

Incidence and Intensity of the Candida in oral submucous fibrosis patients: A case control study.

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Abstract

Background: The aim of this study is to evaluate incidence and severity of Candidal species among oral submucous fibrosis patients and to compare it with the healthy controls so that their role in malignant transformation could better be understood.

Materials and Methods: The comparative study was conducted between 30 oral submucous fibrosis patients and 30 normal healthy individuals. Oral rinse technique was used to collect the samples and immediately sent to the laboratory for culture of Candida on Sabouraud’s agar medium. Buccal smears obtained from both groups were stained using the periodic acid schiff technique to evaluate presence of Candida yeast cells and pseudohyphae.

Result: Out of 30 cases per group, 14 (46.7%) oral submucous fibrosis Patients and 7 (23.3%) controls yielded Candida species on culture. Also the positive Candidal carriage in Oral submucous fibrosis patients increased with increase in severity of the disease and as the oral hygiene status declined which was found to be statistically significant with $p < 0.05$.

Conclusion: The incidence and intensity of Candidal species was quite significantly higher in study group, suggested that patients with oral submucous fibrosis are at high risk to infectious diseases from Candida as compared to healthy individuals, and hence added responsibility upon us in further educating the population to quit the habit.

Keywords: Candida, Incidence, Intensity, Oral Sub mucous fibrosis

Introduction

Candida is predominantly an opportunistic infectious agent that is poorly equipped to invade and destroy tissue. The role of Candida as opportunistic invader versus etiologic agent in patients with oral white lesions, although it is not clearly established. However, the demonstration of the catalytic of some Candida strains in endogenous cellular nitrosamine production, the statistically significant association of certain strains with dysplastic red and white lesions, and the hyperplastic effects on epithelium of Candida in vitro, indicate that Candida may be a carcinogen or promoting agent, rather than only an innocuous opportunistic infectious entity.¹

Of the fungi regarded as human pathogens, members of the genus Candida are the most frequently recovered from disease.² Of the Candida species isolated from humans, Candida albicans is the most prevalent in both health and disease.³

Candida species cause common oral fungal infections in human beings. More recently, it has been suggested that Candida species may be causative agents in some diseases of the mouth other than candidiasis, including oral cancer, burning mouth syndrome, taste disorders and endodontic disease, although the basis of these associations remains uncertain.²

In addition, overgrowth of Candida can lead to local discomfort, an altered taste sensation, dysphagia from oesophageal overgrowth resulting in poor nutrition, slow recovery, and prolonged hospital stay. In immunocompromised patients, infection can spread through the bloodstream or upper gastrointestinal tract leading to severe infection with significant morbidity and mortality.^{4,5}

Oral sub mucous fibrosis (OSMF) has been identified as a high risk precancerous condition and its prevalence has been increasing in young Indian adults due to habit of gutkha chewing.^{6,7} Candidal infection can induce epithelial atypia and lead to malignant transformation through the release of chemical carcinogens like nitrosamine compounds. Similarly, Candida could play a role in malignant transformation of OSMF, but this process has not been confirmed.^{8,9}

The aim of this study is to evaluate incidence and intensity of Candidal species among OSMF patients and to compare it with the healthy controls.

Materials and Methods

The comparative study was conducted between 30 oral sub mucous fibrosis patients and 30 normal healthy individuals. Selection of the patients was made from those attending the outpatient department of oral medicine and radiology department of Darshan dental college and hospital of Udaipur city, Rajasthan, India. Ethical clearance was obtained from ethical committee of Darshan dental college and hospital.

Inclusion criteria for selection of participants

Patients clinically diagnosed as OSMF patient

Exclusion criteria

Patients having known history of systemic illness

Patients ongoing with the steroidal drugs therapy

Lesions and conditions present in oral cavity other than OSMF

The study groups were matched based on their age, sex and their oral hygiene index - score. All the subjects involved in the study were males. Before the collection of the sample, a written informed consent was obtained from all the subjects.

Sample Collection

Oral rinse technique as described by Samaranyake et al.¹⁰ was used to collect the 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 µL 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. The number of yeast colonies was counted and expressed as colony-forming units per milliliter (CFU/mL) of the collected sample.

