Original Article

Evaluation of candidal species among individuals with oral potentially malignant disorders and oral squamous cell carcinoma

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Abstract

Context: Cancer afflicts almost all communities worldwide. Although it arises *de novo* in many instances, a significant proportion of oral squamous cell carcinoma (OSCC) develops from potentially malignant disorders (PMDs). Further, the association of *Candida* with various potentially malignant and malignant lesions has been reported as a causative agent.

Aims: The aim of the study is to evaluate and intercompare the predominant candidal species among individuals with PMD and OSCC.

Subjects and Methods: The swab samples were collected for the microbiological culture followed by incisional biopsy for histopathological confirmation. The swab samples were streaked and incubated on Sabouraud-dextrose agar medium and positive candidal colonies were incubated on CHROM agar for speciation.

Settings and Design: A total of clinically diagnosed 95 subjects of which 25 as normal controls, 30 as PMDs and 40 as OSCC were included. The collected swab samples were initially streaked and incubated on Sabouraud dextrose agar (SDA) medium, and later, only positive candidal colonies were incubated on CHROM agar for speciation.

Statistical Analysis: Chi-square test was utilized.

Results: Positive candidal growth on SDA medium was seen in 24%, 43% and 82% and negative in 76%, 57% and 18% individuals of normal controls, PMDs and OSCC, respectively. On evaluation on Chromagar medium, *Candida* species was present in 20%, 40% and 77% and absent in 80%, 60% and 23% individuals among controls, PMDs and OSCC group, respectively. On speciation of *Candida* in CHROMagar among the controls, PMDs and OSCC, *Candida albicans* species was present in 4 (16%), 7 (23%) and 4 (10%); *Candida krusei* in 1 (4%), 5 (17%) and 10 (25%); *Candida glabrata* in nil, nil and 6 (20%) and *Candida tropicalis* in nil, nil, and 2 (5%) cases, respectively.

Conclusion: There was predominant carriage of candidal species in PMDs and OSCC, but whether *Candida* has specific establishment in PMDs or in malignancy is still a matter of debate.

Keywords: *Candida albicans*, carcinogenesis, CHROMagar, oral squamous cell carcinoma, potentially malignant disorders, Sabouraud dextrose agar

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INTRODUCTION

Oral cancer is currently the most frequent reason of cancer associated deaths among Indian men.[1] Oral squamous cell carcinoma (OSCC) is a leading form of head-and-neck cancer in India, Pakistan, and other Southeast Asian countries. However, oropharyngeal and tongue cancers are common in the Western world.[2] Most oral cancers are squamous cell carcinoma, and some oral carcinomas are preceded by potentially malignant disorders (PMDs) that can present as leukoplakia, erythroplakia or erythroleukoplakia. Microscopically, these lesions may unveil oral epithelial dysplasia (OED), a histological diagnosis characterized by cellular changes and maturation disturbances symbolic of evolving malignancy.[3] The OED is a significant risk factor in predicting consequent development of invasive carcinoma.[4]

The World Health Organization (1978) recommended that clinical presentations of the oral cavity that are recognized as precancer be classified into two broad groups, as lesions and conditions.[5] In a recently held workshop, a recommendation to abandon the distinction between potentially malignant lesions and conditions and to use the term PMDs was proposed, as it expresses that not all lesions and conditions described under this term may transform into cancer.[6]

Oral leukoplakia is the best-known precursor lesion.[7] The clinical type of leukoplakia has a bearing on the prognosis since the nonhomogeneous leukoplakias containing an erythematous, nodular and/or verrucous component have a predisposition for hyphal invasion and have higher malignant potential than the homogeneous ones.[8] Oral submucous fibrosis (OSMF) is a high-risk precancerous condition that predominantly affects Indian youngsters due to the habit of gutkha chewing. Another PMD is oral lichen planus (OLP) which is an immunologically mediated mucocutaneous disease. Usually, they appear bilaterally unlike leukoplakia and are often superimposed with candidal infection.[9] Symptoms of OLP may be exacerbated by *Candida* overgrowth or infection. *Candida albicans* is present in about 37% of OLP lesions. Some *C. albicans* isolates with special genotypic profiles and virulence attributes had been considered to cause development and progression of OLP.[10]

C. albicans has the potential to infect virtually any tissue within the body.[11] The role of *Candida* in oral neoplasia was first reported in 1969. Few studies have also indicated that the presence of candidal infection increases the risk of malignant transformation in premalignant lesions.[9,12,13] It was proved that *Candida* plays a role in the development of cancer production by endogenous nitrosamine production which activates proto-oncogenes.[1]

Therefore, coexistence of *Candida* species within humans either as commensals or as pathogens has been a matter of interest among physicians. Further, the connotation of *Candida* with various potentially malignant and malignant lesions has been reported as a causative agent.[7,11,14] Thus, the present study was undertaken to evaluate and intercompare the different biotypes of *Candida* species associated with the various individuals among PMDs and OSCC.

