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Effects of Locally Delivered Doxycycline on Periodontal Clinical Parameters and Gingival Crevicular Fluid Matrix Metalloproteinase-8

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Abstract:

Background: The aim of this study was to evaluate the effect of local doxycycline (Atridox) as an adjunctive to non-surgical periodontal treatment on clinical parameters and gingival crevicular fluid (GCF) levels of matrix meatalloproteinase-8 in patients with chronic periodontitis.

Materials and Methods: A double-blind spilt mouth; randomized, controlled clinical trial was conducted in 30 patients with moderate to severe chronic periodontitis. Two teeth were selected from similar quadrants with a probing pocket depth ≥5 mm, and randomly allocated into test and control groups. The control group was treated with scaling and root planning (SRP) only; the test group was treated with SRP and received adjunctive local Atridox. Periodontal clinical parameters were recorded and matrix metalloproteinases-8 (MMP-8) level in GCF samples were determined using the enzyme-linked immunosorbent assay at baseline and 1, 3 months.

Results: Statistically significant reduction in probing depth and clinical attachment level and GCF MMP-8 was found in test and control groups compared with baseline, test group showed a significant reduction in clinical parameters and GCF MMP-8 level compared with the control group.

Conclusion: Local delivery doxycycline (Atridox) combined with SRP significantly improved the clinical parameters and decreased GCF level of MMP-8.

Key Words: Doxycycline, matrix metalloproteinase-8, periodontitis, root planning, scaling

Introduction

Chronic periodontitis is one of the most common and progressive diseases, characterized by destruction of the tissues supporting the tooth.[1] However, the presence of bacteria is necessary for disease but not sufficient for the initiation and progression of diseases.[2] It's now well recognized that the releasing of inflammatory factors from host cells in response to the presence of bacterial plaque plays the main role in periodontal tissue destruction.[3] These inflammatory responses lead to the destruction of tooth supporting tissue, alveolar bone, periodontal ligament, and consequently loss of tooth.[4,5] During this event, the most important component of periodontium lost is the collagen Type I, which forms a major part of periodontal ligament and organic matrix of alveolar bone.[6] Four distinct pathways which cause destruction: Plasminogen-dependent, phagocytic, osteoclastic, and matrix metalloproteinases (MMPs). A wide range of evidence demonstrate the major role of MMP in the periodontal tissue destruction.[7] Matrix metalloproteases are a family of zinc-dependent metalloproteinases and a group of at least 23 genetically distinct but structurally related proteases.[8,9] Gingival crevicular fluid (GCF) level of MMP-8 in patients with chronic periodontitis significantly increases compared to healthy tissue, and there is a strong relationship between higher level of gingival tissues MMP-8, probing depth (PD), and clinical attachment level (CAL).[10] Studies showed the fact that neutrophil MMPs (MMP-8, MMP-9) cleave collagens more than other MMPs and play the major role in periodontal diseases.[11,12] Various studies found decrease in level and activity of GCF MMP-8, following successful periodontal treatment.[13] GCF is a modified serum transudate or inflammatory exudates which contains component of connective tissues, epithelial cells, and bacteria. These components of GCF are used in the diagnosis of periodontal disease.[14]

Lee *et al.*[15] study indicated that an increase of 40% was observed in activity level of collagenase during the destruction of periodontal tissues. Their study also confirmed existence of a strong relationship between direct role of active neutrophil collagenase and destruction of periodontal tissues.

The mechanical debridement intervention is the gold standard of periodontal therapy which can be accompanied with the local or systemic use of antibiotics.[16,17] Recently, a new treatment strategy has been provided for host modulation. The aim of

