

Comparative study of *Candida* in oral submucous fibrosis and healthy individuals

Estudo comparativo de *Candida* em fibrose submucosa oral e indivíduos saudáveis

Abstract

Purpose: Oral submucous fibrosis (OSMF) is a high-risk precancerous condition that predominantly affects Indian youngsters due to the habit of gutkha chewing. *Candida* may play a role in the etiopathogenesis of premalignant and malignant lesions. The aim of this study is to compare the incidence, intensity, and species of *Candida* found in OSMF patients and healthy individuals.

Methods: This study included 20 OSMF patients and 20 healthy controls. A detailed history of each patient was recorded along with a clinical examination. Samples were collected with the oral rinse technique and cultured on Sabouraud's agar medium. The isolated yeast species were counted and identified based on Gram staining, a germ tube test, chlamydospore formation and a sugar assimilation test.

Results: In total, 40% of OSMF patients and 15% of healthy controls yielded *Candida* organisms on culture. *C. albicans* was the predominant species isolated, but *C. krusei* and *C. tropicalis* were also identified. Gender, gutkha habit and clinical staging had no influence on the candidal carriage in OSMF patients.

Conclusion: The incidence and intensity of *Candida* (primarily *C. albicans*) was greater in OSMF patients than in healthy controls, but these findings were within the normal limit (3-47%). Therefore, *Candida* may not be an etiologic factor in malignant transformation. However, controversy still exists over whether the chewing of betel quid in cases of OSMF has an inhibitory effect or promotes the adherence and invasion of *Candida*.

Key words: *Candida*; gutkha chewing; oral carriage; oral submucous fibrosis

Resumo

Objetivo: Fibrose submucosa oral (FSO) é uma condição pré-maligna de alto risco que predominantemente afeta jovens da Índia devido ao hábito de mascar 'gutkha'. *Candida* pode ter um papel importante na etiopatogenia de lesões pré-malignas e malignas. O objetivo deste estudo foi comparar a incidência, intensidade e *Candida* spp encontradas em pacientes com FSO e em indivíduos saudáveis.

Metodologia: Este estudo incluiu 20 pacientes com FSO e 20 controles saudáveis. Obteve-se de cada sujeito uma história detalhada e exame clínico. As amostras foram coletadas com uma técnica de enxágue bucal e cultivadas em meio Agar Sabouraud. As espécies isoladas foram contadas e identificadas com base em coloração Gram, teste de tubo de ensaio e teste de assimilação de açúcar.

Resultados: No total, 40% dos pacientes com FSO e 15% dos controles saudáveis apresentaram resultado positivo de cultura para *Candida*. *C. albicans* foi a espécie predominante isolada, mas *C. krusei* e *C. tropicalis* também foram identificados. O sexo, hábito de mascar 'gutkha' e estadiamento clínico não influenciaram a presença de fungos nos pacientes com FSO.

Conclusão: A incidência e intensidade de *Candida* (primariamente *C. albicans*) foi maior nos pacientes com FSO que nos sujeitos controle, mas estes achados estavam dentro dos limites normais (3-47%). Portanto, *Candida* pode não ser um fator etiológico na transformação maligna. Entretanto, ainda há controvérsias se o hábito de mascar 'betel' em casos de FSO teria um efeito inibidor ou promoveria a aderência e invasão de *Candida*.

Palavras-chave: *Candida*; gutkha; fibrose submucosa oral

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Introduction

Candida species cause common oral fungal infections in human beings (1). The presence of *Candida* in the mouth together with epithelial changes may predispose individuals to candidal infection (2). Epithelial changes of the oral mucosa, such as atrophy, hyperplasia, and dysplasia, may compromise the mucosal barrier and facilitate candidal invasion, especially in the event of epithelial atrophy (3).

Candida albicans is the primary cause of oral candidiasis. These opportunistic fungal pathogens may colonize, invade and induce lesions in any part of the oral cavity in both immunocompetent and immunocompromised individuals. However, those who are immunocompromised are affected at a significantly higher frequency than healthy individuals (4). As a result of increasing numbers of immunocompromised individuals within the human population, the incidence of *Candida* infections has increased dramatically in the last decade (5).

