

# Isolation and identification of oral *Candida* species in potentially malignant disorder and oral squamous cell carcinoma

## ABSTRACT

**Context:** Oral cancer is one of the ten most common cancers in the world. More than 95% of the carcinomas of the oral cavity are of squamous cell type in nature. Oral *Candida* is a “yeast-like opportunistic pathogen.” The *Candida* genus is comprised of over 150 species of asporogenous “yeast-like” fungi.

**Aim and Objectives:** The aim of study is to correlate the association of oral fungal infection in progression of oral squamous cell carcinoma (OSCC) and potentially malignant disorders. The current study was undertaken to probe the isolation and identification of oral *Candida* species in potentially malignant disorder and OSCC versus normal oral mucosa.

**Materials and Methods:** Twenty patients for each abovementioned three lesions were randomly selected by using swabs. These swabs were subsequently inoculated in agar medium. *Candida* grows as white, convex colonies. Samples growing 1–3 colony-forming units (CFUs) were considered normal flora of the oral cavity. The specimens showing moderate to heavy growth were subjected to tests for identification of species of *Candida*. The chromogenic medium, HiMedia CHROMagar, has chromogenic substances which helps in the quick detection of *Candida* species, based on the reactions between the extract enzymes of the dissimilar species and the chromogenic substances.

**Statistical Analysis:** Chi-square test, one-way analysis of variance test, and *post hoc* Tukey’s test were utilized.

**Results:** According to our study, *Candida albicans*, *Candida krusei*, *Candida tropicalis*, and *Candida parapsilosis* in the culture were found to be in increasing incidence from healthy, OSCC, and oral potentially malignant disorders (OPMDs). These results clearly indicated that *Candida* species are increasing in the CFUs ( $P < 0.0001$ ).

**Conclusion:** Our study showed a higher intensity of *Candida* in OPMD and squamous cell carcinoma patients with results. The increasing CFU level and hyphae of *Candida* species in individual biopsy tissue with oral potentially malignant lesions to OSCC suggest that this pathogen plays a role in disease development and could aid in identifying the pathogenic commensal.

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

## INTRODUCTION

Oral *Candida* is a “yeast-like opportunistic pathogen.” Depending on the host defense mechanisms or local oral microenvironment, *Candida* can transform from a harmless commensal to pathogenic organism causing oral mucosal infection. Among these more important pathogenic species are *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida lusitanae*, and *Candida glabrata*. *C. albicans* is the primary cause of oral candidiasis.<sup>[1]</sup> Oral

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squamous cell carcinoma (OSCC) accounts for 5% of all cancers in men and 2% in women. *Candida* is among the etiologic risk factors of OSCC, which is part of normal oral flora. *Candida* infection may occur probably due to weakening of the immune system. *Candida* organisms are normal commensals that are found colonizing the oral mucosa frequently.

Oral cancer is a multifactorial disease and besides with many potentially malignant disorders. Chronic oral candidiasis is rarely but can transform into oral cancer. *Candida* as a pathogen can cause both outward and systemic diseases.

*Candida* can then produce carcinogenic compounds, such as nitrosamines and N-nitrosobenzylmethylamine. Strains with high nitrosation potential were isolated from lesions with more advanced precancerous changes. The yeast cells in such cases extend from the mucosal surface to the deeper epithelial cell layers representing transport and deposition of precursors like nitrosamines to deeper layers. This showed that certain strains of *C. albicans* play a key role in the development of dysplasia.<sup>[2]</sup> The possible association between *Candida* species and oral neoplasia was first reported in the 1960s (Cawson, 1969, and Williamson, 1969), with later reports suggesting a link between the presence of *C. albicans* in the oral cavity and the development of OSCC (Bastiaan and Reade, 1982, and Rodriguez *et al.*, 2007).<sup>[3]</sup>

The ability of *C. albicans* to colonize, penetrate, and damage host tissues depends on an imbalance between *C. albicans* virulence factors and host defenses, often due to specific defects in the immune system. *C. albicans* virulence factors and adherence to mucosal or artificial surfaces in the mouth have been extensively reviewed. Two different mechanisms by which *C. albicans* can invade keratinocytes have been proposed. One mechanism involves the secretion of degradative enzymes by the fungus, particularly secreted aspartic proteases that can digest epithelial cell surface components and thereby allow the physical movement of hyphae into, or between, host cells. The second proposed mechanism is the induction of epithelial cell endocytosis.<sup>[4]</sup> Hence, the aim of study is to correlate the association of oral fungal infection in progression of OSCC and potentially malignant disorders.

