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The effect of topical doxycycline usage on gingival crevicular fluid MMP-8 levels of chronic and aggressive periodontitis patients: a pilot study

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Abstract: The aim of this study was to evaluate the efficacy of topical subgingival application of doxycycline hyclate (DH) gel adjunctive to non-surgical periodontal therapy on gingival crevicular fluid (GCF) matrix metalloproteinase (MMP)-8 levels in chronic and aggressive periodontitis patients. Forty teeth of 10 chronic periodontitis patients and 32 teeth of eight aggressive periodontitis patients were screened for 6 months. Scaling and root planing (SRP) was applied to the control sites and DH gel adjunctive to SRP was applied to the test sites of each patient simultaneously. GCF MMP-8 levels were analysed at baseline, 7 days; and at 1, 3 and 6 months by Sandwich Elisa Method. At 1, 3 and 6 months, probing depth ($P < 0.0051$) and plaque scores and bleeding on probing values ($P = 0.000$) significantly decreased in each group when compared with the baseline, but there was no statistically significant difference between the test and control sites. GCF MMP-8 levels reduced presenting statistically significant differences on 7 days, 1, 3 and 6 months in four of the groups ($P < 0.05$); however, intergroup differences were not statistically significant. Developing functional and immunological-based chair-side MMP tests might serve as useful adjunctive diagnostic tools when monitoring the effects of DH gel application.

Key words: doxycycline; gingival crevicular fluid analysis; matrix metalloproteinase-8 and periodontitis

Introduction

The elimination or alteration of microbial pathogens present in subgingival plaque is the primary object of periodontal therapy. If a patient suffering from periodontitis is able to establish and maintain effective individual oral hygiene, subgingival scaling and root planing (SRP) may result in resolving inflammation and reduced probing pocket depths. However, non-surgical periodontal therapy, i.e. SRP, is not found to be successful in all treated sites. Although not all factors influencing failure of non-surgical therapy are known, deep periodontal pockets and furcation involvement (1) are some of them. If the efficacy of non-surgical therapy could be increased, the threshold for surgical treatment might be elevated towards deeper pockets. Advances in understanding the aetiology and pathogenesis of periodontitis have led to increasingly effective pharmacological interventions. In this regard, safe and intrinsically efficacious medications can be delivered into periodontal pockets to suppress or eradicate the pathogenic microbiota or modulate the inflammatory response, thereby, limiting tissue destruction (2). It is beneficial if the agent could be released into the pockets using a controlled-release biodegradable system, because this could provide adequate antimicrobial levels of the active agent directly at the site of infection, without the need for daily applications or subsequent removal (3).

Matrix metalloproteinases (MMPs) are known to be one of the main proteinases related to tissue destruction and remodelling events in periodontal disease (4). It has been shown that an imbalance between activated MMPs and their host-derived endogenous inhibitors leads to pathological breakdown of the extracellular matrix and basement membranes during periodontitis (5). Many studies implicate polymorphonuclear neutrophil (PMN)-type collagenase (collagenase-2, MMP-8) as the main type of interstitial collagenase in gingival crevicular fluid (GCF) from chronic periodontitis patients (6, 7). The specific immunoassay of Söder & Söder (8) for MMP-8 in GCF levels has also shown to reflect the severity of refractory periodontitis. It is believed that, the presence of MMP-8 in GCF correlates with degradation of tissue collagen in the inflamed periodontium. Thus, monitoring the MMP-8 levels will help to give an idea about the disease activity.

Previous studies by Larivee *et al.* (9) and Hakkarainen *et al.* (10) have reported that GCF MMP-8 levels were significantly decreased after mechanical periodontal treatment. Studies focusing on the effects of various anti-inflammatory and antimicrobial agent applications as an adjunct to mechanical therapy revealed varying beneficial results on GCF MMP-8 levels. Investigating the effect of adjunctive use of Meloxicam and initial phase of periodontal therapy on GCF MMP-8 levels, Buduneli *et al.* (11) found out a tendency to reduce in GCF MMP-8 levels *in vivo* within the 10 days. Azmak *et al.* (12) evaluated the efficacy of controlled-release delivery of chlorhexidine gluconate (CHX) on clinical parameters and GCF MMP-8 levels in chronic periodontitis patients and suggested that CHX chip application following SRP is beneficial in improving periodontal parameters and reducing GCF MMP-8 levels for 6-month duration. In an *in vitro* study, Golub *et al.* (13) analysed the gingival extracts of AP patients obtained during periodontal surgery and stated that low-dose doxycycline inhibits MMP-8 and MMP-9 directly.

