Adjunctive Subantimicrobial Dose Doxycycline in the Treatment of Chronic Periodontitis in Type 2 Diabetic Patients: A Unique Combination Therapy

SUMMARY

**Background/Aim:** To evaluate the effectiveness of combination therapy including subantimicrobial dose doxycycline (SDD) and locally delivered doxycycline (LD) as adjuncts to scaling and root planing (SRP) in the treatment of chronic periodontitis in patients with type 2 diabetes mellitus (T2DM). **Material and Methods:** Forty patients with controlled T2DM (HbA1c ≤7%) and chronic periodontitis were selected. They were randomly divided into two groups, twenty patients each: Test group (TG, n=20) patients was treated with combination therapy of full mouth SRP, LD gel 10% and SDD 20 mg twice daily for 6 months. Control group (CG, n=20) patients was treated with full mouth SRP only. The periodontal parameters were recorded at baseline, 3, 6 and 9 months and included periodontal probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP). Gingival crevicular fluid (GCF) samples were collected and a quantitative measurement of matrix metalloproteinase-8 (MMP-8) was carried out by using Enzyme-Linked Immunosorbent Assay (ELIZA) at baseline, 3, 6, and 9 months. **Results:** Statistically significant reduction in all clinical parameters (PPD, CAL, and BOP) was observed at TG over CG at 3, 6, and 9 months (p<0.05). Moreover, combination therapy provided significant reductions in the amount of GCF MMP-8 for the TG compared to CG at 3, 6, and 9 months evaluation period (p<0.05). **Conclusions:** Combination therapy including SRP, SDD, and LD, provided significantly greater clinical benefits than SRP alone in the treatment of chronic periodontitis in patients with controlled T2DM.

**Key words:** Diabetes Mellitus, Gingival Crevicular Fluid, Matrix Metalloproteinase-8, Periodontal Therapy, Subantimicrobial Dose Doxycycline

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Introduction

Type 2 diabetes mellitus (T2DM) and periodontal disease are common chronic diseases in adults. Both diseases are highly prevalent in the world population and constitute a global public health burden. Diabetes affects more than 150 million individuals worldwide and this incidence is increased annually. By the year 2030, it was estimated that about 366 million people worldwide will have diabetes. In addition, more than one in three people worldwide over 30 years of age will have periodontitis. Diabetes mellitus and periodontal disease are assumed to share a common pathogenesis that involves an enhanced inflammatory host response. Investigations have shown that diabetics with long history of diabetes mellitus and poor metabolic control are more likely to have severe periodontal destruction and alveolar bone loss than non-diabetics.

Collagenases are considered the key mediators of inflammatory tissue destruction in periodontal diseases. Among collagenases, matrix metalloproteinase-8 (MMP-8) is the key enzyme in extracellular collagen matrix
degradation and it is the major destructive collagenase that is present in large amounts in gingival crevicular fluid (GCF) of periodontally diseased subjects. Patients with T2DM and chronic periodontitis have been shown to express high levels of active MMP-8 in their GCF. Specific immunoassays targeting MMP-8 were considered to be useful in monitoring the course of periodontitis in patients with diabetes. Evidence based studies have indicated that control of periodontal infection has a positive effect on glycemic control evidenced by a reduction in glycated hemoglobin (HbA1c) levels.

The current therapeutic strategies for the treatment of chronic periodontal disease in T2DM patients have been directed towards two different approaches: antimicrobial therapy and host modulation therapy (HMT). Antimicrobial therapy can be effective in decreasing the total bacterial count in periodontal infection with down-regulation of the inflammatory mediators leading to an improvement of glycemic control in T2DM subjects. However, in HMT excessive and pathologic host inflammatory responses adjusted to provide wound healing and periodontal stability without impairing normal defense mechanisms or inducing inflammation. Subantimicrobial dose doxycycline (SDD) is the only host modulation drug approved by the food and drug administration (FDA) which can inhibited the activity of Matrix metalloproteinases (MMPs) involved in the degradation of collagen in connective tissue as a result of periodontal disease.

