Clinical, Microbial, and Immune Responses Observed in Patients With Diabetes After Treatment for Gingivitis: A Three-Month Randomized Clinical Trial

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Background: Although patients with diabetes are frequently affected by periodontitis, only a few investigations have focused on gingivitis in this at-risk population. This randomized placebo-controlled clinical trial compared the response to a gingivitis treatment protocol that combined mechanical procedures and daily use of an essential oil (EO) mouthrinse between patients with and without diabetes.

Methods: The whole-mouth periodontal probing depth (PD), gingival index (GI), and plaque index (PI) were monitored in gingivitis cases among systemically healthy patients (n = 60) or those with diabetes (n = 60) at baseline and 3 months after treatment. Levels of *Porphyromonas gingivalis*, *Tannella forsythia*, *Aggregatibacter actinomycetemcomitans*, and total bacterial load were determined by a real-time polymerase chain reaction in intrasulci plaque samples. The volume of gingival crevicular fluid (GCF) was quantified, and interleukin-1β (IL-1β) levels were determined in GCF samples. After a full-mouth ultrasonic debridement, patients were randomly assigned to an EO or a placebo rinse for 90 days (40 mL/day). The data were analyzed through repeated-measures analysis of variance and multiple comparisons Tukey tests (P < 0.05).

Results: GI was more severe in the diabetes group. Diabetes impaired GI and reduced GCF volume. PD, bacterial levels, and IL-1β improved similarly in both systemic conditions. The adjunctive use of EO provided greater reductions of PI, GI, total bacterial load, *T. forsythia*, *A. actinomycetemcomitans*, and GCF volume.

Conclusions: Response to gingivitis treatment in patients with diabetes can slightly differ from that in patients without diabetes. Daily use of an EO mouthrinse after ultrasonic debridement benefited patients with and without diabetes.

KEY WORDS
Bacteria; diabetes mellitus; gingivitis; interleukin-1; oils; therapeutics.

In humans, dental biofilms accumulate on a daily basis, leading to different degrees of gingival inflammatory responses. Interestingly, different patients respond differently to the same bacterial challenge.¹ This fact is partially explained by the presence of certain systemic diseases, such as diabetes, that could modify gingival inflammatory response. Gingivitis is a highly prevalent disease worldwide.² The incidence of diabetes has been increasing in many countries, and >370 million people are believed to be affected worldwide.³ Currently, ≈14 million Brazilian people have diabetes, and this number is expected to reach 19 million by the year 2030.³ For this reason, it is important to understand the interaction between gingivitis and diabetes. Periodontitis is a more severe type of periodontal disease that follows gingivitis in the disease course. Interestingly, periodontitis has been shown to be the sixth most common comorbidity associated with diabetes.⁴ The relationship between periodontitis and diabetes has been well studied,⁵,⁶ however, less attention has been given to the gingivitis–diabetes relationship, which represents a lost opportunity to study preventive measures that could increase the quality of life for many patients with diabetes. In this context, a manifesto published this year, which was informed...
by the first joint European Federation of Periodontology and American Academy of Periodontology workshop on periodontitis and systemic health, highlighted the relevance of periodontal health to achieving general health in both systemically healthy and diseased individuals, such as those with diabetes. In addition, it is unclear whether gingivitis progresses to periodontitis more quickly in the presence of diabetes. In the population with diabetes, the nature of the response to gingivitis treatment remains unknown.

Although some authors have reported that the microbial profiles of periodontitis are similar between patients with well-controlled diabetes and patients without diabetes, diabetes seems to lead to differences in the periodontal microbiota. In addition, periodontitis is more severe and shows a faster rate of progression in patients with diabetes compared with their counterparts without diabetes. Poor metabolic control interferes directly with periodontitis. In the population with diabetes, the nature of the response to gingivitis treatment remains unknown.

In addition to differences in the dental biofilm, differences in the genetic profile and immune response should also be considered. Human β-defensins have a strong antibacterial action against periodontal pathogens. Compared with patients without diabetes, patients with diabetes and also with periodontitis and gingivitis showed higher levels of β-defensins 2 and 3 in the gingival crevicular fluid (GCF). Moreover, uncontrolled type 2 diabetes is associated with high levels of proinflammatory cytokines in both periodontally healthy and diseased sites. This increase can contribute to the transition from gingivitis to periodontitis. In patients with type 1 diabetes, Salvi et al. reported that the development of gingivitis was accompanied by higher levels of cytokines after controlling for differences in the mean plaque index (PI) and microbial composition.

