Betel and tobacco chewing habit and its relation to risk factors for periodontal disease

Authors: Maria Laura Giovannoni¹, Iván Valdivia-Gandur², Vicente Lozano de Luaces³, Héctor Varela Véliz⁴, Yamadala Balasubbaiah⁵, Eduardo Chimenos-Küstner¹.

1. Odontostomatology Department, Universitat de Barcelona, Spain.
2. Biomedical Department and Dentistry Department, Universidad de Antofagasta, Chile.
3. Dentistry Coordinator of Rural Development Trust, Vicente Ferrer Foundation, Spain.
4. Mathematic Department, Universidad de Antofagasta, Chile.
5. Director of Kalyandurg Hospital-Kanekal, Anantapur district, India.

Running title: Chewing habit and risk factors for periodontal disease.

Keywords: Periodontal disease, Chewing habit, Community Periodontal index, Oral disease, Betel Quid, Smokeless tobacco.

Corresponding author:

Iván Valdivia Gandur

Biomedical Department and Dentistry Department

Avenida Angamos 601

Universidad de Antofagasta

e-mail: ivan.valdivia@uantof.cl

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/odi.12820

This article is protected by copyright. All rights reserved.
Acknowledgement: We want to thank to the Vicente Ferrer Foundation for their important contribution in this work. We also wish to recognize the Chilean Ministry of Education, Project ANT 1655. This study did not receive any other financial support. The authors declare no conflict of interest.

Conflict of Interest: none to declare.

Date of submission: 29-05-2017.

Abstract

Objectives: To comparatively assess periodontal status in patients who chew different products and patients who do not have this habit and to assess how this practice interacts with predisposing factors and risk indicators of disease.

Methods: Patients included in the dental care carried out in a rural community in India were considered for a cross-sectional study. The Simplified Oral Hygiene Index and the Community Periodontal Index (CPI) were assessed. Furthermore, a validated survey with items concerning chewing habits was administered. Statistical analysis of the effects of age range, gender, chewing products and hygiene status on CPI was performed.

Results: In total, 1,023 patients met the inclusion criteria. The chewer patients (430) exhibited a significantly higher CPI than the non-chewers (593). The chewing habit increased the likelihood of a higher CPI by 6.76-fold, while excellent/good oral hygiene status decreased the probability of a higher CPI by approximately 45%. CPI did not differ significantly among chewers of different products.

Conclusion: In the population studied, a chewing habit was associated with a worse periodontal status, and this association was not modified by gender and age as predisposing factors. Oral hygiene could decrease the effect of chewing habit on periodontal health.
Introduction

Periodontal disease is multifactorial in origin. Infection is always present in the pathology, but many factors might influence its onset and progression (Sanz et al., 2011). These factors include microbiological, genetic, immunological, environmental, and behavioural factors (Clarke et al., 1995; Genco and Borgnakke, 2013; Holtfreter et al., 2015).

Periodontal disease is an event that occurs within a social context and can represent a risk when sociocultural mores include activities that are potentially harmful to the periodontal structure. The betel quid, with or without tobacco, and tobacco by itself are the main substances used for chewing habits in the south and south-east of Asia (Gupta, 2014) and South Africa (VanWyk, 1997), and the use of the betel quid has been described in nearby regions due to migration (Yoganathan, 2002). Additionally, the habit of chewing tobacco has been extended to Europe and North America (Lee and Hamling, 2009). Betel products are the fourth most common psychoactive substance worldwide after caffeine, alcohol, and nicotine (Anand et al., 2014). This habit produces significant modifications of the dental surface and generates wear and stains (Anand et al., 2014). Several studies have demonstrated that the sociocultural habits of chewing betel quid with or without tobacco and tobacco by itself in different presentations have modifying effects on periodontal disease (Gupta and Ray, 2004; Javed et al., 2013; Sumanth et al., 2008; Kulkarni et al., 2016). However, there is limited information available about the interaction of chewing habits and other factors associated with periodontal disease. In health science, risk is defined as the probability that a hazard will cause some harmful event. A risk factor is a characteristic that increases the likelihood of developing a disease or injury. Bouchard et al., (2017) have proposed an epidemiological model for periodontal risk factors, according to the proximity of the factor in the causal chain leading to the diseases. In this context, the concept "true risk factors" is used for proximal risk. For instance, diabetes and smoking are true risk factors for periodontal diseases. Other factors (frequently mentioned in the literature), such as poor hygiene, are “intermediate risk factors” or “risk indicators”, while “age” and “gender” are considered “distal risk factors” or “predisposing factors”. Under the hypothesis that chewing habits are associated with a worse periodontal status, can interact with the risk indicator “hygiene status” or the predisposing factors “age and gender” and are modifiers of periodontal disease expression, the aims of this study were to comparatively assess the periodontal health of patients who routinely chew different products as sociocultural customs and patients who do not have these habits, as well as to assess the influences of gender, age, and hygiene status, when the chewing habits are practised by the patients.

This article is protected by copyright. All rights reserved.
Methodology

The study protocol was reviewed and authorised by the Clinical Research Ethics Committee of the University of Barcelona (CEIC 556) and was authorised by the director of the Hospital Kaliandurg Kanekal. The study was conducted during the dental care activities of the Vicente Ferrer Foundation, whose professionals annually perform oral health promotion activities involving prevention, prophylaxis, and dental treatments when required. The principles of the Declaration of Helsinki were followed during this study. Written consent was obtained from each patient after explaining the objective of the study.

Population characteristics and patient selection

The study population was located in the rural area of the Anantapur state of Andhra Pradesh, India. This area is characterised by low socioeconomic and educational levels and shared sociocultural aspects.

A cross-sectional study was conducted, and the sample was non-probabilistic (the subjects volunteered). The patients included in the social dental care operations promoted and conducted by the Vicente Ferrer Foundation between July and September 2016 were considered. The patients were examined and interviewed at the hospitals of Kanekal, Bathalapalli, and Kalyandurg, and the rural brigades of Anantapur, all under the direction of the Kalyandurg Hospital and Vicente Ferrer Foundation.

The inclusion criteria were as follows:

1. Patients of both genders between 20 and 65 years old;
2. Patients who were currently consuming chewing products;
3. Patients with a sufficient number of teeth for CPI application in each sextant.