The aim of this clinical study was to evaluate the effects of combining systemically administered SDD and locally delivered doxycycline gel (LD) as adjuncts to scaling and root planing (SRP) in the treatment of chronic periodontitis in T2DM patients by assessing the changes in the clinical and biochemical parameters.

Material and Methods

Subjects

Forty patients with T2DM aged 35 to 60 years fulfilling the inclusion criteria were selected from the outpatient clinic of the Department of Oral Medicine, Periodontology, Oral Diagnosis and Oral Radiology, Faculty of Dentistry, Tanta University. The study was reviewed and approved by the University Review Board and the Research Ethical Committee, Faculty of Dentistry, Tanta University. The study was performed in accordance with the Helsinki Declaration of 1975 as revised in 2000. The patients were given written explanations of the study, and they provided written informed consent.

Inclusion criteria

The inclusion criteria for selection were subjects with controlled T2DM that had been established diabetes mellitus at least one year before baseline examination, with at least two consecutive values of glycated hemoglobin (HbA1c) level of ≤ 7%, had been taking a stable dose of oral hypoglycemic medications and/or insulin for at least three months as assessed by their medical records and had been clinically diagnosed with chronic periodontitis. The patients had at least ≥ 10 teeth per dental arch, excluding the third molars and teeth indicated for extraction.

Exclusion criteria

Patients were excluded if they were received antibiotics within 3 months prior to the study, patients subjected to periodontal treatment in the six months period prior to the study, patients with other systemic conditions known to modify periodontal disease expression such as pregnancy, cardiovascular disease, and smokers. Patients in need for prophylactic antibiotics before treatment as well as those hypersensitive to doxycycline or any other drug in the tetracycline class were also excluded. Other exclusion criteria includes: renal impairment, severe liver disease, and grade 3 or 4 retinopathy.

Study design

At the first visit (screening visit), patients were screened for eligibility for the study and they were participated into the study if they met the inclusion/exclusion criteria outlined above. Patients were received oral hygiene instructions (OHI) and their ability to fully comply with OHI was evaluated. Eligible subjects were randomly divided (by tossing a coin) into two equal groups: test group (TG) and control group (CG) of twenty patients each.

At baseline visit, all patients of the TG and CG were received basic periodontal treatment including full mouth scaling and root planing (SRP) using hand and ultrasonic instrumentation followed by tooth polishing with a fluoride containing paste and their OHI was reinforced. Full mouth SRP was conducted in two visits (in two successive days). In each visit one side either right or left (upper and lower) were treated under local anesthesia when indicated. A comprehensive periodontal examination was performed for all patients, including measures of clinical parameters by a single examiner for bleeding on probing (BOP: Ainamo and Bay), PD, and the clinical attachment level (CAL: Ramfjord), using Williams periodontal probe, Hu-friedy, Chicago, IL. Assessments of clinical parameters were performed at baseline, 3, 6 and 9 months after treatment.

Subjects in the TG were treated by full mouth SRP and were received systemic SDD, (doxycycline 20 mg) twice per day at approximately 12 hours intervals, one hour before eating for 6 months. In addition, locally delivered doxycycline gel 10% [LD] (prepared at Pharmaceutical Technology Department, Faculty of...
Pharmacy, Tanta University) was applied using a special syringe with a blunt end that inserted into the periodontal pocket until the gel was extruded from the pocket at selected sites (sites with PD≥ 5mm, with bleeding and/or suppuration on probing). Subjects in the CG were treated by full mouth SRP only. For all subjects, HbA1c level assessments were carried out in a private laboratory to allow for monitoring of the overall glycemic control.

