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Research Article

Assessing the Candida in Healthy Subjects and Subjects with Oral Submucous Fibrosis: A Comparative Clinical Study

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Abstract: One precancerous condition with a high potential to turn into malignancy and pose a high risk is OSMF (oral submucous fibrosis) which mainly affects the young subjects globally as well as in India. The etiology of OSMF is attributed to the gutkha chewing habit. Another predominant etiologic factor governing the etiopathogenesis of malignant and premalignant lesions is the Candida species. Our aim of the present clinical study was conducted to assess and compare the intensity and incidence of Candida species in healthy subjects and subjects with oral submucous fibrosis. This study included 24 healthy controls and 24 subjects with a confirmed diagnosis of OSMF. A detailed history was recorded and a clinical examination was done for all the study subjects. All the samples were obtained from the oral rinse and were cultured using Sabouraud's agar medium followed by identification and counting of isolated yeast species on sugar assimilation test, chlamydospore formation, and germ tube test. In the OSMF group, Candida species were isolated from 41.66% (n=10) of subjects. However, in the control group, Candida species were isolated from 16.66% (n=4) of the collected samples. This identification difference showed that higher species were isolated from the OSMF group compared to the control group (p=0.07). In the OSMF group, CFU/ml had a range of 500-970 with mean CFU values of 721.27±143.88. In the control group, the CFU/ml range was 60-320, whereas, the mean CFU/ml value was 186.64±130.10. The mean value was higher in the OSMF group compared to the control group. This difference was statistically significant with a p-value of 0.005. The present study concludes that the intensity and incidence of *C. Albicans*, candida species was higher in subjects with OSMF compared to healthy controls, however, these species were in the normal range of 3% to 47%. Hence, Candida cannot be considered an etiologic factor for transforming OSMF into a malignant lesion. However, further evaluation is needed to evaluate if betel quid chewing in subjects with OSMF promotes or inhibits the invasion and adherence of Candida species.

Keywords: *Candida*, *C. Albicans*, Gutkha Chewing, Oral Submucous Fibrosis, Oral Carriage.

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1. INTRODUCTION

One of the most common fungal infections seen in the oral cavity of humans is caused by the Candida species. Predisposing factors to the candida infection in the oral cavity are epithelial changes along with the presence of candida species in the mouth of the affected subjects. Epithelial changes in the mucosa of the oral cavity including dysplasia, hyperplasia, and atrophy can facilitate the invasion of candida species by compromising the mucosal barrier with epithelial atrophy being the main predisposing event.[1] The primary etiological species responsible for oral candidiasis is *Candida albicans*. Candida, being opportunistic fungi causing the infection can invade and colonize the oral cavity leading to lesion formation in immunocompromised as well as immunocompetent subjects. Immunocompromised subjects are at a significantly higher risk of getting candida infection compared to immunocompetent subjects. Owing to the increase in immunocompromised subjects, in the last decade, the number of subjects with Candida infection has markedly increased globally as well as in India.[2] Malignant transformations is seen in candida infections with the carcinogenic nitrosamine compound release. The active ingredients in the tobacco-like nitrosoproline, polonium, hydrocarbons which are polycyclic and aromatic, and nicotine provides the nutritive factors to the Candida species and helps in their proliferation which in turn, increases the candida colonization by epithelial changes as dysplasia, hyperplasia, or atrophy with decreased leukocyte functions and salivary IgA levels, an increase in the keratinization of the epithelium.3 With candidiasis, a decrease in salivary flow is also noted. The growth of Candida species in subjects with oral submucous fibrosis is also seen due to decreased salivary flow and reduced mouth opening in the affected subjects.[3] Previous literature data has suggested that colonization of the Candida species on the various surfaces of the oral cavity including dentures can act as a reservoir for infections caused by dissemination including gastrointestinal infections and aspirate pneumonia. In cases with malignancy and premalignant conditions, Candida albicans is one of the most commonly isolated microorganisms. Infection by candida species can lead to epithelial atypia which can further lead to malignant transformation by the release of carcinogens including nitrosamine compounds. Similarly, Candida can also cause malignant transformation in subjects with OSMF. However, this still lacks confirmation by previous literature data and is attempted in the present study.[4] In the recent past, OSMF is suggested to be a precancerous condition with high risk and potential to convert into a malignant lesion. In India, OSMF is seen mainly in young subjects with the main etiologic factor identified for OSMF being gutkha chewing. Despite the variety of etiologic factors considered as predisposing to OSMF, one of the keys and vital predisposing features is epithelial atrophy. One of the reasons favoring the growth of Candida species in OSMF subjects is reduced mouth opening which can further lead to notarization and malignant transformation in OSMF.[5] However, the previous data in the literature does not show a clear picture of assessing the prevalence of Candida species in normal healthy subjects and subjects with OSMF. Hence, the present study aimed to assess and compare the intensity and incidence of Candida species in healthy subjects and subjects with oral submucous fibrosis.

