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# Isolation of Candida Albicans from Subgingival Plaque in Patients with Chronic Periodontitis- A Microbiological Study

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## ABSTRACT

*Chronic periodontitis (CP) results from a complex interplay of mixed polymicrobial infection and host response. The inflammatory mediators released cause destruction of host tissue. The presence of Candida species in the oral cavity of healthy patients varies from 35-60%. Yeasts especially Candida albicans have been recovered from periodontal pockets in patients with CP. Although the role of yeasts in CP is largely unclear, there is evidence to suggest that yeasts can be implicated in the pathogenesis of the tissue destructive periodontal disease. This study was designed to analyze the prevalence and clinical significance of isolation of Candida albicans from subgingival plaque samples of 40 patients with CP.*

## INTRODUCTION

Periodontitis is an infection induced inflammatory disease characterized by irreversible destruction of periodontal ligament and alveolar bone. Untreated periodontitis may eventually lead to tooth loss. Human periodontitis is associated with a widely diverse and complex subgingival microbiota encompassing both Gram positive & Gram negative bacteria, facultative and anaerobic organisms and probably yeasts. At least nearly 500 bacterial strains have been recovered from subgingival crevice. Most of these strains are commensals and a small number are potential pathogens. The ability of one microbe to cause disease is greatly affected by the composition of microbiota of the site. Chronic periodontitis (CP) results from a complex interplay of mixed polymicrobial infection and host response. The adherent microbes evoke release of number of inflammatory mediators in the underlying soft tissues. These activation products ultimately result in the destruction of host tissue. The presence of Candida species in the oral cavity of healthy patients varies from 35-60%. They are commensals in the normal oral flora. Candida albicans constitutes 60-70% of the total isolates of this genus followed by C. tropicalis, C. glabrata, C. parapsilosis, C. krusei, C. dubliniensis. In the oral cavity, yeasts commonly colonize the tongue, palate, and buccal mucosa. Besides oral mucosa, recent studies have shown the presence of C. albicans in other oral sites such as root canal, including persistent infection, carious lesions, and periodontal pockets.[1, 2] C. albicans have also been recovered from subgingival plaque of adults with severe CP.

Candida is one of the commonest opportunistic pathogen that causes disease in compromised hosts. Amongst the important predisposing factors for Candidal colonization are endocrinal disturbances like diabetes, blood diseases, immune deficiencies, antibiotic therapy, use of orthodontic appliances and total prosthesis. There are several important factors affecting the distribution and virulence of Candida like saliva, pH, adhesion, cell surface hydrophobicity, hyphae formation, production of phospholipases, proteinases or other metabolites, synergistic coaggregation or competition with bacteria and mechanisms for adaptation in the host environment.

C. albicans express virulence factors that may have an important role in the pathogenesis of periodontal disease such as the ability of penetrating the epithelium, inhibiting neutrophils and causing lysis of monocytes.

On an average, prevalence of subjects with yeasts in the examined periodontal pockets has been ranging from 7.1- 9.6% to 15.6%. [1, 2, 3] Proportion of Candida species in periodontal pockets has been shown to be similar to that of some bacterial pathogens suggesting a role of Candida species in the pathogenesis of this disease either in development or progression. In immunosuppressed patients, the higher prevalence of Candida species in the oral cavity and specifically in the subgingival biofilm of periodontal pockets, could indicate their coparticipation in the progression of periodontal disease in these patients.[4] The presence of Candida species and the development of opportunistic infections in subgingival sites is also attributed to the use of broad spectrum antibiotics as an adjuvant. We designed a microbial study to isolate C. albicans from subgingival plaque of CP patients with the following

## AIMS AND OBJECTIVES

To study the prevalence and analyze the clinical significance of isolation of C. albicans from subgingival plaque in patients with Chronic Periodontitis

## Material & Method

40 cases of chronic periodontitis were selected for this microbiological study. Healthy individuals without periodontitis served as normal control. Detailed clinical history, medical history & informed written consent were obtained from each patient. Periodontal status of the patient was assessed by using Russell's Periodontal Index. (figure1)

Fig 1- Clinical Photo of CP

Subgingival plaque sample was obtained aseptically from each patient with the help of gracey's curette. (figure 2)

Fig 2- instruments used

2 ml of blood was aseptically withdrawn by venipuncture and processed for random blood sugar. Subgingival Plaque sample

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was smeared on glass slide & fixed immediately with cytospray & stained with Gram's stain. Part of the sample was inoculated on Sabouraud's Dextrose Agar (SDA) with chloramphenicol and incubated at 25- 37$^0$c for 2-3 days. Colony from SDA was inoculated in normal human serum and incubated at 37$^0$c for 90 minutes to demonstrate germ tube formation. Corn Meal agar is a nutritionally deficient medium and highly selective for growth of C. albicans. So positive cultures were sub cultured on Corn Meal agar & incubated at 25-37$^0$ c for 2-3 days. Growth from Corn Meal agar was picked up & teased in a drop of saline water on a glass slide for demonstration of Chlamydospores.