SUBJECTS AND METHODS

The present study was conducted in the Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Bareilly. All the study individuals were selected from the Outpatient Department of Oral Medicine and Radiology and from Keshlata Cancer Institute, Bareilly, Uttar Pradesh, India.

Cooperative individuals confirmed clinically with PMDs and OSCC were included in the study. Patients on topical or systemic corticosteroid therapy, long-term broad-spectrum antibiotic therapy, history of immunocompromised conditions such as HIV, diabetes mellitus and severe anemia, had not underwent any chemotherapy, radiotherapy or antifungal treatment were excluded from the study.

A total number of 95 individuals were included, of which 25 individuals were without any obvious clinically diagnosed lesions as normal healthy controls, 30 individuals clinically diagnosed as PMDs such as oral leukoplakia, OSMF and OLP and 40 individuals clinically diagnosed as OSCC patients.

The ethical clearance was obtained from the institutional ethical committee for human experimentation as per the standard guidelines. All the included individuals were briefed about the procedure, and signed informed consent was obtained. The demographic data of all subjects were recorded on a customized case history pro forma. Detailed oral examination of all the individuals was carried out using diagnostic instruments. Initially, the swab samples were obtained for the microbiological culture technique followed by incisional biopsy of the clinical diagnosed lesion for histopathological confirmation.

For microbiological culture

Initially, the patients were requested to rinse the mouth with distilled water to clear the debris followed by saline water. Sterile cotton swabs (HiMedia, PW1184, Mumbai, Maharashtra, India) with a wooden stick mounted on

a blue-capped high-density polyethylene tube sized 150 mm × 12 mm were used to collect the samples from the buccal mucosa in healthy controls and from the lesional area in case of PMD and OSCC patients. The swab samples were positioned back in the same polyethylene tube and maintained at 40°C until streaking on Sabouraud dextrose agar (SDA) medium (HiMedia MH063, Mumbai, Maharashtra, India) and incubation at 37°C for 24–48 h.

Streaking was done using a sterile tool, such as a cotton swab or commonly an inoculation loop. The inoculation loop was first sterilized by passing it through a flame until red hot. When the loop has cooled, it was dipped into an inoculum such as swab specimen and then dragged across the surface of the agar back and forth in a zigzag motion until approximately 30% of the plate has been covered. The loop was then re-sterilized and the plate was turned to 90°. Starting in the previously streaked section, the loop was dragged through it two to three times continuing the zigzag pattern. The procedure was then repeated once more being cautious to not touch the previously streaked sectors. Each time, the loop gathers fewer and fewer bacteria until it gathers just single bacterial cells that can grow into a colony. Aseptic techniques were used to maintain microbiological cultures and to prevent contamination of the growth medium.[15]

Later, only the creamy/white pasty smooth colonies representative of *Candida* organisms [Figure 1] were further inoculated on HiCrome *Candida* Differential HiVeg agar/CHROMagar (HiMedia MV1297A, Mumbai, Maharashtra, India) and incubated at 37°C for 24–48 h. The microbial-colored colonies representing various species of *Candida* organisms were interpreted for the color and specific colony characteristics as per the guidelines of HiMedia Laboratories.[16]

Biopsy

The most suitable area for incisional biopsy was visually selected and soft tissue specimen was obtained. The tissue specimen was fixed in neutral-buffered formalin, processed as usual and embedded in paraffin wax. The 4–5 μ thick sections were primed and stained with hematoxylin and eosin stain for histopathological confirmation by means of a binocular research microscope (Motic BA400) ensuite with a 5 MP camera for photomicrography (Moticam 2500, USB2.0).

Statistical methods

All the data thus obtained were computed on Microsoft Excel Sheet. Statistical analysis of the data was carried out using the Statistical Package for the Social Sciences (SPSS version 17.0, Chicago, USA) using Chi-square test. A $P < 0.05$ was considered statistically significant.