2. MATERIALS AND METHODS

The present study was conducted to assess and compare the intensity and incidence of Candida species in healthy subjects and subjects with oral submucous fibrosis. The study was conducted after obtaining clearance from the concerned Ethical committee. The study included a total of 48 subjects from both genders. 48 subjects were divided into two groups of 24 subjects each where one group had subjects with OSMF (Figure 1) and the other 24 served as control who were gender and age-matched. After explaining the detailed study design, informed consent was taken from all the subjects in both written and verbal form. The study protocol was approved by the Ethical Committee of the Institute. All procedures performed in the study were following the ethical standard of the Institute (SSIMS/IERBC/2018/03/018333). After explaining the detailed study design, informed consent was taken from all the study subjects.

2.1 Inclusion Criteria

The inclusion criteria for the study were healthy subjects with no systemic disease, no history of oral surgical procedures in the recent past, no treatment of OSMF, subjects of age 18 years and more, and subjects who were willing to participate in the study. The exclusion criteria for the study were subjected less than 18 years, history of malignancy, and the subjects who did not give consent for the study. After the final inclusion of the study subjects, detailed clinical history was recorded for all the subjects followed by a clinical examination. Following this, the clinical staging was done for subjects with OSMF using the Ranganathan et al[6] criteria.

2.2 Exclusion Criteria

The exclusion criteria for the study were subjects having systemic illnesses like diabetes.

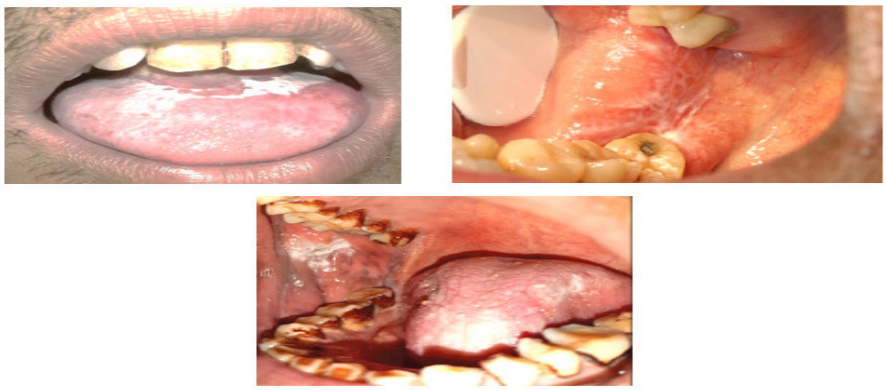

Figure 1: Clinical presentation of study subjects with OSMF

To collect the samples to assess Candida species technique by Samaranayake et al[7] in 1986 was used where all the subjects from both the groups were asked to rinse for 2 minutes using 10ml PBS (phosphate-buffered saline) followed by expectoration in a sterile vessel. The collected sample was subjected to centrifugation for 10 minutes at 2500g. PBS resuspension was again done for the pellet, and 100 µl solution was placed on a plate having SDA (Sabouraud's dextrose agar) followed by incubation at 37° C for 48 hours. After 48 hours, CFU (colony forming units) with yeast resemblance were removed from the culture media and processed for Gram staining, sugar assimilation test, chlamydospore formation, and germ tube test. CFU/ml was contributed by the counted yeast colonies from the sample collected. Growth was assessed on a modified SGA (Sabouraud glucose agar) medium at 45°C to allow easy differentiation of *C. dubliniensis* and *C. Albicans* (Figure 2).

