

Relationship of Clinical Features with Candidal Carriage in Oral Submucous Fibrosis Patients: A Case-control Study

Abstract

Background and Objectives: Oral submucous fibrosis (OSF) is extensively prevalent in India and South-East Asia owing to the habit of arecanut (AN) use. Epithelial atrophy, hyposalivation, and immune alterations in OSF may predispose to increased Candidal carriage. Stomatopyrosis in OSF can result from multiple causes, which may also include Candidal infection. Hence, this study is aimed to assess Candidal carriage, species characterization, salivary flow rate (SFR) and its relationship with the clinical features (stomatopyrosis and mouth opening [MO]) in OSF patients along with the response to antifungal treatment in patients with higher Candidal carriage. **Methodology:** In this case-control study, 60 OSF patients and 30 age- and sex-matched control subjects were enrolled. SFR was assessed using modified Schirmer test. Samples for Candidal assessment were collected with the oral rinse technique and cultured. The isolated yeast species were counted and identified based on Gram staining, germ tube test, and CHROMagar. Data were analyzed with Chi-square test, Pearson's correlation test, and one-way ANOVA test. **Results:** The distribution of mean visual analog scale (VAS) score, SFR and MO was significantly varied ($P < 0.001$) in the study and control groups. *Candida* was found to be present significantly ($P = 0.048$) in OSF group as compared to control group. *Candida albicans* was the predominant species. No statistically significant association was obtained regarding Candidal isolation and SFR, burning sensation and MO in OSF patients. Only 1 patient in the study group yielded a high Candidal carriage (>400 CFU/mL) and reported relief in burning sensation (VAS score) with antifungal therapy. **Conclusions:** OSF patients yielded a significant higher oral Candidal carriage. Although it was not found to be associated directly, its role as a "cause and effect" in SFR and clinical features (stomatopyrosis and MO) of OSF cannot be ignored.

Keywords: *Areca, arecoline, Candida, oral submucous fibrosis, salivary flow rate, stomatopyrosis*

Introduction

A plethora of factors such as genetic, social, occupational, and deleterious lifestyle practices such as smoking, use of smokeless tobacco (SLT), and alcohol abuse, may affect the health of an individual that may reflect in the oral cavity, which is often referred to as the "mirror of the body." Increased practice of SLT habit in the Indian subcontinent and southeast Asia, due to its association with socio-cultural customs, has resulted in various potentially malignant disorders (PMDs) such as leukoplakia and oral submucous fibrosis (OSF). In particular, OSF has shown an alarming rise in its prevalence over the past four decades, from 0.03% to 6.42% in India.^[1]

OSF is classified into different stages, groups, and grades by various researchers

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based on clinical and histological features. It can affect any age/gender. The etiopathogenesis of OSF is not clearly understood but considered as a disease of collagen metabolism with multiple speculated etiological factors ranging from chilies, nutritional deficiency, genetic polymorphism and auto-immune disease.^[2] Recent studies have confirmed that areca nut (AN) is a major causative agent for OSF. AN alkaloid, arecoline can cause fibroblastic proliferation and increased collagen formation leading to the formation of fibrous bands, which ultimately cause reduced mouth opening (MO). Stomatopyrosis, xerostomia, and mucosal rigidity are other clinical features.^[3,4]

Mucosal alterations in OSF such as the tough, leathery, fibrosed oral mucosa, decreased host local immunity, impaired oral hygiene maintenance consequent to restricted MO can provide a favorable

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environment for increased Candidal carriage. Moreover, Candidal infection may play a role in the causation of stomatopyrosis (oral mucosal burning), which is the most common symptom in OSF that prompts an individual to approach an oral physician.

It is a well-known fact that OSF is a preventable PMD with a malignant transformation rate of 7%–30%.^[5] It has been proven that Candidal infection initiates malignant transformation by directly producing carcinogenic nitrosamines or by converting pro-carcinogens into carcinogenic compounds.^[6] Candidal infection may also cause chronic inflammation in the subjacent oral mucosa. Activated innate immune cells in chronic inflammation produce cytokines and chemokines stimulating tumor initiation, growth, and progression, thus, increasing the risk of malignancy. Among the different Candidal species studied, *Candida albicans* is most commonly isolated and is reported to possess maximum carcinogenicity.^[7,8] Hence, identification of higher Candidal carriage and the predominant species of *Candida* in OSF may help in improving the overall prognosis of the disease.

