Role of Candida in oral submucous fibrosis- *A Review*

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Abstract
Oral submucous fibrosis (OSF) is a high-risk potentially malignant disorder that predominantly affects Indians and Southeast Asia due to prevalent habit of smokeless tobacco use. It clinically presents with burning sensation, reduced mouth opening, decreased salivary flow rate and blanching of oral mucosa. The clinical presentation of OSF and its tendency for malignant transformation has been linked directly and indirectly with the presence of Candidal infection in the oral cavity. Features of OSF like altered oral epithelium, decreased salivation, impaired oral hygiene, nutritional deficiencies and altered immunity may play a significant role in the invasion of Candida.
However, it has also been put forward that the Candidal invasion is less in the smokeless tobacco users, which raise a point of ambiguity. There is an enduring discussion whether Candida infection can be a cause of malignant transformation or adds to the clinical features by superimposed infection in a pre-existing OSF condition. This review article highlights the association of Candida in OSF manifestations and its malignant transformation, which may lead to better management and overall improved prognosis.
Key words: Candida; Areca; Oral submucous fibrosis; stomatopyrosis; Malignant transformation.

Introduction

Oral submucous fibrosis (OSF) is described as an insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. It is always associated with a juxtaepithelial inflammatory reaction followed by a fibroelastic change of the lamina propria with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus [1].

It is predominantly a disease of Indian subcontinent and South east Asia, with a prevalence that has increased dramatically from 0.03% to 6.42% in the last four decades [2]. Multifactorial etiology has been proposed for OSF. However, more recent studies have confirmed areca nut as the major etiological factor of OSF, especially among people who possibly have a genetic predisposition to the disease [3].

OSF manifests with burning sensation, reduced mouth opening, decreased salivary flow rate and blanching of oral mucosa [4]. Burning sensation is the most common initial symptom of OSF patients [5] probably contributed by various local factors such as hyposalivation, modified composition, changed rheological properties of saliva, altered epithelium, submucosal changes and Candidal colonisation as well as by systemic factors such as anaemia which may be a cause or effect of OSF.
Indeed, there appears to be an interplay amongst multiple factors which aggravate Candidal colonisation in oral cavity of OSF patients [6] which influences their clinical manifestation as well as their malignant potential. Hence, Candidal colonisation in OSF patients may have a role in its clinical manifestations and prognosis. Candida as a normal commensal is present in the oral cavity with <400CFU/ml of salivary sample [7]. It predominantly harbours the buccal mucosa and followed by tongue region [8] with *C. albicans* being the most common isolate [9].
However the association of candidal colonisation in OSF is not sufficiently documented in the literature. In this view, this review paper aims at analysing the role of Candida in OSF manifestations and in its malignant transformation. If such a role is established, it may serve as a stepping stone in management of OSF symptoms.

Factors affecting the candidal colonisation in OSF
OSF was first associated with Candidal carriage by Ariyawardana et al. in 2007 [6]. Following which multiple factors were proposed which influence this association and are listed below-

Features of OSF influencing the Candidal association-	
Favouring candidal association	**Disfavouring candidal association**
• Altered oral epithelium • Decreased salivation • Impaired oral hygiene due to reduced mouth opening • Nutritional deficiencies • Altered local immune response	• Hypersalivation • Use of lime cause alkalinity • Antifungal effect of arecanut

It is evident that mucosal alterations due to the underlying disease process or betel quid chewing, coupled with other local factors, lead to Candidal colonization, even in the absence of clinically-related mycotic manifestations. Various studies has demonstrated that consumption of tobacco in any manner (smoking, chewing) increases the colonization of Candida due to increased epithelial keratinization, decreased salivary immunoglobulin A and decreased function of leukocytes [10]. It has been recently suggested that the mucosal alterations (especially of the epithelium) like tough and leathery mucosal texture, blanching of oral mucosa, palpable fibrous bands, and restricted mouth opening in OSF, act as a platform for increased Candidal colonization, thus affecting the biological behavior of the disease process[11].