The exclusion criteria were as follows:

1. Patients with physical or mental alterations that modified their feeding system or oral hygiene;
2. Patients with orofacial malformations or pathologies that would alter or increase the difficulty of the examination;
3. Patients who reported habitual alcohol consumption;
4. Patients who were smokers of tobacco and/or other substances regardless of the frequency;
5. Patients who declared that they chewed products only sporadically;
6. Patients with systemic conditions that could modify the course of periodontal disease, such as diabetes, cardiovascular disease, and pregnancy (Clarke et al, 1995; Amarasena et al, 2002; Genco and Börgnakke, 2013; Wu et al, 2015);

7. Patients who did not answer all the questions and those from whom it was difficult to obtain valid information;

8. Patients who attended the dental service with severe pain and required urgent attention.

9. Patients who stated that he/she did not chew any substance but signs of chewing habits were observed during the oral examination.

The patients were divided into three age ranges (20-34, 35-44, and 45-65 years) and were classified into two groups according to their substance chewing habits, i.e., chewers and non-chewers. The chewers were defined as patients who claimed to chew some substance as a sociocultural habit and who had engaged in the habit for at least two years. The non-chewers did not have any chewing habit. Additionally, the chewer group was divided according to the chewed substance as indicated on the survey.

Oral examination and survey used to investigate the habits of chewing substances

Four dentists were trained and calibrated in terms of their diagnoses. The oral examinations recorded the following aspects for simplicity, speed, and uniformity of the measurements (Petersen and Ogawa, 2012): oral hygiene according to the simplified oral hygiene index OHI-S (Greene and Vermillion, 1964) and periodontal status according to the community periodontal index (CPI), which is based on the criteria of the World Health Organization (WHO). In OHI-S, six dental surfaces are selected from four posterior and two anterior teeth. OHI-S has two components, the debris index and calculus index, scored from 0 to 3. Both indexes are combined to obtain OHI-S (range: 0-6), and this score is frequently dichotomised for population studies as OHI-S \( \leq 1 \) (good hygiene) and OHI-S >1 (poor hygiene) (Hermann et al, 2009; Mbawalla et al, 2010). A CPI probe that met the WHO guidelines was used (World Health Organization, 1997). According to this standard, the CPI codes were categorised as normal (CPI 0), gingival bleeding (CPI 1), calculus (CPI 2), shallow periodontal pockets (CPI 3), and deep periodontal pockets (CPI 4). The patients' scores for this index were assigned considering the CPI record of each “sextant” present in the mouth. The Kendall coefficient of concordance (KCC) was used to assess the interobserver agreement. During the training, each examiner applied the CPI and OHI-S to 20 patients. These tests were repeated at three and seven days. The interobserver KCCs varied between 0.888 and 0.925 for the CPI measurements. For the OHI-S scores, the interobserver KCCs varied between 0.822 and 0.913. During the oral examinations, the chewing habits were also confirmed via observations of signs such as stains, the remains of some chewed product, and lesions.
of the oral mucosa. Furthermore, a validated survey was added to the clinical sheet and included questions related to the chewing habits (i.e., the substance used, frequency, and years of consumption).

**Statistical analysis**

The highest CPI value observed for each patient was used for the statistical analysis. With this value, the main explorations were as follows:

1. The association between chewing habits and observed CPI scores.
2. The associations of the age ranges, gender, and CPI scores among the chewers and non-chewers.
3. The association between the type of chewing products used and CPI scores.
4. The associations of oral hygiene aspects (i.e., the OHI-S scores and frequencies of chewing) with the age range, gender, and CPI scores among the chewers and non-chewers.

Additionally, the CPIs of each sextant were considered to assess the local effect of chewing habits, and the highest CPI value from each sextant was considered in this analysis.

The Mann-Whitney-Wilcoxon test was used to compare independent samples, the analysis of variance test (ANOVA), supplemented with a post hoc test, was used to establish differences between several independent data sets, and the Pearson chi-square test, Fisher’s exact test, and odds ratios were used to establish the independence or associations among variables. Additionally, multiple logistic regression was applied with the occurrence of the CPI value as the dependent variable. The CPI and OHI-S values were dichotomised as <3 (lower) and 3-4 (higher) and as “Fair-poor” (score >1) and “Excellent-good” (score ≤ 1). These dichotomisations of the CPI and OHI-S values have been used in the literature and accepted (Angeli et al, 2003; Hermann et al, 2009; Mathur et al, 2016). The significance level used was α<0.05. The statistical software used was IBM SPSS Statistics 22.

**Results**

**General Description**

In total, 1,613 patients were examined, and 1,023 satisfied all the inclusion/exclusion criteria. The distributions of men and women (54.3% and 45.7%, respectively) in the different age ranges did not exhibit significant differences. Considering this sample, the different analyses were associated with statistical powers that varied between 0.82 and 1.0. The distribution of patients by CPI score, chewing habit, and age range is summarised in Figure 1. Of the patients studied, 57.9% (n=593) indicated that

This article is protected by copyright. All rights reserved.
they did not have any chewing habit. Of the chewing patients (n=430), 40.7% chewed betel quid (BQ), 38.1% chewed betel quid with tobacco (BQ+Tb), and 21.2% chewed tobacco alone (Tb). The BQ included the betel leaf, areca nut, slaked lime, and other flavouring elements (e.g., catechu resin, cardamom, and others). The BQ+Tb preparations included the betel leaf and mixtures of the components indicated above plus tobacco (pure or from commercial products). The chewing tobacco (Tb) mainly came from commercial products that were combined with areca nut and slaked lime or just the slaked lime, and four patients used tobacco in their own preparations with other flavour elements. Among the male patients (n=240), BQ+Tb was the most frequently used preparation (61.6%) followed by Tb and BQ (32.1% and 6.3%, respectively). Men in the age range of 20-34 preferred Tb, while men in the older age ranges significantly preferred BQ+Tb (P<0.05). Among the females (n=190), BQ was the most frequently used preparation (84.2%) followed by BQ+Tb and Tb (8.4% and 7.4%, respectively), and there were no differences in the chewing products used between the studied age ranges. The mean chewing habit durations were 9.67 (SD 4.37) years in the patients between the ages of 20 and 34 years, 12.10 (SD 3.46) years in the patients between the ages of 35 and 44 years, and 22.3 (SD 6.33) years in the patients between the ages of 45 and 65 years. The declared frequencies of consumption ranged from three times a week to twice daily for BQ (mean=1.1 daily), from one to four times daily for BQ+Tb (mean=1.8 daily), and from one to eight times daily for Tb (mean=3.8 daily). Significant differences between the chewing products with respect to the frequencies of consumption were observed (P<0.05).

