

Determination , distribution & phenotypic differentiation of Candida : Study in oral precancer and oral cancer

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ABSTRACT

Aim: The present study was aimed at determining the presence of Candida in oral precancer and cancer and differentiation of *C. albicans* and *C. non albicans* in relation to age.

Materials and methods : The study group comprised of patients with oral precancer and cancer , and control group. Scrape cytology was done for smear preparation followed by gram staining to analyze for presence of Candida. Swab method was utilized for culture inoculation followed by germ tube test to differentiate between the *C. albicans* and *C. non albicans*.

Results : A highly significant association of Candida was seen both in precancer and cancer. Statistical comparisons showed significant correlation between age ranges and development of oral precancer and cancer. *Candida albicans* was the most predominantly isolated species. However, statistically significantly more *C. non albicans* were present in the oral cavities of patients over the age of 60 years.

Conclusion : The present results showed a shift in the species of yeast isolated from the oral cavities of patients with precancer and cancer, with an increase in the isolation of *C. non albicans* species with advancing age. Nevertheless, *Candida albicans* was the most predominantly isolated species. Further studies are needed to determine the relationship between distribution of Candida species in relation to age.

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Introduction

Mycotic infections have become a major cause of morbidity and mortality in clinically debilitated or immunocompromised patients .[1]

C. albicans is an opportunistic yeast that is also found as a commensal in the oral cavities of healthy individuals.[2] Healthy individuals as well as patients appear to harbor in the vast majority of cases only a single strain of *C. albicans*.[2] Recently, there has been an increase in the reporting of the isolation of non-*Candida albicans* yeast species, particularly from immunocompromised patients.[3]

Chronic hyperplastic candidosis (CHC) is a form of oral candidosis characterised by hyphal invasion of the oral epithelium and is clinically significant because of its reported association with squamous cell carcinoma (SCC). The development of malignancy at CHC lesional sites has been estimated to occur in up to 10% of untreated cases.[4] Candidal virulence attributes involved in SCC development are unknown and still a matter of debate, although production of endogenous nitrosamines by strains of *C. albicans* has been implicated. The catalytic potential to form the esophageal carcinogen, N-nitrosobenzylmethylamine, from its precursors has been demonstrated in *C. albicans* strains of certain biotypes.[5] The importance of CHC is its reported association with the development

of malignancy at the lesional site.[6] The role of candida in this dysplastic change remains unclear. It has been suggested that the presence of candida represents a secondary infection within a preexisting altered epithelium. However, clinical resolution of CHC and a reduction in the extent of epithelial dysplasia following systemic antifungal therapy have supported a direct role for candida.[7] In addition, a correlation between histologically confirmed fungal invasion and epithelial dysplasia in oral mucosal lesions has been reported.[8] Furthermore, the ability of certain *C. albicans* strains to promote neoplastic changes [9] and to produce carcinogenic nitrosamines from saliva [10] has highlighted the potential role of candida in malignant transformation. The association of Candida with various precancer and cancer lesions has been reported as a causative agent.[1]

There is wide variation in the reported incidence of yeast in the oral cavities of healthy patients, in elderly patients , in patients with oral lichen planus and oral leukoplakia[3] *C. dubliniensis* is phenotypically so similar to *Candida albicans* that identification of the former has proved problematic in clinical samples.[11] However, the recent description of phenotypic tests, including the detection of inability to grow at 45°C [12], may facilitate the identification of this species in oral samples. With the increasing importance of candidosis , there is a need for a practical method for identification of fungus.[1]

The study was planned with the following aims and objectives :

1. To determine the presence of *Candida* in oral precancer and cancer patients using various laboratory tests.
2. To differentiate between *C. Albicans* and non *albicans* species using germ tube test.
3. To rule out *C. dubliniensis* species from *C. Albicans* by detection of their inability to grow at 45°C.
4. To determine the distribution of *C. Albicans* and non *albicans* in relation to age.

Materials and Methods

The sample group consisted of 90 patients (54 men and 36 women) with an age distribution between 20 and 79 years presenting to the Department of Oral and Maxillofacial Pathology, VSPM's Dental College and Research Centre, Nagpur. The subjects were recruited from both inpatients and outpatients.

The inclusion criteria for the research were diagnosis of oral precancer and cancer. The exclusion criteria for the research were estimated prognosis of 1 week, significant cognitive impairment, significant physical impairment, clinical evidence of dehydration, and use of antifungal medication within the previous 2 weeks.