In systemically healthy individuals, mouthwashes are helpful tools for gingivitis control. Among available antimicrobials for daily plaque control, a fixed combination of four essential oils (EO) (timol, menthol, eucalyptol, and methyl salicylate) has demonstrated good antiplaque and antigingivitis properties. Its regular use is accompanied by a reduction in inflammatory levels comparable with those provided by chlorhexidine but with reduced side effects. However, there is no scientific support regarding the adjunctive use of EO among patients with diabetes.

Therefore, the aim of the present placebo-controlled clinical trial is to compare the response to a gingivitis treatment protocol that combined mechanical procedures and daily use of an EO mouthrinse between patients with and without diabetes.

MATERIALS AND METHODS
The present 3-month, double-masked, single-centered, parallel-group, placebo-controlled, randomized clinical trial (RCT) was registered at ClinicalTrials.gov as NCT02123563 and was approved by the Institutional Committee on Research of the University of Taubaté, Taubaté, São Paulo, Brazil (protocol no. 522/10) in accordance with the Declaration of Helsinki of 1975, as revised in 2000. Before selection, oral and written explanations regarding the research protocol were given to the eligible participants. All patients provided written informed consent before enrolling in the present study, which was composed of baseline and 90-day post-treatment appointments.

Study Population
A total of 120 eligible patients were recruited for the study, and 108 underwent the 3-month final examination (Fig. 1). The 49 male and 71 female (aged 20 to 45 years; mean age: 32 ± 6 years) participants in this study were patients with gingivitis and in good general health (control group) or with diabetes (diabetes group) who sought dental care in the Dental Clinic of the University of Taubaté from June 2012 to December 2012. Data and personal information on the medical and dental histories of the patients were obtained by interview.

The initial sample size of 25 to 30 patients per group was chosen considering standard deviations from a previous study of gingivitis treatment. An effect size (gingival index [GI], a minimum detectable change of 10%), a power of 80%, a significance level of 5%, and a loss to follow-up rate of up to 20% of patients.

Inclusion criteria. The inclusion criteria were as follows: 1) plaque-related gingivitis (with no radiographic evidence of periodontal bone resorption and a bleeding site rate of >30%); 2) ≥20 natural teeth; 3) good general health or controlled type 2 diabetes (with a blood glycated hemoglobin level between 6.5% and 7%) that was diagnosed ≥3 years but no more than 5 years before the study; and 4) normal salivary flow. Although the medical records were checked, a physician monitored the diagnosis and the level of diabetes control for the duration of the study.
Exclusion criteria. The exclusion criteria included the following: 1) gingival overgrowth; 2) orthodontic devices, extended fixed prosthetic devices, removable partial dentures, or overhanging restorations; 3) systemic diseases or other conditions that could influence the periodontal status (other than diabetes within the diabetes group); 4) obesity; 5) alcohol abuse; 6) pregnancy or breastfeeding; 7) history of sensitivity or suspected allergies after the use of oral hygiene products; 8) any antibiotic prophylaxis; 9) antibiotics and/or anti-inflammatory drug use in the 6 months before the beginning of the study; 10) regular use of chemotherapeutic antiplaque/antigingivitis products; 11) periodontal treatment performed within 6 months before study initiation; and 12) unwillingness to return for follow-up.

Clinical Examination
A single calibrated examiner (SAR) performed a complete periodontal examination during the screening phase. Initially, measurements of the periodontal probing depth (PD), clinical attachment level (CAL), PI,29 and GI30 were obtained at four sites per tooth using a manual periodontal probe. A panoramic radiograph was obtained for each patient. Each patient with a periodontal diagnosis of plaque-related gingivitis was included. At baseline and at 3 months, the periodontal PD, PI, and GI were monitored as the clinical outcomes by a second masked and calibrated examiner (SCC). The agreement between examiners was high (κ = 0.84 for PD and 0.82 for CAL). At the same visits (baseline and 3 months), microbial and immune parameters were also evaluated.

Microbial Examination
Subgingival samples were obtained from four periodontal sites (one from each quadrant) that exhibited bleeding on probing, as described by Cortelli et al.31 Each selected tooth was isolated with sterile cotton rolls, and the supragingival plaque was removed with sterile curets. A sterilized no. 30 paper point was carefully inserted to the depth of the gingival pocket, maintained in position for 60 seconds, and later stored at –80°C in empty minitubes.