Hence this study was planned to assess Candidal carriage, characterize the Candidal species, and associate the same with salivary flow rate (SFR) and symptoms of OSF patients. Furthermore, this study intended to assess any reduction in burning sensation after antifungal drugs in higher Candidal carriage OSF patients. The null hypothesis states that adopting antifungal agents as adjuvant management in OSF may not decrease oral Candidal loads and may not provide any relief to oral mucosal burning.

Methodology

After obtaining Ethical clearance (Letter no.-GDCRI/ACM (2)/05/2017–2018), the study was conducted in the department of oral medicine and radiology with the collaboration of department of microbiology. Based on the published literature,^[9] *Candida* present in OSF patients was 80%. Assuming 10% absolute difference, 90% power and 5% level of significance, the sample size was estimated to be 60 patients. Hence, on the basis of set inclusion and exclusion criteria, study included 60 OSF patients (study group) and 30 healthy individuals (control group). Controls were further subdivided equally (15 in each group) as Habituees and nonhabituees. The study group consisted of newly diagnosed OSF patients with the age range of 18–50 years with clinical diagnosis as per Pindborg's classification (1989)^[10] [Figure 1a-c] and data pertaining to the presence of stomatopyrosis, maximum MO and SFR was obtained. Patients >50 years were not included in this study as they tend to have undiagnosed comorbid conditions, reduced salivary flow.^[11] Besides, extremes of age is a predisposing factor for oropharyngeal candidiasis.^[12]

Patients with systemic diseases (diabetes, hematinic deficiencies, etc.,) immunocompromised state, salivary

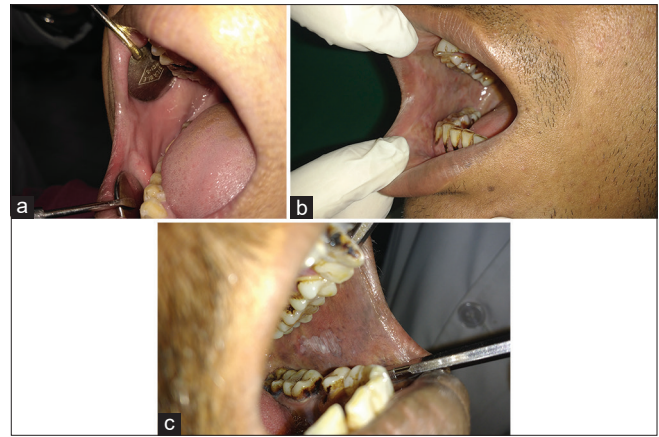


Figure 1: (a) Clinical appearance of buccal mucosa in Stage I oral submucous fibrosis patient. (b) Clinical appearance of buccal mucosa in Stage II oral submucous fibrosis patient. (c) Clinical appearance of buccal mucosa in Stage III oral submucous fibrosis patient

gland disorders/other systemic or local conditions that may affect the salivary flow (like the use of anti-cholinergics, diuretics, anti-histaminics, anti-hypertensives, and psychoactive substances) were excluded. Patients with a history of radiotherapy or trauma to head and neck region or reduced MO due to other causes, antibiotic, antifungal, or steroid usage in the past 1 month and having any other pathological diseases like leukoplakia not associated with OSF in the oral cavity were also excluded, alongwith, pregnant, lactating and denture wearers. All the included patients were explained the details of the study in their known local language, and written informed consent was obtained.

Visual analog scale (VAS) score was obtained regarding stomatopyrosis for the patients who reported burning sensation. MO (in mm) was recorded three times using Digital Vernier caliper (INSIZE CO., LTD; with a resolution of 0.005 mm) and the average of the three recordings was documented. SFR assessment for the unstimulated saliva was carried out between 9.00 a.m. and 12.00 noon to avoid diurnal variation. Subjects were requested not to eat, drink, or perform oral hygiene methods or chew or smoke 60 min before and during the entire procedure. A commercially available 5 mm × 35 mm Schirmer tear test strip (Madhu Instruments, Delhi, India) having a mm scale (0–35 mm) delineating the amount of fluid flow was used. The patient was then asked to swallow all the saliva in the mouth before the test and not to swallow anymore during the test and instructed to rest the tongue on the hard palate so that the modified Schirmer test (MST) strip would not touch the tongue during the test. The distance traveled by saliva up the strip in 3 min were recorded [Figure 2]^[13-15] and an average of three consecutive readings was taken.

