ผลของปริทันต์บำบัดคราวเดียวเสร็จด้วยเครื่องอัลตร้าโซนิกร่วมกับการใช้เจลมิโนไซคลินต่อพารามิเตอร์ทางคลินิกและสารสื่ออักเสบในผู้ป่วยโรคเบาหวานชนิดที่ 2

EFFECTS OF SINGLE-VISIT SUBGINGIVAL ULTRASONIC DEBRIDEMENT WITH ADJUNCTIVE MINOCYCLINE GEL ON PERIODONTAL AND INFLAMMATORY PARAMETERS IN PATIENTS WITH TYPE 2 DIABETES

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บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาผลทางคลินิกและสารสื่ออักเสบของการใช้ยามิโนไซคลินเสริมการรักษาในผู้ป่วยโรคปริทันต์อักเสบและเป็นเบาหวานชนิดที่ 2 วิธีดำเนินการวิจัย: อาสาสมัคร 60 คน ที่ได้รับการวินิจฉัยโรคปริทันต์อักเสบและเป็นเบาหวานชนิดที่ 2 แบ่งเป็นกลุ่มควบคุมและกลุ่มทดลอง จำนวนกลุ่มละ 30 คน อาสาสมัครทั้งหมดได้รับการทำปริทันต์บำบัดคราวเดียวเสร็จด้วยเครื่องอัลตร้าโซนิก แต่เฉพาะอาสาสมัครในกลุ่มทดลองได้รับการทำปริทันต์บำบัดคราวเดียวเสร็จด้วยเครื่องอัลตร้าโซนิกร่วมกับการใช้เจลมิโนไซคลินในครั้งแรกของการรักษาและที่ระยะเวลา 3 เดือน ตรวจพารามิเตอร์ทางคลินิกและสารสื่ออักเสบ (อินเตอร์ลิวคิน-1เบต้า อินเตอร์ลิวคิน-6 ทูเมอร์เน็คโครซิสแฟกเตอร์-แอลฟ่า และโมโนไซต์คีโมแอทแทร็กแทนท์โปรตีน-1) อะดิโพเนคตินและพอร์ไฟโรโมแนส จิงจิวาลิส แอนติบอดี ก่อนและหลังการรักษา 3 และ 6 เดือน ผลการศึกษา: อาสาสมัครทั้ง 2 กลุ่ม มีพารามิเตอร์ทางคลินิกดีขึ้นอย่างมีนัยสำคัญทางสถิติหลังการรักษา 3 และ 6 เดือน ในกลุ่มทดลองมีค่าเฉลี่ยของร่องลึกปริทันต์ลดลงและค่าเฉลี่ยของระดับยึดเกาะทางคลินิกเพิ่มขึ้นมากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติหลังการรักษา 3 เดือน ($p<0.05$) อาสาสมัครทั้ง 2 กลุ่มมีอินเตอร์ลิวคิน-6 ทูเมอร์เน็คโครซิสแฟกเตอร์-แอลฟ่าและโมโนไซต์คีโมแอทแทร็กแทนท์โปรตีน-1 ลดลงอย่างมีนัยสำคัญทางสถิติหลังการรักษา 3 เดือน อาสาสมัครในกลุ่มทดลองมีอะดิโพเนคตินเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติหลังการรักษา 6 เดือน นอกจากนี้ยังพบระดับพอร์ไฟโรโมแนส จิงจิวาลิส แอนติบอดีของอาสาสมัครทั้ง 2 กลุ่มลดลงอย่างมีนัยสำคัญทางสถิติ สรุป: ปริทันต์บำบัดคราวเดียวเสร็จด้วยเครื่องอัลตร้าโซนิกร่วมกับการใช้เจลมิโนไซคลินในผู้ป่วยโรคปริทันต์อักเสบและเป็นเบาหวานชนิดที่ 2 เป็นวิธีการรักษาที่มีประสิทธิภาพ ทำให้พารามิเตอร์ทางคลินิกและสารสื่ออักเสบดีขึ้นภายหลังการรักษา 3 เดือน

คำสำคัญ: เจลมิโนไซคลิน โรคปริทันต์อักเสบ ปริทันต์บำบัดด้วยอัลตร้าโซนิก โรคเบาหวานชนิดที่ 2

