Peri-implant clinical and radiographic status and whole salivary cotinine levels among cigarette and waterpipe smokers and never-smokers

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Abstract: The aim was to compare the peri-implant clinical and radiographic inflammatory parameters and whole salivary cotinine levels among cigarette smokers (CS), waterpipe smokers (WS) and never-smokers (NS). Thirty-four CS (Group 1), 33 WS (Group 2), and 31 NS (Group 3) were included. Peri-implant plaque index (PI), bleeding-on-probing (BOP), and probing depth (PD) were measured, and crestal bone loss (CBL) was assessed on standardized digital radiographs. Unstimulated whole saliva samples were collected and whole salivary cotinine levels were measured. Peri-implant PI and PD were higher in Groups 1 ($P < 0.05$) and 2 ($P < 0.05$) than in Group 3. Peri-implant BOP was significantly higher in Group 3 than in Groups 1 ($P < 0.01$) and 2 ($P < 0.01$). Peri-implant MBL was significantly higher in Groups 1 ($P < 0.05$) and 2 ($P < 0.05$) than in Group 3. There were significant differences in PI, BOP, PD, and CBL between Groups 1 and 2. There was no significant difference in the whole salivary cotinine levels in Groups 1 and 2. Peri-implant sites with plaque accumulation, PD, CBL, and whole salivary cotinine levels were higher in CS and WS than in NS, but did not differ between CS and WS.

Keywords: bone loss; clinical; cotinine; peri-implantitis; smoking; waterpipe.

Introduction

Cigarette smoking is a traditional risk factor for peri-implant diseases (peri-implant mucositis and peri-implantitis) (1,2). Although cigarette smoking is not an absolute contraindication to dental implant therapy (3,4), studies (5,6) have shown that smoking retards bone healing around dental implants and significantly increases the risk of peri-implant crestal bone loss (CBL). Another form of tobacco smoking that is a cultural norm in many Middle Eastern countries (such as Lebanon, Iraq, Qatar, and Saudi Arabia) is waterpipe smoking. Waterpipes (also known as hookah, narghile, and sheesha) work on the principle of charcoal-heated air passing through perforated aluminium foil and across tobacco (usually flavored), producing smoke that bubbles through water before being inhaled. There is a general misconception that waterpipe smoking is less hazardous to health than conventional cigarette smoking as the water through which the waterpipe smoke bubbles filters most of the toxic chemicals present in smoke, such as carbon monoxide (CO), tar, and nicotine (7). However, it has been shown that individuals smoking waterpipes have oral and systemic health conditions similar to those of cigarette smokers, such as periodontal disease, asthma, respiratory infections, hypertension, and persistent coughing (7,8). To our knowledge, no previous studies have investigated peri-implant clinical and radiographic...
Cotinine, a major alkaloid in tobacco, is a metabolite of nicotine that can be measured in unstimulated whole saliva (UWS) and other biological fluids including blood, gingival crevicular fluid and urine (9). Cotinine has a longer durability of approximately 20 h in comparison with nicotine (nearly 2 h) and therefore remains in the body for a longer period. This property of cotinine allows it to be used as a biomarker of tobacco exposure (10,11). It has been reported that cotinine levels are higher in UWS of WS and CS than in never-smokers (NS) (12). This suggests that assessment of whole salivary cotinine levels would be a useful and non-invasive means of assessing exposure to waterpipe and cigarette smoke. Higher whole salivary cotinine levels have been independently and positively associated with the prevalence of periodontal disease (13); however, no previous studies have assessed salivary cotinine levels in CS and WS with peri-implant diseases.

We hypothesized that (a) peri-implant clinical and radiographic inflammatory parameters are worse in WS and CS than in NS; and (b) whole salivary cotinine levels are significantly higher in WS and CS than in NS. The aim of the present retrospective study was to compare the peri-implant clinical and radiographic inflammatory parameters and whole salivary cotinine levels in CS, WS, and NS.

Materials and Methods
The study was approved by the Research Review Board of the Jinah Medical Centre, Karachi, Pakistan (ID: JMC-2016-046). Volunteering individuals were requested to read and sign a consent form before being included. All participants reserved the right to retire from the study at any stage of the investigation. The present observational case-control study was conducted at the Department of Dentistry, Jinah Medical Center, Karachi, Pakistan, between November 2016 and March 2017. All participants were residents of Karachi.