To ascertain the oral Candidal carriage, patients were asked to rinse their mouth with 10 ml of phosphate-buffered



Figure 2: Method for assessment of salivary flow rate using Schirmer strip

saline (PBS) (AVICHEM, Avinash Chemicals, Bangalore) for 2 min and expectorate into a sterile container. The obtained sample was immediately and adequately transported to the laboratory in an icebox, where it was centrifuged at 2500 rpm for 10 min to remove the supernatants, and the deposits were re-suspended in PBS.^[9] A known volume (40 microlitre-volume of inoculating loop) of this concentrate was inoculated onto Sabouraud's Dextrose Agar media (HiMedia Laboratories, Mumbai) and incubated at 37° for 24–48 h to get quantitative culture of *Candida*. The appearance of white to cream colored and smooth growths signifies candidal growth [Figure 3a and b]. Obtained colonies were counted by the manual method and expressed in colony-forming units per mL (CFU/mL).^[9] Small inoculums from these colonies were subjected to the Germ Tube test, Gram staining, and CHROMagar (HiMedia Laboratories, Mumbai) [Figures 4 and 5]. The inoculated plates were incubated at 37°C and observed up to 7 days. *Candida* species recognition was done based on the morphology of the colonies and color produced.^[16] All the patients were managed according to standard treatment protocol for OSF. The patients who had high Candidal carriage (>400 CFU/ml) as per Epstein *et al.*,^[17] were initially treated with antifungal therapy i.e., Tablet Fluconazole 200 mg on 1st day, followed by 100 mg for 13 days and thereafter managed with standard protocol. After antifungal therapy, burning sensation was re-evaluated.

Data were entered in Microsoft Excel and analyzed using SPSS (Statistical Package for Social Science, version 10.0.5) package. The results were averaged for continuous data, and the number and percentage for dichotomous data are presented in Tables and Figures. Chi-square test was applied to sets of categorical data to evaluate how likely it is that any observed difference between the sets arose by chance. One-way ANOVA was used to compare means of two or more samples.

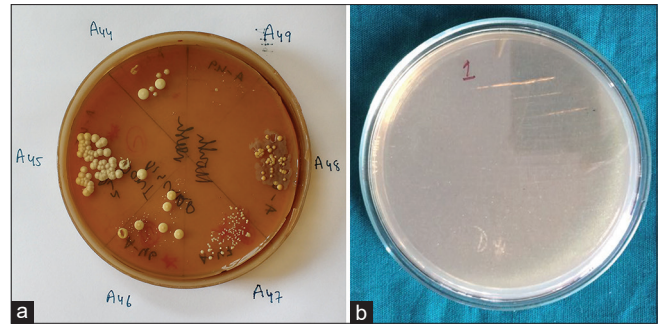


Figure 3: (a) Sabouraud dextrose agar plates with white to cream colored smooth candidal colonies (*Candida* positive). (b) No Candidal colony growth (*Candida* negative)

Results

Among the 90 patients, 69 (76.7%) were male and 21 (23.3%) were female. In the study group, 49 (81.7%) patients were male and 11 (18.3%) were female. In nonhabitueés and habitueés control group, 8 (53.3%) and 2 (13.3%) were female and 7 (46.7%) and 13 (86.7%) were male, respectively. The mean age of study groups was in the 4th decade. Eleven (18.3%) study patients had combined habits, whereas 49 (81.7%) were using only SLT. On the other hand, 7 (46.7%) patients of the habitueés control group had combined habits (smoking and smokeless forms) and 8 (53.3%) were using only SLT. All patients in the study group gave a positive history of use and commercial tobacco preparations were the most frequent used form. The frequency of tobacco use per day ranged from 3 to 22 times, with an average of 7.70 ± 4.33 . The duration of tobacco use varied from 3 to 24 years with a mean of 7.76 ± 5.06 years. Highly significant ($P = 0.000$) distribution of frequency and duration of tobacco use was found among the study group and habitueés control group. The maximum number of the study participants (95%) was in Stage II OSF while one (1.7%) and two (3.3%) patients were in Stage I and III, respectively.

A highly significant ($P < 0.001$) association was found between the SFR and the perception of xerostomia among the study participants. One hundred percent ($n = 2$) patients with SFR < 12 mm reported xerostomia. Fifty-five percent ($n = 11$) of the study participants with SFR between 13 and 24 mm reported xerostomia, whereas only about 11% of the study participants with SFR above 25 mm perceived xerostomia.

The distribution of mean VAS score, SFR and MO was significantly varied ($P < 0.001$) among the study and control groups [Table 1]. However, their relationship among the different clinical stages of OSF was not significant ($P > 0.05$) [Table 2].