## MATERIALS AND METHODS

The current study was undertaken to probe the isolation and identification of oral *Candida* species in potentially malignant disorder and OSCC versus normal oral mucosa. Twenty patients for each abovementioned three lesions were randomly selected as provided in institute ethical clearance certificate No. 811718/OP/EC.

Salivary swab was collected from the patients who have been clinically/histopathologically confirmed with oral potentially malignant disorder (OPMD) and OSCC. These swabs were subsequently inoculated in Sabouraud dextrose agar (SDA) medium. The SDA is inoculated aerobically for 24–48 h. *Candida* grows as white, convex colonies. Samples growing 1–3 colony-forming units (CFUs) were considered normal flora of the oral cavity. The specimens showing moderate to heavy growth were subjected to tests for identification of species of *Candida*<sup>[5]</sup> [Figure 1].

The chromogenic medium, HiMedia CHROMagar, has chromogenic substances which helps in the quick detection of *Candida* species, based on the reactions between the extract enzymes of the dissimilar species and the chromogenic substances<sup>[6]</sup> [Figure 2].

## RESULTS

Inferential statistics were performed using Chi-square test and one-way analysis of variance (ANOVA) test. Chi-square test is used to compare categorical data. Intergroup comparison of colony counts is compared using one-way ANOVA test. *Post hoc* pairwise comparison was done using *post hoc* Tukey's test.

### Demographic distribution of study participants according to gender [Table 1 and Graph 1]

In this study, 60 cases were included, out of which 20 cases were of OPMD, out of which 14 were males and 6 were females. Out of 20 cases of OSCC, there were more male patients than females that included 17 males and 3 females.

### Mean age distribution of study participants [Table 2 and Graph 2]

Patients in this study ranged from 14 to 78 years. OPMD patients were in the age range of the 3<sup>rd</sup>–6<sup>th</sup> decade of life (mean age: 45 years). OSCC patients were older in their 6<sup>th</sup>

**Table 1: Demographic distribution of study participants according to gender**

		Sex		Total
		Males	Females	
OSCC	<i>n</i>	17	3	20
	%	85.0	15.0	100.0
PMD	<i>n</i>	14	6	20
	%	70.0	30.0	100.0
Normal	<i>n</i>	10	10	20
	%	50.0	50.0	100.0
Total	<i>n</i>	41	19	60
	%	68.3	31.7	100.0
<i>P</i> value		0.058, NS		

OSCC: Oral squamous cell carcinoma, PMD: Potentially malignant disorder, NS: Not significant

decade of life (mean age: 54 years). This distribution was statistically highly significant with a probability value of 0.001.

**Comparison of the presence of *Candida* species among oral potentially malignant disorder, oral squamous cell carcinoma, and healthy control [Table 3 and Graph 3]**

When the presence of *Candida* species among study groups was compared, the most prevalent *Candida* species was *C. albicans* (100.0%) in OSCC patients.

In the OSCC Group, *C. albicans* was the most commonly cultured species accounting for 100.0%, followed by *C. krusei* (78.3%), *C. tropicalis* (55.0%), and *C. parapsilosis* (35%).

In the OPMD group, the most common cultured species was *C. albicans* (100.0%), followed by *C. krusei* (95%), *C. tropicalis* (60%), and *C. parapsilosis* (30%). In healthy individuals, the most commonly cultured species was *C. albicans* and *C. krusei* (40%) followed by *C. tropicalis* (20%) and *C. parapsilosis* (10%).