Inclusion and exclusion criteria
The inclusion criteria were as follows: (a) CS, individuals who reported to have been smoking at least one cigarette daily for at least 12 months and had never consumed tobacco in any other form (14) (Group 1); (b) WS, individuals who reported to have been smoking exclusively waterpipes at least once daily for a minimum period of 12 months and had never consumed tobacco in any other form (8) (Group 2); and (c) NS, individuals who reported to have never used to tobacco in any form (15) (Group 3). The exclusion criteria were: (a) dual smokers (individuals smoking cigarettes and waterpipes); (b) patients with self-reported systemic conditions including diabetes mellitus, human immunodeficiency virus infection or acquired immunodeficiency syndrome, cardiovascular diseases, hepatic disorders, and renal disease; (c) edentulous patients; (d) patients with crowded teeth; (e) self-reported alcohol users and smokeless tobacco chewers; (f) participants who had used antibiotics, non-steroidal anti-inflammatory drugs, and/or steroids within the past 90 days or had undergone periodontal therapy (such as ultrasonic scaling) within this period; and (g) third molars.

Questionnaire
Data on age, gender, duration, and daily frequency of CS and WS, duration of each session (in minutes) of WS and CS, and family history of smoking were collected using a questionnaire, which was administered to all participants by a trained investigator.

Peri-implant clinical and radiographic parameters
Peri-implant plaque index (PI), bleeding-on-probing (BOP), and probing depth (PD) ≥ 4 mm were measured as described elsewhere (16,17). These parameters were measured at six sites per implant (mesiobuccal, mid-buccal, distobuccal, distopalatal, mid-palatal, and mesiopalatal) and presented as mean percentages per individual. In each group, the mean mesial and distal CBL was recorded in millimeters on digital peri-apical radiographs (Belmont ACURAY 071A Intra Oral X-Ray System, Hudson, FL, USA) using a software program (Scion Image, Scion Corp., Fredrick, MD, USA). The long cone paralleling technique was used to standardize the angulations of the radiographs (18). The total CBL was presented as the mean of the mesial and distal CBL. All clinical and radiographic assessments were performed by one experienced and calibrated investigator blinded to the study groups.

Collection of unstimulated whole saliva samples and assessment of whole salivary cotinine levels
All UWS samples were collected from fasted individuals during early morning hours by a trained and calibrated investigator. Each participant was seated on a chair in a quiet room and requested to allow saliva to accumulate in the mouth for 5 min, avoiding swallowing and lip movement. They were then instructed to expectorate the saliva into a funnel connected to a gauged measuring cylinder. The amount of saliva expectorated was recorded in milliliters (19-21). The UWS samples were then transferred to plastic tubes with a lid and stored at −70°C. For each
individual per group, the unstimulated whole salivary flow rate (UWSFR) was determined.

The frozen UWS samples from all groups were thawed and centrifuged at 3,000 rpm for 15 min in a centrifuge machine (OverStock Lab, Hampton, NH, USA). Salivary cotinine analysis was performed via enzyme linked immunosorbent assay (ELISA) using an immunoassay kit (Dako Cytomation A/S, Glostrup, Denmark) as described elsewhere (22). In summary, 96-well ELISA plates (Nunc, Roskilde, Denmark) were coated (0.1 mL/well) with a solution of rabbit polyclonal anti-goat IgG (10 μg/mL) (Dako Cytomation) in Tris buffer, pH 8.4, and incubated at 4°C for 12 h. The plates were then blocked with 0.2 mL of 10 mM phosphate buffer, pH 7.5, containing 0.1% BSA (phosphate-BSA buffer) and incubated for 60 min at room temperature and stored at 4°C. A standard inhibition curve was constructed using serial dilutions (1:2) of a solution consisting of cotinine (160 ng/mL) in phosphate-BSA buffer to obtain seven dilutions of known concentration. Each dilution was investigated in duplicate by addition of 50 μL of cotinine solution, 50 μL of (1/10,000) goat polyclonal anti-cotinine reagent (Affiniti Research Product Ltd., Exeter, UK) and 50 μL of cotinine conjugated with horseradish peroxidase (Aldrich Chemical Co., Milwaukee, WI, USA) and horseradish peroxidase (Sigma Co., St Louis, MO, USA). A substrate solution (100 μL) containing tetramethylbenzidine was then added, and plates were incubated in the dark at 25°C for 30 min. Color development was terminated by addition of 100 μL of 1 M phosphoric acid, and the optical density of each well was assessed at 450 nm using a microplate reader. The minimum detection limit for whole salivary cotinine levels was 1 ng/mL.