Bacteria from the sterile paper point were dispersed in Tris-EDTA solution (0.01 M, pH 8) using a vortex mixer at the maximal setting for 1 minute.
and then maintained at −80°C until laboratory processing. A genomic DNA purification kit was used to extract the DNA from the samples following the instructions of the manufacturer.

To quantify the total bacteria load and the periodontopathogens A. actinomycetemcomitans, P. gingivalis, and Tannerella forsythia, the real-time quantitative polymerase chain reaction technique was performed in a 25-μL reaction volume containing 12.5 μL of 2X master mix, 300 nM forward and reverse primers, 250 nM hydrolysis probes, and 2.5 μL DNA sample in a thermal cycler. The cycling conditions were 95°C for 10 seconds, followed by 40 cycles at 95°C for 15 seconds and at 60°C for 1 minute. In the negative control, the DNA sample was replaced by sterile ultrapure water. The primer/probe sequences were as follows: for A. actinomycetemcomitans, forward, CAAGTGTATTAGGTAGTTGGGGG; and reverse, TTCATTACCGCGCATGGG (probe, ATCGCTAGCGGCATGGC); for P. gingivalis, forward, ACCTAACCAGGATTGAAAAATGG; and reverse, CAACCATGCAGCCTACATAGAA (probe, ATGACTGTTGAAAAACCGTCTTCCTTC; reference strain, W83); for T. forsythia, forward, AGCGATGGTACAAAAACCTGAC; and reverse, TGCGGGACTTAACCCAACA (probe, CACAGGGACTTAACCCAACA; reference strain, ATCC 43037); and for total bacterial load (universal primer), forward, TGGACATGTGTTTAATTCGA and reverse, TGGCCGGGTATCCCTC; reference strain, C. jejuni ATCC 25922). Their specificity was checked using a basic local alignment search tool.

For standard curves, known amounts of each bacterial species (10^1 to 10^8 cells) were used to convert cycle threshold values into the number of bacterial cells in the samples.

### Immunoassay

GCF was collected from the same sites that were microbiologically sampled according to Casarin et al. After an interval of 90 seconds after subgingival biofilm collection, the teeth were washed, isolated with sterile cotton rolls, and gently dried. GCF was collected by placing filter paper strips into the gingival pocket until slight resistance was felt and then leaving them there for 15 seconds. After filter paper removal, the volume of the sample was immediately measured with the aid of a calibrated electronic GCF measuring device. The strips were then placed in sterile tubes containing 400 μL phosphate-buffered saline with 0.05% polysorbate 20. Strips visually contaminated with blood were discarded, and a new collection was performed after 30 seconds. During collection, the GCF samples were kept on ice and then stored at −80°C.

The laboratory aliquots of each GCF sample were combined, yielding a single observation per patient at each time point. These samples were assayed by an enzyme-linked immunosorbent assay using a commercially available kit for interleukin-1β (IL-1β), according to the instructions of the manufacturer. Samples were diluted with the diluent included in the kit. This dilution step was used to calculate the concentration of IL-1β in the GCF. The concentration was calculated with a standard curve, which was prepared using the standard proteins in the kit. The immune assays were run in duplicate, and the mean values were used to calculate the concentrations of each cytokine.

### Gingivitis Treatment

According to their systemic condition, two blocks of patients were allocated randomly to one of two groups by a closed-envelope system. Previously, opaque envelopes containing identifications for either the EO or placebo groups were closed, mixed, and then numbered. Each selected participant took a single envelope, which was opened by a masked researcher (AG-F) who then assigned the patient to a specific group.

After baseline examinations, the EO group underwent a one-stage ultrasonic debridement to remove plaque, stain(s), and dental calculus. The EO group also received a 90-day supply of mouthrinse (twice daily use; 20 mL for 30 seconds) containing a fixed combination of four EO (0.092% eucalyptol, 0.042% menthol, 0.060% methyl salicylate, and 0.064% thymol), zinc chloride, and sodium fluoride (0.0221%). After identical mechanical procedures, the placebo group followed the same rinsing regimen with a placebo solution (15% sorbitol solution; 21.6% ethanol, United States Pharmacopeia grade; 0.05% sodium saccharin; 0.1% benzoic acid; mint flavoring; sodium benzoate; purple dye; and water enough for 1 L). One independent research assistant (GCNF) dispensed 1,500 mL of either the EO or placebo in identical sets of three 500-mL bottles, labeled only with the randomization code on the label and no other identifying information. Researchers who had any contact with the study participants were masked to their treatment assignment for the duration of the study and the completion of statistical analysis. Each month, participants received a set of mouthrinse bottles and plastic cups marked to indicate...
a 20-mL volume. The first rinse was performed under supervision (SAR) at the study center, and the remaining rinses were performed unsupervised at home. Patients were instructed to rinse in both the morning and evening. In addition, the patients received fluoride dentifrice, toothbrush, and dental floss on a monthly basis.

