

Candida species in Oral Submucous fibrosis and healthy individuals

Dr.Samatha.K.S¹, Dr.Usha.Chikkaiah², Dr. Sujata Mohan Byahatti³, Dr Renuka Ammanagi⁴, Dr Praveena Tantradi⁵

^{1.} Assistant professor, Department of Oral Medicine and Radiology. Sharavathi Dental college & Hospital, Shivamogga, Karnataka, India.

^{2.} Professor and Head, Department of Oral Medicine and Radiology. Sharavathi Dental college & Hospital, Shivamogga, Karnataka, India

^{3.} Professor, Department of Oral Medicine and Radiology. M M's N.G. Halgekar Institute of Dental sciences and research centre, Belgaum, Karnataka, India

^{4.} Professor and Head, Department of Oral Medicine and Radiology at M M's N.G. Halgekar Institute of Dental sciences and research centre, Belgaum, Karnataka, India

^{5.} Professor, Department of Oral Medicine and Radiology. M M's N.G. Halgekar Institute of Dental sciences and research centre, Belgaum, Karnataka, India

Corresponding Author: Dr.Usha.C,

Professor & HOD, Department of Oral Medicine and Radiology Sharavathi Dental college & Hospital, TH Road, Alkola, Shivamogga 577204, Karnataka, India.

Abstract: Background:- Oral submucous fibrosis (OSMF) is a well-known precancerous condition. Presence of *Candida* in the mouth along with epithelial changes may predispose to candidal infection. The purpose of this study was to compare isolate, quantify, speciate the Candidal species in Oral submucous fibrosis and in healthy individuals in the Indian patients.

Methods: - This study included 20 OSMF patients and 20 healthy individuals. A detailed clinical history with relevant medical history and deleterious habits were recorded. Sample collection was done by scrapping the superficial mucosal layer for estimation of candidal growth, quantification of candidal colony count and to speciate the different species of candida cultured on Sabouraud's dextrose agar (SDA) and CHROM agar.

Results: In total, 53.3% of OSMF patients and 6.7% of healthy controls yielded candida growth on culture. *C. albicans* was the predominant species isolated, but *C.albicans* & *C.dubliences* were also speciated. Gender, gutkha habit had no influence on the candidal growth in OSMF patients.

Conclusion: The probable role of *Candida* in oral carcinogenesis remains the subject of considerable debate. Studies in this field are fraught with difficulty as *Candida* organisms are commensals in the oral cavity; thus, establishing their role in carcinogenesis is challenging. The present study revealed, evaluation of growth of candida by using sterile cotton swab wherein candidal colony forming units (CFU) were calculated by using SDA. We isolated combination of *C.albicans* & *C.dubliences* in OSMF patients for the first time in the Indian patient's. The candidal colonies were higher in the OSMF group than compared to healthy controls. However the candidal carriage in OSMF group was not statistically significant when compared with the control group.

Date of Submission: 13-05-2019

Date of acceptance: 30-05-2019

I. Introduction

Oral submucous fibrosis (OSMF) is a chronic, disabling condition of the oral mucosa affecting any part of the mouth and rarely the pharynx, larynx and esophagus.^{1,2} Oral submucous fibrosis has been identified as a high-risk precancerous condition that affects young Indians due to their habit of gutkha chewing.^{3,4} Chewing tobacco is highly prevalent in India, Pakistan, Bangladesh, Myanmar, Taiwan and Sri Lanka. Betel chewing has strong association between most of the religious and cultural rituals of ethnic communities in the Indian subcontinent including Sri Lanka.⁵ The precancerous nature of OSMF has been well established with a frequency of malignant transformation rate of 3–6%.⁶ Epithelial atrophy is one of the key features of OSMF.⁷ *Candida* species are normal oral commensals, along with epithelial changes may predispose to candidal infection. Reduced mouth opening in OSMF might predispose candidal growth, and this *Candida* can further predispose the mucosa for malignant transformation through the process of nitrosation. Candidal carriage can induce epithelial atypia and progress to malignant transformation by the release of chemical carcinogens like nitrosamine compounds. *C.albicans* is the predominant species isolated in premalignancy and carcinoma.⁸ The

aim of the present study was to compare the isolates, quantify, speciate the Candidal species in Oral submucous fibrosis and healthy individuals in North Karnataka, Indian patients.