In the present study, doxycycline hyclate (DH) (Atridox, Atrix Laboratories, Inc., Fort Collins, CO, USA) was chosen as an antimicrobial substance, because of its mechanism of action, its activity against putative periodontal pathogens, its known effectiveness in the management of periodontitis in humans (14) and because that very few studies have been met investigating the effect of doxycycline on GCF MMP-8 levels of periodontitis patients. Multicentre trials evaluating the drug delivery systems as a monotherapy demonstrated that periodontal pockets responded favourably to such treatment with doxycycline and that the response was similar to that obtained with SRP (14, 15). However, whether locally delivered doxycycline may act synergistically with mechanical instrumentation to enhance the outcome of non-surgical treatment of chronic periodontitis has not been evaluated. Therefore, this study was designed to evaluate the clinical effects of controlled-release DH as an adjunct to SRP in the treatment of chronic and aggressive periodontitis patients and to assess the changes in GCF collagenase-2 (MMP-8) levels.

Materials and methods

Study design

The trial was designed as a randomized, blinded, controlled split-mouth study of 6-month duration and conducted at Ege University, School of Dentistry, Izmir, Turkey. Forty teeth of 10 chronic periodontitis patients (six men, four women; mean age 55 years; range 41–69 years) and 32 teeth of eight aggressive periodontitis patients (four men, four women; mean age 25 years; range 19–31 years), a total of 72 teeth took part in the study. Chronic periodontitis and aggressive periodontitis patients were diagnosed as described by Flemmig (16) and Tonetti and Mombelli (17). The study comprised of four groups: (1) SRP applied teeth of chronic periodontitis patients (Ch-SRP) ($n = 20$); (2) SRP + DH applied teeth of chronic

periodontitis patients (Ch-SRP + DH) ($n = 20$); (3) SRP applied teeth of aggressive periodontitis patients (A-SRP) ($n = 16$); (4) SRP + DH applied teeth of aggressive periodontitis patients (Ch-SRP + DH) ($n = 16$). Subjects must have at least two qualifying periodontal sites (i.e. probing pocket depth ≥6 mm, attachment loss ≥3 mm and bleeding following probing) located in two different quadrants or jaws, meaning non-adjacent sites and the teeth diagnosed as having periodontitis should be single-rooted. Also, subjects must be in good health according to medical history, blood pressure and clinical judgment and must voluntarily sign an informed consent agreement. Subjects who received subgingival instrumentation, subgingival antimicrobial application or systemic antibiotics <6 months prior to the baseline examination; and subjects who used mouthwash or subgingival irrigation with agents having known antibacterial properties within 1 month prior to baseline examination are excluded. Pregnant or lactating females or patients with the history of allergies to DH or other tetracyclines are also not included.

After completion of initial periodontal treatment including oral hygiene instructions and SRP, the patients who fulfilled the inclusion criteria are informed about the study protocol, risks and benefits of the procedure and informed consent was obtained. Test and control sites were randomized at the split-mouth level by the flip of a coin. In each patient, one site received SRP + doxycycline while the other site received SRP alone. At the baseline visit, the following procedures were performed for each test and control teeth: (1) Probing depth (PD) and clinical attachment level (CAL) measurements were assessed at six sites (mesiobuccal, midbuccal, distobuccal, distolingual, midlingual, mesiolingual); (2) plaque scores (PI) (18) and probe bleeding index (PBI) (19) scores were recorded; (3) GCF samples were collected; (4) SRP was performed at all test and control teeth under local anaesthesia with hand instruments; (5) mechanical debridement of one tooth was limited to 10 min (20) in the SRP + doxycycline group DH gel in the isolated pocket(s). Five examination intervals were defined as baseline, 1 week, 1, 3 and 6 months.