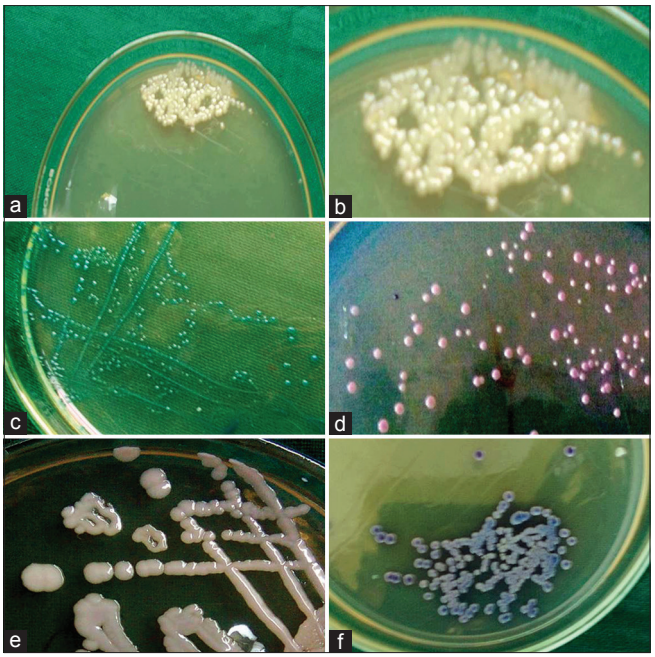

Figure 1: The Sabouraud dextrose agar media showing white creamy pasty colonies representative of candidal species (a and b). The HiCROM *Candida* differential agar media showing green color colonies representative of *Candida albicans* (c). Purple color colonies representing *Candida krusei* (d). Creamy white colony representing *Candida glabrata* (e). and blue colour colonies representing *Candida tropicalis* (f)

RESULTS

The distribution of the individuals in controls, PMD and OSCC has an age range of 17–63, 21–65 and 27–75 years, respectively, and the mean age ± standard deviation was 31.72 ± 12.76, 42.6 ± 5.09 and 49.27 ± 13.46 years, respectively. The distribution of the individuals among males and females in controls was 23 (92%) and 2 (8%), in PMD was 19 (63%) and 11 (37%) and in OSCC was 37 (92%) and 3 (8%), respectively.

On evaluation on SDA medium in controls, PMD and OSCC groups, *Candida* was present in 6 (24%), 13 (43%) and 33 (82%) and absent in 19 (76%), 17 (57%) and 7 (18%) individuals, respectively. On statistical analysis of intergroup comparison, an extremely significant difference ($P = 0.000$) was noted [Table 1]. Intragroup comparison also showed an extremely significant difference with $P = 0.000$ among both controls versus OSCC and PMD versus OSCC. However, controls versus PMD showed a nonsignificant, $P = 0.1332$ [Table 2].

On evaluation on CHROMagar media among controls, PMD and OSCC groups, *Candida* species was present in 5 (20%), 12 (40%) and 31 (77%) and absent in 20 (80%), 18 (60%) and 9 (23%) individuals, respectively. On

statistical analysis, an extremely significant difference was noted ($P = 0.000$) [Table 3].

On speciation of *Candida* in CHROMagar among the controls, PMD and OSCC groups, *C. albicans* species was present in 4 (16%), 7 (23%) and 4 (10%), *Candida krusei* in 1 (4%), 5 (17%) and 10 (25%), *Candida glabrata* in 0, 0, and 6 (20%) and *Candida tropicalis* in 0, 0 and 2 (5%) cases, respectively [Figure 2]. However, only OSCC group showed a combination of species such as *C. glabrata* and *C. krusei* [Figure 3] was present in 1 (3%) case, *C. tropicalis* and *C. krusei* in was present 3 (7%) cases, *C. tropicalis* and *C. glabrata* was present in 1 (3%) case, *C. albicans* and *C. tropicalis* was present in 3 (7%) cases and *C. krusei*, *C. glabrata* with *C. albicans* was present in 1 (3%) case, respectively [Table 4 and Figure 3].

All the other forms of fungi, except *Candida*, were considered as contamination. On evaluation on SDA medium, contamination in the form of fungal molds was present in 10 (40%) in control, 8 (27%) in PMD and 6 (15%) in OSCC groups [Table 5].

