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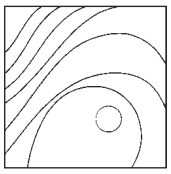
Immunohistochemical Expression of Matrix Metalloproteinase 13 in Chronic Periodontitis



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The extracellular matrix is a complex integrated system responsible for the physiologic properties of connective tissue. Collagen is the major extracellular component that is altered in pathologic conditions, mainly periodontitis. The destruction involves proteolytic enzymes, primarily matrix metalloproteinases (MMPs), which play a key role in mediating and regulating the connective tissue destruction in periodontitis. The study group included 40 patients with clinically diagnosed chronic periodontitis. The control group included 20 patients with clinically normal gingiva covering impacted third molars undergoing extraction or in areas where crown-lengthening procedures were performed. MMP-13 expression was demonstrated using immunohistochemistry in all the gingival biopsies, and the data were analyzed statistically. MMP-13 expression was observed more in chronic periodontitis when compared with normal gingiva. MMP-13 expression was expressed by fibroblasts, lymphocytes, macrophages, plasma cells, and basal cells of the sulcular epithelium. Comparative evaluation of all the clinical and histologic parameters with MMP-13 expression showed high statistical significance with Spearman correlation coefficient. Elevated levels of MMP-13 may play a role in the pathogenesis of chronic periodontitis. There is a direct correlation of increased expression of MMP-13 with various clinical and histologic parameters in disease severity. (Int J Periodontics Restorative Dent 2014;34:e79–e84. doi: 10.11607/prd.1922)

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Periodontitis is an infectious disease characterized by gingival inflammation and loss of supporting periodontal tissues, comprising periodontal ligament, cementum, and alveolar bone, leading to bone destruction. Local factors such as microbial plaque, calculus, food impactions, and overhanging restorations play a very important etiologic role for the development of periodontitis. Chronic periodontitis is predominantly associated with *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Eikenella corrodens*. Clinically, the disease manifests as a simple marginal gingivitis followed by ulceration of the crevicular epithelium and deepening of the crevice, leading to bleeding, tooth mobility, and gingival recession. Histopathologically, gingival epithelium appears hyperplastic with adjacent connective tissue showing increased vascularity and inflammatory cell infiltrate, predominantly of lymphocytes, plasma cells, and a variable number of neutrophils.[1]

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Collagen is the major extracellular component of gingiva and periodontal ligament and is altered in pathologic conditions like periodontitis. During active periodontitis, degradation of collagen is due to matrix metalloproteinases (MMPs).[2] Based on the substrate specificity and cellular localization, MMPs are divided into collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), matrilysins (MMP-7 and -26), stromelysins (MMP-3, -10, -11, and -19), and other membrane-associated MMPs (MMP-14, -15, -16, -17, -24, and -25). Collagenases are part of a family of MMPs that degrade collagen. There are three principal types of specific collagenases: MMP-1 (collagenase 1), MMP-8 (collagenase 2), and MMP-13 (collagenase 3).[3] Their activity can be detected in gingival tissue, saliva, and gingival crevicular fluid and can be assayed biochemically with various methods such as immunohistochemistry, in situ hybridization, Western blot, and enzyme-linked immunosorbent assay.[4]

Review of the literature revealed numerous studies involving MMP-1 and -8 in chronic periodontitis, and the role of collagenase is well understood. Research has been oriented toward collagenase 3 (MMP-13), which was cloned from breast carcinoma. The present study aims to immunohistochemically evaluate the role of MMP-13 expression in chronic periodontitis and improve the understanding of periodontitis.

Method and materials

Sixty patients who reported to the Department of Oral Pathology, Vishnu Dental College, Bhimavaram, India, were included in the study. The gingival tissues were obtained after ethical clearance from the institute and the Department of Periodontics and Oral Surgery. Of the 60 included patients, 40 (23 women and 17 men; age range: 39 to 63 years, mean: 51 years) were clinically diagnosed with chronic periodontitis. The remaining 20 patients (12 women and 8 men; age range: 18 to 28 years, mean: 23 years) were included in the control group. Gingival biopsy site in the control group included clinically normal gingiva covering impacted third molars that underwent surgical extraction under local anesthesia and in sites undergoing crown-lengthening procedures. Clinical parameters included tooth mobility, probing depth, and clinical attachment loss. Each tooth was examined at six sites: mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual prior to the treatment.

The inclusion criteria followed in case selection included clinical evidence of chronic periodontitis (test group) and normal gingiva in patients undergoing crown lengthening or surgical extraction of impacted teeth (control group). Exclusion criteria included antibiotic therapy during the past 4 to 6 weeks, periodontal treatment in the past 6 months, a history of systemic diseases, tobacco use, pregnancy, and use of hormonal contraceptives.