this method is to inhibit the MMPs activity and to prevent tissue destruction.[18,19] Figueredo *et al*.[20] in a study demonstrated clinical improvements and a reduction in MMP-8 level of GCF after non-surgical treatment in both gingivitis and periodontitis patients. Golub *et al*.[21] showed that subantimicrobial doses of doxycycline improved clinical parameters. Tetracycline has the inhibitory effect on neutrophil collagenases such as MMP-8; it reduced the MMP level without creating microbial resistance. In another study performed by Crout *et al*.,[22] a 20 mg dose of doxycycline twice a day, decreased neutrophil collagenase, and improved attachment level and PD in the periodontal patients. The use of systemic antibiotics is accompanied with side effects such as hypersensitivity and gastrointestinal intolerance.[23] Hence, the local route of antibiotics and administration can accomplish with the following advantages: Higher therapeutic doses in the subgingival site, decreasing side effects of systemic antibiotics, no drug intervention with other drugs, reducing advent risk of bacterial resistance, not depend on patient's cooperation.[24] The use of micro-doxycycline local systems is one of local delivery antimicrobial systems.[25,26] Salvi *et al*.[27] evaluated the clinical effects of metronidazole benzoate 25% (dental gel elyzol), cholorhexidine gluconate (perichip), and hyclate doxycycline 8.5% (Atridax) into periodontal pockets following initial periodontal therapy. The best results were reported in use of Atridox system. Oringer *et al*.[28] indicated that the use of local minocycline microspheres lead to reduction in GCF interleukin-1 and pyridinoline cross-linked carboxyterminal telopeptide of Type I collagen levels. The purpose of this study was to identify effect of locally delivered doxycycline microspheres in combination with scaling and root planning (SRP) in GCF levels of MMP-8 and clinical parameters in patients with chronic periodontitis.

Materials and Methods

This double-blind spilt mouth; randomized, controlled clinical trial was conducted between 2015 and 2016 in Tabriz, Iran. The protocol employed for this study was approved by the research council and Ethical Committee of Tabriz University of Medical Sciences, Iran. This study was registered at Iranian registry of clinical trial and was IRCT 201601173690N6.

About 32 patients with moderate to severe chronic periodontitis with a mean age of 35-50 years old were selected. They were referred to the periodontics department of Tabriz dental faculty. All patients met the inclusion and exclusion criteria. Inclusion criteria included moderate to severe chronic periodontitis, having at least four teeth with PD ≥5 located in the contralateral quadrants of the same jaw in the maxilla and mandible. Selected quadrants randomly divided into control (SRP) and test groups (SRP + Atridox) by flipping a coin. Smokers, pregnant, patients allergic to antibiotics, patients with poor compliance, and patients who used any herbal drug with effect on MMPs and patients received SRP or periodontal surgery in the last 6 months were excluded from the research. Two teeth were selected randomly as test group, and the other two teeth were considered as control group at the contralateral quadrants. Clinical parameters such as PD and CAL at four areas of the teeth (i.e., mesiobuccal, buccal, distobuccal, and lingual), bleeding index,[29] were evaluated. PD was measured from the gingival margin to pocket base and CAL was defined as the distance from the cementoenamel junction to the base of the pocket by UNC-15 (Hu-Fridey Instruments, Chicago, IL, USA). All subjects received a clinical examination by a single examiner who was an expert periodontist and blinded to the type of treatment. Intra-examiner reliability was tested by examining five patients in an identical manner 1 h apart and observing more than 95% of recordings being within 1 mm. The selected sites were isolated with cotton rolls followed by removal of saliva and supragingival plaque. The baseline sampling was performed by a #25 paper point placed in the gingival sulcus of each selected pockets and was removed after 4 min, and the paper point was placed into test tube full of normal saline. The test tube was kept in a box filled with dried ice with ≤20°C temperature, and then, it was transferred to the laboratory. Sub and supra-gingival treatments were conducted for all participants. In two groups, patients subjected to full-mouth SRP using an ultrasonic device (Various 350, NSK, Japan) and standard gracey periodontal curettes (Hu-Fridey Instruments, Chicago, IL, USA) with no time limitation. Patients in both groups received standard oral hygiene instructions. Instantly, locally doxycycline (Atridox) was placed in the pockets in the test group. The Atridox (doxycycline hyclate) (Atrix Laboratories, NJ) product is a subgingival controlled-release device including two syringe mixing systems. Syringe A contains 450 mg of the ATRIGEL® Delivery System, which is a flowable, bioabsorbable polymeric formulation composed of 36.7% poly(DLlactide) dissolved in 63.3% N-methyl-2-pyrrolidone. Syringe B contains 50 mg of doxycycline hyclate which is equivalent to 42.5 mg doxycycline. This product is a pale yellow to yellow viscous liquid with a concentration of 10% of doxycycline hyclate. In contact with GCF, the liquid state solidifies and led to release of drug within 7 days. The two components of this product were provided in two separate syringes that were coupled together 15 min before use and mixed for 100 cycles. After completion of mixing, the coupled syringes are placed at room temperature to set for 15 min and then mixed for another 10 cycles before use. Using of 2/3 gauge cannula attached to the delivery syringe, the prepared product was slowly transferred into the periodontal pocket. Injection of local antibiotic started from the base of the pocket until it reached the gingival margin. After removing of the cannula tip from the pocket, a wet curette was used to pack any overflow of Atridox into the pocket. Then, treated sites were covered with periodontal dressing to keep the material inside the pocket; this is while the control group received no intervention after SRP. After application of doxycycline gel, the patients were instructed to avoid brushing or flossing on the treated site for 7 days, lack of use hard or sticky foods for 7 days and not to

