Impact of scaling and root planing with adjunct essential-oil-based mouthwash usage on whole salivary IgG levels in patients with periodontal inflammation

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Abstract: Objective: The aim 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 immunoglobulin G (IgG) levels in patients with periodontal inflammation. Methods: Fifty patients with periodontal inflammation were included. Treatment wise, these patients were randomly divided into two groups. In group 1, patients underwent SRP and were instructed to rinse with 10 mL of an EOBM twice daily for 30 days, whereas those 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 at baseline and after 40 days of treatment. P-values < 0.05 were considered statistically significant. Results: At baseline, whole salivary IgG levels were comparable among patients in groups 1 (60.5 ± 5.5 mg/dL) and 2 (57.3 ± 2.4 mg/dL). After 40 days of follow-up, there is a significant decrease in whole salivary IgG levels among patients in group 1 (7.2 ± 2.4 mg/dL) as compared to those in group 2 (26.6 ± 4.6 mg/dL) (P < 0.001). Conclusions: SRP, when performed with adjunct use of an EOBM, is more effective in reducing whole salivary IgG levels as compared to when SRP is performed without adjunct use of an EOBM.

Keywords: immunoglobulin G, essential oil, mouthwash, periodontal inflammation, scaling and root planing

Introduction

It has been reported that scaling and root planing (SRP), when performed with adjunct use of an essential-oil based mouthwash (EOBM), is more effective in the treatment of periodontal inflammation than when SRP is performed as the sole therapeutic strategy in patient with and without systemic diseases [1]. In a recent clinical study, Alshehri et al. [1] showed 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 SRP alone. These results may be explained by the fact that EOBM exhibits a broad antimicrobial spectrum, thereby minimizing the pathogenicity of Gram-positive as well as Gram-negative microbes. It has been suggested that EOBM ruptures bacterial cell walls, thereby reducing the counts of pathogenic microbes [2].

Unstimulated whole saliva (UWS) is a complex oral fluid, which can be collected non-invasively [3, 4]. The most abundant antibody in serum is immunoglobulin G
IgG), accounting for about 80% of the entire immunoglobulin content [5]. IgG is monomeric, is a "Y" shaped molecule, and has a serum half-life of approximately 21 days [6]. The concentration of IgG in normal saliva is approximately 20 mg/L, which is about 0.1% to 0.2%, of its serum concentration [7]. However, in patients with periodontal disease, salivary IgG may leak from the serum into the oral environment via the gingival crevicular fluid. Studies [8–10] have shown that whole salivary IgG levels are significantly higher in patients with periodontal disease as compared to healthy controls. In this regard, evaluation of IgG levels in UWS can reveal pertinent information regarding the severity of periodontal inflammation in vulnerable population.

Since SRP with adjunct use of an EOBM reduces periodontal inflammation to a much greater extent as compared to when SRP is performed as a sole therapeutic strategy [1, 11, 12], we hypothesize that SRP with adjunct use of an EOBM reduces whole salivary IgG levels to a significantly greater extent as compared to when SRP is performed alone. With this background, the aim was to assess the effect of SRP with and without adjunct use of an EOBM on whole salivary IgG levels in patients with periodontal inflammation.

Materials and Methods

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 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) edentulism; and e) self-reported habitual tobacco smoking and/or chewing, f) alcohol consumption, g) history of periodontal treatment within 6 months, and h) pregnancy and/or lactation.

Participants and groups

Fifty patients with periodontal inflammation were included. These individuals were recruited from an oral healthcare center located in Riyadh, Saudi Arabia. Treatment wise, these patients were randomly divided into two groups. In group 1 (n = 25) 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, whereas those in group 2 (n = 25) underwent SRP and were instructed to rinse with 10 mL of water twice daily for 30 days.

Collection of unstimulated whole saliva samples

UWS samples were 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. In both groups, whole salivary IgG levels were measured at baseline and 40 days after treatment. All UWS samples analyzed within 6 months of collection.

