ORIGINAL PAPER

Correlations between the gingival crevicular fluid MMP8 levels and gingival overgrowth in patients with fixed orthodontic devices

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Abstract

Levels of metalloproteinase 8 (MMP8) in gingival crevicular fluid were studied in case of treatment with fixed orthodontic appliances. It were found a relationship between its levels and stages of the treatment under a good control of the bacterial plaque. Gingival overgrowth (GO) during the orthodontic treatment was traditionally considered as an inflammatory reaction consecutive of bacterial plaque accumulation because of difficult hygiene in those patients. Our study starts from the hypothesis that the gingival volume growth during the fixed orthodontic treatment appears at the beginning, without any inflammatory signs, as a result of the mechanical stress and periodontal remodeling during the orthodontic dental movement, the MMP8 acting as an indicator of this situation. *Material and Methods*: Twenty-two patients received a fixed orthodontic treatment. Periodontal examination took place one hour before and one hour, four and eight hours and weekly after until eight weeks. At each session gingival crevicular fluid (GCF) was sampled and the level of MMP8 was determined. At the appearance of gingival overgrowth (GO) gingivectomy was performed. *Results*: In the 13 patients that did not develop gingival overgrowth, the levels of MMP8 increased in the first 4–8 hours after orthodontic appliance and then fall to the initial level. In the nine patients with gingival overgrowth, the MMP8 levels in GCF continued to rise until the appearance of GO. In cases of GO with inflammation the levels of MMP8 were significantly higher than in cases of GO without inflammation. The expression of MMP8 in hypertrophied gingiva was more intensive in cases of GO with inflammation. *Conclusions*: It is possible that the MMP8 values in GCF to be a marker of the GO onset. MMP8 determination and monitoring at shorter time intervals may lead to a better control of the bacterial plaque and avoidance of gingival inflammation.

***Keywords*:** gingival crevicular fluid, matrix metalloproteinase, orthodontic treatment, gingival overgrowth.

Introduction

Matrix metalloproteinase 8 (MMP8) is a part of the zinc-dependent endopeptidases family with role in collagen breakdown [1, 2] and is produced mainly by the polymorphonuclear leucocytes but also by the epithelial cells and fibroblasts [3–5].

Levels of MMP8 in gingival crevicular fluid were studied in case of treatment with fixed orthodontic appliances. It were found a relationship between its levels and stages of the treatment under a good control of the bacterial plaque [3, 6] and also a relationship with the pain reported by some patients in the first hours from the insertion of the orthodontic device [7, 8]. Both were explained by the involvement of MMP8 in the induction of periodontal ligaments remodeling (PDL) during dental movement. Other authors [9] also sustain that the matrix metalloproteinases are implicated in the regulation of bone remodeling in the marginal periodontium. During this process, the extracellular matrix components, including the collagen, are broken down and removed and new components are synthesized and deposited. Experiments on laboratory rats about the MMP production in PDL during the orthodontic dental movement revealed [10] that the MMP8 is produced by the cells in the cemental surface and by the osteocytes in the alveolar bone but not in the other PDL cells.

Gingival overgrowth (GO) during the orthodontic treatment was traditionally considered as an inflammatory reaction consecutive of bacterial plaque accumulation because of difficult hygiene in those patients. The inflammation of GO determines the orthodontist to temporize the treatment until its removal, especially when its size and inflammatory symptoms are important [11]. There are less studies in the literature about this subject proven scientifically [12], the relationship between the onset of the GO and bacterial plaque accumulation being more a clinical evidence. However, our clinical observations showed a gingival volume growth in patients with good dental hygiene, without any clinical signs of gingival inflammation.

Our study starts from the hypothesis that the gingival volume growth during the fixed orthodontic treatment appears at the beginning, without any inflammatory signs, as a result of the mechanical stress and periodontal remodeling during the orthodontic dental movement, the MMP8 acting as an indicator of this situation. The inflammation is, in our opinion, an added process because of bacterial plaque accumulation under

the shelter of the hypertrophied gingiva. We try to demonstrate that the mechanical stress is leading to MMP8 accumulation in the gingival crevicular fluid and in the hypertrophied gingiva as an expression of collagen remodeling. The monitoring of the MMP8 levels in GCF during the initial treatment may be a marker for the next evolution of the gingival mucosa.

Material and Methods

Twenty-two patients (12 females and 10 males) with ages between 12 and 29 years, mean age 15.32±3.99 years, with fixed orthodontic appliances for dental anomalies, were included in the study. The patients were informed in detail about the study and formal consent was obtained from the patient or from its tutors.

From the 22 patients, nine developed a gingival volume increase between the third and the eight week from the orthodontic appliance.