Cannon et al. have suggested that *Candida* colonization of oral surfaces, including the denture-fitting surface, can serve as a reservoir for disseminated infections such as aspirate pneumonia and gastrointestinal infections (6). *C. albicans* is the predominant species isolated in premalignancy and carcinoma. Candidal infection can induce epithelial atypia and lead to malignant transformation through the release of chemical carcinogens like nitrosamine compounds (2). Similarly, *Candida* could play a role in malignant transformation in OSMF, but this process has not been confirmed.

Recently, oral submucous fibrosis (OSMF) has been identified as a high-risk precancerous condition that affects young Indians due to their habit of gutkha chewing (7,8). Several predisposing factors may be present in patients with OSMF, but epithelial atrophy is considered one of the key features of OSMF. Decreased mouth opening may predispose an individual to candidal growth, and this *Candida* can further predispose the mucosa for malignant transformation through the process of nitrosation (2,9). The aim of the present study was to determine the incidence, intensity and species of *Candida* present in the oral cavity of oral submucous fibrosis patients and healthy controls.

Methods

The study included a total of 40 male and female participants. Patients were selected from among those visiting the outpatient department of oral medicine and radiology at SDM Dental College and Hospital in Dharwad. The study protocol was approved by the institutional ethics committee.

The study group included twenty cases of oral submucous fibrosis and twenty age- and gender-matched healthy controls. Informed consent was obtained from all participants prior to inclusion in the study. A detailed clinical history was obtained from each participant and clinical staging was noted using the criteria established by Ranganathan et al. (10). OSMF patients with systemic illnesses like diabetes were excluded from the study.

Microbiology

The oral rinse technique described by Samaranayake et al. (11) was used to collect samples. Subjects in both groups were asked to rinse their mouth with 10 mL of phosphate buffered saline (PBS) for 2 min and expectorate into a sterile container. The sample was immediately transported to the laboratory where it was centrifuged at 2,500 g for 10 min. The pellet was resuspended in PBS; 100 μ L of this solution were plated onto Sabouraud's dextrose agar and incubated for 48 h at 37°C. Colony forming units resembling yeast growth were removed from the plates and processed further for identification using Gram staining, a germ tube test, chlamyospore formation and sugar assimilation tests (3). The number of yeast colonies was counted and expressed as colony-forming units per milliliter (CFU/mL) of the collected sample. To facilitate the differentiation of *C. albicans* and *C. dubliniensis*, we investigated the growth at 45°C on modified Sabouraud glucose agar (SGA).

Cytology

Buccal smears were obtained from both groups and stained using the periodic acid schiff (PAS) technique. The stained smears were examined under a microscope (original magnification X40). The presence of *Candida* yeast cells or yeast cells and pseudohyphae was recorded as a positive finding and classified as follows: group 1 = none, group 2 = yeast cells only, group 3 = a few yeast cells and pseudohyphae (2-3 cells), and group 4 = many yeast cells and pseudohyphae.

Statistical analysis

Group analyses were performed with the chi-square test and the differences between two independent samples were analyzed with the Student's *t*-test. A *P*-value < 0.05 was considered statistically significant.

Results

The mean age of both groups was 26.7 years, and 90% of the participants were male. Of the 20 individuals with OSMF, 18 (90%) chewed gutkha (areca nut and tobacco) and only 2 (10%) chewed areca nut alone. The average mouth opening of patients with OSMF was 19.75 mm.

In total, 40% (8/20) of the OSMF patients and 15% (3/20) of the controls yielded *Candida* organisms on culture. However, the difference between the two groups was not statistically significant ($\chi^2=3.135$; *P*=0.770) (Table 1).

Table 1. Presence of *Candida* in the OSMF and control groups.

Groups	No. of cases	Frequency of <i>Candida</i> isolation	χ^2	<i>P</i> -value*
Control Group	20	3 (15%)	3.135	0.077
OSMF Group	20	(40%)		

* Chi square test

The mean counts of *Candida* colonies in the OSMF and control groups were 721.25 CFU/mL and 186.7 CFU/mL, respectively. However, the difference between the two groups was not statistically significant ($t=5.6030$; $P=0.0053$) (Table 2).

Gender had no influence on the yeast carriage in the mouth ($\chi^2=0.0930$, $P=0.761$) (Fig. 1). The habit of betel chewing (Fig. 2) and clinical staging (Fig. 3) had no influence on the candidal carriage status of OSMF patients. Cytological buccal smears detected no yeast cells or pseudohyphae in either group.