II. Materials and methods

This study consisted of total 40 male and female patients. Case history along with informed consent was taken. The total sample included 20 OSMF patients and 20 healthy individuals with no deleterious habits and no known observable oral clinical lesions within the age group ranging between 20 to 60 years were selected. Patients above 40 years were evaluated to rule out the underlying systemic diseases. We excluded the patients on any medications especially h/o topical or systemic corticosteroid therapy or who are on long term broad spectrum antibiotic therapy, patients with any medical disorder or history of immunocompromised conditions like Diabetes Mellitus, HIV, severe anemia, etc and denture patients and patients with removable /fixed partial dentures. This study was approved by the Institutional Review Board. For microbiological analysis, samples collected from lesions as well as from healthy individuals with sterile cotton swabs, immediately placed in transport medium, and processed for the inoculation onto Sabouraud's dextrose agar (SDA) and incubated for 48 h at 37⁰C, for noting the CFU. Later CHROM agar was used to isolate the different candidal species based on the change in the colour as follows.

C. albicans appear as green, smooth colonies. *C. tropicalis* appear as blue, smooth colonies with pink halos. *C. krusei* appear as rough, spreading pale pink with white borders. *C. Glabrata* appears as dark pink colonies with pale edges.

Statistical analysis: The data was statistically analyzed with the t-test

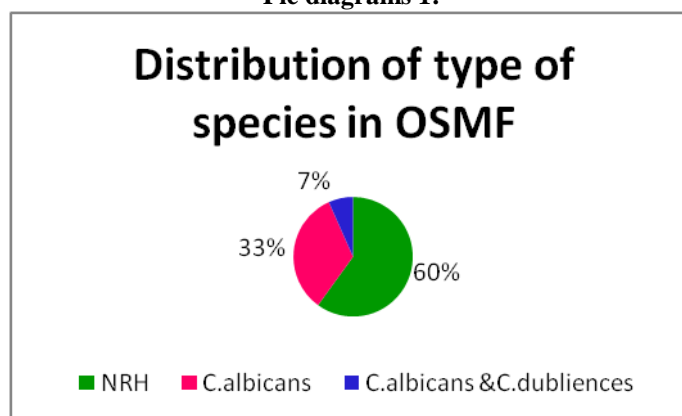
III. Results

The mean age of the case was 30 years and the control was 28 years. There was a male preponderance with 93.3% of them were males and 6.7% were females in OSMF patients and in the control group 86.7% of them were males and 13.3% were females. The growth of *Candida* organisms on culture was 53.3% of the OSMF patients and 6.7% of the controls. In OSMF patients 72 x10³ CFU in 1 patient i.e., which is highest number and least number being 2x10³CFU and in controls 13x10³CFU and 0x10³CFU respectively. The mean was 13.15 and 1.25 in case and controls. The Std.Deviation was 16.19 and 2.12 in case and controls. However, the difference between the two groups was not statistically significant with the p-value of 0.004

T-test

	group	Mean	Std. Deviation	p-value
OSMF	case	13.15	16.194	0.004
	control	1.25	2.124	

Pie diagrams 1:



IV. Discussion

According to WHO (1978), OSMF is defined as “A slow growing progressive disease in which fibrous bands form in the blanched oral mucosa resulting in severe restriction of movement of mouth”. The most accepted definition is the one stated by Pindborg and Sirsat (1966), “OSMF is an insidious chronic disease affecting any part of oral cavity and sometimes pharynx although occasionally preceded by and/ or associated with juxta epithelial inflammatory reaction followed by fibro elastic changes in the lamina propria with epithelial atrophy leading to stiffness of oral mucosa and causing trismus and inability to eat”.⁹