touch the area with tongue or finger. Oral hygiene instructions were given to each participant. Furthermore, the patients were also asked to report immediately, if the periodontal dressing is dislodged before the scheduled recall visit or if pain, swelling or any other problem occurs. The patients were recalled after 7 days for the removal of periodontal dressing. The patients were evaluated 1 month later. Resampling and measurement of PD, bleeding on probing (BOP) and CAL clinical parameters was repeated in this session. SRP was conducted at both groups and Atridox was placed inside pockets of test group's patients. The patients were revisited after 3 months and resampling and measurement clinical parameters were carried out. The samples level of MMP-8 was studied in the laboratory by enzyme-linked immunosorbent assay. The study method of using kits made in R and D Systems, Inc. USA and Canada with catalogue No: DMP800 for MMP-8 was based on proposed instructions of the manufacture.

The collected data were analyzed statistically in SPSS. 21. Descriptive statistical methods (average and standard deviation) and paired *t*-test (considering normality of data distribution based on Kolmogorov-Smirnov test) were used in this study. In this study, $P < 0.05$ was considered as statistically significant.

Results

All patients completed the study period. The analysis of result of the baseline indicated that the test and control groups share the same profile for CAL, PD, BOP, and MMPs level and had no statistically significant difference. PD, BOP, and CAL significantly decreased in the 1 month interval compared to the baseline in both groups ($P < 0.001$). These parameters significantly decreased in the 3 months compared to 1 month in the test group ($P < 0.001$) but not significantly decreased in the 3 months interval compared to 1 month at control group. Improvements in evaluated clinical parameters in the treated with Atridox group were significantly higher than the control group, 1 and 3 months after treatment ($P < 0.05$) (Table 1 and Graphs 1-4). The control group level of MMP-8 significantly decreased from baseline (83.66 ± 3.5 ng/ml) to 1 month (53.93 ± 5.47 ng/ml) ($P < 0.001$), but in this group MMP-8 level changes were not significant from 1 month (53.93 ± 5.47 ng/ml) to 3 months (49.49 ± 12.04 ng/ml) ($P = 0.056$) (Graph 1). In test group, the level of MMP-8 significantly decreased from baseline (85.34 ± 3.49 ng/ml) to one and 3 months (51.16 ± 5 ng/ml) ($P < 0.001$). Furthermore, reductions were significant from 1 month (51.16 ± 5 ng/ml) to 3 months (28.35 ± 3.35 ng/ml) ($P < 0.001$) in this group (Table 1).

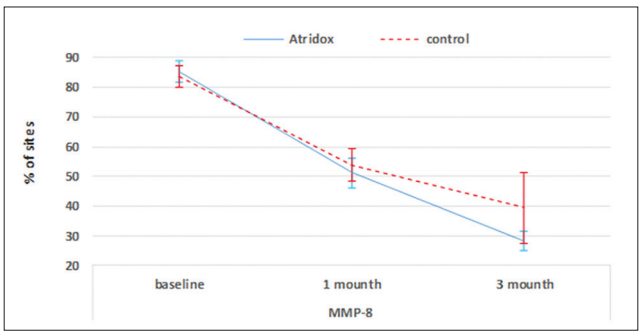
Discussion

The results of this study indicate that local administration of Atridox combined with SRP, significantly reduced GCF level of MMP-8 and improved clinical periodontal parameters in patients with chronic periodontitis.