**CPI scores of the chewers and non-chewers considering gender, age range, and chewing products**

The general distributions of chewers and non-chewers according to CPI score and age are summarised in Table 1. The patients with a chewing habit were significantly more likely to have CPI values of 3-4. Similar results were produced when each age range and gender were analysed separately. These observations were corroborated by the ANOVA test, which revealed significant differences in the CPI scores of the non-chewers compared with those in the BQ, BQ+Tb, and Tb chewers. Tukey’s HSD post hoc test indicated that the non-chewers had significantly lower CPI scores than the chewers (P<0.05). There were no differences in the CPI scores according to the age ranges among either the chewers or non-chewers (gender independent). Considering gender, only the non-chewer men exhibited a difference that indicated a possible association between a higher age range and a CPI of 3-4. Considering only the chewers, the CPIs of the BQ, BQ+Tb, and Tb chewers exhibited no significant differences. The same result was observed when this analysis was performed considering gender and each age range separately. Overall, the effects of chewing habits on the CPI values were independent of the chewed substance (Table 2).
CPIs of the chewer and non-chewer groups in relation to the observed hygiene and declared hygiene frequency

During the clinical inspections, 91.3% (n=934) of the patients presented with an OHI-S that was considered "fair-poor". Of these patients, 45.1% (n=421) reported a chewing habit, and these patients were significantly associated with CPI values of 3-4. The latter result was confirmed by the Mann-Whitney test, which indicated that the CPI values were higher among the chewing group with “fair-poor” hygiene (P<0.05). Regarding the patients with "excellent-good" hygiene, 89.8% (n=80) indicated that they did not chew substances, and these patients were significantly associated with CPI values <3. By contrast, the chewer patients with “excellent-good” hygiene were associated with CPI values of 3-4. Concerning the non-chewer patients, those with “fair-poor” hygiene were associated with CPI scores of 3-4 values, while those with “excellent-good” hygiene were associated with CPI scores <3, and the odds ratio adjusted by gender was 1.74 (95% confidence interval 1.07-2.82).

Regarding the hygiene frequency reported by the patients, 84.85% (n=868) indicated that they performed oral hygiene at least once daily, and the remaining patients reported practising oral hygiene two or more times per day. Oral hygiene performed only once per day was significantly associated with CPI scores of 3-4. Additionally, 46.19% (n=401) of these patients reported chewing some substance, and this subgroup of patients was significantly associated with CPI scores of 3-4. Considering the patients who declared that they engaged in oral hygiene practices more than once a day (n=155), 16.7% (n=26) indicated that they chewed some substance. Table 3 presents the results of a statistical analysis of the patients according to hygiene (OHI-S and frequency), CPI, and chewed substances.

CPI analysis of the sextants within the different groups

The sextants 1, 3, 4, and 6 of the chewers exhibited a predominance of CPI 3-4 scores, whereas the non-chewers exhibited a predominance of CPI scores <3 in all sextants. The same analysis that separately considered gender and each age range produced similar results. The CPI analysis of each sextant revealed a significant association between CPI 3-4 scores and chewers in all sextants, and the odds ratio indicated stronger effects in sextants four and six. Additionally, higher prevalences of CPI 3-4 scores were associated with the mandibular teeth in both the chewers and non-chewers (Table 4). Finally, the analysis of the effects of chewing products in each sextant only revealed a strong association of CPI scores of 3-4 in the second and fifth sextants using BQ+Tb products (P<0.05).
Multiple logistic regression was used to observe the effect of the main variables studied (age range, gender, chewing products and hygiene status) on CPI. The main finding was that chewing habit practice increased 6.76 times the likelihood of CPI scores of 3-4 (confidence interval: 4.72-9.67; \( P<0.00 \)), keeping the other variables fixed. On the other hand, the oral hygiene excellent/good decreased in approximately 45% the probability to obtain CPI scores of 3-4 (odds ratio: 0.55; confidence interval: 0.34-0.88). This latter result did not match that described above with respect to chewing habits and observed hygiene.

**Discussion**

This study analysed how different products used for chewing habits affect the periodontal health of a rural population in India and how such habits could interact with other factors, including gender, age and hygiene status. The principal observed effect was the significantly higher CPI values among the patients in the chewer group than in the non-chewer group (Table 1 and 3). In this regard, our results agree with the reports from different studies that have been performed in Taiwan (Jeng et al, 1996), Sri Lanka (Amarasena et al, 2002, 2003), Thailand (Chatrchaiwiwatana, 2007), Bangladesh (Akher et al, 2008), India (Mehta et al, 1955; Choudhury et al, 2003), and other regions in which betel quid with or without tobacco is consumed. Compared with reports for tobacco chewers, our results agree with studies from the USA (Robertson et al, 1990), Yemén (Al-Tayar et al, 2015), and India (Parmar et al, 2008; Anand et al, 2012), among others.

Gender is considered a non-modifiable predisposing factor or distal risk because it is in the rear area of the causal chain (Bouchard et al, 2017). Several prevalence studies have shown that male gender greatly increases the risk of periodontal disease (Albandar, 2002; Hermann et al, 2009; Eke et al, 2015). In our study, no significant differences in the CPI scores were observed between the males and females among either the chewers or the non-chewers (Table 1). Socioeconomic and sociocultural factors (including chewing habits) associated with the particular lifestyle of the population studied could explain the observed results. These factors have been suggested as relevant elements within the chain of risks that affect the prevalence of periodontal disease (Genco and Borgnakke, 2013; Holtfretter et al, 2015). Additionally, it is interesting to consider the significant difference detected in the preferences for chewed substances according to gender; although BQ was preferred by the females, and BQ+Tb was preferred by the males, no differences in the CPI scores were detected between the genders in the chewer group (Table 1).
Age has been described as a non-modifiable predisposing factor (Bouchard et al., 2017), a confounding factor for periodontal disease (Genco and Borgnakke, 2013) and an important variable in reports related to periodontal status (Holtfretter et al., 2015). As observed in the results, the chewing habits of the males seemed to generate a transverse consequence on periodontal status that was independent of the age range and modified the effect of this variable as observed in non-chewer males. However, the result of multiple logistic regression analysis makes this finding questionable. In regions in which chewing habits are sociocultural customs, such as southern Asia, northern Oceania, and India, these practices begin at an early age (Talonu, 1989; Philip et al., 2013; Singhvi et al., 2016). This fact helps to explain the significantly higher CPI scores of the chewer patients than those of the non-chewer patients in the younger group (20-34 years, Table 1). Nevertheless, no specific antecedents were found in the literature concerning the initiation of chewing habits in the region included in our study. Only the patients who expressed declarations regarding this issue were considered (with its inherent limitations).

