Assessment of interleukin-1β, interleukin-6, and tumor necrosis factor-A levels in the peri-implant sulcular fluid among waterpipe (narghile) smokers and never-smokers with peri-implantitis

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Abstract

Background: It is hypothesized that levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α are significantly higher in the peri-implant sulcular fluid (PISF) of waterpipe-smokers (WS) compared with never-smokers with peri-implantitis.

Purpose: The aim of the present convenience sample case-control study was to compare the levels of IL-6, IL-1β, and TNF-α in the PISF of WS and never-smokers with peri-implantitis.

Materials and methods: Demographic data was collected using a questionnaire. Peri-implant probing depth (PPD) was measured and crestal bone loss (CBL) was measured on digital bitewing radiographs. PISF samples were collected using paper strips and the collected PISF volume was determined. Levels of IL-6, IL-1β, and TNF-α were measured using enzyme linked immunosorbent assay. Study sample-size was estimated and statistical analysis was performed. P values less than .05 were considered statistically significant.

Results: Sixty-six individuals (33 individuals in group-1 and 33 in group-2) were included. In groups 1 and 2, 41 and 44 implants, respectively were placed. The mean total PPD (P < .001) and peri-implant CBL (P < .001) was statistically significantly higher around implants affected by peri-implantitis in group-1 compared with group-2. The PISF volume (P < .05) collected and levels of IL-1β (P < .01), IL-6 (P < .01), and TNF-α (P < .01) were statistically significantly higher among individuals in group-1 compared with group-2.

Conclusion: WS with peri-implantitis present increased expression of local proinflammatory cytokines in the PISF than never-smokers.

KEYWORDS
alveolar bone loss, cytokine, inflammation, peri-implant diseases, smoking

1. INTRODUCTION

Habitual tobacco smoking is a classical risk factor of soft tissue inflammation and alveolar bone loss around natural teeth and dental implants. It has also been reported that tooth loss (as a consequence of periodontitis) and implant failure (an aftermath of peri-implantitis) is more often manifested in cigarette-smokers than never-smokers. Another form of smoking that originated from Middle-Eastern countries (such as Saudi Arabia, Qatar, and Lebanon) and has gained acceptance in many Western countries (including the United Kingdom and the United States) is waterpipe (synonyms, narghile, hookah, and shisha) smoking. A general delusion among waterpipe-smokers (WS) is that this form of tobacco smoking is less hazardous to health than conventional cigarette-smoking as the smoke is filtered through water,
which absorbs toxic chemicals before the smoke is inhaled. However, clinical evidence has shown that oral inflammatory conditions (such as periodontitis, peri-implantitis, and oral cancer); systemic diseases (such as bronchitis, lung cancer, and cardiovascular diseases) are more often manifested in WS than never-smokers.

Traditionally, clinical and radiographic examinations are performed for the assessment of peri-implant soft tissue status and crestal bone loss (CBL), respectively. However, laboratory-based investigations, based on the assessment of proinflammatory cytokines (such as interleukin [IL]-1β, IL-6, and tumor necrosis factor (TNF-α)) in the oral fluids (saliva, gingival crevicular fluid [GCF], and peri-implant sulcular fluid [PISF]) may also yield pertinent information regarding the progression of oral inflammatory conditions including periodontitis and peri-implantitis. Fonseca and colleagues compared the levels of IL-1β in the PISF of patients with peri-implant mucositis and peri-implantitis. The results showed that levels of IL-1β were statistically significantly higher among patients with peri-implantitis compared with peri-implant mucositis. The authors suggested that raised levels of IL-1β in PISF is a characteristic trait of patients with peri-implantitis. Similarly, in the study by Ata-Ali and colleagues, PISF levels of IL-1β, IL-6, and TNF-α were statistically significantly higher among cigarette-smokers than never-smokers. To our knowledge, there are no studies in indexed literature that have assessed the levels of proinflammatory cytokines in the PISF of WS and never-smokers with peri-implantitis. It is hypothesized that levels of IL-6, IL-1β, and TNF-α are significantly higher in the PISF of WS compared with never-smokers with peri-implantitis.

The aim of the present convenience sample case-control study was to compare the levels of IL-6, IL-1β, and TNF-α in the PISF of WS and never-smokers with peri-implantitis.

2 MATERIALS AND METHODS

2.1 Ethical guidelines

The study protocol was reviewed and approved by the research ethics committee of the College Dentistry, King Saud University, Riyadh, Saudi Arabia. The individuals were informed of the study design and the potential risks and benefits associated with the study. Consenting individuals were requested to read and sign an informed consent. All participants were informed that they reserved the right to withdraw from the present research study at any stage of the investigation without consequences.