Selection criteria

Absence of general diseases that may be associated with periodontal lesions with or without GO (diabetes, hematologic diseases, HIV infection, hypovitaminosis).

Exclusion criteria

Patients that received, in the last 30 days, general treatment with antiepileptics, calcium antagonists, cyclosporines, antibiotics and anti-inflammatory drugs.

Oral clinical examination

Orthodontic

The patients were explained: the nature of the orthodontic treatment, estimated length of time, frequency. A clinical observation chart was filled. An initial classification of the cases was made in function of age and sex. Fixed orthodontic device with straight-wire technique with Balance brackets were placed.

Periodontal

In the periodontal department, the selected patients were informed and instructed upon oral hygiene maintenance through dental brushing and auxiliary means and a good control of the bacterial plaque. A clinical periodontal observation chart was filled including:

- Stilness and Löe plaque index (IP) [13] as an indicator of the dental hygiene. Scores of 2–3 were considered as high;
- Muhleman bleeding index (IM) as an indicator of gingival inflammation. Scores as 2–3–4 were considered as high;
- Presence/absence of gingival overgrowth;
- History of smoking.

Samples of GCF were obtained according to previously described techniques [14] using paper strips maintained for 30 seconds in the gingival sulcus one hour before the placement of the orthodontic device, and then at one hour, four hours, eight and 24 hours after. Then, the GCF was sampled weekly for eight weeks. The samples were introduced in polypropylene tubes with 0.1 mL PBS and stored at -20^{0}C prior to its use (measure of MMP8 levels). Clinically, the patients were examined weekly for eight weeks, measuring IP, BOP and IM. On those occasions, the patients in whom the gingival overgrowth was noticed, a gingivectomy was performed and the material obtained was used for histologic and immunohistochemical study.

Immunohistochemical examination

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

The histologic examination was performed using classic Hematoxylin–Eosin technique. For the immunohistochemical examination the antigenic recovery was achieved by boiling for 11 minutes in the microwave oven in buffered citrate solution with pH of 6.

Immunomarking for MMP8 was achieved using LSAB technique. It was used the primary antibody Rabbit anti Human MMP* (Abcam ab81286) in 1:500 dilution and for detection, the Dako Envision system, the chromogen used being 3.3'-diaminobenzidine (DAB).

The plates were analyzed with a Nikon Eclipse 90i microscope with 5 megapixels CCD camera. Random images of the tissue were captured with 20× plan apochromatic objective. The images were saved as .tiff files and analyzed with the dedicated software NIS-Elements (Nikon). MMP8 expression was estimated on groups of six images on each studied case by calculating the total integrated optical density (IOD). This was achieved by preliminary calibration of the CCD sensor for absolute white and black signals, and then levels of real intensity corroborated with signal area (cumulated as IOD) were calculated for each image. It was then calculated the average of IOD for each case and compared afterwards.

Immunological examination

For GCF determination we used the commercial tests Quantikine Elisa Kit (R&D Systems, USA) for MMP8 using polypropylene tubes to avoid contamination. Every kit component was used according to the manufacturer's indications. Reading was performed with Elisa tests PR 2100 reader (BioRad, USA) at 450 nm with a correction at 540 nm to reduce optical imperfections on the reading plate.

Statistical analysis

Both the results of the clinical orthodontical and periodontal examination and those of the immunological and immunohistochemical examination were statistically analyzed using ANOVA and Mann–Whitney U-test for significant correlations (p<0.05). It was also used the average ± standard deviation (SD).

Results

Determination of MMP8 levels in GCF according to the previously described technique, in the first few hours following orthodontic appliance indicated an increase of 3.5–6 folds reaching a maximum level of 36±18.32 μg/L at eight hours, in all 22 cases.

The other nine cases presented a continuous increase

of MMP8 levels, maximal values (723.11±762.66 μg/L) were reached in the week of clinical increase of gingival volume. In those nine cases, the GO occurred between the third and the eight-week with an average of 5.85 ±1.81 weeks.

The high level for the standard deviation of the MMP8 level – 762.66 μg/L – is explained by the fact that in three cases, the MMP8 levels reached a maximum of 1640.66±627.82 μg/L and in the rest of six the maximum level was 264.33±123.55 μg/L.

In those three cases, there were also signs of gingival inflammation with high levels of IP and IM values of 2.67±0.35 and 3.83±0.63 respectively. The GO appeared in the first three weeks after orthodontic appliance.

In the rest of six cases of GO without clinical signs of gingival inflammation, lower levels for IP and IM were recorded 1.21±0.18 and 1.28±0.06 respectively. Average level of MMP8 in GCF was 264.33±123.55 μg/L. In those patients GO appeared later, in the 6–7th week.