Buccal smears were obtained from both groups using a wooden tongue spatula and stained using the periodic acid Schiff (PAS) technique. The stained smears were examined under 40X magnification to evaluate presence of Candida yeast cells and pseudohyphae.

Analyses were performed using chi-square test and the differences between two independent samples were analyzed using Student's *t*-test. A *P*-value < 0.05 was set as statistically significant. Statistical analysis was performed using SPSS version 17.0.

Results

Table 1: shows the presence of Candida in controls and cases. Out of 30 cases per group, the control group yielded 23.3% (7/30) of the Candida species on culture while the OSMF group yielded 46.7% (14/30) Candida organisms on culture. However the difference between the two groups was not statistically significant i.e. $P > 0.05$

Table 2: shows the intensity of Candida in cases and controls. The mean colony count of the controls was 48.67 colony formation unit/milliliter (CFU/ml) while the mean colony count of the OSMF group was 292.17 CFU/ml. The difference between the two groups was statistically significant. ($t = 3.627$) and ($p = 0.00$).

Fig 1 shows the comparison of the Candidal carriage positive and Candidal carriage negative OSMF patients classified according to their clinical staging of OSMF. The difference between the two group was statistically significant i.e. $p = 0.009$.

Fig 2 shows the comparison of the oral hygiene status of Candidal carriage positive and Candidal carriage negative OSMF subjects. The difference between the two was statistically significant i.e. $p = 0.001$

Discussion

Several studies have reported the association between the Candida and pre-cancerous and cancerous lesions. Candida albicans which is the most prominent species among the Candidal species are known to infect any tissue within the body. Studies have proved the histologically presence of the Candida albicans in dysplastic lesions. Candida is also found in patient with lichen planus and lichen planus patients undergoing topical steroid therapy.¹¹

The present study found the prevalence of Candidal species 46.7% in OSMF patients and 23.3% in control group. A similar study done by Anila K⁸ showed prevalence rate 40% and 15% in OSMF and control group respectively. The higher prevalence of the Candida in OSMF patient as compared to the healthy individuals may be attributing to their decreased immunity and reduced mouth opening.

Recently, a study done by Preeti Sharma and Susmita Saxena on correlation between Candida albicans and oral epithelial neoplasia showed presence of Candida albicans in precancerous lesions, conditions and malignant disorders with prevalence rate of 84.4%, 44.4% and 91.1% respectively.³ A study by Ariyawardana et al. determined the prevalence of oral yeast in OSMF patients and healthy individuals. Candida was isolated from 63.6% of the OSMF group and 50% of the control group.¹²

In present study, we observed mean intensity of Candida carriage in study group and control group to be 292.17 CFU/ml and 48.17 CFU/ml respectively which was statistically highly significant ($p=0.00$). Anila K⁸ found similar results with mean intensity 186.6667CFU/ml in control and 721.2500CFU/ml in OSMF patients.

In this study the OSMF group was divided into three groups according to their clinical staging given by Mahtur and Jha.¹³ Study subjects with grade 1 OSMF had 12.5%, grade 2 had 47.1% and grade 3 had 100% positive Candidal carriage which suggested that positive Candidal carriage increased with increase in severity of the disease, which helped in explanation that Candida albicans may be secondary predisposing factor for invasion of potentially malignant disorders.

We also found the co-relation between the Positive Candidal carriage and the oral hygiene status of the study group, which was not, assessed by the previous other similar studies. Percentage of positive Candidal carriage was proportional to the oral hygiene status of the individuals of OSMF group. Candidal carriage was greater in patients with poor oral hygiene, which was found to be statistically significant ($p = 0.001$).

Buccal smears were obtained to evaluate yeast cells and pseudohyphae, which may anchorage Candidal species in local invasion. A number of putative virulence factor have been suggested in the enhancement of C. albicans pathogenesis. These includes yeast to hyphal form transition, phenotype switching mimicry, adhesion factor or surface hydrophobicity, secretion of phospholipids and aspartyl proteinase.^{14,15,16,17,18} Oral yeast species carry a significant risk of malignant transformation. There appears to be a significant relationship between histologically determined fungal infection and epithelial dysplasia; about 21.9% of Candidal infected lesions showed more severe epithelial dysplasia.^{8, 9, 19} Field et al

proposed that nitrosamine compounds produced by Candida species may directly or in combination with other chemical carcinogens, activate specific proto-oncogenes and thus lead to initiation of the development of a malignant lesion.²⁰

The mortality rate of oral submucous fibrosis is significant and its malignant transformation is noticed in 2.3-7.6% of the cases.²¹ Only few studies have revealed the association between Candida and oral submucous fibrosis. The present undertaken study aimed to find the incidence and intensity of the Candida in the oral submucous fibrosis patient in view that role of Candida in its malignant transformation could better be understood.

