Effect of scaling and root planing with and without adjunct use of an essential-oil-based mouthwash on whole salivary interleukin-1β levels in patients with periodontal disease: A short-term follow-up study

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Abstract: Objective: The aim of the present short-term follow-up study was to assess the effect of scaling and root planing (SRP) with and without adjunct use of an essential-oil-based mouthwash (EOBM) on whole salivary interleukin (IL)-1beta (β) levels in patients with periodontal disease. Methods: Ninety individuals with periodontal disease were divided into two groups. Patients in group 1 underwent SRP and were instructed to rinse with 10 mL of an EOBM twice daily for 30 days. Patients in group 2 underwent SRP and were instructed to rinse with 10 mL of water twice daily for 30 days. Whole saliva samples were collected, and IL-1β levels were measured at baseline and after 60 days of treatment. P-values < 0.05 were considered statistically significant. Results: At baseline, whole salivary IL-1β levels were comparable among patients in groups 1 (135.6 ± 13.5 μg/mL) and 2 (141.2 ± 5.4 μg/mL). After 60 days of follow-up, there is a significant decrease in whole salivary IL-1β levels among patients in group 1 (10.2 ± 6.4 μg/mL) as compared to those in group 2 (56.6 ± 10.2 μg/mL) (P < 0.01). Conclusion: SRP, when performed with adjunct use of an EOBM, is more effective in reducing whole salivary IL-1β levels as compared to when SRP is performed without the use of an EOBM.

Keywords: IL-1β, essential oil, mouthwash, periodontal disease, scaling and root planing

Introduction

In our recent clinical studies [1, 2], we demonstrated that scaling and root planing (SRP) when performed with adjunct use of an essential-oil-based mouthwash (EOBM) is more effective in reducing periodontal inflammation as compared to when SRP is performed as the sole therapeutic strategy in systemically healthy and medically compromised patients. In a recent study, Alshehri et al. [1] demonstrated significant reductions in plaque index, bleeding on probing (BOP), and probing depth (PD) in patients treated with SRP with adjunct use of an EOBM as compared to patients that underwent traditional SRP. An explanation in this regard may be associated with the fact that EOBM exhibits a broad antimicrobial spectrum, thereby minimizing the pathogenicity of Gram-positive as well as Gram-negative bacteria. Moreover, EOBM has also been reported to retard microbial colonization and multiplication on the teeth surfaces [3]. It has been proposed that EOBM reduces the counts of pathogenic microbes by rupturing bacterial cell walls and enzymatic inhibition [3].

Unstimulated whole saliva (UWS) is a complex oral fluid, which can be collected non-invasively [4]. Studies [5–7] have shown that a variety of inflammatory biomarkers (such as interleukin [IL]-1beta (β), IL-6,
and immunoglobulins) are expressed in the UWS of patients with oral and systemic disorders [5, 6, 8, 9]. In this regard, evaluation of UWS can reveal pertinent information and can be used for monitoring the severity of periodontal inflammation. IL-1β is a proinflammatory cytokine that increases osteoclastic activity, thereby enhancing bone resorption [10]. Moreover, IL-1β has also been reported to mediate periodontal soft tissue destruction via the stimulation of proteases [10–12].

Since SRP with adjunct use of an EOBM reduces periodontal inflammation to a much greater extent as compared to when SRP is performed, a sole therapeutic strategy [1, 2], we hypothesized that SRP with adjunct use of an EOBM reduces whole salivary IL-1β to a significantly greater extent as compared to when SRP is performed alone. With this background, the aim of the present study as to assess the effect of SRP with and without adjunct use of an EOBM on whole salivary IL-1β levels in patients with periodontal disease.

Materials and Methods

Ethical guidelines

The study protocol was reviewed and approved by the College of Dentistry Research Center at King Saud University, Riyadh, Saudi Arabia (NF 2336). Consenting individuals were requested to read and sign a consent form. Participation in the present study is completely voluntary, and all participants were given the freedom to retire from the project at any stage of the study without any penalty.

Inclusion and exclusion criteria

The following inclusion criteria were imposed: a) self-reported systemically healthy individuals; b) patients with bleeding on probing (BOP) in at least 30% sites; and c) patients with a probing depth (PD) of at least 4 mm in 30% sites. Exclusion criteria were as follows: a) patients with self-reported systemic diseases such as prediabetes, such as type 1 diabetes mellitus (DM), type 2 DM, HIV, acquired immune deficiency syndrome, cardiovascular disorders, epilepsy, hepatic disorders, and renal disorders; b) antibiotic and/or steroid intake within the past 90 days; c) overlapping teeth; d) smoking, and e) self-reported habitual tobacco smoking and/or chewing, f) alcohol consumption, g) history of periodontal treatment within 6 months, and h) pregnancy.

Participants and groups

In total, 90 self-reported systemically healthy individuals were included. All individuals had periodontal disease (in accordance with the eligibility criteria reported above). These individuals were equally divided into two groups based upon the treatment strategy adopted for the treatment of periodontal disease. In group 1 (n = 45), patients underwent SRP and were instructed to rinse with 10 mL of an EOBM (Listerine, Johnson & Johnson Middle East FZ-LLC) twice daily for 30 days. In group 2 (control-group) (n = 45), patients underwent SRP alone and were instructed to rinse with 10 mL of water twice daily for 30 days.

The study participants were recruited from an oral healthcare center located in Riyadh, Saudi Arabia. Baseline and follow-up examinations were performed at the same oral healthcare center by one investigator.