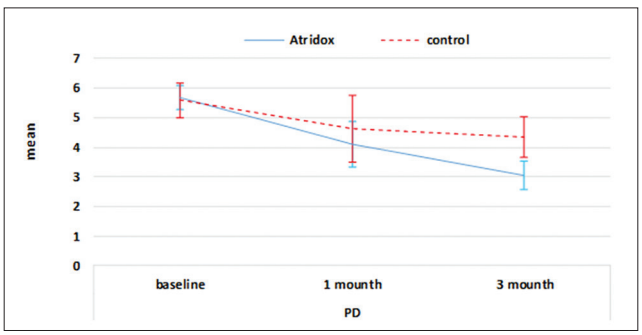
Table 1: Comparison GCF level of MMP-8, PD, BOP and CAL in group treated with Atridox and control group.

Indexes	Baseline	1 month	*P* value	3 months	*P* value*
MMP-8 (ng/ml)					
Control	83.66±3.5	53.93±5.47	<0.001	49.49±12.04	0.056
Atridox	85.34±3.49	51.16±5	<0.001	28.35±3.35	<0.001
P value**	0.059	0.039		<0.001	
PD (mm)					
Control	5.59±0.41	4.62±0.77	<0.001	4.35±0.48	0.213
Atridox	5.69±0.59	4.10±1.14	<0.001	3.05±0.68	<0.001
P value**	0.216	0.037		0.000	
BOP (%)					
Control	76.02±10.23	47.85±12.54	<0.001	41.56±15.62	0.202
Atridox	78.27±11.95	41.85±8.07	<0.001	14.37±10.49	<0.001
P value**	0.421	0.025		0.000	
CAL (mm)					
Control	5.58±1.13	4.70±1.56	0.045	4.16±1.62	0.305
Atridox	5.60±0.85	3.98±1.18	<0.001	3.04±1.05	0.001
P value**	0.927	0.044		0.002	

P value: Paired sample *t*-test for comparison baseline and 1 month, **P* value: Paired sample *t*-test for comparison 1 and 3 months, ***P* value: Paired sample *t*-test for comparison control and Atridox, CAL: Clinical attachment level, BOP: Bleeding on probing, PD: Probing depth, MMP-8: Matrix metalloproteinases 8

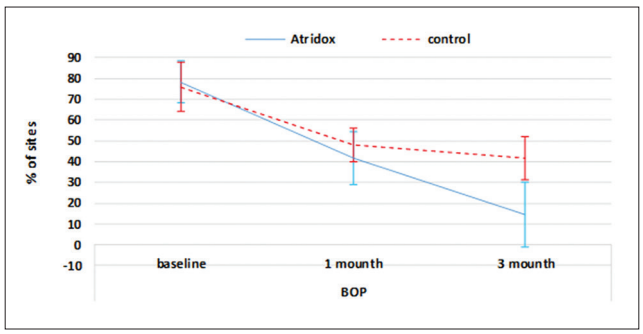


Graph 1: Comparison of gingival crevicular fluid level of matrix metalloproteinases 8 in the treated with Atridox group and control group.

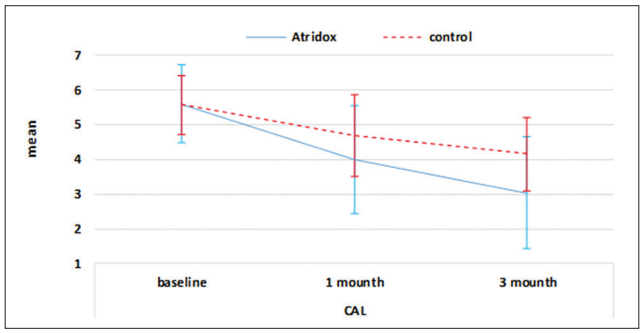


Graph 2: Comparison level of probing depth in treated with Atridox group and control group.

In spite of existence of relationship between periodontal diseases and specific pathogen bacteria, study in recent two decades have shown that majority of periodontal tissue destruction is caused by an exaggerated host response to infection.[30] Release of inflammatory factors from host cells in response to the presence of bacteria plays the main role in periodontal tissues destruction and a wide range of evidence



Graph 3: Comparison level of bleeding on probing in treated with Atridox group and control group.



