Assessment of matrix metalloproteinase-8 and -9 levels in the peri-implant sulcular fluid among waterpipe (narghile) smokers and never-smokers with peri-implantitis

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Assessment of matrix metalloproteinase-8 and -9 levels in the peri-implant sulcular fluid among waterpipe (narghile) smokers and never-smokers with peri-implantitis

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ABSTRACT

Objectives: It is hypothesized that levels of matrix metalloproteinase (MMP)-8 and MMP-9 are significantly higher in the peri-implant sulcular fluid (PISF) of waterpipe-smokers (WS) compared with never-smokers with peri-implantitis. The aim of the present convenience sample case-control study was to compare the levels of MMP-8 and MMP-9 in the PISF of WS and never-smokers with peri-implantitis.

Material and Methods: Individuals smoking waterpipe (Group 1) and never-smokers (Group 2) were included. 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 MMP-8 and MMP-9 were measured using enzyme-linked immunosorbent assay. Study sample-size was estimated and statistical analysis was performed. P values < .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 MMP-8 (p < .01) and MMP-9 (p < .01) were statistically significantly higher among individuals in Group 1 compared with Group 2.

Conclusion: PISF levels of MMP-8 and MMP-9 are significantly higher among WS compared with never-smokers with peri-implantitis.

Introduction

Habitual tobacco smoking is a classical risk factor of soft tissue inflammation and alveolar bone loss around natural teeth and dental implants (de Araujo Nobre and Malo, 2017). It has also been reported that tooth loss (as a consequence of periodontitis) and implant failure (after an aftermath of peri-implantitis) is more often manifested in cigarette-smokers than never-smokers (Chrcanovic et al., 2015; de Araujo Nobre & Malo, 2017; Veitz-Keenan, 2016). 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 UK and USA) is waterpipe (synonyms, narghile, hookah and shisha) smoking (Akl et al., 2011; Jawad et al., 2013; Salloum et al., 2015). A general misconception 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 (ALHarthi et al., 2017; Jukema et al., 2014). However, clinical evidence has shown that oral inflammatory conditions (such as periodontitis, peri-implantitis and oral cancer) (ALHarthi et al., 2017; Bibars et al., 2015; Warnakulasuriya, 2011) and systemic diseases (such as bronchitis, lung cancer and cardiovascular diseases) are more often manifested in WS than never-smokers (Warnakulasuriya 2011; Waziry et al., 2017).

Dental implants are used to replace missing teeth. These are placed inside the jaw bones to support artificial tooth (dental crown). Peri-implantitis is defined as inflammatory process with loss of supporting bone in the tissues surrounding functioning dental implants. Traditionally, clinical and radiographic examinations are performed for the assessment of peri-implant soft tissue status and crestal bone levels, respectively (ALHarthi et al., 2017). However, laboratory-based investigations, based on the assessment of modulators of proinflammatory activity [such as matrix metalloproteinase (MMP)-8 and MMP-9] 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 (Belstrom et al., 2017; Javed et al., 2011; Kivelä-Rajamäki et al., 2003; Romano et al., 2018). PISF is an inflammatory exudate that occurs in minute amounts in the peri-implant sulcus and is believed by researchers that biomarkers and enzymes in this fluid helps to differentiate peri-implant disease condition from health (Akram et al. 2018). Arakawa et al. (2012) compared the levels of MMP-8 in the PISF of patients with peri-implantitis and healthy patients. The results showed that levels of MMP-8 were statistically significantly higher among patients with peri-implantitis compared with healthy patients (Arakawa et al., 2012). The authors suggested that raised levels of MMP-8 may indicate as a possible marker for progressive bone loss in peri-implantitis (Arakawa et al., 2012). Similarly, in the study by Maraccini et al. (2009), levels of MMP-8 and MMP-9 were statistically significantly higher among patients with chronic periodontitis than healthy patients. To our knowledge, there are no studies in indexed literature that have assessed the levels of MMP-8 and MMP-9 in the PISF of WS and never-smokers with peri-implantitis. It is hypothesized that levels of MMP-8 and MMP-9 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 MMP-8 and MMP-9 in the PISF of WS and never-smokers with peri-implantitis.

Materials and methods

**Ethical guidelines**

The study protocol was reviewed and approved by the research ethics review committee of the College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia. Potential risks and benefits related to the present study were discussed with volunteering individuals. A consent form written in simple English and Arabic was presented consenting individuals.

**Eligibility criteria**

Information regarding age, gender, duration of waterpipe smoking habit, daily frequency of waterpipe smoking, duration of implants in function and daily tooth brushing and flossing was collected using a questionnaire. The inclusion criteria were as follows: (i) WS; (ii) never-smokers (individuals who reported to have never consumed any form of tobacco product); (iii) individuals having undergone dental implant therapy; and (iv) individuals diagnosed with peri-implantitis around at least one dental implant. The exclusion criteria were as follows: (i) cigarette smokers, (ii) dual smokers (individuals smoking cigarettes and waterpipe), (iii) 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; (iv) patients without peri-implantitis; (v) self-reported alcohol users and smokeless tobacco chewers; (vi) participants who had used antibiotics, non-steroidal anti-inflammatory drugs and/or steroids within the past 90 days or (vii) participants who had undergone surgical and/or non-surgical periodontal therapy within this time duration.