Joshi S G (1953) first described the condition in India and suggested the name “submucous fibrosis” of palate and pillars.¹⁰ **Pindborg J J et al** (1984) found that the rate of malignant transformation to be 4.5% out of 89 patients with the disease in Ernakulum district, Kerala.¹¹ 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.¹² 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. 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.¹³ Healthy individuals carry 3-47% of candidal species as a component of normal oral flora. Oral candidiasis 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.¹⁴ In our current study candidal carriage noted among 20 patients with OSMF compared with 20 healthy controls. The sample size was similar to the study conducted by Saigal S *et al*¹⁵, Singh SK *et al*¹⁶, Hongal BP *et al*¹⁷, Beena George.¹⁸ Study conducted by Ariyawardana A *et al*¹⁹, Kumar RS *et al*²⁰, Anila K *et al*¹⁴, Kamat MS *et al*²¹ had higher sample size when compared to our current study. The age group in our present study ranged from 20-60 years with the mean age of 29.87 years for OSMF patients where as the average age for healthy subjects was 28.33 years. This was similar to Anila K *et al*¹⁴, Kamat MS *et al*²¹ and Hongal BP *et al*¹⁷ Whereas the study conducted by Ariyawardana A *et al*¹⁹ included younger age group with the mean age of 44.2 years, Kumar RS *et al*²⁰, {mean age for OSMF(39.53±16.50), and control (58.53± 20.55)}, Singh SK *et al*¹⁶ included higher age group when compared to our present study. In the current study, the numbers of male patients with OSMF were 93.3% and female was 6.7% respectively. This gender distribution was in contrast to study conducted by Ariyawardana A *et al*¹⁹ (M-21, F-29), Anila K *et al*¹⁴ (M-20, F-20), Hongal BP *et al*¹⁷ (M-13, F-3). Beena George.¹⁸ study included only male patients (M-60) in her study group. In our study, out of 20 patients with OSMF, 53.3% of them showed growth of candida which was higher than the study conducted by Kumar RS *et al*²⁰, Sharma P *et al*²², Anila K *et al*¹⁴, Kamat MS *et al*²¹ and Singh SK *et al*¹⁶ But growth of candida noted in the current study was lower than the study conducted by Sharma P *et al*²², Beena George¹⁸, Hongal BP *et al*¹⁷. Whereas healthy subjects in the current study, 6.7% individual revealed growth of candida which was lower than the study conducted by Kumar RS *et al*²⁰, Anila K *et al*¹⁴, Kamat MS *et al*²¹, Beena George.¹⁸ This variation in the results obtained could be due to difference in the sample size, gender distribution and the method of collection of sample. Candidal colonies were counted and expressed in colony forming units (CFU) among OSMF patients. In the current study patients with OSMF had the mean candidal count which was 10333 CFU noted to be higher than the study conducted by Anila K *et al*¹⁴, Kamat MS *et al*²¹, Sharma P *et al*²², Beena George.¹⁸ This could be due to difference in the method of inoculation. Growth of candida species i.e., *C. albicans* alone noted in 33.3% OSMF patients, followed by combination of *C. albicans* & *C. dubliniensis* in 6.7% individual But the remaining 60% patients showed no candidal growth noted. Isolation of 33.3% candida albicans species was noted both in the study group, which was similar to other study conducted by Ariyawardana A *et al*¹⁹, Anila K *et al*¹⁴, Kamat MS *et al*²¹, Sharma P *et al*²², Saigal S *et al*¹⁵ and Beena George.¹⁸ *C. dubliniensis* noted in our current study samples were similar to the other study conducted by Ariyawardana *et al*¹⁹ Apart from above mentioned species other different species which were isolated varied when compared to our study as given by Ariyawardana *et al*¹⁹, Anila *et al*¹⁴, Kamat MS *et al*²¹. This difference in the identification of other species is due to variation in the method of isolation and different identification kits used in their study. But however among these various species of candida we were able to isolate *C. albicans*, *C. tropicalis*. Therefore the future research should include studies with large sample size to isolate, quantify and to speciate the candida in patients with OSMF and healthy individuals.

V. Conclusion

Hence, the present study mainly emphasizes on most reliable, easy and simple method of isolation of candidal species i.e. by swabbing the lesion and later inoculation on to SDA and the different species identification by CHROM agar. Although very few literatures mentions regarding usage of swab method of collection, SDA and CHROM agar for candidal isolation and species identification together, it was difficult for us to discuss and compare our results with the previous literature. Till date no study was found on these three techniques together, which would be the first of its kind going to add knowledge in the field of research.

References

- [1]. Pindborg JJ, Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Mehta FS. Oral submucous fibrosis as a precancerous condition. Scand J Dent Res 1984; 92: 224–9.
- [2]. Maher R, Ahmed W, Qureshi H, Zuberi SJ, Syed S. Oesophageal changes in oral submucous fibrosis using fiberoptic endoscopy – a pilot study. J Pak Med Assoc 1991; 41: 312–3.
- [3]. Nair U, Bartsch H, Nair J. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and *pan masala*: a review of agents and causative mechanisms. Mutagenesis 2004;19:251-62.
- [4]. Pillai R, Balaram P, Reddiar KS. Pathogenesis of oral submucous fibrosis. Relationship to risk factors associated with oral cancer. Cancer 1992;69:2011-20.