Three different candidal species were isolated from the OSMF group (*C. albicans*, *C. krusei* and *C. tropicalis*) and two species were isolated from the control group (*C. albicans* and *C. tropicalis*). *Candida albicans* was the most common isolate in OSMF patients ($n=7$, 87.5%), while *Candida krusei* (37.5%) and *Candida tropicalis* (12.5%) were less common (Fig. 4). In addition, two species were simultaneously present in three OSMF patients; *C. albicans* and *C. krusei* were present in two patients, and *C. albicans* and *C. tropicalis* were present in one patient.

Table 2. Intensity of *Candida* in the OSMF and control groups.

Group	Range CFU/mL	Mean CFU/mL	SD	t-value	P-value*
Control group	60-320	186.6667	130.1281	-5.6030	0.0053
OSMF group	500-970	721.2500	143.8687		

* Student's t-test

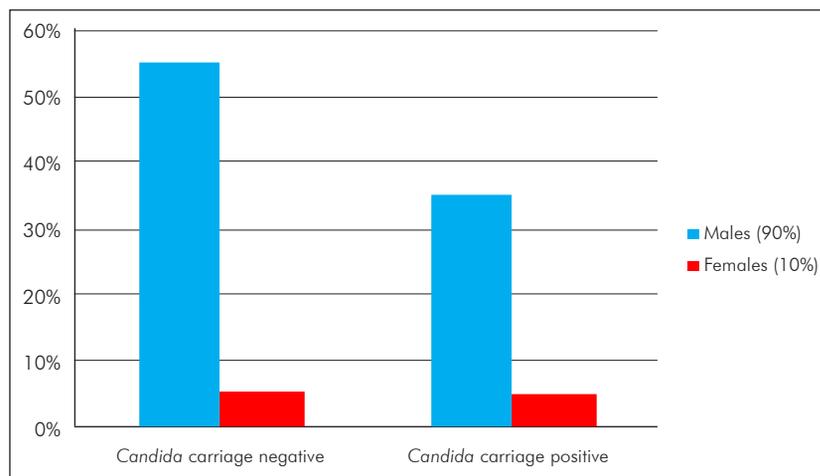


Fig. 1. Gender distribution and candidal carriage in patients with oral submucous fibrosis.

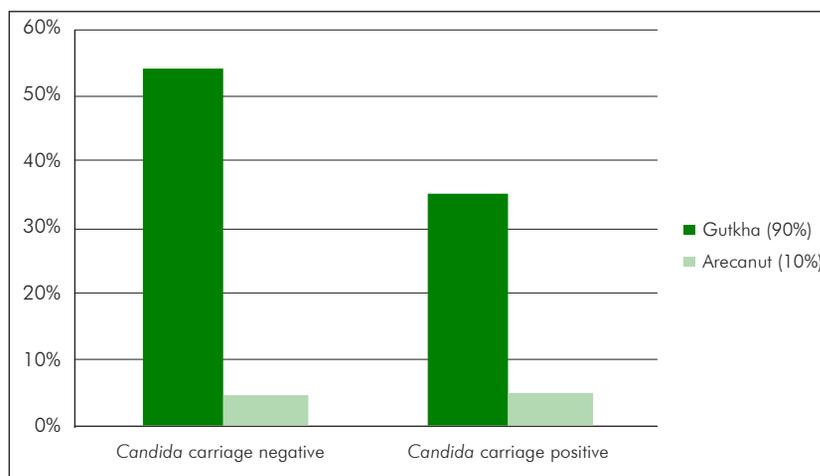


Fig. 2. Chewing habit and candidal carriage in patients with oral submucous fibrosis.