Measurement of IgG levels in unstimulated whole saliva

Levels of whole salivary IgG were determined by direct enzyme-linked immunosorbent assay as described elsewhere [8]. In summary, microrotter plates (Corning Inc. NY, USA) were coated with 100 μL per well of anti-human IgG and anti-human IgA (DAKO A/S, Denmark) in coating buffer (0.05 M carbonate–bicarbonate buffer, pH 9.6) and incubated at room temperature for 24 h. After washing, 100 μL/well of appropriately diluted IgG (Human serum protein calibrator DAKO A/S, Denmark) standard, and patient saliva samples were added to the respective microplate wells. After incubation at room temperature, the microplates were washed to remove unbound proteins. Purified alkaline phosphatase conjugated anti-human IgG were added (100 μL/well), and the microplates were incubated for 3 h at room temperature. After washing, 100 μL/well of appropriately diluted IgG (Human serum protein calibrator DAKO A/S, Denmark) standard, and patient saliva samples were added to the respective microplate wells. After incubation at room temperature, the microplates were washed to remove unbound proteins. Purified alkaline phosphatase conjugated anti-human IgG were added (100 μL/well), and the microplates were incubated for 3 h at room temperature. After washing, 100 μL/well of substrate (p-nitrophenyl phosphate) in 1.0 M diethanolamine, 0.5 mM magnesium chloride, pH 9.8 (Sigma S-0942) was added. The absorbance was read at 405 nm in a microtiter plate photometer (Molecular Devices, Vmax, Sunnyvale, CA, USA).

Statistical analysis

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

The study protocol was reviewed and approved by the College of Dentistry Research Center at King Saud University, Riyadh, Saudi Arabia (NF2336). Consenting individuals were requested to read and sign a consent form.

Results

Characteristics of the study cohort

Mean ages of patients in groups 1 and 2 were 44.6 ± 3.5 years and 48.5 ± 3.1 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). At follow-up, UWSFR was comparable between patients in groups 1 and 2 (0.52 ± 0.1 mL/min and (0.51 ± 0.1 mL/min, respectively).

Whole salivary IgG levels

At baseline, whole salivary IgG levels were comparable among patients in groups 1 (60.5 ± 5.5 mg/dL) and 2 (57.3 ± 2.4 mg/dL). After 40 days of follow-up, there was a significant decrease in whole salivary IgG levels among patients in group 1 (7.2 ± 2.4 mg/dL) as compared to those in group 2 (26.6 ± 4.6 mg/dL) (P < 0.001).

Discussion

Recent studies [1, 14] have shown 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. In this context, the present study was based on the hypothesis that SRP with adjunct use of an EOBM reduces whole salivary IgG levels to a significantly greater extent than when SRP is performed alone. To our knowledge from indexed literature, this is the first study that has assessed the effect of SRP with and without the use of an EOBM on whole salivary IgG levels.

The present results showed that, after follow-up, whole salivary IgG levels were significantly reduced in patients that were recommended to rinse with an EOBM after SRP as compared to controls. This reflects that EOBM exerts 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 [15]. Moreover, EOBM presents anti-inflammatory and prostaglandin synthetase inhibitor activity, which can occur at concentrations lower than that needed for antibacterial activity [15]. Moreover, EOBM is also capable of extracting bacterial endotoxins that theoretically may reduce plaque pathogenicity [16]. Furthermore, in vitro and in vivo studies have shown that EOBM penetrates the plaque biofilm and is active against biofilm-embedded bacteria [17, 18]. These characteristics may support the potentiality of EOBM as a subgingival irrigating agent and at the same time act as an explanation for the present results in which whole salivary IgG were 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 [19, 20] 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 chronic periodontitis (CP) is worse in prediabetic females compared to males with prediabetes. In a recent study, Javed et al. [21] showed that SRP reduces hyperglycemia and periodontal inflammation in patients with prediabetes. Moreover, studies [22–24] have also shown that, besides cigarette smoking, other tobacco habits such as waterpipe smoking and smokeless tobacco chewing can also enhance periodontal inflammation. It is speculated that SRP and regular oral hygiene maintenance reduces hyperglycemia and severity of CP in tobacco users and nonusers with prediabetes; however, most favorable outcomes may be achieved via patient education, strict glycemic maintenance, and quitting the smoking habit.

Within the limits of the present study, it is concluded that, SRP when performed with adjunct use of an EOBM, is more effective in reducing whole salivary IgG levels as compared to when SRP is performed without the use of an EOBM.

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