2.2 Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) WS; (2) never-smokers (individuals who reported to have never consumed any form of tobacco product); (3) individuals having undergone dental implant therapy; and (4) individuals diagnosed with peri-implantitis around at least 1 dental implant. The exclusion criteria were as follows: (1) cigarette smokers (2) dual smokers (individuals smoking cigarettes and waterpipe), (3) patients with self-reported systemic conditions such as diabetes mellitus, human immunodeficiency virus infection, or acquired immunodeficiency syndrome, cardiovascular diseases, hepatic disorders, and renal disease; (4) patients without peri-implantitis; (5) self-reported alcohol users and smokeless tobacco chewers; (6) participants who had used antibiotics, nonsteroidal anti-inflammatory drugs and/or steroids within the past 90 days or had undergone surgical and/or nonsurgical periodontal therapy within this time duration.

2.3 Study participants

The study population comprised of WS (individuals smoking waterpipe 1 daily since at least at least 12 month, group-1); and never-smokers (individuals who reported to have never used any form of tobacco product, group-2).

2.4 Questionnaire

Information regarding age, gender, duration of waterpipe smoking habit, daily frequency of waterpipe smoking, duration of implants in function, and daily toothbrushing and flossing was collected using a questionnaire.

2.5 Assessment of peri-implantitis

Peri-implant probing depth (PPD) was measured as described elsewhere, and digital bitewing radiographs (Ektaspeed plus; Kodak, Rochester, New York) were taken and viewed on a calibrated computer screen (Samsung SynchronMaster digital TV monitor, Suwon City, Gyeonggi-do, Korea). CBL was measured as the linear distance from 2 mm below the implant-abutment interface to the most crestal part of the alveolar bone. Peri-implantitis was defined as the presence of at least 1 peri-implant site with a PPD of ≥ 4 mm; and radiographic mesial or distal CBL of ≥ 3 mm around the implant. Total CBL was defined as the average of the CBL on the mesial and distal surfaces of the implant.

2.6 Collection of peri-implant sulcular fluid

After carefully removing the supragingival oral biofilm, the peri-implant sites were isolated with sterile cotton rolls and dried gently with an air syringe. Two PISF samples were collected by inserting standard paper strips (Periopaper, Interstate Drug Exchange, Amityville, New York) 1–2 mm into the peri-implant sulcus or pocket for 30 seconds. PISF samples contaminated with blood were discarded, and fresh samples from the same site were collected after an interval of 10 minutes. In total, 4 and 9 blood contaminated samples from groups 1 and 2, respectively were discarded. The collected PISF volume was measured using a calibrated electronic gingival fluid measuring device (Periotron 8000, Oraflow, Inc, New York). The 2 samples from the same side were pooled and eluted in 1 ml phosphate buffered saline for 60 minutes prior to freezing at −80°C.
2.7 Measurement of interleukin-6, interleukin-1β, and tumor necrosis factor-alpha in peri-implant sulcular fluid

All laboratory-based investigations were performed by a trained and calibrated technician, who was blinded to the study groups. The overall kappa score if the intra-examiner reliability was 0.88. The PISF samples were centrifuged at 5000g for 15 minutes at 4°C. Aliquots of each PISF sample were assayed by enzymatic immunosorbent assay (ELISA) to determine the levels of IL-6, IL-1β, and TNF-α, according to the manufacturer’s recommendations (Quantikine, R&D Systems, Minneapolis, Minnesota). In summary, 100 μl of detection antibody was added to all wells, except blank, mixed gently, and incubated overnight (16–24 hours) at room temperature. Plates were washed 3 times and standards and PISF were added in the respective wells in duplicate. After the incubation time, the plates were washed again and incubated with 100 ml of conjugate for 60 minutes at room temperature. Plates were washed 3 times again and 100 ml of substrate was added and incubated for 15 minutes at room temperature in the dark. The reaction was stopped by the addition of 50 ml stop solution, and color was measured in an automated microplate spectrophotometer (Microplate Reader/Model 3550, Bio Rad, Hercules, California). The total amounts of cytokines were determined as pictograms per milliliters (pg/ml). Results were calculated using the standard curves created in each assay.

