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Comparision of Prevalance of Candida Species in Healthy Individuals, Patients with Habits, Oral Leukoplakia and Oral Cancer

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Original Research Article

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Abstract: Candida species constitute part of the oral harmless commensal flora in about 2–70% of the general population but disequilibrium in the homeostasis between Candida, host immune system and normal oral bacterial flora favors Candida virulence which causes damage to the tissues which may increase the risk of a premalignant and malignant lesion. The aim of this study is to evaluate the prevalence of Candida infections in patients with history of habits, Oral leukoplakia and history of Oral Squamous cell carcinoma in comparision to healthy indiviuals. The present study included 20 patients with habits, 20 patients with premalignant lesions, 20 patients with known case of cancer and 20 healthy controls. A detailed history of each patient was recorded along with a clinical examination. Samples were collected with the swab and oral rinse technique, cultured on Sabouraud's agar medium. The isolated yeast species were identified based on Gram staining, a Germ tube test, Hichrome Candidal differential agar for speciation. Of the 80 samples collected Candida was isolated in 30 specimens. Out of 30 positive Candida species 21 was Candida albicans 06 was Candida glabrata and 03 were Candida tropicalis. Candida species were most isolated in Group IV ie Oral Carcinoma 11 (36.6%) followed by group III ie Oral leukoplakia. The incidence of Candida (primarily C. albicans) was maximum in Oral Carcinoma 11 (36.6%) followed by Oral leukoplakia. The findings of the present study suggest that elevated Candidal carriage were seen in patients with potentially malignant disorders and OSCC. Hence there must be a possible association between the developments of oral squamous cell carcinoma in potentially malignant oral lesions with chronic candidal infection due to Candida-associated malignant transformation"

Keywords: Candida; gutkha chewing; oral carriage; Oral leukoplakia; Oral Squamous cell carcinoma.

INTRODUCTION

Normal oral flora comprises a diverse array of organisms which includes eubacteria, archaea, fungi, mycoplasmas and protozoa [1]. Among these, fungi are classified as eukaryotes, and the most important to dentistry belong to the genus Candida [3]. The organism is unicellular yeast of the Cryptococcus family. Several *Candida spp.* are common commensal fungi that frequently can be found colonizing the oral mucosa. From cross-sectional studies, asymptomatic carriage rates in healthy individuals range from 3 to 70% [2]. The presence of *Candida* in mouth together with epithelial changes may predispose to Candidal infection which together with other cofactors may also induce epithelial dysplasia leading to malignant change [3].

Oral cancer encompasses all malignancies originating in the oral cavity. It is often grouped together with cancers of pharynx as "Oropharyngeal" cancer [7]. These patient groups have been reported to have impairment of host resistance, mucosal damage and prolonged neutropenia resulting in an increased prevalence of OC. The incidence of oral Candidal infection in these patients can range from 30 to 94% [1]. The oral mucosa is compromised in potentially malignant lesions, it can be argued that this species may be involved in carcinogenesis by elaborating the nitrosamine compounds which either act directly on oral mucosa or interact with other chemical carcinogens to activate specific proto-oncogenes and thereby initiate oral neoplasia.

Oral Candidal carriage has been reported by various studies in healthy as well as in immunocompromised individuals. This study is undertaken to analyse oral Candida colonization and identification of the species of Candida in patients attending Sri Sai College of Dental Surgery, Vikarabad

MATERIALS AND METHODS

The present study was a Prospective non-randomized study, conducted in 80 patients attending the outpatient department of Oral Medicine and Radiology. Patients were selected based on the determined criteria and were divided into four groups: Group-I: Control group ie healthy individuals with no habits -20; Group-II: Patients with habits such as smoking or gukta-20; Group-III: known case of oral leukoplakia-20; Group IV: known case of oral carcinoma.-20. The clinical details of all the patients were obtained and documented.

Patient's oral examination was conducted and method of collection or sampling was decided depending upon the presence of obvious lesion or white patch or its absence. Where an accessible and defined lesion is evident; a direct sampling approach that is the use of a sterile swab was preferred as this will provide information of the organisms present at the lesion itself. Two swabs were collected aseptically. Swab used was a nontoxic, sterile disposable cotton swab and a wooden applicator.