Although conflicting results exists regarding the salivary flow rate in OSF [12-18], numerous studies have established a decreased SFR. A decrease in SFR among OSF subjects could be due to conversion of arecoline by lime to Arecaidine which lacks parasympathomimetic effect of arecoline [19] or due to atrophy of the acinar cells as disease progresses. This may lead to oral candidiasis due to lack of salivary dilution effect, salivary flushing of microorganisms away from the oral cavity and salivary antimicrobial proteins [4, 20]. On the contrary, certain studies state that 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 of *Candida* growth [21].

Reduced mouth opening 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 candidosis 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 [22]

Arecanut is said to possess a medicinal value due to its bactericidal and fungicidal ability. The effectiveness of phenolic contents as fungicidal in areca nut is due to their ability to denature the protein binding in cell membrane of fungi, so that the cell lysis can occur [23]. The role of slaked lime as antifungal agent has been highlighted in literature. The slaked lime, whenever added in quid creates an alkaline pH in the oral cavity; this pH is not favourable 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 [24].

However, in OSF patients with smoking habit, this antifungal effect may be masked by the smoking tobacco products. Various studies has demonstrated that smoking increases the colonization of Candida, owing to the presence of the aromatic hydrocarbons in tobacco, which have been shown to act as nutrients to the yeast cells. In addition, smoking may indirectly increase the level of salivary glucose, which enhances yeast growth. Further, tobacco use can facilitate candidal colonisation by increasing epithelial keratinisation and by depressing the activity of oral leucocytes, salivary immunoglobulin A and other non-specific immune defences [10, 22].

Interplay of Candidal infection and clinical manifestations in OSF

Role in stomatopyrosis-

Burning mouth sensation (stomatopyrosis) might be regarded as a single symptom related to different causes. The detection of painful stimuli occurs primarily at the peripheral terminals of specialised sensory neurons called nociceptors. These small-diameter neurons transduce signals of chemical, mechanical or thermal nature into action potentials and transmit this information to the central nervous system, ultimately eliciting a perception of pain or discomfort [25]. Candida metabolites induce burning by 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. Patients with burning mouth sensations could possibly have a reduced sensitivity threshold to react to candidosis, respectively, to metabolic products of yeasts [25]. Several studies have reported the role of Candida in stomatopyrosis [25, 26] and hence associated the burning sensation of oral cavity with presence of Candida. Burning sensation which is a common symptom of OSF patients, hence was associated with presence of Candida in oral cavity.

Conversely, some studies [11, 27] have concluded that the OSF patients with burning sensations and negative Candidal carriage outnumbered OSF patients with burning sensations and positive Candidal carriage. This observation suggested that the sensation of a burning mouth is influenced not only by the presence and colonization of Candida, but by other factors too [11, 27].

Role in malignant transformation-

OSF is a preventable, potentially malignant disorder with a malignant transformation rate of 7-30% [28]. It is noteworthy that association of Candida as carcinogen is well established. Candida infection together with other cofactors may induce epithelial atypia and dysplasia leading to malignant change [6].

Association of Candida and its role as carcinogen is well established in potentially malignant disorders [29]. OSF is a high risk precancerous condition which favours the colonization of *Candida* [10].

Characteristic of Candidal species that contribute to the virulence of Candida-

- **Adhesion**
  - Mannoprotein layer of cell wall
  - Biofilm formation
- **Invasion**
  - Degradative enzymes
  - Epithelial cell endocytosis – phenotypic switching
- **Pathogenecity**
  - Destruction of host tissues by hydrolytic enzymes (Saps, LIP's)

Adhesion: Candidal infection together with other co-factors may also induce epithelial atypia and dysplasia leading to malignant change [6] or Candida might initiate malignant transformation by directly producing carcinogenic compounds, for example, nitrosamines [30]. Such a carcinogenic potential of Candida assumes greater significance in relation to OSF which is a high risk precancerous condition.