Abstract

Objective: To evaluated the clinical periodontal and inflammatory outcomes of adjunctive minocycline in patients with both periodontitis and type 2 diabetes mellitus (T2DM). Methods: Sixty patients with T2DM diagnosed with periodontitis were recruited and divided into control and test groups. All participants underwent a single-visit subgingival ultrasonic debridement, but only the test group received adjunctive sustained-release minocycline gel immediately afterward. Periodontal parameters, cytokines (IL-1β, IL-6, TNF-α and MCP-1), adiponectin, and *Porphyromonas gingivalis (Pg)* antibody were measured at baseline, 3 and 6 months after treatment. Results: Patients in both groups showed significant improvement in all clinical parameters after three and six months. The mean probing depth reduction and clinical attachment level gain after three months were significantly improved in the test group compared to the control group ($p<0.05$). IL-6, TNF-α, and MCP-1 were significantly reduced at the 3-month visit in both groups. Adiponectin was significantly increased at 6 months only in the test group. Moreover, *Pg* antibody was significantly reduced in both groups. Conclusion: Subgingival ultrasonic debridement combined with locally administered minocycline gel was effective in improving periodontal and inflammatory parameters in patients with T2DM at 3 months after treatment.

Keywords: Minocycline Gel, Periodontitis, Subgingival Ultrasonic Debridement, Type 2 Diabetes

Introduction

Periodontitis is an inflammatory disease that destroys tooth-supporting apparatuses and is mostly caused by bacterial biofilm accumulation [1]. Periodontitis and diabetes mellitus (DM) have a bi-directional relationship [2]. Periodontitis also contributes to poor glycemic control and insulin resistance in patients with DM [3]. These reciprocal influences associate with dysregulation of the host immune responses by upregulation of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) [4], mainly released by monocytes. Monocyte chemoattractant protein-1 (MCP-1/CCL2) is an important chemokine that controls the recruitment of monocytes and macrophages [5]. MCP-1 is a reported biomarker of pathogenesis in patients with both DM with periodontitis. Inhibition of MCP-1 has been reported to diminish inflammation in many models and, additionally, mitigate DM-associated periodontitis in mouse models [5]. Both pro- and anti-inflammatory molecules play important roles in the pathogenesis of periodontitis. Recently, adiponectin (ADP), an adipokine mainly secreted by adipose tissue into the circulation with anti-inflammatory and metabolism regulation functions, has been revealed as a link between periodontitis and other systemic diseases, including DM [6]. ADP is decreased in DM patients with periodontitis but increases after periodontitis treatment [7]. Inflammatory-related molecules are becoming an emerging field for therapeutic targets in the treatment of periodontitis.

Periodontitis treatment primarily aims to eliminate the bacteria on the root surface by removing calculus and infected cementum [8]. Conventional hand instrumentation is one treatment of choice. However, recently, the ultrasonic device has become the most preferred instrumentation for periodontitis

treatment, due to its evidenced efficacy and efficiency and its comfort for patients [9]. Single-visit full-mouth disinfection has been introduced by aiming to prevent re-infection from untreated sites during multiple visits [10]. Despite its additional advantages, especially shorter time, mechanical debridement alone cannot achieve successful treatment in some areas with difficult access such as deep periodontal pockets and furcation [11].

Many adjunctive therapies have been employed to overcome this limitation, including the use of antimicrobial agents to eradicate periodontopathogens and support the healing process [12]. A locally used minocycline has been reported to promote the improved outcomes of periodontitis treatments, at the same time, reduce the side effects from the use of systemic antibiotics [13]. A minocycline gel for subgingival application, containing 2% minocycline has been developed and globally used for periodontitis treatment. Multiple studies demonstrate its positive outcomes on periodontal-related parameters in DM patients with periodontitis [14-16]. Nonetheless, the effects of minocycline gel as an adjunctive treatment with scaling and root planning (SRP) compared to sole SRP on inflammatory-related cytokines and chemokines is very limited.

In this study, we modified the uses of minocycline gel from the manufacturer's recommendations to make the treatment protocol practical with the socioeconomic conditions of the participants. Consequently, we have designed a single-visit SRP protocol with an ultrasonic device followed by adjunctive 2% minocycline gel at the initial treatment and 3 months later.

Objectives

Our objective was to investigate the effects of single-visit subgingival debridement with adjunctive minocycline on periodontal and serum biomarkers including IL-1β, IL-6, TNF-α, MCP-1, *Porphyromonas gingivalis* antibody, and ADP in comparison with sole subgingival debridement in periodontitis patients with type 2 DM (T2DM).

Methods

Study Design

This randomized, single-blinded, controlled trial was conducted in the dental department of Buntharik Hospital, Ubonrachathani Province, Thailand from July 2020 to January 2021. The study protocol was approved by the Ethical Committee of Ubonrachathani Health Provincial Office (SSJ.UB2563-036). One investigator was responsible for all measurements and subgingival debridement, and another was responsible for subgingival administration of minocycline gel directly into all periodontal pockets after debridement.

