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RESEARCH ARTICLE

ISOLATION OF CANDIDA SPECIES, IDENTIFICATION OF CANDIDA ALBICANS AND THEIR CHARACTERIZATION AMONG BROCNCHODIALATOR USERS

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ABSTRACT

Oral candidal infection is common among bronchodialator users irrespective of whether they use it as puff or as nebulizer. There are several theories and hypothesis on the role of *Candida albicans* in the oral cancer and initiation of oral malignancy. Though Candida has a pathogenic role in the oral cavity, C.albicans nitrosation are able to convert chemical compounds which are able to trigger proto oncogenes and initiate malignancy[2]

Aim: In the current study we aim to find out the different species of Candida that colonize the oral cavity of patients on broncho dialtors.

Material and Methods: Saliva sample of the patients (n=40) were collected and was serially diluted, inoculated onto SDA. Isolates were then sub cultured onto HiChrome Candida Agar for identification. Other tests of identification like germ tube test and carbhohydrate fermentation tests were also carried out.

Results: It was noted that out of 40 samples 37 samples had Candida growth. In the present study it can be seen that Candida albicans is isolated from 29 samples from 30 puff users and 2 samples from the 10 nebulizer users.

Conclusion: In our study we found that 97 % patients who are puff users had *Candida albicans.* The chances of the yeast triggering proto oncogene cannot be ignored.

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INTRODUCTION

Candida albicans is yeast like fungi which has become a significant pathogen in the oral cavity. This yeast shows increased pathogenecity in the case of immuno compromised patients, those who are undergoing radiation therapy and in patients who take corticosteroid treatments. During yesteryears Candidal infection was considered to be an indicator of a much severe underlying diseases like malignancy, diabetes and other chronic infections (Treatment Guidelines for Candidiasis, 2009). There are several theories and hypothesis on the role of *Candida albicans* in the oral cancer and initiation of oral malignancy. Though Candida has a pathogenic role in the oral cavity, C.albicans nitrosation are able to convert chemical compounds which are able to trigger proto oncogenes and initiate malignancy (Sanjaya, 2011). The most common condition among corticosteroid patients with asthma is oropharngeal candidiasis. Since only a minimal of 10 to 20 percent of the inhaled puff goes into the lungs, and the remaining 80 percent which acts as a topical application on the mucosal layer of orapharynx region, supports the growth of Candida (Thomas, 2010). Another reason for Candida colonization in the oral cavity among pouf users is may be due to the presence of Lactose monohydrate as the carrier vehicle in many of the puffs which uses dry powder. The high sugar content in these puffs favours the proliferation of Candida (Samaranayake *et al*., 1986). In a case report by Suyama *et al*., it was reported that a 63 year old man who had expiratory wheeze resembling asthmatic attack died due to respiratory failure even after the administration of bronchodialtors for four days. An autopsy result showed that the majority of the bronchioles were filled with Candida psuedohyphae (Suyama *et al*., 1992). From the above case report it can be assumed that bronchodialators are not able to dilate obstructed bronchial pathways due to Candidal infection. In the current study we attempt to characterize *Candida albicans* from patients who use broncho dialotors.

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MATERIAL AND METHODS

A total of 40 saliva samples were collected from patients who were using bronchodialators for a period of minimum 6 months from Dept. of Oral Pathology, SRM Dental College, Ramapuram Ch:89. The patients were classified based on the type of bronchodialators used.

Sample processing

The collected sample was serially diluted and inoculated onto Sabourauds Dextrose Agar by spread plate method. From 40 saliva samples 37 Candida species was isolated. The isolated Candida species was sub cultured onto Hi Chrome Candida Agar. The colony morphology and the colour of the colony, was noted. From each isolate Grams stain was done to study the morphology and psuedohyphal formation and germ tube test for confirmation of *Candida albicans* was carried out. For further confirmation of the isolated strains, Carbohydrate fermentation test was carried out by using a modified method of PMV Charles *et al*[6] . 8 test tubes each was used for each isolate. 2 test tubes each for a single carbohydrate. Sucrose, glucose, lactose and maltose were taken for the fermentation study. 3 ml of basal media containing 2% of the test carbohydrate and phenol red indicator was taken in each test tube. Yeast suspension of No: 1 Mc Farland standard was prepared and inoculated into each test tube. The tubes were incubated at 37^0C for 72 hrs. Colour change to yellow was considered positive.

