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Levels of Gingival Crevicular Metalloproteinases-8 and -9 in Periodontitis

Níveis de Metaloproteinases da Matriz 8 e 9 na Periodontite

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RESUMO

Objetivo: Determinar os níveis de matriz GCF metaloproteinases 8 (MMP-8) e metaloproteinase 9 (MMP-9) em indivíduos saudáveis e com periodontite.

Método: Dez pacientes com doença periodontal e 10 sujeitos saudáveis compuseram a amostra. As análises foram realizadas no Jain Diagnostic Centre, Nova Déli, Índia. O consentimento informado foi obtido para todos os sujeitos. Níveis presentes no sulco crevicular de MMP-8 e MMP-9 foram determinados por meio do ELISA em ambos os grupos. Dois examinadores analisaram os parâmetros clínicos periodontais e a saliva não estimulada foi coletada. A perda de inserção óssea clínica foi determinada por meio da mensuração dos sítios interproximais.

Resultados: Valores significativamente elevados de MMP-8 e MMP-9 foram diagnosticados nos portadores de periodontite quando comparado aos indivíduos saudáveis.

Conclusão: A presença no fluido crevicular de MMP-8 e MMP-9 servem como biomarcadores da doença periodontal e auxiliam na detecção precoce da periodontite.

ABSTRACT

Objective: To determine the levels of GCF matrix metalloproteinase-8 (MMP-8) and metalloproteinase-9 (MMP-9) patients with periodontitis and in healthy controls.

Methods: Ten patients with periodontitis, and 10 healthy controls were included in this study. Samples were collected and processed at the Jain Diagnostic Centre, New Delhi. Informed consent was taken from subjects. Levels of crevicular MMP-8 and MMP-9 were determined by ELISA in subjects with healthy gingiva (n=10) and periodontitis (n=10). Two examiners recorded the clinical periodontal parameters and unstimulated saliva was collected. Clinical loss of attachment was determined by measuring the interproximal sites only.

Results: Significantly higher crevicular MMP-8 and-9 were observed in cases of periodontitis compared to healthy adults.

Conclusion: Crevicular MMP-8 and 9 may serve as biomarkers of periodontal disease and aid in early detection of periodontitis.

DESCRITORES

Doenças periodontais; Metaloproteinases da Matriz; Fluido do Sulco Gingival.

DESCRIPTORS

Periodontal diseases; Matrix Metalloproteinases; Gingival Crevicular Fluid.

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 periodontitis¹⁻³.

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, and they degrade type IV collagen present in gingival tissues (basement membrane remodeling)⁴⁻⁶.

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.

METHODS

Ten patients with periodontitis, and 10 healthy controls were included in this study. Samples were collected and processed at the Jain Diagnostic Centre, New Delhi. Informed consent was taken from subjects.

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 Navazesh⁷. 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 (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-8 and MMP-9 were measured by Enzyme-linked immunosorbent assays (R&D systems for MMP-8,-9 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 periodontitis patients (Table 1), but were clearly distinct in terms of the clinical parameters measured ($p < 0.005$). The crevicular MMP-8,-9 levels in periodontitis were higher than those in healthy subjects (Table 2, $p < 0.05$).

Table 1. Clinical Parameters in Subjects (Mean + SD).

Variable	Healthy	Periodontitis
Age (year)(Range)	17-56	18-59
Age(Years)	34.1 + 8.7	36.3 + 9.6
Number of teeth	20.3 + 2.2	18.2 + 3.5
Probing depth (mm)	2.5 + 1.9	6.9 + 1.9
Clinical loss of attachment (in mm)	1.9 + 0.5	5.5 + 1.3
Bleeding on probing (in %)	5.32 + 3.71	57.9 + 14.6
No. of teeth with periodontitis	4.12 + 1.32	10.31 + 1.32

Table 2. Crevicular MMP-8 and MMP-9 (Mean + SD).

Variable	Healthy	Periodontitis
Crevicular MMP-8 (pg/ul)	4.13 + 12.32	15.13 + 12.46
Crevicular MMP-9 (pg/ul)	37.8 + 24.31	59.42 + 22.32

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 degradation⁹⁻¹¹.

Elevated MMP levels have been observed in inflamed human gingiva and GCF in subjects with adult periodontitis¹¹⁻¹⁴. 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}. Gingival crevicular MMP- 8,-9 levels in periodontitis were higher than

those in healthy subjects. Our results are in agreement with previous findings¹⁷⁻¹⁹.

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.

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-8 and 9 in periodontitis, as together they can degrade most of extracellular matrix components. Conventional periodontal treatment efficiently reduces the levels of MMP-8 and 9. Hence, MMP-8 and 9 in GCF were studied to study their correlation with periodontitis.

CONCLUSION

MMP-8,-9 levels in GCF were higher in patients with chronic periodontitis than in healthy subjects.

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