Original Article

Levels of gingival crevicular fluid matrix metalloproteinases in periodontally compromised teeth under orthodontic forces

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ABSTRACT

Objective: To examine levels of matrix metalloproteinases (MMPs)-1, -2, -3, -7, -8, -12, and -13 in the gingival crevicular fluid (GCF) of periodontally compromised teeth at different time points during orthodontic movement.

Materials and Methods: Ten controlled periodontitis subjects were submitted to orthodontic treatment. One dental arch was subjected to orthodontic movement, and teeth in the opposite arch were used as controls. GCF samples were collected from the lingual sites of two movement and two control incisors 1 week before orthodontic activation (−7 d), immediately after orthodontic activation, and after 1 hour, 24 hours, and 7, 14, and 21 days. Multiplexed bead immunoassay was used to measure MMPs in GCF. Data were analyzed using Friedman and Wilcoxon statistical tests.

Results: The only significant change found over time was in the levels of MMP-1 in the movement group ($P < .05$). When the two groups were compared after activation, the only statistically significant difference found was in levels of MMP-12 24 hours after activation ($P < .05$).

Conclusions: Our findings suggested that the orthodontic movement of periodontally compromised teeth without active pockets did not result in significant changes in the GCF levels of MMPs. (*Angle Orthod.* 2015;85:1009–1014.)

KEY WORDS: Gingival crevicular fluid; Matrix metalloproteinases; Orthodontics; Tooth movement; Periodontal disease

INTRODUCTION

Matrix metalloproteinases (MMPs) are believed to be the main endogenous mediators of the pathologic tissue destruction in periodontitis.[1,2] The levels of MMPs have been studied extensively in gingival crevicular fluid (GCF)[3,4] and saliva[5,6] and have been shown to be elevated in patients with periodontitis compared to periodontally healthy subjects. Further, periodontal treatment resulted in decreases in GCF levels of MMPs.[7] GCF levels of MMP-8,[8,9] MMP-3,[10] and MMP-13[11,12] have also been associated with periodontal disease progression.

MMPs also play a central role in periodontal ligament (PDL) remodeling during orthodontic tooth movement. Redlich et al.[13] demonstrated an increase in the messenger RNA (mRNA) levels and activity of MMP-1 in the compression side of the gingiva during orthodontic tooth movement in dogs. An increased expression of MMP-8 and MMP-13 mRNA in the PDL of rats during active tooth movement has been demonstrated.[14] Orthodontic tooth movement can be delayed or prevented in mice and rats by the use of MMP inhibitors.[15,16] A few human studies[17–19] have quantified the presence of MMPs in GCF during orthodontic tooth movement and have reported alterations in their levels during the application of orthodontic forces. Further, total collagenase activity in the GCF of orthodontic patients treated with fixed appliances has been shown to be 10-fold that of control GCF.[20]

The effects of orthodontic forces on teeth affected by periodontal disease have not been extensively studied. However, a few longitudinal and retrospective studies[21,22] indicated that periodontally compromised teeth

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can withstand orthodontic forces without additional damage to the periodontium. Since the levels of MMPs seen to be associated with an increased risk of progression of periodontal disease and with orthodontic tooth movement, the aim of this study was to evaluate the changes in the GCF levels of MMPs in patients with controlled periodontal disease submitted to orthodontic tooth movement.

MATERIALS AND METHODS

The study population was composed of 10 periodontitis subjects submitted to orthodontic treatment at the dental school of the Rio de Janeiro State University (UERJ), Brazil. There were eight females and two males—eight blacks and two whites—and their mean age (±standard deviation [SD]) was 46.2 ± 10.4 years. The subjects were required to have their periodontal condition under control with radiographic evidence of bone loss and they had to present flared incisors requiring orthodontic retraction. Subjects had to be in good general health and were excluded if they had any systemic condition that would influence the course of periodontal disease or treatment and if they were smokers. Individuals who had taken antibiotics or any anti-inflammatory drug in the previous month or were either pregnant or nursing were also excluded.[23] The Ethics Committee of the Pedro Ernesto University Hospital (UERJ, Brazil) approved the protocol, and all participants signed an informed consent form prior to enrollment in the study.