To express and statistically analyze the intensity of the MMP8 expression in the hypertrophied gingival, we used the IOD method for quantification of the optical integrated density. The values obtained served for MMP8 comparison in GO with and without clinical signs of inflammation. The same method was used to established a statistical correlation between the inflammation indicators (IP and IM) and MMP8 expression in GO.

On every histologic sample (HE stain) in those nine cases, we identified in chorion the presence of the chronic inflammatory infiltrate, weakly represented in the six cases without any clinical sign of inflammation (Figure 1) and richly represented in the other three (Figure 3). Gingival mucosa presents overgrowth upon hyperplasia of the basal lamina and hypertrophy of the intermediary layer, with foamy lower acidophilic cytoplasm cells.

In the three cases with GO with clinical signs of inflammation, the average IOD was 161881.3±139737. In the six cases of GO without clinical signs of inflammation the average IOD was 24354.9±11138.36. The difference was significant (p<0.01). We noticed an increased expression of MMP8 (Figure 2) in cases of GO without clinical signs of inflammation with low IP and IM (<2) but a statistically significant difference in favor of MMP8 expression (Figure 4) in the three cases of GO with inflammation compared to the other six. This high expression of MMP8 in the three cases is correlated with high IP and IM (>2).

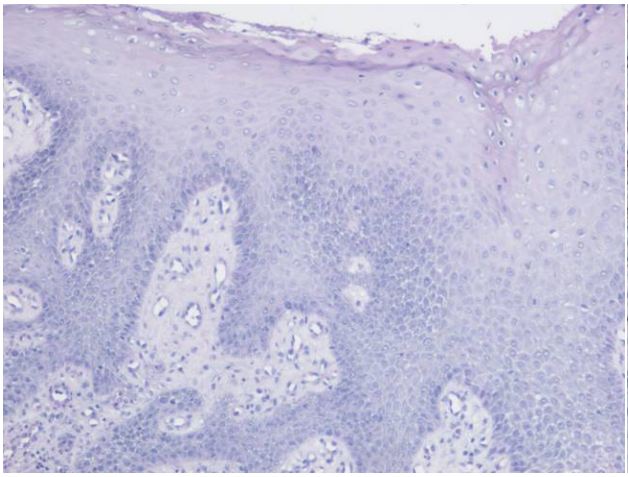

Figure 1 – ***Gingival mucosa with hypertrophy and hyperplasia, chorion with discreet fibrosis and rich in small caliber capillaries with neoforming aspect (HE stain, ×100).***

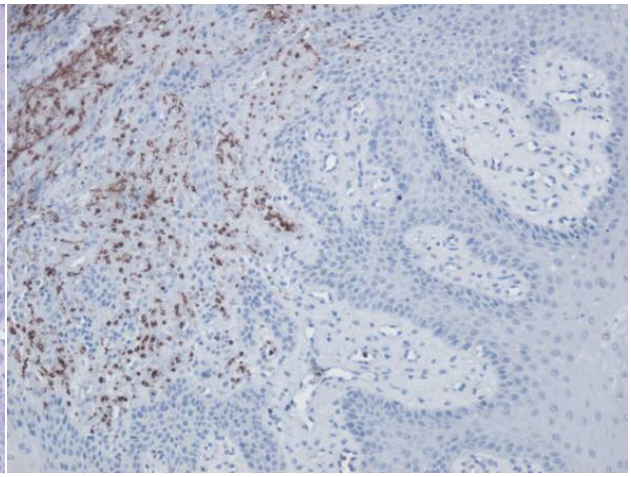

Figure 2 – ***Immunomarking for MMP8 in papillary chorion with high intensity. Gingival mucosa with overgrowth, intense regional vascularisation, capillary type (DAB, ×100).***

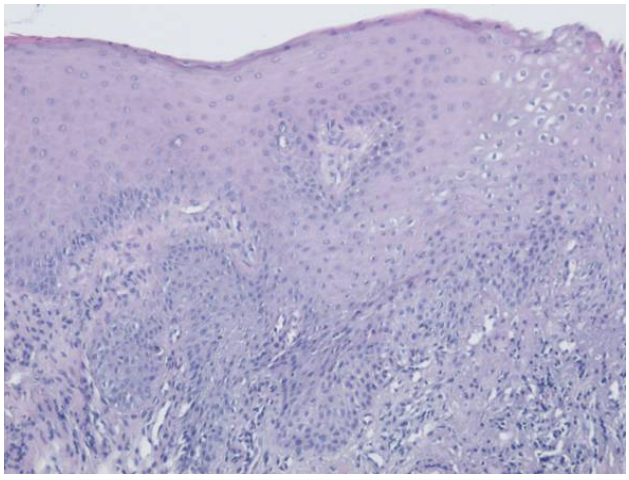

Figure 3 – ***Gingival mucosa with overgrowth upon hyperplasia especially of the basal lamina and hypertrophy of the intermediary layer, with foamy low acidophilic cytoplasm cells; chronic inflammatory infiltrate of lympho-plasmocytic type; islands of connective tissue that disorganize the stratified structure of the epithelia; thick bands of collagenous fibers with quasiordered arrangement perpendicular on the basal layer of the epithelia (HE stain, ×100).***