No statistically significant association was obtained among *Candida* positive and *Candida* negative OSF patients with respect to age, gender, type, and duration of habit and perception of xerostomia. However, the frequency

Table 1: Mean distribution of burning sensation (using visual analogue scale score), salivary flow rate and mouth opening in study group (oral submucous fibrosis) and control groups

Sign and symptoms	Group	n	Mean±SD	Minimum	Maximum	F	P
VAS score (burning sensation)	Study group (OSF)	60	27.72±22.27	0	80	20.895	0.000**
	Non-Habitués control	15	0.67±2.58	0	10		
	Habitués control	15	1.33±5.16	0	20		
SFR	Study Group (OSF)	60	26.28±7.17	12	35	10.157	0.000**
	Non-Habitués control	15	32.87±2.64	26	35		
	Habitués control	15	32.13±3.7	23	35		
MO	Study group (OSF)	60	27.42±10.15	9	48	29.713	0.000**
	Non-Habitués control	15	43.13±4.4	36	49		
	Habitués control	15	41.40±3.01	35	47		

**Highly significant; P>0.05 (not significant). VAS: Visual analogue scale; SFR: Salivary flow rate; SD: Standard deviation; OSF: Oral submucous fibrosis; MO: Mouth opening

Table 2: Comparison of mouth opening, burning sensation (using visual analogue scale score) and salivary flow rate in different stages of oral submucous fibrosis

Clinical features	Stage	n	Mean±SD	Minimum	Maximum	F	P
MO	I	1	41.00±0.00	41	41	0.908	0.409
	II	57	27.19±10.48	9	48		
	III	2	27.00±11.31	19	35		
VAS score	I	1	24.00±0.00	24	24	0.136	0.873
	II	57	28.05±22.48	0	80		
	III	2	20.00±28.28	0	40		
SFR	I	1	35.00±0.00	35	35	1.74	0.185
	II	57	25.89±7.14	12	35		
	III	2	33.00±0.00	33	33		

P>0.05 (not significant). VAS: Visual analogue scale; SFR: Salivary flow rate; SD: Standard deviation; MO: Mouth opening



Figure 4: Candidal spores on gram staining

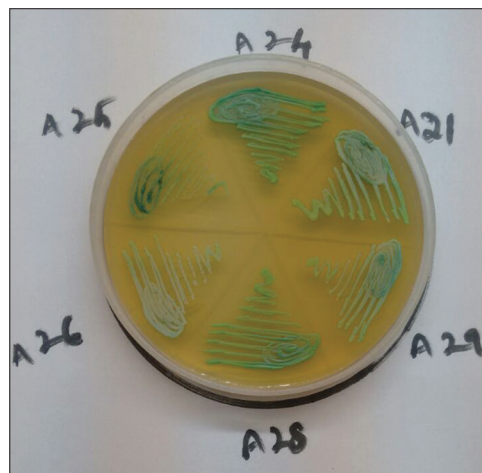


Figure 5: Varied colour growth of different Candida species on CHROMagar from oral submucous fibrosis patients

of habit was found to have a statistical significance association among Candida positive and negative OSF patients [Table 3].

Candida was found to be present significantly (P = 0.048) in the OSF group as compared to the control group. Eleven patients (18.3%) were Candida positive in the OSF group and only one (3.33%) patient was positive in the control group [Graph 1]. The mean counts of Candida colonies in the OSF group were 227.27 CFU/mL. Candidal carriage with

a colony count of 1–200 CFU/mL was attained in 6 (60%) patients. Three (30%) patients of the study group had a colony count of 201–400 CFU/mL. Notably, only 1 (10%) patient of the study group had colony count of >400 CFU/mL. Among controls, only 1 patient (nonhabitués) was Candida positive with a count of 175 CFU/mL [Graph 2].

Species characterization using CHROMagar revealed C. albicans in 6 OSF patients and 1 control group patients.

Candida dubliniensis and *Candida parapsilosis* were present in 1 and 4 patients, respectively [Graph 3].

Only one patient who was SLT user had colony count >400 CFU/mL for whom VAS score had reduced to 10 from 25 after antifungal treatment.

No Candidal organisms were isolated from Stage I OSF in this study group. 10 (17.54%) patients in Stage II OSF had positive Candidal carriage in their oral cavity. The distribution of candidal organisms in Stage II OSF patients ranged from 50 CFU/ml to 725 CFU/ml. One hundred and fifty CFU/mL of Candidal organisms were isolated from oral rinse samples in 1 (50%) patient of Stage III who practised combined habits. No significant association could be established regarding the Candidal isolation in the various stages of OSF as $P > 0.05$.

