

Collagen IV and MMP-9 expression in hypertrophic gingiva during orthodontic treatment

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Abstract

Introduction: In the present study, we aimed to assess MMP-9 and type IV collagen in the gingival tissue in patients with gingival overgrowth (GO) after orthodontic treatment, with or without clinical signs of inflammation to appreciate the role of balance between those two markers in the onset of GO during tooth movement. **Materials:** Gingival tissue was harvested from 45 patients divided in three groups: 15 patients with orthodontic appliance that developed GO on at least two teeth; 15 patients with chronic gingivitis with GO; 15 patients (control group) with healthy periodontal tissues, without clinical gingival changes, in whom the first premolar was planned for extraction for orthodontic purposes (canine distalization). **Methods:** The tissue samples obtained by gingivectomy were fixed in 10% neutral formalin solution for 48 hours and then included in paraffin with the usual technique. The histologic examination was performed using classic Hematoxylin–Eosin technique. Double immunofluorescence was performed for anti-MMP-9 and anti-collagen IV antibodies. **Results:** MMP-9 / collagen IV double stainings showed an increase of fibrosis and inflammation in different degrees. **Conclusions:** The mechanical stress induced by the orthodontic devices, might be the key players in driving both the inflammation and the fibrotic reaction.

Keywords: gingival crevicular fluid, matrix metalloproteinase, orthodontic treatment, gingival hypertrophy.

Introduction

Matrix-metalloproteinases (MMP) are a family of zinc-dependent endopeptidases that mediates degradation of extracellular matrix. Those enzymes are involved in oral pathologic processes such as destruction of periodontal tissue [1, 2], tumoral invasion or temporo-mandibular joint dysfunctions [3]. Every MMP contain Zn^{2+} at the level of the catalytic site and requires Ca^{2+} for stability and activity. In plus, MMP-2 and MMP-9 may present a binding domain for gelatin [4], inserted between the catalytic domain and the active one, reason for which MMP-9 is called the gelatinase-B.

MMP-9 or gelatinase B is a protein with many active forms, weighting 82–130 kD. The activity of MMP-9 is related to inflammation, wound healing [5, 6] and tumor growth [7]. The involvement of MMP-9 in periodontal disease [8] was already shown as it was in the orthodontic periodontal remodelling [9, 10].

Gingival overgrowth (GO) during orthodontic treatment is usually considered to be the result of the inflammation emerging as a reaction to the accumulation of bacterial plaque caused by the difficult keeping of oral hygiene in those patients. Gingival overgrowth appears also in patients with good oral hygiene and the inflammatory signs appear later as an accumulation of the bacterial plaque favored by the presence of GO [11]. A possible explanation for GO

without any clinical sign of inflammation could be the reaction of gingiva to the mechanical stress induced by the orthodontic forces, considering that MMP-8 may be marker in GCF and gingival tissue for this situation.

Patients and Methods

Patients

The protocol for gingival tissue harvesting was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania and informed consent of the patients or their parents was obtained. Gingival tissue was harvested from 45 patients divided in three groups:

- 15 patients with orthodontic appliances that developed a gingival overgrowth in at least two teeth;
- 15 patients with chronic gingivitis and GO;
- 15 patients with healthy periodontal tissues (control group), without any clinical gingival changes, in whom extraction of the first premolar was planned for orthodontic purposes (canine distalization).

Every 45 patients fulfilled the following criteria: (1) patients in general good health, (2) without general diseases that may be associated with periodontal lesions with GO (diabetes, hematologic diseases, HIV infection, hypovitaminosis), (3) no general treatment with anti-epileptics, calcium antagonists, cyclosporines, (4) with

no radiological signs of bone loss, (5) no therapy with antibiotics in the last three months, and (6) no use of anti-inflammatory drugs in the previous 30 days. Pregnancy was excluded for the female patients included in the study. None of the patients was smoker.

Clinical recordings

Before the sampling of gingival tissue there were recorded:

- Silness and Loe plaque index (IP) as an indicator of dental hygiene and accumulation of plaque. Scores of 2–3 were considered as high;
- Bleeding on probing index (BOP) as an indicator of gingival inflammation, could be 0 (absence of bleeding) or 1 (presence of bleeding).

Immunohistochemical examination

The tissue samples obtained by gingivectomy were fixed in 10% neutral formalin solution for 48 hours and then included in paraffin with the usual technique.

The histologic examination was performed using classic Hematoxylin–Eosin technique.

