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ORIGINAL ARTICLE

Levels of gingival crevicular metalloproteinases-8 and -9 in periodontitis

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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 aim of this study was planned to determine the levels of GCF (gingival crevicular fluid) matrix metalloproteinase-8 (MMP-8) and metalloproteinase-9 (MMP-9) patients with periodontitis and in healthy controls. Levels of crevicular MMP-8 and -9 were determined by ELISA in subjects with healthy without any periodontal disease ($n = 10$) and periodontitis ($n = 10$). Significantly higher crevicular MMP-8 and -9 were observed in cases of periodontitis compared to healthy adults. Crevicular MMP-8 and -9 may serve as biomarkers of periodontal disease and aid in early detection of periodontitis.

1. Introduction

Periodontitis is an inflammatory disease which affects the supporting tissues of teeth, leading to progressive destruction of connective tissue attachment and alveolar bone. Matrix metalloproteinases (MMPs) are the major group of enzymes responsible for degradation of ECM (extracellular matrix). 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 (Ingman et al., 1994; Alpagot et al., 2001; Kinane, 2001; Hutter et al., 2003; Cota et al., 2006). Current information indicates that bacterial infection may be the primary causative agent of periodontitis (Alpagot et al., 2001; Kinane, 2001; Hutter et al., 2003). 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

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and MMP-9 is mainly secreted by polymorphonuclear leukocytes, and they degrade type IV collagen present in gingival tissues (basement membrane remodeling) (Ingman et al., 1994; Alpagot et al., 2001; Chang et al., 2000; Kinane, 2001).

The aim of the present study was to compare the levels of MMP-8 and MMP-9 in gingival crevicular fluid (GCF) from healthy subjects and periodontitis.

2. Materials and methods

Ten patients with periodontitis (M:F;5:5, aged between 29 and 34 years), and 10 healthy controls (M:F;5:5 aged between 28 and 34 years) were included in this study. Samples were collected and processed at the Jain Diagnostic Centre, New Delhi. Informed consent was taken from subjects.

2.1. 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.

2.2. Selection criteria of healthy

The inclusion criteria were absence of any periodontal disease, 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.

First author 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 (2003). Probing depths at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual) were measured using a manual probe (Hu-Friedy, Chicago, 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 (the procedures done on GCF avoiding contamination), and the crevicular area was air-dried. GCF was collected by inserting Periopaper strips (PerioCol Paper Strips, Oraflow Inc., USA) in gingival pocket for 45s. The strips contaminated by blood or saliva were discarded. The strips were placed into 50 µl of 0.9% phosphate-buffered saline. After 1 h in ice, the GCF samples were centrifuged at 9220*g* for 15 min. In collecting GCF, the levels of MMP-8 and -9 were measured by Enzyme-linked immunosorbent assays (R&D systems for MMP-8, -9 ELISA kit, Minneapolis, MN, USA).

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.

3. Results

The control subjects were demographically similar to periodontitis patients (Table 1), but were clearly distinct in terms of the clinical parameters measured ($P < 0.05$). The crevicular MMP-8, -9 levels in periodontitis were higher than those in healthy subjects (Table 2, $P < 0.05$).

4. 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 (Woessner, 1994; Delaissé et al., 2000; Miller et al., 2006; Rai et al., 2007). Elevated MMP levels have been observed in inflamed human gingiva and GCF in subjects with adult periodontitis (Villela et al., 1987; Gangbar et al., 1990; Sorsa et al., 1990). MMP-8 has the unique ability to breakdown type I and III collagen, which is critical for periodontal destruction. Subantimicrobial

Table 1 Clinical parameters in subjects (mean ± SD).

