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Prevalence of Oral Candidasis in Oral Cancer Expansion

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Abstract:

Oral cancer is a multifactorial disease and beside with many potentially malignant disorders, chronic oral Candidiasis is rarely but can transfer into oral cancer. Oral Candidiasis includes the exterior of whitish, velvety plaques on the mucous membranes of the mouth and tongue. Candida as a pathogen can cause both outward and grave systemic disease. The finding of its deep seated infections remains a great challenge. Identification and characterization of antigens would be useful in the diagnosis and treatment of Candida infections. Present study was aimed to culture and identify C. albicans from patients with suspected oral cancer and separate proteins put in on SDS-PAGE to identify its patterns. The prominently were resolved single polypeptide band of approximately 46 KDa.

***Keyword:** Oral Cancer • Candida albicans • SDA • SDS-PAGE.*

Introduction

Infection has a considerable role in cancer expansion, causing about one in five malignancies global.[1,2] Bacteria similar to Helicobacter pylori, viruses like Human Papilloma Virus (HPV), chronic hepatitis B and C infections, and herpes virus, Epstein-Barr virus (EBV), causes cancer due to chronic infections[3] and most observe in Candida in carcinogenesis is related to oral and esophageal carcinoma. The promising connection between Candida species and oral neoplasia was first reported in the 1960s with later reports signifying a link between the presence of *Candida albicans* in the oral cavity and the development of oral squamous cell carcinoma.[4]

Oral cancer is a multifactorial disease and beside with many potentially malignant disorders, chronic oral Candidiasis is rarely but can transfer into oral cancer. Oral Candidiasis includes the exterior of whitish, velvety plaques on the mucous membranes of the mouth and tongue. An disparity between *Candida albicans*, virulence factors and host defenses frequently due to precise defects in the immune system causes *Candida albicans* to colonize, penetrate, and scratch host tissues[5] increasing the incidence of infections caused by Candida especially by Non *Candida albicans*[6] though Candida as a pathogen can cause both outward and grave systemic disease.[7]

The finding of its deep seated infections remains an immense challenge. One of the major reasons for the increase in *Candida infections* is the growth of it's opposed to strains to azole drugs, such as fluconazole used in the prophylaxis and treatment of candidiasis.[8,9] The identification of persistent or disseminated candidiasis is based on clinical symptoms that are spread and not simply differentiated from those manifested by other infectious agents. Therefore substantial interest in the identification and characterization of antigens would be useful in the diagnosis and treatment of *Candida infections*.

The present study was aimed to culture and identify *C. albicans* from patients with suspected oral cancer and separate put in on SDS-PAGE to identify its patterns and add in the literature of chronic oral Candidiasis which converts into oral cancer.

2. Materials and methods

C. albicans strains were isolated from patients with medical symptoms of oral Candidiasis. Samples were collected and processed as per the standard microbiological procedures. Sterile cotton swabs were prepared, gently smeared over the oral region and the swabs were immediately transferred to Sabouraud's Dextrose broth (pH, 5.6). They were screened for budding yeast like cells with the help of Gram stain, 10% KOH. The candida isolates which were obtained were further speciated by the germ tube test, chlamydospore formation on corn meal agar and inoculation on chromogenic medium.[10] The chromogenic medium, HiMedia CHROM agar, has chromogenic substances which helps in the quick detection of the candida species, based on the reactions between the exact enzymes of the dissimilar species and the chromogenic substances.

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Fermentative yeasts recovered from clinical specimens produce carbon dioxide and alcohol. Production of gas rather than a pH shift is indicative of fermentation. The 5 ml of carbohydrate (pH, 7.4) containing 1 % peptone, 1 % sugar, 0.3 % beef extract and 0.5 % NaCl, 0.2 % Bromothymol blue in distilled water medium was dispensed in sterilized Durham tube and 0.2 ml of saline suspension of the test organism was added and incubated at 370 C for 10 days.