An important factor that contributes to the adhesion of C. albicans to mucosal surfaces is a fibrillar surface component of the yeast cell wall, a strain-specific mannoprotein layer [31].

Adherence of Candida to mucosal epithelium is followed by cell division, proliferation and subsequent biofilm development [32]) and thus invading the mucosal surface.

Invasion: Two different mechanisms by which C. albicans can invade keratinocytes have been proposed. One mechanism involves the secretion of degradative enzymes by the fungus, particularly secreted aspartic proteases that can digest epithelial cell surface components and, thereby, allow the physical movement of hyphae into, or between, host cells. The second proposed mechanism is the induction of epithelial cell endocytosis [31].

Pathogenecity: Destruction of host tissues by *Candida* species may be facilitated by the release of hydrolytic enzymes into the local environment. Secreted aspartyl proteinases (Saps), phospholipases, lipases (LIPs) and haemolysins are the enzymes most frequently implicated in *Candida* species pathogenicity [32].

Further Phenotypic switching of Candida helps in invasion of oral mucosa and is noticed when they become pathogenic [33]. Phenotypic switching' in Candida albicans was first defined in 1985 as the capacity to undergo spontaneous, reversible transitions between a set number of colony morphologies [34].

Candida can act as a carcinogen due to the following features-

a. Production of NBMA
b. Tubular hyphal structure
c. Ability to bind with DNA adducts that result in point mutations
d. Conduciveness to cell proliferation
e. Ability to metabolize pro-carcinogens to carcinogens
f. Alteration of micro-environment and onset of chronic inflammation

C. albicans proteinases have keratinolytic activity that can both serve to facilitate initial penetration of keratinised cells as well as providing a valuable source of nitrogen during colonisation. In terms of virulence, secreted aspartyl proteinases (SAPs) activity can therefore directly induce damage to host cells, facilitate hyphal growth for invasion of tissue, increase adherence following exposure of receptor sites, and also degrade host immunoglobulins and other defence proteins. Another group of hydrolytic enzymes produced by Candida species are the phospholipases (PLs). Through the hydrolysis of ester linkages of phospholipids, PLs can effectively degrade the membrane of host cells leading to cell lysis and death. By this process, both adherence of Candida to receptor sites and its subsequent penetration of damaged tissue can be facilitated [35].

Krogh et al. [36] showed that some Candida species isolated from potentially malignant disorders lesions were able to produce the potent carcinogen N-nitrosobenzylmethylamine(NBMA).Strains with the highest potential to produce NBMA were isolated from advanced, potentially malignant, oral mucosal lesions rather than early lesions or normal oral mucosa. It was suggested that the tubular hyphal structure of *C.albicans* might be important as this structure allows ingress of precursors from saliva and a release of the nitrosamine product to keratinocytes, potentially initiating oral carcinoma [36].

Candida will bind with DNA to form adducts with bases, phosphate residues, and/or hydrogen bonding sites that could cause miscoding or irregularities with DNA replication. Point mutations thus induced may activate specific oncogenes and initiate the development of oral cancer [31].

Also, it was postulated that Candida creates an environment conducive to cell proliferation that may lead to clonal expansion of genetically altered cells, thus demonstrating its ability to promote carcinogenesis in initiated epithelium [31]. Other proposed mechanism includes-Ability to metabolize procarcinogens, i.e., conversion of alcohol into acetaldehyde, hydroxyethyl radicals, ethoxy radicals, and hydroxyl radicals in alcohol users [37]. Further, Ability to modify the microenvironment and onset of chronic inflammation as C. albicans has been shown to secrete specific proteinases, capable of degrading basement membrane and extracellular matrix [31] will promote the malignant transformation. Thus, The understanding the role of Candida in clinical manifestations of OSF and in its subsequent malignant transformation may lead to a better prognosis of OSF.

Association between Candida and OSF revisited-

The fact that OSF is associated with Candidal carriage was first reported by Ariyawardhane et al. 2007 [6] who demonstrated a higher Candidal carriage in patients with OSF, though, it was not statistically significant.