Oral hygiene is a modifiable risk indicator and plays an important role in the prevention and treatment of periodontal disease (van der Weijden, 2011). In the group studied, the observed effects of chewing habits and hygiene status on CPI were ambiguous. Table 3 shows that the effect of the chewing habit was not affected by hygiene status or its frequency; even the chewer patients with “excellent-good” hygiene or hygiene frequencies of “twice a day or more” exhibited significant associations with CPI scores of 3-4 (Table 3). These results agree with those of Parmar et al. (2008), who reported that the oral hygiene statuses of chewers are significantly deteriorated compared with those of non-chewers. However, multiple logistic regression analysis showed a different behaviour of these variables when they are considered in conjunction with other data. According to the latter finding, excellent/good oral hygiene decreased the probability to obtain a CPI of 3-4. A specific study design may be necessary to confirm these results because, according to this finding, habitual oral hygiene practice could limit the deleterious effect of the chewing habit.

The general results revealed no significant differences in the comparisons of the CPI scores of those chewed BQ, BQ+Tb, or Tb (Table 2). However, the analysis by gender revealed an effect of the chewing product on the CPI scores among the female chewers of Tb and male chewers of BQ (Table 2). These minority groups represent only 6.7% of chewers, and, consequently, the effect of chewing habits on periodontal health was independent of the chewed products used by the population studied. This result does not agree with other reports in the literature in which different effects of two chewing products have been observed (Sumanth et al., 2008; Javed et al., 2013). Nevertheless, our results agree with those of another study that compared periodontal inflammatory conditions between habitual
gutka chewers and betel quid chewers (Javed et al, 2015). The common components detected in the chewed substances could have influenced these results. For example, calcium hydroxide (slaked lime) was present in all the chewed products, and calcium hydroxide is a strong alkali that can irritate the oral mucosa (Dunham et al, 1996; Javed et al, 2010). Another common element observed in the chewing products was the areca nut, which has been demonstrated to alter gingival keratinocytes and fibroblasts (Chang et al, 1998; Jeng et al, 1999) and modify the antimicrobial function of neutrophils (Lee et al, 2014). Furthermore, the effect of tobacco used locally (chewed tobacco) causes hyperaemia in the gingiva (Mavropoulos et al, 2001) and increases the levels of periodontitis and gingival bleeding (Amarasena et al, 2002, 2003). Therefore, a more detrimental effect on periodontal status could theoretically be generated by the joint use of slaked lime, areca nut, and tobacco (BQ+Tb and commercial Tb chewers) compared with BQ alone. However, no differences among these chewer groups were observed in our study.

The analysis of the effects of chewing habits on periodontal status considering each sextant revealed greater CPI scores in the mandibular sextants, especially in the fourth and sixth sextants (Table 4). Antecedents regarding local effects on periodontal status caused by chewing habits were not found. However, the literature describes that the chewing substances are commonly placed between mandibular teeth and the buccal mucosa (Reichart and Phillipsen, 1998), which likely explains our finding. Moreover, BQ+Tb generated a significantly worse effect on sextant five. The habit of chewing this product generates parasympathetic stimulation and increases the rate of salivary secretion (Mehta et al, 1955; Boucher and Mannan, 2002). The above actions contribute to supragingival calculus formation on the lingual surfaces of the mandibular anterior teeth, and greater accumulations of calculi are frequent in this area (Jin and Yip, 2002). Other antecedents to consider are the higher levels of calcium that have been described in the saliva of long-term tobacco chewers (Khan et al, 2005) and the characteristically high levels of fluoride in the drinking water of India (Jagtap et al, 2012). The presence of these elements in the saliva could facilitate calculus formation (Jin and Yip, 2002). Specific studies are needed to confirm this conjecture.

Several limitations must be considered regarding this study. The activities of the Vicente Ferrer Foundation range from prevention to the application of treatments when needed; therefore, although several inclusion/exclusion criteria were applied, the interpretation of the results should be performed with caution because the sample included volunteer patients with different treatment needs. The CPI score was used because it provides an objective classification system for a suitable cross-sectional view of the periodontal status of the population. However, this scoring system could be insufficient for measurements of the cumulative damage to periodontal tissues (Leroy et al, 2010) and, thus, could

This article is protected by copyright. All rights reserved.
have led to underestimations of the extents and severities of periodontal destruction that have previously occurred in the mouths of the volunteers. Moreover, this report did not consider aspects such as the method of hygiene or other risk factors for periodontal disease. Randomised stratified studies that consider these aspects are needed to complement and deepen the results reported in our study.

Within the inherent limitations of this report, we concluded that, in the studied population, the chewing habits were associated with a higher prevalence of CPI 3-4 scores. This observation was independent of the chewing products used by the patients and was not influenced by factors such as age or gender. The CPI values were greater in all sextants of the chewers than in the non-chewers, but sextants four and six were observed to be the most detrimentally affected. The habitual oral hygiene practice could decrease the effect of chewing habits on periodontal health. Chewing habits have deep-seated cultural roots in populations in which it is customary, and educational or sanitary initiatives must be sensitive to the community’s belief systems. From the clinical perspective, sanitary personnel must be aware that chewers will present with worse periodontal health, and this type of patient will have had a modified periodontal condition from an early age (among other oral complications). Finally, due to the specific sociocultural aspects of the community studied, complementary prevalence studies, including other distal, intermediate or proximal risk factors for periodontal disease are needed. This information could contribute to regional public decisions and the direction of the actions of international aid agencies.