Double immunofluorescence was performed for anti-MMP-9 (mouse anti-human, Millipore, 1:100) and anti-collagen IV antibodies (rabbit anti-human, Novus Biologicals, 1:500). After citrate buffer antigen retrieval, the slides were blocked for one hour in 4% skim milk, and then both antibodies were added in a cocktail for overnight incubation. Next day, the slides were incubated with another cocktail of anti-mouse biotinylated (Dako, 1:300) and respectively anti-rabbit Alexa 488 (Millipore, 1:400) conjugated secondary antibodies for 30 minutes. After thorough washing, the slides were incubated for another 30 minutes with Alexa 594 conjugated Streptavidin (Millipore, 1:300). Last, the slides were counterstained with DAPI (Millipore) and coverslipped in an anti-fading medium (Dako).

The sections were imaged with an Eclipse 90i microscope (Nikon, Apidrag, Romania) equipped with a QImaging Rolera cooled CCD camera and with narrow-band fluorescent filters centered for Alexa 594, Alexa

488 and DAPI excitation and emission wavelengths. Images were captured and archived using the Image ProPlus 7 AMS software (Media Cybernetics Inc., Buckinghamshire, UK).

Statistical analysis

We used the mean \pm standard deviation (SD) to express the values of MMP-9 in GCF. We also performed Wilcoxon test to compare the means ($p < 0.05$ for significantly statistical differences) and Pearson's and Spearman's tests for statistical correlations using a professional software (SPSS 16.0, Chicago, USA).

Results

Because in the group with gingival overgrowth during orthodontic treatment, values such as IP < 1 and BOP = 0 were recorded in nine cases, and IP > 1 and BOP = 1 in six cases, it was divided accordingly in two subgroups. In the nine cases, there was no clinical sign of inflammation but those were present in the other six. Unlike the control cases (Figure 1A), in all histologic samples (HE stain) from the patients in the GO group, there was chronic inflammatory infiltrate in the chorion, weakly represented in the nine cases without any clinical sign of inflammation (Figure 1B) and richly represented in the others (Figure 1, C and D). Gingival mucosa presented overgrowth upon hyperplasia of the basal lamina and hypertrophy of the intermediate layer, with foamy less acidophilic cytoplasm cells.

Fluorescence double labeling studies showed that in incipient, moderate and aggressive fibroblastic reactions of the gingiva, MMP-9 positive cells originate around the dermal papillary vessels. Compared to the gingiva of control cases (Figure 2A), MMP-9/collagen IV double stainings showed an increase of fibrosis and inflammation in different degrees (Figure 2, B–D). Given the high degree of fibrosis concurring with the MMP-9 positive cells accumulations, no direct influence could be assessed for the enzymes on the vascular basement membranes.

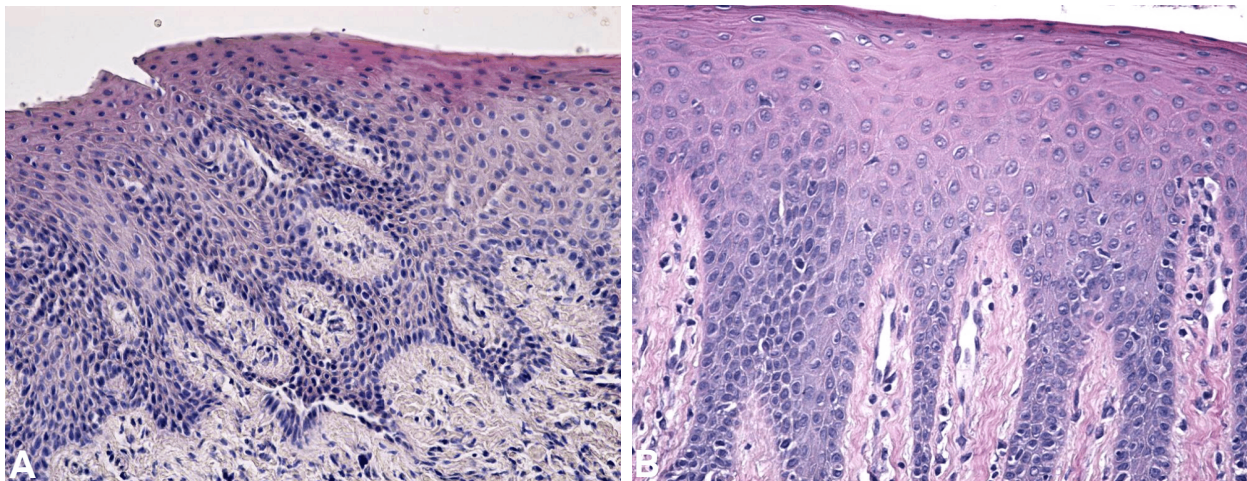


Figure 1 – Histologic samples (HE stain) from control cases (A) and from patients with GO without any clinical sign of inflammation (B).