Study conducted by Kamat et 2011[11] reflected that the value of CFU/mL increased with an increased duration of betel quid chewing habit and affirmed the finding that OSF favours the colonization of Candida.

Anila et al [24] conducted a study in 2011 which resurfaced the findings of Ariyawardhane et al with the conclusion that the incidence and intensity of *Candida* (primarily *C. albicans*) was greater in OSF patients than in healthy controls, but these findings were within the normal limit.

These contradictory findings gained the attention of the researchers and further studied were initiated in this direction.

A study conducted by Beena George in 2015 [10] concluded that Smokers with OSF are more easily prone to develop *Candida albicans* infection as compared to the other groups. Cigarette smokers had a significantly increased carrier rate, compared with non-smokers as smoking increases the proinflammatory factors, oxidative stress and depresses the immune mechanism of the oral cavity. Consumption of tobacco in any manner (smoking,chewing) increases the colonization of Candida due to increased epithelial keratinization, decreased salivary immunoglobulin A and decreased function of leukocytes.

Further, Gupta et al in 2015 [4] suggested a higher Candidal carriage in grade II and grade III OSF patients and affirmed the finding that OSF favours the colonization of Candida.

Recent literature documenting the Candidal carriage in OSF patients-

Study	No. Of OSF patients	Age distribution	Sex distribution	Key finding	Results
Ariyawardhane et al(2007) [6]	30 histologically proven OSF patients 30 controls	Age range (14->64 years) Mean age – 44.2 years	M:F 2.3:1		The carriage of yeast in the OSF group was higher (63.6%) than control group (50%) but it was not statistically significant.
Kamat et al(2011) [11]	30 clinically-diagnosed and -staged OSF Patients 20 age- and sex-matched controls.	20-40 years mean ages of OSF and control groups were 26.7 years and 26.9 years respectively	-	All Candida-positive OSF patients complained of a burning sensation.	Candida carriage in OSF patients (36.67%), as compared to controls (10%), and the difference was statistically significant
Anila et al(2011) [24]	20 clinically diagnosed OSF patients and 20 healthy controls	Mean age of both groups was 26.7	90% of the participants were male.		40% (8/20) of the OSF patients and 15% (3/20) of the controls yielded *Candida* organisms on culture. But the difference was not statistically significant.
George et al(2015) [10]	60 patients who were divided into three groups : Controls- 20 Smokers- 20 OSF patients-20		All male patients		80% of the OSF patients showed the presence of *C. albicans,* which was statistically significant as compared to the other groups
Gupta et al (2015) [4]	30 OSF patients and 30 controls			salivary flow was reduced in OSF patients as compared to control and difference was	Candidal carriage was seen in grade II and grade III OSF patients

				statistically significant	

Despite of the conclusion of the above mentioned studies, *C. albicans*, which is the predominant species isolated in premalignancy and carcinoma [24, 29, 31], followed by *Candida tropicalis* were the most common species in the OSF cases [6, 8, 10, 11, 24].

Earlier *in-vitro* studies have reported *C. albicans* to have greater adhesion to oral epithelial cells followed by *C. tropicalis* and *C. parapsilosis.* The presence of more α-L fucose remnants promotes greater adhesion of *C. albicans.* Betel quid with tobacco chewing is reported to cause an increase in salivary pH of 8-10 due to chemical constituents such as slaked lime and nicotine, a component of tobacco. *C. albicans* is capable to adapt to pH of 2-10 by actively alkalizing surrounding environment, release of ammonia after amino acid degradation and by phenotypic switching. Also *C. albicans* biofilms are resistant to neutrophils due to the presence of β-glucans in the extracellular matrix [8], thus supporting the *C. albicans* as a predominant species.

Conclusion- Although a higher incidence and intensity of *Candida* was observed in OSF patients when compared to healthy individuals. However, controversy still exists over whether arecanut chewing in OSF inhibits or promotes the adherence and invasion of Candida and hence, the role of Candida in malignant transformation of OSF is also questioned.

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