Author contributions

All authors have contributed to the work substantially. Giovanonni L. and Chimenoz E. conceived and designed the study; patient and examiner (dentists) recruitment was carried out by Lozano de Luaces V. and Balasubbaiah Y.; Valdivia I., Varela H., and Chimenoz E. performed the analysis and interpretation of the data. Varela H., and Valdivia I. performed the statistical analysis. All authors have been significantly involved in revising the article and have read and approved the final version of the manuscript.

This article is protected by copyright. All rights reserved.
References.

Akhter, R., Hassan, N. M., Aida, J., Takinami, S., & Morita, M. (2008). Relationship between betel quid additives and established periodontitis among Bangladeshi subjects. *J Clin Periodontol*, 35, 9-15. doi: 10.1111/j.1600-051X.2007.01164.x.

Albandar, J.M. Global risk factors and risk indicators for periodontal diseases. (2002). *Periodontol 2000*, 29, 177-206. doi: 10.1034/j.1600-0757.2002.290109.x

Al-Tayar, B., Tin-Oo, M. M., Sinor, M. Z., & Alakhali, M. S. (2015). Prevalence and association of smokeless tobacco use with the development of periodontal pocket among adult males in Dawan Valley, Yemen: a cross-sectional study. *Tob Induc Dis*, 4, 13-35. doi: 10.1186/s12971-015-0061-8.

Amarasena, N., Ekanayaka, A. N., Herath, L., & Miyazaki, H. (2002). Tobacco use and oral hygiene as risk indicators for periodontitis. *Community Dent Oral Epidemiol*, 30, 115-123. doi: 10.1034/j.1600-0528.2002.300205.x.

Amarasena, N., Ekanayaka, A. N., Herath, L., & Miyazaki, H. (2003). Association between smoking, betel chewing and gingival bleeding in rural Sri Lanka. *J Clin Periodontol*, 30, 403-408. doi: 10.1034/j.1600-051X.2003.20010.x.

Anand, P. S., Kamath, K. P., Shekar, B. R., & Anil, S. (2012). Relationship of smoking and smokeless tobacco use to tooth loss in a central Indian population. *Oral Health Prev Dent*, 10, 243-252. doi:10.3290/j.ohpd.a28520.

Anand, R., Dhingra, C., Prasad, S., & Menon, I. (2014). Betel nut chewing and its deleterious effects on oral cavity. *J Cancer Res Ther*, 10, 499-505. doi: 10.4103/0973-1482.137958.

Angeli, F., Verdecchia, P., Pellegrino, C., Pellegrino, R.G., Pellegrino, G., Prosciutti L., … Bentivoglio, M. (2003). Association between periodontal disease and left ventricle mass in essential hypertension. *Hypertension*, 41, 488–492. doi: 10.1161/01.HYP.0000056525.17476.D7.

Boucher, B. J., & Mannan, N. (2002). Metabolic effects of the consumption of Areca catechu. *Addict Biol*, 7, 103-110. doi: 10.1080/13556210120091464.

Bouchard, P., Carra, M.C., Boillot, A., Mora, F., Rangé, H. (2017). Risk factors in periodontology: a conceptual framework. *J Clin Periodontol*, 44, 125-131. doi:10.1111/jcpe.12650.

Chang, M. C., Kuo, M. Y., Hahn, L. J., Hsieh, C. C., Lin, S. K., & Jeng, J. H. (1998). Areca nut extract inhibits the growth, attachment, and matrix protein synthesis of cultured human gingival fibroblasts. *J Periodontol*, 69, 1092-1097. doi: 10.1902/jop.1998.69.10.1092.

This article is protected by copyright. All rights reserved.
Chatrchaiwiwatana S. (2007). Factors affecting tooth loss among rural Khon Kaen adults: analysis of two data sets. *Public Health*, 121, 106-112. doi: 10.1016/j.puhe.2006.06.010.

Choudhury, C. R., Choudhury A. D., Alam, S., Markus, A. F., & Tanaka, A. (2003). Presence of *H. pylori* in the oral cavity of betel-quid (‘Paan’) chewers with dyspepsia: relationship with periodontal health. *Public Health*, 117, 346–347. doi: 10.1016/S0033-3506(03)00104-5.

Clarke, N. G., & Hirsch, R. S. (1995). Personal risk factors for generalized periodontitis. *J Clin Periodontol*, 22, 136–145. doi: 10.1111/j.1600-051X.1995.tb00125.x.

Dunham, L. J., Muir, C. S., & Hamner, J.E. III. (1996). Epithelial atypia in hamster cheek pouches treated repeatedly with calcium hydroxide. *Br J Cancer*, 20, 588-593.

Eke, P. I., Dye, B. A., Wei, L., Slade, G. D., Thornton-Evans G. O., Borgnakke, W.S., … Genco, R.J. (2015). Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. *J Periodontol*, 86, 611-622. doi: 10.1902/jop.2015.140520.

Genco, R. J., & Borgnakke, W. S. (2013). Risk factors for periodontal disease. *Periodontol 2000*, 62, 59-94. doi: 10.1111/j.1600-0757.2012.00457.x.

Gupta, B., & Johnson, N.W. (2014). Systematic review and meta-analysis of association of smokeless tobacco and of betel quid without tobacco with incidence of oral cancer in South Asia and the Pacific. *PLoS One*, 20(9), e113385. doi: 10.1371/journal.pone.0113385.

Greene, J., Vermillion, J. (1964). The Simplified Oral Hygiene Index. *J Am Dent Assoc*, 68, 7-13.

Gupta, P. C., & Ray, C. S. (2004). Epidemiology of betel quid usage. *Ann Acad Med Singapore*, 33, 31s-36s.

Hermann, P., Gera, I., Borbély, J., Fejérly, P., & Madléna, M. (2009). Periodontal health of an adult population in Hungary: findings of a national survey. *J Clin Periodontol*, 36, 449-457. doi: 10.1111/j.1600-051X.2009.01395.x.

Holtfreter, B., Albandar, J. M., Dietrich, T., Dye, B. A., Eaton, K. A., Eke, P. I., … Joint EU/USA Periodontal Epidemiology Working Group. (2015). EU/USA Periodontal Epidemiology Working Group. *J Clin Periodontol* 42, 407-412. doi: 10.1111/jcpe.12392.

Jagtap, S., Yenkie, M. K., Labhsetwar, N., & Rayalu, S. (2012). Fluoride in drinking water and defluoridation of water. *Chem Rev*, 112, 2454-2466. doi: 10.1021/cr2002855.