GCF sampling

So as to avoid blood contamination and possible stimulation of GCF flow during clinical measurement (probing, etc.), GCF samples were collected before any other clinical recordings. The sampling sites were selected from buccal aspects of the mesial and distal surfaces at the interproximal sites of single-rooted teeth. Prior to GCF sampling, the surfaces were isolated with cotton rolls and dried with a gentle air stream. GCF samples were collected by inserting standardized filter paper strips (Periopaper, ProFlow Inc., Amityville, NY, USA) for 30 s (21). During this process, care was taken to avoid mechanical injury and strips contaminated with blood were discarded. GCF volume on the strips were measured after sampling by electron impedance (Periotron 6000, Proflow Inc.). The paper strips were immediately placed in polypropylene tubes and stored at −40°C until MMP-8 analysis.

Antimicrobial therapy

The components of the biodegradable controlled-release delivery system containing DH (10% w/w) are packed in two separate syringes that are mixed just prior to use. The mixture was slowly expressed into the periodontal pocket, starting from the base of the pocket, until it reached the gingival margin. Following the withdrawal of the cannula tip from the pocket, a curette was used to pack any overflow of material down into the pocket. Periodontal dressing (Peripac; Dentsply DeTrey, Konstanz, Germany) was used to increase the retention of the gel in the pocket. The patients were instructed not to perform oral hygiene measures in treated areas during a period of 1 week (22).

MMP-8 analysis

Matrix metalloproteinase-8 quantification was performed on the elutes of GCF and all constituents were assayed by means of sandwich ELISA's (Human MMP-8 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, USA) based on a modification of the method of Haerian *et al.* (23). Standards and GCF supernatants were incubated for 2 h at room temperature in microtitre wells (Immulon 4 Dynateh Laboratories, Billinghurst, Sussex, UK) precoated with MMP-8 antibody. After five washing steps with buffer solution to remove the unbounded antigen, peroxidase-labelled antibody was added to the medium to detect the unlabelled MMP-8. Visualization was achieved by incubation with *p*-nitrophenylphosphate and the colour development was terminated by the addition of 2% oxalic acid. The amount of MMP-8 was measured at 450 nm in an EC 312 Microplate Reader (Bio-Tek Instruments, Winonski, VT, USA). The total amount of MMP-8 was expressed as per site ng/2 sites, and the concentrations expressed as ng μl^{-1}.

Statistical analysis

In the present study, data analysis and statistical tests were subjected to each treatment protocol on a patient-level basis.

Taking into account the paired nature of the split-mouth design, intergroup differences in the PBI and PI scores between treatment groups were tested at each time point by the Wilcoxon signed rank test (confidence interval of $P < 0.05$). To test the differences between SRP + DH and SRP-alone groups, changes from baseline for PD and CAL scores and GCF MMP-8 levels at each time point were analysed by the repeated measures analysis of covariance (ANOVA) (confidence interval of $P < 0.05$). Statistical models were adjusted for relevant covariates (e.g. baseline). Intergroup comparisons in clinical measurements between baseline and at months 1, 3 and 6 were performed using Friedman and Bonferroni corrected Wilcoxon signed rank test and $P < 0.01$ were considered statistically significant. Intergroup comparisons of GCF MMP-8 levels between baseline and at each time point were performed using repeated measures of ANOVA and Bonferroni-corrected paired t-test and $P < 0.01$ were considered statistically significant.

Results

At baseline, there was no statistically significant difference between the clinical parameters of the four groups (Table 1).

Table 1. **Clinical periodontal data at baseline**

	PD (mm)	CAL (mm)	PBI	PI
Chronic periodontitis				
(1) SRP + DH ($n = 20$)				
Mean	7	8.27	3.70	3.76
SD	0.62	1.14	0.48	0.65
Median	7	9	3	4
Minimum	6	7	2	3
Maximum	8	11	4	5
(2) SRP alone ($n = 20$)				
Mean	7.21	8.72	3.57	4.12
SD	0.67	0.94	0.53	0.82
Median	7	9	3	3
Minimum	6	7	2	3
Maximum	8	10	4	5
Aggressive periodontitis				
(3) SRP + DH ($n = 16$)				
Mean	6.99	8.05	3.63	3.63
SD	0.68	0.64	0.52	0.74
Median	7	9	3	3
Minimum	6	7	2	3
Maximum	8	11	4	5
(4) SRP alone ($n = 16$)				
Mean	6.94	7.94	3.54	3.51
SD	0.76	0.82	0.53	0.76
Median	7	9	3	4
Minimum	6	7	2	3
Maximum	8	10	4	5

No statistically significant difference was noted between the PD, CAL, PBI and PI scores of the four groups. PD, probing depth; CAL, clinical attachment level; PBI, bleeding on probing; PI, plaque scores; SRP, scaling and root planning.