Table 1: Evaluation of *Candida* in Sabouraud dextrose agar media among the study groups

Study groups	*Candida* in Sabouraud dextrose agar		*P*
	Present (%)	Absent (%)	
Control	6 (24)	19 (76)	0.000 (highly significant)
PMD	13 (43)	17 (57)	
OSCC	33 (82)	7 (18)	

OSCC: Oral squamous cell carcinoma, PMDs: Potentially malignant disorders

Table 2: Inter-group comparison of *Candida* in Sabouraud dextrose agar media

Study groups	*P*
Control versus PMD	0.1332 (nonsignificant)
Control versus OSCC	0.000 (highly significant)
PMD versus OSCC	0.000 (highly significant)

PMD: Potentially malignant disorders, OSCC: Oral squamous cell carcinoma

Table 3: Evaluation of *Candida* in chrome agar media among study group

Study group	*Candida* in chrome agar		*P*
	Present (%)	Absent (%)	
Control	5 (20)	20 (80)	0.000 (highly significant)
PMD	12 (40.0)	18 (60.0)	
OSCC	31 (77)	9 (23)	

OSCC: Oral squamous cell carcinoma, PMD: Potentially malignant disorders

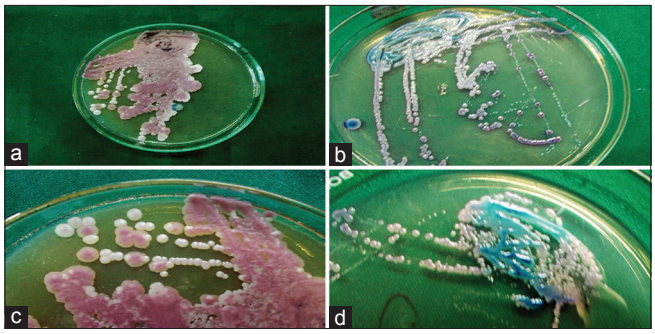

Figure 2: The HiCROM *Candida* differential agar media showing a combination of purple white colonies representing *Candida krusei* and *tropicalis* (a and b). Blue and creamy white colony representing *Candida tropicalis* and *glabrata* (c). And green, blue and white colonies representing *Candida albicans*, *Candida tropicalis* and *Candida glabrata* (d)

DISCUSSION

Oral cancer is one of the most familiar forms of cancer in the Indian subcontinent. From the beginning of the twentieth century, frequency of oral cancer is known to be high in India.[17] Although arising *de novo* in many instances, a significant proportion of OSCC develops from PMDs such as leukoplakia, OSMF and lichen planus. The strong association between cancers of the oral cavity and pharynx with use of smoking, snuff and chewing tobacco is well established. Few investigators have also indicated that the presence of candidal infection increases the risk of malignant transformation in premalignant lesions.[12] Yeasts are common commensal organisms found in approximately 40% of individuals, the predominant species isolated being *C. albicans*. *C. albicans* predominantly affects oral mucosa.[18]

The possible association between *Candida* species and oral neoplasia was initially reported in the 1960s.[19,20] Candidal species may be capable of metabolizing ethanol to carcinogenic acetaldehyde and can thus progress oral

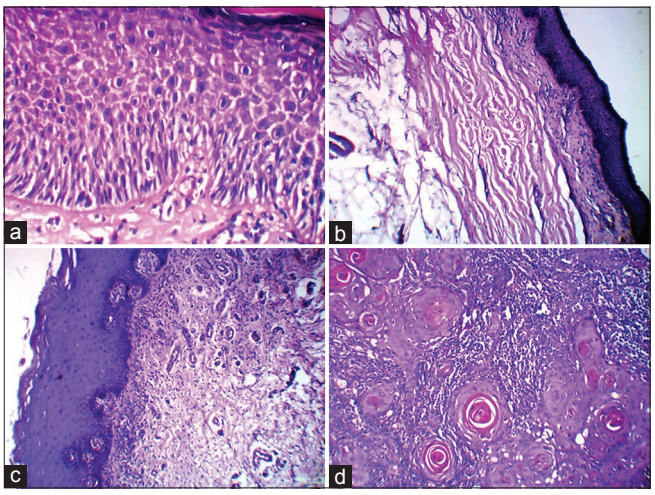

Figure 3: The photomicrograph showing hyperkeratinised mucosa with moderate epithelial dysplasia as in leukoplakia (a). Atrophic epithelium and dense collagen fiber bundles in oral submucous fibrosis (b). Basal cell degeneration and juxta-epithelial inflammatory infiltrate as in lichen planus (c). And epithelial cells in the form of islands, sheets, cords and strands with numerous keratin pearl formation as in well differentiated squamous cell carcinoma (d). (H&E, ×40)