The study group comprised of 60 cases of oral precancer and cancer, diagnosed on the basis of clinical and histological features, were selected. The precancer group comprised of Oral submucous fibrosis (OSMF), and Oral leukoplakia (OLP). The control group comprised of 30 cases of normal patients.

A complete medical history was taken to ensure that no individual had any medical condition or was taking medication predisposing toward opportunistic fungal infection. All of the subjects granted informed consent.

Sample Collection

The clinical component of the protocol involved examination of the oral cavity, followed by wiping a sterile cotton swab across the affected mucosal site of patients with evidence of oral precancer and cancer. Smears were prepared by scrape cytology using wooden stick moistened in normal saline. For histopathological examination, a tissue was obtained by using a 7 mm punch.

Method of Identification

For the control group, smears were obtained from the posterior dorsal surface of the tongue and buccal mucosa of normal healthy individuals and for the study group, smears were prepared from the lesional site. The smears were Gram stained and observed under a light microscope where *Candida* was confirmed as gram-positive, dark blue colored hyphae and yeasts.

The swabs were inoculated immediately on the Sabouraud's slope and incubated at 37°C for 48 hrs. (Figure 1) From those samples demonstrating positive fungal growth, single colonies were isolated, transferred to Sabouraud agar and incubated at 37°C

hrs. The pure colonies were tested for germ tube production in serum after 3 hrs of incubation at 37°C.

Growth at 45°C

Ability to grow at 45°C was assessed for all germ tube-positive isolates by removing a small portion of a single colony and streaking it over the surface of two separate Sabouraud dextrose agar plates, which were incubated at 37°C and at 45°C, respectively, for 48h. Colony formation on the last three quadrants of the plate was scored as good growth, while growth on the first quadrant only was considered poor growth.[12]

Written consents were obtained from the patients prior to the biopsy procedure. Local anesthesia was achieved by infiltration / block technique ; given away from the site of biopsy. Punch biopsies were obtained by using a 7mm punch.



Figure 1 : Candidal growth on Sabouraud's agar slope

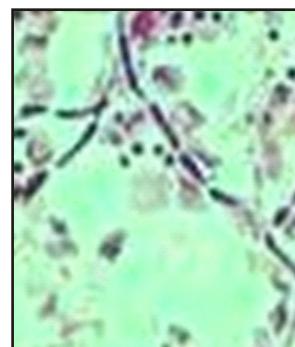


Figure 2 : Gram Staining showing *C. albicans*

Results

The clinical and laboratory data were collated and grouped into specific age ranges (20 - 40 years, 40 - 60 years, 60 - 80 years).

Statistical comparisons amongst these groups showed significant correlation between age ranges and development of oral precancer and cancer. There was a highly significant association of *Candida* more often in oral cancer than in oral precancer [Chart 1]. The mean age of females in the oral precancer and oral cancer groups were higher than that of males.

Among the 60 samples of study groups, 37 (61.66%) samples showed candidal colonies, fulfilling all the diagnostic criteria, whereas only one (3.33%) sample from the control group was positive for candidal growth.

Among the 37 culture positive samples of study groups, 29 samples and culture-positive sample of the control group both were Germ Tube test positive.

By using the GT test, the species identified was predominantly *Candida albicans* in 30 cases (78.94%), whereas the remaining eight cases (21.05%) belonged to non *albicans*. (Figure 3-6)

All the germ tube positive isolates were submitted to tests for detection of inability to grow at 45°C. Four of the 30 germ tube positive isolates were not able to grow at 45°C and one exhibited highly restricted growth. *C. dubliniensis* was isolated from the oral cavities of five out of 90 (5.56%) patients four of whom were female.

C. dubliniensis occurred significantly more often ($P = 0.001$) in patients between the ages of 60 and 80 years.

Similarly, statistically significantly more *C. non albicans* were present ($P = 0.003$) in the oral cavities of patients belonging to age group of 60 - 80 years. However, significantly more *C. albicans* were present in the oral cavities of patients belonging to age group of 40 - 60 years than in any other age group. [Chart 2]



Figure 3 : Germ tube of *C. albicans* (20 minutes old)

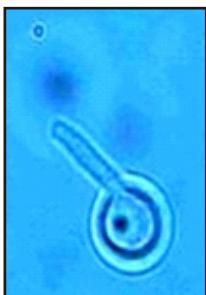


Figure 4 : Germ tube of *C. albicans* (2 hours old)



Figure 5 : Germ tube of *C. albicans* (3 hours old)

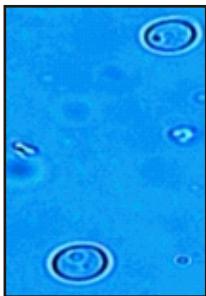


Figure 6 : *Candida* non *albicans* (GT test Negative - After 4 hours)

Discussion

Medical mycology is a growing field of interest because an increased number of clinical diseases are associated with pathogenic fungi.