Probing depth reduction and clinical attachment level improvements

The mean pocket depths of Ch-SRP group at baseline, 1, 3 and 6 months are 7.21, 3.83, 4 and 4.15 mm respectively. The mean pocket depths of Ch-SRP + DH group at baseline, 1, 3 and 6 months are 7.14, 3.75, 3.85 and 4 mm respectively. The mean pocket depths of A-SRP group at baseline, 1, 3 and 6 months are 6.94, 3.84, 4 and 4.19 mm respectively. The mean pocket depths of A-SRP + DH group at baseline, 1, 3 and 6 months are 6.99, 3.51, 3.63 and 3.88 mm respectively. The reduction of PD (mm) at 1, 3 and 6 months with respect to the baseline was statistically significant in all of the groups ($P = 0.0051$); but there was no statistically significant difference between the mean pocket depth reduction of the four groups at each time point. The mean CAL (mm) improvements of the groups Ch-SRP, Ch-SRP + DH, A-SRP and A-SRP + DH on first month were 1.63, 1.73, 1.60 and 1.73; on third month 1.50, 1.60, 1.50 and 1.63; and on sixth month 1.44, 1.56, 1.40 and 1.55 respectively. The improvements of CAL did not have statistically significant difference either between the groups at each time points, or when compared with the baseline.

Plaque scores and bleeding on probing

Bleeding on probing and PI scores were similar in Ch-SRP, Ch-SRP + DH, A-SRP and A-SRP + DH at baseline. The mean PBI and PI scores were significantly reduced at 1, 3 and 6 months for both treatment groups when compared with baseline ($P = 0.000$); but there were no significant differences between the PBI and PI scores of the four groups at any visit.

Effect of SRP and SRP + DH on MMP-8 levels

Matrix metalloproteinase-8 total amounts reduced presenting statistically significant differences on 7 days, 1, 3 and 6 months in four of the groups ($P < 0.05$) (Table 2, Fig. 1). When concentration of MMP-8 in ng μl^{-1} was considered, the difference between the baseline and the time points of all the groups were statistically significant ($P < 0.05$) (Table 3, Fig. 2). However, when focused on intergroup MMP-8 levels, it has been found out that the differences between the total amount and concentration values of each group did not reach statistical significance.

Discussion

The advantages of controlled delivery devices are described as (1) better subject compliance; (2) enhanced or improved

Table 2. **GCF MMP-8 total amounts (ng/2 sites) in SCR + DH and SRP-alone groups of chronic periodontitis and aggressive periodontitis patients**

	Baseline	7 days	1 month	3 months	6 months
Chronic periodontitis					
(1) SRP + DH					
Mean	3.85	1.93*	1.24*	0.65*	1.02*
SD	1.49	0.93	0.89	0.13	0.24
Median	2.04	1.06	0.81	0.43	0.92
Minimum	1.13	0.8	1.01	0.45	0.98
Maximum	8.1	5.43	4.49	4.27	4.58
(2) SRP alone					
Mean	4.04	2.16*	1.88*	0.92*	1.02*
SD	1.11	0.87	0.76	0.54	0.61
Median	3.01	1.99	1.05	0.67	0.75
Minimum	1.67	0.75	1.05	0.55	0.34
Maximum	9.78	4.97	4.36	4.95	4.77
Aggressive periodontitis					
(3) SRP + DH					
Mean	3.39	1.79*	0.87*	0.69*	0.90*
SD	1.80	0.74	0.65	0.38	0.31
Median	2.46	1.55	0.65	0.51	0.78
Minimum	1.67	0.75	0.58	0.34	0.66
Maximum	8.75	5.21	4.87	4.13	4.87
(4) SRP alone					
Mean	3.77	1.91*	1.11*	0.68*	1.04*
SD	1.58	0.61	0.35	0.25	0.25
Median	2.95	1.23	0.89	0.46	0.67
Minimum	1.70	0.44	0.58	0.36	0.28
Maximum	8.56	5.13	4.78	4.21	3.95

*Statistically significant difference from the baseline within the same group ($P < 0.05$). GCF, gingival crevicular fluid; MMP, matrix metalloproteinase; DH, doxycycline hyclate; SRP, scaling and root planning.