Table 4: Identification of various candidal species in CHROM agar among the study groups

Candida species	Control (%)	PMD (%)	OSCC (%)
C. albicans	4 (16)	7 (23)	4 (10)
C. krusei	1 (4)	5 (17)	10 (25)
C. glabratta	Nil	Nil	6 (20)
C. tropicalis	Nil	Nil	2 (5)
C. krusei and *C. tropicalis*	Nil	Nil	3 (7)
C. krusei and *C. glabratta*	Nil	Nil	1 (3)
C. glabratta and *C. tropicalis*	Nil	Nil	1 (3)
C. albicans and *C. tropicalis*	Nil	Nil	3 (7)
C. albicans C. krusei, and *C. glabratta*	Nil	Nil	1 (3)
Total	5 (20)	12 (40)	31 (77)

C. albicans: *Candida albicans*, *C. krusei*: *Candida krusei*, *C. glabratta*: *Candida glabratta*, *C. tropicalis*: *Candida tropicalis*, OSCC: Oral squamous cell carcinoma, PMD: Potentially malignant disorder

Table 5: Contaminations in control, potentially malignant disorders and oral squamous cell carcinoma groups

Contamination	Control (%)	PMD (%)	OSCC (%)
Molds	10 (40)	8 (27)	6 (15)

OSCC: Oral squamous cell carcinoma, PMD: Potentially malignant disorder

and upper gastrointestinal tract cancer.[21] Later reports suggests link between the presence of *C. albicans* in the oral cavity and the development of OSCC. There are seven *Candida* species of major medical importance; *C. albicans*, *C. tropicalis* and *C. glabrata* are frequently isolated; while the sparingly isolated forms from the medical specimens are *Candida parapsilosis*, *Candida stellatoidea*, *Candida guilliermondii*, *C. krusei* and *Candida pseudotropicalis*.[13]

In the present study, the samples were collected using sterile swab. Contrastingly, Saigal *et al*.[1] used saliva for the culture technique instead of swab. Swab technique was simple to use; viable cells were isolated from the specific site, while saliva technique was not so.[22]

The collected swab sample was inoculated primarily on SDA medium followed by CHROMagar (a differential medium) for speciation. The same combination of culture media were also used by Nadeem *et al*.[23] and Odds and Bernaerts.[24] Contrastingly, Saigal *et al*.[1] used corn meal agar as primary culture media, followed by germ tube test, which identifies only *C. albicans* and *Candida dubliniensis*, and are unable to identify other *Candida* species.[1]

In the present study, the presence of *Candida* in SDA medium showed maximum specimens from OSCC followed by PMD and least in controls. Similarly, the results were demonstrated by various investigators such as Hongal *et al*.,[12] Saigal *et al*.,[1] Anila *et al*.[25] and Vučković *et al*.[7] *Candida* plays a fundamental role in the development of oral cancer, by means of endogenous nitrosamine, oligosaccharide and lectin-like component production.[1]

In the present study, the most prevalent *Candida* species identified under CHROMagar was *C. albicans* in control and PMD group, while OSCC group showed *C. krusei*. This was in consistent to the study by Gall *et al*.,[11] who have proved that there was a shift in distribution of *Candida* species with PMD undergoing malignant transformation from *C. albicans* to nonalbicans species such as *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis*.[25]

In the present study, all the other forms of fungi, except *Candida*, were considered as contamination in SDA medium. No such evidence of contamination was reported in other studies. This can be due to moist environment and improper sterilization technique followed.

CONCLUSION

However, further studies constituting larger sample size, various race and long-term follow-up studies need to be conceded to establish the exact role of *Candida* in carcinogenesis. Consequently, more focus should be placed on diagnosis and treatment of oral *Candida* infections, especially of nonalbicans species, which may cause systemic infections and are often resistant to commonly used antifungal agents such as fluconazole. The importance of antimycotic therapy before or associated with any other treatment and the importance of oral hygiene maintenance can be emphasized.

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Conflicts of interest

There are no conflicts of interest.

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