2.8 Statistical analysis

Statistical analyses were carried out using a the SPSS software (Version 20.0 for Windows, IBM, Chicago, Illinois). Data were expressed as means and standard deviations. Comparison of cytokine levels between groups was performed using Mann-Whitney U test. Stepwise logistic regression analysis was employed to identify explanatory variables for cytokine levels, controlling for jaw location. Power analysis was performed with a computer software (nQuery Advisor 5.0, Statistical Solutions, Saugus, Massachusetts). It was estimated that with the inclusion of at least 33 individuals per group, the study will achieve 85% power with a .05 two-sided significance level. P values less than .05 were considered statistically significant.

3 | RESULTS

3.1 | Demographic characteristics

In total, 66 individuals (33 individuals in group-1 and 33 in group-2) were included. All participants were male with mean ages of 48.6 ± 6.9 and 51.2 ± 2.2 years in groups 1 and 2, respectively. The mean duration and daily frequency of waterpipe smoking among individuals in group-1 was 16.2 ± 5.1 years and 5.3 ± 0.2 times daily, respectively. The average duration of each waterpipe smoking session was 35.6 ± 7.5 minutes (Table 1). Twenty-six (78.8%) individuals in group-1 and 24 (72.7%) in group-2 reported to brush their teeth once daily. None of the individuals in either group reported to have ever used a dental floss.

| TABLE 1 | General characteristics of individuals in groups 1 and 2 |
|---------|-------------|-------------|
|          | Group-1     | Group-2     |
| Number of individuals | 33          | 33          |
| Gender (male) | 33          | 33          |
| Mean age ± SD (in years) | 48.6 ± 6.9 | 51.2 ± 2.2 |
| Mean duration of waterpipe smoking ± SD (in y) | 16.2 ± 5.1 | NA          |
| Mean daily frequency of waterpipe smoking ± SD (no. of times/d) | 5.3 ± 0.2 | NA          |
| Mean duration of each waterpipe smoking session (in min) | 35.6 ± 7.5 | NA          |

Abbreviation: NA, not applicable.

3.2 | Implant related characteristics

All implants were platform-switched with moderately rough surfaces and were placed in the regions of missing molars. In both groups, the implants were placed at bone level using insertion torques ranging between 30 and 35 NCM. The diameters and lengths of implants placed in both groups ranged between 3.8-4.1 mm and 11-14 mm, respectively. Prosthetic rehabilitation of all implants was done within 4 months of placement using screw-retained restorations. In groups 1 and 2, 41 and 44 implants, respectively were placed. In group-1, 24 implants were placed in maxilla and 17 in the mandible. In group-2, 29 implants were placed in the maxilla and 15 in the mandible (Table 2). In groups 1 and 2, all implants were in function since 6.5 ± 0.8 and 7.1 ± 0.3 years, respectively.

3.3 | Peri-implant clinical and radiographic parameters

The mean total PPD (P < .001) and peri-implant CBL (P < .001) was statistically significantly higher around implants affected by peri-implantitis in group-1 compared with group-2. There was no statistically significant difference in total PPD and peri-implant CBL in implants placed in the maxilla and the mandible among individuals in groups 1 and 2 (Table 2).

3.4 | Levels of IL-1β, IL-6, and TNF-α in PISF

The PISF volume (P < .05) collected and levels of IL-1β (P < .01), IL-6 (P < .01), and TNF-α (P < .01) were statistically significantly higher among individuals in group-1 compared with group-2 (Table 3). There was no statistically significant difference in the PISF levels of IL-1β, IL-6, and TNF-α with reference to jaw location among individuals in groups 1 and 2 (Figure 1A,B).

4 | DISCUSSION

In this study, strict eligibility criteria were imposed. For instance, cigarette-smokers, dual smokers, and patients with self-reported systemic diseases (such as diabetes mellitus) and patients having undergone any form of periodontal therapy and antibiotic/steroid therapies
within the past 3 months were excluded. Moreover, it is already known that levels of proinflammatory cytokines in the PISF are markedly lower in patients without peri-implantitis as compared to individuals with peri-implantitis. In this context, in the current study, only individuals (WS and never-smokers) with peri-implantitis were included. This was primarily done in an attempt to control the potential risk-factors that could bias the reported results.