This article is protected by copyright. All rights reserved.
Javed, F., Chotai, M., Mehmood, A., & Almas, K. (2010). Oral mucosal disorders associated with habitual gutka usage: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 109, 857-864. doi: 10.1016/j.tripleo.2009.12.038.

Javed, F., Tenenbaum, H. C., Nogueira-Filho, G., Qayyum, F., Correa, F. O., Al-Hezaimi, K., & Samaranayake, L. P. (2013). Severity of periodontal disease in individuals chewing betel quid with and without tobacco. Am J Med Sci, 346, 273-278. doi: 10.1097/MAJ.0b013e31827333fb.

Javed, F., Vohra, F., Al-Kheraif, A. A., Malmstrom, H., & Romanos, G. E. (2015). Comparison of periodontal inflammatory conditions among habitual gutka chewers and betel quid chewers. Oral Dis, 21, 437-442. doi: 10.1111/odi.12295.

Jeng, J. H., Hahn, L. J., Lin, B. R., Hsieh, C. C., Chan, C. P., & Chang, M. C. (1999). Effects of areca nut, inflorescence piper betle extracts and arecoline on cytotoxicity, total and unscheduled DNA synthesis in cultured gingival keratinocytes. J Oral Pathol Med, 28, 64-71. doi: 10.1111/j.1600-0714.1999.tb01998.x.

Jeng, J. H., Lan, W.H., Hahn, L.J., Hsieh, C.C., & Kuo, M.Y. (1996). Inhibition of the migration, attachment, spreading, growth and collagen synthesis of human gingival fibroblasts by arecoline, a major areca alkaloid, in vitro. J Oral Pathol Med, 25, 371-375. doi: 10.1111/j.1600-0714.1996.tb00281.x.

Jin, Y., & Yip, H. K. (2002). Supragingival calculus: formation and control. Crit Rev Oral Biol Med, 13, 426-441.

Khan, G. J., Mehmood, R., Salah-ud-Din, Marwat, F. M., Ihtesham-ul-Haq, & Jamil-ur-Rehman. (2005). Secretion of calcium in the saliva of long-term tobacco users. J Ayub Med Coll Abbottabad, 17, 60-62.

Kulkarni, V., Uttamani, J. R., & Bhatavadekar, N. B. (2016). Comparison of clinical periodontal status among habitual smokeless-tobacco users and cigarette smokers. Int Dent J, 66, 29-35. doi: 10.1111/idj.12192.

Lee, Y. Y., Lin, Mo, Cheng, C. F., Chang, L. Y., Liu, T. Y., & Hung, S. L. (2014). Inhibitory effects of areca nut extract on expression of complement receptors and fc receptors in human neutrophils. J Periodontol, 85, 1096-1106. doi: 10.1902/jop.2013.130498.

Leroy, R., Eaton, K.A., Savage, A. (2010). Methodological issues in epidemiological studies of periodontitis--how can it be improved? BMC Oral Health. 21, 10:8. doi: 10.1186/1472-6831-10-8.

This article is protected by copyright. All rights reserved.
Mathur, M. R., Tsakos, G., Parmar, P., Millett, C. J., & Watt, R. G. (2016). Socioeconomic inequalities and determinants of oral hygiene status among Urban Indian adolescents. *Community Dent Oral Epidemiol*, 44, 248-254. doi: 10.1111/cdoe.12212.

Mavropoulos, A., Aars, H., & Brodin, P. (2001). The acute effects of smokeless tobacco (snuff) on gingival blood flow in man. *J Periodontal Res*, 36, 221-226. doi: 10.1034/j.1600-0765.2001.036004221.x.

Mehta, F. S., Sanjana, M. K., & Barretto, M.A. (1955). Relation of betel leaf chewing to periodontal disease. *J Am Dent Assoc*, 50, 531-536. doi: 10.14219/jada.archive.1955.0098.

Parmar, G., Sangwan, P., Vashi, P., Kulkarni, P., & Kumar, S. (2008). Effect of chewing a mixture of areca nut and tobacco on periodontal tissues and oral hygiene status. *J Oral Sci*, 50, 57-62. doi: 10.2334/josnusd.50.57.

Petersen, P.E., & Ogawa, H. (2012). The global burden of periodontal disease: towards integration with chronic disease prevention and control. *Periodontol 2000*, 60, 15-39. doi: 10.1111/j.1600-0757.2011.00425.x.

Philip, P. M., Parambil, N. A., Bhaskarapillai, B., & Balasubramanian, S. (2013) Evaluation of a specially designed control program to reduce tobacco use among school children in Kerala. *Asian Pac J Cancer Prev*, 14, 3455-3459. doi: 10.7314/APJCP.2013.14.6.3455

Reichart, P.A., & Phillipsen, H.P. (1998). Betel chewer's mucosa--a review. *J Oral Pathol Med*, 27, 239-242. doi: 10.1111/j.1600-0714.1998.tb01949.x.

Robertson, P. B., Walsh, M., Greene, J., Ernster, V., Grady, D., & Hauck, W. (1990). Periodontal effects associated with the use of smokeless tobacco. *J Periodontol*, 61, 438-443. doi: 10.1902/jop.1990.61.7.438.

Sanz, M., van Winkelhoff, A. J., & Working Group 1 of Seventh European Workshop on Periodontology. (2011). Periodontal infections: understanding the complexity--consensus of the Seventh European Workshop on Periodontology. *J Clin Periodontol*, 38, 3s-6s. doi: 10.1111/j.1600-051X.2010.01681.x.

Singhvi, A., Joshi, A., Bagul, N., Bhatia, S., Singh, G., & Gupta, R. (2016). The Insight for Initiation and Maintenance of Areca nut chewing Habit and its Effects on Oral Health Status among School Age Population in Western Rajasthan, India. *J Clin Diagn Res*, 10, ZC15-ZC18. doi: 10.7860/JCDR/2016/21010.8789

This article is protected by copyright. All rights reserved.
Sumanth, S., Bhat, K. M., & Bath, G. S. (2008). Periodontal health status in pan chewers with or without the use of tobacco. *Oral Health Prev Dent*, 3, 223-229. doi: 10.3290/j.ohpd.a13969.

