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

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[illegible]

Abstract

[illegible]

Introduction

Periodontitis [illegible] is a chronic infection [illegible] disease that can see tooth [illegible] Periodontal disease is a [illegible] of the periodontal tissues that results in [illegible] and destruction of alveolar bone. Several pathogens are [illegible] with periodontitis [illegible] gingivitis [illegible] periodontitis [illegible]. The natural history of periodontal disease [illegible] an [illegible] tooth loss. Periodontal disease, however, [illegible] spectrum of diseases [illegible] periodontitis and the [illegible] of these diseases [illegible] a diagnosis be made [illegible]. Periodontology [illegible] today is very different from what it was 20 years ago, and will most likely

become as different over the next 20 years [illegible]. [illegible] of patients will be [illegible] and the [illegible] of risk profiles will allow identification of individuals [illegible] greater or lesser amounts of care [illegible] status will be assessed by sensitive tests that [illegible] predict [illegible] of an active phase of disease so that appropriate interceptive treatment can be provided [illegible]. The "gold standard" [illegible] periodontal [illegible] inflammation [illegible] gingivitis and periodontal [illegible] which [illegible] attachment loss, is a potential [illegible] of any test [illegible] measuring the host response [illegible]

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

[illegible]

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[illegible]

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 sulcus and tissued 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.[3] Matrix metalloproteinases (MMPs) are a family of 24 proteinases that act in physiological and pathological conditions.[4] They can degrade almost all extracellular matrix constituents and regulate inflammatory processes. They are mainly inhibited by tissue inhibitors of metalloproteinases.[5] MMP-8 (collagenase 2) is a collagenolytic enzyme that can initiate the digestion of type 1 collagen, the predominant interstitial collagen type in the periodontal tissues. Collagen degradation is considered as one of the key factors in the uncontrolled tissue destruction in periodontitis.[6] 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 registration number 247/KEP/FK/2013. The researchers are trained dentists who are competent in their respective fields. The sample size was calculated by the formula:

nl = n2 = n3 = 2 [(Zα + Zβ) s / (X1 − X2)]²

n1 = n2 = n3 = 2 [(1.96 + 0.842) (50.7) / (2350 − 1850)]²

n1 = n2 = n3 = 2 (1.42)²

(5)

n1 = n2 = n3 = 4.54

The study samples includes 20 patients with moderate gingivitis, 20 with mild periodontitis and 20 healthy others. Subjects 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 was determined by periodontal tissue screening. Gingival or periodontal tissue was inspected by using an instrument named a periodontal probe (Chung). This tool was used to measure the depth of the sulcus gum, which are V-shaped gaps between the teeth and gums. The normal depth of gum 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 on 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, human MMP-8 RPN2639 Human Biotrak System by Amersham Biosciences Reagents samples and a number of wells (that which was not used was stored back at 4°C temperatures) were prepared. A volume of 150 ml assay diluent was pipetted into each well. A 50 ml standard and the samples were pipetted into each well that has been set. The plate was closed with a plastic cover that was incubated for 2 h at room temperature on a 50-500 rpm shaker. As 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 μl of MMP-8 Conjugate was added to each well and was incubated for 2 h at room temperature. A volume of 200 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. Then plate 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 at 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 X ± 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 = 40.18 ± 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 in oral fluid (GCF, peri-implant sulcular fluid and saliva) and [illegible] diagnostics to predict, diagnose and differentiate the progressive phases of [illegible] periodontitis and peri-implantitis as well as to monitor the treatments and medications. 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 was statistically significant ($P < 0.05$). This is in line with the study of [illegible] *et al.* who identified the diagnostic accuracy of GCF biomarker candidates 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 ($P = 0.0001$). The study of Kim *et al.* (2007) on the other hand shows that the percentage of MMP-8 enzyme correlates significantly with gingival and bleeding index ($P < 0.05$). 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 non-surgical therapy shows that higher levels of MMP-8 are found in periodontitis patients compared with controls ($P < 0.001$) and these levels decreased after 3 months periodontal therapy ($P < 0.01$). Kiili *et al.* compared MMP-8 level in GCF in 18 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 nature 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 tissues. Type I collagen is the major extracellular matrix component of gingiva. It acquires a significant

Table 1: Periodontal disease index criteria

0	Negative. There is neither overt inflammation in the gingiva nor loss of function due to destruction of supporting tissues
	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 attachment
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 be loose, may have drifted, may sound dull on percussion with a metallic instrument, may be depressible in its socket

20-35	Male	14	21.15±31.18	0.14
17-35	Female	46	23.39±4.39	

Table 3: Value of the average levels of MMP-8 significance between healthy, mild gingivitis and mild periodontitis based on periodontal disease index