Patients

A total of 60 patients (44 females, 16 males; aged 45–73 years, mean age 56 years) with T2DM, diagnosed with periodontitis by the American Academy of Periodontology (AAP)/European Federation of Periodontology (EFP) 2018 classification, were recruited for this study. All patients received medical care for diabetes control by physicians at Buntharik Hospital. The criteria for enrollment were: 1) age $\geq$45 years;

2) ≥16 remaining teeth; 3) diagnosis of periodontitis stage III or IV and grade B or C. Patients treated with antibiotics during the six months before and during the study were excluded. The usage of any anti-inflammatory drugs was not permitted during the clinical trial. All patients provided written informed consent to participate in the study, and 30 patients were enrolled in the control and test group. All participants were treated with single-visit subgingival ultrasonic debridement, but only participants in the test group received adjunctive minocycline gel immediately and three months after debridement.

Treatment

Each participant was given the oral hygiene instruction at baseline and three months after debridement, and no extensive specific oral hygiene instructions were given. All participants were treated with subgingival piezoelectric ultrasonic debridement (P5 Newton, Acteon, Merignac, France) with a special tip design (H3, H4R, H4L), but only participants in the test group received adjunctive sustained-release minocycline gel (Periocline®, Sunstar, Osaka, Japan) 1-2 cartridges (each cartridge contained 0.5 mg minocycline gel) into all sulcus around the teeth immediately after debridement. Subgingival debridement was repeated after all the clinical measurements were performed at three months in both groups, but only participants in the test group received adjunctive sustained-release minocycline gel.

Periodontal Examination

The periodontal parameters included probing depth (PD), clinical attachment level (CAL), plaque index (PI), gingival index (GI), and bleeding on probing (BOP). PD was determined using periodontal probes (PCPUNC 15, Hu-Friedy, Chicago, IL) at six locations per tooth: mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual. CAL measurements were conducted on each tooth at the same six points as the PD measurements. PI was evaluated on the buccal, lingual, mesial, and distal surfaces of each treated tooth and graded using the Silness and Löe scale [17]. The gingival index (visual only) was evaluated at the same six sites on each treated tooth according to Löe and Silness [18]. BOP was evaluated for each treated tooth using the scale developed by Ainamo and Bay [19]. All clinical parameters were measured at baseline, three and six months after treatment.

Measurement of serum immunoglobulin G titer against *P. gingivalis*

Serum immunoglobulin G (IgG) antibody titers against *P. gingivalis* were measured by chemiluminescent enzyme immunoassay using the N-terminus of arginine gingipain RgpA as the antigen. Readings were automatically calculated into specific IgG antibody titer against *P. gingivalis* (U/mL).

Measurement of serum cytokines and ADP

All participants were requested not to have any food or drink for 8 h prior to the blood collection. A 5-mL volume of blood was collected for quantitative measurements. All samples were stored at −80°C and subjected to two freeze-thaw cycles. Before measurement, serum samples were centrifuged 1000 ×g for 15 min at 4°C and supernatants were taken. The samples were then diluted 4 times (×4) with sample diluent included in the measurement assay. TNF-α, IL-1β, IL-6, MCP-1, and ADP were measured at baseline, 3 and 6 months after the treatment using BioPlex human cytokine panel assay (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. A single operator blinded to the

clinical groupings performed all the measurements. Data were collected and analyzed using a BioPlex 200 system instrument equipped with analysis software (Bio-Rad Laboratories).

Statistical Analysis

To compare mean periodontal parameters between the two groups, we used an independent sample t-test and the Mann–Whitney U test. The Wilcoxon signed-rank test, repeated-measures analysis of variance and the Friedman test were applied to compare the mean serum biomarkers among the three timepoints within each group. Pearson's correlation analyses between serum biomarkers and periodontal parameters were performed using the R package "ggpubr" (R version 4.0.2, R Foundation, Vienna, Austria). In the present study, p-values < 0.05 were considered statistically significant. SPSS Statistics for Windows version 26.0 (IBM, Armonk, NY) and GraphPad Prism version 9.1.1 (GraphPad Software, San Diego, CA) were used for statistical analysis.

Results

Two subjects were excluded from this study as they dropped out during follow-up due to inconvenient transportation. The final enrolled subjects were 58 subjects consisting of 28 subjects in the control group and 30 subjects in the test group. At the baseline, we did not observe any differences in sex, age, remaining teeth, mean site PD $\geq$ 5 mm, HbA1c, or fasting plasma glucose (FPG) between the two groups. The demographic information of the patients is presented in Table 1.

Table 1. Demographic information of patients.