In cases where there was no obvious lesions or in instances where the lesion is difficult to access, an indirect sample based on culturing saliva specimens or an oral rinse was undertaken**.**

Direct examination of smears

The first swab collected was used for 10% KOH Mount preparation on a clean glass slide and a direct smear which was stained by Jensen's modification of Gram's stain.

10% KOH mount: Wet mount was screened under low power objective (1OX) and observed under high power (40X) to note: Presence/absence of budding yeast cells & pseudo hyphae

GRAM'S STAIN: After allowing the smear to air dry, the smears were observed under oil immersion objective to note:
Number of epithelial cells, polymorphs/HPF
Presence/absence of Gram positive budding yeast cells(4-81Am)
Presence/absence of pseudohyphae.

Fig-1:10% KOH MOUNT under 40x showing presence of budding yeast cells

Concentrated Oral Rinse: The oral rinse technique involves the patient holding 10 mL of sterile phosphate buffered saline (0.01 M, pH 7.2) in the mouth for 1 minute.The solution is then concentrated (10-fold) by centrifugation1700 rpm for 10 min and a known volume, usually 50 µL, inoculated on an agar medium. After 24–48 hrs incubation at 37◦C, growth is assessed by enumeration of colonies and expressed as candidal colony forming units per mL (cfu mL−1) of rinse.

Fig-2: Phosphate buffer saline

CULTURE OF CANDIDA: Sabouraud's dextrose agar plate (SDA Becton Dickinson & BBL ™) is the most frequently used primary isolation medium for Candida. The swab was inoculated on SDA slopes and incubated at 37ºC for 24 hours to 48 hours. The colony characteristics were recorded and compared with known colony characters of various candidal species. If no colonies were seen on Sabouraud's agar after 48hrs incubation, culture plate was kept for seven days in incubator before being considered as negative.

Fig-3: Sda slants with candida colonies growth

If the growth is creamy, smooth, pasty convex colonies on SDA then a Gram stain is performed on a colony by smearing on a clean sterile glass slide and stained by grams technique.

Fig-4: Growth on sda plates

Presence of violet coloured Gram positive budding yeast cells indicates towards Candida species.

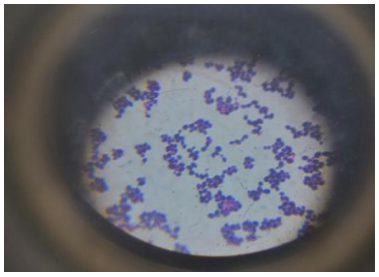

Fig-5: Gram positive budding yeast cells on gram stain

Identification of Candida species: Very small inoculum from an isolated candidal colony was picked up with a sterile inoculating loop and was suspended in a test tube containing normal human serum (0.3-0.5 ml). The mixture was incubated at 42°C for 2-3 hrs. A drop of mixture was placed in a clean glass slide and covered with a clean cover slip. This was first examined under a low-power objective to locate the group of cells and later, the presence of germ tube was confirmed under high-power objective of the microscope.

Positive Test: A short hyphal (filamentous) extension arising laterally from a yeast cell, with no constriction at the point of origin

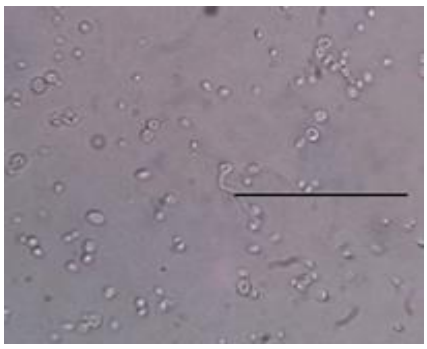

Fig-6: Germ tube method showing pseudohyphae

DIFFERENTIAL MEDIA: HiCrome Candida differential agar M1456A facilitate the isolation and presumptive identification of some clinically important yeast species was obtained commercially from Hi Media, CHROMagar Candida medium was prepared according to the manufacturer's instructions

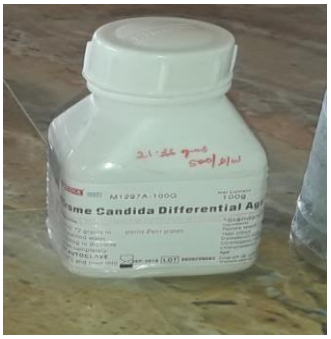

Fig-7: Hicrome candida differential agar base

Culture was inoculated and incubation was done at 37°C. The appearance of colonies, including colour, size, and textures on CHROMagar Candida was analyzed. Different species were identified based on the colour produced by the organism**.** Different species were identified based on the colour produced by the organism. C. albicans-blue colonies; Candida tropicalis-Blue with pink halo etc.

