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ABSTRACT

Objective: The aim of the present split-mouth retrospective study was to compare the clinical periodontal parameters among gutka-chewers and naswar-dippers.

Methods: A structured questionnaire was used to record demographic information. Jaw quadrant in which gutka or naswar were placed were defined as “test-sites”. The remaining jaw quadrants were designated as “control-sites”. Clinical [plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment loss (CAL)] and radiographic [alveolar bone loss (ABL)] parameters were compared among gutka-chewers and naswar-dippers in the test- and control sites. Data were analysed using non-parametric Wilcoxon signed rank test followed by post hoc analyses with the Bonferroni correction. p < 0.05 were considered statistically significant.

Results: Seventy-five gutka-chewers and 72 naswar-dippers with mean ages of 45.2 ± 0.8 years and 47.3 ± 2.8 years, respectively were included. Gutka-chewers and naswar-dippers were consuming their respective smokeless tobacco product 10.2 times and 8.5 times daily for 15.1 ± 2.7 and 12.3 ± 3.4 years, respectively. Among gutka-chewers and naswar-dippers, scores of PI, BOP, PPD ≥ 4 mm, CAL and ABL were significantly higher in the test-sites compared with their respective control-sites (p < .01).

Conclusions: Periodontal inflammatory parameters were poorer in the test-sites among gutka-chewers and naswar-dippers compared with the control-sites.

Introduction

Gutka is one of the several smokeless tobacco (ST) formulations that is generally a mixture of powdered tobacco, areca-nut, slaked lime (aqueous calcium hydroxide paste) and fragrances such as menthol and saffron (Figure 1(A)) [1]. Gutka is commercially available in sachets (average weight 3.5 g) [1]; and is commonly consumed in South Asian countries including India, Pakistan, Bangladesh, and Sri Lanka [2,3]; however, due to its global export, gutka is also consumed by immigrants in countries such as United Kingdom and the United States [4–6]. Gutka is placed in the buccal vestibule and gently chewed and sucked. It is then held in the buccal vestibule for a prolonged duration and continued to be gently chewed and sucked intermittently. The contents may either be swallowed or spat out when desired. Likewise, Naswar (also known as Nass or Niswar) is a form of dipping ST product and is an amalgam of ground tobacco leaves (Nicotina rustica), slaked-lime, wood ash and flavourings such as cardamom and menthol [7]. Naswar also contains a small quantity of water and colouring agents such as indigo (average weight 9.2 g) [7,8]. Naswar is commonly sold in small polythene pouches (almost the size of a tea bag) (Figure 1(B)) [9]. Usually a small bolus of naswar is manually shaped into a ball and placed in the buccal vestibule between the mandibular premolars and first molar teeth. It is gently sucked intermittently for extended periods of time and spat out when desired.

Habitual use of ST products is a significant risk factor for periodontal inflammatory conditions, such as gingivitis and periodontitis [10–12]. Several studies [3,13] have reported that scores of clinical (plaque index [PI], bleeding on probing [BOP], probing pocket depth [PPD] and clinical attachment loss [CAL]) and radiographic (alveolar bone loss [ABL]) parameters of periodontal inflammation are significantly higher among habitual ST product users compared with individuals not using tobacco in any form. To our knowledge from indexed literature, there is a death of studies that have compared periodontal inflammatory parameters among individuals using different types of ST products using a split-mouth design. It is hypothesized that (i) among gutka-chewers and naswar-dippers, periodontal inflammatory parameters are worse in the test-sites (buccal vestibules in which, gutka or naswar are placed, respectively) compared with the control-sites (buccal vestibules in both arches in which, gutka or naswar are not placed, respectively); (ii) since gutka has more dry
weight of tobacco than naswar, clinical periodontal parameters are worse among gutka-chewers than naswar-dippers.

The aim of the present split-mouth retrospective study was to compare the clinical periodontal parameters among gutka-chewers and naswar-dippers.

Materials and methods

Ethical guidelines

The present study was performed in accordance with the Declaration of Helsinki. The study was approved by the research ethics committee of the College of Dentistry, Ziauddin Medical University, Karachi, Pakistan. Volunteering individuals were requested to read and sign a consent form, which was printed in simple English and Urdu (national language of Pakistan). All participants were informed that participation in the present study is completely voluntary and that they reserved the right to terminate their participation at any stage of the investigation without penalty.