	Control group	Test group	p-value
Number of participants	28	30	
Sex (female : male)	23 : 5	21 : 9	0.280
Age (years)	56.29 ± 6.87	56.67 ± 7.39	0.840
Remaining teeth	25.82 ± 4.78	26.07 ± 3.84	0.963
Mean site PD $\geq$ 5 mm	30.68 ± 18.82	40.17 ± 36.29	0.767
HbA1c (%)	8.93 ± 2.49	9.04 ± 2.00	0.856
FPG (mg/dL)	154.17 ± 59.18	165.77 ± 48.44	0.196

PD, probing depth; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose.

Effects of periodontal treatment with adjunctive 2% minocycline gel on periodontal parameters and inflammation parameters

Periodontal parameters

Table 2 shows mean PD (mPD), mean CAL (mCAL), mean PI (mPI), mean GI (mGI), and %BOP at baseline and 3 and 6 months after treatment. The single-visit subgingival ultrasonic debridement showed a significant improvement in all clinical parameters in both groups at both 3 and 6 months compared to baseline. Furthermore, mPD, mPI, mGI, and %BOP at 6 months were also significantly improved compared to 3 months after the treatment in both groups. However, there were no significant differences between control and test groups.

Table 2. The mean ± standard deviation of baseline, 3 and 6 months treatment effect on probing depth, clinical attachment level, plaque index, gingival index, and the percentages of bleeding on probing.

	Baseline	3 months	6 months
mPD (mm)			
Control group	3.66 ± 0.59	2.91 ± 0.57†	2.56 ± 0.40†,††
Test group	3.81 ± 0.76	2.80 ± 0.51†	2.57 ± 0.48†,††
p-value	0.663	0.379	0.797
mCAL (mm)			
Control group	4.22 ± 0.94	3.84 ± 0.99†	3.73 ± 0.90†
Test group	4.46 ± 1.10	3.81 ± 0.93†	3.78 ± 0.88†
p-value	0.560	0.950	0.692
mPI (mm)			
Control group	2.90 ± 0.36 (96.67%)	2.43 ± 0.47$^{£}$ (81%)	2.11 ± 0.41$^{£,££}$ (70.33%)
Test group	2.94 ± 0.20 (98%)	2.53 ± 0.40$^{£}$ (84.33%)	2.09 ± 0.41$^{£,££}$ (69.67%)
p-value	0.674	0.383	0.869
mGI (mm)			
Control group	2.01 ± 0.13 (67%)	1.72 ± 0.26$^{£}$ (57.33%)	1.59 ± 0.22$^{£,££}$ (53%)
Test group	1.99 ± 0.07 (66.33%)	1.77 ± 0.22$^{£}$ (59%)	1.64 ± 0.21$^{£,££}$ (54.67%)
p-value	0.490	0.460	0.435
BOP %			
Control group	98.31 ± 5.74	73.88 ± 22.71$^{£}$	59.51 ± 21.68$^{£,££}$
Test group	98.11 ± 6.46	75.93 ± 19.01$^{£}$	64.28 ± 20.71$^{£,££}$
p-value	0.906	0.711	0.395

mPD, mean probing depth; mCAL, mean clinical attachment level; mPI, mean plaque index; mGI, mean gingival index; BOP, bleeding on probing.

[†]Statistically significant within-group difference by Wilcoxon signed-rank test ($p<0.05$) compared to baseline data.

[††]Statistically significant within-group difference by Wilcoxon signed-rank test ($p<0.05$) compared to data at 3 months.

[£]Statistically significant within-group difference by repeated-measures analysis of variance ($p<0.05$) compared to baseline data.

[££]Statistically significant within-group difference by repeated-measures analysis of variance ($p<0.05$) compared to data at 3 months.

The mean PD reduction ($m\Delta PD$) and mean CAL gain ($m\Delta CAL$) after treatment are shown in Table 3. $m\Delta PD$ and $m\Delta CAL$ significantly improved in the test group after 3 months compared to those in the control group ($p<0.05$), but these parameters showed no significant differences between the two groups after 6 months. Furthermore, Table 4 shows the reduction in the number of sites of initial PD (IPD) $\geq$ 5 mm, IPD 5–6 mm, or IPD $\geq$ 7 mm. The mean reduction in the number of sites of IPD $\geq$ 5 mm and IPD 5–6 mm but not IPD $\geq$ 7 mm were significantly higher in the test group than in the control group after 3 months.

Table 3. The mean ± standard deviation of probing depth reduction and clinical attachment level gain after treatment.

