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Effect of khat chewing on 14 selected periodontal bacteria in sub- and supragingival plaque of a young male population

N. N. Al-Hebshi, N. Skaug
Department of Odontology–Oral Microbiology, Faculty of Dentistry, University of Bergen, Norway

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Background/aims: The habit of chewing khat (*Catha edulis*) for its amphetamine-like effects is highly prevalent in Yemen and east Africa, and has expanded to Western countries. The purpose of this study was to estimate and compare the prevalence and levels of 14 periodontal bacteria in gingival plaque of khat chewers and khat nonchewers, as well as of khat chewing sides and khat nonchewing sides.
Methods: A total of 408 sub- and supragingival plaque samples were collected from 51 young males (29 khat chewers and 22 khat nonchewers; age range 19–28 years) and analyzed using whole genomic DNA probes and checkerboard DNA–DNA hybridization. Clinical parameters were recorded for all teeth at six sites per tooth.
Results: *Streptococcus intermedius* and *Veillonella parvula* were significantly more prevalent in the subgingival plaque of chewers, which also showed significantly higher levels of *V. parvula* and *Eikenella corrodens.* Similar results were found for the subgingival plaque of the chewing sides compared to the nonchewing sides. However, there was a significantly higher prevalence and higher levels of *Tannerella forsythia* in the subgingival plaque of the nonchewing sides. No significant differences were observed for the supragingival plaque between the two study groups. There was a significantly lower prevalence of *Capnocytophaga gingivalis* and *Fusobacterium nucleatum* in the khat chewing sides, and higher levels of *V. parvula* and *Actinomyces israelii*.
Conclusion: The data suggest that khat chewing induces a microbial profile that is not incompatible with gingival health.

Key words: *Catha edulis*; gingival plaque; khat; microbiology; periodontal bacteria

Nezar Noor Al-Hebshi, Laboratory of Oral Microbiology, Armauer Hansens Hus, N-5021 Bergen, Norway
Tel.: +47 55975784;
fax: +47 55974979;
e-mail: Nezar.Al-hebshi@student.uib.no
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Khat is the name generally used for *Catha edulis*, an evergreen shrub of the plant family *Celastraceae*. It is cultivated but also grows wild in East Africa and Yemen. The fresh leaves and young shoots of khat are habitually chewed for their amphetamine-like effects (10). This habit is prevalent in geographic areas close to where khat grows, particularly Yemen, Somalia, Ethiopia, Djibouti, and Kenya. However, khat is also imported legally or illegally from these areas to Western countries where it is chewed by immigrants from khat-chewing countries. Several million people are estimated to be frequent users of khat (11).

Khat chewing is predominantly a male habit, but women do practice it as well (13). Khat is chewed at social gatherings (khat sessions) where the plant leaves are chewed on one side of the mouth, the juice being swallowed. The residue is retained as a bolus on the inside of the cheek and is ejected at the end of the session (10). This is typical for male khat chewers, for whom the chewing is the main event. At female khat sessions, however, the social gathering is more important than the chewing itself and much smaller quantities are chewed and for shorter periods (13).

Three groups of alkaloids are present in khat: phenylalkylamines, phenylpentylamines, and cathedulins (1). Cathinone

belongs to the first group and is the pharmacologically active constituent of the fresh leaves to which the amphetamine-like effects are attributed (28). Khat also contains tannins and ascorbic acid as well as small amounts of essential oils, sterols, triterpenes, thiamine, riboflavin, niacin, iron and amino acids (16).

There is an extensive literature on khat. Only a few studies, however, have investigated the effect of khat chewing on oral health (2, 8, 9, 17–19, 25). Epidemiologic investigations on a possible association between khat chewing and periodontal diseases have reported conflicting results. Two studies indicated no such detrimental effects and suggested a beneficial influence on the periodontium (8, 9). A larger study concluded that khat chewers had more clinical periodontal attachment loss than had nonchewers, a finding that was significant only for the 12–24 years age group (18). One of these studies showed, in addition, a lower prevalence of caries among khat chewers (8). The effects of khat on the oral mucosa include frictional keratosis (8), genotoxicity (12) and possibly malignancy (2, 19, 25).