				P
MMP8	Healthy	20	4.15±4.41	0.001
	Mild gingivitis	20	21.69±3.94	
	Mild periodontitis	20		
	Total	60		

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

Table 4: Results of analysis of the post hoc test average level and mild-mild healthy, mild gingivitis between periodontal disease index

Healthy			0.00
Mild gingivitis	0.00		0.06
Mild periodontitis	0.0	0.06	

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, 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. MMP-8 is the most effective in hydrolyzing Type I collagen. MMP-8 has 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. Thus, synthesis and activation of MMP-8 are important steps in the pathological extracellular matrix destruction associated with the inflammatory periodontal disease. 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. 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. This may explain the elevated levels of MMP-8 in periodontitis compared to gingivitis and healthy controls.

Recent studies have shown that high level of MMP-8 can result in increased inflammation. [illegible] the [illegible] of MMP-8 in GCF represents a potential diagnostic tool to identify patients and sites at risk for periodontal disease progression and predict [illegible] response.

Conclusion

Periodontitis ranges from gingival inflammation to serious disease that results in major damage to tissue and bone that support the teeth. When not properly treated, periodontitis can lead to tooth loss. This study has shown that assessing the level of MMP-8 with comfortable instruments and sensitive detection can determine the progression of the periodontal disease for better treatment. The findings of this study can be used by clinicians to diagnose and treat periodontal disease by measuring the scale of MMP-8 to [illegible] further complication in periodontitis patients.

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Conflicts of interest

There are no conflicts of interest.

References

1. Kinane [illegible] T, [illegible] Sorsa [illegible] AM, [illegible] M, [illegible] et al. Local and systemic [illegible] matrix metalloproteinase-8 [illegible] during [illegible] periodontitis [illegible] 2009.
2. Highfield J. Diagnosis and classification of periodontal disease. Aust Dent J 2009; 54 Suppl 1: S11-26.
3. Lamont [illegible] Current concepts and future trends for periodontal disease and periodontal therapy. Part 1: Classification, diagnosis [illegible] and surgical therapy. [illegible] 2001; 28: 88-91.
4. Kinane DF. Periodontal diagnosis. Ann R Australas Coll Dent Surg 2000; 15: 34-41.
5. [illegible] in the diagnosis of periodontal disease. [illegible] 2008; 181: 298-303.
6. [illegible] M, [illegible] M, [illegible] (MMP)-8 [illegible] periodontitis. [illegible] 2010.
7. [illegible] B, Rydén L, et al. Salivary [illegible] MMP-8 and [illegible] in [illegible] periodontal disease [illegible] the PAROKRANK study [illegible]. PLoS One 2016 [illegible].
8. [illegible] Tervahartiala T, [illegible] MMP-8 in gingival crevicular fluid [illegible] for monitoring periodontal disease. Periodontol [illegible].
9. [illegible] M, [illegible] PA, [illegible] MMP-8 [illegible] gingival crevicular fluid [illegible] purposes. J Clin Periodontol 2014; 41: 548-56.
10. [illegible] M, [illegible] W, Chen BY, Wahlgren J, Maisi P, [illegible] et al. Collagenase-2 (MMP-8) [illegible] (MMP-13) [illegible] gingival crevicular fluid [illegible] J Clin Periodontol 2000; 27: 224-32.
11. [illegible] Khan S, [illegible] of periodontal [illegible]. J Oral Sci 2008; 50: 35-6.
12. [illegible] in [illegible] and gingival crevicular fluid [illegible] 2012; 34: 33-40.
13. Lee [illegible] J, [illegible] JC. Characteristics of [illegible]
14. [illegible]

of matrix metalloproteinases-8 and -9 in gingival crevicular fluid of smokers and non-smokers with chronic periodontitis using ELISA. J Int Oral Health 2014;6:67-71.

15. Bizzaki T, Ozenka Cakmaneyeva B, Nalbantsoy A, Akcah A, Bizzaki N, et al. Effects of potassium aluminium sulfate on TNF-a, MMP-1 and MMP-8 levels of gingival crevicular fluid in periodontally healthy subjects: a pilot study. Turk J Biochem 2012;37:315-21.

16. Bembaid E, N Raghavendra B, Pelam A, Ramamurthy B, Sankaralingam T, Cherian K. Role of matrix metalloproteinases (MMPS) in periodontitis and its management. J Indian Acad Dent Spec Res 2014;1:65.

17. Uitto VJ, Overall CM, McCulloch C. Proteolytic host cell enzymes in gingival crevice fluid. Periodontol 2000 2003;31:77-104.

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