	0–3 months	0–6 months	3–6 months
$m\Delta PD$ (mm)			
Control group	0.75 ± 0.20	1.10 ± 0.32[†]	0.34 ± 0.29 [†,††]
Test group	1.01 ± 0.37	1.24 ± 0.45[†]	0.24 ± 0.15 [†,††]
p-value	**0.005**[‡]	0.262	0.379
$m\Delta CAL$ (mm)			
Control group	0.38 ± 0.22	0.49 ± 0.36	0.11 ± 0.32[£,££]
Test group	0.65 ± 0.40	0.68 ± 0.48	0.03 ± 0.22[£,££]
p-value	**0.002**[¶]	0.105	0.251

$m\Delta PD$, mean probing depth reduction; $m\Delta CAL$, mean clinical attachment level gain.

[†]Statistically significant within-group difference by Wilcoxon signed-rank test ($p<0.05$) compared to data at 0–3 months.

[††]Statistically significant within-group difference by Wilcoxon signed-rank test ($p<0.05$) compared to data at 0–6 months.

£Statistically significant within-group difference by repeated-measures analysis of variance (p<0.05) compared to data at 0–3 months.

££Statistically significant within-group difference by repeated-measures analysis of variance (p<0.05) compared to data at 0–6 months.

‡Statistically significant between-group difference by Mann–Whitney U Test (p<0.05)

¶Statistically significant between-group difference by independent-samples t-test (p<0.05)

Table 4. The mean ± standard deviation of the number of sites with reduction of initial probing depth ≥5 mm, 5–6 mm, and ≥7 mm after treatment.

	0–3 months	0–6 months	3–6 months
mΔSitePD ≥ 5 mm			
Control group (sites)	17.04 ± 8.90	25.21 ± 15.67†	8.18 ± 11.49†,††
Test group (sites)	30.77 ± 23.85	34.13 ± 27.43†	3.37 ± 4.91†,††
p-value	**0.016‡**	0.334	0.115
mΔSitePD 5–6 mm			
Control group (sites)	15.36 ± 8.82	22.61 ± 15.13†	7.25 ± 10.95†,††
Test group (sites)	26.03 ± 18.31	29.07 ± 21.04†	3.03 ± 4.13†,††
p-value	**0.011‡**	0.272	0.165
mΔSitePD ≥ 7 mm			
Control group (sites)	2.76 ± 2.17	4.29 ± 4.81†	1.53 ± 3.26†,††
Test group (sites)	8.88 ± 10.03	9.50 ± 10.48†	0.63 ± 1.31†,††
p-value	0.059	0.155	0.514

†Statistically significant within-group difference by Wilcoxon signed-rank test (p<0.05) compared to data at 0–3 months.

††Statistically significant within-group difference by Wilcoxon signed-rank test (p<0.05) compared to data at 0–6 months.

‡Statistically significant difference between group by Mann–Whitney U test (p<0.05).

Inflammation Parameters

Although we found no significant intergroup difference of the candidate serum biomarkers at any timepoint, except that of MCP-1 at 3 months after baseline (p=0.038), we observed intragroup changes in IL-6, TNF-α, MCP-1, and ADP during the period of study, while a significant intragroup change of IL-1β was not observed in either group. IL-6 significantly decreased at 3 and 6 months after baseline in both groups. TNF-α significantly decreased at 3 and 6 months after baseline in the test group, while a significant decrease in TNF-α was only found at 3 months after the initial treatment in the control group. MCP-1

significantly decreased at 3 months after baseline in both groups. However, in the test group, there was a significant upregulation of MCP-1 at 6 months after baseline, compared to that at 3 months after baseline. ADP did not significantly change at 3 or 6 months after baseline in the control group, but there was a significant increase at 6 months after baseline compared to 3 months after baseline only in the test group (Fig. 1).

Effects of periodontal treatment with adjunctive 2% minocycline gel on serum P. gingivalis antibody

While we observed significant intra-group decreases of serum P. gingivalis antibody during the period of study in both control and test groups, no significant difference between control and test groups was found at any timepoint (Fig. 1).

