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Identification and Quantification of Candida Species in Oral Squamous Cell Carcinoma

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ABSTRACT

Background and objectives: Candida infection has been associated with malignancy in the oral cavity ever since it was found to cause candidal oral leukoplakia to correlate with oral epithetlial dysplasia. The fact that epithelial dysplasia improves after elimination of candida from infected tissue also support the casual link. Oral carcinoma is the fifth most common cancer in the world and one of the 10 most common causes of death. The encouraging evolvement in oral cancer management has increased the cancer survival rate over the past decades. Recently,it has been observed that Candida albicans is much more frequently detected in the biofilms of oral squamous cell carcinoma sites as well as some premalignant Candida albicans is much more frequently detected in biofilms of oral squamous cell carcinoma, lesions, than in control areas. A literature review showed that only few studies have been assessed the role of candida species with oral squamous cell carcinoma. Therefore more detailed studies are required to understand the relationship between the candida species and oral squamous cell carcinoma [OSCC]. The objective of this study is to identify and estimate the colony forming unit of different Candida species (Candida albicans, Candida tropicalis, Candida krusei, Candida glabarata) in OSCC patients and correlation of identification and quantification of candidal species among age and gender matched patient with OSCC and control group. **Methodology:** 40 patients reporting to the Department of Oral Medicine and Radiology at Bangalore Institute Of Dental Sciences Outpatient department who were histopathologically diagnosed with oral carcinoma were selected for the study with a control group of 40 otherwise healthy patients reporting to the department were included. Oral rinse was collected in a sterile and disposable container by oral rinse technique method. Sample collected were then centrifuged for 10 minute and then inoculated on the chrome agar and incubated at 37˚C for 24 hours. The different candida colonies were counted based on the colour chart provided by the supplier. (Green – Candida albicans, metallic blue – Candida tropicalis, pink – Candida krusei, white – Candida glabarata) and then Colony forming units (CFU) per ml of saliva (cfu/ml) were calculated. **Results:** Candida albicans was the predominant species to be isolated in control group and study group OSCC patients. Other common Candida species isolated from the study group OSCC were Candida krusei, C. tropicalis, and Candida glabarata. Incidentally C. Krusei was found in large CFU in the study group. There was no significant association between CFU of the different candidal species and the clinical staging. **Interpretation and Conclusion:** The findings of the present study suggest that the elevated candidal species especially if based on the CFU of C. albicans in OSCC patients could be a cause of OSCC.

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INTRODUCTION:

Oral cancer, especially oral squamous cell carcinoma (OSCC), is a major global health issue. It is the fifth most common cancer worldwide. Over 90% of oral cancers are OSCC[1]. OSCC is currently the most frequent cause of cancer related death among Indian men[2]. While factors like tobacco and alcohol are known causes, microbial agents, particularly *Candida* species, are increasingly suggested as contributors to oral cancer[3]. *Candida* species are often present as harmless commensals in the mouth of up to 50% of healthy people[2]. However, conditions in the mouth can change, allowing *Candida* to become pathogenic and express virulence factors[4]. Some *Candida* species can produce N-nitroso compounds, which are carcinogenic, and may interact with other carcinogens to activate proto-oncogenes, starting malignant growth[5]. This study aimed to evaluate the identification and quantification of *Candida* species in oral cancer patients compared to healthy control subjects. It also sought to correlate these findings between the two groups.

Aims & objectives:

1. To evaluate the identification and quantification of candidal species among the oral cancer patients.
2. To evaluate the identification and quantification of candidal species among the control subjects.
3. To evaluate and correlate the identification and quantification of candidal species among both the groups.

METHODOLOGY:

Study Design: This was a comparative clinical study. Institutional ethical board clearance was obtained.

Study Participants: A total of 80 age and gender-matched individuals were included.

Study group: 40 patients clinically and histopathologically diagnosed with oral squamous cell carcinoma (OSCC). Patients were recruited from the Out Patient Department of Bangalore Institute of Dental Sciences and Hospital and Post Graduate Research Centre.

- **Inclusion criteria:** Patients aged 20-80 years, with clinically and histopathologically proven squamous cell carcinoma, and willing to participate.
- **Exclusion criteria:** Patients with systemic diseases, immunocompromised states, on steroid therapy, on antibiotic, antifungal, or chemo-radiotherapy, or receiving treatment for recurrence of oral cancer.

Control group: 40 routine patients visiting the Department of Oral Medicine and Radiology at the same institute, selected as a control.

- **Inclusion criteria:** Clinically healthy volunteers who were age and gender matched.
- **Exclusion criteria:** Patients with systemic diseases, immunocompromised states, on steroid therapy, on antibiotic, antifungal, or chemo-radiotherapy, or with other mucosal lesions.

Study Setting: Bangalore Institute of Dental Sciences and Hospital and Post Graduate Research Centre

Sample Collection & Processing:

- **Tissue Biopsy:** A punch biopsy was taken from the most representative lesion site for histopathological analysis.
- **Oral Rinse:** Age and gender-matched subjects from both study and control groups performed an initial rinse with distilled water, followed by a 2-minute rinse with 10 ml of sterile phosphate buffer saline. The expectorate was collected in a sterile container between 8:30 a.m. and 2:00 p.m. to account for diurnal variations. Samples were immediately centrifuged for 10 minutes upon reaching the lab.