Cell fractionation were suspended in a breaking buffer (pH, 6.8) containing 62.5 mM Tris-HCl buffer, 15 % glycerol, 1mM dithiotheritol and 20 mg of phenyl methyl sulfonyl flouride and broken by mechanical disruption in a Braun's homogenizer for 2 min with irregular cooling in the presence of glass beads 0.45 mm in dia. After disruption of the cells, the glass beads and unbroken cells were removed by centrifugation at 500 x g. The homogenate was then centrifuged at 6000 x g for 20 min and the pellet was collected. The supernatant containing the soluble protein fraction was recovered and passed through a 0.45 m filter membrane. The filtrates were then extracted with an equal volume of chilled chloroform. Following centrifugation at 40 C for 15 min at 2000 x g, the upper aqueous phase was aspirated and transferred to a dialysis tube. The crude protein fraction was dialyzed in column binding buffer (20 mM Bis-Tris, pH6.5) for 24 hr. The dialysed crude enolase was subjected to Sodium Dodecyl Sulphate - Poly Acrylamide Gel

Electrophoresis (SDS - PAGE). SDS - PAGE was performed following the method of [11] Equal amount of sample loading buffer was added to the enolase samples (20 µg protein). The samples were boiled for 1 min and loaded on 12% SDS - PAGE.

3. Results and Discussion

Cancer is the second most leading cause of death in economically developed countries and the third most leading cause of death in developing countries.[12] Yeasts are commensal organisms found in about 40% of individuals, the mostly species being *C. albicans*. It has the potential to infect virtually any tissue within the body, however, it's predominantly found on oral mucosa.[13]

An imperative increase in the incidence of fungal infections, particularly those due to yeasts, mainly *Candida* species is being seen in recent times. *Candida albicans* is considered the most pathogenic member of the genus *Candida* and is the species most frequently isolated from clinical materials, although infections with other species of *Candida* have been noticed in recent times.[14,15]

The present study evaluated the association of *C. albicans* with oral patients, potentially malignant for which we used oral pus culture technique and took biopsy from the suspected abrasion. Previous study also shown that Candida has capacity to induce oral cancer by directly producing carcinogenic compounds, like nitrosamines[16] Such a carcinogen attach with DNA to form adducts with bases, phosphate residues, and/or hydrogen bonding sites which leads to miscoding or irregularities with DNA replication.[17] The point mutations thus induced may triggers the precise oncogenes and bring about the expansion of oral cancer.[18]

After the 72 hours of cultivation of the *candida* at the 37˚C, white creamy colonies appear on the petridish of SDA media. **Fig. 1**

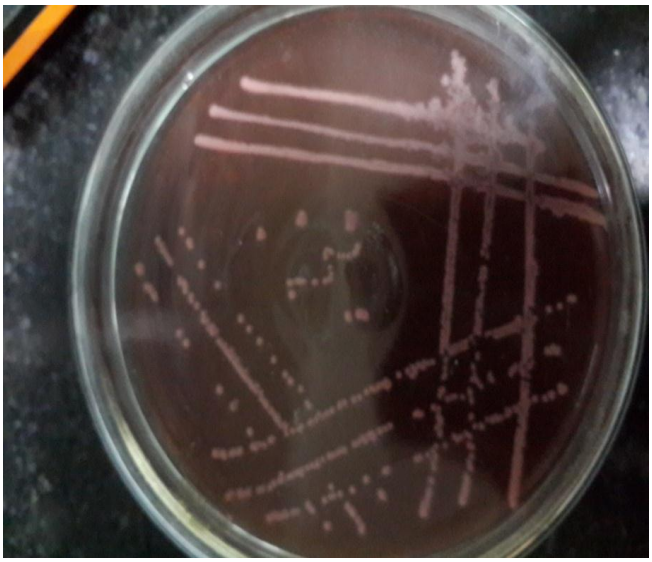

Figure 1: Microscopic view of *Candida albicans*

By the Gram staining of the *Candida albicans* strains Isolated from the sample, apparent that it belongs to the gram positive category of microorganisms because in the microscopy its colonies appeared purple-bluish in color. **Fig.2**

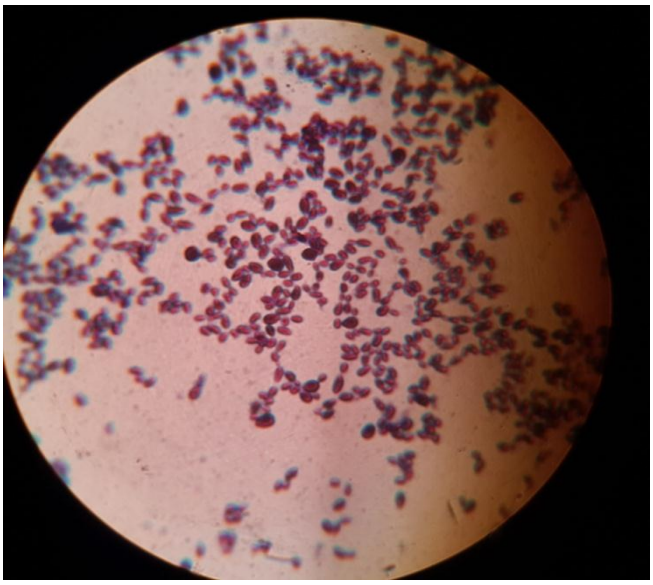

Figure 2: Microscopic view of the *Candida albicans*

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Formation of germ tubes was seen as long tube like projections extending from the yeast cells. There was no constriction at the point of attachment to the yeast cells. The germ tubes were formed within two hours of incubation in *C. albicans.* **Fig. 3**