Clinical data involving periodontal status were recorded, including three major clinical parameters, ie, tooth mobility, pocket depth, and clinical attachment loss, and were graded as suggested by Itioz and Carranza.[1] Patients were evaluated clinically for tooth mobility, pocket depth, clinical attachment level, bleeding on probing, and the presence of supra- and subgingival calculus deposits.

The surgical procedure was explained to the patients included in the study, and gingival biopsies were obtained. Paraffin-embedded tissue sections of healthy and diseased gingiva were stained with hematoxylin and eosin, and serial sections were stained with immunohistochemical reagent (MMP-13 mouse monoclonal antibody, ImmunoCruz Mouse LSAB Staining System, Santa Cruz Biotechnology). Positive control included tissue sections of human breast carcinoma.

All the specimens were fixed in 10% neutral buffered formalin and were processed and embedded in paraffin wax. Tissue sections of 4 µm were obtained and deparaffinized, and endogenous peroxidase activity was blocked with 10% hydrogen peroxide for 5 minutes. After washing, sections were treated with serum block for 20 minutes and blot drained. Sections were incubated for 2 hours with 1:200 diluted primary MMP-13 mouse monoclonal antibody in 0.1% phosphate buffered saline. After washing, sections were incubated with goat anti-mouse immunoglobulin G secondary antibody for 30 minutes. A brown color was developed

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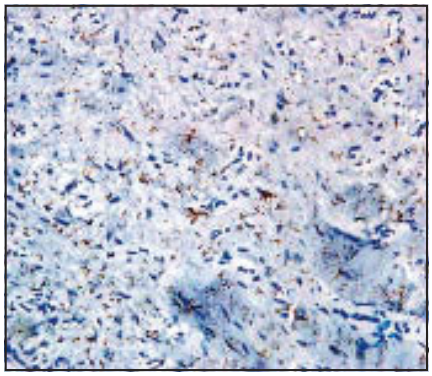

Fig 1 *Dense, diffuse expression of MMP-13 in gingiva with chronic periodontitis (original magnification ×40).*

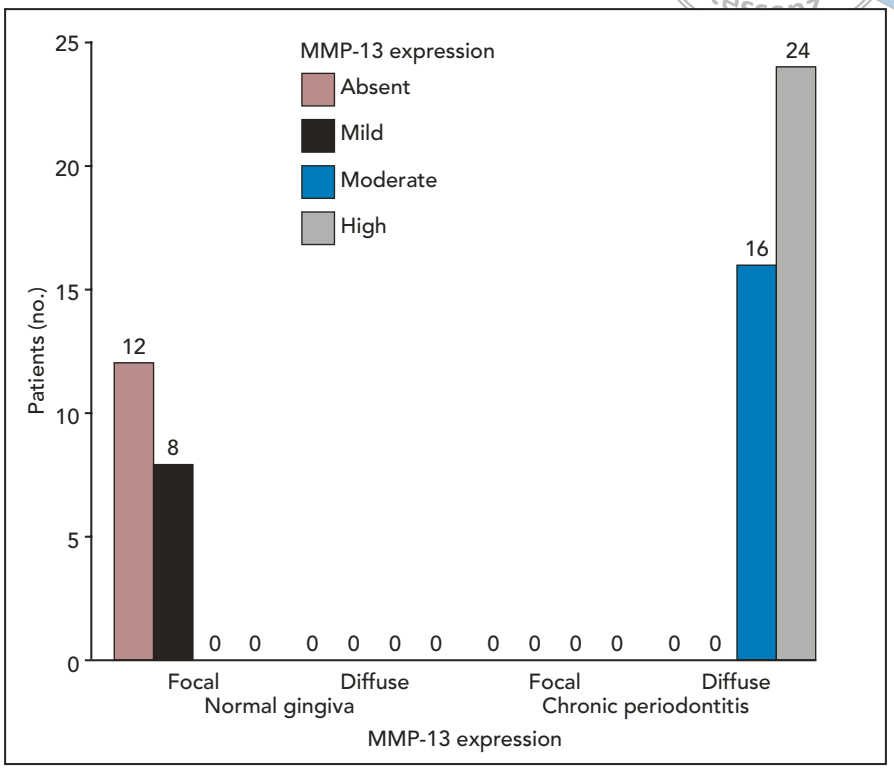

Fig 2 *Increased MMP-13 expression in gingiva with chronic periodontitis when compared with normal gingiva.*

by exposure to 3,3′-diaminobenzidine chromogen for 20 minutes. Sections were counterstained with Gill hematoxylin, dehydrated, and mounted using distrene 80, dibutyl phthalate, and xylol.

