Comparison of oral Candida species prevalence and carriage among gutka-chewers and betel-quid chewers
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Abstract

Objective: To compare prevalence and carriage of Candida species among gutka-chewers and betel-quid-chewers.
Methods: The cross-sectional case-control study was conducted between January and December, 2015 at the Oral Surgery department of Abbasi Shaheed Hospital and the Dental department of Jinnah Postgraduate Medical Centre, Karachi, and comprised oral yeast samples of gutka-chewers, betel-quid-chewers, and non-chewers. A standardised questionnaire was used to gather demographic data and oral hygiene maintenance information. Oral Candida strains were collected, cultured and identified using standard techniques and yeast identification system. In all groups, unstimulated whole saliva flow rate was determined. Lesions on the tongue and oral mucosa were clinically investigated and numbers of missing teeth were recorded. SPSS 20 was used for data analysis.
Results: Of the total 185 samples, 50(27%) were from gutka-chewers, 50(27%)betel-quid-chewers, and 85(46%) non-chewers. Oral Candida carriage was comparable among betel-quid-chewers (18 [36%]) and gutka-chewers (20 [40%]), but it was significantly higher than the non-chewers (11 [12.9%]) (p<0.05). Candida species were isolated from 45 (90%) of gutka-chewers and 45 (90%) of BQ-chewers. Among the groups, Candida albicans was the most commonly isolated yeast species (38% in gutka-chewers and 12.9% non-chewers). Mean numbers of missing teeth were significantly higher among BQ-chewers (6.8±0.4 teeth [range: 5-10]) (p<0.01) and gutka-chewers 6.8±0.6 teeth (range: 5-10) (p<0.01) than non-chewers (2.2±0.3 teeth [range: 0-3 teeth]). There was no significant difference in unstimulated whole saliva flow rate and the number of missing teeth among gutka-chewers and betel-quid-chewers (p>0.05).

Conclusion: Prevalence and carriage of Candida species were comparable between betel-quid-chewers and gutka-chewers compared to non-chewers.

Keywords: Lime-piper betel-quid, Candida, Prevalence, Tobacco, Smokeless. (JPMA 67: 350; 2017)
The current study was planned to compare prevalence and carriage of Candida species among gutka-chewers and BQ-chewers.

**Subjects and Methods**

The cross-sectional case-control study was conducted between January and December, 2015 at the Oral Surgery department of Abbasi Shaheed Hospital and the Dental department of Jinnah Postgraduate Medical Centre, Karachi, and comprised oral yeast samples of gutka-chewers, betel-quid-chewers, and non-chewers. The study was approved by the research ethics review committee of Karachi Medical and Dental College (KMDC) and Abbasi Shaheed Hospital. Written consent was obtained from all participants.

Smokers, AN-chewers, tobacco-free BQ-chewers, subjects with systemic diseases, including cardiovascular disorders, hepatitis B and C, diabetes and acquired immune deficiency syndrome (AIDS), denture wearers and individuals who had taken antifungals and antibiotics within the preceding 3 months were excluded. Individuals chewing a minimum of one gutka sachet for a minimum of one year were defined as gutka-chewers. Individuals consuming at least 1 BQ daily since a minimum of one year were included as BQ-chewers. Individuals with no history of using any type of tobacco products were categorised as non-chewers.

Information regarding age and gender from gutka-chewers, BQ-chewers and non-chewers was gathered using a standardised questionnaire. Duration and daily frequency of BQ and gutka-chewing, duration of gutka and BQ placement in the mouth (in minutes), intra-oral site of gutka or BQ placement, tooth-brushing frequency, brushing of tongue and oral rinsing after chewing tobacco was also gathered.