Inclusion and exclusion criteria

The following inclusion criteria were entailed: (a) self-reported gutka-chewers (subjects who reported to be consuming one gutka-sachet daily since ≥12-months); and (b) self-reported naswar-dippers (subjects who reported to be dipping naswar once daily since ≥12-months). Subjects with the following characteristics were excluded: (a) refusal to sign the consent form (b) habitual tobacco-smokers and alcohol-users; (c) patients with self-reported systemic diseases such as acquired immune deficiency syndrome, diabetes mellitus, hepatitis B and/or hepatitis C and cardiovascular diseases; (d) patient that reported to have used antibiotics within the past three months; and (e) patients that reported to have undergone periodontal therapy (surgical or non-surgical) within the past three months. In addition, third molars were also excluded.

Study participants and grouping

A cohort study was performed between October 2015 and July 2016 at the College of Dentistry, Ziauddin Medical University, Karachi, Pakistan. Individuals who reported to be consuming one gutka sachet daily since ≥12-months were Gutka-chewers. Individuals who reported to be dipping naswar at least once daily since ≥12-months were Naswar-dippers. Among gutka-chewers and naswar-dippers the jaw quadrant in which, gutka and naswar, respectively were placed were defined as “test-sites”. The remaining jaw quadrants in which, the participants were not placing their respective ST product were designated as “control-sites”.

Interview questionnaire

A trained interviewer (TA) presented a structured written questionnaire to all participants. Information regarding age, gender, education status (undergraduate level education – primary school and college education for a combined period of 12 years; graduate level education – post-4-year college degree) were recorded in a questionnaire sheet. The questionnaire sheet also recorded duration and daily frequency of gutka or naswar usage, location of gutka or naswar placement in the mouth, family history of tobacco use, reason for gutka or naswar usage, and daily tooth brushing (once, twice or more).

Assessment of periodontal parameters

Periodontal parameters (PI [14], BOP [15], PPD (4–6 and >6 in mm) [16] and CAL [17]) were assessed at six sites per tooth (mesio-facial, mid-facial, disto-facial, mesio-lingual, mid-lingual and disto-lingual). BOP and PI were recorded at 6 sites per tooth for absence or presence of bleeding, based on dichotomous scoring system. The scores ranged as follows; 0 – no visible bleeding/plaque, 1 – visible bleeding/plaque. PPD was measured to the nearest mm using a graded periodontal probe (Hu-Friedy, Chicago, IL, USA). Number of missing teeth were also recorded. All periodontal examinations were carried out by a trained examiner (ZA) who was blinded to the study groups (gutka-chewers or naswar-dippers) and sites (test- or control-sites). The kappa value for intra-assessor reliability with respect to CAL was 0.91.
Assessment of alveolar bone loss

Digital full-mouth radiographs were taken using a dental radiography machine (CareStream Dental LLC, Atlanta, GA, USA). The radiographs were viewed on a calibrated computer screen (Samsung SyncMaster digital TV monitor, Seoul, Korea) using a software program (Image Tool 3.0 Program; Department of Dental Diagnostic Science, University of Texas Health Science Centre, San Antonio, TX, USA). ABL was defined as the vertical distance from 2 mm below the cementoenamel junction (CEJ) to the most crestal part of marginal bone. ABL was presented as the average of the sum of the mean mesial and distal ABL [13]. All radiographic examinations were performed by a trained and calibrated investigator (ZA) (kappa = 0.89). Third molars and teeth surfaces on which, the CEJ and/or the crestal bone were not clearly visible due to of technical reasons (such as dental restorations, interproximal caries, overlapping of teeth, and/or poor radiographic quality) were not assessed.

Statistical analyses

Statistical analyses were carried out using a statistical software (SPSS, v.20.0 for Windows, IBM, Chicago, IL, USA). The alpha level was set at 0.05. Normality of distribution of the variables was tested with Kolmogorov–Smirnov and Shapiro–Wilk tests and confirmed with Q–Q plots. The significance of differences in periodontal parameters between gutka-chewers and naswar-dippers and the test- and control-sites within each group were determined using the non-parametric Wilcoxon signed rank test. Bonferroni post hoc adjustment test was used for multiple comparisons. Stepwise logistic regression analysis was employed to identify explanatory variables for periodontal outcomes, controlling for the effect of possible covariate such as age, education status, daily frequency, duration of habit, and duration of placement of ST products. The direction and strength of association between periodontal outcomes and covariates were assessed by generating odds ratios, the precision of which could be measured by 95% confidence intervals. Power analysis was performed with a computer software (nQuery Advisor 5.0; Statistical Solutions, Saugus, MA, USA). Power analysis was based on the supposition that a mean difference of 0.5 mm and 1 mm in ABL and PPD, respectively should be detected at a significance level of 0.05 and a desired study power of at least 80%. It was estimated that a sample size of 68 individuals per group will achieve 95% power with a 0.05 two-sided significance level.