**MMP-8 assay**

Supragingival plaque was removed, and the area was isolated with cotton rolls and gently dried before sampling. GCF samples were collected from all patients from areas of deepest pocket depth by a single examiner using sterile prefabricated paper points prior to recording the clinical parameters at baseline, 3, 6, and 9 months. The absorbed GCF volume of each point was measured using a calibrated device (Periotron 8000, ProFlow Inc., NY, USA). All samples were then immediately placed in an Eppendorf tube® containing 200 μl phosphate buffered saline (PBS pH 7.2), and stored at −20°C until analyzed®. MMP-8 levels in the GCF samples were determined by Enzyme-Linked Immunosorbent Assay (ELISA) test kit (Quantikine®, R&D Systems, Minneapolis, MN, USA) according to the instructions of the manufacturer. The specific kit can detect total MMP-8 (pro- and active forms of MMP-8) in saliva, GCF, serum and plasma. The levels of MMP-8 in each sample were calculated based on the dilutions, and the results were expressed as the concentration in GCF sample.

**Statistical Analysis**

The Student’s t-test (two tailed, independent) was used for comparison between the two groups (intergroup analysis) and a Student’s t-test (two tailed, dependent) was used to determine the significance of study parameters within each group (intragroup analysis). Chi-square and Fisher exact tests were used to test the significance of study parameters on a categorical scale. All statistical tests were conducted at a significance level of \( p \leq 0.05 \). The Student’s t-test (two tailed, independent) was used for comparison between the two groups (intergroup analysis). Chi-square and Fisher exact tests were used to test the significance of study parameters on a categorical scale. All statistical tests were performed using computer software program SPSS ver. 20.0 (SPSS Inc., Chicago, IL, USA).

**Results**

A total of 40 patients (26 males and 14 females) were included and completed the study in adherence to the prescribed protocol. Demographic data, clinical parameters, and GCF MMP-8 levels of the patients were summarized in (Table 1). At baseline, the TG and CG had similar mean values for age and gender distribution. The types and doses of hypoglycemic agents and/or insulin were constant during the 9 months study period as verified by the patients’ physician and their glycemic control were maintained throughout the study. For the TG, there were no adverse events reported during the study and the treatment appeared to be well tolerated.

**Periodontal and biochemical parameters**

As previously stated, all clinical and biochemical parameters were assessed in both groups at baseline and again at 3, 6 and 9 months after treatment. At baseline, there were no statistically significant differences among patients of both groups in clinical parameters and GCF MMP-8 levels (Table 1). However, both TG and CG showed significant improvement in clinical and biochemical parameters over the study period.

The mean PD at baseline in the TG was (3.97±0.64) and in the CG was (4.19±0.23) which was not statistically significant (\( P=0.06 \)). During the study, a statistically significant reductions in PD compared to baseline were seen in both groups at 3, 6, and 9 months (\( P<0.05 \)). Comparison between the TG and CG, showed a greater reduction in mean PD in the TG than in the CG at 3 months (TG 3.00±0.47, CG 3.71±0.22), 6 months (TG 3.04±0.39, CG 3.68±0.24) and 9 months (TG 2.95±0.40, CG 3.66±0.22) from baseline (\( P<0.05 \)) (Table 2).

Compared to baseline, the CAL also showed significant reductions at 3, 6 and 9 months (\( P<0.05 \)) in both groups. Comparison between the TG and CG, showed a greater reduction in CAL in the TG than in the CG at 3 months (TG 3.99±0.64, CG 2.99±0.39), 6 months (TG 3.69±0.62, CG 2.77±0.38) and 9 months (TG 3.51±0.52, CG 2.64±0.49) from baseline (\( P<0.05 \)) (Table 2).