No statistically significant association of Candidal isolation was established with SFR, different grades of MO, and stomatopyrosis, as $P > 0.05$ [Table 4 and Graphs 4-6]. However, Pearson’s correlation test reported a significant negative correlation between MO and candidal isolation in OSF patients.

Discussion

The oral cavity has an established microbiome that includes numerous bacteria, fungi, and viruses.^[18] Oral candidal carriage denotes colonization of the mouth by yeast without overt infection.^[8] OSF is associated with the altered salivary flow and restricted MO that may facilitate Candidal growth, which in turn may contribute to stomatopyrosis.

The overall mean age of study participants was 33.48 years ranging from 18 to 50 years, highlighting the prevalence of OSF in young adults. A significant male predominance was noted in the present study with a ratio of 4:1. Similar findings were noted in previous studies.^[8,19-21] This could be attributed to increased access and decreased inhibition to procure SLT products and a general social acceptance of the practice of such habit by men in Indian society compared to females. However, in a 10-year prospective intervention study by Murti *et al.* in 1990 found a higher incidence of OSF in females.^[22] Various studies have reiterated the contribution of SLT in commercial tobacco preparations along with AN for causation of OSF.^[11,23,24] Similar findings were reproduced in the present study.

Table 3: Association of demographic characteristics and tobacco habit among Candida positive in study group (oral submucous fibrosis)

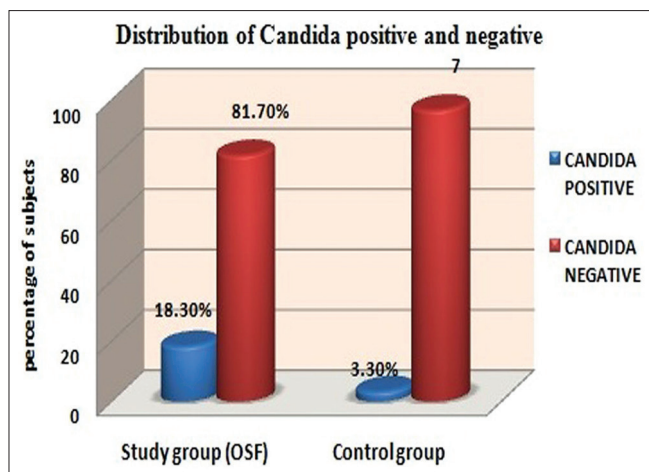
Candidal carriage	Age (in years)				Gender		Habit		Frequency (per day)					Duration (in years)				Xerostomia		
	<20	20-30	30-40	>40	M	F	SLT	Combined	1-5	6-10	11-15	16-20	>20	1-5	6-10	11-15	16-20	>20	Present	Absent
Candida positive																				
<i>n</i>	0	3	5	3	8	3	8	3	1	5	1	3	1	3	1	4	2	1	4	7
%	0	14.3	23.8	18.8	16.3	27.3	16.3	27.3	6.7	16.7	11.1	75	50	15.0	4.8	33.3	50.0	33.3	23.5	16.3
Candida negative																				
<i>n</i>	2	18	16	13	41	8	41	8	14	25	8	1	1	17	20	8	2	2	13	36
%	100	85.7	76.2	81.3	83.7	72.7	83.6	72.7	93.3	83.3	88.9	25.0	50.0	85.0	95.2	66.7	50.0	66.7	76.5	83.7
<i>P</i>		0.777			0.321		0.497		0.020*					0.105				0.377		

*Significant; $P > 0.05$ (not significant). SLT: Smokeless tobacco

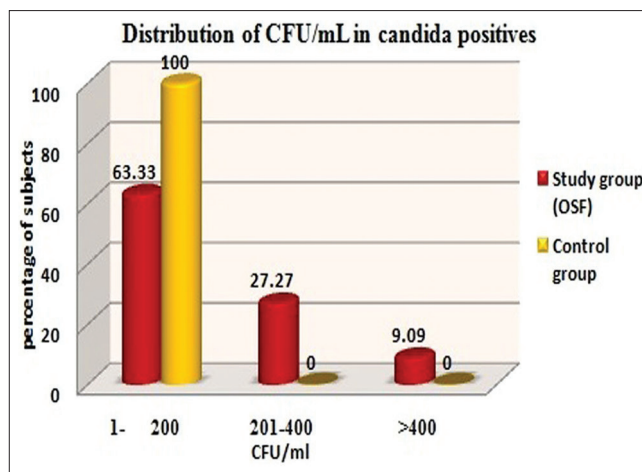
Table 4: Association of salivary flow rate, mouth opening and burning sensation (using visual analogue scale score) with candidal isolation in study group (oral submucous fibrosis)

