Effectiveness of 0.12% chlorhexidine and a Salvadora persica-based mouthwash in reducing periodontal inflammation and whole salivary IL-1β levels after non-surgical periodontal therapy in young light cigarette-smokers

A.M. BASUDAN1, A.S. AL-ZAWAWI1, M.Y. SHAHEEN1, D.D. DIVAKAR2,3, H.A. ALDULAIJAN1

1Department of Periodontics and Community Dentistry, College of Dentistry, King Saud University, Riyadh, Saudi Arabia
2Department of Oral Medicine and Radiology, Sharavathi Dental College and Hospital, Shivamogga, Karnataka, India
3Department of Oral Medicine and Radiology, Faculty of Dentistry, Levy Mwanawasa Medical University (LMMU), Ministry of Health, Lusaka, Zambia

Abstract. – OBJECTIVE: The aim was to assess the effectiveness of 0.12% chlorhexidine (CHX) and a Salvadora persica (SP)-based mouthwash in reducing periodontal inflammation and whole salivary interleukin (IL)-1β levels after non-surgical periodontal therapy in young light cigarette-smokers.

PATIENTS AND METHODS: Self-reported current cigarette-smokers and never-smokers with periodontal inflammation were included. All patients underwent non-surgical periodontal therapy. Patients were divided into 2 subgroups. In the test- and control-group, patients were advised to rinse with a Salvadora persica-based mouthwash and a non-alcoholic 0.12% CHX twice daily for 2-weeks, respectively. Full-mouth plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment loss (AL) and whole salivary IL-1β were measured at baseline and at 3-months’ follow-up. Significance was set at p<0.01.

RESULTS: 34 cigarette-smokers and 34 never-smokers were included. At 3-months of follow-up, PI, PD, and clinical AL were comparable with their respective baseline values among cigarette-smokers. In never-smokers, there was a significant reduction in scores of PI (p<0.01), GI (p<0.01) and PD (p<0.01) at 3-months of follow-up compared with baseline. At 3-months of follow-up, there was no significant difference in PI, GI, PD, and clinical AL among never-smokers in the test- and control-groups. Among never-smokers, there was a significant reduction in whole salivary IL-1β levels at 3-months’ follow-up (p<0.01).

CONCLUSIONS: Usage of 0.12% CHX or a S. persica-based mouthwash following NSPT is ineffective in controlling periodontal inflammation and reducing whole salivary IL-1β in young light cigarette-smokers compared with never-smokers.

Key Words: Chlorhexidine, Non-surgical periodontal therapy, Periodontal inflammation, Salvadora persica, Smoking.

Introduction

Mechanical debridement (MD) of teeth surfaces and periodontal pockets or non-surgical periodontal therapy (NSPT) is the classical treatment for the management of periodontal inflammation1. Following NSPT, routine oral hygiene maintenance instructions are enforced, and mouthwashes or oral rinses are often prescribed as measures of post-therapy care2,3. Chlorhexidine gluconate (CHX) at a concentration of 0.12% is frequently used as an adjunct to routine toothbrushing or/and after NSPT due to its antibacterial, anti-inflammatory, and anti-biofilm characteristics4-6. Results from a meta-analysis7 showed that rinsing with 0.12% CHX after NSPT reduces probing depth (PD) to a greater extent compared with NSPT alone. In a recent laboratory-based investigation, Coelho et al8 investigated the cytotoxic effects of CHX on human gingival fibroblasts (HGF). The
results showed that CHX exerts cytotoxic effects on HGF at concentrations that are lower than those used in clinical scenarios. Moreover, CHX allergy, is a clinical situation that results in type-IV hypersensitivity reactions including burning sensation in the mouth, erythema in gingival tissues and stomatitis challenges clinicians as well as patients. Scientific evidence has shown that herbal-based healthcare products are effective in reducing periodontal inflammation and promoting healing and are devoid of side-effects that are often associated with pharmacological medications. The plant Salvadora persica, commonly known as “miswak”, is well-known for its oral-health related benefits. In a systematic review and meta-analysis, Jassoma et al reported that S. persica-based mouthwashes significantly reduce the plaque index (PI), gingival index (GI) and bacterial counts compared with CHX. It has also been documented that herbal-based mouthwashes are as effective as CHX in terms of their antiplaque and anti-inflammatory properties.