The mean BOP values of both TG and CG were significantly lower at 3, 6 and 9 months compared to baseline (\( P<0.05 \)). The reduction was more significant in the TG compared to the CG at 3 months (TG 34.94±14.30, CG 57.21±06.96), 6 months (TG 27.41±14.29, CG 54.16±10.29) and 9 months (TG 26.62±13.70, CG 52.78±10.37) (\( P<0.05 \)) (Table 2). From baseline to 9 months, GCF MMP-8 levels were significantly reduced in both groups (\( P<0.05 \)). However, GCF MMP-8 levels in the TG was significantly lower than that of the CG at 3 months (TG 34.40±5.23, CG 52.80±8.26), 6 months (TG 25.00±7.72, CG 44.20±8.44), and 9 months (TG 18.60±8.26, CG 36.40±3.44) (\( P<0.05 \)) (Table 2).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TG (n=20)</th>
<th>CG (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.33±4.34</td>
<td>44.12±5.20</td>
</tr>
<tr>
<td>Probing depth</td>
<td>3.97±0.64</td>
<td>4.19±0.23</td>
</tr>
<tr>
<td>Clinical attachment level</td>
<td>4.42±0.82</td>
<td>4.20±0.39</td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td>74.22±22.88</td>
<td>77.61±13.94</td>
</tr>
<tr>
<td>MMP-8</td>
<td>65.80±6.56</td>
<td>67.20±9.13</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. There were no significant differences between groups (\( P>0.05 \)).
Table 2. Results of periodontal and biochemical parameters at baseline and at 3, 6, and 9 months in the Test group (TG), and control group (CG)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TG (n=20)</th>
<th>CG (n=20)</th>
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</thead>
<tbody>
<tr>
<td>Probing depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.97±0.64</td>
<td>4.19±0.23</td>
</tr>
<tr>
<td>3 months</td>
<td>3.00±0.47</td>
<td>3.71±0.22</td>
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<td>6 months</td>
<td>3.04±0.39</td>
<td>3.68±0.24</td>
</tr>
<tr>
<td>9 months</td>
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<td>9 months</td>
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</tr>
<tr>
<td>Bleeding on probing</td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>74.22±22.88</td>
<td>77.61±13.94</td>
</tr>
<tr>
<td>3 months</td>
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<td>57.21±06.96</td>
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<tr>
<td>6 months</td>
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</table>

a) P < 0.05 compared to the baseline, b) P < 0.05 compared to the control group.

Discussion

The present study was designed to evaluate the effectiveness of combining SDD and LD as adjuncts to SRP in the treatment of chronic periodontitis in T2DM patients. The severity of chronic periodontitis was similar in TG and CG at the beginning of the present study. Significant improvements in clinical and biochemical parameters were observed after treatment in both groups, patients were evaluated every 3 months until the end of the study at 9 months and improvements were maintained throughout the study period. The selected patients were all controlled T2DM patients with HbA1c levels of ≤7%. A follow-up period of 9 months was selected for the evaluation of clinical, and biochemical responses to the treatment which was considered a sufficiently long period for observation of any possible relapse.

To the best of our knowledge no study has compared SRP alone to SRP in addition to SDD and LD in T2DM patients with chronic periodontitis. Our results of combination therapy with SRP, SDD and LD compared favorably to the use of SRP alone, SRP and SDD or SRP and LD. SRP has been considered the gold standard for non-surgical periodontal therapy. It induces the resolution of inflammatory processes and reduction of progression of the periodontal disease, hence it results in reduction of PD and gain of clinical attachment. In the present study, patients of the CG received only SRP, OHI and the results showed significant reduction in PD and gain of CAL beside the improvements of gingival inflammation at 3, 6, and 9 months compared to the baseline. This was expected as sufficient time was spent for thorough SRP in addition to adequate periodontal maintenance given to the patients at recall visits. These findings were consistent with the results of previously reported studies that have been evaluated the efficiency of conventional SRP in patients with T2DM and chronic periodontitis which has been revealed a significant improvement of clinical periodontal parameters including PD, CAL, and BOP.