These findings were statistically significant with a probability value of 0.001.

**Table 2: Mean age distribution of study participants**

	N	Mean	Age (years)		
			Std. deviation	95% confidence interval for mean	
				Lower bound	Upper bound
OSCC	20	55.8000	13.50867	49.4777	62.1223
PMD	20	43.7500	11.02091	38.5921	48.9079
Normal	20	39.3500	11.92642	33.7683	44.9317
P value			<0.0001, S		
P value of post hoc pairwise comparison			1>2,3 1*2-0.008 1*3-<0.0001 2*3-0.493		

OSCC: Oral squamous cell carcinoma, PMD: Potentially malignant disorder, S: Significant, SD: Standard deviation, CI: Confidence interval

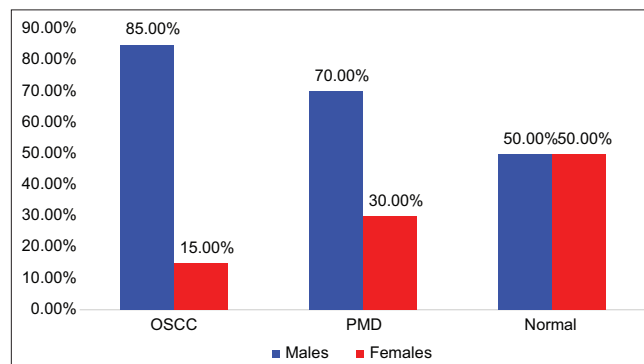
**Table 3: Comparison of the presence of candida species among oral potentially malignant disorder. Oral squamous cell carcinoma, and healthy control**

	N	Growth of <i>C. albicans</i>	
		Present	Total
OSCC	N	20	20
	%	100.0%	100.0%
PMD	N	20	20
	%	100.0%	100.0%
Normal	N	20	20
	%	100.0%	100.0%
Total	N	60	60
	%	100.0%	100.0%
P value			

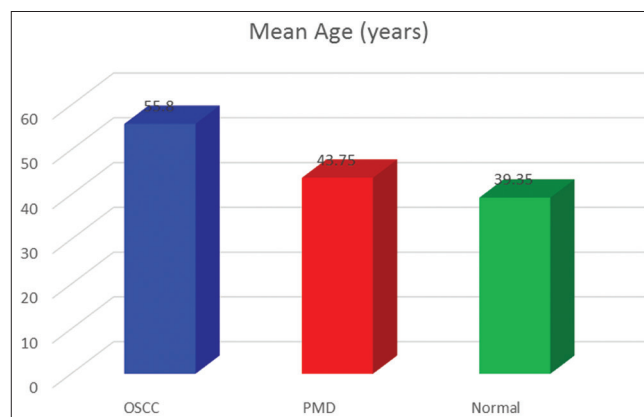
OSCC: Oral squamous cell carcinoma, PMD: Potentially malignant disorder

**DISCUSSION**

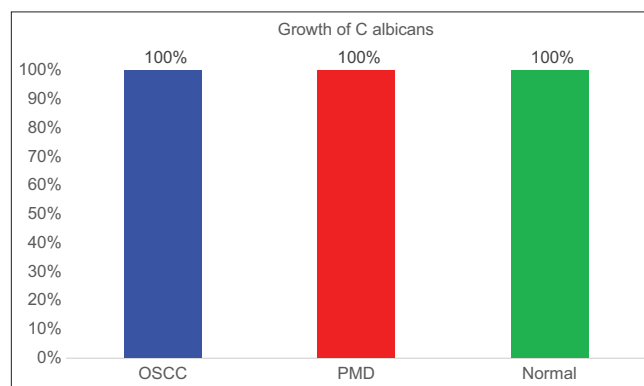
*C. albicans* is the most commonly implicated organism in this condition. *C. albicans* is carried in the mouths of about 50% of the world's population as a normal commensal component of the oral microbiota. Oral cancer is one of the ten most common cancers in the world. The term oral cancer includes a diverse group of tumors arising from the oral cavity. More than 95% of the carcinomas of the oral cavity are of squamous cell type in nature.<sup>[7]</sup>