Clinical features	Subgroups (mm)	Study group (OSF), <i>n</i> (%)	Candida positive, <i>n</i> (%)	Candida negative, <i>n</i> (%)	χ^2	<i>P</i>
SFR	0-12	2 (3.3)	1 (50)	1 (50)	1.541	0.463
	13-24	20 (33.3)	4 (20)	16 (80)		
	25-35	38 (63.3)	6 (15.8)	32 (84.8)		
MO	1-10	2 (3.3)	1 (50.0)	1 (50)	4.503	0.342
	11-20	15 (25.0)	3 (20.0)	12 (80)		
	21-30	24 (40.0)	6 (25.0)	18 (75)		
	31-40	11 (18.33)	1 (9.09)	10 (90.91)		
	>40	8 (13.33)	0	8 (100)		
VAS score	Zero	14 (23.33)	4 (28.57)	10 (71.43)	3.977	0.409
	1-20	12 (20.0)	1 (8.3)	11 (91.7)		
	21-40	18 (30.0)	2 (11.11)	16 (88.89)		
	41-60	11 (18.33)	2 (18.18)	9 (81.82)		
	61-80	5 (8.33)	2 (40)	3 (60)		
	81-100	0	0	0		

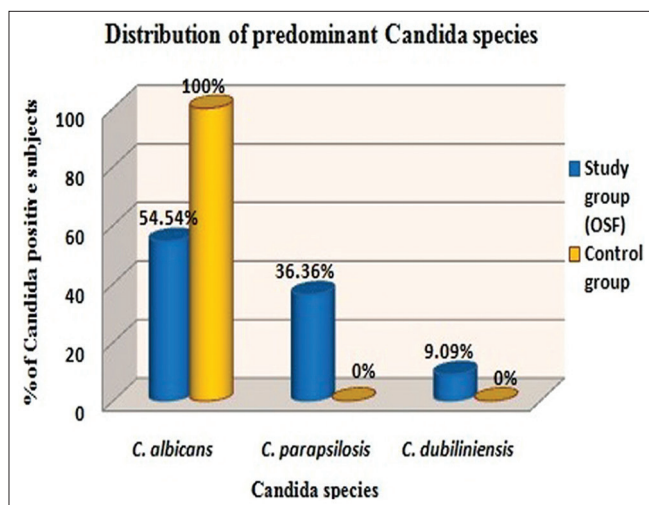
$P > 0.05$ (not significant). VAS: Visual analogue scale; SFR: Salivary flow rate; OSF: Oral submucous fibrosis; MO: Mouth opening



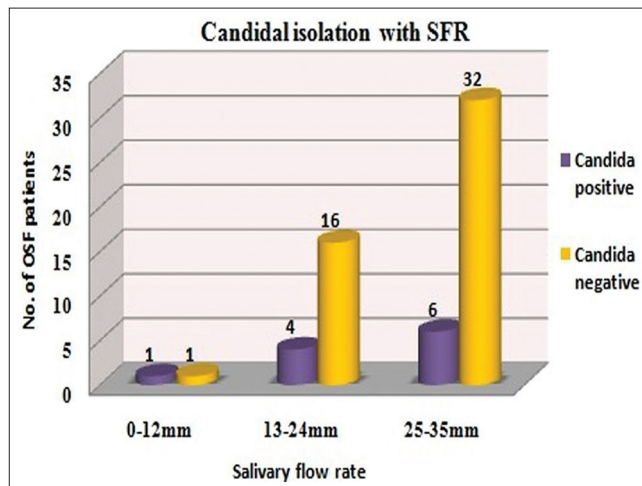
Graph 1: Showing distribution of *Candida* positive and negative patients of study group (oral submucous fibrosis) and control group



Graph 2: Distribution of Candidal CFU/mL in study group (oral submucous fibrosis) and control group



Graph 3: Showing distribution of predominant *Candida* species among the study group (Oral submucous fibrosis) and control group



Graph 4: Association of salivary flow rate with Candidal isolation in study group (oral submucous fibrosis)