Fig-8: Colored colonies on chrome agar

OBSERVATION AND RESULTS

In the present study total study population size was 80, out of these 80 patients 44 (55%) were males while 36(45%) were female. Candida species was isolated in 30 (37.5%) and no growth was observed in 50 (62.5%). The prevalence of Candida was significantly higher in oral cancer patients (11(36.6%) patients).

Germ tube (GTT) test was performed on all growth positive cases in serum media to differentiate between Candida albicans and non albicans group. . HiCrome Candida differential agar M1456A was used as a differential media for species identification based on the colour produced by the organism. Of the 30 Candida species isolated Candida albicans (21) was the predominant species to be isolated followed by Candida glabrata(06), Candida tropicalis (03).

Table-1

Factor	Total no of pts	Positive growth	No growth	%
Healthy – Group I	20	03	17	10%
Habits – Group II	20	07	13	23.3%
Oral leukoplakia- Group III	20	09	11	30%
Oral Cancer – Group IV	20	11	09	36.6%
Total	80	30	50	100%

P-value = 0.058; Sig; Chi-square = 7.467

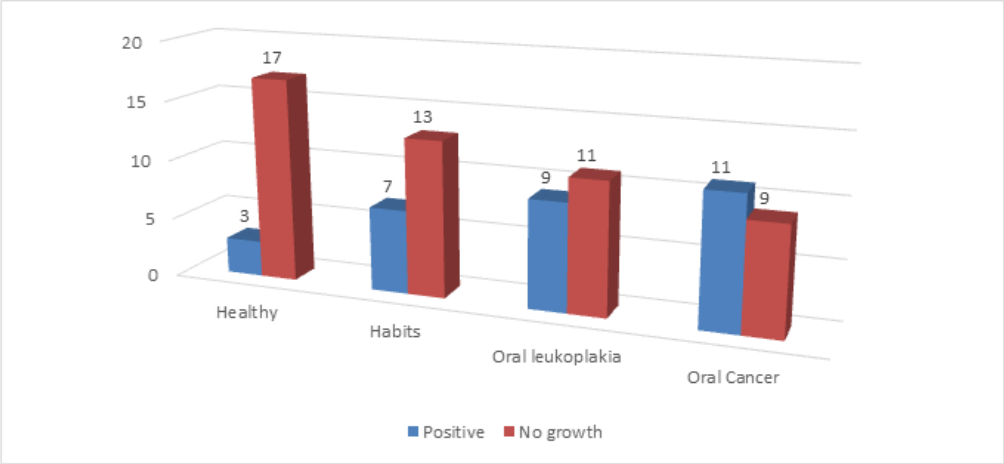

Fig-9: Overall results,of the study were out of the 80 patients Candida species was isolated in 30(37.5%)and no growth was observed in 50(62.5%).The prevalence of Candida was significantly higher in oral cancer patients

Table-2

Candida types	Number	Percentage
No Candida	50	62.5%
Candida albicans	21	26.25%
Candida glabrata	06	7.5%
Candida tropicalis	03	2.66%
Total	80	100%