Variable	Healthy ($n = 10$)	Periodontitis ($n = 10$)
Age (year) (range)	17–56 (32.3)	18–59 (34.5)
Age (year)	34.1 ± 8.7	36.3 ± 9.6
Number of teeth	20.3 ± 2.2 (18–24)	18.2 ± 3.5 (19–24)
Probing depth (mm)	2.5 ± 1.9 (1.2–4.1)	6.9 ± 1.9 (5.2–7.9)
Clinical loss of attachment (mm)	1.9 ± 0.5 (1.2–2.4)	5.5 ± 1.3 (3.5–6.9)
Bleeding on probing (%)	5.32 ± 3.71 (3.78–8.56)	57.9 ± 14.6 (44.2–68.8)
No. of teeth with periodontitis	4.12 ± 1.32 (3.24–6.89)	10.31 ± 1.32 (9.06–12.08)

Table 2 Mean and standard deviation of crevicular MMP-8 and -9 in various studies.

Variable	Healthy ($n = 10$)	Periodontitis ($n = 10$)
Crevicular MMP-8 (pg/ul)	4.13 ± 12.32 (3.87–15.96)	15.13 ± 12.46 (11.06–28.36)
Crevicular MMP-9 (pg/ul)	37.8 ± 24.31 (21.56–68.87)	59.42 ± 22.32 (34.86–78.87)

$P < 0.05$.

doses of doxycycline inhibit MMP-8 and reduce periodontal disease activity (Caton et al., 2001; Novak et al., 2002). Gingival crevicular MMP-8, -9 levels in periodontitis were higher than those in healthy subjects. Our results are in agreement with previous findings (Mäkelä et al., 1994; Korostoff et al., 2000; Maeso et al., 2007).

Further studies may be required to address the role of GCF 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. The role of gene polymorphism in relation to MMP expression is unclear and so far, there are no published reports about MMP-8 gene polymorphism in periodontal disease. However, there are studies concerning several cytokine gene polymorphisms associated with an increased risk of periodontal disease. Increased production of a given cytokine is associated with carriage of a risk genotype. Previously, the risk of having periodontal disease has been related to carriage of rare alleles of single cytokine and receptor molecule genes such as IL-1, TNF-α, IL-6 and CD-14 (Caton et al., 2001; Novak et al., 2002; Miller et al., 2006; Rai et al., 2007). MMPs are derived predominantly from polymorphonuclear leukocytes during acute stages of periodontal diseases. 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-8 and -9 in periodontitis, as together they can degrade most of extracellular matrix components. Recently, it has been reported that vivo immune cell interactions in the presence of plasma proteins to show that TC (tetracycline), doxy (doxycycline), and CMT-3 (chemically modified tetracycline-3) can reduce the production of pro-inflammatory mediators in periodontitis (Passoja et al., 2008; Cazalis et al., 2009).

5. Conclusion

Conventional periodontal treatment efficiently gave a conclusion that has more impact on reducing the levels of MMP-8 and -9. Hence, MMP-8 and -9 in GCF were studied to study their correlation with periodontitis. MMP-8, -9 levels in GCF were higher in patients with chronic periodontitis than in healthy subjects.

References

Alpagot, T., Bell, C., Lundergan, W., Chambers, D.W., Rudin, R., 2001. Longitudinal evaluation of GCF MMP-3 and TIMP-1 levels as prognostic factors for progression of periodontitis. J. Clin. Periodontol. 28, 353–359.

Caton, J.G., Ciancio, S.G., Blieden, T.M., Bradshaw, M., Crout, R.J., Hefti, A.F., Massaro, J.M., Polson, A.M., Thomas, J., Walker, C., 2001. Subantimicrobial dose doxycycline as an adjunct to scaling and root planing: post-treatment effects. J. Clin. Periodontol. 8, 782–789.

Cazalis, J., Tanabe, S., Gagnon, G., Sorsa, T., Grenier, D., 2009. Tetracyclines and chemically modified tetracycline-3 (CMT-3) modulate cytokine secretion by lipopolysaccharide-stimulated whole blood. Inflammation 32 (2), 130–137.

Chang, Y.C., Yang, S.F., Lai, C.C., Liu, J.Y., Hsieh, Y.S., 2000. Regulation of matrix metalloproteinase production by cytokines, pharmacological agents and periodontal pathogens in human periodontal ligament fibroblast cultures. J. Periodontal Res. 37, 196–203.

