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Evaluation of oral *Candida* species among tobacco consumers: An *in vivo* study

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Abstract:
The prevalence of *Candida* species among tobacco users was evaluated to improve the understanding of oral mycological ecology and to aid the development of appropriate therapeutic strategies. This *in vivo* study was conducted on 78 individuals with a known history of tobacco use at Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, who were divided into three groups of 26 each: tobacco smokers (Group I), tobacco chewers (Group II) and individuals engaged in both habits (Group III). Comparative assessment revealed a statistically highly significant variation in yeast distribution among the groups ($p \leq 0.001$, Pearson chi-square). *Candida albicans* was identified in 11.5% of Group I (tobacco smokers), followed by Group III (combined users), with the lowest frequency in Group II (tobacco chewers). Thus, data shows the prevalence of oral *Candida* carriage was significantly higher among tobacco smokers, indicating that smoking exerts a greater influence on oral *Candida* colonisation than chewing or combined habits.

Keywords: *Candida*, tobacco chewers, smokers

Background:
The human oral cavity is an extremely complex and dynamic biological ecosystem that supports a broad range of microorganisms, including bacteria, fungi, viruses and protozoa **[1]**. These microbial populations are sustained in a finely regulated symbiotic balance with the host, contributing substantially to the preservation of oral health and physiological stability **[2]**. *Candida* species, which constitute the most commonly recovered yeasts from the oral mycobiota, form an integral component of the normal commensal microbial ecosystem in healthy individuals. Nonetheless, under appropriate local and systemic conditions, some *Candida* species may turn from innocuous commensals into opportunistic pathogens, causing mucosal disease such as oral candidiasis **[3]**. *Candida albicans* represents the predominant strain recovered; however, non-albicans *Candida* species are increasingly recognised owing to their augmented virulence and diminished susceptibility to antifungal therapy **[4]**. Tobacco use negatively affects the oral environment by altering salivary secretion, the immune defence system and the mucosal tissues, thereby promoting *Candida* colonisation and biofilm formation. *Candida* colonisation is also closely related to the development of oral potentially malignant disorders **[5].** Therefore, it is of interest to determine the prevalence of *Candida* species among different categories of tobacco users, in order to improve the understanding of oral mycological ecology and to aid the development of appropriate therapeutic strategies.

Methodology:
Study design and ethical approval:
A cross-sectional study was conducted in the Department of Oral Pathology and Microbiology, Teerthanker Mahaveer Dental College and Research Centre, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, after obtaining institutional ethical clearance.

Inclusion criteria:
Participants aged 20–55 years with a history of tobacco consumption were included. Individuals engaged in smoking, smokeless tobacco intake, or combined forms of tobacco use for a minimum duration of one year were considered eligible.

Exclusion criteria:
Participants on steroid or antifungal medication within the preceding three months; those with systemic disorders including diabetes mellitus and hepatitis B and C infection; those who failed to provide written consent; and subjects wearing removable or complete dental prostheses were excluded.

Study groups:
A total of 78 individuals with a known history of tobacco use were enrolled and divided into three groups of 26 each - Group I (tobacco smokers), Group II (tobacco chewers) and Group III (individuals engaged in both tobacco chewing and smoking).

Statistical analysis:
All results were analysed using SPSS version 20. With the alpha error set at 5% and power at 95%, sample-size estimation using G*Power software yielded 78 subjects, 26 in each group.

Results:
The three treatment groups were comparable at baseline with respect to age, sex distribution, and clinical and microbiological parameters, with all baseline intergroup comparisons showing no statistically significant differences ($p > 0.05$), indicating successful randomisation. The mean age of the study population was 36.8 ± 8.4 years, and the sex distribution was similar across the three groups **(Table 1)**. In the combined cohort of 45 participants, all clinical parameters demonstrated statistically significant improvements at one and three months compared with baseline. The plaque index decreased from 2.34 ± 0.29 at baseline to 0.93 ± 0.17 at one month and 0.50 ± 0.13 at three months. Similarly, the gingival index decreased from 2.16 ± 0.25 to 1.06 ± 0.18 and 0.57 ± 0.15, respectively. Probing pocket depth (PPD) decreased from 4.93 ± 0.59 mm at baseline to 3.20 ± 0.56