The subjects were refrained from eating or drinking 2 hours prior to clinical appointment for data collection and unstimulated whole saliva (UWS) samples were collected. For collection of UWS samples, participants were instructed to pool saliva intra-orally for 5 minutes following which they expectorated saliva into a gauged measuring cylinder. UWS samples were collected at early morning hours between 8 am and 9.30 am and the unstimulated whole salivary flow rate (UWSFR) was recorded in millilitres per minute (mL/min). Samples of oral Candida were collected as described in an earlier study. Scraping of the tongue and buccal mucosa with a cotton swab (sterile) (Biome´rieux S.A., Montalieu-Vercieu, France) was utilised to collect microbiological samples. After sampling, swabs were replaced in containment tubes immediately.

Sabouraud dextrose agar at 37°C was used to culture Candida species. For assessment of yeast growth, the cultures were examined until seven days of incubation. A yeast identification system (API 32-C System bioMériux, Lyon, France) was utilised, but in case of non-identification of yeast, molecular identification was performed. Deoxyribonucleic acid (DNA) isolation was performed by suspension of yeast cells in 200μl sterile Polymerase Chain Reaction (PCR) water. Using MagNA pure (Roche Diagnostics GmbH, Mannheim, Germany), genomic DNA was prepared. DNA sequencing and PCR analysis was performed, the details of which have been presented in a previous study.

The clinical diagnosis of tongue lesions was performed using the World Health Organisation (WHO) criteria. Other mucosal lesions, including white and red lesions, along with number of missing teeth were also assessed and recorded clinically. Simple descriptive statistical tests were used in the form of percentage using SPSS 20. Kruskal-Wallis test was performed to see if there were differences in mean age, UWSFR and number of missing teeth between gutka-chewers, BQ-chewers and controls. Fisher’s exact test was used to determine the significant differences in the prevalence rates of oral candida carriage between the groups. For multiple comparisons, Bonferroni Post-hoc test was used. $P<0.05$ was considered statistically significant.

**Results**

Of the total 185 samples, 50(27%) were from gutka-chewers, 50(27%)betel-quid-chewers, and 85(46%) non-chewers. Most of the participants 160(86.4%) were male. The mean ages of non-chewers (44.6±0.8 years), BQ-chewers (44.4±2.7 years) and gutka-chewers (40.7±4.1 years) were statistically similar ($p>0.05$). On average, gutka-chewers consumed 4±1.5 packets of gutka daily since 10.5±2.3 years (range 3-14 years); and BQ-chewers reported chewing 6.3±0.5 quids daily for 9.3±3.5 years (range: 7-12 years). Gutka-chewers and BQ-chewers reported to place these products in the buccal vestibule for an average duration of 7.1±0.5 minutes (range: 5-15 minutes) and 7.5±1.1 minutes (range: 5-20 minutes), respectively. Mouth rinsing after consumption of ST products was performed by 22% of BQ-chewers and 16% of gutka-chewers (Table-1).

Besides, 92% gutka-chewers, 84% BQ-chewers and 68.2% non-chewers brushed their teeth once daily. In all study groups, none of the participants brushed the dorsum of their tongue during daily tooth brushing. There was no statistically significant difference in the mean UWSFR among BQ-chewers (0.56±0.1 mL/min [range: 0.5-0.6

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In general, Candida species were isolated from 80% and 74% of gutka-chewers and BQ-chewers respectively. Candida albicans was the most commonly isolated yeast species (38% in gutka-chewers and 12.9% non-chewers). Candida species prevalence and carriage among gutka-chewers and BQ-chewers was statistically comparable (Table-2) (p=0.12). No oral mucosal lesions were found in non-chewers, gutka-chewers and BQ-chewers. The mean numbers of missing teeth were significantly higher among BQ-chewers (6.8±0.4 teeth [range: 5-10]) (p<0.01) and gutka-chewers 6.8±0.6 teeth (range: 5-10) (p<0.01) compared to non-chewers (2.2±0.3 teeth [range: 0-3 teeth]). There was no statistically significant difference in the number of missing teeth among BQ-chewers (6.8±0.4 teeth [range: 5-10]) and gutka-chewers (6.8±0.6 [range: 5-10]) teeth (p=0.11).