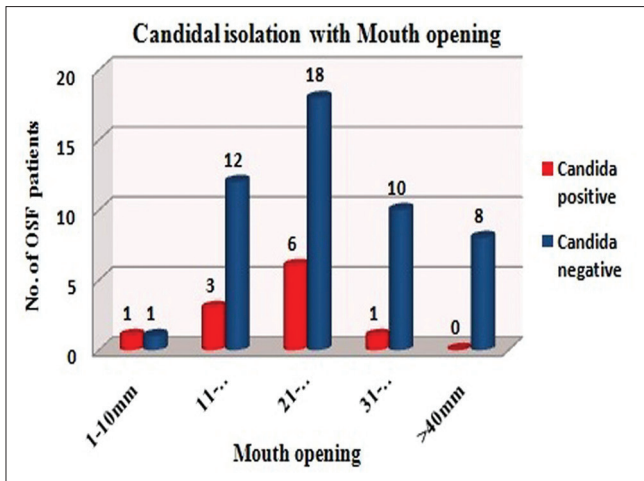
The average duration of AN preparations chewing habit, in the present study, among all the study participants was 7.76 years with average frequency of 7.70 chews/day matched the findings of other studies where it has been identified that the relative risk of OSF increased with increase in the frequency as well as the duration of chewing habits.^[4,24-26]

In this study, maximum subjects (95%) were categorized into stage 2, highlighting the role of the classification system in the subject distribution in different stages.^[27]

Forty-six (76.6%) patients in the study group suffered from stomatopyrosis, which is more compared to the findings of Pandya *et al.*^[27] Oral mucosal burning sensation, especially on eating spicy foods, has been attributed to the lack of protective effect by salivary mucins in OSF. Fibrosis and hyalinization in and around minor salivary glands (MSGs) due to OSF leads to reduction in the secretion of saliva. MSGs are a major source of membrane associated mucin (MAM) which plays a very important role in the

protection and lubrication of oral mucosa. MAM attaches to the “microplicae” and act as scaffold for the formation of highly hydrated and viscous gel called salivary mucous gel (SMG). The reduction of SMG formation in OSF hampers the “protective diffusion membrane” function and causes their rapid exfoliation even by normal physiologic friction. Epithelial atrophy in OSF reduces the distance of intra-epithelial nerve endings from the surface, making it more sensitive against irritation from food substances, for example, spicy and hot food, thus resulting in burning sensation.^[28] The MO findings in the present study are comparatively greater than the previous studies.^[29,30] This might have facilitated adequate oral hygiene maintenance in the current study population.

The present study revealed a statistically significant higher Candidal carriage in OSF patients (18.3%), as compared to controls (3.3%). This was in accordance with the findings of More *et al.* and Gupta *et al.* (16.66%) but much less than isolated by Sankari and Mahalakshmi, George (80%).^[9,31-33] However, some studies reported an increased but statistically

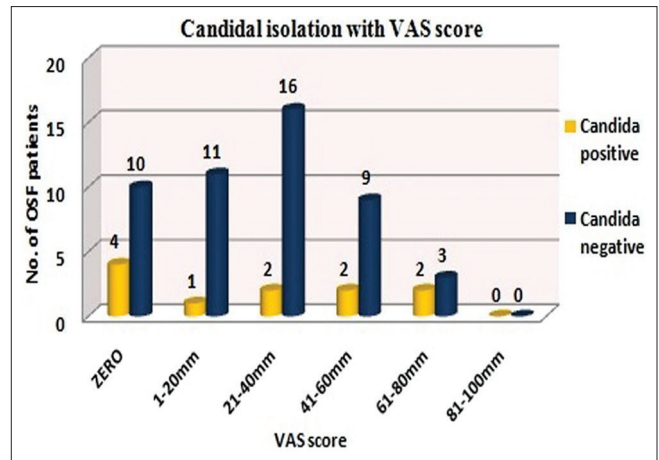


Graph 5: Association of mouth opening with Candidal isolation in study group (oral submucous fibrosis)

insignificant Candidal carriage.^[8,18,21] Such discrepancies are probably due to variations in the technique and the site of sample collection and the disease process itself, along with other local and systemic factors.^[18] Although oral environment and tissue characteristics in OSF favor increased Candidal loads, the low Candidal carriage (18.3%) found in OSF patients of the present study could be due to the slaked lime content of gutkha that creates an alkaline pH in the oral cavity; which is not favorable for *Candida* growth because *Candida* is best able to adhere to epithelial cells at an acidic pH. In addition, bioactive chemical compounds present in AN have been shown to have inhibitory effects on Candidal carriage.^[34]

