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Matrix metalloproteinase 9 levels in gingival crevicular fluid in patients after periodontal microsurgery for orthodontic induced gingival hypertrophy

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Abstract

Introduction: In this study, we aim to compare the levels of matrix metalloproteinase 9 (MMP9) in the gingival crevicular fluid (GCF), as indicators for healing, in two groups of patients – operated with a classic periodontal surgical technique and the same technique but using a dental microscope. *Materials*: We included 14 patients with ages between 12 and 26 years, average 14±6.2 years. Eight patients were women and six men. All patients presented gingival hypertrophy because of the orthodontic treatment on the mandibular arch. We performed gingivectomy on one-half of the mandibular arch by classic periodontal surgery and on the other half of the mandibular arch by a microscope-assisted gingivectomy. *Methods*: In the hypertrophied gingiva, the expression of MMP9 was identified using immunohistochemical-staining techniques. For immunological determination of MMP9 in GCF we performed Elisa tests. *Results*: We found different levels in different moments of the healing process for the two hemiarcades. *Conclusions*: We consider that faster healing in case of microscope-assisted gingivectomy may be related to the expression of MMP-9 in the GCF.

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

Introduction

Periodontal microsurgery imposed itself in practice due to its clinical advantages determined by the good postoperative clinical course [1].

Clinical observations indicated a faster healing of the gingival mucosa compared to same periodontal techniques performed without microscope and less pain for the patient. Those findings were reproduced also for other tissues submitted to microsurgery, also with good healing [2, 3]. There are studies trying to bring scientific proofs for the clinical observations by determining the levels of MMP9, TGF-1beta, and TNF-alpha [4] to emphasize their role in the healing process.

In this study, we aim to compare the levels of MMP9 in the gingival crevicular fluid (GCF) as indicators for healing, in two groups of patients – operated with a classic periodontal surgical technique and with the same technique using a dental microscope.

Materials and Methods

Materials

We included 14 patients with ages between 12 and 26 years, average 14±6.2 years. Eight patients were women and six men. All patients presented gingival hypertrophy because of the orthodontic treatment on the mandibular arcade. The hypertrophic gingiva was removed from one-half of the mandibular arch by a classic gingivectomy and from the other half by a gingivectomy performed under vision from a dental microscope. In the hypertrophied gingiva, the expression of MMP9 was identified using immunohistochemical-staining techniques.

Methods

GCF sampling

Samples of GCF were obtained according to previously described techniques [5] using paper strips maintained for 30 minutes in the gingival sulcus one hour before the gingivectomy and then at 24, 48, 72 hours and one week after. After weighting, the samples were introduced in polypropylene tubes with 0.1 mL PBS and stored at -20^{0}C prior to its use (measure of MMP9 levels).

Immunohistochemical examination

The tissue samples obtained by gingivectomy were fixed in neutral 10% formalin solution for 48 hours and then included in paraffin with the usual technique. The histological 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 6.

Immunomarking for MMP9 was achieved using LSAB technique, with primary antibody Rabbit anti Human MMP9 (Abcam ab38898) in 1:200 dilution. For detection we used the Dako Envision system, with 3.3'-diaminobenzidine (DAB) as chromogen.

Immunological examination

For MMP9 determination in GCF we used the commercial tests Quantikine Elisa Kit (R&D Systems, USA) for MMP9, 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

The results were statistically analyzed using Mann–Whitney U-test for significant correlations ($p<0.05$). We also used the mean ± standard deviation (M±SD).

Results

MMP9 levels in the GCF before gingivectomy was 1.6 µg/mL in the mandibular hemiarcade with classic periodontal intervention, 1.5 µg/mL in the other. At 24 hours postoperatively, the MMP9 levels in GCF was greater in both hemiarcades compared with preoperative levels ($p<0.05$). There was no significant difference between the hemiarcades at that moment.

After 48 hours, the level of MMP9 in the GCF in the microsurgical operated hemiarcade was inferior to the 24 hours level, but still higher than the preoperative value.

In the classically operated hemiarcade the levels of MMP9 in GCF continued to raise, reaching 3.2 µg/mL at 72 hours, and while in the micro surgically operated hemiarcade the values went well under the preoperative values, to a level not far from the level registered a week after surgery.

For the hemiarcade with classic intervention, the levels of MMP9 in the GCF reached the values before the gingivectomy one week after surgery, the maximal value being recorded at 72 hours. The MMP9 immunostaining was positive both in the chorion and in the deep structures of the overlaying epithelia (Figure 1). MMP9 positive cells were observed at the level of each component of the gingival mucosa. The highest intensity of staining was noted in the blood vessels and perivascular (Figure 2).