Talonu, N. T. (1989). Observations on betel-nut use, habituation, addiction and carcinogenesis in Papua New Guineans. *PNG Med J*, 32, 195-197.

van der Weijden, F., & Slot, D. E. (2011). Oral hygiene in the prevention of periodontal diseases: the evidence. *Periodontol 2000*, 55(1), 104-23. doi: 10.1111/j.1600-0757.2009.00337.x.

World Health Organization. (1997). Oral Health Surveys, Basic Methods, World Health Organization, Geneva, Switzerland, 4th edition.

Wu, Y. Y., Xiao, E., & Graves, D.T. (2015). Diabetes mellitus related bone metabolism and periodontal disease. *Int J Oral Sci*, 7, 63-72. doi: 10.1038/ijos.2015.2.

Yoganathan, P. (2002). Betel chewing creeps into the New World. *NZ Dent J*, 2002, 98(432), 40-5.

**Figure Caption**

Figure 1. The distribution of the patients included in the study is shown. In the graph, the study groups present differences in the distribution of the CPI values according to the habit and age range of the study. In the groups that chewed betel quid (BQ), betel plus tobacco (BQ+Tb) and tobacco preparations (Tb), there is a predominance of CPI 3 and 4 values in all age ranges, unlike in the non-chewer group.
Table 1. Analysis of patients distribution considering CPI, gender, age range and chewing habits (n=1023).

|                          | Ch   | n-Ch  | P value  | OR (CI 95%)         |
|--------------------------|------|-------|----------|---------------------|
| **Chewers (Ch) and Non-chewers (n-Ch)** |      |       |          |                     |
| Ch                       | 51   | 379   | <0.001*  | 7.71 (5.5 to 10.77) |
| n-Ch                     | 302  | 291   |          |                     |
| Ch Females               | 22   | 169   | <0.001*  | 8.08 (4.89 to 13.36) |
| n-Ch Females             | 142  | 135   |          |                     |
| Ch Males                 | 29   | 210   | <0.001*  | 7.43 (4.75 to 11.61) |
| n-Ch Males               | 160  | 156   |          |                     |
| Ch Females 20-34 age range | 8   | 57   | <0.001*  | 7.21 (3.25 to 15.98) |
| n-Ch Females 20-34 age range | 89 | 88   |          |                     |
| Ch Females 35-44 age range | 4  | 46   | <0.001*  | 13.53 (4.42 to 41.44) |
| n-Ch Females 35-44 age range | 40 | 34   |          |                     |
| Ch Females 45-60 age range | 10 | 66   | <0.001*  | 6.6 (2.39 to 11.42)  |
| n-Ch Females 45-60 age range | 13 | 13   |          |                     |
| Ch Males 20-34 age range  | 14   | 68   | <0.001*  | 6.06 (3.21 to 11.42) |
| n-Ch Males 20-34 age range | 121 | 97   |          |                     |
| Ch Males 35-44 age range  | 6    | 68   | <0.001*  | 7.3 (2.81 to 19.01)  |
| n-Ch Males 35-44 age range | 29 | 45   |          |                     |
| Ch Males 45-60 age range  | 9    | 74   | <0.001*  | 5.87 (2.02 to 17.06) |
| n-Ch Males 45-60 age range | 10 | 14   |          |                     |
| **Chewers**              |      |       |          |                     |
| Females                  | 22   | 169   | 0.881    |                     |
| Males                    | 29   | 210   |          |                     |
| Females 20-34 age range  | 8    | 57   | 0.490    |                     |
| Males 20-34 age range    | 14   | 68   |          |                     |
| Females 35-44 age range  | 4    | 46   | 1.000    |                     |
|              | CPI <3 | CPI 3-4 | P value | OR (CI 95%) |
|--------------|--------|---------|---------|-------------|
| Chewer Groups|        |         |         |             |
| BQ chewers   | 27     | 148     |         |             |
| BQ+Tb chewers| 17     | 147     | 0.135   |             |
| Tb chewers   | 7      | 84      |         |             |
| Non-chewers versus: | 302    | 291     |         |             |
| BQ chewers   | 27     | 148     | <0.001* | 5.59 (3.66 to 8.84) |

*P value by Fisher's exact test. * Significant association.

**Table 2. Association among the chewing products and CPI.**

a CPI values were dichotomised in <3 and 3-4 values for test and Fisher's exact test.

b P value by Fisher's exact test. * Significant association.

c Odds ratio with 95% confidence interval (CI).
| Group                        | Count | Total | P-value | OR (CI)  |
|------------------------------|-------|-------|---------|----------|
| BQ+Tb chewers                | 17    | 147   | <0.001* | 8.97 (5.29 to 15.20) |
| Tb chewers                   | 7     | 84    | <0.001* | 12.45 (5.67 to 27.38) |
| Female non-chewers versus:   | 142   | 136   |         |          |
| Female chewers               | 22    | 168   | <0.001* | 7.97 (4.82 to 13.18) |
| Female BQ chewers            | 17    | 143   | <0.001* | 8.78 (5.04 to 15.30) |
| Female BQ+Tb chewers         | 2     | 14    | 0.003*  | 7.31 (1.63 to 32.76) |
| Female Tb chewers            | 3     | 11    | 0.051   | 3.83 (1.05 to 14.02) |
| Male non-chewers versus:     | 160   | 155   |         |          |
| Male chewers                 | 29    | 211   | <0.001* | 7.51 (4.81 to 11.74) |
| Male BQ chewers              | 10    | 5     | 0.293   | 0.52 (0.17 to 1.54)  |
| Male BQ+Tb chewers           | 15    | 133   | <0.001* | 9.15 (5.14 to 16.31) |
| Male Tb chewers              | 4     | 73    | <0.001* | 18.84 (6.72 to 52.79) |

*a* CPI values were dichotomised in <3 and 3-4 values for Odds Ratio (OR), Fisher's exact test and Pearson chi-square test.

*b* P value by Fisher's exact test and Pearson chi-square test. * Significant association.