Cigarette-smoking is a global risk factor of several systemic diseases including cardiovascular, respiratory, and cerebrovascular diseases that may have a lethal outcome. From a dental perspective, the risk of oral diseases such as periodontitis, peri-implantitis and oral cancer, are significantly higher in cigarette-smokers than never-smokers (individuals that have never used any combustible or non-combustible nicotinic product). Studies have shown that habitual smoking compromises healing and jeopardizes the periodontal ligament cells and HGF. Moreover, smoking is linked to an increased formation and accumulation of advanced glycation end products (AGEs) in periodontal tissues. An interaction between these endproducts and their receptors induces a state of oxidative stress (OS) in oral tissues including those of the periodontium, which in turn compromises healing. Results from a systematic review of 24 clinical studies showed that cigarette-smoking negatively influences the outcomes of periodontal interventions. Unstimulated whole saliva (UWS) is a complex oral fluid that expresses raised levels of destructive inflammatory cytokines, such as interleukin (IL) 1β (β) under periodontal inflammatory conditions. Mokeem et al reported that the levels of whole salivary IL-1β levels are significantly higher in cigarette-smokers compared with never-smokers. Due to its high specificity and sensitivity, IL-1β is considered to be a biomarker of periodontal status before and after NSPT. It is hypothesized that outcomes of NSPT are compromised and whole salivary IL-1β levels remain high in cigarette-smokers compared with never-smokers, and this relationship is independent from the post-operative use of 0.12% CHX or S. persica-based oral rinses.

The aim was to assess the effectiveness of 0.12% CHX and a S. persica-based mouthwash in reducing periodontal inflammation and whole salivary IL-1β levels after NSPT in young light cigarette-smokers.

Patients and Methods

Ethical Statement

The present study was carried out in accordance with the Declaration of Helsinki, as revised in 2013, and the Guidelines involving human subjects. Volunteers were asked to sign an informed consent form. Withdraw did not bear any penalization and/or consequences. Ethical approval was obtained from the Ethics Research Committee of the Sharavathi Dental College and Hospital, Shivamogga, Karnataka, India. All individuals were also given verbal and written information about brushing and flossing techniques.

Inclusion and Exclusion Criteria

Self-reported current cigarette-smokers and never-smokers were included. Cigarette-smokers were defined as individuals who were currently smoking and had smoked at least 100 cigarettes during their life-time. Never-smokers were defined as volunteers who have never consumed combustible or non-combustible nicotinic products in any form. Periodontal inflammation was defined as the presence of dental plaque, gingival bleeding, PD ≥ 3 mm and clinical AL ≥ 1 mm in at least 30% sites. Dual-smokers, habitual alcohol users, and patients with self-reported systemic diseases (such as cardiovascular diseases, diabetes mellitus and cancer) were excluded. Patients that had used antibiotics, bisphosphonates, probiotics, steroids and non-steroidal analgesics within the past 90 days were not sought. Moreover, third molars, nursing and pregnant females and patient undergoing cancer treatment were also excluded.

Questionnaire

A questionnaire was used to gather demographic data pertaining to patients’ age and gender. Information regarding, family history of smoking, duration of cigarette-smoking habit and number of cigarette/packs smoked daily was also collected. The participants were also inquired how often
they brushed teeth daily (once, twice or more) and when did they last visit their oral healthcare provider. The questionnaire was administered to all participants by a trained investigator.

**Classification of Cigarette-Smokers**

Based on the calculated pack-years, cigarette-smokers were classified as never-smokers (0 pack-years), light-smokers (0.1 to 20 pack-years), moderate-smokers (20.1 to 40 pack-years), or heavy-smokers (over 40 pack-years).

**Randomization, Study Group Allocation Concealment and Blinding**

Randomization was done using a site-specific randomization assignment sequence generated prior to initiation of the present study, and allocation to the study-groups was done via block randomization. Following the confirmation of patient eligibility, a trained investigator produced a randomization assignment electronically and notified the principal investigator, who then communicated with the participant with the assigned treatment group and this information was concealed throughout the study duration. Besides the principal investigator, all investigators (including the statistician) were blinded to the study groups.