Candida Identification & Quantification:

- **Culture:** A 0.01 ml oral rinse sample (using a 4mm inoculating loop) was inoculated onto Candida CHROMagar culture media and incubated at 37∘C for 48 hours.
- **Identification:** Colonies were identified by color based on the supplier's code (e.g., green for *Candida albicans*, metallic blue for *Candida tropicalis*). Gram staining confirmed yeast growth.
- **Quantification:** Colonies were counted, and Colony Forming Units (CFU/ml) were estimated using the formula: CFU/ml=Colonies counted×(1/0.01).

Statistical Analysis:

Independent sample 't' tests and ANOVA tests were used for statistical analysis between the study and control groups.

RESULTS:

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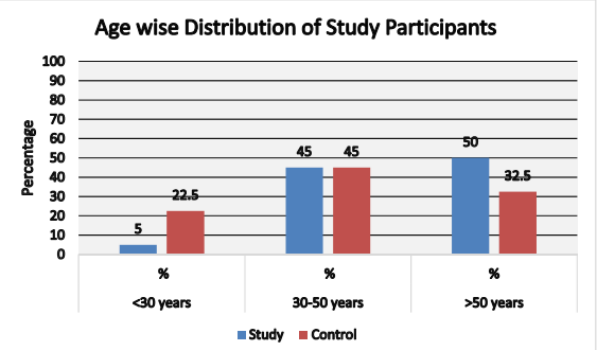
This study compared Candida species in 80 subjects: 40 with oral squamous cell carcinoma (OSCC) (study group) and 40 controls. OSCC patients of both genders, aged from their twenties to eighties, were included. The study aimed to quantify Candida colony-forming units (CFU/ml) in saliva for different species in both groups. Statistical analysis, including Independent T-test and ANOVA, was performed on Mean±SD (min-max) and Median values.

Isolated Candida Species: The study identified *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida glabrata*. These species' presence was compared between the study and control groups, and among different subgroups within the study patients.

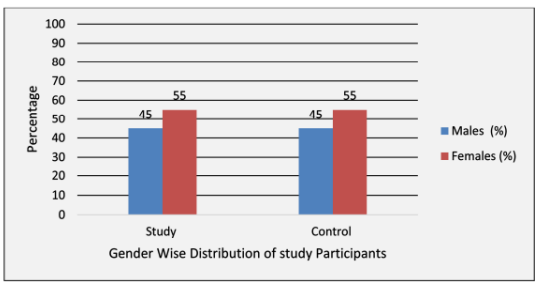
Age and Gender Distribution:

- **Age:**
- Study group: 2 (5%) were under 30 years, 18 (45%) were between 30-50 years, and 20 (50%) were above 50 years.
- Control group: 9 (22.5%) were under 30 years, 18 (45%) were between 30-50 years, and 13 (32.5%) were above 50 years.
- Overall: 11 (13.75%) participants were under 30 years, 36 (45%) were between 30-50 years, and 33 (41.25%) were above 50 years.
- **Gender:**
- Both study and control groups had 18 (45%) males and 22 (55%) females.
- Overall: 36 (45%) males and 44 (55%) females participated.

Graph1. AGE WISE DISTRIBUTION OF STUDY PARTICIPANTS



GRAPH 2.GENDER WISE DISTRIBUTION OF STUDY PARTICIPANTS



Candida CFU in Relation to Gender:

- **Control Group:** Only *Candida albicans* was isolated. There was no statistically significant difference in

C. albicans CFU/ml between males (1.6250±471.699) and females (95.883±142.88) (P-value = 0.518).
C. tropicalis, *C. krusei*, and *C. glabrata* had 0 CFU/ML for both genders in the control group.

- **Study Group:**
- *C. albicans*: Males 938.8±821.8 CFU/ml, Females 640.9±848.8 CFU/ml (p-value = 0.26).
- *C. tropicalis*: Males 533.3±418.6 CFU/ml, Females 854.4±2086.6 CFU/ml (p-value = 0.488).
- *C. krusei*: Males 466.6±474.03 CFU/ml, Females 1400±4177.6 CFU/ml (p-value = 0.31).
- *C. glabrata*: Males 88.8±177.85 CFU/ml, Females 136.63±317.04 CFU/ml (p-value = 0.55).
- No statistically significant difference was found in Candida species CFU based on gender in the study group.

Types of Oral Squamous Cell Carcinoma (OSCC) and Candida CFU (Study Group): Among the 40 study participants, various types of OSCC were observed. The most common was buccal mucosa carcinoma (25%), followed by lateral border of tongue (15%).