Following immunohistochemical staining, all the stained sections of gingiva were studied under a light microscope. The immunopositive stain of the controls from human breast carcinoma showed strong positivity for MMP-13 in the epithelial tumor cells, inflammatory cells, and fibroblasts. Cells with dark brown stain within the gingival connective tissue of the study sample were considered as MMP-13 positive cells, which included inflammatory cells like plasma cells, macrophages, lymphocytes, polymorphonuclear cells, and mast cells. Sections were evaluated for MMP-13 positivity in terms of location (epithelium or connective tissue), intensity of staining (mild, moderate, or severe) pattern of distribution (focal or diffuse), and type of inflammatory cells (lymphocytes, macrophages, or plasma cells).

Results

There was a significant increase in the expression of MMP-13 in gingiva with chronic periodontitis (Fig 1) compared with that in normal gingiva (Fig 2). In normal gingiva there was lack of MMP-13 expression in 12 cases (Fig 3), whereas the remaining cases showed mild, focal expression in the connective tissue mainly limited to the fibroblasts (Fig 4).

MMP-13 expression in chronic periodontitis was observed in the connective tissue of 22 patients, and of these 18 showed expression in both the epithelium and connective tissue. In the epithelium, the basal layer expressed MMP-13 (Fig 5), whereas in the connective tissue the inflammatory cells such as macrophages, lymphocytes, and plasma cells showed dense and diffuse expression (Fig 6).

Comparison of clinical parameters like tooth mobility, pocket depth, and clinical attachment loss

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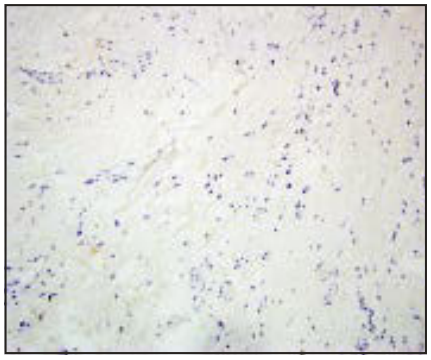

Fig 3 (left) *Lack of MMP-13 expression in normal gingiva (original magnification ×40).*

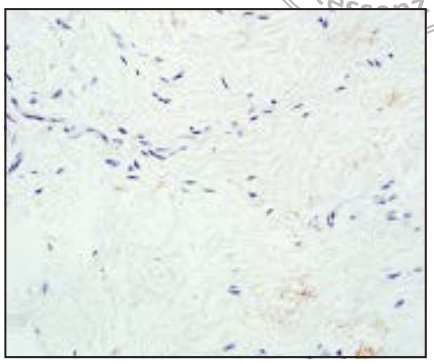

Fig 4 (right) *Mild focal expression of MMP-13 in normal gingiva (original magnification ×40).*

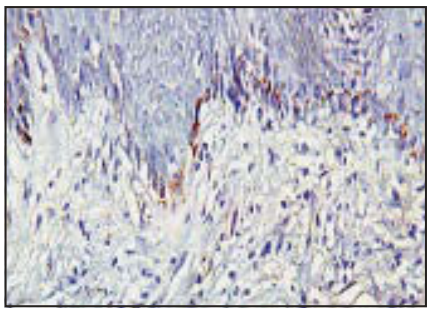

Fig 5 (left) *MMP-13 expression by the basal layer of epithelium in chronic periodontitis (original magnification ×40).*

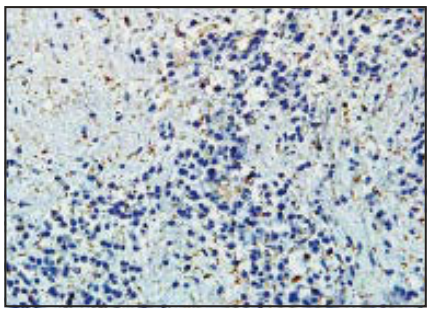

Fig 6 (right) *MMP-13 expression by lymphocytes, macrophages, and plasma cells in chronic periodontitis (original magnification ×40).*

with MMP-13 expression in normal gingiva were insignificant, whereas the intensity, pattern, type, and location of MMP-13 staining were highly significantly different ($P \leq .001$) than the clinical parameters observed in the chronic periodontitis group.