**Study participants**

The study population comprised of WS (individuals smoking waterpipe one 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) (Daood et al., 2018; Javed et al., 2007).

**Assessment of peri-implantitis**

Peri-implant probing depth (PPD) is a space present between dental implant and gingiva. PPD was measured as described elsewhere (Al Amri et al., 2017a). It is determined by measuring the distance from the crest of gingival margin to the base of the sulcus using a manual probe (University of North Carolina-15 mm graded probe); and digital bitewing radiographs helps to assess extensive bone loss. The films are situated with their longer dimension in the vertical axis so as to better visualize bone levels in relation to the teeth or implants.

The amount of bone loss around dental implants is the crestal bone loss (CBL). This was measured as the linear distance from 2 mm below the implant–abutment interface to the most crestal part of the alveolar bone (Al Amri et al., 2017b). Peri-implantitis was defined as the presence of at least one peri-implant site with a PPD of at least 4 mm; and radiographic CBL of ≥3 mm on the mesial and distal surfaces of the implant was measured (Lang and Berglundh 2011; Zani et al., 2016; Zitzmann and Berglundh 2008). Total CBL was defined as the average of the CBL on the mesial and distal surfaces of the implant (Figure 1).

**Collection of PISF**

All PISF samples were collected between 7:00 am and 8:00 am. All participants were requested beforehand to be in a fasting state during their visit for PISF collection. 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 using standard paper strips (Periopaper®, Interstate Drug Exchange, Amityville, NY) which are special absorbent paper used to collect PISF. These strips are inserted 1–2 mm into the peri-implant sulcus or pocket for 30 s. PISF samples contaminated with blood were discarded, and fresh samples from the same site were collected after an interval of 10 min. In total, four and nine 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., Plainview, NY). The two samples from the same side were pooled and eluted in 1 ml phosphate-buffered saline for 60 min until the desired concentration of PISF is obtained. By making this dilution the sample volume can be saved. These samples are kept in freezer at −80°C until further analysis.

**Measurement of MMP-8 and MMP-9 in peri-implant crevicular fluid**

All laboratory-based investigations were performed by a trained and calibrated technician, who was blinded to the study groups. The PISF samples were centrifuged at 5000g for 15 min at 4°C. Aliquots of each PISF sample were assayed by enzyme-linked immunosorbent assay (ELISA) to determine the levels of MMP-8, and MMP-9, according to the manufacturer’s recommendations (Quantikine, R&D Systems, Minneapolis, MN). In summary, 100 μl of detection antibody was added to all wells, except blank, mixed gently and incubated overnight (16–24 h) at room temperature. Plates were washed three 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 μl of conjugate for 60 min at room temperature. Plates were washed three times again and 100 μl of substrate was added and incubated for 15 min at room temperature in the dark. A stop solution (50 μl) was used to halt the reaction and color intensity was gauged in an automated microplate spectrophotometer (Microplate Reader 3550; Bio-Rad, Hercules, CA). The total amounts of cytokines were determined as ng/ml. Results were calculated using the standard curves created in each assay.

**Statistical analysis**

A computer software program (SPSS 20 for Windows; IBM, Chicago, IL) was used to perform statistical analysis of the data. The results were presented as mean and standard deviation (SD). Comparison of cytokine levels between groups was performed using Mann–Whitney U-test. Power analysis was performed with a computer software (nQuery Advisor 5.0, Statistical Solutions, Saugus, MA). It was estimated that with the inclusion of at least 33 individuals per group, the study will achieve 85% power with a 0.05 two-sided significance level. Statistical significance was established at $p < .05$.

**Results**

**Demographic characteristics**

A total of 66 individuals (33 individuals in Group 1 and 33 in Group 2) were included in the present study. All participants were male. The mean age of Group 1 and Group 2 patients was 48.6 ± 6.9 and 51.2 ± 2.2 years, 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 waterpipe smoking session was 35.6 ± 7.5 min (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.

**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 0.30 and 0.35 N·m. 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

<table>
<thead>
<tr>
<th>Table 1. General characteristics of individuals in Groups 1 and 2.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
</tr>
<tr>
<td>Number of individuals</td>
</tr>
<tr>
<td>Gender (male)</td>
</tr>
<tr>
<td>Mean age ± SD (years)</td>
</tr>
<tr>
<td>Mean duration of waterpipe smoking ± SD (years)</td>
</tr>
<tr>
<td>Mean daily frequency of waterpipe smoking ± SD (no. of times/day)</td>
</tr>
<tr>
<td>Mean duration of each waterpipe smoking session (min)</td>
</tr>
</tbody>
</table>