**Statistical analysis**

Statistical analysis was performed using a software program (SPSS Version 18, Chicago, IL, USA). To evaluate differences in periodontal status and salivary cotinine levels among individuals in Groups 1, 2, and 3, Kruskal-Wallis and Wilcoxon rank-sum tests were performed. The Bonferroni adjustment *post-hoc* test was performed for multiple comparisons. Sample size estimation was based on the supposition that a mean difference of 0.5 mm and 1 mm in CBL and PD, respectively, should be detected at a significance level of 0.05. It was estimated that inclusion of at least 30 individuals per group was necessary to attain a study power of 85%. *P* values of <0.05 were considered to be statistically significant.

**Results**

**General characteristics of the study groups**

In total, 98 male individuals (34 in Group 1, 33 in Group 2, and 31 in Group 3) participated. The mean age of individuals in Groups 1, 2, and 3 were 50.3 ± 3.5, 44.5 ± 2.8, and 42.6 ± 0.7 years, respectively. In Groups 1 and 2, the mean duration of cigarette and waterpipe smoking among individuals was 22.3 ± 3.7 and 14.5 ± 2.8 years, and the daily frequency of smoking was 17.4 ± 2.2 and 5.2 ± 0.4 times, respectively. The duration of each smoking session among individuals in Groups 1 and 2 was 8.5 ± 0.6 and 29.5 ± 2.1 min, respectively. A family history of smoking was more often reported by individuals in Groups 1 and 2 than by those in Group 3 (Table 1).

**Implant-related characteristics**

A total of 113 platform-switched implants with moderately rough surfaces were placed in the study groups. In Groups 1, 2, and 3, 41 (maxilla: 12 implants; and mandible: 29 implants), 37 (maxilla: 14 implants; and mandible: 23 implants), and 35 implants (maxilla: 6 implants; and mandible: 29 implants), respectively, were placed. All implants were placed at bone level in the regions of missing premolars and molars using an insertion torque of 30 to 35 Ncm. The diameters and lengths of the implants used ranged between 4.1 to 4.8 mm and 10 to 16 mm, respectively.

**Peri-implant clinical and radiographic parameters**

Peri-implant PI and PD were significantly higher among individuals in Groups 1 (*P* < 0.05) and 2 (*P* < 0.05)
compared with Group 3. Peri-implant BOP was significantly higher in Group 3 than in Groups 1 ($P < 0.01$) and 2 ($P < 0.01$). Peri-implant total MBL was significantly higher in Groups 1 ($P < 0.05$) and 2 ($P < 0.05$) than in Group 3. There was no statistically significant difference in PI, BOP, PD, and CBL among the participants in Groups 1 and 2 (Table 2).

**Salivary flow rate and whole salivary cotinine levels**

There was no statistically significant difference in the UWSFR among individuals in Groups 1, 2, and 3 ($0.6 \pm 0.1$ mL/min, $0.5 \pm 0.05$ mL/min, and $0.5 \pm 0.04$ mL/min, respectively). Whole salivary cotinine levels among individuals in Groups 1 and 2 were $181.2 \pm 6.9$ μg/mL and $169.4 \pm 4.2$ μg/mL, with no statistically significant difference between them. All individuals in Group 3 had non-detectable levels of cotinine in UWS.

**Discussion**

It has been shown that waterpipe and cigarette smoking cause levels of periodontal destruction (in terms of increased clinical attachment loss, PD, and CBL) comparable to those in NS (8); however, to our knowledge, this is the first study to have compared peri-implant clinical and radiographic inflammatory parameters and whole salivary cotinine levels among CS, WS, and NS.