Discussion

Chronic periodontitis is an infectious disease characterized by intense inflammatory infiltrate associated with irreversible loss of alveolar bone and connective tissue attachment in the periodontium, which ultimately results in loss of teeth.[5] Excessive breakdown of connective tissue components plays an important role in destruction of functional tissue architecture. MMPs belong to a family of zinc- and calcium-dependent proteases that play a role in the degradation and remodeling of matrix proteins during different physiologic and pathologic processes.[6]

Previous studies by Hernandez et al,[2] Kiili et al,[7] and Silva et al[8] demonstrated lack of MMP-13 expression in normal gingiva, but the present study demonstrated limited MMP-13 expression in 8 out of 20 patients with normal gingiva. Among those eight patients, seven showed expression in the fibroblasts, and only one case showed fibroblast and lymphocyte expression. This expression could be attributed to a process of normal homeostasis in which continuous remodeling of tissues takes place, ie, deposition and degradation of collagen fibers. MMP-13 expression in lymphocytes could be related to the site of biopsy, which involved pericoronal tissue at the third molar region.

Degradation of collagen fibrils takes place both by intracellular and extracellular pathways. The intracellular pathway is responsible for localized and physiologic collagen removal, whereas in the extracellular pathway collagen is degraded outside the cell by secreted MMPs. The external pathway causes rapid and widespread collagen destruction mainly in an inflammatory reaction.[9] Higher levels of MMP-13 in chronic periodontitis indicates the presence of the active form of the enzyme, which is responsible for degradation of connective tissue, thereby leading to bone loss and tooth loss in the disease process. The available evidence in the

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present study suggests that MMP-13 may be involved in the initiation and progression of bone resorption.

The pattern of MMP-13 expression in chronic periodontitis is affected by several cytokines present in inflamed tissue, such as interleukin 1, tumor necrosis factor α, and prostaglandin E_2.[5] In the present study, 22 patients showed intense to moderate MMP-13 expression in the connective tissue, whereas 18 patients showed expression in the basal cell layer of epithelium and connective tissue. The staining of the basal cell layer of epithelium could be attributed to the ability of MMP-13 to degrade Type VII collagen in the basement membrane zone. Sulcular epithelium proliferating into the connective tissue in the form of fingerlike projections showed MMP-13 expression in the basal layer of the chronic periodontitis study group. This finding was consistent with the studies of Kiili et al,[7] Silva et al,[8] Sorsa et al,[10] and Meikle et al.[11]

Distinct MMP species are produced by various periodontal ligament cell types, and these cells have a clear role during health and disease. MMP-13 is secreted by epithelial cells,[11] endothelial cells, fibroblasts,[12] lymphocytes, macrophages, plasma cells, mast cells,[13] and neutrophils,[10] which plays a significant role in the initiation and progression of bone resorption. The present study also showed a similar staining pattern in fibroblasts, lymphocytes, plasma cells, and macrophages in different grades of chronic periodontitis. The intensity of staining has increased with the severity of the disease, suggesting involvement of MMPs in tissue remodeling in periodontal disease, which is in correlation with the findings of Hernandez et al[2] and Meikle et al.[11]

Clinical parameters assessed in the present study included tooth mobility, pocket depth, and clinical attachment loss. These parameters were compared with the MMP-13 staining, and the disease severity was categorized based on MMP-13 expression (mild, < 25%; moderate, 25% to 50%; and severe, > 50%) along with all the clinical parameters. The results of the current study are in correlation with those of Hernandez et al,[2] Kiili et al,[7] Silva et al,[8] Meikle et al,[11] Goncalves et al,[14] Kumar et al,[15] and Kardesler et al,[16] who report a combined effect of inflammatory cells and fibroblasts in periodontal destruction, but not concurrent with the findings of Lee et al,[17] who mainly stressed the role of neutrophils, which have a direct influence on the pathologic destruction of periodontal ligament connective tissue.

The type of inflammatory cell infiltrate is a key factor in any pathology. In the present study MMP-13 is expressed by macrophages, lymphocytes, and plasma cells, thereby suggesting the coordinated work of inflammatory cells and their released products along with cytokines, which play a major role in the destruction of periodontal ligament tissue with minimal repair of tissues, resulting in the loss of supporting tissue and bone.

MMP-13 has been involved in the degradation of connective tissue matrix, tumor invasion, and metastasis. It was also observed that MMP-13 near the vicinity of bone-lining cells causes exposure of mineralized bone matrix by removing nonmineralized collagen fibrils, which are essential for the differentiation and activation of osteoclasts. Hence, the available evidence suggests the role of MMP-13 in the degradation of Type I, II, and III collagens and aggrecan as well as in creating a microenvironment for degradation of organic components of bone by activating MMP-9.

Conclusions

MMP-13 is considered to have an important role in skeletal biology during embryonic development and can also be detected in pathologic conditions such as chronic periodontitis, rheumatoid arthritis, periapical granuloma, squamous cell carcinoma, and breast carcinoma. Hence, further studies are required to focus on the inflammatory cells and their products responsible for causing the destruction and early loss of attachment in chronic periodontitis.

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