- [5]. Ariyawardana A, Vitanaarachchi N. Awareness of oral cancer and precancer among patients attending a hospital in Sri Lanka. *Asian Pacific J Cancer Prev* 2005; 6: 58–61.
- [6]. Rajendran R. Oral submucous fibrosis: etiology, pathogenesis and future research. *Bull World Health Organ* 1994; 72: 985–96.
- [7]. Johnson NW, Maher R, Trivedy S, Warnakulasuriya S. The clinical condition and pathology of oral submucous fibrosis (abstract). *Oral Dis* 1997; 3: 278–9.
- [8]. Sitheequ MAM, Samaranyake LP. Chronic hyperplastic candidosis/candidiasis. *Crit Rev Oral Biol Med* 2003; 14:253–67.
- [9]. Shafer W.G. Hine, M.K and Levy B.M. Text book of Oral Pathology, 4th Edition. Philadelphia. W.B. Saunder's Company 1983: 109.
- [10]. V.Jayanthi et al. Oral submucous fibrosis – A preventable disease. *Gut* 1992; 33:4-6.
- [11]. Pindborg JJ and Sirsat SM. Oral Submucous Fibrosis. *Oral Surg Oral Me Oral Pathol* 1966; 22(6): 764-779.
- [12]. Ho P, Chen P, Warnakulasuriya S, Shieh T, Chen Y, Huang I. Malignant transformation of 22. oral potentially malignant disorders in males: a retrospective cohort study. *BMC Cancer* 2009; 9:260-7.
- [13]. Lai DR, Chen HR, Lin LM, Huang YL, Tsai CC. Clinical evaluation of different treatment 23. methods for oral submucous fibrosis. A 10-year experience with 150 cases. *J Oral Pathol Med* 1995;24:402-6.
- [14]. Anila K, Hallikeri K, Shubhada C, Naikmasur VG, Kulkarni RD. Comparative study of Candida in oral submucous fibrosis and healthy individuals. *Rev Odonto Cienc* 2011; 26(1):71-76.
- [15]. Saigal S, Bhargava A, Mehra SK, Dakwala F. Identification of candida albicans by using different culture medias and its association in potentially malignant and malignant lesions. *Contemp Clin Dent* 2011;2 (3):188-93.
- [16]. Singh SK, Gupta A, Rajan SY, Padmavathi BN, Mamatha GP, Mathur H et al. Correlation of Presence of Candida and Epithelial Dysplasia in Oral Mucosal Lesions Correlation of Presence of Candida and Epithelial Dysplasia. *Journal of Clinical and Diagnostic Research*. 2014 ;8(10): ZC31-ZC35.
- [17]. Hongal BP, Kulkarni VV, Deshmukh RS, Joshi PS, Karande PP, Shroff AS. Prevalence of fungal hyphae in potentially malignant lesions and conditions—does its occurrence play a role in epithelial dysplasia?. *J Oral Maxillofac Pathol*.2015; 19(1): 10–17.
- [18]. Beena George. Evaluation of the Prevalence of Candida albicans Infection in Patients with Oral Sub Mucous Fibrosis In Comparison With Healthy Individuals. *International Journal of Bioassays* 2015; 4(10): 4411-4413.
- [19]. Ariyawardana A, Panagoda GJ, Fernando HN, Ellepola AN, Tilakaratne WM, Samaranyake LP. Oral submucous fibrosis and oral yeast carriage-A case control study in Sri Lankan patients. *Mycoses*. 2007; 50:116–20.
- [20]. Kumar RS, Ganvir SM, Hazarey VK Candida and calcofluor white: Study in precancer and cancer. *J Oral Maxillofac Pathol*. 2009 ; 13(1): 2–8.
- [21]. Kamat MS, Vanaki SS, Puranik RS, Puranik SR, Kaur R. Oral Candida carriage, quantification and species characterization in oral submucous fibrosis patients and healthy individuals. *Journal of investigative and clinical dentistry* 2011;2:1-5.
- [22]. Sharma P Saxena s. Candida albicans and its correlation with oral epithelial neoplasia. *Int J Oral-Med Sci* 2011; 10(3):140- 48.

Dr.Samatha.K.S. “Candida species in Oral Submucous fibrosis and healthy individuals.” *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, vol. 18, no. 5, 2019, pp 18-21.