In the current study, 62 % of the patients with oral precancer and cancer had microbiological evidence of fungi, of which *C. albicans* was by far the most frequent isolated yeast species (66% of all isolates). These results are in agreement with previous report of Krogh et al. where *C. albicans* was found to be the dominating species in oral leukoplakia and lichen planus. A slightly

higher percentage of positive fungal cultures in the male patients (73% compared with 52% among female patients) was found in the current study which was in contradiction with the findings of Al-Karaawi Z M et al. who reported no statistically significant differences in the male-to-female ratio.[3]

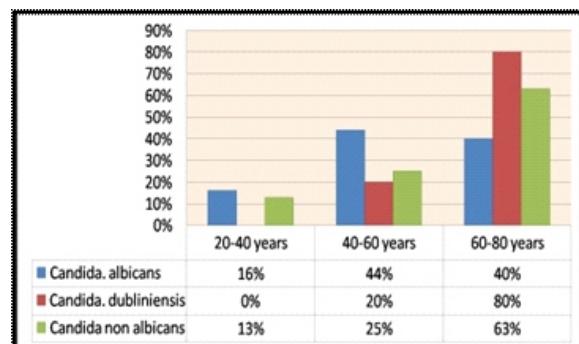


Chart 2 : Distribution of Candida in relation to age groups

Since the majority of clinical laboratories use the germ tube test as their sole method for the identification of *C. albicans*, isolates of *C. dubliniensis* have been misidentified as the former species.[11] The main problem in the detection of *C. dubliniensis* in oral samples is the lack of a simple, reliable, and inexpensive phenotypic identification protocol. However, inability to grow at 45° C proved to be useful for presumptive identification of *C. dubliniensis* in our study. Similar phenotypic differentiation of *C. dubliniensis* from *C. albicans* had been suggested in a study carried by Giannanco GM, et al.[14] Five of the isolates in the current study were *C. dubliniensis*, four of whom were female patients. A previous study of Davies AN & Brailsford S involving patients with advanced cancer reported isolation of a possible *C. dubliniensis* species.[13]

Interestingly, *C. dubliniensis* was isolated from the patients with oral precancer and cancer and the strong statistical association found in the present study between patients 60-80years of age [Chart 2], and the oral isolation of *C. dubliniensis* may not be merely circumstantial. The adult oral acquisition of this newly described species of yeast would indicate that the spread of *C. dubliniensis* might well be horizontal, rather than vertical. Nevertheless, *C. dubliniensis* has been isolated from the oral cavities of healthy individuals [11,12], and from those of patients with a variety of different diseases.[11,12]

A greater degree of *Candida* isolates were seen to occur more often with the advancing age in the current study [Chart 2]. Furthermore, there was a highly significant association of *Candida* more often in oral cancer than in oral precancer [Chart 1], which was in accordance with the findings of Rashmi Santosh Kumar et al. [1] A shift in the species of yeast isolated from the oral cavities of patients with precancer and cancer, with an increase in the isolation of *C. non albicans* species with advancing age was seen [Chart 2]. The outcomes were in agreement with previous findings of Al-Karaawi Z M et al. who reported statistically significantly more non-*C. albicans* *Candida* present in the oral cavities of patients over the age of 70years than in any other age group.[3]

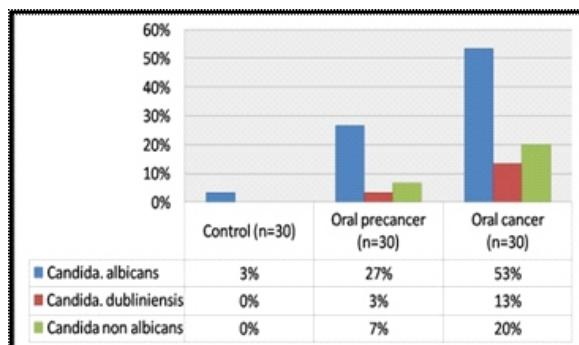


Chart 1 : Distribution of *Candida* among Control & Study group

Conclusion

The present results showed a shift in the species of yeast isolated from the oral cavities of patients with precancer and cancer, with an increase in the isolation of *C. non albicans* species with advancing age. Nevertheless, *Candida albicans* was the most predominantly isolated species. Further studies are needed to determine the relationship between distribution of *Candida* species in relation to age.

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