Candidal count between the study and control group was not found to be statistically significant, and this finding was in concurrence with the study conducted by Anila et al.^[21] On the contrary, a higher Candidal carriage was obtained by Kamat et al.^[18] Previous studies have reported *C. albicans* as the most common isolate in OSF patients.^[8,9,21,31,33] The present study also concurs with their findings. This is owing to the ability of phenotypic switching, biofilm, and hyphae formation by *C. albicans*.^[16] It has been identified to have maximum carcinogenic potential and also possesses a greater adherence capability to buccal epithelial and vascular endothelial surfaces and increased secretion of proteinases as compared to other Candidal species.^[35] However, the presence of non-*albicans* species indicates an alteration in oral micro-flora, which may not be adequately treated with regular antifungal agents.^[31]

Association of SFR with Candidal presence in the present study highlights the importance of reduced SFR in increasing the probability of higher oral Candidal carriage. Previous studies also suggested an inverse relationship between salivary flow and Candidal colony.^[33,36] Impaired salivary gland function can predispose to oral Candidiasis due to the lack of salivary dilution effect, salivary flushing of microorganisms, and salivary antimicrobial proteins. Antimicrobial proteins in the saliva interact with the oral



Graph 6: Association of burning sensation (using visual analogue scale score) with Candidal isolation in study group (oral submucous fibrosis)

mucosa and prevent overgrowth of *Candida*. Therefore, conditions that can reduce salivary secretions, such as OSF, can lead to an increased risk of oral Candidiasis.^[12]

The present study also reported a negative correlation regarding MO and candidal isolation among OSF patients. Reduced MO which is an important manifestation in OSF patients can lead to poor oral hygiene, which has been frequently claimed to be a local predisposing factor for increased oral Candidal carriage and Candidiasis in dentate subjects. *Candida* cells may preferentially colonize the dental plaque in subjects with poor oral hygiene who have higher level of dental plaque and gingival inflammation.^[37] On the contrary, it resurfaced the findings of Ariyawardana et al. 2007 who reported grade of MO had no influence on the Candidal carriage in the patients with OSF.^[8] The maintenance of oral hygiene and absence of comorbid conditions in the present study population could be the probable factors for this finding.

Stomatopyrosis in OSF has been conventionally associated with hyposalivation and epithelial atrophy. Association of Candidal organisms with stomatopyrosis has been explained on the basis of Candidal metabolites causing stimulation of capsaicin (vanilloid) receptors of nerve endings, which is responsible for the detection of pain-producing chemical and thermal stimuli. The induction of burning sensations by fungal products indicates such a probability also in cases of *Candida* multiplication.^[38,39] Forty-six cases in the OSF group who complained of burning sensation, 7 yielded Candidal growth and 39 were negative. Similar finding was observed by Kamat et al., where OSF patients with burning sensations and negative *Candida* carriage outnumbered those of OSF patients with burning sensations and positive *Candida* carriage.^[18] This observation suggests that the sensation of a burning mouth is influenced not only by the presence and colonization of *Candida*, but other factors, such as a dry mouth, saliva, and systemic conditions as suggested by Vitkov et al.^[39]

Only one patient in this study group was found to have higher candidal carriage, i.e., >400 CFU/mL^[17] for whom antifungal treatment with oral fluconazole tablets was given following which he reported a marked decrease in burning sensation with the VAS score reducing from a pretreatment score of 24 mm to a posttreatment score of 10 mm on the scale. This improvement of symptom on VAS scale was previously studied and reported by Terai and Shimahara in 2010, who associated glossodynia with *Candida* and reported a marked improvement on VAS scale after antifungal treatment.^[40]

Strength and limitations of the study

The strengths of the study were that besides assessing the Candidal presence and identifying the species, it also determined candidal carriage quantitatively, which can help recognize the onset of Candidiasis in OSF patients.

There were certain limitations to this study, as the maximum number of study subjects was categorized into Stage 2 of Pindborg's clinical criteria. A large sample size including other stages of OSF would have allowed for better assessment of oral Candidal carriage and its association with signs and symptoms of OSF.

Conclusion and Future Scope

Mucosal and salivary flow alterations in OSF favor the Candidal colonization and the symptoms of OSF can be secondary to the cause and effect of the Candidal infection. However, studies with larger sample size with a functional and histological staging of OSF are required to affirm these findings. It also provides a gateway regarding the use of antifungal agents in OSF patients to treat stomatopyrosis, once increased candidal carriage is determined in such patients. Furthermore, this halts the role of *Candida* in malignant transformation of OSF and could improve the overall prognosis of the disease.

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Conflicts of interest

There are no conflicts of interest.

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