Cota, L.O.M., Guimarães, A.N., Costa, J.E., Lorentz, T.C.M., Costa, F.O., 2006. Association between maternal periodontitis and an increased risk of preeclampsia. J Periodontol 77, 2063–2069.

Delaissé, J.M., Engsig, M.T., Everts, V., del Carmen Ovejero, M., Ferreras, M., Lund, L., Vu, T.H., Werb, Z., Winding, B., Lochter, A., Karsdal, M.A., Troen, T., Kirkegaard, T., Lenhard, T., Heegaard, A.M., Neff, L., Baron, R., Foged, N.T., 2000. Proteinases in bone resorption: obvious and less obvious roles. Clin. Chim. Acta 291, 223–224.

Gangbar, S., Overall, C.M., McCulloch, C.A., Sodek, J., 1990. Identification of polymorphonuclear leukocyte collagenase and gelatinase activities in mouthrinse samples: correlation with periodontal disease activity in adult and juvenile periodontitis. J. Periodontal Res. 25, 257–267.

Hutter, G., Schlagenhauf, U., Valenza, G., Horn, M., Burgemeister, S., Claus, H., Vogel, U., 2003. Molecular analysis of bacteria in periodontitis: evaluation of clone libraries, novel phylotypes and putative pathogens. Microbiology 149, 67–75.

Ingman, T., Sorsa, T., Michaelis, J., Konttinen, Y.T., 1994. Matrix metalloproteinases-1, -3, and -8 in adult periodontitis in situ. An immunohistochemical study. Ann. NY Acad. Sci. 732, 459–461.

Kinane, D.F., 2001. Causation and pathogenesis of periodontal disease. Periodontol 25, 8–20.

Korostoff, J.M., Wang, J.F., Sarment, D.P., Stewart, J.C., Feldman, R.S., Billings, P.C., 2000. Analysis of in situ protease activity in chronic adult periodontitis patients: expression of activated MMP-2 and a 40 kDa serine protease. J. Periodontol. 1, 353–360.

Maeso, G., Bravo, M., Bascones, A., 2007. Levels of metalloproteinase-2 and -9 and tissue inhibitor of matrix metalloproteinase-1 in gingival crevicular fluid of patients with periodontitis, gingivitis, and healthy gingiva. Quintessence Int. 38, 247–252.

Mäkelä, M., Salo, T., Uitto, V.J., Larjava, H., 1994. Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relationship to periodontal status. J. Dent. Res. 73, 1397–1406.

Miller, C.S., King Jr., C.P., Langub, M.C., Kryscio, R.J., Thomas, M.V., 2006. Salivary biomarkers of existing periodontal disease: a cross-sectional study. J. Am. Dent. Assoc. 137, 322–329.

Navazesh, M., 2003. Methods for collecting saliva. Ann. NY Acad. Sci. 694, 72–77.

Novak, M.J., Johns, L.P., Miller, R.C., Bradshaw, M.H., 2002. Adjunctive benefits of subantimicrobial dose doxycycline in the management of severe, generalized, chronic periodontitis. J. Periodontol. 73, 762–769.

Passoja, A., Ylipalosaari, M., Tervonen, T., Raunio, T., Knuuttila, M., 2008. Matrix metalloproteinase-8 concentration in shallow crevices associated with the extent of periodontal disease. J. Clin. Periodontol. 35 (12), 1027–1031.

Rai, B., Kharb, S., Anand, S.C., 2007. Thiocynate, a salivary marker of periodontitis among smokers and nonsmokers: a pilot study. J. Pak. Dent. Assoc. 16, 35–37.

Sorsa, T., Suomalainen, K., Uitto, V.J., 1990. The role of gingival crevicular fluid and salivary interstitial collagenases in human periodontal diseases. Arch. Oral Biol. 35 (Suppl.), 193S–196S.

Villela, B., Cogen, R.B., Bartolucci, A.A., Birkedal-Hansen, H., 1987. Collagenolytic activity in crevicular fluid from patients with chronic adult periodontitis, localized juvenile periodontitis and gingivitis, and from healthy control subjects. J. Periodontal Res. 22, 381–389.

Woessner Jr., J.F., 1994. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J. 5, 2145–2154.