One week prior to the beginning of the orthodontic movement all subjects received oral hygiene instructions on the correct tooth-brushing technique and use of interdental cleaning devices. In addition, all participants received a bottle (250 mL) of chlorhexidine gluconate 0.12% mouth rinse and were instructed to rinse twice a day with 15 mL of the solution for 30 seconds for the duration of the study (28 days). During this session (day −7) orthodontic brackets were bonded in the upper arch of five subjects and in the lower arch of the remaining five participants to guarantee an even distribution of movement and control sites in both arches. Control and treated teeth had similar periodontal conditions. GCF samples were collected from the midpalatal and midlingual sites of one central and one lateral incisor of each arch from each subject, for a total of four individual samples per subject. These sites were selected to represent the pressure side of the applied forces (movement sites). Samples obtained from the arch without the appliance served as controls. On day 0, the orthodontic appliances were activated to start the retraction of the flared incisors. The orthodontic treatment plan was specific for each patient, but all plans involved fixed appliances with metallic brackets (Morelli, Sorocaba, Brazil) and had as the first alignment arch a stainless-steel stranded arch wire measuring 0.0155 inches (Highland Metals Inc, San Jose, Calif) with activated tie back loops. Additional GCF samples were collected immediately after activation (time 0) and 1 hour, 24 hours (24 h), 7 days (7 d), 14 days (14 d), and 21 days (21 d) after application of the orthodontic force. The presence or absence of plaque (PI) and bleeding on probing (BOP) (0 or 1) were recorded at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) at visits −7 d, 0, 24 h, 7 d, 14 d, and 21 d (Table 1).

Prior to GCF sample collection, sampled sites were isolated with cotton rolls and dried gently with an air syringe. Supragingival plaque was carefully removed before sampling. Thirty-second GCF samples were collected using paper strips (Periopaper®, Interstate Drug Exchange, Amityville, NY), which were inserted 1 to 2 mm into the gingival crevice. The volume of GCF in each strip was measured with a calibrated GCF meter (Periotron 8000®, Oraflow Inc, Plainview, NY), and individual samples were transferred to Eppendorf tubes and stored at −20°C until assayed.

Levels of MMP-1, -2, -3, -7, -8, -12, and -13 were determined using the multiplexed bead immunoassay technique and a commercially available kit (Human 7-Plex MMP Fluorokine® MultiAnalyte Profiling [R&D Systems, Minneapolis, Minn]). Prior to assay the GCF contents were eluted from the periopaper strips by immersing each strip into 60 μL of the assay buffer provided in the kit. Samples were then vortexed for 30 minutes, centrifuged at 9300 g for 10 minutes, and the entire supernatant was used in the assay, representing the content of one site. The assay was conducted according to the manufacturer's instructions; briefly, 96-well filter plates were pre-wetted with washing buffer and the solution was aspirated from the wells using a vacuum manifold (Millipore Corp, Billerica, Mass). Microspheres coated with monoclonal antibodies against the seven different target analytes were added to the wells. Samples and standards were pipetted into the wells and incubated overnight at 4°C. The upper and lower limit of the standard curves varied according to the different MMPs being measured; the lowest limit of detection was 11 pg/mL for MMP-1 and the highest 74,500 pg/mL for MMP-7. The wells were washed using a vacuum manifold, and a mixture of biotinylated secondary antibodies was added. After incubation for 1 hour, streptavidin conjugated to the fluorescent protein, R-phycoerythrin (streptavidin-RPE), was added to the beads and incubated for 30 minutes. After washing to remove unbound reagents, sheath fluid was added to the wells, and microspheres (minimum of 100 per analyte) were

Table 1. Medians and Interquartile Ranges for Gingival Crevicular Fluid (GCF) Levels of Matrix Metalloproteinases (MMPs) in the Two Clinical Groups at the Different Time Points. M Represents Movement Group, and C Represents Control Group, Followed by the Time Points of the Study (−7 days [−7 d], time 0 [0], 1 hour [1 h], 24 hours [24 h], 7 days [7 d], 14 days [14 d], and 21 days [21 d])