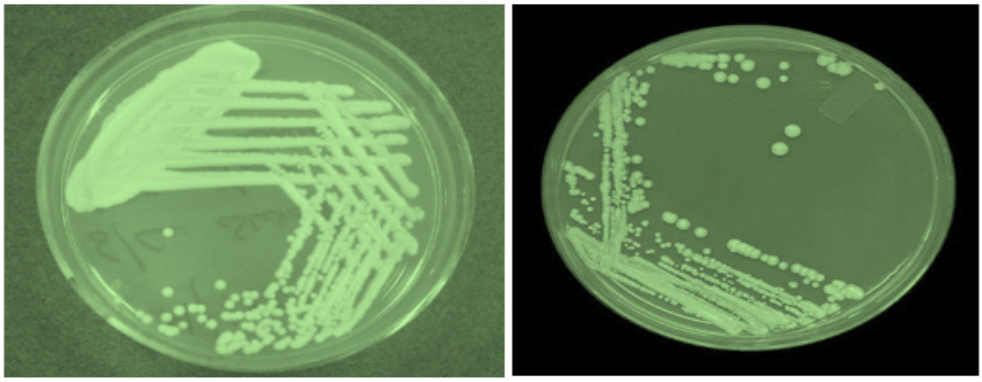

Figure 2: Culture plate with candida species in the study subjects

From the subjects of both the groups, buccal smears were taken and stained with PAS (periodic acid Schiff) stain technique. The smears that were stained were evaluated under the microscope at 40X magnification. A positive finding was recorded for the presence of pseudohyphae and yeast cells or candida yeast cells which were further classified as group 1 = none, group 2 = yeast cells only, group 3 = a few yeast cells, and pseudohyphae (2-3 cells), and group 4 = many yeast cells and pseudohyphae.

3. STATISTICAL ANALYSIS

The statistical analysis of the collected data was done using SPSS software version 21 (Chicago, IL, USA) and one-way ANOVA and t-test for results formulation. The data were expressed in percentage and number, and mean and standard deviation. The level of significance was kept at $p<0.05$.

4. RESULTS

The present study was conducted to assess and compare the intensity and incidence of Candida species in healthy subjects and subjects with oral submucous fibrosis. The study included a total of 48 subjects from both genders. 48 subjects were divided into two groups of 24 subjects each where one group had subjects with OSMF and the other 24 served as control who were gender and age-matched. The mean age of the study subjects was 26.5±3.46 years. The study had 89.58% (n=43) males and 10.41% (n=5) females. Among 24 subjects having OSMF, the etiologic factor in 91.66% (n=22) subjects was gutkha chewing, whereas, in 8.33% (n=2) subjects, it was areca nut chewing alone. The mean mouth opening in the subjects was 19.75±1.12 mm (Table 1).

Table 1: Demographic characteristics of the study subjects

Characteristics		Percentage (%)	Number (n)
Mean age (years)		26.5±3.46	
Age range (years)		18-35	
Gender	Males	89.58	43
	Females	10.41	5
Etiology	Gutkha chewing	91.66	22
	Areca nut chewing	8.33	2
	Mouth opening (mm)	19.75±1.12	

On identifying the presence of Candida species in OSMF and control groups of the study subjects, it was seen that in the OSMF group, Candida species were isolated from 41.66% (n=10) subjects. However, in the control group, Candida species were isolated from 16.66% (n=4) of the collected samples. This identification difference showed that higher species were isolated from the OSMF group compared to the control group. This difference was statistically significant with p=0.07 as shown in Table 2 and Figure 3. The candida species isolated from the control group were *C. tropicalis* and *C. Albicans,* whereas, from the OSMF group, *C. tropicalis, C. krusei, and C. Albicans* were isolated. The most commonly isolated species in the OSMF group was Candida albicans in 87.5% (n=21) subjects followed by *C. Krusei* in 37.5% (n=9) subjects and *C. tropicalis* in 12% (n=3) subjects. In 6 subjects with OSMF, the concomitant presence of multiple candida species was seen.

Table 2: Identification of Candida species in the two groups of the study subjects (p=0.07)			
Groups	**Total number (n)**	**Candida isolation (%)**	**Candida isolation (n)**
OSMF	24	41.66	10
Control	24	16.66	4
p-value		0.07	

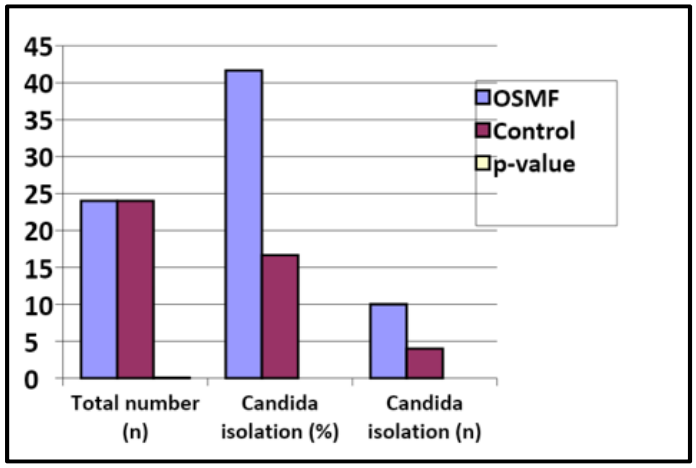

Fig 3: Candida species isolated in the two groups of the study subjects

On counting the mean numbers of colonies in the two groups of the study subjects, it was seen that in the OSMF group, CFU/ml had a range of 500-970 with mean CFU values of 721.27±143.88. In the control group, the CFU/ml range was 60-320, whereas, the mean CFU/ml value was 186.64±130.10. The mean value was higher in the OSMF group compared to the control group. This difference was statistically significant with a p-value of 0.005 as depicted in Table 3. No influence of gender was seen on the yeast carriage. Also, clinical staging and betel chewing habits showed no influence on candida carriage in subjects with OSMF. No pseudohyphae or yeast cells were detected on the buccal cytologic smears of either group.