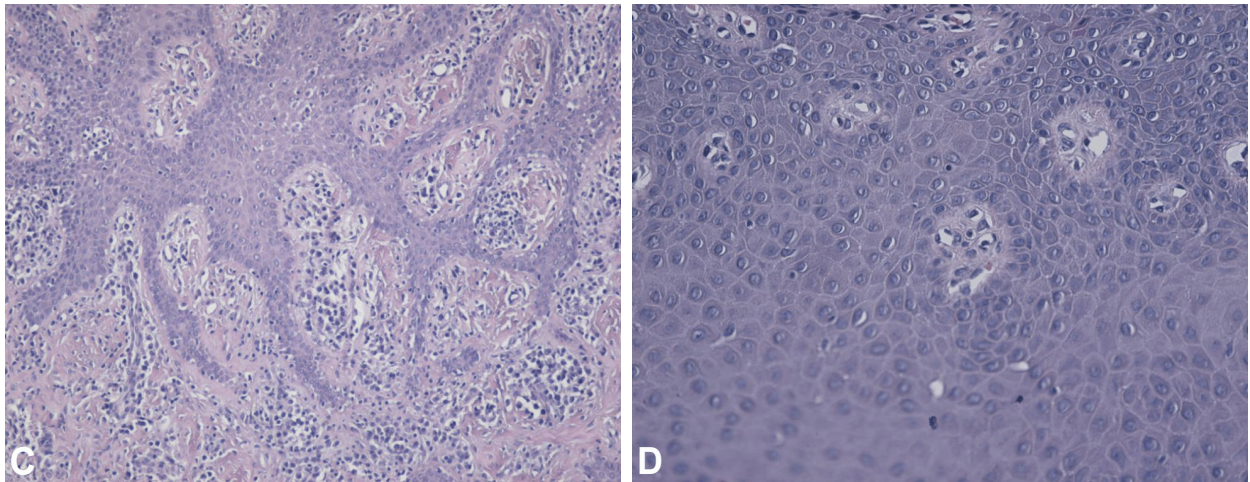


Figure 1 – (C and D) Histologic samples (HE stain) from patients with GO and inflammation.