	25th	Median	75th
MMP1_M_−7 d	0.525	1.245	1.9925
MMP1_C_−7 d	1.4	2.76	7.3125
MMP1_M_0	0.745	1.745	3.345
MMP1_C_0	0.7875	1.595	3.09
MMP1_M_1 h	0.9275	1.715	2.25
MMP1_C_1 h	1.5875	5.52	12.1575
MMP1_M_24 h	3.5	6.5	9.25
MMP1_C_24 h	1.985	4.545	9.4725
MMP1_M_7 d	1.7425	3.995	7.1525
MMP1_C_7 d	2.325	3.64	14.5875
MMP1_M_14 d	0.9825	3.325	7.6625
MMP1_C_14 d	3.1725	7.03	9.6325
MMP1_M_21 d	0.8925	3.25	8.1975
MMP1_C_21 d	1.3075	3.525	5.41
MMP12_M_−7 d	1.4725	3.735	10.98
MMP12_C_−7 d	3.6925	10.04	24.485
MMP12_M_0	4.44	9.84	17.23
MMP12_C_0	3.32	6.175	10.9675
MMP12_M_1 h	5.6475	8.01	11.845
MMP12_C_1 h	2.5375	5.75	8.55
MMP12_M_24 h	13.1925	16.895	19.31
MMP12_C_24 h	4.355	6.77	11.895
MMP12_M_7 d	4.42	6.485	15.455
MMP12_C_7 d	10.08	14.635	15.34
MMP12_M_14 d	2.2025	9.36	19.6325
MMP12_C_14 d	2.5025	14.905	32.035
MMP12_M_21 d	4.3875	6.725	13.655
MMP12_C_21 d	3.65	4.38	18.685
MMP13_M_−7 d	1.87	3.835	8.8575
MMP13_C_−7 d	4.1675	9.44	17.0125
MMP13_M_0	2.4025	4.635	11.4175
MMP13_C_0	3.7125	5.67	11.07
MMP13_M_1 h	1.5675	2.08	3.3925
MMP13_C_1 h	2.2575	2.95	11.95
MMP13_M_24 h	1.9525	3.72	8.15
MMP13_C_24 h	1.825	5.6	10.4875
MMP13_M_7 d	1.6475	4.32	11.3975
MMP13_C_7 d	5.0275	7.75	14.5625
MMP13_M_14 d	0.7525	3.955	8.665
MMP13_C_14 d	3.53	7.215	10.695
MMP13_M_21 d	1.945	5.16	7.865
MMP13_C_21 d	3.2	4.375	11.25
MMP2_M_−7 d	4.965	14.73	22.5925
MMP2_C_−7 d	7.5075	14.66	25.535
MMP2_M_0	6.005	10.585	16.84
MMP2_C_0	5.0175	11.78	14.725
MMP2_M_1 h	8.065	10.7	13.515
MMP2_C_1 h	6.615	9.985	18.1725
MMP2_M_24 h	7.46	10.725	16.375
MMP2_C_24 h	5.2525	12.845	21.625
MMP2_M_7 d	5.285	10.23	25.0075
MMP2_C_7 d	7.88	9.03	32.295
MMP2_M_14 d	5.1275	8.28	19.08
MMP2_C_14 d	6.475	15.08	27.84
MMP2_M_21 d	5.25	11.06	19.6575
MMP2_C_21 d	6.255	14.8	20.5775
MMP3_M_−7 d	0.8325	1.94	10.21
MMP3_C_−7 d	1.0225	1.83	2.7025
MMP3_M_0	0.6025	1.61	2.1375
MMP3_C_0	0.46	1.255	2.6175
MMP3_M_1 h	0.7675	0.98	2.9
MMP3_C_1 h	0.815	1.525	2.545
MMP3_M_24 h	1.295	1.675	3.8875
MMP3_C_24 h	0.8375	4.675	7.9175
MMP3_M_7 d	0.72	1.15	11.0275
MMP3_C_7 d	0.91	1.47	3.3825
MMP3_M_14 d	0.7125	1.995	4.0325
MMP3_C_14 d	0.735	1.795	4.3275
MMP3_M_21 d	0.5475	0.985	2.1375
MMP3_C_21 d	0.905	1.425	1.9875
MMP7_M_−7 d	1.1225	3.09	12.905
MMP7_C_−7 d	2.29	4.58	8.075
MMP7_M_0	2.1375	2.695	9.125
MMP7_C_0	1.8525	3.845	5.08
MMP7_M_1 h	1.755	5.08	7.6275
MMP7_C_1 h	1.2625	3.135	11.78
MMP7_M_24 h	2.47	4.695	8.88
MMP7_C_24 h	1.8	3.975	6.5025
MMP7_M_7 d	2.6575	4.23	7.3425
MMP7_C_7 d	2.23	5.5	8.7575
MMP7_M_14 d	1.38	2.62	5.82
MMP7_C_14 d	1.035	5.54	17.3275
MMP7_M_21 d	1.235	3.59	9.715
MMP7_C_21 d	1.3725	3.895	5.51
MMP8_M_−7 d	486.5475	1399.12	2820.53
MMP8_C_−7 d	1034.03	2334.87	3350
MMP8_M_0	778.02	1505.57	2587.16
MMP8_C_0	1041.438	1785.49	2805.745
MMP8_M_1 h	1177.785	2224.855	3350
MMP8_C_1 h	750.87	1962.005	2729.068
MMP8_M_24 h	948.265	2296.47	3107.518
MMP8_C_24 h	846.9425	1887.615	2620.783
MMP8_M_7 d	803.2825	1967.355	3350
MMP8_C_7 d	808.9325	1674.855	2795.15
MMP8_M_14 d	486.03	1708.06	2611.15
MMP8_C_14 d	771.12	1540.62	2647.423
MMP8_M_21 d	892.9225	2015.87	3350.62
MMP8_C_21 d	512.995	2582.555	3072.023