Collection of unstimulated whole saliva samples

UWS samples were collected by a single trained and calibrated investigator. UWS was collected at early morning hours as described elsewhere [13]. Briefly, all participants were comfortably seated on a chair and requested to spit (without swallowing) into a funnel connected to a gauged measuring cylinder for five continuous minutes. Unstimulated whole salivary flow rate (UWSFR) was measured and recorded in milliliters per minute (mL/min). Immediately after collection, UWS samples were immediately transferred to disposable Eppendorf tubes and placed on ice. UWS samples were aliquoted and frozen at −80°C. All UWS samples analyzed within 6 months of collection.

Measurement of interleukin-1β and interleukin-6 levels in unstimulated whole saliva

The laboratory based investigations were performed by a single trained and calibrated investigator. Unstimulated whole salivary levels of IL-1β were investigated in duplicates using enzyme-linked immunosorbent assay (ELISA). Human IL-1β kits (Salimetrics LLC, Salivary interleukin 1β, Carlsbad, CA) were used according to the manufacturers’ instructions. In summary, a standard curve was constructed using standards provided with the IL-1β kits, and protein concentrations were calculated from the standard curve. A total of 100 μL diluted standards with samples was dispensed, in duplicate, into the wells coated with a specific protein antibody. The plates were incubated at room temperature for 60 min, following which, they were washed three times with a wash solution. One hundred microliters of conjugate solution was added, and the plates were incubated at room temperature for another 120 min. The wells were washed once again three times with a wash solution, and 100 μL substrate solution was added. The plates were incubated for 20 min at room temperature, following which, 50 μL
of stop solution was added to terminate color development. Absorbance was determined by reading the plate at 450 nm in a spectrophotometer.

**Statistical analysis**

Statistical analysis was performed using a software program (SPSS Version 18, IL, USA). Whole salivary IL-1β concentrations were assessed using one-way analysis of variance. *P*-values less than 0.05 were considered statistically significant.

**Results**

**Characteristics of the study cohort**

Mean ages of patients in groups 1 and 2 were 34.6 ± 6.3 years and 38.5 ± 2.8 years, respectively. All participants were male.

**Unstimulated whole salivary flow rate**

At baseline, there was no statistically significant difference in UWSFR between patients in group 1 (0.52 ± 0.1 mL/min) and group 2 (0.51 ± 0.1 mL/min).

**Whole salivary interleukin-1β levels**

At baseline, whole salivary IL-1β levels were comparable among patients in groups 1 (135.6 ± 13.5 μg/mL) and 2 (141.2 ± 5.4 μg/mL). After 60 days of follow-up, there was a significant decrease in whole salivary IL-1β levels among patients in group 1 (10.2 ± 6.4 μg/mL) as compared to those in group 2 (56.6 ± 10.2 μg/mL) (*P* < 0.01).

**Discussion**

Results from our recent studies [1, 2] showed that SRP, when performed with adjunct use of an EOBM, is more effective in reducing periodontal inflammation as compared to when SRP is done alone. We therefore hypothesized that SRP with adjunct use of an EOBM reduces whole salivary IL-1β to a significantly greater extent as compared to when SRP is performed alone. To our knowledge from indexed literature, this is the first study that has assessed whole salivary IL-1β levels following SRP with and without adjunct use of an EOBM.

The present results showed that after 60 days of follow-up, whole salivary IL-1β levels were nearly 5 times higher among patients in group 2 (SRP alone) as compared to those in group 1 (SRP + EOBM). This reflects that EOBM exert an anti-inflammatory effect, thereby enhancing the overall efficacy of SRP. It has been reported that EOBM denatures bacterial membrane protein and inhibits bacterial enzyme action [14]. Moreover, EOBM presents anti-inflammatory and prostaglandin synthetase inhibitor activity, which can occur at concentrations lower than that needed for antibacterial activity [14]. Moreover, EOBM is also capable of extracting bacterial endotoxins that theoretically may reduce plaque pathogenicity [15]. Furthermore, *in vitro* and *in vivo* studies have shown that EOBM penetrates the plaque biofilm and are active against biofilm-embedded bacteria [16, 17]. These characteristics may support the potentiality of EO as a subgingival irrigating agent and, at the same time, act as an explanation for the present results in which IL-1β was significantly lower in patients treated with SRP + EOBM as compared to those treated with SRP alone.

A limitation of the present study is that all participants were males. Studies [18, 19] have reported that multiple episodes of pregnancy, recurrent gestational diabetes, and obesity are significant risk factors of prediabetes among females. Therefore, it is hypothesized that the severity of periodontal disease is worse in pre-diabetic females compared to males with prediabetes. In a recent study, Javed et al. [20] showed that SRP reduces hyperglycemia and periodontal inflammation in patients with prediabetes. Moreover, studies [4, 21–23] have also reported that periodontal inflammation is worse in smokers and individuals using smokeless tobacco products as compared to individuals not using tobacco in any form. It is speculated that SRP and regular oral hygiene maintenance reduces hyperglycemia and severity of periodontal disease in tobacco product users with as well as without prediabetes; however, most favorable outcomes may be achieved via patient education, strict glycemic maintenance, and quitting the tobacco smoking and chewing habits. Further long-term follow-up studies are needed in this regard.

Within the limits of the present short-term follow-up study, it is concluded that SRP, when performed with adjunct use of an EOBM, is more effective in reducing whole salivary IL-1β levels as compared to when SRP is performed without the use of an EOBM.

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References