The primary function of dental plaque in the etiology of periodontal diseases is well established. Accumulating evidence on the role of specific bacterial species has led to a consensus implicating *Porphyromonas gingivalis*, *Tannerella forsythia* and *Actinobacillus actinomycetemcomitans* as etiologic agents and suggesting that a number of other species may also have an etiologic role (3). Socransky et al. (23) found that these established and putative pathogens as well as other species existed as bacterial complexes in subgingival plaque. According to this, the disease-associated species belong to the so-called red and orange complexes. Bacteria of the other complexes did not show an association with periodontitis and seemed to be compatible with periodontal health. Periodontal pathogens at low levels can also be found in supragingival plaque and their presence there is related to periodontitis (31, 32).

Some oral habits have been shown to influence the microbial composition of gingival plaque and this may explain their influence on the periodontal condition. Cigarette smoking (6) and betel nut chewing (14) are such examples. To our knowledge, the effects of khat chewing on the oral microbiota or the microbial composition of gingival plaque have never been reported. Since khat leaves contain various chemical substances, chewing these may affect oral bacteria directly or indirectly by inducing local environmental changes. The purpose of our study was to assess and compare the prevalence and levels of selected periodontal bacteria in both sub- and supragingival plaque of khat chewers and khat nonchewers as well as of khat chewing sides and khat nonchewing sides.

Material and methods

Study subjects

Twenty-nine male khat chewers (mean age 23.7 ± 2.3 years) and 22 male khat nonchewers (mean age 21.8 ± 2.1 years) were recruited from among dental students and new graduates at the Dental College, University of Science and Technology, Sana'a, Yemen. A habitual khat chewer was defined as a person who chewed khat at least once weekly. Based on chewing frequency, a khat chewer was categorized as a heavy chewer if he chewed khat more than 3 days a week, and as a light chewer if chewed less often. The aims and procedure of the study were explained to each participant and oral consent was obtained. Exclusion criteria included the presence of aggressive periodontitis or necrotizing ulcerative gingivitis, a history of periodontal therapy or use of antibiotics during the previous 3 months, and any systemic condition, e.g. diabetes, known to affect the periodontal status. A questionnaire constructed by one of the authors (N.N.A.) was used to obtain information on oral hygiene practices, the duration and frequency of khat chewing, cigarette smoking and other relevant oral habits.

Clinical monitoring

The plaque index (21), gingival index (15), pocket depth and clinical loss of attachment were recorded for each subject. Measurements were done at six sites (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) for all teeth except third molars. Pocket depth and clinical loss of attachment were measured as the distance from the free gingival margin to the bottom of the pocket and from the cementoenamel junction to the bottom of the pocket, respectively.

Measurements were recorded to the nearest mm using a North Carolina periodontal probe. The clinical monitoring was done by the same examiner (N.N.A.).

Microbiological sampling

For each study subject, the tooth with the highest plaque index that did not have an artificial crown or cervical caries was sampled in each quadrant. Thus, four supra- and four subgingival plaque samples were obtained from each subject. A sterile curette was used to sample as much supragingival plaque as possible from around each tooth. Using sterile cotton pellets, any remaining supragingival plaque was removed before subgingival plaque was similarly sampled. Each plaque sample was transferred into a separate tube containing 150 μl TE buffer (10 mM Tris, 0.1 mM EDTA, pH 7.6). The samples were kept at 4°C until transported to the Laboratory of Oral Microbiology, University of Bergen, Norway, where they were analyzed within 6 months.

Table 1. Probe strains

Red complex*	
Porphyromonas gingivalis	ATCC 33277
Tannerella forsythia	FDC 2008
Orange complex*	
Prevotella intermedia	VPI 4197
Camylobacter rectus	ATCC 33238
Eubacterium nodatum	CCUG 15996
Streptococcus constellatus	ATCC 27823
Peptostreptococcus micros	CCUG 17638
Fusobacterium nucleatum	ATCC 23726
Yellow complex*	
Actinobacillus actinomycetemcomitans	ATCC 33384
Capnocytophaga gingivalis	ATCC 33624
Eikenella corrodens	ATCC 23834
Green complex*	
Streptococcus intermedius	ATCC 27335
Purple complex*	
Veillonella parvula	ATCC 10790
*Actinomyces**	
Actinomyces israelii	CCUG 18307

*Microbial complexes described by Socransky et al. (23).
ATCC: American Type Culture Collection. FDC: Forsyth Dental Center. VPI: Virginia Polytechnic Institute and State University. CCUG: Culture Collection, University of Gothenburg.