Patients were encouraged to comply with the study protocol. The compliance, desirable effects, and undesirable side effects were assessed by a monthly interview.

**Statistical Analyses**
The primary outcomes were PI and GI improvements. For analytic purposes, PI and GI were reclassified as 0 (absence of plaque and bleeding) and 1 (presence of plaque and bleeding). All periodontal measurements were averaged for each participant.

To evaluate the effects of diabetes, treatment, and interactions between these two factors on monitored parameters over time, a two-factor repeated-measures analysis of variance (ANOVA) was performed. A time x group interaction term was the primary test. Microbiologic parameters were log transformed, and a Q–Q plot (in which Q is quantile) of residual values showed acceptable levels of normality and a small range of variances. Based on a statistically significant effect of the independent variables, a multiple comparisons Tukey test was used. Dependent variables were periodontal measurements, whereas independent variables were time (with repeated measures) and its interaction with diabetes, treatment, and both. All tests considered a significance level of 5% (P <0.05).

**RESULTS**
Baseline clinical data from all recruited volunteers are shown in Table 1. The final group with diabetes was composed of well-controlled patients (with glycated hemoglobin values from 6.5% to 7%) with gingivitis who were assigned randomly to either the EO (n = 25) or placebo (n = 26) group. The final control group was composed of systemically healthy patients with gingivitis who were similarly randomized into the EO (n = 28) or placebo (n = 29) group. Tobacco use was not an exclusion criterion, but no more than 10% of patients per group were smokers. For this reason, patients who smoked were still included in the statistical analysis.

Independently of diabetes and the type of rinse, mean PD, PI, and GI were higher at baseline than at 3 months. PD was only affected by time. GI was more severe among patients with diabetes at the two evaluation times. Diabetes also negatively influenced GI reductions; at 3 months, the lowest values were found among systemically healthy individuals. Regarding treatment, EO provided the greatest PI and GI reductions. When all interactions were tested, results revealed that clinical improvements occurred in both groups of patients (those with and without diabetes) (Table 2).

Table 3 shows the microbial data. Independently of systemic status and type of rinse, time affected total bacterial load and levels of *A. actinomycetemcomitans* that were higher at baseline when compared with 3 months. Levels of *P. gingivalis* and *T. forsythia* were not significantly different between the times. In fact, *P. gingivalis* seemed to increase in all groups; however, this finding was not statistically significant. Diabetes did not affect bacterial reductions, but treatment did. At 3 months, patients who rinsed with EO demonstrated lower levels of total bacterial, *T. forsythia*, and *A. actinomycetemcomitans*. These last two bacterial species were also reduced in the group that rinsed with a placebo after ultrasonic debridement.

IL-1β and GCF volume showed higher levels at baseline compared with 3 months. However, IL-1β reductions were not influenced by systemic condition and type of rinse. On the contrary, diabetes affected GCF reductions, with the lowest levels after treatment being observed among systemically healthy controls. The placebo group exhibited a reduction in GCF

<table>
<thead>
<tr>
<th>Table 1. Descriptive Periodontal Clinical Parameters at Baseline for the Whole Population (N = 120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinse</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Non-diabetes</td>
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</tbody>
</table>
volume. Although EO provided GCF improvements for patients with and without diabetes, the greatest GCF improvements occurred in the absence of diabetes and after the use of EO (Table 4).

**DISCUSSION**

Both diabetes and gingivitis are highly prevalent diseases. Gingivitis is a risk factor for periodontitis, which widely affects patients with diabetes. Therefore, the appropriate management of gingivitis is critical for the prevention of periodontitis in both systemically healthy patients and those with diabetes. A method for identifying patients with gingivitis who will experience periodontal breakdown has not yet been discovered. Unfortunately, the available literature regarding gingivitis and diabetes is scarce. Based on knowledge of diabetes and periodontal status, patients with diabetes have higher amounts of bacteria and more significant biofilm accumulation. They also have more severe inflammation, including higher levels of cytokines. Their poorer periodontal status seems to be affected by their glycemic control.