analyzed in the bead analyzer (Luminex 100™, Luminex®, MiraiBio, Alameda, Calif). The concentrations of the unknown samples (antigens in GCF samples) were estimated from the standard curve using commercial software (Prism 5 for Windows, version 5.04, GraphPad Software Inc, La Jolla, Calif) and the MMP levels expressed as the total amount (pg) per site.

Mean values were calculated for each subject and averaged across subjects for each time point in each clinical group separately. The D'Agostino and Pearson omnibus normality test was used to determine if the data had a normal distribution. Significance of statistical differences over time was tested using the repeated-measures analysis of variance (ANOVA) for the mean clinical data. For the levels of MMPs, significance of statistical differences over time in each

group separately was tested using the Friedman test, and differences between groups at each time point were tested using the Wilcoxon test.

RESULTS

The results from the repeated-measures ANOVA revealed that both clinical parameters decreased statistically significantly over time ($P < .001$), the mean ($\pm$SD) plaque index was reduced from 48% $\pm$ 17.3% to 17% $\pm$ 10.2%, and the mean percentage of BOP was reduced from 20% $\pm$ 10.5% to 4% $\pm$ 7.3% from day -7 to day 21. These data indicate that the oral hygiene regimen was able to reduce plaque accumulation and gingival inflammation to a minimum.

A total of 280 GCF samples (10 subjects $\times$ 4 samples $\times$ 7 time points) were processed for the levels of seven MMPs. The wide dynamic range of the multiplex bead immunoassay employed allowed us to quantify the MMPs examined in the vast majority of samples tested. The detection frequencies of MMP-1, -2, -3, -7, -8, -12, and -13 were 93%, 98%, 99%, 76%, 100%, 100%, and 80%, respectively.

Table 1 illustrates the median values and the interquartile range for MMPs in both groups. When the statistical significance of changes over time was tested the only significant change found was in the levels of MMP-1 in the movement group ($P < .05$). When the two groups were compared at each time point, the only statistically significant differences found were in levels of MMP-1 at day -7 ($P < .05$) and 1 hour after activation ($P < .05$) and in MMP-12 at day -7 ($P < .05$).

DISCUSSION

To our knowledge this is the first time that the multiplex bead immunoassay has been employed for the simultaneous quantification of multiple MMPs in the GCF of periodontally compromised teeth treated orthodontically. The results demonstrated that although the levels of these MMPs fluctuated during the 21 days of application of orthodontic forces, these changes were for the most part non–statistically significant. The only statistically significant difference between orthodontically moved and control teeth was in the level of MMP-1 at the 1-hour time point.

Previous research[17–20] has indicated that GCF levels of MMPs and activation of collagenases can be altered by orthodontic forces. Apajalahti et al.[19] and Ingman et al.[18] found that using an immunofluorometric assay significantly elevated MMP-8 immunoreactivity in GCF samples collected after force application. Neither study could detect immunoreactivity to MMP-1 using Western blot analysis. Those findings are in agreement with previous results[20] that have shown significantly elevated total collagenase activity in the GCF of orthodontic patients 24 hours after retractor activation.