**Non-Surgical Periodontal Therapy**

In all patients, full-mouth non-surgical mechanical debridement of teeth surfaces, and periodontal pockets were performed at baseline by a trained and calibrated investigator (Kappa score = 0.88), using sterile manual (Hu-Friedy Mfg., EverEdge® 2.0 scalers, Chicago, IL, USA) and ultrasonic scalers (Dentamerica® Scalex 880 Plus, Green Bay, WI, USA). In all patients, full-mouth NSPT was done in a single visit by an investigator who was blinded to the study groups.

**Grouping and Concealment of the Type of Mouthwash Prescribed**

Cigarette-smokers and never-smokers were divided into test- and control-groups. In the test-group, participants were instructed to rinse twice daily for 2 weeks with 10 ml of a *S. persica*-based mouthwash (HiOra, Himalaya Drug Company, Bengaluru, India). In the control-group, patients were instructed to rinse twice daily for 2 weeks with 10 ml of non-alcoholic 0.12% CHX mouthwash (Paroex®, Sunstar Americas Inc., IL, USA). In the test- and control groups, the prescribed mouthwashes were placed in an opaque container which had a standard shape and dimensions. The mouthwashes were provided to the participants by an investigator who was blinded to the study groups. The patients were unaware of the type of prescribed oral rinse.

**Periodontal Parameters**

In all patients, PI, GI, clinical attachment loss (AL) and PD were assessed on each tooth. Bitewing radiographs were taken and viewed on a calibrated computer screen. The MBL was examined on radiographs using a software program (Image Tool 3.0, Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA). The MBL was defined as the vertical distance from 2 mm below the cementoenamel junction to alveolar crestal bone. Clinical periodontal parameters (PI, GI, PD and clinical AL) were assessed at baseline and at 3-months’ follow-up. The MBL was only assessed at baseline to determine the radiographic periodontal status of all participants. Radiographic evaluation was not performed at 3-months of follow-up to avoid undue radiation exposure to the study participants.

**Collection of Unstimulated Whole Saliva and Assessment of Whole Salivary IL-1β Levels**

The UWS samples were non-invasively collected from all individuals as described in a previous study. In summary, UWS were collected during early morning hours with the participants being in a fasting state. The patients were comfortably seated on a chair in a quiet room and requested to allow saliva to accumulate in the mouth for 5 continuous minutes. Patients were advised to refrain from swallowing and jaw movements. After 5-minutes, the participants drooled the UWS into a disposable plastic funnel that was attached to a gauge disposable measuring cylinder. The unstimulated whole salivary flow rate (UWSFR) was recorded in milliliters per minute (ml/min). The UWS samples were immediately transferred to a plastic tube with lid and placed on an ice bath. The UWS samples were transferred to a cold room (4°C) where they were centrifuged in a 1.5 mL microcentrifuge tube at 10,000 g for 5 minutes. The supernatant was collected and stored at -80°C until further analysis. All UWS samples were collected by one trained and calibrated investigator (Kappa = 0.88) and analyzed within 48 hours of collection. Commercially available kits (SALIMETRICS LLC, Salivary interleukin 1 beta, Carlsbad, CA, USA) were used to measure
whole salivary IL-1β levels using enzyme-linked immunosorbent assay (ELISA). All UWS samples were assessed in duplicate wells according to manufacturers’ instructions and expressed in picograms per milliliter (pg/ml). Whole salivary IL-1β levels were measured at baseline and at 3-months’ follow-up.

Statistical Analysis and Sample-Size Estimation

Statistical analyses were done using a software program (SPSS Version 20, IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to assess normality of the data. The clinico-radiographic and immunological parameters were compared using the analysis of variance and Bonferroni post-hoc adjustment tests. Correlation of IL-1β with clinical AL and PD was assessed using logistic regression analysis. Probability-values (p-values), which were less than 0.01 were selected as statistically significant. Power and sample sizes were determined via data collected from a pilot investigation using a computer software (nQuery Advisor 6.0, Statistical Solutions, Saugas, MA, USA) with an alpha and effect size of 0.01 and 0.3, respectively. It was estimated that with the inclusion of at least 17 individuals in the test- and control-groups among cigarette-smokers and never-smokers, the study would have a power of 92% in order to detect a 1 mm difference in clinical AL and PD in the study groups.