Results

Characteristics of the included study sample

In total, 147 male subjects (75 gutka-chewers and 72 naswar-dippers) were included. The mean ages of gutka-chewers and naswar-dippers were 45.2 ± 0.8 years and 47.3 ± 2.8 years, respectively. Gutka-chewers and naswar-dippers were consuming their respective ST product 10.2 times and 8.5 times daily for 15.1 ± 2.7 and 12.3 ± 3.4 years, respectively. A family history of tobacco use was reported by 80% gutka-chewers and 81.9% naswar-dippers. Sixteen percent gutka-chewers and 37.5% naswar-dippers were illiterate. Management of psychological stress was the most common reason for ST consumption, which was reported by 68.1% gutka-chewers and 75% naswar-dippers (Table 1). Tooth brushing once daily was reported by 92% gutka-chewers (n = 69) and 86.1% naswar-dippers (n = 62). Gutka-chewers were placing gutka in the right buccal vestibule and naswar-dippers were dipping the ST product in the in the left buccal sulcus between the mandibular premolars and first molar teeth.

Clinical periodontal and radiographic parameters

Among gutka-chewers, scores of PI (p < .001), BOP (p < .001), PPD ≥ 4 mm (p < .001) and CAL (p < .001) were significantly higher in the test-sites compared with the control-sites. Among naswar-dippers, scores of PI (p < .001), BOP (p < .001), PPD ≥ 4 mm (p < .001) and CAL (p < .001) were significantly higher in the test-sites compared with the control-sites. Scores of PI, BOP, PPD ≥ 4 mm and CAL were comparable among test-sites in gutka-chewers and naswar-dippers. There was no statistically significant difference in the scores of PI, BOP, PPD ≥ 4 mm and CAL in the control-sites among gutka-chewers and naswar-dippers (Table 2).

Alveolar bone loss

Among gutka-chewers and naswar-dippers, scores of ABL was significantly higher in the test-sites compared with their respective control-sites (p < .001). There was no statistical difference in scores of total ABL in the test-sites among gutka-chewers and naswar-dippers. In the control-sites, scores of ABL were 1.8 mm and 2 mm among gutka-chewers and naswar-dippers, respectively (Figure 2).

Stratification of periodontal parameters for gutka-chewers and naswar-dippers according to the daily frequency, duration of placement and habit of smokeless-tobacco

There was no statistically significant difference in the scores of PI, BOP, PPD ≥ 4 mm, CAL and ABL among gutka-chewers and naswar-dippers.
scores of PI, BOP, PPD for up to 10 min. There was no statistically significant influence of habit, and duration of placement of ST products (Figure 3(a–d)).

Scores of PI, BOP, PPD ≥4 mm and CAL were statistically significantly higher among individuals carrying gutka in the buccal vestibule for 21–30 min (p < .05) and more than 30 min (p < .05) compared with those carrying gutka in the buccal vestibule for up to 10 min. ABL was significantly higher among gutka-chewers carrying gutka in the buccal vestibule for more than 21–30 min (p < .05) and more than 30 min (p < .05) compared with individuals placing gutka in the buccal vestibule for up to 10 min. Among naswar-dippers, scores of PI, BOP, PPD ≥4 mm and clinical AL were statistically significantly higher among individuals dipping naswar for more than 30 min compared with individuals dipping naswar for up to 10 min (p < .05). ABL was significantly higher among individuals dipping naswar in the buccal vestibule for more than 30 min (p < .05) compared with individuals dipping naswar for up to 10 min. There was no statistically significant influence of the duration of gutka or naswar usage on the severity of PI, BOP, PPD ≥4 mm, CAL and ABL among gutka-chewers and naswar-dippers, respectively (data not shown).

Regression analysis to control confounders

The logistic regression analysis revealed that periodontal outcomes showed statistically significant difference even after adjusting for age, education status, daily frequency, duration of habit, and duration of placement of ST products (p > .05) (Table 3).