Western blotting analysis was also used by Cantarella et al.[17] to examine the expression of MMP-1 and -2 in GCF samples from the compression and tension sides during force application. The levels of MMP-1 increased after 1 hour of activation of the orthodontic device in both sides, which was also found in this study. MMP-2 was induced by compression, reaching a peak after 8 hours of force application, while on the tension side it increased after 1 hour but returned to baseline values within 8 hours. Gelatin zymography has also been used to detect active and latent MMPs in GCF samples collected after orthodontic movement.[24] Partially active MMP-1 was found in GCF in both tension and compression sites but was never detected in control teeth.

There are several differences in the methodology employed in the above-mentioned studies compared to the current report that could justify the differences in the results. Our study examined treated periodontitis subjects under maintenance, while other authors[17–19,24] studied periodontally healthy individuals. However, using multiplex bead immunoassay we have also examined the effects of orthodontic forces on periodontally healthy teeth and found MMPs changes for the compression sites but not for the tension sites as a result of mechanical forces.[25]

In the current study, the lower limit of detection for the MMPs examined ranged from 11 pg/mL for MMP-1 to 102 pg/mL for MMP-7. All MMPs examined, including MMP-1, were detected by the bead immunoassay in the majority of samples analyzed, while previous studies[17–19] failed to detect MMP-1 in GCF samples. Data reported for MMP-8 using immunofluorometric assays suggested a lower limit of detection of approximately 1 ng/mL.[1,18] In multiplex bead immunoassay, the sensitivity of the assay for MMP-8 was 0.03 ng/mL, and 90% of GCF samples had MMP levels above 7.2 ng/mL (0.36 ng/site). Therefore, the sensitivity of the multiplex bead immunoassay used in our study seems to be adequate to detect even small differences in GCF levels of MMPs. The method for GCF sampling might also have influenced the difference in the results between previous reports and the present study. For instance, some authors[18,19] have kept two paper strips within the sulcus for 3 minutes in order to collect GCF samples. This might have resulted in approximately a 12-fold increase in the amount of GCF collected per sample when compared to the 30-second samples obtained in our study.

In previous articles[1,18] in which increases in GCF levels of MMP-8 were detected as a result of the application of orthodontic forces to periodontally healthy teeth, the levels detected were still below those found in gingivitis and periodontitis. This

indicates that elevations of GCF MMP-8 were likely still compatible with periodontal health. However, since MMP-8 has been proposed as a biomarker of periodontal disease progression,[4,8,9] one of our goals was to test if the application of orthodontic forces in subjects with a history of periodontitis would not induce elevations in its level, resulting in additional risk to periodontally involved teeth. Our findings could not confirm an increase in GCF MMP-8 as a result of orthodontic movement.

The fact that previous studies have found increases in GCF MMP levels in teeth affected by periodontal disease and in teeth submitted to orthodontic treatment shows the importance of this study, which combined the two factors, controlled periodontal disease and orthodontic movement.

The variability on the MMPs GCF levels was notable, which is partially due to the limited sample size and may be the reason that we did not find a significant difference in the results. The lack of a method error analysis is a limitation of this study and of others based on GCF. According to Perinetti et al.,[26] the method error on GCF evaluation ranged from 40% to 58%, so reliable use of the GCF collection and quantification should take into account relevant errors. Although this study sample was small, we have recently examined GCF levels of MMPs in periodontitis and periodontally healthy individuals using the same multiplex bead immunoassay employed here and also with a relatively small sample, and these unpublished data demonstrated that the levels of MMP-2, -3, and -8 were higher in periodontitis subjects compared to periodontally healthy subjects but that these levels decreased 3 months after scaling and root planing. The GCF MMP levels found in the periodontal maintenance subjects studied here were below the values found in these periodontitis subjects even after periodontal therapy. These data reinforce the notion that the periodontal condition of our study population was under control and that the application of orthodontic forces was well tolerated by teeth with a reduced but healthy periodontium.

CONCLUSIONS

- Our findings suggest that the orthodontic movement of periodontally compromised teeth without active pockets does not result in significant changes in the GCF levels of MMPs.

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