Graph 4: Comparison level of clinical attachment level in treated with Atridox group and control group.

have shown that MMPs have the main role in periodontal destruction.[2,3,7] Studies have shown that MMP-8 is a key marker in chronic periodontitis, and there is a strong relationship between higher level of GCF MMP-8, PD, CAL, and BOP.[31]

According to various evidence MMP-8 has exclusive ability in the destruction of collagens type I and III.[32] Kuula *et al.*[33] demonstrated MMP-8 has intensive Collagenase activity and increased level of this enzyme is related to intensity of periodontal inflammation. In other studies, Rathnayake *et al.* and Gursoy *et al.* reported MMP-8 as an important diagnostic index in determination severity of periodontal diseases and alveolar bone destruction.[34,35]

Non-surgical periodontal treatment including through removal of sura and subgingival deposits by SRP is considered to be the gold standard for the prevention and treatment of periodontal disease.[36] In addition to achieving a high level of personal oral hygiene, SRP induces the improvement of inflammation and stops the progression of periodontal destruction, which leads to a decrease in PD, reduction in the number of gingival sites with BOP, attachment gain, and a shift from a predominantly Gram-negative anaerobe to a gram-positive aerobe subgingival microbiota.[37] However, SRP is not to be successful at all treated sites. Various systemic and local antimicrobial agents to provide additional benefits that reinforce non-surgical therapy effectiveness.[38] The use of tetracycline family especially doxycycline with subantimicrobial doses is effective along with non-surgical treatments and is mainly effective in cases of localized acute lesions or single locations insensitive to the causative therapy.[39,40] Salvi *et al.* in 2002 investigated the effects of different drugs after non-surgical treatments on clinical parameters; the best results were reported with using Atridox.[27] Due to advantages of local delivery antibiotics compared to systemic therapy and also considering that doxycycline has aboard spectrum of bacteriostatic action and ability to maintain effective concentration at target site, Atridox was chosen in the present study.

In this study, significant improvements in clinical measurements were observed in chronic periodontitis patients after SRP, SRP+ Atridox. This was not surprising since mechanical SRP procedure as the main treatment modality was included in two groups. Nevertheless, the highest reduction in PD and the highest gain in attachment level were observed in the SRP plus local Atridox group. This finding confirms previous studies of Emingil *et al.* and Gürkan *et al.* who demonstrated favorable treatment outcomes in the group of SRP and systemic low-dose doxycycline.[41,42]

To the best of our knowledge, this is the first study to investigate the effects of local doxycycline on the GCF levels of MMP-8 in chronic periodontitis patients. MMP-8 plays a central role in the turnover and degradation of periodontal tissues, especially in the degradation of type I collagen. Since higher levels of MMP-8 have been found in GCF of periodontitis patients, this enzyme has been suggested to be suitable for monitoring periodontal conditions.[43,44] The pre-treatment GCF level of MMP-8 has been shown to decrease to levels found in periodontal healthy GCF, after non-surgical periodontal treatment.[41,44,45] Our data have shown that local usage of Atridox as an adjunct to SRP resulted in GCF MMP-8 levels comparable to that of the SRP alone. Emingil *et al.*[41] indicated that low-dose doxycycline therapy in combination with SRP can reduce GCF MMP-8 levels and improve clinical periodontal parameters in patients with chronic periodontitis. Crout *et al.* and Golubs *et al.* also obtained similar results in their studies demonstrated that use of subantimicrobial dose of doxycycline improves clinical parameters such as attachment level and PD in patient with periodontitis and decreasing level of MMP-8 without any microbial resistance.[21,22] Results of the present study were in agreement with the results of other studies that evaluated the effect of non-surgical periodontal treatment along with systemic doxycycline, GCF level of MMP-8 was significantly decreased. However, amount of improvement in this study was higher than previous studies; these differences can be related to higher concentrations of doxycycline in local application compared to systemic administration. Adjunctive treatment with Atridox caused a significant decrease of GCF level of MMP-8, PD, BOP and CAL in the test group compared to control group in the 1 month interval compared to baseline and 3 months compared to 1 month interval. An additional advantage of this study was its split mouth design that eliminated the effect of confounding factors.

Conclusion

The findings of this study indicate that local administration of doxycycline with SRP can improve clinical periodontal parameters, reduce the GCF MMP-8 level. Further studies with larger samples are required to establish the effects of 10% doxycycline hyclate gel in different periodontitis cases.

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