Results

Characteristics of the Study Groups

34 self-reported current cigarette-smokers (18 in the test and 17 in the control-group) and 34 never-smokers (17 in the test and 17 in the control-group) were included. In all groups and sub-groups, there was no significant difference in the mean age of patients and majority of the participants were male. Cigarette-smokers had a mean smoking history of 5.7±0.7 pack-years. A family history of tobacco-smoking was more often reported by cigarette-smokers (85.3%) than non-smokers (45.7%). None of the participants reported to have ever used a dental floss and 26.5% and 28.6% cigarette-smokers and never-smokers, respectively reported to brush twice daily. None of the participants reported to have visited a dentist and/or dental hygienist within 2-years (Table I).

Periodontal Parameters

At baseline, there was no statistically significant difference in PI, PD, clinical AL and mesial and distal MBL among cigarette-smokers and non-smokers in their respective test- and control-groups. Never-smokers displayed significantly higher scores GI (p<0.01) compared with cigarette-smokers. Among cigarette-smokers, there was no significant difference in PI, PD, and clinical AL in the test- and control-groups at 3-months of follow-up. At 3-months of follow-up,
scores of PI, PD, and clinical AL were comparable with their respective baseline values among cigarette-smokers. In never-smokers, there was a statistically significant reduction in scores of PI \((p<0.01)\), GI \((p<0.01)\) and PD \((p<0.01)\) at 3-months of follow-up compared with baseline. Three-month follow-up scores of clinical AL were comparable with their baseline values among never-smokers. At 3-months of follow-up, there was no significant difference in PI, GI, PD, and clinical AL among never-smokers in the test- and control-groups (Table II).

**Unstimulated Whole Salivary Flow Rate and Whole Salivary IL-1β Levels**

At baseline, there was no statistically significant difference in UWSFR and whole salivary IL-1β levels in cigarette-smokers and never-smokers. At baseline, whole salivary IL-1β levels were comparable among cigarette-smokers and never-smokers. There was no significant difference in whole salivary IL-1β levels among cigarette-smokers in the test- and control-groups at 3-months' follow-up. Among never-smokers, there was a significant reduction in whole salivary IL-1β levels at 3-months' follow-up. There was no significant difference in whole salivary IL-1β levels in the test- and control-groups among cigarette-smokers and never-smokers at baseline and at 3-months' follow-up.

**Correlation Between Periodontal Parameters and Whole Salivary IL-1β Levels**

Among cigarette-smokers, there was no statistically significant correlation between PD and clinical AL at baseline and at 3-months of follow-up (Figure 1). Among never-smokers, there was no statistically significant correlation between PD and clinical AL at baseline. At 3-months of follow-up, PD was significantly correlated with whole-salivary IL-1β levels among never-smokers (Figure 2).

**Discussion**

Cigarette-smoking is a well-known risk factor of periodontitis\(^{25,39}\), and several studies\(^{39-41}\) have shown that PI, PD and clinical AL are higher

![Figure 1. Correlation between probing depth and clinical attachment loss and whole salivary IL-1beta levels among cigarette-smokers at baseline and 3-months' follow-up.](image-url)
Table II. Periodontal parameters (mean ± standard deviation) at baseline among cigarette-smokers and never-smokers.

<table>
<thead>
<tr>
<th>Periodontal parameters</th>
<th>Baseline</th>
<th>3-months' follow-up</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Cigarette-smokers</td>
<td>Never-smokers</td>
</tr>
<tr>
<td></td>
<td>All individuals (n=34)</td>
<td>S. Persica-group (n=18)</td>
</tr>
<tr>
<td>PI</td>
<td>2.3±0.02</td>
<td>2.2±0.04</td>
</tr>
<tr>
<td>GI</td>
<td>0.7±0.04†</td>
<td>0.9±0.05</td>
</tr>
<tr>
<td>PD (in mm)</td>
<td>5.3±0.2 mm</td>
<td>5.4±0.1 mm</td>
</tr>
<tr>
<td>Clinical AL (in mm)</td>
<td>0.4±0.005 mm</td>
<td>0.3±0.002 mm</td>
</tr>
<tr>
<td>MBL (mesial)</td>
<td>1.05±0.05 mm</td>
<td>1.2±0.03 mm</td>
</tr>
<tr>
<td>MBL (distal)</td>
<td>1.1±0.06 mm</td>
<td>1.1±0.02 mm</td>
</tr>
</tbody>
</table>