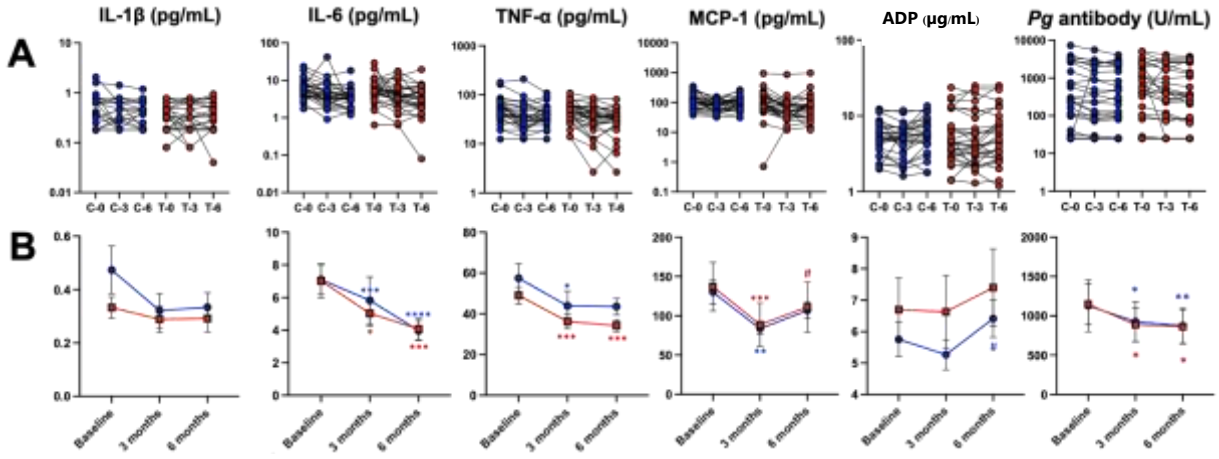

Figure 1. Changes in serum IL-1β, IL-6, TNF-α, MCP-1, adiponectin (ADP), and *Porphyromonas gingivalis (Pg)* antibody at baseline, 3 and 6 months after treatment.

(A) Blue and red dots represent the individual values of the control group and the test group, respectively, with connecting lines representing the same individuals. Data are shown on a logarithmic scale. C-0, baseline data of the control group; C-3, 3 months data of the control group; C-6, 6 months data of the control group; T-0, baseline data of the test group; T-3, 3 months data of the test group; T-6, 6 months data of the test group.

(B) Blue and red colors represent the control and test groups, respectively. Data is presented as mean and standard error of the mean (SEM) of serum biomarkers.

* $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ Statistically significant difference within group compared to baseline data analyzed by Friedman test.

$^{\#}p<0.05$ Statistically significant difference within group compared to 3 months data analyzed by Friedman test.

Correlation between serum biomarkers and periodontal parameters during periodontal treatment

To assess whether there is a correlation between the inflammation markers and the periodontal parameters during periodontitis treatment, we performed Pearson's correlation analyses between target serum biomarkers (IL-1β, IL-6, TNF-α, MCP-1, and ADP) and periodontal parameters, including mPD and mCAL, by using individual data at every timepoint. As shown in Table 5, there were no significant associations between IL-6, MCP-1, and ADP and periodontal parameters. Although some associations were not significant, there were some weak-to-moderate associations between IL-1β and periodontal parameters. However, the association in the control group was stronger than that in the test group. We observed moderate significant association between TNF-α and periodontal parameters. In addition, the correlation coefficient between TNF-α and periodontal parameters in the test group was stronger than that in the control group. According to the color-grouped dot plot, TNF-α was downregulated with decreased mPD and increased mCAL gain in the test group (Fig. 2).

Table 5. Pearson correlation coefficient (R) and *p*-value between periodontal and inflammatory parameters at baseline, 3 and 6 months of treatment.

Serum biomarker	Mean PD				Mean CAL			
	Control group		Test group		Control group		Test group	
	R	*p*-value	R	*p*-value	R	*p*-value	R	*p*-value
IL-1β	0.23	**0.035**	-0.11	0.29	0.17	0.13	-0.13	0.21
IL-6	0.19	0.087	0.1	0.34	0.046	0.68	0.024	0.82
TNF-α	0.31	**0.0038**	0.4	**0.0001**	0.21	0.053	0.33	**0.0017**
MCP-1	0.084	0.45	0.0061	0.95	-0.024	0.83	0.054	0.61
ADP	-0.057	0.61	-0.099	0.35	-0.19	0.079	-0.093	0.38

PD, probing depth; CAL, clinical attachment level; IL, interleukin; TNF, tumor necrosis factor; MCP, monocyte chemoattractant protein; ADP, adiponectin.