There are many gaps in the present knowledge that have yet to be investigated. For example, it is unknown whether gingivitis progresses to periodontitis faster in the presence of diabetes, which could partially explain, for example, the greater difficulty observed in the present study regarding GI reduction among patients with diabetes. Furthermore, the effects of gingivitis treatment have never been studied specifically in a diabetes population. Thus, the expectation that patients with diabetes will have a worse response to treatment is currently only speculation.

This 3-month RCT was designed to evaluate the response to gingivitis treatment in patients with diabetes by assessing several clinical, microbial, and immune indicators, using gingivitis patients without diabetes as the control group. In systemically healthy patients with mild to moderate gingivitis, a fixed combination of four EOs demonstrated clear

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**Table 2.**

**Effects of Time (baseline/3 months), Diabetes (yes/no), Treatment (EO/placebo), and Their Interactions on Clinical Parameters**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean PD (mm)</th>
<th>Mean PI (0/1)</th>
<th>Mean GI (0/1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time Point</strong></td>
<td>Baseline</td>
<td>3 Months</td>
<td>Baseline</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.75 ± 0.71</td>
<td>1.64 ± 0.63</td>
<td>0.63 ± 0.36</td>
</tr>
<tr>
<td>Non-diabetes</td>
<td>1.84 ± 0.61</td>
<td>1.59 ± 0.56</td>
<td>0.56 ± 0.38</td>
</tr>
<tr>
<td><strong>p</strong>&lt;sup&gt;±&lt;/sup&gt;</td>
<td>0.14</td>
<td>0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EO</td>
<td>1.77 ± 0.70</td>
<td>1.55 ± 0.58</td>
<td>0.60 ± 0.37&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.82 ± 0.62</td>
<td>1.67 ± 0.60</td>
<td>0.60 ± 0.37&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>p</strong>&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>0.17</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.72 ± 0.75</td>
<td>1.58 ± 0.63</td>
<td>0.61 ± 0.34</td>
</tr>
<tr>
<td>EO</td>
<td>1.78 ± 0.68</td>
<td>1.70 ± 0.63</td>
<td>0.66 ± 0.37</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.83 ± 0.66</td>
<td>1.52 ± 0.54</td>
<td>0.58 ± 0.39</td>
</tr>
<tr>
<td>Non-diabetes</td>
<td>1.86 ± 0.57</td>
<td>1.65 ± 0.58</td>
<td>0.54 ± 0.36</td>
</tr>
<tr>
<td><strong>p</strong>&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0.92</td>
<td>0.69</td>
<td>0.007</td>
</tr>
<tr>
<td>Time</td>
<td>1.80 ± 0.66</td>
<td>1.61 ± 0.59</td>
<td>0.60 ± 0.37</td>
</tr>
<tr>
<td><strong>p</strong>&lt;sup&gt;§&lt;/sup&gt;</td>
<td>0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Different lowercase letters (a, b) within rows indicate differences between times (baseline versus 3 months) by multiple comparisons Tukey tests. Different capital letters (A, B) within columns indicate differences between diabetes (yes/no) and treatment (EO/placebo) groups by multiple comparisons Tukey tests. *P* values set in boldface are significant (*P* < 0.05).

* Time × diabetes interactions by repeated-measures ANOVA.
† Time × treatment interactions by repeated-measures ANOVA.
‡ Time × diabetes × treatment interactions by repeated-measures ANOVA.
§ Effect of time by repeated-measures ANOVA.
### Table 3.
Effects of Time (baseline/3 months), Diabetes (yes/no), Treatment (EO/placebo), and Their Interactions on Microbiologic Parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Bacterial Load</th>
<th>P. gingivalis</th>
<th>T. forsythia</th>
<th>A. actinomycetemcomitans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>3 Months</td>
<td>Baseline</td>
<td>3 Months</td>
</tr>
<tr>
<td>Diabetes</td>
<td>762.18 ± 1,070,162.65</td>
<td>212.35 ± 500.12</td>
<td>271.34 ± 61.36</td>
<td>199.27 ± 558,470.46</td>
</tr>
<tr>
<td>Non-diabetes</td>
<td>799.87 ± 1,124,528.86</td>
<td>198.67 ± 371,975.91</td>
<td>226.07 ± 407.77</td>
<td>230.75 ± 653,860.88</td>
</tr>
</tbody>
</table>