**Graph 1: Demographic distribution of study participants according to gender**



**Graph 2: Mean age distribution of study participants**



**Graph 3: Comparison of the presence of candida species among oral potentially malignant disorder. Oral squamous cell carcinoma, and healthy control**

The *Candida* species are indigenous to the human oral cavity and can produce a variety of oral infections. These findings are consistent with the results of the present study, in which OPMD and OSCC samples gave positive colonies from saliva and swab samples of the oral cavity. Studies have shown that *C. albicans* is the most commonly isolated pathogen from the oral cavity and that it is the most virulent of the *Candida* species. This may be because *C. albicans* is also able to produce various virulence factors.<sup>[8]</sup> These results clearly indicated that *Candida* spp. are increasing in the colony forming units. This study showed that *Candida* is more prevalent in healthy, oral squamous cell carcinoma (OSCC) Potential Malignant Disorders (PMD). Also the fact that healthy case has increasing prevalence of *Candida albicans* *Candida krusei* [Figure 3].

The intergroup comparison of colony counts of *C. albicans* was done using one-way ANOVA. Overall, a statistically significant difference was found in colony counts of *C. albicans* among cases of OSCC, potentially malignant disorder (PMD), and normal. *Post hoc* pairwise comparison was done using *post hoc* Tukey's test, and it was found that mean colony counts of *C. albicans* among PMD cases were significantly higher than that among PMD, which also showed a statistically significant difference as compared to that among normal controls.

Diagnosis of oral candidiasis is not always performed, and a presumptive diagnosis is often the only one made, based on the patient's history, clinical presentation, and response to antifungal treatment rather than on cultural and histological methods. In the OSCC Group, *C. albicans* was the most commonly cultured species accounting for 100.0%, followed by *C. krusei* (78.3%), *C. tropicalis* (55.0%), and *C. parapsilosis* (35%). In the OPMD group, the most common cultured species was *C. albicans* (100.0%), followed by *C. krusei* (95%), *C. tropicalis* (60%), and *C. parapsilosis* (30%). In healthy individuals, the most commonly cultured species was *C. albicans* and *C. krusei* (40%), followed by *C. tropicalis* (20%) and *C. parapsilosis* (10%).

In case of *Candida* species, we found that the incidence of *Candida albicans* is positivity was significantly higher in Squamous Cell Carcinoma group as compared to Oral Potentially Malignant Disorders and healthy groups ( $p < 0.0001$ ). Minimum positivity for *Candida* species in dish was in Healthy group.

Our results related to counting of colony formation of *Candida* species in healthy, OSCC, and OPMD were found that *Candida* species was significantly associated with potentially malignant disorders and oral cancers (OSCC).



Figure 1: *Candida albicans* colonies on Sabouraud agar



Figure 2: Cell culture plate containing *Candida parapsilosis*, *Candida tropicalis*



Figure 3: Is *Candida krusei*

## CONCLUSION

The aims of our study were to evaluate CFUs of *Candida* spp. in the healthy, OSCC and OPMD patients.



*C. albicans* and *C. krusei*, *C. tropicalis*, *C. parapsilosis* were seen on the cultured media petri dish that include HiCrome agar media and CFU is counted by using Chi-square method that included counting of CFU in four segments of petri dish.

In case of *Candida* species, we found that the incidence of candida albicans positivity was significantly higher in Squamous Cell Carcinoma group as compared to Oral Potentially Malignant Disorders and healthy groups ( $P < 0.0001$ ). Minimum positivity for *Candida* species was in Healthy group. Minimum positivity for *Candida* spp. in dish was in the healthy group.

The present study suggests that the increasing CFU level and hyphae of *C. albicans* in individual biopsy tissue with oral potentially malignant lesions to OSCC suggest that this pathogen plays a role in disease development and could aid in identifying the pathogenic commensal.

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Nil.

#### Conflicts of interest

There are no conflicts of interest.

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