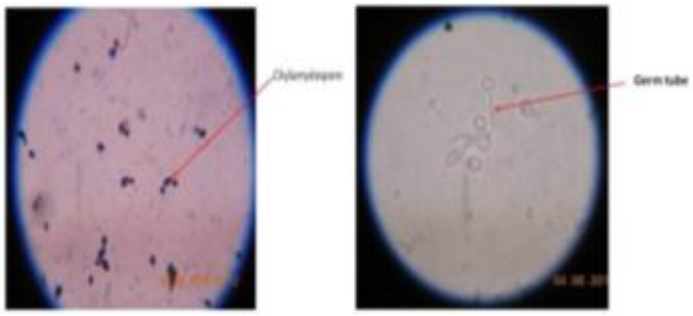

Figure 3: Microscopic view of Formation of germ tubes.

On Hi-chrome Candida agar, *Candida glabrata* shown white smooth colony, *Candida krusei* give purple colony and *Candida albicans* gives green colony. In present study, isolate of *C. albicans* was used. **Fig. 4**

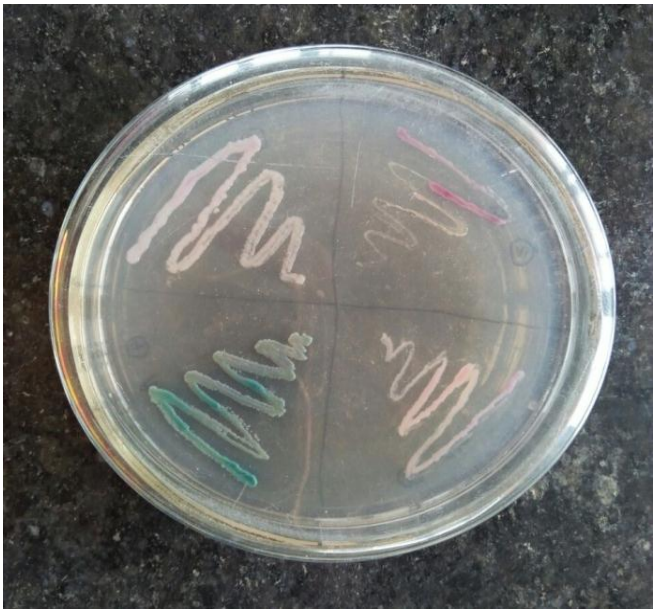

Figure 4: On Hi-chrome Candida agar media.

The *Candida albicans* fermented the carbohydrate sugars (Dextrose, Fructose, Lactose and Sucrose). In this process color of the broth is changed red to yellow because of the accumulation of the acidic end product and observed the bubbles that mean production of the Carbon dioxide and alcohol. **Fig. 5 & 6** It is also clear that this dimorphic fungus gives the process of sugar fermentation or the production of carbon dioxide and alcohol.

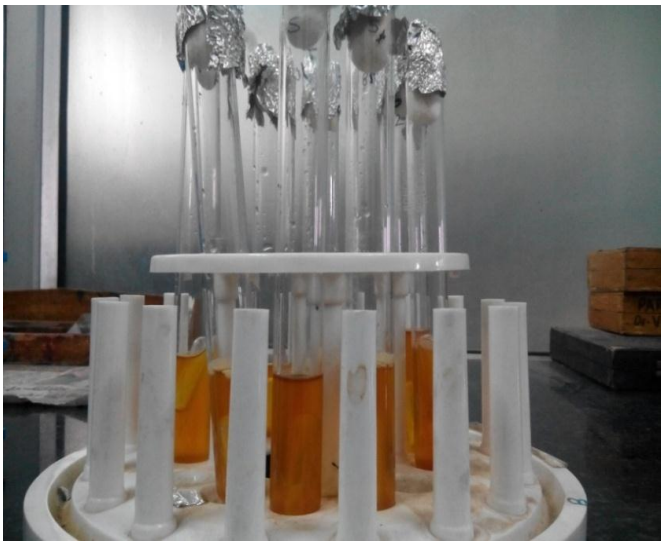

Figure 5: Carbohydrate Fermentation.