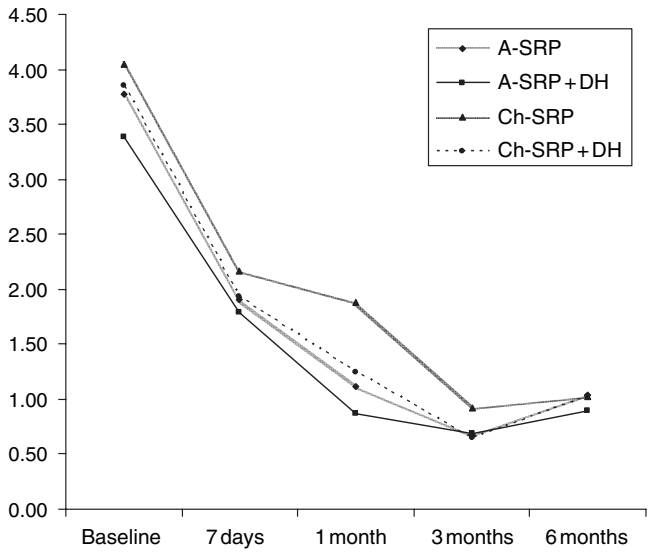

Fig 1. GCF MMP-8 total amounts (ng/2 sites) in SRP + DH and SRP-alone groups of chronic periodontitis and aggressive periodontitis patients (statistically significant difference from the baseline to every time point within the same group, $P < 0.05$). GCF, gingival crevicular fluid; MMP, matrix metalloproteinase; SRP, scaling and root planing; DH, doxycycline hyclate.

pharmacokinetic response; (3) greater advantage in positioning the active agent in proximity to the disease; and (4) delivery of a lower total dose of drug at a more controlled concentration (14, 24). No complications or complaints were reported during this study; therefore, it may be concluded that subgingival application of 10% DH is safe. Sustained devices generally provide delivery for <24 h and controlled delivery devices should provide delivery for more than 1 day (25). Stoller *et al.* (26) have stated that a controlled delivery system of doxycycline functions with a minimum inhibitory concentration (MIC_{90}) well above the (MIC_{90}) for suspected periodontal pathogens for 7–10 days. As prolonged delivery at high concentrations is quite important in periodontitis-involved sites, it is thought that a single application of DH is a good treatment of choice.

Previously published studies by Garret *et al.* (14) and Polson *et al.* (15) focused on the effects of locally delivered DH when used as a monotherapy and compared the results with SRP alone. Results of these trials demonstrate that the treatment of periodontitis with subgingivally delivered DH is equally effective as SRP and superior in effect to placebo control and oral hygiene in reducing the clinical signs of adult periodontitis over a 9-month period. In contrast, a multicentre trial designed by Wennström *et al.* (21) evaluated the effect of DH as an adjunctive therapy to mechanical instrumentation and concluded that DH + SRP application in deep periodontal pockets can be considered as a justified approach for non-surgical treatment of periodontitis. A similar multicentral study by Eickholz *et al.* (20) evaluating the clinical effect of topical application of DH adjunctive to non-surgical periodontal therapy concluded that adjunctive subgingival application of DH is safe and provided more favourable CAL gain and PD reduction than SRP alone. In addition to Eickholz *et al.*'s (20) and Wennström *et al.*'s (21) study design, the present study included aggressive periodontitis patients. Unlike the previous studies, however, our results showed that the reduction of PD (mm) and mean PI and PBI scores at 1, 3 and 6 months with respect to baseline in Ch-SRP, Ch-SRP + DH, A-SRP and A-SRP + DH groups was statistically significant but there was no statistically significant difference at each time points between the aforementioned parameters of the four groups. This might be due to the relatively small number of patients in our study groups, as this is a single clinical centre design. Besides, in his two consecutive reviews, Cobb (27) has stated that the analysis of Egyptian hieroglyphics and medical papyri indicates that non-surgical periodontal treatment was common 3000–4000 years ago and is still the traditional method of controlling sub-gingival microbiota. The results of the present study are in accord-

Table 3. **GCF MMP-8 concentrations in SCR + DH and SRP-alone groups of chronic periodontitis and aggressive periodontitis patients**