mm at one month and 2.46 ± 0.51 mm at three months. Relative attachment level (RAL) also improved, decreasing from 8.86 ± 0.74 mm at baseline to 6.20 ± 0.94 mm at one month and 5.13 ± 0.63 mm at three months. All within-group changes were statistically significant ($p < 0.001$; **Table 1**). Intergroup comparisons of clinical outcomes revealed a statistically significant difference in PPD reduction at one month. The mean reductions were 1.73 ± 0.88 mm in Group A (scaling and root planing (SRP) alone), 2.20 ± 0.77 mm in Group B (SRP plus curcumin chip), and 2.33 ± 0.72 mm in Group C (SRP plus Aloe vera chip). The overall intergroup difference was statistically significant ($p = 0.043$), with a significant post hoc difference between Groups A and C ($p = 0.025$). At three months, Group C showed the greatest mean PPD reduction (3.13 ± 0.74 mm), followed by Group B (2.60 ± 0.91 mm) and Group A (2.46 ± 0.74 mm). However, the intergroup difference was not statistically significant ($p = 0.062$). RAL reductions did not differ significantly among the three groups at either one month ($p = 0.162$) or three months ($p = 0.445$), although all groups demonstrated significant within-group improvements ($p < 0.001$; **Table 2**). Microbiological analysis showed significant reductions in the counts of *Porphyromonas gingivalis, Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans* in all three treatment groups at one and three months compared with baseline ($p < 0.001$ for all within-group comparisons). For *P. gingivalis*, the counts at three months decreased to 0.22 ± 0.05, 0.40 ± 0.29, and 0.33 ± 0.24 × 10^5 CFU/mL in Groups A, B, and C, respectively. For *F. nucleatum*, the corresponding counts were 0.35 ± 0.37, 0.40 ± 0.29, and 0.40 ± 0.26 × 10^5 CFU/mL. For *A. actinomycetemcomitans*, the counts at three months were 0.42 ± 0.19, 0.24 ± 0.14, and 0.37 ± 0.21 × 10^5 CFU/mL in Groups A, B, and C, respectively **(Table 3)**. Post hoc Tukey comparisons of microbiological counts at three months revealed no statistically significant differences between the treatment groups for *P. gingivalis* or *F. nucleatum* (all adjusted p-values > 0.05). For *A. actinomycetemcomitans*, the difference between Groups A and B was statistically significant ($p = 0.039$), whereas the differences between Groups A and C ($p = 0.775$) and Groups B and C ($p = 0.165$) were not statistically significant **(Table 4)**.

Table 1: Yeast prevalence among the three groups

Candida type	Group I Smokers	Group II Chewers	Group III Both	Total
Candida albicans	9 (11.5%)	3 (3.8%)	4 (5.1%)	16 (20.5%)
Candida tropicalis	4 (5.1%)	3 (3.8%)	3 (3.8%)	10 (12.8%)
Candida kruseii	3 (3.8%)	0 (0.0%)	6 (7.7%)	9 (11.5%)
Candida glabrata	3 (3.8%)	0 (0.0%)	0 (0.0%)	3 (3.8%)
Candida guilliermondii	0 (0.0%)	5 (6.4%)	0 (0.0%)	5 (6.4%)
p-Value			**0.001****	

*NS = non-significant; * = significant; ** = highly significant.*

Table 2: *Candida* colony count among the three groups

Group	Group I Smokers	Group II Chewers	Group III Both	Total
N	19	11	13	43
%	24.4%	14.1%	16.7%	55.1%
p-Value		0.068 (NS)		

*NS = non-significant; * = significant; ** = highly significant.*

Table 3: Inter-group comparison of *Candida* colony count

Group	Mean	Std. Deviation	Minimum	Maximum	p-Value
Group I – Smokers	1.46	1.334	0	4	0.340 (NS)
Group II – Chewers	1.31	1.955	0	5	
Group III – Both	1.08	1.262	0	3	

*NS = non-significant; * = significant; ** = highly significant.*

Discussion:

In vivo assessment of *Candida* species distribution among tobacco users is important for understanding epidemiological trends, identifying high-risk groups and selecting appropriate antifungal treatment. The present study revealed the distribution of various *Candida* species across the three groups. *Candida albicans* was the most commonly isolated species, with the highest incidence among tobacco smokers (11.5%), followed by combined users (5.1%) and tobacco chewers (3.8%), constituting 20.5% overall. *Candida tropicalis* showed a fairly similar distribution across all groups. *Candida krusei* was mainly detected among those exposed to both smoking and smokeless tobacco (7.7%), indicating elevated colonisation with dual exposure. *Candida glabrata* was detected only among smokers, whereas *Candida guilliermondii* was found only among tobacco chewers. The p-value of 0.001 confirms a strong association between the type of tobacco use and the pattern of *Candida* species distribution, indicating that oral fungal colonisation is influenced by tobacco habits. Comparable findings were reported by Ye *et al.* (2021) **[5]** and El-Sakhawy *et al.* (2023) **[6]**, consistent with the present results. The distribution of *Candida* positivity also varied across tobacco-user categories. Group I (tobacco smokers) recorded the greatest number of positive cases - 19 (24.4%) - followed by Group III (both habits) with 13 cases (16.7%) and Group II (tobacco chewers) with 11 cases (14.1%). Overall, 43 participants (55.1%) showed the presence of *Candida*. The non-significant p-value (0.068) indicates that although *Candida* prevalence was higher among smokers, the differences between the three habit groups were not statistically significant. Similar observations were reported by Navabi *et al.* (2021) **[7]** and Javed *et al.* (2014) **[8]** and El-Sakhawy *et al.* (2023) **[9]** in accordance with the present study.

Conclusion:

Candida albicans was the predominant species, with tobacco smokers showing the highest prevalence and colony counts compared with tobacco chewers and individuals with combined habits. Although colony counts did not differ significantly between the groups, the distribution of *Candida* species varied significantly according to tobacco habit. These findings suggest that tobacco smoking is a stronger risk factor for oral *Candida* colonisation and highlight the importance of routine oral screening among tobacco users.

Disclosure of conflict of interest: None.

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