RESULTS

Confluent growth was observed in 30 samples. 7 saliva samples showed 10^4 cfu/ml to 10^3 cfu/ml of Candida sps. growth pattern and 3 saliva samples showed no growth.

Table 1.

No:of patients	Candida species isolated	Candida species not isolated
40	37	03
Percentage	92.5%	7.5%

In the current study it was noted that out of the 40 patients who were using bronchodialators Candida species was isolated from 37 patients. Candida species was found to be absent in 3 patients. From Table 1 it can be seen Candida growth was seen among 92.5% of patients. It was observed from our study that the samples which had confluent Candida sps growth belonged to *Candida albicans*.

Table 2. Colony colour on Hi Chrome agar

Difference in colony morphology in Chrome agar	No:of samples	Germ tube appearance	Puedohyphae presence.
Green	31	31	30
Blue	4	Absent	Absent
White	2	Absent	Absent

According to the Hi Chrome Candida Agar results (Table 2) it was observed that from the 37 Candida isolates, 31 isolates showed Green colouration, which indicates that the Candida species belongs to *Candida albicans*. All the 31 isolates showed positive results for Germ Tube test. 30 Green coloured Candida isolates from the Hi Chrome agar had Psuedohyphae, whereas 01 isolate even though it had green colour colony morphology on Hi Chrome Candida agar indicating *C.albicans*, did not show any psuedohyphae. The remaining 6 isolates showed Blue and white coloured colonies. 6 isolates belonged to the species *Candida tropicalis* (n=4) and *Candida glabarata* (n= 2), blue and white respectively. In all the 6 isolates the Germ tube test was negative and did not show any psuedohyphal appearance in Grams staining (Table 2).

Table 3. Fermentation of sugars

Species	Germ tube	Sucrose	Glucose	Lactose	Maltose
Candida albicans	+ve	-ve	+ve	-ve	+ve
Candida tropicalis	-ve	+ve	+ve	-ve	+ve
Candida glabrata	-ve	-ve	-ve	-ve	+ve

The sugar fermentation test shows the fermentation of the test carbohydrate with the production of Carbon dioxide and and alcohol. A positive result is indicated by gas production and no shift in the pH ()[7]. Change in the colour to yellow indicates that the fermentation has taken place. In the current study it was noted that *C.albicans* fermented Glucose and maltose while *C. tropicalis* fermented Sucrose, glucose and maltose. It was also noted that Lactose was not fermented by all the three Candida sps. in the study. Maltose was fermented by all the the three Candida sps. in the study (Table 3).

Table 4

S. No:	Bronchodialators used		Candida species grown		
	Puffs/Nebulizer	n	C.albicans n (%)	Other Candida sps n (%)	No growth n (%)
1	No: of patients using puffs	30	29 (97)	01(3)	Nil
2	No: of patients using Nebulizer	10	2 (20)	05(50)	03(30)

Among the 40 bronchodialator users we had 30 patients using puffs and the rest were using Nebulizers. An evaluation on the Candida isolated and the mode of bronchodialators used it shows that among puff users 29 patients had Candida albicans. 02 patients showed other species of Candida. While, among the patients who used nebulizers 30% (n=03) showed no growth on SDA. 50% (n=5) of the Candida isolated belonged to *C. tropicalis* and C.glabrata. Among the 10 nebulizer users only 20% (n= 20) patients had *C. albicans* (Table 4).

DISCUSSION AND CONCLUSION

According to Cawson RA *et al*., Candida has the ability to convert a non homogenous leukoplakia into a homogenous lesion. It is reported in study by Krogh P *et al*., Candida infected lekoplakia shows a higher risk of becoming malignant. Since the chances of Oral leukoplakia getting infected with Candida are high, regular monitoring is required by the physician. In a comparative study conducted by Shruthi *et al* of Candida by conventional method and CHROM agar method among denture wearers showed that the usage of conventional method was time consuming when compared with CHROM agar. It also stated that the specificity and sensitivity of CHROM agar was 100% when compared with fermentation test and SDA. Similarly in our study, it was noted that the fermentation results and Chrom Agar results were in concordance with the above study. In a review done by Leena S *et al*., it is highlighted that Candida as a factor for the development of Potentially Malignant Disorder (PMD). PMD is a word that was proposed in the year 2007 by World Health Organization for a clinical state that may lead to malignancy. In our study we found that 97 % patients who are on puffs had Candida albicans. The chances of the yeast triggering proto oncogene cannot be ignored.

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