Cultivation of probe bacteria

Eight of the 14 selected probe species belong to the red or orange bacterial complexes described by Socransky et al. (23). Each of the other bacterial complexes proposed by these authors (see Table 1) is also represented in our probe panel by at least one species. The 14 bacterial strains used to prepare DNA probes are listed in Table 1. The streptococci species and *Eikenella corrodens* were grown on Colombia blood agar (Acumedia Manufacturers, Lancing, MI). All other strains were cultivated on fastidious anaerobic blood agar (Lab M, Bury, UK) except *T. forsythia* (formerly *Bacteroides forsythus*) and *Campylobacter rectus. T. forsythia* was grown on NAM medium (26) with the addition of 0.2 μg/ml menadion. Wolinella medium (7) supplemented with 1% yeast, 25 μg/ml hemin and 0.1 μg/ml menadion was used for the cultivation of *C. rectus*. Incubations at 35°C for 2–5 days were done aerobically for streptococci, microaerophilically for *E. corrodens, A. actinomycetemcomitans* and *Capnocytophaga gingivalis, and* anaerobically for the remaining strains. The Anoxomat System™ (MART Microbiology BV, the Netherlands) connected to a gas source (80% N_2, 10% H_2 and 10% CO_2) was used to create microaerophilic and anaerobic conditions.

DNA extraction and preparation of whole genomic probes

For each probe, bacteria were harvested from one to three plates and suspended in 1 ml TE buffer. Gram-positive bacteria were pelleted by centrifugation at 16,000 g for 1 min, resuspended in 600 ml TE buffer (pH 8) containing 1 mg/ml lysozyme, and incubated at 37°C overnight. Thereafter, gram-positive and gram-negative strains were treated similarly and DNA was extracted using the method described by Smith et al. (22). The quantity of extracted DNA was determined spectrophotometrically as the absorbance at 260 nm and its purity as the ratio of absorbance at 260 nm and 280 nm (a ratio of 1.8 corresponds to DNA of 100% purity). DNA of at least 90% purity was accepted for probe preparation. Whole genomic DNA probes were prepared from each strain by labeling 1–3 μg DNA with digoxigenin (DIG-high prime, Roche Diagnostic, Basel, Switzerland) using the random primer technique (4).

Sample preparation and checkerboard DNA–DNA hybridization

A total of 408 sub- and supragingival plaque samples were analyzed by checkerboard DNA–DNA hybridization (24). In brief, plaque samples were lysed and vacuum filtered onto a 15 × 15 cm positively charged nylon membrane using the 'Minislot-30' device (Immunetics, Cambridge, MA). Two pooled standards containing 1 ng and 10 ng DNA (corresponding to 10^5 and 10^6 bacteria, respectively) of each probe species were included in each membrane. The membranes were allowed to dry at room temperature and samples were fixed by exposure to 70 mJ/cm^2 of UV light. The membranes were prehybridized and then hybridized with the digoxigenin-labeled whole genomic DNA probes using the 'Miniblot 45' device (Immunetics). Hybrids were detected by chemiluminescence as described by Wall-Manning et al. (30) except that skim milk was used instead of casein in the blocking solution and the stringency washes were performed in SDS buffer (0.1% SSC, 0.1% SDS). Signals from DNA hybrids were read as spot darkness, i.e. a figure between 0 (pure black) and 255 (pure white), using the Jasc PAINT SHOP PRO 8 image software (Jasc Software Inc., USA). The readings were adjusted by subtracting the signal of the adjacent background. In this way, false-positive and false-negative spot readings due to varying background darkness along the probe lanes were avoided. Adjusted readings were compared with those of the two pooled DNA standards (see above) and scored as $\sim 10^5$, 10^5–10^6 and $\sim 10^6$ bacterial cells. The latter two scores were used to demonstrate presence at high levels.

Statistical analysis

Data on the prevalence of each test species, expressed as the number of sites (0–4) colonized by each species, were available for each study subject. This was averaged across subjects to obtain the mean prevalence (mean number of sites colonized) of each species in both study groups. The significance of differences between the two groups regarding the prevalence of each bacterial species in sub- and separately supragingival plaque samples were determined using ordinal logistic regression analysis with khat and smoking as covariates to adjust for a possible effect of cigarette smoking. Similarly, among khat chewers, the mean number of sites colonized by each species was calculated for the khat chewing and khat nonchewing sides and the significance of differences was assessed by the Wilcoxon signed rank test. All the analyses were repeated after the exclusion of signals scoring $\sim 10^5$ bacterial cells to test for significant differences in the prevalence of each species at high levels. These were described as differences in the levels of species between the two groups/sides. For probe species that showed a significant association with khat chewing, light chewers and heavy chewers were examined separately and differences in prevalence and levels of those species among the three groups (heavy chewers, light chewers and nonchewers) were estimated using ordinal logistic regression analysis as described above to demonstrate the effect of khat chewing frequency (dose–response).