- CFU ranges for different Candida species varied widely depending on the OSCC site. For example, in right buccal mucosa carcinoma,

C. albicans ranged from 700−1300 CFU/ml. In left retromolar area carcinoma,
C. krusei ranged from 600−20000 CFU/ml.

Clinical Staging and Histological Grading of OSCC:

- **Clinical Staging:** Out of 40 study participants, 5 (12.5%) were Stage I, 7 (17.5%) were Stage II, 15 (37.5%) were Stage III, 9 (22.5%) were Stage IVA, and 4 (10%) were Stage IVB.
- **Histological Grading:** 28 (70%) were Grade I, 6 (15%) were Grade II, and 6 (15%) were Grade III.
- **CFU vs. Histological Grading:** No statistically significant association was found between different Candida species and histological grading of OSCC.
- **CFU vs. Clinical Staging:** *Candida krusei* was found to be statistically significant with a p-value of 0.03 in clinical staging of OSCC.

Table 1: Grading VS CFU

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		C.albicans	C.tropicalis	C.krusei	C.glabarata
Grading	I	753±903	417±401	1146±3706	85.7±224.2
	II	816±921.2	833±420	583±752.1	83.3±116.1
	III	833±484	1950±3949	600±715	283±441.1
F		0.029	2.58	0.127	1.49
P value		0.97	0.08	0.881	0.23

Table 2: Staging VS CFU

TABLE 26 STAGING VS CFU

		C.albicans	C.tropicalis	C.krusei	C.glabarata
Staging	I	1300±1564.1	500±312.6	5650±9603	300±469.3
	II	685.7±589.9	200±244.1	400±310.7	14.2±37.7
	III	860±891.1	573±393.8	440±503	80±185
	IVA	670±696.1	1516±3039.8	560.5±313	110±228.6
	IVB	350±412	325±298.6	400±244.8	250±500
F		0.72	0.94	3.01	1.10
P value		0.5	0.45	0.03*	0.37

Comparison of Candida CFU between Study and Control Groups: The control group predominantly showed

Table 3: Comparison of CFU Among Study and Control Group

	STUDY Mean±SD	CONTROL Mean±SD	T	P value
C.albicans	7.8±8.3	1.2±3.1	4.6	0.000*
C.tropicalis	4.5±4.2	0	6.8	0.000*
C.krusei	5.3±4.9	0	6.7	0.000*
C.glabarata	1.15±2.6	0	2.7	0.05*

Candida albicans, while the study group also had *C. tropicalis*, *C. krusei*, and *C. glabrata*.

Statistically significant differences were observed for all isolated Candida species between the study and control groups:

- *C. albicans* (p-value = 0.000)
- *C. tropicalis* (p-value = 0.000)
- *C. krusei* (p-value = 0.000)
- *C. glabrata* (p-value = 0.05)

DISCUSSION:

In the present study, the candidal species did not show any significant change in its presence, between the genders and age in both study group and the control group. This is in agreement with Siddharth Kumar Singhetal[6] who had found in their study that there was no correlation of Candidal growth in the oral cavity with gender/age. Candida is the commensals of oral cavity with the normal colony forming unit ranging from 300-500 cfu/ml of saliva, and above which the count is considered pathogenic[7].

Based on the colour that developed on CHROMagar Candida medium, four different types of candidal species were isolated. In the study group, along with Candida albicans, other pathogenic strains were also identified namely Candida krusei, Candida tropicalis and Candida glabrata. Whereas in the control group, only Candida albicans were isolated. Supporting our study, there were previously conducted studies by P Sanjay etal[8] and other researchers who had shown similar result with increased candidal isolation in OSCC patients. According to a study conducted by P Sanjaya et al[8] 43.3% were found to be culture positive. There was a definite increase in the candidal carriage rate in OSCC patients when compared to healthy controls as demonstrated in his study; this is correlating with the present study.

In the present cross-sectional study, all patients in the study group (100%) were positive for candida, [500-10000] CFU/ml, whereas in control group, only 15(37.5%) patients were found to have Candida [100-500] CFU/ml. This was in correlation to a study conducted by Jobbins et al[9] where they identified substantial changes in the oral flora of patients with advanced cancer and reported this to be due to lowered resistance of the individual, causing high level of candidal carriage and candidiasis on oral mucosa.

Cawson et a1[10] had suggested that presence of candida can be regarded as coincidental that is an infection of an already diseased surface with little relevance to the pathogenic nature of the lesion as a whole[1]. However, in our study, it was found that candida species had statistically significant presence in OSCC as compared to the control healthy group individuals.

CONCLUSION:

This study compared Candida species in 80 individuals (40 OSCC patients and 40 healthy controls). It aimed to identify and quantify different Candida species in saliva.

Key Observations:

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- **Candida Presence:** All four studied *Candida* species (*C. albicans, C. tropicalis, C. krusei, C. glabrata*) were found in OSCC patients, whereas only *C. albicans* was consistently isolated in healthy controls.
- **No Link to Staging/Grading:** The number of Candida colony-forming units (CFUs) did not correlate with the clinical stage or histological grade of OSCC.
- **Elevated CFUs in OSCC:** OSCC patients exhibited significantly higher CFU counts for all four *Candida* species compared to healthy individuals.

Further investigation with larger sample sizes and molecular techniques is recommended to understand the role of Candida in OSCC progression.

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