

Biomarkers of periodontitis in oral fluids

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Abstract: Matrix metalloproteinases (MMPs), the key enzymes responsible for matrix degradation, are derived from polymorphonuclear leukocytes during the early stages of periodontitis. The present study determined the levels of GCF matrix metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) and salivary MMP-8 in patients with gingivitis and periodontitis and in healthy controls. Levels of crevicular MMP-2, MMP-9 and salivary MMP-8 were determined by ELISA in subjects with healthy gingiva (n = 15), gingivitis (n = 18) and periodontitis (n = 20). Significantly higher salivary MMP-8 and crevicular MMP-9 were observed in cases of periodontitis compared to gingivitis and healthy adults. On the other hand, crevicular MMP-2 levels in periodontitis subjects were lower than those in gingivitis and healthy subjects. The three MMP levels were highly correlated to probing depth, and bleeding on probing. Salivary MMP-8, crevicular MMP-2 and 9 may serve as biomarkers of periodontal disease and aid in early detection of periodontitis or gingivitis. (*J. Oral Sci.* 50, 53-56, 2008)

Keywords: periodontal disease; metalloproteinase; biomarkers.

Introduction

Periodontitis is an inflammatory disease which affects the supporting tissues of teeth, leading to progressive destruction of connective tissue attachment and alveolar bone. Current information indicates that bacterial infection

may be the primary causative agent of periodontitis (1-3). In periodontal disease, a number of proteases degrade collagen and extracellular matrix. Matrix metalloproteinases (MMPs) are the major group of enzymes responsible for degradation of ECM. The onset of collagen destruction in periodontitis is caused by the action of collagenases, a subgroup of MMPs. In the healthy condition, the periodontal ligament apparatus is protected from matrix metalloproteinases (MMP)-mediated proteolytic attack by TIMPs (tissue inhibitors of metalloproteinases). In chronic periodontitis, levels of TIMP are low and thus inadequate to inhibit the elevated MMPs, activated neutrophil pro-collagenase (Pro-MMP-8) and progelatinase (pro-MMP-9). Furthermore, mobilization and activation of inflammatory cells such as lymphocytes and neutrophils, alteration of immunomodulators and secretion of inflammatory proteases occur (4,5). MMPs-1, 2, 3, 8 and 9 have been found in biopsy specimens of human inflammatory periodontal tissues, whereas healthy gingiva contains only pro MMP-2. MMP-2 is secreted by gingival fibroblasts and MMP-9 is mainly secreted by polymorphonuclear leukocytes (5), and they degrade type IV collagen present in gingival tissues (basement membrane remodeling) (6).

The aim of the present study was to compare the levels of MMP-2 and MMP-9 in gingival crevicular fluid (GCF) and MMP-8 in saliva samples from healthy subjects, and patients with gingivitis and periodontitis.

Materials and Methods

Twenty patients with periodontitis, 18 patients with gingivitis and 15 healthy controls were included in this study. Samples were collected and processed at the Jain Diagnostic Centre, New Delhi. Informed consent was obtained from subjects.

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Selection criteria of Periodontitis patients

The selection criteria for this group were as follows: at least 18 teeth had to be present, excluding third molars, of which at least 12 had to be posterior teeth; presence of moderate to advanced chronic periodontitis (at least 7 teeth with periodontal pockets deeper than 6 mm); absence of systemic disease; no history of medication in the previous 5 months and no previous periodontal treatment. Women who were pregnant or receiving hormone or vitamin treatment were excluded.

Selection criteria of Gingivitis patients

The inclusion criterion was presence of generalized gingival inflammation with bleeding on probing. Exclusion criteria included no attachment loss more than 15 mm, any characteristics of periodontitis, any history of previous scaling or root planing or any systemic disease.

Two examiners recorded the clinical periodontal parameters (probing pocket depth, bleeding on probing and clinical loss of attachment) in each subject after the collection of saliva. Unstimulated saliva was collected from each subject according to a modified version of the method described by Navazesh (7). Probing depths at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual) were measured using a manual probe (Hu-Friedy, Chicago, IL, USA). Clinical loss of attachment was determined by measuring the interproximal sites only.

One to 4 sites per patient were randomly selected for GCF collection. The respective tooth was isolated with cotton after removing the supragingival plaque with curettes (avoiding contact with the gingival margin), and the crevicular area was air-dried. GCF was collected by inserting Periopaper strips in gingival pocket for 45 seconds. The strips contaminated by blood or saliva were discarded. After collecting GCF, the levels of MMP-2, MMP-9 and salivary MMP-8 were measured by Enzyme-linked immunosorbent assays (R&D systems for MMP-9 and Biosource International for MMP-2, Human Quantikine MMP-8 ELISA kit).

The description of the variables and the correlation of MMPs with the clinical parameters were analyzed in these patients using Mann-Whitney and Wilcoxon tests with SPSS version 11.0.

Results

The control subjects were demographically similar to the gingivitis and periodontitis patients (Table 1), but were clearly distinct in terms of the clinical parameters measured ($P < 0.005$).

Elevated salivary levels of MMP-8 were observed in the

periodontitis and gingivitis subjects compared to the controls (Table 2, $P < 0.001$). The crevicular MMP-9 levels in periodontitis and gingivitis were higher than those in healthy subjects (Table 2, $P < 0.001$). The crevicular MMP-2 levels were lower in gingivitis and periodontitis as compared to healthy controls (Table 2, $P < 0.001$).