Table 2. Clinical characteristics of the study groups (mean ± SD)

	Chewers ($n = 28$)	Nonchewers ($n = 22$)
Age (years)**	23.7 ± 2.3	21.8 ± 2.1
Smokers (%)***	52	14
Plaque index	0.80 ± 0.32	1.00 ± 0.48
Gingival index	1.14 ± 0.20	1.19 ± 0.23
Pocket depth (mm)	1.66 ± 0.18	1.72 ± 0.19
Clinical attachment loss (mm)	0.05 ± 0.07	0.04 ± 0.05

**$P < 0.01$, Mann-Whitney test. ***$P < 0.005$, Chi-squared test.

Table 3. Clinical parameters of khat chewing and khat nonchewing sides (mean ± SD)

	Chewing sides	Nonchewing sides
Plaque index	0.79 ± 0.32	0.82 ± 0.32
Gingival index	1.17 ± 0.24	1.13 ± 0.19
Pocket depth (mm)*	1.62 ± 0.20	1.68 ± 0.18
Clinical attachment loss (mm)	0.06 ± 0.09	0.03 ± 0.05

*$P < 0.05$, Wilcoxon signed ranks test.

P-values ≤ 0.05 were considered statistically significant.

Results

Clinical characteristics

The clinical characteristics of the two study groups and of the two study sides are presented in Tables 2 and 3, respectively. The study groups did not differ significantly in any of the clinical parameters measured. However, smoking was significantly ($P = 0.007$) more prevalent in the khat chewers, who also had a significantly ($P = 0.005$) higher mean age. The khat chewing sides showed a slightly but significantly ($P = 0.01$) lower mean pocket depth than did the khat nonchewing sides. Sixty-eight percent of the khat chewers used their left side for khat chewing ($P = 0.015$).

Microbial findings

All probe bacteria were detected in both sub- and supragingival plaque. *Actinomyces israelii* was the most prevalent species in both habitats. *Veillonella parvula, Fusobacterium nucleatum* and *E. corrodens* were also among the species commonly detected. Compared to the subgingival plaque, the supragingival plaque showed a significantly higher prevalence of *C. gingivalis* and significantly higher levels of this species and *V. parvula*. *T. forsythia* and *F. nucleatum* were significantly more prevalent and at significantly higher levels in the subgingival plaque, in which a significantly higher level of *Peptostreptococcus micros* was also found.

Subgingival plaque

Figure 1 shows the mean prevalence and levels of each species in the subgingival plaque of the two study groups and the two study sides. There were no significant differences for most of the probe species including the red and orange complex species and *A. actinomycetemcomitans*. However, a few significant differences did exist. The khat chewers had a significantly higher prevalence of *Streptococcus intermedius* ($P = 0.002$) and *V. parvula* ($P = 0.01$), and significantly higher levels of *V. parvula* ($P = 0.036$) and *E. corrodens* ($P = 0.037$). Consistently, there was a significantly higher prevalence of *S. intermedius* ($P = 0.014$), *V. parvula* ($P = 0.027$), and *E. corrodens* ($P = 0.01$) in the chewing sides, but a significantly lower prevalence and levels ($P = 0.018$ and $P = 0.008$, respectively) of *T. forsythia*.

Figure 2 presents the mean prevalence and levels of *V. parvula, S. intermedius* and *E. corrodens* in the subgingival plaque of the khat nonchewers, light chewers and heavy chewers. All three species demonstrated a khat chewing frequency-dependent response. This was most pronounced with *V. parvula* with respect to both prevalence and bacterial levels. For *S. intermedius* and *E. corrodens*, the khat chewing frequency-dependent response was valid only for prevalence and bacterial level, respectively. Regression results showed that only heavy chewing explained the significant differences for these three species between khat chewers and nonchewers.

Supragingival plaque

The mean prevalence and levels of each species in the supragingival plaque of the two study groups and the two study sides are presented in Fig. 3. Neither the prevalence nor the levels of any probe species differed significantly between the study groups. The chewing sides, however, showed significantly higher levels of *V. parvula* and *A. israelii* ($P = 0.01$ and $P = 0.007$, respectively), and the khat nonchewing sides had significantly higher prevalence of *C. gingivalis* and *F. nucleatum* ($P = 0.003$ and $P = 0.045$, respectively).