	Baseline	7 days	1 month	3 months	6 months
Chronic periodontitis					
(1) SRP + DH					
Mean	1652.60	1175.00*	906.00*	438.20*	691.20*
SD	652.46	399.28	217.53	270.54	361.45
Median	945.45	674.67	545.12	312.45	399.34
Minimum	334.75	228.67	189.36	195.22	178.54
Maximum	9793.45	5349.32	4651.41	5491.45	4892.51
(2) SRP alone					
Mean	1505.00	1240.60*	1069.50*	504.90*	700.00*
SD	527.91	495.74	468.80	241.15	385.09
Median	845.77	567.44	616.22	344.12	427.13
Minimum	413.27	310.23	221.75	271.38	201.45
Maximum	8941.57	6461.38	5451.93	4018.22	4983.58
Aggressive periodontitis					
(3) SRP + DH					
Mean	1186.75	946.50*	676.13*	342.38*	495.00*
SD	463.33	402.48	280.72	146.35	215.44
Median	778.13	349.47	245.77	212.45	312.56
Minimum	332.19	245.55	201.11	189.17	188.19
Maximum	9125.49	4312.77	4011.49	3991.46	3891.44
(4) SRP alone					
Mean	1188.38	1057.38*	745.38*	353.88*	529.25*
SD	551.37	417.86	184.63	177.65	244.02
Median	712.46	415.45	313.57	198.45	345.19
Minimum	391.58	209.15	198.77	176.49	298.57
Maximum	8563.75	5983.22	5012.54	5924.78	4289.22

*Statistically significant difference from the baseline within the same group ($P < 0.05$). GCF, gingival crevicular fluid; MMP, matrix metalloproteinase; DH, doxycycline hyclate; SRP, scaling and root planning.

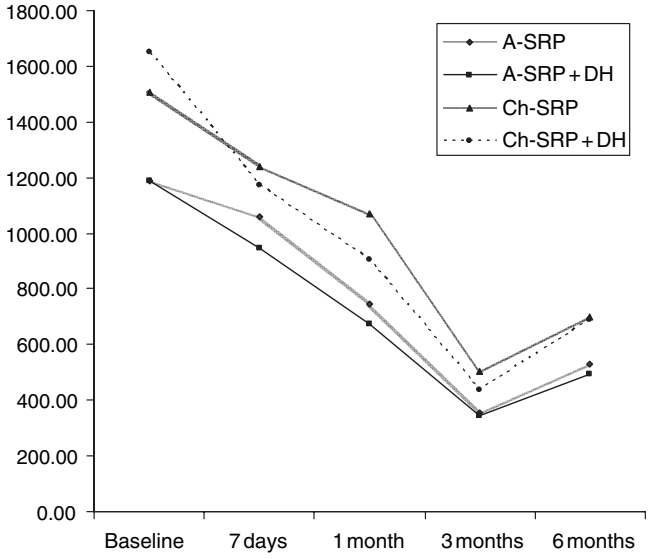

Fig 2. GCF MMP-8 concentrations in SRP + DH and SRP-alone groups of chronic periodontitis and aggressive periodontitis patients (statistically significant difference from the baseline to every time point within the same group, $P < 0.05$). GCF, gingival crevicular fluid; MMP, matrix metalloproteinase; SRP, scaling and root planing; DH, doxycycline hyclate.

ance with Cobb's (1, 27) comments, as locally delivered DH did not act synergistically with mechanical instrumentation to enhance the outcome of non-surgical treatment of chronic and aggressive periodontitis. This may be due to the fact that even today, SRP remains as the 'gold standard' in successful periodontal therapy, and its victory over the subgingival microbiota especially on single-rooted teeth might mask the success of secondary treatment modalities.

Results of the present study showed that better improvements of CAL and more reduction in PD were found in DH applied groups of chronic and aggressive periodontitis patients but the differences were not statistically significant either at each time points or when compared with the baseline. This finding can be improved if new and similar multicentral studies are planned in the future, including aggressive periodontitis patients.