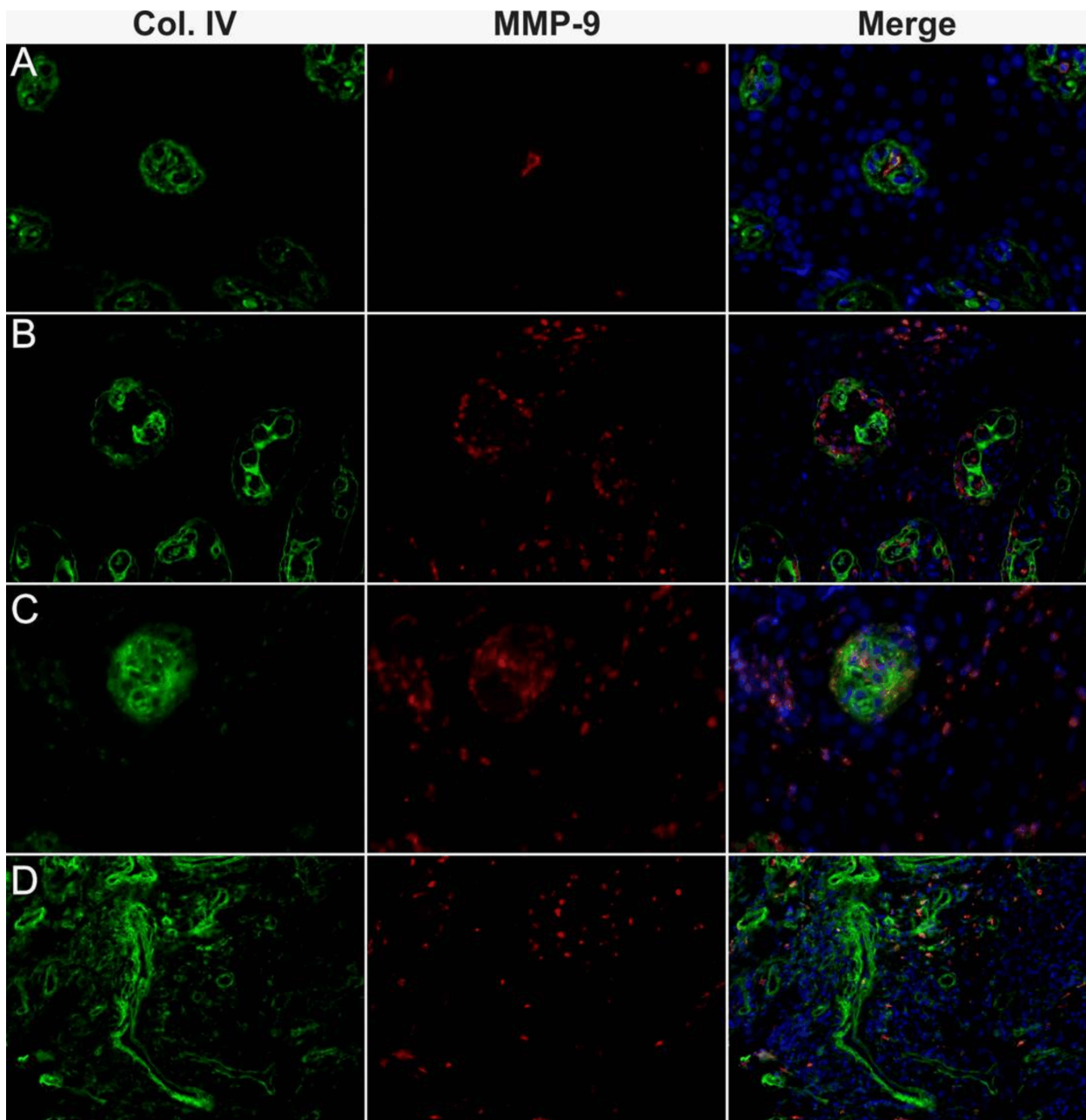


Figure 2 – MMP-9 / collagen IV fluorescence double stainings in the gingiva of control cases (A), and patients with GO without or with inflammation (B–D).

Discussion

Expressed at basal-line levels or completely absent in physiological conditions, up-regulation of MMP-9 has been widely studied in stroke pathology, and especially in ischemic lesions prone to develop a secondary hemorrhage [12–15]. MMP-9 seem to increase in the event of brain hemorrhages, showing thus the extent of its contribution to infarct extension and blood brain barrier (BBB) leakage [16]. The loss of type IV collagen in basal membrane along with elevations in MMP-9 expression, especially the activated forms, was identified during colorectal tumorigenesis. These data suggest that control of type IV collagenase activation may be beneficial in preventing human colorectal tumor progression [17]. Other studies showed that the degradation of type IV collagen by the MMP-9 is modulated by MMP-2 [18–20]. Although MMP-9 expression is high, in the gingiva of patients with GO and increased level of bacterial plaque and BOP<1, it does not harm yet the type IV collagen in the basal membrane. In the present study, the elevated levels of MMP-9 in gingiva do not seem to alter the pattern of collagenous depositions regardless of their increasing density. We may say that during orthodontic treatment, the mechanical stress is one of the factors determining the increase of MMP-9 in GCF and the onset of GO. The presence of bacterial plaque superimposed over the mechanical effect of the orthodontic appliance leads to the occurrence of clinical signs of inflammation and MMP-9 values higher than in case of chronic gingivitis, but lower than in profound periodontal disease. Results of double immunomarking for MMP-9 and type IV collagen show that these values are not enough to determine any damage of this type of collagen.

In our study, increasing MMP-9 in gingiva of patients with GO did not seem to decrease the pattern of collagen IV expression. Moreover, it seemed that both MMP-9 and collagen IV expressions increased in a parallel fashion. It could be that other factors, like the mechanical stress induced by the orthodontic devices, might be the key players in driving both the inflammation and the fibrotic reaction, thus overdriving the direct influence of the enzyme on collagen remodeling. Also, as the antibody used here detects a sequence of amino acids near the C-terminal tail of MMP-9 (and thus a fragment present in both the pro-enzyme and its active form), we cannot rule out that this abundant enzyme is in fact in the inactive form. Further studies will be needed to clarify this issue.

Conclusions

This study showed that the mechanical stress could be involved in both inflammation and fibrotic reactions in gingiva during orthodontic treatment.

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