Discussion

It is well-known that clinical periodontal parameters is worse in ST product users compared with individuals not using tobacco in any form [13,18]. However, to our knowledge, this is the first study that compared periodontal inflammatory parameters among individuals consuming different types of ST products (gutka and naswar) in relation to the site of ST placement (test-site) and control-sites (buccal vestibules in which, ST was not placed). The present retrospective clinical split-mouth study was based on two hypotheses. According to the first hypothesis, scores of PI, BOP, PPD, CAL and ABL are significantly higher in the test-sites among gutka-chewers and naswar-dippers compared with their respective control-sites. The second hypothesis was that the above-mentioned clinical periodontal parameters are worse among gutka-chewers than naswar-dippers since gutka has more dry weight of tobacco than naswar. The results reported in the present investigation are in accordance with the first hypothesis.

This could be explained by the essential constituents of gutka and naswar such as slaked lime and powdered tobacco that increase the expression of reactive oxygen species in the periodontal tissues and proinflammatory cytokines (such as tumour necrosis factor-alpha, interleukin (IL)-6, IL-1β and matrix metalloproteinase-8 and 9) in whole saliva of tobacco users compared to subjects not using any form of tobacco [19]. Moreover, nicotine in tobacco induces hyperaemia in the gingival blood vessels possibly by stimulation of sensory nerves and the consequent release of vasodilatory peptides from their peripheral endings [20]. Furthermore, in-vitro studies have indicated that nicotine impairs the function and proliferation of gingival fibroblasts [21], increases integrin beta-1 expression [22], reduces collagen production [23] increases the fibroblast collagenase activity [24] and enhances alveolar bone loss [25]. These are possible factors that contributed towards significantly higher scores of PI, BOP, PPD ≥4 mm, CAL and ABL in the test-sites compared with the control-sites among gutka-chewers and naswar-dippers. Since gutka has additional dry weight of tobacco compared with naswar, it is postulated that clinical periodontal parameters are worse in gutka-chewers than naswar-dippers (second hypothesis of the present findings). Interestingly, the results indicated no statistical difference in PI, BOP, PPD ≥4 mm, CAL and total ABL upon comparison of the test-sites among gutka-chewers and naswar-dippers. One explanation for these results is that irrespective of whether a ST product is in moist form or dry, the detrimental effects of nicotine (such as those described above) on periodontal tissues remain persistent. This suggests that gutka and naswar are hazardous to oral health and neither ST product is considered less hazardous than the other. Therefore, public health awareness programs should regularly be performed to discourage the consumption of nicotine containing products and educate the public about the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test-site</th>
<th>Control-site</th>
<th>Test-site</th>
<th>Control-site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque index</td>
<td>57.6 ± 5.2a</td>
<td>20.5 ± 4.1</td>
<td>52.2 ± 10.3b</td>
<td>25.6 ± 3.9</td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td>63.4 ± 6.8a</td>
<td>30.3 ± 7.1</td>
<td>56.6 ± 9.3b</td>
<td>28.5 ± 5.7</td>
</tr>
<tr>
<td>Probing pocket depth ≥4 mm</td>
<td>22.4 ± 5.1a</td>
<td>4.2 ± 1.1</td>
<td>25.6 ± 4.7b</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>Clinical attachment loss (in millimetres)</td>
<td>5.5 ± 1.2a</td>
<td>0.5 ± 0.2</td>
<td>4.8 ± 1.3b</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

*Compared with the control-sites among gutka-chewers (p < .001).

*Compared with the control-sites among naswar-dippers (p < .001).
Figure 3. (a) Dot plots and median values of plaque index (PI) (Y-axis) with reference to the daily frequency of smokeless tobacco consumption (X-axis) among gutka-chewers and naswar-dippers. Plaque index was presented as the percentage of sites that displayed dental plaque upon clinical examination. (b) Dot plots and median values of bleeding on probing (BOP) (Y-axis) with reference to the daily frequency of smokeless tobacco consumption (X-axis) among gutka-chewers and naswar-dippers. BOP was presented as the percentage of sites that displayed BOP upon clinical examination. (c) Dot plots and median values of probing pocket depth (PPD) of $\geq 4$ mm (Y-axis) with reference to the daily frequency of smokeless tobacco consumption (X-axis) among gutka-chewers and naswar-dippers. Probing pocket depth was presented as the percentage of sites that displayed a PPD $\geq 4$ mm. (d) Dot plots and median values of clinical attachment loss (AL) (Y-axis) with reference to the daily frequency of smokeless tobacco consumption (X-axis) among gutka-chewers and naswar-dippers. Clinical AL was presented in millimeters. (e) Dot plots and median values of alveolar bone loss (ABL) (Y-axis) with reference to the daily frequency of smokeless tobacco consumption (X-axis) among gutka-chewers and naswar-dippers. ABL was presented in millimeters.
harmful effects of tobacco-based products on oral and general health. It is worth mentioning that in the present study, 16% of gutka-chewers and nearly 37% of naswar-dippers were illiterate.