*c* Odds ratio with 95% confidence interval (CI).
Table 3. Analysis of chewers and non-chewers patients distribution considering oral hygiene status (OHI-S), hygiene frequency, gender, chewing habits and CPI (n=1023). BQ=Betel Quid chewers; BQ+Tb=Betel Quid and Tobacco chewers; Tb= Tobacco chewers.

| CPI and Hygiene by OHI-S | CPI and Hygiene by Frequency |
|-------------------------|-------------------------------|
|                         | CPI <3<sup>a</sup> | CPI 3-4<sup>b</sup> | P value<sup>c</sup> | OD<sup>d</sup> (CI 95%) | CPI <3<sup>a</sup> | CPI 3-4<sup>b</sup> | P value<sup>c</sup> | OD<sup>d</sup> (CI 95%) |
| CPI and Hygiene          |                         |                     |                   |                         |                         |                     |                   |                         |
| OHI-S "fair-poor"       | 300                     | 634                 | P<0.001<sup>*</sup> | 3.11                    | (1.99 to 4.86)          | 269                 | 599                 | P<0.001<sup>*</sup> | 2.63                    | (1.86 to 3.73)          |
|                         |                         |                     |                   |                         |                         |                     |                   |                         |                         |
| OHI-S "excellent-good"  | 53                      | 36                  | P<0.001<sup>*</sup> | 7.27                    | (5.15 to 10.26)         | 44                  | 357                 | P<0.001<sup>*</sup> | 7.54                    | (5.25 to 10.83)         |
|                         |                         |                     |                   |                         |                         |                     |                   |                         |                         |
| CPI and Hygiene          |                         |                     |                   |                         |                         |                     |                   |                         |                         |
| OHI-S "fair-poor"       | 132                     | 289                 | P=0.672           |                         |                         | 121                 | 273                 | P=0.883               |                         |                         |
|                         |                         |                     |                   |                         |                         |                     |                   |                         |                         |
| OHI-S "excellent-good"  | 168                     | 345                 | P=0.089           |                         |                         | 148                 | 326                 | P=0.420               |                         |                         |
|                         |                         |                     |                   |                         |                         |                     |                   |                         |                         |

This article is protected by copyright. All rights reserved.
|                          | Female chewers |     |     | Female non-chewers |     |     |
|--------------------------|----------------|-----|-----|--------------------|-----|-----|
| **OHI-S “fair-poor”**    |                |     |     |                    |     |     |
| Female chewers           | 21             | 166 |     | P < 0.001*         | 7.13| (4.23 to 12.02) |
| Male chewers             | 28             | 206 |     | P < 0.001*         | 7.41| (4.68 to 11.73) |
| Male non-chewers         | 140            | 139 |     | P < 0.001*         | 6.16| (1.2 to 31.61)  |
| **Hygiene “once a day”** |                |     |     |                    |     |     |
| Female chewers           | 19             | 160 |     | P < 0.001*         | 7.6 |     |
| Male chewers             | 25             | 197 |     | P < 0.001*         | 7.51|     |
| Male non-chewers         | 123            | 129 |     | P < 0.001*         | 4.94| (1.96 to 12.43) |
| **OHI-S “excellent-good”** |              |     |     |                    |     |     |
| Female chewers           | 2              | 7   |     | P = 0.027*         | 6.16| (1.2 to 31.61)  |
| Male chewers             | 51             | 29  |     |                    |     |     |
| Male non-chewers         | 77             | 49  |     |                    |     |     |
| **Hygiene twice a day or more** |            |     |     |                    |     |     |
| Female chewers           | 7              | 22  |     | P < 0.001*         | 4.94| (1.96 to 12.43) |
| Male chewers             | 16             | 144 |     | P = 0.146          |     |     |
| Male non-chewers         | 16             | 136 |     | P = 0.458          |     |     |
| **Tb**                   | 7              | 83  |     |                    |     |     |
| **BQ**                   | 26             | 145 |     |                    |     |     |
| **BQ+Tb**                | 16             | 144 |     |                    |     |     |
| **Tb**                   | 7              | 83  |     |                    |     |     |

---

\(^a\) CPI values were dichotomised in <3 and 3-4 values, and OHI-S in “fair-poor” and “excellent-good” for Pearson chi-square test and Fisher's exact test.

\(^b\) P value by Fisher's exact test and Pearson chi-square test. * Significant association; \(^c\) Odds ratio with 95% confidence interval (CI).
Table 4. Analysis of sextants by CPI considering chewers and non chewers patients (n=6138).

| Sextants between chewers and non-chewers | CPI <3<sup>a</sup> | CPI 3-4<sup>a</sup> | P value<sup>b</sup> | OR (CI 95%)<sup>c</sup> |
|-----------------------------------------|--------------------|----------------------|---------------------|------------------------|
| Sextant 1 (chewers)                     | 147                | 282                  | P<0.001*            | 5.07 (3.88 to 6.64)    |
| Sextant 1 (non-chewers)                 | 431                | 163                  |                     |                        |
| Sextant 2 (chewers)                     | 308                | 121                  | P<0.001*            | 5.91 (3.99 to 8.77)    |
| Sextant 2 (non-chewers)                 | 557                | 37                   |                     |                        |
| Sextant 3 (chewers)                     | 127                | 302                  | P<0.001*            | 5.98 (4.55 to 7.86)    |
| Sextant 3 (non-chewers)                 | 425                | 169                  |                     |                        |
| Sextant 4 (chewers)                     | 99                 | 330                  | P<0.001*            | 7.37 (5.55 to 9.79)    |
| Sextant 4 (non-chewers)                 | 409                | 185                  |                     |                        |
| Sextant 5 (chewers)                     | 281                | 148                  | P<0.001*            | 3.94 (2.87 to 5.42)    |
| Sextant 5 (non-chewers)                 | 524                | 70                   |                     |                        |
| Sextant 6 (chewers)                     | 98                 | 331                  | P<0.001*            | 7.41 (5.57 to 9.85)    |
| Sextant 6 (non-chewers)                 | 408                | 186                  |                     |                        |

Dental arcades from chewers

| Maxillary sextants                      | 582                | 708                  | P<0.001*            |                        |
| Mandibular sextants                    | 478                | 812                  |                     |                        |

Dental arcades from non-chewers

| Maxillary sextants                      | 1413               | 366                  | P=0.004*            |                        |
| Mandibular sextants                    | 1341               | 438                  |                     |                        |

<sup>a</sup> CPI values were dichotomised in <3 and 3-4 values for Odds Ratio (OR) and Fisher's exact test.

<sup>b</sup> P value by Fisher's exact test. * Significant association.

<sup>c</sup> Odds ratio with 95% confidence interval (CI).

This article is protected by copyright. All rights reserved.