*p* 0.22 0.69 0.87 0.46

EO 743.85 ± 1,043,440.65aA 80.50 ± 207,119.80bB 91.63 ± 319,453.33bA 71.05 ± 118,789.23bA 7.64 ± 13,063.91bB

Placebo 818.20 ± 1,148,459.17aA 344.24 ± 482,833.52aA 153.68 ± 493,571.77bB 158.31 ± 266,581.66aA 88.69 ± 172,514.45bB

*p†* <0.001 <0.001 <0.001 <0.001

Different lowercase letters (a, b) within rows indicate differences between times (baseline versus 3 months) by multiple comparisons Tukey tests. Different capital letters (A, B) within columns indicate differences between diabetes (yes/no) and treatment (EO/placebo) groups by multiple comparisons Tukey tests. *P* values set in boldface are significant (*P*<0.05).

* Time × diabetes interactions by repeated-measures ANOVA.
† Time × treatment interactions by repeated-measures ANOVA.
‡ Time × diabetes × treatment interactions by repeated-measures ANOVA.
§ Effect of time by repeated-measures ANOVA.
antiplaque and antigingivitis effects.22-24,35,36 Because ultrasonic debridement is believed to be an appropriate alternative to manual instrumentation,37,38 the therapeutic approach selected for the present study involved the daily use of EO after ultrasonic debridement in a group of patients with diabetes. To the best of the authors’ knowledge, having a placebo as the comparator, the design of the present study also answered for the first time whether the adjunctive use of EO provides additional benefits based on different gingivitis indicators, even in the presence of diabetes.

Investigating an antimicrobial that could be used on a daily basis seemed to be very helpful for patients with diabetes, considering their susceptibility to gingival inflammation. Although Novaes Júnior37 reported that a single session of ultrasonic prophylaxis combined with oral hygiene instructions was sufficient to treat gingivitis in adolescents (as assessed 15 and 30 days after treatment), this result is not observed in the present study. At 3 months, no clinical improvements were detected in the patients with or without diabetes who only received ultrasonic debridement (placebo groups), although it cannot be confirmed that improvements did not occur in the first month. In future studies, the present authors plan to evaluate the effect of multiple prophylaxis regimens or oral hygiene reinstructions, which seem to positively affect gingivitis.39 The effects of manual instrumentation should also be tested. In a previous study, the present authors manually treated gingivitis diagnosed in systemically healthy patients and observed clinical and bacterial improvements.26 Although a single session of ultrasonic debridement alone provided no clinical benefits, it was accompanied by some bacterial and GCF reductions (Tables 3 and 4, respectively).

Systemically healthy participants benefited from the daily use of EO. This group showed improvements in their PI and GI (Table 2), total and specific bacterial levels (Table 3), and IL-1β and GCF (Table 4). The present findings are supported by previous studies. Cortelli et al.40 and Goutham et al.41 also reported

| Table 4. Effects of Time (baseline/3 months), Diabetes (yes/no), Treatment (EO/placebo), and Their Interactions on Immunologic Parameters |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Mean IL-1β (concentration in fluid: pg/mL/total amount = pg) | Mean GCF (μl)   |                      |
|                 | Baseline        | 3 Months        | Baseline        | 3 Months        |
| Diabetes        | 20.40 ± 5.16    | 16.66 ± 5.02    | 111.90 ± 21.80aA| 90.83 ± 21.73bA |                      |
| Non-diabetes    | 21.89 ± 6.66    | 17.67 ± 5.50    | 104.82 ± 20.45bA| 70.97 ± 13.75dB |                      |
| p*              | 0.36            |                 | <0.001          |                 |                      |
| EO              | 21.16 ± 5.32    | 16.25 ± 4.87    | 108.41 ± 21.02aA| 74.46 ± 14.9gbB |                      |
| Placebo         | 21.13 ± 6.62    | 18.09 ± 5.52    | 108.31 ± 21.86aA| 87.33 ± 23.53bA |                      |
| p†              | 0.34            |                 | 0.01            |                 |                      |
| Diabetes        | 21.47 ± 4.21    | 16.20 ± 4.92    | 109.50 ± 21.47aA| 78.44 ± 15.38bB |                      |
| EO              | 19.33 ± 5.88    | 17.13 ± 5.21    | 114.31 ± 22.42aA| 103.21 ± 20.24aA|                      |
| Placebo         | 20.86 ± 6.34    | 16.30 ± 4.95    | 107.33 ± 21.05aA| 70.47 ± 13.82bB |                      |
| p‡              | 0.19            |                 | 0.01            |                 |                      |
| Time            | 21.15 ± 5.97    | 17.17 ± 5.26    | 108.36 ± 21.31  | 80.90 ± 20.64   |                      |
| p§              | <0.001          |                 | <0.001          |                 |                      |