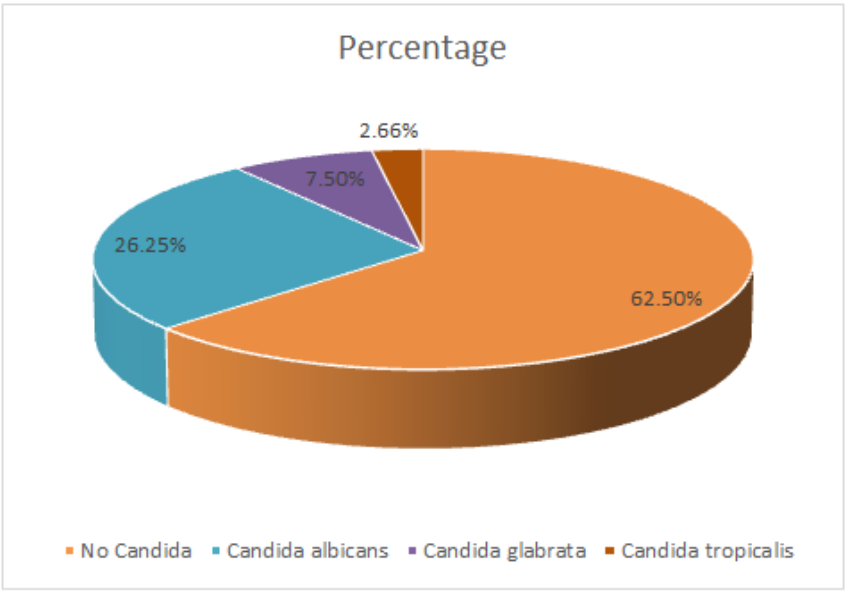

Fig-10: Of the 30 Candida species isolated Candida albicans was the predominant species

DISCUSSION

An increasing emergence in oral Candidal and non-Candidal fungal infections is evident in the past decade owing to the rise in the immunodeficient and immunocompromised population globally". Depending on the host defense mechanisms or local oral microenvironment, Candida can transform from a harmless commensal to the pathogenic organism causing oral mucosal infection.

Microbiological analysis is a reliable method to assess the presence of Candida spp. in advance and possibly establish a treatment for pre-cancerous lesions on the basis of the antifungal susceptibility patterns shown by isolated strains [8].

The present study evaluated the association of Candida species with normal controls, patients with habits, potentially malignant and malignant patients for which we used a sterile swab method and oral rinse method for isolation of Candida species. Specimen obtained was subjected for direct examination and was inoculated in SDA media for fungal growth at 37°C - 2 days. Positive fungal growth cases were undergone for germ tube test and CHROM agar to identify C. albicans at 42°C.

The entire data was entered in MS Excel master sheet and analyzed using Statistical Package for Social Sciences (SPSS). Chi-square test of independence of attributes was used to find out the significant differences and linearity trend of proportion was tested using Chi-square test to find out association. The level of significance was taken at $P < 0.05$.

Candida species was isolated in 11 (55%) of the 20 patients with oral carcinoma. Our finding of increased candidal isolation in OSCC patients is in accordance with the majority of findings by other researchers**.** Sanjaya P R. *et al*. in 2015 in their study- 21 cases out of 30 clinically known OSCC (oral squamous cell carcinoma) patients, 70% were found to be positive for *Candida* isolation. Saigal S *et al.* in [7] 2011 in their study- concluded that 20(66%) cases showed fungus growth out of 30 malignant cases cases(3)

The association of *C. albicans* with potentially malignant and malignant cases has been investigated by various authors under microbiological, cytological and histopathological studies. The possible association between Candida spp. and oral neoplasia was first reported in the 1960s [21, 22], with later reports suggesting a link between the presence of C. albicans in the oral cavity and the development of oral squamous cell carcinoma (OSCC) [23, 24, 8].

Most oral *Candida* are 'opportunistic pathogens' where, depending on the local oral microenvironment or whether host defense mechanisms are compromised, they can transform from a harmless commensal to an organism causing oral mucosal infection leading to a range of conditions. Epidemiological studies have shown that when host defenses are compromised most candidal infections arise from an endogenous commensal strain rather than an exogenous strain.

Our results support the frequent presence of Candida spp. in the cancerous and precancerous lesions of the oral cavity. Considering the research studies from various articles that certain strains of C. albicans probably have properties that are important in the development of pathological conditions and premalignant changes it is hence advisable for all patients with history of habits and in patients with compromised oral mucosa to take up regular screening of oral cavity for Candida overgrowth and maintenance of Oral hygiene in order to prevent opportunistic Candidal infections in precancerous lesions in oral cavity; thus accentuating their progression to oral neoplasia.

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