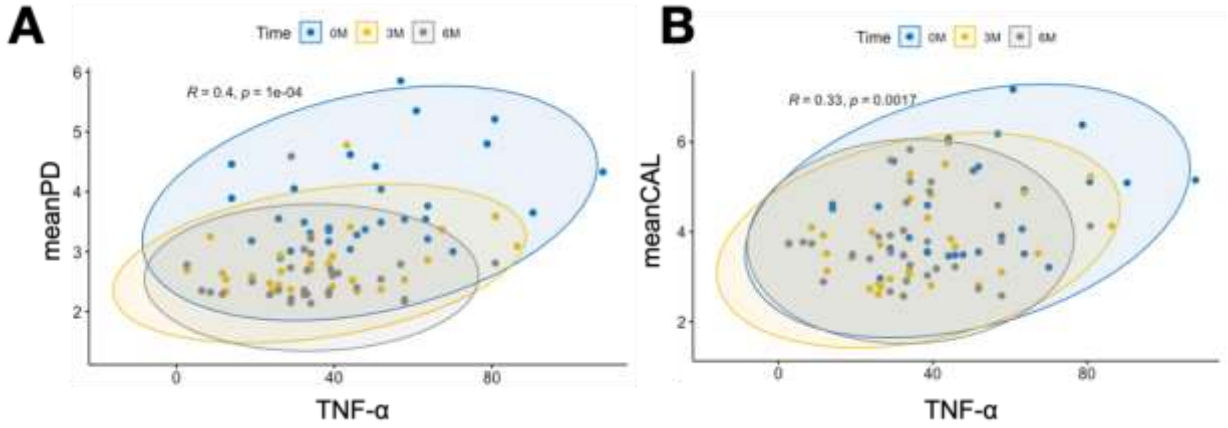

Figure 2. Dot plot shows the correlation between TNF-α and periodontal parameters including (A) mean probing depth (PD) and (B) mean clinical attachment level (CAL) in the test group.

Conclusions and Discussion

The main aim of periodontitis treatment is to reduce the number of periopathogenic bacteria through mechanical debridement, such as SRP [20]. In clinical practice, several studies have shown improvements in clinical periodontal parameters through the adjunctive administration of antibiotics such as metronidazole [21] and azithromycin [22] after SRP in patients with T2DM; however, the adjunctive effect of locally administered minocycline with SRP on periodontal and systemic inflammatory parameters is not fully understood.

In the present study, a significant PD reduction and CAL gain were observed after 3 months in the test group compared to the control group ($p<0.05$), ranging between 1.01 and 0.75 mm and 0.65 and 0.38, respectively. Interestingly, in patients without DM, a study showed that SRP with subgingival minocycline administration led to significantly improved PD and CAL compared to SRP alone [23]. In contrast to our findings, a study in patients with both T2DM and periodontitis found that instrumental periodontal therapy with or without subgingival minocycline administration may achieve significant periodontal improvement but had no significant additive effect of subgingival minocycline administration [14]. This inconsistency regarding the additive effect of minocycline might be due to poor glycemic control in patients with DM with higher HbA1c levels, leaving no room for further improvement by minocycline. Therefore, the present study is the first randomized control study showing evidence of the adjunctive effect of minocycline gel with SRP to treat periodontitis in patients with T2DM.

The current study showed a significant decrease in the number of sites with IPD $\geq$ 5 mm and IPD 5–6 mm ($p<0.05$), but not those with IPD $\geq$ 7 mm in the test group compared to the control group. According to meta-analyses, both SRP alone and SRP combined with a surgical flap are effective methods for chronic periodontitis treatment. Moreover, open-flap debridement results in pocket depth reduction and attachment gain in deep pockets (PD > 6 mm) [24]. In patients with deep PD $\geq$7 mm, osseous surgery also significantly reduced PD compared to SRP [25]. Therefore, surgical or non-surgical interventions with

adjunctive antibiotic treatment might be necessary for treating severer periodontal pockets in patients with T2DM.

Hyperglycemia in DM is associated with higher levels of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and MCP-1 [5, 26]. On the other hand, those cytokines are significantly increased in periodontitis [27]. The local increase of these cytokines may result in increased serum levels where they can induce systemic effects. The current study assesses the potential additive effect of minocycline in reducing biochemical inflammatory markers in T2DM patients with periodontitis. The present study showed significant decreases of IL-6, TNF-α, and MCP-1 in both groups at 3 months after baseline, although no significant differences were found between groups. In line with our results, a previous study reported decreased levels of both IL-6 and TNF-α in patients with T2DM and periodontitis [28]. Interestingly, we previously found that HbA1C level, indicating hyperglycemia, was reduced in poorly controlled T2DM patients with periodontitis after 3 months of minocycline treatment [29]. In contrast, another study showed no significant reduction in systemic IL-6 and TNF-α after SRP treatment [30]. Among the studied cytokines, only MCP-1 was further reduced in the test group at 3 months after baseline ($p=0.038$). Systemic MCP-1 is known to be increased in patients with DM [31] and may play a role in the onset of complications in DM [32]. A previous study has reported the detection of MCP-1 in the serum of patients with DM and periodontitis and its positive correlation with HbA1c, thus serving as a biomarker in patients with DM and chronic periodontitis [5]. Interestingly, studies showing the effect of local administration of minocycline on MCP-1 are limited. These enhanced effects could be attributed to the antibacterial effect of minocycline that leads to a lower pathogenic bacterial load in the pockets and consequently less induction of MCP-1.