Different lowercase letters (a, b) within rows indicate differences between times (baseline versus 3 months) by multiple comparisons Tukey tests. Different capital letters (A, B) within columns indicate differences between diabetes (yes/no) and treatment (EO/placebo) groups by multiple comparisons Tukey test. P values set in boldface are significant (P<0.05).

* Time x diabetes interactions by repeated-measures ANOVA.
† Time x treatment interactions by repeated-measures ANOVA.
‡ Time x diabetes x treatment interactions by repeated-measures ANOVA.
§ Effect of time by repeated-measures ANOVA.
reductions in the PI and GI in systemically healthy gingivitis patients who rinsed with EO. In addition, Cortelli et al., Haffajee et al., and Fine et al. observed a decrease in the bacterial load associated with the use of EO, whereas Sharma et al. reported a decrease in the proinflammatory cytokines IL-2 and interferon-γ in patients with gingivitis.

Fortunately, patients with diabetes who rinsed with EO at 3 months also showed good clinical responses, as demonstrated by improvements in their PI, GI (Table 2), total load, and bacterial species (Table 3). In addition, the specific reduction in GI and GCF among EO users with diabetes should be mentioned. This is a particularly relevant finding, because patients with diabetes tend to exhibit more severe inflammation. In the present study, they showed higher GI levels compared with patients without diabetes. The ability of EO to penetrate the biofilm and promote anti-inflammatory effects can partially explain the ability of EO to promote beneficial effects in the diabetes group. Even when ultrasonic debridement alone produced improvements in the parameters assessed in both groups, the largest improvements tended to occur in the EO groups. This result indicates that rinsing with EO provides additional antimicrobial benefits beyond those provided by the mechanical procedures alone. However, inflammation was more difficult to control among patients with diabetes, who therefore finished the study with higher GI levels than patients without diabetes.

Regarding the PI, patients with and without diabetes who rinsed with EO completed the study with similar mean values (Table 2). In the present study, diabetes does not influence the magnitude of the measured bacterial changes. Therefore, under controlled conditions, diabetes would not represent a limitation in reducing the bacterial load in gingivitis cases. In addition, the tendency to increase demonstrated by P. gingivalis should be mentioned because it was suggested that the persistence of this pathogen could interfere with glycemic control in patients with diabetes. Such interference is not observed in the present 3-month study based on glycemic levels. However, in future studies, other systemic parameters could be monitored between periodontal examinations to clarify some unanswered questions.

Changes in GCF volume also demonstrated a negative effect of diabetes (Table 4). The lowest GCF volumes were observed among patients without diabetes who rinsed with EO. However, it cannot be ensured that, over a longer period, the same level of benefit would not be reached, considering that EO rinse shows a cumulative beneficial effect over 6 months. Therefore, in the future, an additional 6-month study should be conducted with patients with gingivitis and diabetes.

Ultrasonic debridement alone did not provide 3 months of sustained clinical benefits. In general, the benefits were caused by the daily use of the chemical agent. Diabetes influenced the clinical inflammatory status of gingival tissues and impaired improvements in searched inflammatory parameters. Both hypotheses—that diabetes could negatively affect the response to treatment and that EO would be superior to a placebo in the presence of diabetes—were confirmed.

CONCLUSIONS
After ultrasonic debridement, routine rinsing with EO provided a therapeutic benefit to patients with and without diabetes. Responses to gingivitis treatment are not completely similar between patients with well-controlled diabetes and systemically healthy controls.

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REFERENCES


36. Neely AL. Essential oil mouthwash (EOMW) may be equivalent to chlorhexidine (CHX) for long-term control of gingival inflammation but CHX appears to perform better than EOMW in plaque control. *J Evid Based Dent Pract* 2012;12(Suppl. 3):69-72.


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