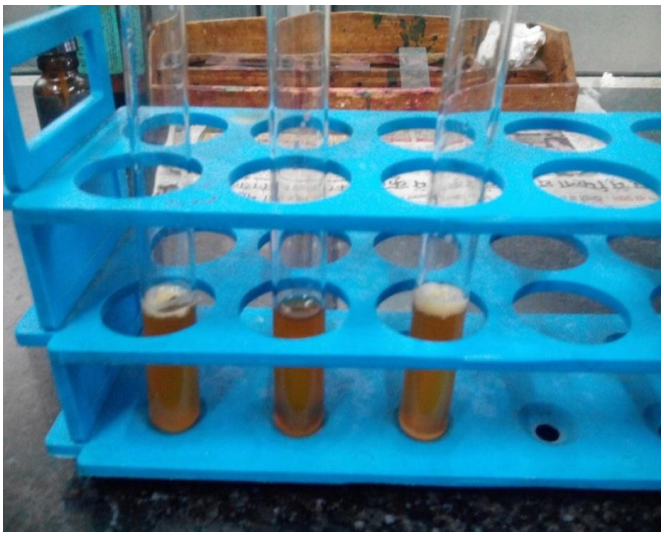

Figure 6: Gas production

In present study supports that association of yeast and its role in malignant transformation of oral sub mucous fibrosis. Other studies also supports that there may be a connection of yeast and its part in malignant transformation of oral sub mucous fibrosis.[19]

Due to the accepted significance of these agents as nosocomial and opportunistic pathogens, it is important to have a simple, effective and relevant typing method for the characterization of these yeasts. Individual such technique could be the creation of protein profiles, which involves solubilisation of microbial proteins and electrophoresis in gel matrices such as polyacrylamide. The use of SDS-PAGE in epidemiological typing of nosocomial infection in neonates by *Klebsiella* spp. has been reported.[20]

This study also confirmed that the use of SDS-PAGE showed a high degree of protein comparison among isolates of *C.albicans.* A similar finding has also been reported by other workers.[21]

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The SDS-PAGE profile of the crude sample prepared from *C. albicans* for enclose showed several polypeptide bands from 205 kDa to 26 kDa. **Fig.7** these, the significantly resolved single polypeptide band of around 46 KDa. This support was based on the reports in the literature.[22,23,24]

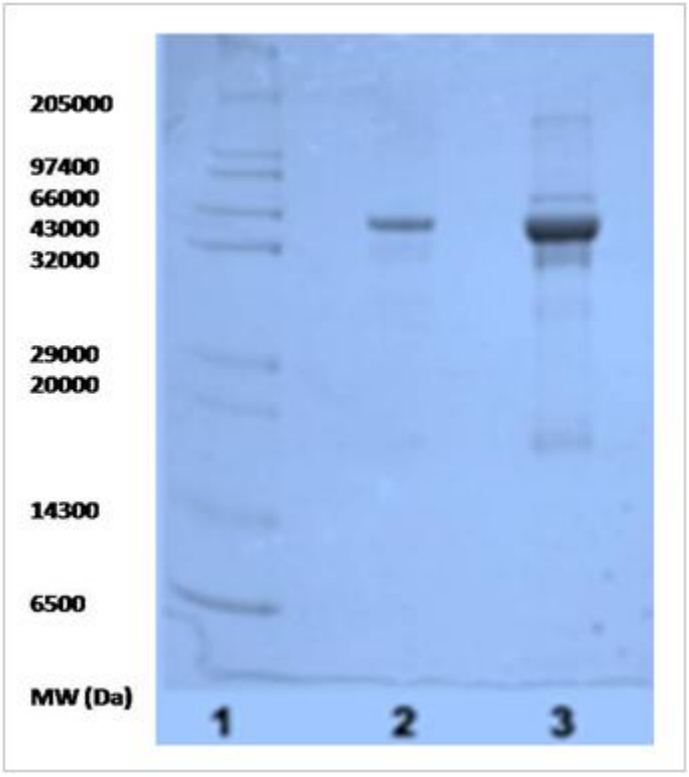

Figure 7: SDS-PAGE PROFILE

Conclusion

Isolation and screening of *Candida albicans* from the sample of Oral Candidiasis was done, as it is well recognized in support of an association of *Candida* and in malignant transformation of oral sub mucous fibrosis.

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