Conclusion

The incidence and intensity of Candidal species was quite significantly higher in study group, suggested that patients with OSMF are at high risk to infectious diseases from Candida as compared to healthy individuals, and hence added responsibility upon us in further educating the population to quit the habit. However, further study with greater sample size is recommended to better know the mechanism underlying relation between Candida and potentially malignant disorders.

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Legend Table and Figure

Table 1: Presence of Candida in cases and controls

Groups	Cases	Frequency of Candida isolation
Control group	30	7 (23.3%)
OSMF group	30	14 (46.7%)

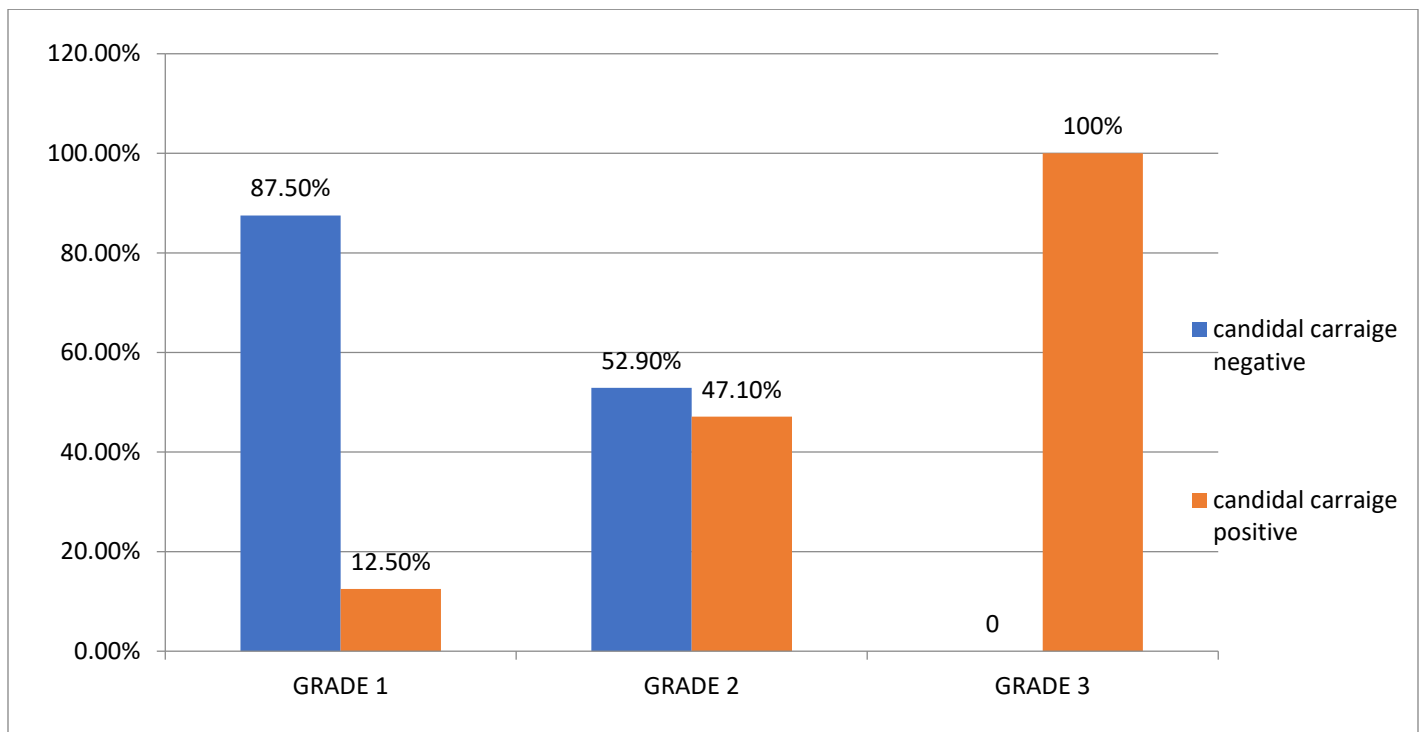
$X^2 = 3.590, P = 0.058$

Table 2: Intensity of Candida in two groups

Groups	Range CFU/ml	Mean CFU/ml	Standard Deviation
Control group	65-525	48.67	123.050
OSMF group	350-1050	292.17	346.499