Discussion

To the best of our knowledge, this is the first publication assessing the possible effects of khat chewing on oral bacteria. The aim was to examine whether habitual khat chewing influenced colonization of gingival plaque. To answer this question, we assessed and compared the prevalence and levels of 14 selected periodontal bacteria, associated with periodontal diseases or periodontal health, in sub- and supragingival plaque of khat chewers and khat nonchewers. In addition, since khat chewers use predominantly the same side of the mouth for khat chewing, comparison of the microbiota in the khat chewing and khat nonchewing sides could also be performed, constituting a study model similar to the split-mouth technique. We found that 68% of the khat chewers used their left side for khat chewing. This finding is very similar to that reported by Hill et al. (8).

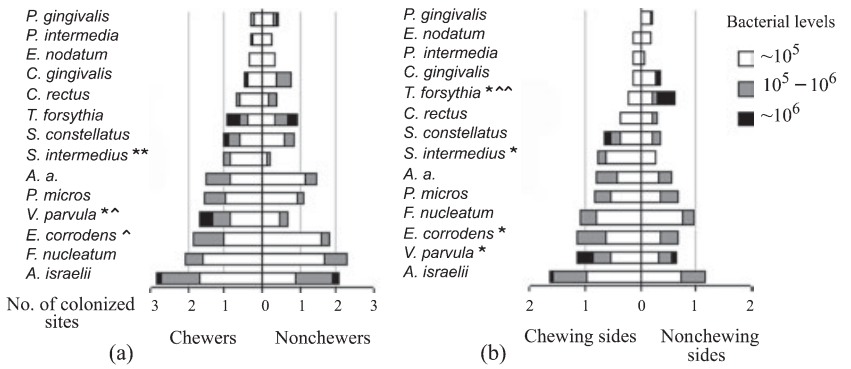

Fig. 1. Stacked bars of the mean prevalence and levels of 14 periodontal bacteria in subgingival plaque of (a) khat chewers and khat nonchewers, and (b) khat chewing sides and khat nonchewing sides. *****$P < 0.05$ and ******$P < 0.01$ for differences in mean prevalence. ^$P < 0.05$ and ^^$P < 0.01$ for differences in levels. A.a.: *Actinobacillus actinomycetemcomitans*.

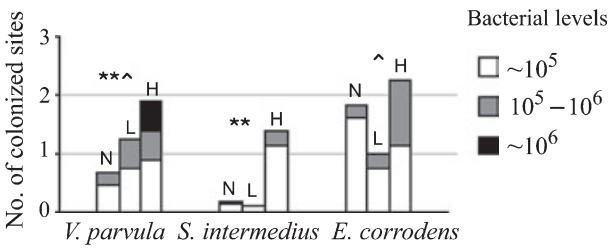

Fig. 2. Stacked and clustered bars of the mean prevalence and levels of three species in subgingival plaque of nonchewers (N), light chewers (L) and heavy chewers (H). ******$P < 0.01$ for differences in mean prevalence. ^$P < 0.05$ for differences in levels.

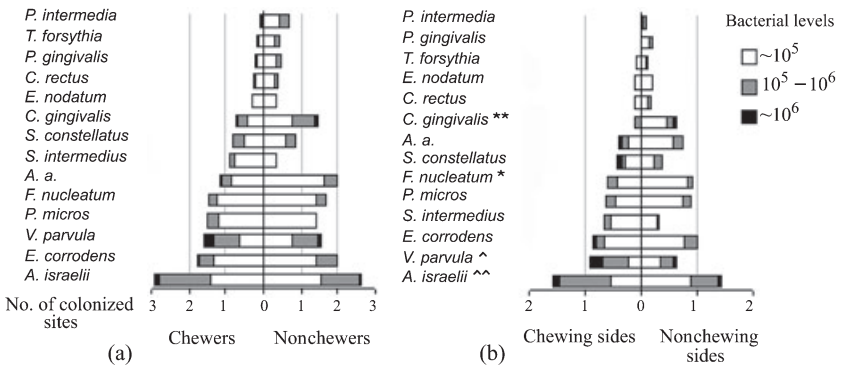

Fig. 3. Stacked bars of the mean prevalence and levels of 14 periodontal bacteria in supragingival plaque of (a) khat chewers and khat nonchewers, and (b) khat chewing sides and khat nonchewing sides. $^{*}P < 0.05$ and $^{**}P < 0.01$ for differences in mean prevalence. $^{\wedge}P < 0.05$ and $^{\wedge\wedge}P < 0.01$ for differences in levels. A.a.: *Actinobacillus actinomycetemcomitans*.