Salivary MMP-8 and crevicular MMP-2 and -9 levels were significantly correlated to the clinical parameters of the patients. Probing depth, clinical loss of attachment, bleeding on probing and age were significantly correlated with elevated levels of MMP-8 (Table 3). Clinical loss of attachment and bleeding on probing were significantly correlated with elevated levels of MMP-2 and MMP-8 (Table 3).

Discussion

MMPs are zinc-dependent endopeptidases derived predominantly from polymorphonuclear leukocytes during acute stages of periodontal disease and are the key enzymes responsible for extracellular collagen matrix degradation (9-11). Elevated MMP levels have been observed in inflamed human gingiva and GCF in subjects with adult periodontitis (11-14). MMP-8 has the unique ability to breakdown type I and III collagen, which is critical for periodontal destruction. Subantimicrobial doses of doxycycline inhibit MMP-8 and reduce periodontal disease activity (15,16). In the present study, salivary MMP-8 levels were higher in periodontitis and gingivitis patients compared to the healthy subjects and this is in agreement with previous studies (11,13). 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 periodontal disease (Table 3).

The crevicular MMP-9 levels in periodontitis and gingivitis were higher than those in healthy subjects. Our results are in agreement with previous findings (17-19). Elevated levels of crevicular MMP-9 were highly correlated with clinical loss of attachment and bleeding on probing.

The crevicular MMP-2 levels were lower in gingivitis and periodontitis as compared to healthy controls (17). Reduced levels of GCF MMP-2 were highly correlated with clinical loss of attachment and bleeding on probing. Latent MMP-2 and MMP-9 have been detected in healthy and diseased subjects, but the active form of MMP-2 is found only in patients with clinical disease (18). Further studies may be required to address the role of salivary MMPs in periodontitis. Also, it remains to be determined whether the GCF and salivary biomarkers analyzed are capable of distinguishing health from disease when the nature of disease is less generalized in subjects.

Table 1 Mean and Standard Deviation of clinical parameters in subjects

Variable	Healthy (n = 15)	Gingivitis (n = 18)	Periodontitis (n = 20)
Age range (years)	18-58	16-58	19-58
Age (years)	35.1 ± 8.7	36.1 ± 9.3	35.3 ± 9.6
Number of teeth	21.3 ± 2.2	21.2 ± 2.3	19.2 ± 3.5
Probing depth (mm)	2.1 ± 1.9	3.2 ± 2.3	6.8 ± 1.9
Clinical loss of attachment (mm)	1.8 ± 0.5	2.3 ± 0.2	5.3 ± 1.3
Bleeding on probing (%)	4.32 ± 3.71	29.8 ± 13.8	53.9 ± 14.6
Number of teeth with periodontitis	2.12 ± 1.32	2.32 ± 1.62	9.31 ± 1.32

Table 2 Mean and Standard Deviation of crevicular MMP-2, MMP-9 and salivary MMP-9 in various studies

Variable	Healthy (n = 15)	Gingivitis (n = 18)	Periodontitis (n = 20)
Crevicular MMP-2 (pg/μl)	29.13 ± 12.32	28.12 ± 12.43	18.13 ± 12.46
Crevicular MMP-9 (pg/μl)	38.8 ± 24.31	42.31 ± 21.35	56.42 ± 22.32
Salivary MMP-8 (ng/ml)	95.2 ± 70.2	312.8 ± 301.8	428.6 ± 432.4

$P > 0.001$

Table 3 Correlation between clinical profile parameter and salivary MMP-8, crevicular MMP-2 and crevicular MMP-9

Parameter	Salivary MMP-8		Crevicular MMP-2		Crevicular MMP-9	
	Correlation	<i>P</i> value	Correlation	<i>P</i> value	Correlation	<i>P</i> value
Probing depth	0.42	0.001	0.31	0.009	0.31	0.009
Clinical loss of attachment	0.52	0.0001	0.51	0.0001	0.53	0.0001
Bleeding on probing	0.43	0.001	0.41	0.001	0.47	0.001
Number of teeth involved	0.32	0.008	0.33	0.008	0.27	0.07
Age	0.41	0.001	0.23	0.04	0.07	0.54

MMPs are derived predominantly from polymorphonuclear leukocytes during acute stages of periodontal diseases. Several of the 25 types of MMPs have been detected in inflamed human gingiva and GCF in periodontitis subjects.

Non-neutrophil cells, such as gingival and periodontal ligament fibroblasts, release MMP-8, whereas monocytes and macrophages form a potential source of MMP-9. MMP-2 (gelatinase) is produced by various cells in the oral cavity, and MMP-9 is found in acinar epithelial cells. MMPs have been less frequently detected in saliva. However, recent reports indicated the roles of oral fluid MMP-2 and 9 in periodontitis, as together they can degrade most of extracellular matrix components. Conventional periodontal treatment efficiently reduces the levels of MMP-2 and 9. Hence, MMP-2 and 9 in GCF were studied to study their correlation with periodontitis.

MMP-8 has the unique ability to break down type I and III collagen, which is critical for periodontal destruction in periodontitis but not for normal gingival tissue remodeling. This may explain the elevated MMP-8 levels in periodontitis.

MMP-9 levels in GCF were higher in patients with chronic periodontitis than in patients with gingivitis and healthy subjects, while MMP-2 levels in GCF were lower in patients with chronic periodontitis than in patients with gingivitis and healthy subjects. Salivary MMP-8 levels were higher in patients with chronic periodontitis than in patients with gingivitis and healthy subjects. MMP-2, MMP-8 and MMP-9 levels were highly correlated with the clinical loss of attachment and probing depth.

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