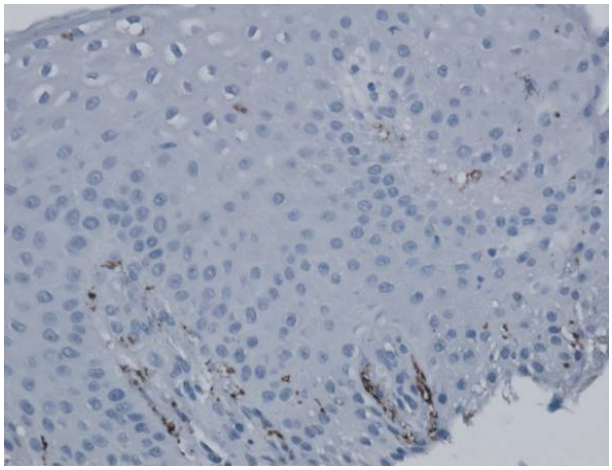

Figure 4 – ***Immunomarking for MMP8 of high intensity, present among the hyperplastic epithelial cells, at the level of the connective tissue outlined by epithelial overgrowth (DAB, ×100).***

Discussion

Regarding the MMP8 levels in GCF immediately after the initiation of the orthodontic treatment, the results that we obtained are similar to those previously found by other authors [3, 4, 6]. It showed that there is significant increase of the MMP8 levels in GCF in patients with fixed orthodontic appliances in the first 4–8 hours from insertion.

In a study from 2003, Mäntylä P *et al.* [5], trying to establish the level of reference of MMP8 values in GCF from which this could become an indicator for periodontal changes, indicated a threshold of 100 μg/L for the healthy periodontium, 750 μg/L for gingivitis and 2500 μg/L in periodontitis. In our study, the situation of average ± SD for MMP8 in GCF of the nine patients with GO, beyond the threshold for healthy periodontium is given by the values of the MMP8 in GCF in those three patients in whom the GO had an inflammatory origin. Migration of SD beyond the average (723.11±762.66 μg/L) is explained by the very high values of MMP8 in those three cases compared to the other cases in the same group.

The presence of MMP8 in the cases of GO without gingival inflammation (IP and IM <2), sustains the hypothesis that their presence in the gingival tissue is related to the remodeling process of PDL [15] and not to the presence of the bacterial plaque. We noticed a significant difference in the favor of MMP8 expression in the three cases of GO with clinical inflammation in comparison with the other six without inflammation. This high expression of MMP8 in those three cases is correlated with IP and IM (>2) probably because of the inflammatory process induced by the presence of the bacterial plaque.

It is already known the implication of a smoking history in the presence of the marginal periodontal changes [16–18]. Recent studies correlate the expression of some biomarkers as a nuclear factor kB ligand (RANKL) and osteoprotegerin (OPG) in GCF and in the gingival tissue [19, 20] with smoking in patients with periodontitis. In the present study, we could not find a relationship between smoking and the levels of MMP8 in GCF and GO in the processes of orthodontic periodontal remodeling with GO because all the patients included in the study declared themselves as non-smoking.

Conclusions

The increase of MMP8 levels in GCF in patients with fixed orthodontic devices continues even after the first 4–8 hours from appliance in patients that will further develop a GO even in the absence of a bacterial plaque. In this patients, GO does not have clinical features of inflammation in the conditions of a good control of the bacterial plaque through mainly mechanical means (dental brushing) and supportive means (water pick and mouth wash). When, because of GO and orthodontic appliance components, the bacterial plaque accumulation occurs, the levels of MMP8 increase significantly and the GO takes a clinical inflammatory aspect, imposing the temporizing of the orthodontic treatment. Thus, is possible that the MMP8 values in GCF to be a marker of the GO onset. MMP8 determination and monitoring at shorter time intervals may lead to a better control of the bacterial plaque and avoidance of gingival inflammation.

We consider that the extension of the research to a larger group of patients and other possible biomarkers in GCF and GO may lead to results with higher statistic significance; therefore, this study must be continued.

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