as initiators of the majority of extracellular matrix destruction in periodontal disease.

MMP-8 (collagenase-2) is synthesized by differentiating granulocytes in the bone marrow and stored in specific granules of circulating neutrophils.[14] Thus, synthesis and activation of MMP-8 are important steps in the pathological extracellular matrix destruction associated with the inflammatory periodontal disease.[15] Inflammatory cells such as neutrophils and macrophages produce MMPs, with neutrophils being the major source of collagenase and gelatinase in inflammatory diseases such as periodontitis. Epithelial cells can also produce elevated levels of these enzymes, which may facilitate the apical migration and lateral extension of the junctional epithelium and the subsequent loss of connective tissue attachment. Inflammatory cells, particularly neutrophils, are thought to play a particularly important role in the MMP-mediated periodontal destructive lesion.[16] The other cellular sources are sulcular epithelial cells, gingival and periodontal ligament fibroblasts, monocytes/macrophages, and plasma cells. MMP-8 is detected as a latent proenzyme in the GCF of shallow pockets, but in deep periodontal pockets, it is converted to the active form.[17] This may explain the elevated levels in mild periodontitis compared to gingivitis and healthy controls.

Recent studies have shown that high level of MMP-8 can result in more severe inflammation. Overall, the testing of MMP-8 in GCF represents a promising diagnostic tool to identify patients' and sites at risk for periodontal disease progression and prediction of treatment response.

Conclusion

Periodontitis ranges from gum inflammation to serious disease that results in major injuries to tissue and bone that support the teeth. When not properly treated, periodontitis can lead to tooth lost. This study has shown that assessing the level of MMP-8 with comfortable methods and no tissue intervention can determine the progression of the periodontal disease for better treatment. The findings of this study can be used by dentists to diagnose and treat periodontal disease by measuring the scale of MMP-8, to prevent or to minimize further complication in periodontitis patients.

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Conflicts of interest

There are no conflicts of interest.

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