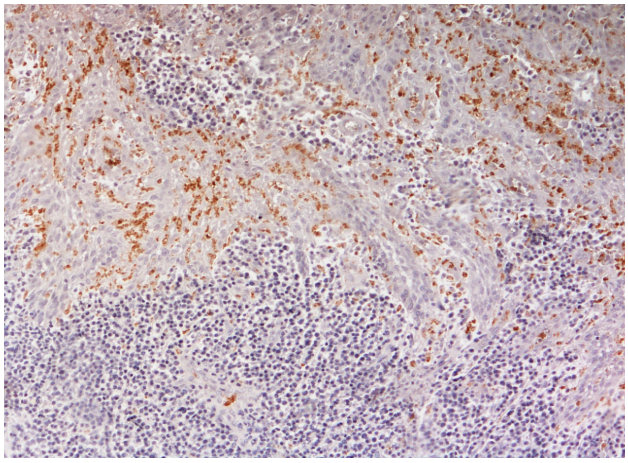

Figure 1 – ***Positive intense immunostaining for MMP9 in the areas with disorganized epithelial layers.***

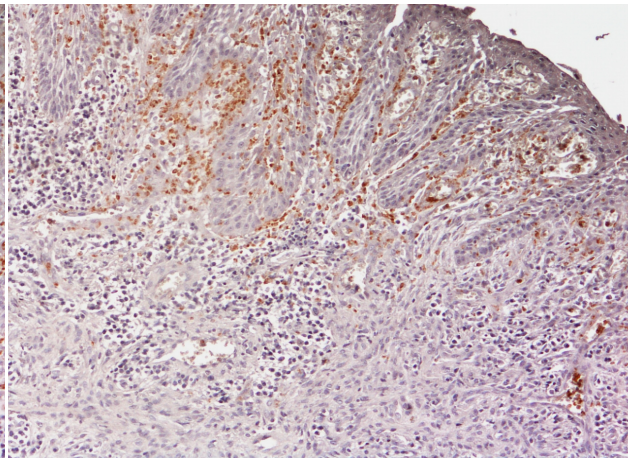

Figure 2 – ***Gingival hypertrophy with subepithelial inflammatory infiltrate and positive immunostaining for MMP9 disposed under the basal epithelial layer and perivascular.***

Discussion

Metalloproteinases are a family of Zn-dependent endopeptidases that degrade the extracellular matrix. Those enzymes are implied in the pathologic processes of the oral cavity as the destruction of the periodontal tissues, tumoral invasion or the temporo-mandibular joint dysfunctions [6]. All MMP contain Zn^{2+} [7, 8] at the level of the catalytic site and need Ca^{2+} for stability and activity [9]. The MMP9 also has a gelatin-binding domain, inserted between the catalytic and the active domains [10] reason for which the MMP9 is also known as gelatinase B.

The MMP9 is a 92-kDa molecule with a specific substrate of gelatin, fibronectin, elastin, type IV, V, VII and X collagen and type I denaturized collagen. The MMP9 role in healing of the wounds was studied for different reasons. Studying patients with diabetes mellitus with foot ulcers, Liu Y *et al.* [11] monitored the levels of MMP9 in the ulcerous lesions for 12 weeks. The patients with high levels of MMP9 had a delayed healing of the lesions.

Other authors showed that the 92-kDa gelatinase (MMP9) is linked to the epithelisation process and early repair events [12]. During wound healing, MMP9 is suggested to be involved in keratinocyte migration and granulation tissue remodeling [4].

Measuring MMP9 after surgical interventions in the maxillofacial area (sinus surgery) leads to the same conclusion, that the high levels of MMP9 indicate a delayed healing [13]. Also, increased expression of

MMP-9 was found in the periodontal disease, decreasing after the treatment, higher MMP9 levels in the GCF being associated with the severity of the disease [14, 15]. Levels of MMP9 in GCF were determined during the orthodontic treatment along with other MMPs, their role and activating cascade being still insufficiently clarified [16, 17]. The MMP values that we found in the GCF after gingivectomy are significantly higher (p<0.05) after classic surgery compared to microsurgery. Also, those values increase in time, with a maximum at 72 hours for classic surgery. For microsurgery, the MMP9 values reached a maximum in 24 hours and those values were significantly lower than those registered after classic surgery (Table 1).

Table 1 – ***MMP9 levels in GCF [ng/mL]***

MMP9 levels (M±SD)	Classical gingivectomy	Microsurgery
Before gingivectomy	1.6±0.3	1.5±0.1
+24 hours	2.1±0.7	2.2±1.2
+48 hours	2.8±1.2	1.8±1.2
+72 hours	3.2±1.1	0.8±1.0
+1 week	1.5±0.6	0.7±0.4

This particular expression of MMP9 may be due to both the different invasiveness of the two interventions and the accuracy of the surgery performed under dental microscope. Better visualization allows more precise and less aggressive maneuvers. In the same time, tissues with clinical signs of inflammation are more precisely removed. On the other hand, high levels of MMP9 induce a loss of balance between the processes of production and degradation of collagen in the wall of the blood vessels, leading to the degrading of vascularity in that area. The reduction in tissue trophicity may be the cause of the delayed healing of the wound after periodontal classic surgical act, unlike those assisted by the microscope in which the MMP9 is lower.

Conclusions

We consider that faster healing in case of gingivectomy performed under visualization through a dental microscope may be related to the expression of MMP9 in the crevicular fluid.

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