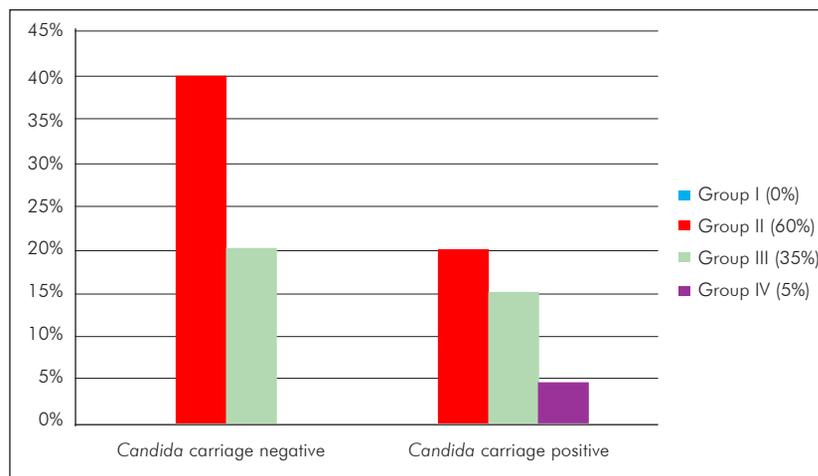


Fig. 3. Clinical staging and candidal carriage in patients with oral submucous fibrosis.

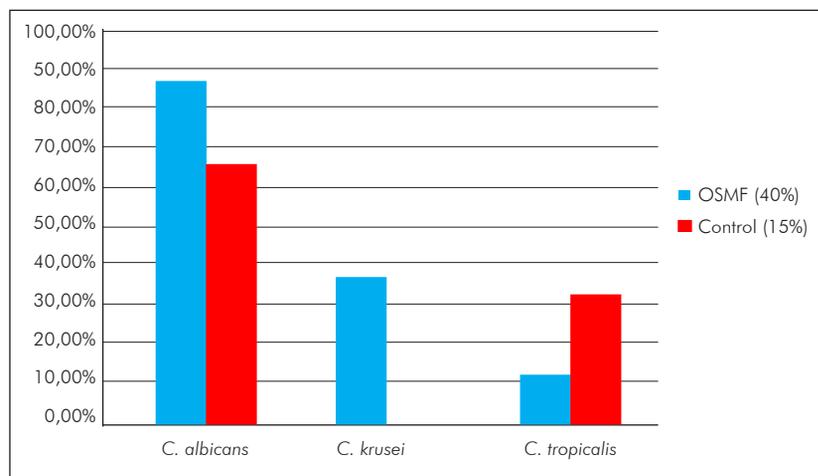


Fig. 4. Distribution of *Candida* species in OSMF and control group.

Discussion

Healthy individuals carry 3-47% of *Candida* species as a component of normal oral flora (11). Oral yeasts are known to be associated with systemic and localized oral disease. The predominant species is *Candida albicans*, which has the potential to infect any tissue within the body (6). Other *Candida* species that have been isolated are *C. krusei*, *C. guilliermondii* and *C. tropicalis* (12).

An association between *Candida* and various pre-cancerous and cancerous lesions has been reported in the literature (13-15). *Candida* has also been reported in cases of lichen planus, and its prevalence has increased in lichen planus patients undergoing topical steroid therapy (16).

Studies of various populations have recently linked the use of betel quid to the presence of candidal species. Reichart et al. has studied oral candidal species in betel quid chewers and identified associated oral lesions in Cambodian females. *Candida albicans* was the most commonly isolated species

and *C. tropicalis* was the second most isolated species. A single isolate of the newly described *C. dubliniensis* was found in the control population (17).

In another study conducted in isolated populations of Padaung, *C. parapsilosis* was the most common *Candida* species isolated from both betel quid chewers (46%) and non-chewers (44%). *Candida albicans* was identified in 24% of betel quid chewers and 18% of non-chewers (18).

A study by Ariyawardana et al. sought to determine the prevalence of oral yeast in OSMF patients and healthy individuals. *Candida* was isolated from 63.6% of the test group and 50% of the control group. This study also reported *C. dubliniensis* for the first time in both groups (19). However, none of these studies revealed statistically significant differences in the prevalence of *Candida* species between the two groups.

Our study revealed a higher candidal prevalence in OSMF patients (40%) when compared to a control group (15%), and mean scores of candidal growth were also higher in OSMF patients than controls; however there was no statistically

significant difference between the two groups. The results of the present study were similar to those presented by Ariyawardana et al. (19). In addition, *C. albicans* was the predominant species isolated (87.5%) in OSMF patients in this study; other identified species were *C. krusei* and *C. tropicalis* (Fig. 4).

