Candida species in Oral Submucous fibrosis and healthy individuals

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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. dubliences & 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.

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. Oral submucous fibrosis has been identified as a high-risk precancerous condition that affects young Indians due to their habit of gutkha chewing. 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. The precancerous nature of OSMF has been well established with a frequency of malignant transformation rate of 3–6%. Epithelial atrophy is one of the key features of OSMF. 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.
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 37°C, 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 x10^3 CFU in 1 patient i.e., which is highest number and least number being 2x10^3 CFU and in controls 13x10^3 CFU and 0x10^3 CFU 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

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<th>Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p-value</th>
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<tr>
<td>control</td>
<td>1.25</td>
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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. 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 Ernakulam district, Kerala. 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. 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. 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. 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. Study conducted by Ariyawardana A et al 18, Kumar RS et al 20, Anila K et al 4, Kamat MS et al 21 had higher sample size when compared to the present study. The age group in our present study ranged from 20-60 years with the mean age of 29.87 years for OSMF patients whereas 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 only male patients 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 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 18 (M-20, F-20), Hongal BP et al 17 (M-13, F-3). Beena George.14 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 candid 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 George 18, 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 patients 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.topicalis. Therefore the future research should include studies with large sample size to isolate, quantify and to speculate 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


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