The present study was based on two hypotheses. The first hypothesis was that peri-implant clinical and radiographic inflammatory parameters would be worse among individuals in Groups 1 and 2 than among those in Group 3. Previously reported outcomes are in accordance with this hypothesis. One explanation is that smoke from waterpipes and cigarettes exposes consumers to the same toxic chemicals, for example CO, nicotine and tar (23-25). It has been suggested that nicotine upregulates the secretion of proinflammatory cytokines (such as interleukin-1 beta and tumor necrosis factor-alpha), which play a role in increasing alveolar bone loss around teeth (26). Raised levels of these proinflammatory cytokines have also been identified in the peri-implant sulcular fluid of patients with peri-implantitis (27). Moreover, the results of a recent systematic review and meta-analysis indicated that nicotine from tobacco smoke impairs new bone formation around dental implants and bone-to-implant contact (28). Furthermore, nicotine has also been reported to reduce the cellular healing response and increase the accumulation of oral biofilm (a potential risk factor that may led to peri-implantitis) in smokers (29). The present results support the study by Barao et al. (29), since the number of peri-implant sites with plaque accumulation was significantly higher among individuals in Groups 1 and 2 than in Group 3. These are possible explanations for the similarity in PI, PD, and CBL among individuals in Groups 1 and 2. Among participants in Group 3, the most likely reason for the occurrence of peri-implantitis appeared to be poor oral hygiene maintenance, as dental plaque harbors pathogenic microbes (such as Porphyromonas gingivalis and Treponema denticola) that are associated with oral soft tissues inflammation and alveolar bone loss around teeth and implants (30,31).

The present results are in accordance with the second hypothesis, according to which whole salivary cotinine levels would be significantly higher among individuals in Groups 1 and 2 than in Group 3. It has been shown that raised levels of salivary cotinine are associated with an increased prevalence of periodontal disease in CS (13). The same explanation may be account for the increased degree of soft tissue inflammation and CBL around implants in Groups 1 and 2. An interesting finding in the present study was that although the daily frequency of smoking was nearly 4 times higher in CS than in WS, levels of cotinine in UWS were comparable among individuals in Groups 1 and 2. However, it is noteworthy that with reference to the daily frequency of smoking and the duration of each smoking session, CS and WS would have been exposed to tobacco smoke for comparable periods, i.e., approximately 147 min and 130 min,
respectively. This may explain the similarity of whole salivary cotinine levels among individuals in Groups 1 and 2. Moreover, this outcome supports a previous study in which direct laboratory-based comparison of toxicants in smoke from waterpipes and cigarettes demonstrated similar levels of cotinine in CS and WS (12). This refutes the general perception that waterpipe smoking is less harmful than smoking cigarettes as water in the former filters out chemicals including nicotine. These results suggest that assessment of UWS is a useful and non-invasive method for assessing exposure to waterpipe and cigarette smoke.

A limitation of the present study was that patients with systemic diseases were excluded. It is well known that immunocompromised individuals such as those with poorly controlled diabetes mellitus and prediabetes are more susceptible to oral soft tissue inflammation and bone loss around teeth and implants compared with systemically healthy individuals (14,32,33). It is therefore likely that CS and WS with poorly controlled DM are more susceptible to peri-implant soft tissue inflammation and CBL than systemically healthy CS and WS. Moreover, dual-smokers were not assessed in the present study. It is hypothesized that peri-implant clinical and radiographic status and whole salivary UWS are poorer in dual-smokers than in individuals who smoke exclusively cigarettes or waterpipes. Further studies are needed to test this hypothesis.

In conclusion, peri-implant sites with plaque accumulation, PD, CBL, and whole salivary cotinine levels are more susceptible to peri-implant soft tissue inflammation and CBL than systemically healthy CS and WS. Moreover, dual-smokers were not assessed in the present study. It is hypothesized that peri-implant clinical and radiographic status and whole salivary UWS are poorer in dual-smokers than in individuals who smoke exclusively cigarettes or waterpipes. Further studies are needed to test this hypothesis.

In conclusion, peri-implant sites with plaque accumulation, PD, CBL, and whole salivary cotinine levels are higher in CS and WS than in NS. There is no difference in these parameters among CS and WS.

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Conflict of interest
The authors have no conflict of interest to declare.

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