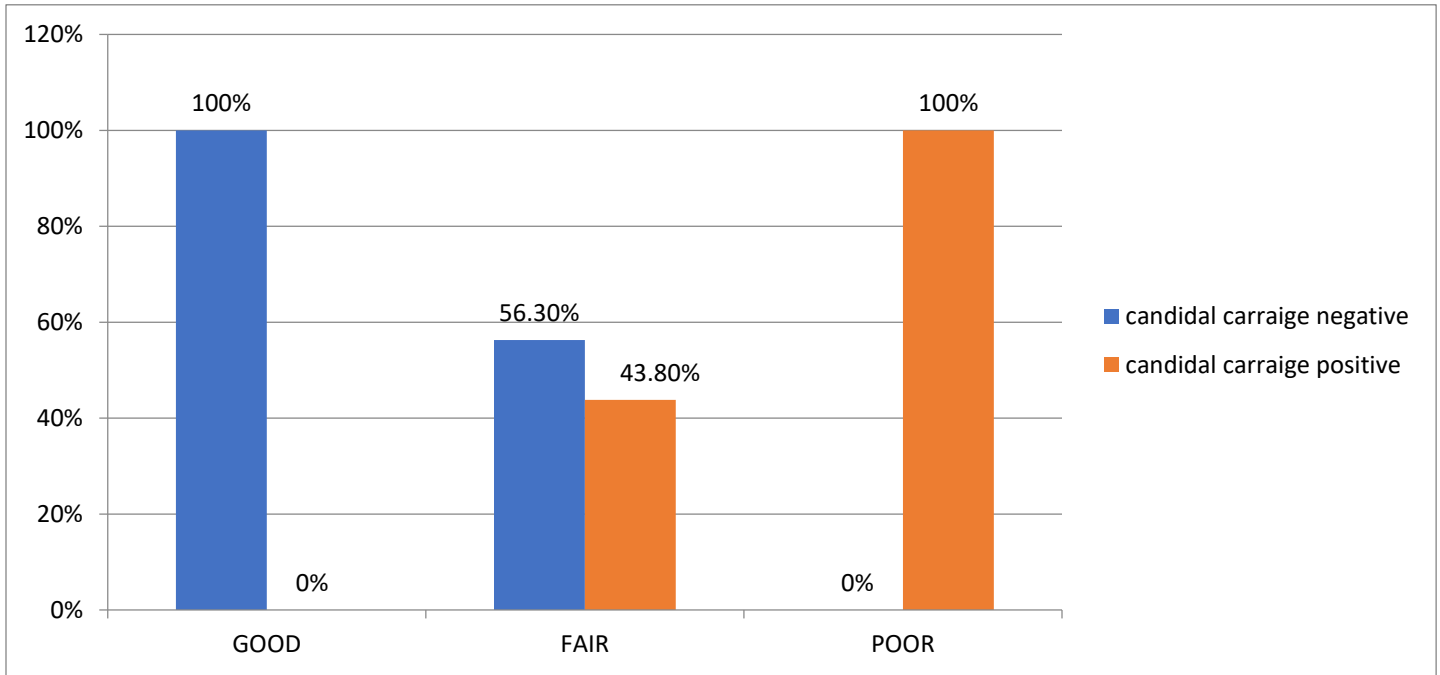
$t = 3.627, p = 0.00$

Fig. 1: Comparison of the Candidal carriage positive and Candidal carriage negative OSMF patients classified according to their clinical staging of OSMF.



$X^2 = 9.468, p = 0.009$

Fig. 2: Comparison of the oral hygiene status of Candidal carriage positive and Candidal carriage negative OSMF subjects.



$X^2 = 14.180, p = 0.001$