Mm: millimeters; NA: Not applicable; CHX: Chlorhexidine; S. Persica: Salvadora persica. *Compared with all never-smokers at 3-months of follow-up (p<0.01). †Compared with all never-smokers at baseline (p<0.01). ‡Compared with never-smokers in the S. Persica-group at baseline (p<0.01). §Compared with never-smokers in the CHX-group at baseline (p<0.01). ††Compared with all never-smokers in the S. Persica-group at 3-months of follow-up (p<0.01). †‡Compared with all never-smokers in the CHX-group at 3-months of follow-up (p<0.01). 

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CHX, herbal-mouthwash, smoking and periodontal inflammation

Figure 2. Correlation between probing depth and clinical attachment loss and whole salivary IL-1beta levels among never-smokers at baseline and 3-months’ follow-up.

among smokers than never-smokers. Moreover, from an immunoinflammatory point of view, there is sufficient scientific evidence in indexed literature\textsuperscript{42,43} to confirm that proinflammatory cytokines such as IL-1β are expressed in significantly higher concentrations in the UWS and gingival crevicular fluid of smokers than in never-smokers. One justification for this is that habitual smoking increases OS in periodontal tissues due to an increased interaction between AGEs and their receptors\textsuperscript{24}. This interaction in conjunction with the state of OS increases the production of destructive inflammatory cytokines that worsen periodontal inflammation and attract osteoclasts towards the inflamed tissues. Therefore, the authors of the present study anticipated that baseline scores of PI, PD, clinical AL and MBL would be worse in cigarette-smokers compared with never-smokers. Astoundingly, the results of the present investigation showed no statistically significant difference in the aforementioned parameters among cigarette-smokers and never-smokers at baseline (as shown in Tables II and III). There are a number of factors that may have influenced the results. Firstly, it is known that severity of periodontal increases with advancing age\textsuperscript{30}. According to Mullally\textsuperscript{44}, prevalence of cigarette-smoking is high among young individuals, which makes them more susceptible to periodontal destruction compared with never-smokers. However, based upon the self-reported smoking history, all cigarette-smokers were “light-smokers” as they had had a smoking history of approximately 6 pack-years. Here, it is worth mentioning that individuals with a smoking history of up to 20 pack-years are considered “light-smokers\textsuperscript{32}”. According to Zambon et al\textsuperscript{45}, the risk of MBL and clinical AL are 2x higher in heavy cigarette-smokers than light-smokers. With reference to never-smokers, the chief justification that can be posed for increased periodontal inflammation is poor routine oral hygiene maintenance (OHM). Our results suggest that poor routine OHM can present as periodontal inflammation that is comparable with that observed in light cigarette-smokers. A short cigarette-smoking history and younger age of participants may explain why none of the cigarette-smokers or never-smokers had periodontitis. It is hypothesized that periodontal inflammatory parameters are poorer in heavy cigarette-smokers (>40 pack-years history).
Table III. Unstimulated whole salivary flow rate and whole salivary IL-1β levels among cigarette-smokers and never-smokers in test- and control-groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>3-months’ follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cigarette-smokers</td>
<td>Never-smokers</td>
</tr>
<tr>
<td>All patients (n=34)</td>
<td>tower</td>
<td>tower</td>
</tr>
<tr>
<td>S. Persica-group (n=17)</td>
<td>tower</td>
<td>tower</td>
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<tr>
<td>CHX-group (n=17)</td>
<td>tower</td>
<td>tower</td>
</tr>
<tr>
<td>All patients (n=35)</td>
<td>tower</td>
<td>tower</td>
</tr>
<tr>
<td>S. Persica-group (n=17)</td>
<td>tower</td>
<td>tower</td>
</tr>
<tr>
<td>CHX-group (n=17)</td>
<td>tower</td>
<td>tower</td>
</tr>
<tr>
<td>Unstimulated whole salivary flow rate (ml/min)</td>
<td>0.13±0.003 ml/min</td>
<td>0.14±0.02 ml/min</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>199.5±17.5 pg/ml</td>
<td>207.2±19.8 pg/ml</td>
</tr>
</tbody>
</table>

CHX: Chlorhexidine; S. Persica: Salvadora persica. *Compared with all never-smokers at 3-months of follow-up (p<0.01). †Compared with all never-smokers in the S. Persica-group at 3-months of follow-up (p<0.01). ‡Compared with all never-smokers in the CHX-group at 3-months of follow-up (p<0.01).
compared with never-smokers with periodontal inflammation.