**Discussion**

In the present investigation, the most common Candida species isolated from all groups was C. albicans, which is in accordance with previous studies.1,8 The second most common species isolated from 20% of both gutka-chewers and 20% BQ-chewers was C. parapsilosis. This result is in contrast to the study by Reichart et al.23 in which C. parapsilosis was isolated from 44% of BQ-chewers. In addition, in the study by Reichart et al.23 C. parapsilosis was the most commonly isolated Candida species. To date, there is a shortage of studies that have focused on the prevalence of oral yeasts in gutka and BQ chewing populations. It is therefore difficult to estimate a
The precise prevalence of oral Candida carriage among gutka-chewers and BQ-chewers.

In the present study Candida carriage was significantly higher in ST chewers (gutka-chewers and BQ-chewers) than non-chewers. These results are in contrast with a previous study, which reported comparable levels of oral Candida carriage among ST product users than controls. An explanation for this may be derived from the fact that the duration of gutka-chewing habit was ~10 years compared to the study by Javed et al. in which chewers had been using ST products for relatively shorter durations (~4 years).

Interestingly, in the present study oral Candida carriage and species prevalence was comparable between gutka-chewers and BQ-chewers. One explanation could be associated with the duration of intra-oral gutka or BQ placement and frequency of ST consumption. In the current study, the mean duration of gutka-chewing and BQ-chewing was ~10 years and ~9 years respectively. In addition, on average gutka-chewers reported consuming 4 gutka sachets daily; whereas BQ-chewers reported chewing ~6 betel-quids daily. Furthermore, both gutka-chewers and BQ-chewers were placing gutka and quid in their buccal vestibule for approximately 7 minutes. It is therefore speculated that the quantity of ST consumed by gutka-chewers and BQ-chewers was similar. This may be the possible cause for the comparable oral Candida carriage among gutka and BQ-chewers.

A poor oral hygiene status and reduced salivary flow rate have been associated with an increased oral Candida carriage. In the present study, UWSFR among gutka-chewers and BQ-chewers was comparable. This factor may also possibly explain why oral Candida carriage among gutka-chewers and BQ-chewers was also comparable. Muzurovic et al. assessed the relationship between oral hygiene and oral Candida colonisation. The results showed that poor oral hygiene (increased plaque index, oral hygiene index and dental calculus index) was significantly associated with an increased oral Candida carriage, predominantly C. albicans. Likewise, in a recent clinical study, periodontal parameters among gutka-chewers and BQ-chewers was also comparable.

In the present study, more than 80% of the tobacco chewers (gutka and BQ) brushed their teeth once daily and nearly 20% in either group reported to rinse their mouth with water after consuming their respective forms of ST products (gutka or BQ). This suggests that gutka-chewers and BQ-chewers overall had poor oral hygiene. Thus, the contribution of poor oral hygiene in Candida prevalence and carriage in the present study subjects cannot be disregarded.

Self-reported tobacco habits, relatively young study population and short history and duration of ST product consumption are among the limitations of the present study. It is therefore hypothesised that oral Candida carriage may differ among elderly individuals using more than one form of ST (for example gutka and BQ) and placing such products for prolonged durations in the mouth compared to the population assessed in the present study. Further studies are warranted to test this hypothesis.

Conclusion

The prevalence and carriage of oral Candida species was similar in gutka-chewers and BQ-chewers, suggesting that individuals chewing either forms of ST are susceptible to oral Candida infections than non-chewers.

Acknowledgement

We are grateful to the Deanship of Scientific Research at King Saud University for its support.

Disclaimer: None.

Conflict of Interest: None.

Funding Source: The authors extend their sincere appreciations to Deanship of Scientific Research at King Saud University for its funding of this prolific research group (PRG-1437-38).

References