We also isolated two species of *Candida* at the same time in 3 of the 20 OSMF patients. Most people carry a single strain of *Candida* at different body sites for a long time. For instance *C. albicans* is most prevalent on the palate, tongue and gingiva. However, this study shows that a few individuals may harbor more than one strain or species of *Candida* at the same time. This observation suggests that several ecological niches exist in the oral cavity that harbor different *Candida* species. However, this occurs more commonly in hospitalized and immunocompromised patients (14,20).

In this study, mouth opening was included in the assessment of clinical staging, and gutkha was most commonly chewed by these patients; gutkha is known to cause early clinical symptoms and early malignant transformation (tobacco is one component of gutkha). However, this study found no correlation between candidal carriage and gender, gutkha habit or clinical staging in these OSMF patients.

In order to proliferate in the oral cavity, yeast cells must adhere to oral surfaces; otherwise, they are washed away by salivary flows. One of the most important factors affecting the virulence of *Candida* species is therefore their ability to use a variety of mechanisms to anchor to a site and colonize (20). In betel quid chewers and OSMF patients, the constant chewing of betel quid increases the salivary flow rate and should lead to the loss of superficial colonization and *Candida* growth. In addition, the hydrophilic solvents present in betel quid have inhibitory effects and lead to a decreased oral carriage rate of *Candida* (17). The slaked lime content of gutkha creates an alkaline pH in the oral cavity; this pH is not favorable for *Candida* growth because *Candida* is best able to adhere to epithelial cells at an acidic pH. All of these factors could explain the low candidal carriage found in OSMF and betel quid chewers. Our study therefore shows similar results as previous studies.

In contrast, Reichart et al. reported that the chemical constituents of betel quid have no overall effect on the oral carriage of *Candida* and do not preferentially affect the specific *Candida* species present in the oral environment. Although betel quid has some antibacterial effects, it does not affect oral *Candida* species (17).

Oral yeast species carry a significant risk of malignant transformation. There appears to be a statistically significant relationship between histologically determined fungal infection and epithelial dysplasia; about 21.9% of candidal infected lesions showed more severe epithelial dysplasia (2,13). The possible association between

Candida species and oral neoplasia was first reported in the 1960s, and later reports suggested a link between the oral mucosal presence of *C. albicans* and the development of oral squamous cell carcinoma (13). Field et al. (1989), after reviewing evidence for the role of *Candida* in oral epithelial neoplasia, postulated that nitrosamine compounds produced by *Candida* species may directly, or in concert with other chemical carcinogens, activate specific proto-oncogenes and thus initiate the development of a malignant lesion (21).

Oral submucous fibrosis has a significant mortality rate as it is a premalignant condition and malignant transformation has been noticed in 2.3-7.6% of cases (22). As the oral mucosa is compromised in OSMF, it can be argued that the presence of *Candida* may predispose the individual to candidal infection and invasion, although our results were not statistically significant.

OSMF does not regress spontaneously or on cessation of gutkha chewing. Once the disease is present, it either persists or becomes more severe with the involvement of additional areas of the oral mucosa. There is currently no successful treatment available for OSMF. Local and systemic applications of glucocorticoids and placental extracts and the intralesional injection of hyaluronidase (which breaks down the components of connective tissue) have been used for OSF therapy (23). Muzyka and Glick surmised that corticosteroids lower host resistance to *Candida* by suppressing both nonspecific inflammatory responses and cell-mediated immunity (24). *Candida* colonization that is not clinically evident may be easily overlooked, leading to ineffective treatment of the underlying lesions. It is therefore beneficial to examine the yeast cultures in the oral lesions of patients during prolonged steroid therapy. Until now very few papers have focused on the prevalence of oral *Candida* in OSMF patients. Therefore the present study made an attempt to observe the presence and intensity of *Candida* in OSMF patients.

Conclusions

A higher incidence and intensity of *Candida* was observed in OSMF patients when compared to healthy individuals, but these findings were within normal limits (3-47%). The predominant species isolated was *C. albicans*. *Candida* may not be an etiologic factor in malignant transformation in OSMF patients. However, controversy still exists over whether betel quid chewing in OSMF inhibits or promotes the adherence and invasion of *Candida*. Further research with larger sample sizes is warranted to elucidate the factors that may predispose individuals with OSMF to oral candidal colonization. Phenotypic characteristics may endow certain *Candida* species or strains with a competitive advantage in the oral cavity.

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