Matrix-degrading metalloproteinases are key mediators of connective tissue destruction characteristics of chronic periodontitis (28). Pathological elevation in levels and activity of MMP-8 in the GCF and surrounding tissues appear to drive primarily from infiltrating PMN (6, 7); and other collagens including MMP-1 (i.e. fibroblast-type collagenase) (5) and MMP-13 (i.e. bone-type collagenase) may also be involved in periodontal destruction (29). MMP-8 is released by degranulating PMNs and also secreted by certain non-PMN lineage cells like gingival fibroblasts, endothelial cells, sulcular epithelial cells and plasma cells (30, 31). PMNs are the main infiltrating cells immediately after an injury occurs and also during the

first week of wound healing, when the number of accumulating fibroblasts increases as well (32). Elevated MMP-8 levels in GCF have been found in periodontitis sites when compared with healthy sites and reduction of MMP levels in GCF follows successful periodontal treatment (9, 23). In a clinical study, Kinane *et al.* (33) aimed to investigate the effect of SRP and the maintenance phase of treatment on the GCF MMP-8 levels and found that clinical improvement following SRP was associated with significant reduction in MMP-8 levels. Furthermore, they stated that GCF concentration of MMP-8 decreased after initial therapy but reduced even more dramatically following a 3-month period of maintenance. Chen *et al.* (34) also reported that following a successful periodontal treatment including SRP and oral hygiene instructions, MMP-8 levels have been reduced significantly. In the present study, there was a significant reduction in the total and concentration MMP-8 values of the SRP-alone groups of aggressive and chronic periodontitis patients at each time points when compared with the baseline. These data are in agreement with previous reports (33, 34).

In their study of 6-month duration, Radvar *et al.* (35) evaluated the efficacy of three commercially available periodontal systems for local delivery of antibiotics as adjunct to SRP and Kinane and Radvar (36) compared the three periodontal local antimicrobial therapies in persistent periodontal pockets. The common results of these two studies indicate that the frequency of sites with suppuration was markedly reduced following all antimicrobial therapies. In the present study, PBI, which is the major sign of inflammation, was examined instead of suppuration; however, the results were similar to the findings of Radvar *et al.* (35) and Kinane and Radvar (36), meaning that the mean PBI scores were significantly reduced at 1, 3 and 6 months for both treatment groups when compared with baseline ($P = 0.000$); but there were no significant differences between the scores of the four groups at any visit. Pourtaghi *et al.* (37) studied the effect of subgingival microbial therapy on the levels of stromelysin and tissue inhibitor of metalloproteinases in GCF and as a result, they stated that the use of adjunctive locally delivered antimicrobial systems, especially the tetracycline family, may offer an advantage in changing the metalloproteinase profile of the GCF to one that is more compatible with periodontal health. The findings of Pourtaghi *et al.* (37) supports the results of the present study, meaning that, when compared with baseline, the MMP-8 concentration at the time points of all the groups were statistically significant ($P < 0.05$) (Table 3, Fig. 2). However, when focused on intergroup MMP-8 levels, it has been found out that the differences between the total amount and concentration values of chronic and aggressive periodontitis groups did not reach statistical significance.

Golub *et al.* (13, 38) have reported that specially formulated low-dose doxycycline regimens can inhibit collagenase activity both in gingival tissue and GCF of adult periodontitis patients when used as an adjunctive to SRP. The results of the present study have shown that the inhibitory effect of DH on MMP-8 levels in chronic and aggressive periodontitis groups was more apparent but the differences were not statistically significant when compared with the results of SRP-alone groups. When searching Medline, no parallel study, investigating the effect of local DH application on GCF MMP-8 levels, has been met. Therefore, further studies are needed to clarify the effects of locally delivered controlled-release DH on GCF MMP-8 levels.

There are many potential markers of periodontal disease based on the MMP-family members. The reduction in GCF MMP-8 levels following therapy indicates that MMP-8 is one molecule, which may eventually prove useful as an indicator of current disease status and possibly as a predictor of future disease. Sorsa *et al.* (39) and Mäntylä *et al.* (40) have developed a MMP-based-point-of-care test stick which can be used for clinical diagnosis and chair-side monitoring of periodontal disease. However, functional and immunological-based MMP tests are under development and studies remain to be performed to fully validate their utility (38, 41). A dream will come true if, in the near future, new developed tests based on MMP's in patients prior to treatment, might indicate their likely response to therapy and thus the need for adjunctive therapeutic agents such as systemic or locally delivered antibiotics or more novel technologies to reduce pathologically elevated MMPs.

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