The frequency and duration of ST usage has been reported to influence the severity of oral inflammation including periodontal disease and malignant transformations [26,27]. Singh et al. [26] reported that periodontal inflammation was worse in individuals using ST products for over five years compared with individuals with a shorter history of T usage (<5 years). The present findings corroborates with the study by Singh et al. [26] since the average duration of ST usage among gutka-chewers and naswar-dippers was approximately 15 and 12 years, respectively. Moreover, when the present data was stratified with relation to the duration of ST usage, it was observed that there was no statistical difference in the severity of periodontal conditions among gutka-chewers and naswar-dippers who had been consuming ST for up to five years and those who had a longer history of gutka and naswar use (≥10 years). This suggests that individuals with a shorter duration of ST consumption (up to five years) of ST usage are as susceptible to periodontal disease as are individuals with a significantly longer history of ST use (≥10 years). Data stratification also showed that either chewing gutka or dipping naswar at least three times daily for at least 10 min exposed the consumers to periodontal inflammation. This suggests that a time duration as short as 10 min is sufficient to induce an inflammatory effect of ST products in the mouth. Nevertheless, placement of ST products in the mouth for durations less than 10 min and less frequently (<3 times daily) should not be considered as safe.

Few limitations that may be taken into regard is that tobacco smokers and patients with systemic diseases were excluded. It is well-recognized that cigarette smoking and systemic diseases such as diabetes mellitus are known risk factors of periodontal disease [28,29]. It is postulated that (a) clinical periodontal parameters are poorer in cigarette smokers with gutka-chewing and/or naswar-dipping habits compared to individuals smoking solely cigarettes; (b) clinical periodontal parameters are worse in immunosuppressed gutka-chewers and naswar-dippers as compared to systematically healthy gutka-chewers and naswar-dippers. Further studies are warranted to test these hypotheses. All included subjects were approximately 45-years-old. It is well-recognized that advancing age is a risk-factor for periodontitis [29].

Table 3. Logistic regression model for age, education status, daily frequency, duration of habit, and duration of placement of ST products influencing periodontal outcomes with odds ratios, 95% confidence intervals (CI) and p-values.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2.03</td>
<td>0.97, 5.48</td>
<td>.046</td>
</tr>
<tr>
<td>Education status</td>
<td>2.95</td>
<td>1.26, 4.83</td>
<td>.028</td>
</tr>
<tr>
<td>Daily frequency</td>
<td>2.22</td>
<td>1.09, 5.34</td>
<td>.031</td>
</tr>
<tr>
<td>Duration of habit</td>
<td>2.88</td>
<td>1.2, 6.12</td>
<td>.016</td>
</tr>
<tr>
<td>Duration of placement</td>
<td>1.76</td>
<td>1.3, 4.1</td>
<td>.025</td>
</tr>
</tbody>
</table>

Covariates were simultaneously adjusted for all variables. Bold denotes statistically significant result at p < .05 in direction of increased risk.
It is therefore assumed that old-aged subjects (e.g. over 70 years old) who utilise ST products experience worse periodontal inflammation as compared to relatively younger individuals (such as those reported in this study, 45 years old. Further investigations are needed in this regard. Another important limitation is the carry-over effect. There is certainly a possibility that the ST products (gutka and naswar) can induce an inflammatory response in the control-sites (buccal vestibule opposite to the vestibule in which such products are placed). It seems difficult for ST users to prevent the tobacco products from reaching the control-sites. In order to determine the inflammatory response of ST products in the control-sites, a separate research study based on the following groups would be needed: Group 1: Individuals using ST products (Group 1a: test sites and Group 1b: control sites) and Group 2: individuals not using tobacco in any form (both buccal vestibules may serve as control-sites). It is hypothesized that periodontal inflammatory parameters (such as PI, BOP, PPD ≥ 4 mm and CAL) are poorer in the control-sites in ST product users compared with individuals not using tobacco in any form. However, further studies are warranted to test this hypothesis. It is imperative for clinicians and health care providers to be aware of the deleterious effects of ST products and educate the community about the detrimental effects of tobacco products on health and simultaneously emphasize on the importance of regular oral hygiene maintenance, dental visits towards a better quality of life.

This study concludes that clinical periodontal parameters were poorer in the test-sites among gutka-chewers and naswar-dippers compared with the control-sites.

Disclosure statement
No potential conflict of interest was reported by the authors.

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