From a therapeutic perspective, the 3-month follow-up results present results showed a statistically significant reduction in clinical periodontal inflammatory parameters and whole salivary IL-1β levels among never smokers in the test- and control-groups compared with their respective baseline values (Tables II and III). These results suggest that prescription of 0.12% CHX or *S. persica* based mouthwash is reliable for periodontal health maintenance following NSPT in never-smokers. Our results further demonstrate that use of 0.12% CHX or a *S. persica* based mouthwash twice daily for 14 days helps reduce periodontal inflammation and maintain periodontal health for at least 90 days in never-smokers. The authors support the results of a randomized controlled trial, in which 0.12% was reported to be as effective as *S. persica*-based mouthwash in terms of reducing periodontal inflammation. Based upon the current results, it is also speculated that prescription of herbal-mouthwashes could be a potential replacement to 0.12% CHX particularly among patients with CHX-allergy. With regards to cigarette-smokers, the present results showed that periodontal parameters and whole salivary IL-1β levels assessed at 3-months of follow-up were comparable with their respective baseline values among patients in the test- and control-groups. It is well-known that habitual cigarette-smoking enhances plaque accumulation and compromises the integrity of HGF and the extracellular matrix. Moreover, it has been reported that habitual smoking delays healing and compromises the outcomes of oral therapeutic interventions. It is speculated that self-reported current cigarette-smokers included in the present study continued to smoke after NSPT. This may have compromised periodontal healing, and this seems to be independent of whether the patients were using 0.12% CHX or *S. persica*-based mouthwash. It is therefore mandatory to educate the population (particularly tobacco-smokers) about the deleterious effects of smoking on health and wound healing. Routine community-based oral health programs and anti-tobacco campaigns can play a role in this regard. A notable clinical observation was that baseline GI was significantly higher in never-smokers than cigarette-smokers. It is known that nicotine exerts a vasoconstrictive effect on gingival blood vessels, which masks the clinical sign of periodontal inflammation (bleeding gums). Therefore, cigarette-smokers may remain unaware of the ongoing periodontal inflammation compared with never-smokers. This finding is consistent with previous studies.

**Limitations**

One limitation of the present study is that immunosuppressed patients, such as individuals with DM, were excluded. Since hyperglycemia, which is often manifested in patients with DM is by itself a risk-factor of periodontal disease, it is hypothesized that diabetic smokers are more susceptible to periodontal inflammation and express raised whole salivary IL-1β levels compared with non-diabetic cigarette-smokers. Another limitation is that all cigarette-smokers were light-smokers. This was merely a coincidence as there were no stringent criteria to encompass patients based on smoking history. It is speculated that heavy smokers express raised whole salivary IL-1β levels and poorer clinicoradiographic parameters compared with light-smokers. Nevertheless, a shorter history of smoking is by no means a safe strategy from an oral and systemic health perspective.

**Conclusions**

Usage of 0.12% CHX or a *S. persica*-based mouthwash following NSPT is ineffective in controlling periodontal inflammation and reducing whole salivary IL-1β in young light cigarette-smokers compared with never-smokers.

**Conflicts of Interest**

The authors declare that they have no competing interests.

**Ethics Approval**

The present study was carried out in accordance with the Declaration of Helsinki, as revised in 2013, and the guidelines involving human subjects. Ethical approval was obtained from Ethics Research Committee of the Sharavathi Dental College and Hospital, Shivamogga, Karnataka, India.

**Informed Consent**

Volunteers were asked to sign an informed consent form. Withdraw did not bear any penalization or consequences.
Availability of Data and Materials
Data is available on reasonable request (Contact Dr. Amani Basudan).

Funding
The authors would like to thank the Deanship of Scientific Research and College of Dentistry Research Center (CDRC) at King Saud University, Riyadh, Saudi Arabia for their help and support.

Acknowledgements
The authors would like to thank the Deanship of Scientific Research at the College of Dentistry at King Saud University, Riyadh, Saudi Arabia, for supporting during the completion of this research project.

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