To minimize possible effects of socioeconomic differences, food habits and age-related periodontitis on the results, the influence of khat chewing on the gingival microbiota was studied in a young population consisting of dental students and recently graduated dentists. A previous study on the microbial effect of cigarette smoking used periodontally healthy young study subjects to avoid the effect of age-related periodontitis on the gingival microbiota (20). Haffajee et al. (6) demonstrated that cigarette smoking has a significant effect on colonization patterns of subgingival plaque only in pockets < 4 mm, suggesting the difficulty of demonstrating the effect of smoking (and probably other oral habits) in deeper pockets.

Khat chewing is typically a male habit, which is why previous studies were conducted on exclusively male populations (8, 12). Furthermore, the exclusion of females from our study was necessary because it would have been very difficult to recruit a matching number of young female habitual khat chewers as there are relatively few in Yemen. They are not willing to expose themselves by participating in a study on khat chewing because their habit is not socially accepted.

There was a very high association between khat chewing and cigarette smoking in our study subjects. Such an association is very important when studying the relationship between khat chewing and periodontitis or mucosal lesions since cigarette smoking is a strong risk factor for both (27). Due to the small sample size and few smokers among the khat nonchewers, possible interactions between khat chewing and smoking could not be tested in our study.

The general microbial findings of the present investigation are in accordance with those from previous studies that found detectable levels of established and putative periodontal pathogens in both sub- and supragingival plaque from periodontally healthy subjects (5, 31). According to those studies, *Actinomyces* species were the most prevalent in both habitats, which is also consistent with our findings. The presence of *T. forsythia* at high levels in subgingival plaque of some subjects in both study groups indicates that sites harboring this species may be at risk of developing periodontal disease (5). Tanner et al. (26) reported that *T. forsythia* is a prominent member of subgingival plaque in initial periodontal lesions in subjects with minimal attachment loss.

Khat chewing did not significantly increase the prevalence or levels of any of the examined periodontal pathogens even without adjustment for cigarette smoking. In a number of recent studies, cigarette smoking has been shown to increase the prevalence and/or levels of many of these bacteria (6, 20, 29, 33). Interestingly, compared with the khat nonchewing sides, the khat chewing sides showed a significantly lower prevalence and levels of *T. forsythia* (a red complex member) in the subgingival plaque and a significantly lower prevalence of *F. nucleatum* (an orange complex member) in the supragingival plaque.

Three species in subgingival plaque showed a strong association with khat chewing. *V. parvula*, *S. intermedius* and *E. corrodens* were significantly more prevalent and/or at significantly higher levels in the subgingival plaque of the khat chewers than of the khat nonchewers and of the khat chewing sides compared to the khat nonchewing sides. A significant dose–response relationship further supported this association. These bacteria were, in many studies, shown to be among the species compatible with periodontal health (5, 23). On the other hand, there is also some evidence to suggest that *S. intermedius* and *E. corrodens* may play a role in periodontal diseases (3). An association between khat chewing and health compatible species can also be concluded from the presence of significantly higher levels of *V. parvula* and *A. israelii* in the supragingival plaque of the khat chewing sides.

The two study groups did not differ significantly in any clinical parameter. This is consistent with previous findings (9), but not with the report of Mengel et al. (18), which found that in a 12–24 years age group, the khat chewers had significantly more attachment loss than the khat nonchewers. However, they also showed that the khat chewing sides demonstrated lower pocket depth compared to the khat nonchewing sides, which is in agreement with findings of the current study as well as of a previous one (8). This may, at least, be explained by the current finding that the subgingival plaque of khat nonchewing sides harbored significantly higher levels of *T. forsythia*.

In conclusion, khat chewing does not seem to increase the colonization of gingival plaque by periodontal pathogens that would have represented a risk factor for periodontal disease. It seems instead to favor the presence of species that are compatible with periodontal health. The microbiological and clinical findings suggest that khat chewing has no detrimental periodontal effects. *In vitro* studies are needed to elucidate the biological basis for the microbial shifts produced by khat.

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