Data from previous studies showed that the addition of SDD to SRP resulted in significant clinical improvement in terms of CAL gain and PPD reduction over SRP alone in T2DM patients with periodontal disease following 3 and 6 months of therapy. Similarly, the beneficial effects of combining LD to SRP have been supported by studies on different patient groups. In a clinical study evaluated the effect of application of a single dose LD as an adjunct to SRP in the treatment of periodontitis in type 1 diabetes mellitus patients (T1DM), Lima et al. have reported a statistically significant PD reduction and CAL gain which were maintained during the entire 12 months study period. Moreover, a study with 2 years follow up by Machion et al. the adjunctive use of LD to SRP in the periodontal treatment of non-diabetic smokers were evaluated and the results showed a significant PD reduction, CAL gain at 6, 18, and 24 months. In this study, the addition of SDD, LD to SRP in the treatment of chronic periodontitis in T2DM patients (TG) was provided a significant improvement of the clinical periodontal parameters (PPD, BOP reductions, and CAL gain) at 3 months and maintained at 6, 9 months beyond that obtained by SRP alone (CG). These results suggested that a synergy of action possibly exists between systemically administrated SDD and locally delivered LD which could be represented in their combined potential for modifying the local, systemic inflammation and tissue destruction.

In the present study, the levels of MMP-8 in GCF were measured by using ELIZA test and high concentrations of MMP-8 were reported at baseline in both study groups. The levels of GCF MMP-8 showed marked reductions following treatment in both groups which maintained throughout the study period but the results were statistically significant for the TG rather than CG. Hence decreased levels of MMP-8 in both groups might suggest the effectiveness of conventional periodontal therapy represented by full mouth SRP and OHI in reducing the bacterial loads in periodontal environment. Furthermore, these reductions could be related to the combined anticollagenase activity of SDD and antibacterial efficiency of LD for patients of TG. Previous study reported significant reduction...
in the levels of GCF MMP-8 in T2DM patients with chronic periodontitis after conventional SRP for 3 months treatment period\(^6\). Furthermore, in a randomized controlled double blind study by Gilowski et al.\(^{12}\), the effectiveness of short term SDD as an adjunct to SRP in patients with T2DM and chronic periodontitis were studied by evaluating MMP-8 levels in GCF. The results revealed statistically significant reduction of GCF MMP-8 levels only in the test group received SDD for 3 months. Our results of combination therapy of SRP, SDD, and LD support and further extend the findings of these studies with a different treatment protocol, for a different follow up period and in correlation with changes in the clinical and biochemical parameters.

In our study design, only controlled T2DM patients have been participated based on their consecutive assessments of HbA1c levels \(\leq 7\). Previous studies investigating the effect of periodontal treatment on metabolic control of T2DM patients and presented a conflicting results. A number of studies have shown positive effects of periodontal treatment on metabolic control\(^{13,20,26}\), however, other studies reported the contrary\(^{12,27,28}\). In a recent systematic review, Pérez-Losada et al.\(^{29}\) stated that there was no clear evidence of a relation between periodontal treatment and improved metabolic control in patients with T2DM.

In brief, the results of current study suggested that greater benefits for improving the periodontal status of T2DM patients may be achieved by a combination therapy of SRP, SDD and LD. These improvements represented a significant clinical benefit to T2DM patients and reduced the need for further treatment provided that the patients maintain their glycemic control and continued their oral hygiene measures.

Conclusions

In conclusion, combination therapy of SDD and LD has a synergistic effect as an adjunct to SRP in the treatment of chronic periodontitis in T2DM patients. It was effective in suppressing pathologically excessive MMP-8 associated with chronic periodontitis in patients with T2DM.

References


9. Correa FO, Gonçalves D, Figueiredo CM, Gustafsson A, Orrico SR. The short term effectiveness of non-surgical treatment in reducing levels of Interleukin-1ß (IL-1ß) and proteases in gingival crevicular fluid from patients with type 2 diabetes mellitus and chronic periodontitis. J Periodontal, 2008;79:2143-2150.