Chronic periodontitis is caused by a group of pathogenic bacteria called the red complex, which includes *P. gingivalis, Tannerella forsythia*, and *Treponema denticola*. *P. gingivalis* is a Gram-negative bacterium and is considered one of the keystone pathogens in periodontitis [33]. Hence, the progress of periodontitis is linked to high serum IgG antibody levels against *P. gingivalis* [34]. In this regard, a study reported that full-mouth SRP resulted in significantly decreased IgG titers against *P. gingivalis* 6 weeks after treatment [34] and another study showed that the local application of minocycline adjunctive to SRP suppressed regrowth of periodontal pathogens including *P. gingivalis* in the subgingival plaque taken from patients with chronic periodontitis [35]. In our study, both groups showed significantly decreased serum IgG antibody levels against *P. gingivalis*, although there was no difference in this change between groups.

ADP is an adipokine secreted by adipocytes and is a key component in the association between adiposity, insulin resistance, and inflammation [36]. Previous studies have showed conflicting results regarding the beneficial effect of periodontal treatment in increasing the serum level of ADP. In patients with DM and chronic periodontitis, periodontal treatment significantly increased serum ADP after 3 months [7] while no significant change was reported in another study [28]. In the present findings, ADP was significantly increased after 6 months only in the test group but no change was detected in the control group. The enhancing effect of minocycline on ADP production may result from decreased oral bacterial burden and consequently the systemic inflammation that favors the production of ADP. Nevertheless, further studies are needed to investigate the mechanism behind this observation. Moreover, evidence

exists that TNF-α levels are regulated by ADP and vice versa [37]; thus, ADP suppresses the production of TNF-α both in vivo and in vitro [38-39]. On the other hand, TNF-α inhibits the production of ADP [40]. In our study, TNF-α was decreased only after 3 months in the control group while significant reduction continued to 6 months in the test group in parallel with increased production of ADP, which might indicate a possible inhibition by ADP. In addition, TNF-α is one of the major cytokines involved in the pathogenesis of periodontitis. Elevated levels of TNF-α may promote the release of collagenase, leading to collagen destruction and bone resorption [41]. In this study, we found that TNF-α was downregulated with decreased mPD and increased mCAL in both groups with a stronger association in the test group. These results suggest a positive correlation between periodontitis and increased serum TNF-α. Supporting our conclusion, a study showed that serum TNF-α was strongly significantly correlated with PI, pocket PD, CAL, and GI in patients with chronic periodontitis [42].

In summary, we demonstrated that the addition of minocycline led to PD reduction, CAL gain, and number of sites with IPD reduction compared to ultrasonic debridement alone. These reported periodontal parameter improvements were reflected at the systemic level by decreasing the serum inflammatory cytokines and *P. gingivalis* antibody while increasing ADP.

Minocycline, an antibiotic belonging to the tetracycline family, shows a broad antibacterial effect and can be used to treat periodontitis [20]. The clinical efficacy of local minocycline administration could be explained by its anti-inflammatory properties and gradual release. Moreover, minocycline can alter immune response and produce beneficial effects on periodontal health [43]. For successful periodontal treatment, a continuous release of the drug is needed [20], and the active ingredient, minocycline, used in this study is gradually released from the microsphere formulation in situ after injection into the gingival sulcus. This gradual release of the drug enhances the clinical antibacterial effect because of its longer duration. The gel was administered in all subgingival pockets around the teeth only once after piezoelectric ultrasonic debridement at baseline and after 3 months.

Our protocol is simple and convenient as an initial treatment for periodontal disease in patients with T2DM which can be adopted to minimize the risk of reinfection and to reduce the repeated trauma that causes maintained inflammatory status. Some studies have proposed subgingival administration of minocycline gel directly into the periodontal pockets. However, the differences in the beneficial effects of this administration might result from different study designs, patient selection criteria, or heterogeneity among those studies [13-14, 44].

Recent evidence clearly shows that periodontal treatment is effective for diabetic control to reduce HbA1c in patients with T2DM [28]. In relation to this, a study reported the effect of local minocycline adjunctive therapy with SRP on the improvement of HbA1c in patients with T2DM with high-sensitivity C-reactive protein (hs-CRP) >500 ng/mL [15]. Therefore, it will be necessary to evaluate the effects of this protocol on blood examination, including HbA1c, fasting plasma glucose, and inflammatory markers such as hs-CRP after treatment in future studies.

Within the limitations of this study, periodontitis treatment with a single-visit subgingival ultrasonic debridement and local adjunctive minocycline gel improved the status of periodontal and inflammatory parameters in patients with T2DM and chronic periodontitis at the first 3 months after treatment.

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