NA: not applicable.
Table 2. Implant-related characteristics and PPD and CBL in Groups 1 and 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of individuals</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Number of implants placed</td>
<td>41</td>
<td>44</td>
</tr>
<tr>
<td>Number of implants placed in the maxilla</td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td>Number of implants placed in the mandible</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Number of maxillary implants with peri-implantitis</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Number of mandibular implants with peri-implantitis</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Mean total PPD around implants affected by peri-implantitis (mm)</td>
<td>6.7 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Mean PPD around maxillary implants affected by peri-implantitis (mm)</td>
<td>7.1 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8 ± 0.7</td>
</tr>
<tr>
<td>Mean PPD around mandibular implants affected by peri-implantitis (mm)</td>
<td>6.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Mean total CBL around implants affected by peri-implantitis (mm)</td>
<td>5.8 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Mean total CBL around maxillary implants affected by peri-implantitis (mm)</td>
<td>6.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>Mean total CBL around mandibular implants affected by peri-implantitis (mm)</td>
<td>5.5 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>PPD: peri-implant probing depth; CBL: crestal bone loss.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Compared with Group 2 (p < .001).

<sup>b</sup>Compared with Group 2 (p < .05).

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).

Table 3. Levels (mean ± SD) of MMP-8 and MMP-9 in the PISF among individuals in Groups 1 and 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of PISF collected (μl)</td>
<td>3.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Level of MMP-8 (ng/ml)</td>
<td>128.3 ± 12.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.6 ± 19.3</td>
</tr>
<tr>
<td>Level of MMP-9 (ng/ml)</td>
<td>98.2 ± 6.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.7 ± 10.4</td>
</tr>
</tbody>
</table>

PISF: peri-implant sulcular fluid.

<sup>a</sup>Compared with Group 2 (p < .05).

<sup>b</sup>Compared with Group 2 (p < .01).

The mean PISF volume (p < .05) collected and levels of MMP-8 (p < .01) and MMP-9 (p < .01) were significantly higher among individuals in Group 1 compared with Group 2 (Table 3).

Discussion

In the present 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 pro-inflammatory cytokines [such as tumor necrosis factor-α, interleukin (IL)-6, IL-1β] 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 the present study, it was hypothesized that levels of MMP-8 and MMP-9 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 (MMP-8 and MMP-9) were statistically significantly higher among individuals in Group 1 compared with Group 2. Several experimental studies (Biswas et al., 2013; Katz et al., 2007, 2005; Xu et al., 2015) have shown that exposure to tobacco smoke augments the formation and accumulation advanced glycation endproducts (AGEs) in periodontal tissues. Interactions between AGEs and their receptors produces a state of oxidative stress within gingival tissues (Al-Sowygh et al., 2018; Gorudko et al., 2012); impairs the function of polymorphonuclear leukocytes (Gorudko et al., 2012); and produce destructive inflammatory cytokines (such as MMP-8 and MMP-9) in the serum and GCF (fluid present inside the gingival sulcus around natural tooth) (Gurav 2013; Javed et al., 2012; Kardesler et al., 2010). It has also been reported that AGEs mediate inflammation of human periodontal tissues via the endoplasmic reticulum stress-induced nuclear factor-kB pathway (Xu et al., 2015). Furthermore, nornicotine (a metabolite of nicotine), upregulates the expression of RAGE in the gingival tissues of smokers and stimulates the formation reactive oxygen species, which jeopardize the oral soft tissues and increases bone loss around teeth (Katz et al., 2005). 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 MMP-8 and MMP-9 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.

Individuals in Group 2, despite being self-reported never-smokers, demonstrated a mean CBL of ~4.5 mm around dental implants. Studies have shown that poor oral hygiene is a classical risk factor of periodontitis (Lertpinmonchai et al., 2017; Nanaiah et al., 2013) as well as peri-implantitis (Konstantinidis et al., 2015; Ogata et al., 2017). In the present study, nearly 72% of the individuals in Group 2 reported to brush their teeth once daily and none in either group reported to have ever used a dental floss. Studies have reported that an inadequate plaque control is associated with an increased frequency of pathogenic microbes (such as Prevotella intermedia and Aggregatibacter actinomycetemcomitans), which are associated with the etiology of periodontitis as well as peri-implantitis (Cortelli et al., 2013; Gürlek et al., 2017). It is also notable that although the number of individuals in Group 1 (~78%) that reported to brush their teeth once daily was similar to those in Group 2 (~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, which were 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 towards a better oral health and quality of life. In addition, our findings present an association between hookah smoking and increased peri-implant disease. To bring improvement, it is imperative to quit narghile/hookah smoking so as to avoid further peri-implant tissue destruction.

One limitation of the present study is that individuals in Groups 1 and 2 were ~50 years old. Elderly individuals (≥60 years) have been shown to be more susceptible to oral inflammatory conditions and CBL compared with individuals <50 years old (Javed et al., 2007). It is therefore hypothesized that peri-implant PPD is worse and levels of cytokines in the PISF are significantly higher among elderly 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 cytokines in the PISF are significantly higher among WS with poorly controlled diabetes mellitus compared with systemically healthy WS. This requires further investigations.

Conclusion

Within the limits of the present study, it is concluded that PISF levels of MMP-8 and MMP-9 are significantly higher among WS compared with never-smokers with peri-implantitis.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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