Table 3: Intensity of Candida species in the two groups of the study subjects (p=0.005)			
Groups	**CFU/ml (range)**	**CFU/ml (Mean± S.D)**	**p-value**
OSMF	500-970	721.27±143.88	**0.005**
Control	60-320	186.64±130.10	

5. DISCUSSION

The present study was conducted to assess and compare the intensity and incidence of Candida species in healthy subjects and subjects with oral submucous fibrosis. The study included a total of 48 subjects from both genders. 48 subjects were divided into two groups of 24 subjects each where one group had subjects with OSMF and the other 24 served as control who were gender and age-matched. The mean age of the study subjects was 26.5±3.46 years. These demographics were comparable to the studies by Henriques M et al[8] in 2006 and Reichart PA et al[9] in 2005 where authors assessed subjects with comparable demographics as in the present study. For identification of the Candida species in OSMF and control groups of the study subjects, it was seen that in the OSMF group, Candida species were isolated from 41.66% (n=10) subjects. However, in the control group, Candida species were isolated from 16.66% (n=4) of the collected samples. This identification difference showed that higher species were isolated from the OSMF group compared to the control group. This difference was statistically significant with p=0.07 showing that in subjects with OSMF presence of candida is further leading to candidiasis and complicating the situation. Isolation of the higher species from the OSMF group was similar to the studies of Anila K et al[10] in 2011 and Reichart PA et al[11] in 2002 where similar species isolation was seen in healthy subjects and subjects with OSMF. Also, these results were supported by the results of Mech F et al[12] in 2014 and Sudarshan R et al[13] in 2012 where high candida prevalence was shown in subjects with OSMF. The Candida species isolated from the control group were *C. tropicalis and C. Albicans,* whereas, from the OSMF group, *C. tropicalis, C. krusei, and C. Albicans* were isolated showing that different species and more candida species are isolated from OSMF subjects compared to healthy controls. These results were consistent with the findings of Ariyawardana GA et al[14] in 2007, Lima-nato RG et al[15] in 2011, and Ho P et al[16] in 2009 where a similar proportion of the candida species was identified in their study subjects of OSMF. Concerning the mean numbers of colonies in the two groups of the study subjects, it was seen that in the OSMF group, CFU/ml had a range of 500-970 with mean CFU values of 721.27±143.88. In the control group, the CFU/ml range was 60-320, whereas, the mean CFU/ml value was 186.64±130.10. The mean value was higher in the OSMF group compared to the control group. This difference was statistically significant with a p-value of 0.005. These findings were in agreement with the studies of Jain Kittiwong A et al[17] in 2007, McCullough M et al[18] in 2002, and Nair U et al[19] in 2004 where the comparable number of colonies were counted in subjects with OSMF. The most commonly isolated species in the OSMF group was *Candida albicans* in 87.5% (n=21) subjects followed by C. Krusei in 37.5% (n=9) subjects and C. tropicalis in 12% (n=3) subjects. In 6 subjects with OSMF, the concomitant presence of multiple candida species was seen.

No influence of gender was seen on the yeast carriage. Also, clinical staging and betel chewing habits showed no influence on candida carriage in subjects with OSMF. No pseudohyphae or yeast cells were detected on the buccal cytologic smears of either group. These findings were in agreement with the studies of Shinozaki S et al[20] in 2017, Gupta B et al[21] in 2015, and Kamat MS et al[22] in 2011 where authors reported similar candida species isolation in their studies.

6. CONCLUSION

Within its limitations, the present study concludes that the intensity and incidence of *C. Albicans*, candida species was higher in subjects with OSMF compared to healthy controls, however, these species were in the normal range of 3% to 47%. Hence, candida cannot be considered as an etiologic factor for transforming OSMF into a malignant lesion. However, further evaluation is needed to evaluate if betel quid chewing in subjects with OSMF promotes or inhibits the invasion and adherence of Candida species. The present study had a few limitations including a small sample size, shorter monitoring period, and geographical area biases. Hence, more longitudinal studies with a larger sample size and longer monitoring period will help reach a definitive conclusion.

7. AUTHOR'S CONTRIBUTION STATEMENT

Dr. Smita Deshkar and Dr. Anushree S. Gaigawale designed and conceptualized the study along with collecting the data. Dr. Priyadarshini Rangari and Dr. Anil Raj discussed the methodology and assessed data to formulate the results. Also, the manuscript was designed by both Dr. Charul Aggarwal and Dr. Garima Tyagi. The final version of the manuscript was approved by both authors.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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