ORIGINAL ARTICLE

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

Tariq Abduljabbar,[1] Mudassir Hussain,[2] Tariq Adnan,[3] Fahim Vohra,[4] Fawad Javed[5]

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 salivary 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 salivary 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)

Introduction

Chewing tobacco which may include gutka and/or betel-quid (BQ) is a common oral habit in South Asian countries, including Pakistan, Bangladesh, Sri Lanka and India.[1-5] Gutka is a kind of smokeless tobacco (ST) containing a blend of slaked lime, powdered tobacco and ground areca-nut (AN).[5,6] BQ is a mixture of areca-nut, lime and ground tobacco enveloped in a Piper betleleaf.[7,8] In addition, components such as saffron, artificial fragrances and menthol are common both in BQ and gutka. Gutka is available in sachets with net weight of 3.5gm and comprises 7% water; whereas BQ contains 70% water and an approximate weight of 4gm (1.14 gm of tobacco).[9] Therefore, individuals chewing BQ consume less dry weight of tobacco and AN than gutka-chewers.

Oral yeasts species (particularly Candida albicans [C. albicans]) are a part of normal oral flora; and the prevalence of oral Candida among healthy humans ranges between 40% and 60%. However, under opportunistic conditions, such as immunosuppression and tobacco chewing, these fungi become opportunistic pathogens.[10-12] It is recognised that oral Candida carriage in habitual tobacco consumers is higher than non-tobacco users.[8,11,13] A possible explanation for this association is the presence of nicotine and hydrocarbons such as polycyclic aromatic hydrocarbons in ST, acting as nutrients for oral yeasts and intern facilitating their growth.[12]

Studies[8,11] have reported that prevalence and carriage of oral Candida is higher in BQ-chewers and gutka-chewers than non-chewers, but none of the studies has compared oral Candida carriage and species prevalence between BQ-chewers and gutka-chewers. Since BQ-chewers consume less dry weight of powdered tobacco than gutka-chewers,[9] it is hypothesised that(a) oral Candida carriage is higher in gutka-chewers than BQ-chewers, and (b) oral Candida species prevalence also differs among gutka-chewers and BQ-chewers. The

[1,4]Prosthetic Dental Science, College of Dentistry, King Saud University, Riyadh, Saudi Arabia, [2]Oral Surgery, [3]Community Medicine, Karachi Medical and Dental College, Karachi, Pakistan, [5]Department of General Dentistry, Eastman Institute for Oral Health, University of Rochester, NY, USA.

Correspondence: Tariq Abduljabbar. Email: academicksa1@gmail.com

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.[14] 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.[10] 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.[10] After sampling, swabs were replaced in containment tubes immediately.

Sabouraud dextrose agar at 37°C was used to culture Candida species.[15,16] 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.[17] DNA sequencing and PCR analysis was performed, the details of which have been presented in a previous study.[11]

The clinical diagnosis of tongue lesions was performed using the World Health Organisation (WHO) criteria.[18-22] 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

Table-1: Features of the study cohort.

	All chewers	Gutka-chewers	Betel-quid chewers	Non-chewers
Number of subjects	100	50	50	85
Gender (male)	80	39	41	80
Mean±SD age in years (range)	42.5±3.6 (33-60)	40.7±4.1 (33-51)	44.4±2.7 (35-60)	44.6±0.8 (31-54)
Mean±SD period of gutka chewing habit (years - range)	10.5±2.3 (3-14)	10.5±1.8 (3-14)	-	-
Mean±SD period of betel-quid chewing habit (years - range)	9.3±3.5 (7-12)	-	9.3±3.5 (7-12)	-
Mean±SD numbers of gutka sachets consumed daily (range)	4±1.5 (2-6)	4±1.5 (2-6)	-	-
Mean±SD numbers of betel-quids consumed daily (range)	6.3±0.5 (1-8)	-	6.3±0.5 (1-8)	-
Mean±SD period of intra-oral gutka placement (minutes-range)	7.1±0.5 (5-15)	7.1±0.5 (5-15)	-	-
Mean±SD period of intra-oral betel-quid placement (minutes-range)	7.5±1.1 (5-20)	-	7.5±1.1(5-20)	-
Daily oral hygiene maintenance				
Tooth brushing (once daily) (%)	88 (88%)	46 (92%)	42 (84%)	58 (68.2%)
Tooth brushing (twice daily)(%)	12 (12%)	4 (8%)	8 (16%)	27 (31.8%)
Tongue brushing after tooth brushing (%)	0	0	0	0
Rinsing the mouth with water after betel-quid consumption (%)	19 (38%)	8 (16%)	11 (22%)	-

Table-2: Oral candida species isolated from individuals chewing betel-quid with and without tobacco and non-chewers.

	All chewers (n=100) n (%)	Gutka-chewers (n=50) n (%)	Betel-quid chewers (n=50) n (%)	Non-chewers (n=85) n (%)
Candida albicans	38 (38%)*	20 (40%)†	18 (36%)‡	11 (12.9%)
Candida tropicalis	7 (7%)	4 (8%)	3 (6%)	5 (5.8%)
Candida albicans + Candida tropicalis	20 (20%)*	8 (16%)†	12 (24%)‡	5 (5.8%)
Candida parapsilosis§	20 (20%)*	10 (20%)†	10 (20%)‡	5 (5.8%)
Candida albicans + Candida parapsilosis§	5 (5%)	3 (6%)	2 (4%)	—
Candida krusei§	—	—	—	—
Candida luscitanie§	—	—	—	—
Candida glabrata§	—	—	—	—
Candida gullerimondi§	—	—	—	—
No candida species isolated	10(10%)*	5 (10%)†	5 (10%)‡	59 (69.4%)

*With Fisher's exact test, a significant difference was found when compared with non-chewers (P<0.05)
† With Fisher's exact test, a significant difference was found when compared with non-chewers (P<0.05)
‡ With Fisher's exact test, a significant difference was found when compared with non-chewers (P<0.05)
§These Candida species were investigated using polymerase chain reaction.

mL/min]) (p=0.17) and gutka-chewers (0.57±0.1 mL/min [range: 0.5-0.6 mL/min]) (p=0.14) compared to controls (0.56mL/min [range: 0.5-0.6 mL/min]).

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

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,[11] 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.[11] 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.[10,24] 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.[24] 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.[24] Likewise, in a recent clinical study, periodontal parameters among gutka-chewers and BQ-chewers was found to be comparable.[25] 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.

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