In this study, it is hypothesized that levels of IL-6, IL-1β, and TNF-α are significantly higher in the PISF of WS (group-1) compared with never-smokers (group-2) with peri-implantitis. The present result support this hypothesis as levels of destructive inflammatory cytokines (IL-6, IL-1β, and TNF-α) were statistically significantly higher among individuals in group-1 compared with group-2. One explanation in this regard is that habitual smoking increases the formation and accumulation of advanced glycation endproducts (AGEs) in oral tissues including gingival tissues and periodontal fibroblasts. Increase interactions between AGEs and their receptors (receptors for AGEs [RAGE]) have been associated with oral soft tissue inflammation and alveolar bone loss around teeth.25–28 It has also been shown that smoking increases the production of reactive oxygen species that impairs the chemotactic and phagocytic function of polymorphonuclear leukocytes,29,30 and produce proinflammatory cytokines (such as interleukin IL-1β, IL-6, and TNF-α) in the serum and GCF.21–32 It has also been reported that AGEs mediate inflammation of human periodontal tissues via the endoplasmic reticulum stress-induced nuclear factor-kappa-B pathway.28 Furthermore, nicotine (a metabolite of nicotine), up regulates the expression of RAGE in the gingival tissues of smokers and stimulate the formation reactive oxygen species, which jeopardize the oral soft tissues and increases bone loss around teeth.26 These mechanisms have been associated with oral soft tissue inflammation and alveolar bone loss around teeth in cigarette-smokers. It is speculated that the same mechanisms are associated with raised levels of IL-1β, IL-6, and TNF-α in the PISF of individuals with group-1 compared with group-2. However, to our knowledge there are no studies in indexed literature that have assessed markers of oxidative stress and AGEs-RAGE interactions in the periodontal and peri-implant tissues among WS. Hence, further studies are warranted in this regard.

Individuals in group-2, despite being self-reported never-smokers, demonstrated a mean CBL of approximately 4.5 mm around dental implants. The most logical explanation for this is poor oral hygiene. Studies have shown that poor oral hygiene is a classical risk factor of periodontitis as well as peri-implantitis.34,35 As well as peri-implantitis.36,37 In the present study nearly 72% individuals in group-2 reported to brush their teeth once daily and none of the individuals in either group reported to have ever used a dental floss. Studies have reported that a poor plaque control is associated with an increased frequency of pathogenic microbes (such as Prevotella intermedia, Treponema denticola, and Aggregatibacter actinomycetemcomitans) that have been associated with the etiology of periodontitis as well as peri-implantitis. It is also notable that although the number of individuals in group-1 (approximately 78%) that reported to brush their teeth once daily was similar to those in group-2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group-1</th>
<th>Group-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of PISF collected (in µl)</td>
<td>3.2 ± 0.6a</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Level of interleukin 1-beta (in pg/ml)</td>
<td>264.3 ± 16.5b</td>
<td>160.6 ± 9.6</td>
</tr>
<tr>
<td>Level of interleukin-6 (in pg/ml)</td>
<td>3047.5 ± 102.5b</td>
<td>1423.2 ± 87.2</td>
</tr>
<tr>
<td>Level of tumor necrosis factor-alpha (in pg/µl)</td>
<td>103.6 ± 25.2b</td>
<td>66.3 ± 10.8</td>
</tr>
</tbody>
</table>

Abbreviation: PISF, peri-implant sulcular fluid; µl, microliters; pg/ml, picograms per milliliter.

aCompared with group-2 (P < .05).
bCompared with group-2 (P < .01).
(approximately 72%), peri-implant PPD and CBL were significantly higher among individuals in group-1 than group-2. It has been suggested that smoking influences the peri-implant microbiomes by supporting a pathogen-rich community including those belonging to the genera *Prevotella*, *Treponema*, *Lactobacillus*, *Propionibacterium*, and *Pseudomonas*. It is hypothesized that colony forming units of pathogenic microbes are significantly higher in the peri-implant oral biofilm among individuals in group-1 than group-2, that was associated with a significantly higher PPD and CBL among individuals in group-1. Further microbiological studies are needed to test this hypothesis. It is therefore imperative for clinicians and health care providers to be aware of the deleterious effects of tobacco habits (including waterpipe smoking) and simultaneously educate the community about the importance of regular oral hygiene maintenance, dental visits toward a better oral health and quality of life.

One limitation of this study is that individuals in groups 1 and 2 were approximately 50 years old. It is known that advancing age is a significant risk factor for oral soft tissue inflammation and CBL. It is therefore hypothesized that peri-implant PPD is worse and levels of proinflammatory cytokines in the PISF are significantly higher among elderly (>70 years old) WS and never-smokers compared with relatively younger individuals (≤50 years old). Moreover, since chronic hyperglycemia is a risk factor for peri-implant diseases, it is speculated that peri-implant PPD is worse and levels of proinflammatory cytokines in the PISF are significantly higher among WS with poorly controlled diabetes mellitus compared with systemically healthy WS. Additional research is needed to test these hypotheses.

5 | CONCLUSION

WS with peri-implantitis present increased expression of local proinflammatory cytokines in the PISF than never-smokers.

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CONFLICT OF INTEREST

None declared.

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