**RESULTS**

**Clinical data analysis is presented in Table 1.**

| Age groups | Sex | | Total |
|---|---|---|---|
| | M | F | |
| ≤40 years | 1 | | 1 |
| 41-50years | 10 | 11 | 21 |
| 51-60years | 15 | 3 | 18 |
| Total | 26 | 14 | 40 |

Mean Age--51.30, SD – 5.67

Table 1- Clinical Data Analysis

Mean Russell's periodontal index score of 5.75 denoted that the periodontal disease was advanced.

Of the 40 smears, 3 were positive for Candida, with budding yeast cells & pseudohyphae (figure3). Remaining 37 smears were negative for candida.

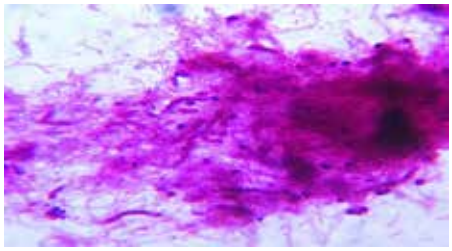

Fig 3- Gram's stain (40 X)

All smears consisted of epithelial cells, few leucocytes, gram+ve & –ve bacilli around debris, gram+ve cocci in clusters, discrete forms & in chains. Colonies on SDA appeared as creamy white coloured, smooth with yeasty odour (figure4).

Fig 4- growth on SDA

Colony smears on Gram's staining showed gram positive ovoid budding yeast cells (figure5).

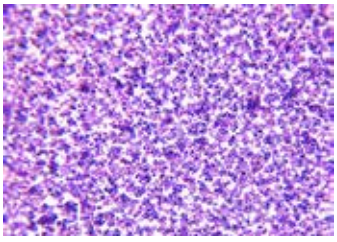

Fig-5 Gram +ve yeast cells (40X)

Germ Tube test was positive in the 3 suspected positive samples & did not show any constriction at its origin from mother cell so it was a true germ tube (figure 6).

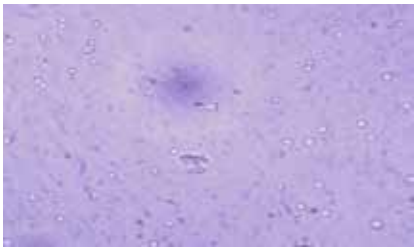

Fig 6 - +ve Germ Tube (40X)

Subculture on Corn Meal agar presented with colonies similar to that on SDA with chloramphenicol. (figure7)

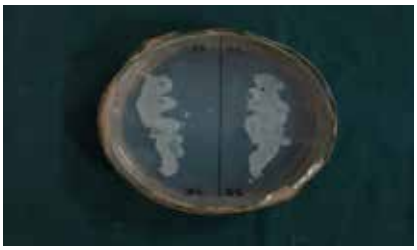

Fig 7- growth on Corn Meal agar

3 positive smears demonstrated Chlamydospores (figure 8).

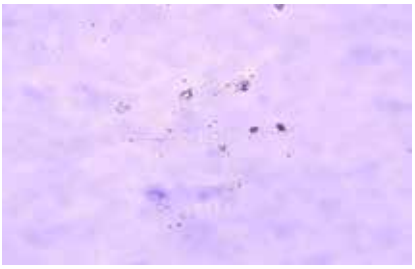

Fig 8 – Chlamydospores (40X)

Positive Germ Tube test & demonstration of Chlamydospore confirmed the species of yeast as Candida albicans. 3 positive cases also had diabetes as associated finding.

Random Blood Sugar of positive cases was 180, 160 & 210mg\dl respectively.

Statistical Analysis- Using Z test of Proportionality with P value at 5% level of significance (P= -1.645); the results were not statistically significant.

**Discussion**

Extensive studies on Candidal Carriage in patients with periodontitis have been documented. The results of Candidal isolation in the previous studies have been shown to vary from 7.1 to 69.2%.[1,2,3,5,6] In the present study Candidal Carriage in patients with periodontitis was found to be 7.5%. Javed et al[7] in 2009 have showed that clinical & salivary parameters of periodontal inflammation were higher in type II diabetic patients with oral C. albicans colonization. Lema –Neto et al[8] in 2009, have showed a higher affinity of C. albicans for epithelial cells than C. parapsilosis. Brusca et al[9] in 2010, have found a significant association between Candida and periodontitis only for C. parapsilosis. Our results are in accordance with the study conducted by Dahlen G et al[10] (7.3%). Dahlen G et al [10] studied frequency and prevalence of enteric rods, staphylococci and Candida in subgingival plaque samples from patients subjected to different periodontal treatment procedures. Fungi were recovered from 7.3% of samples.

In a study conducted by Jarvensivu A et al[3] Candida was typically found to be presents in the outer layers of the plaque and

seemed to act as a barrier between the host immunity and inner layers of the mixed biofilm. They have inferred that C. albicans may thus play role in the immune evasion of the plaque in periodontal infection.

Clinical Analysis of our 3 positive cases revealed that these patients had confounding local factors. Two of three positive cases had habits like alcohol consumption and tobacco chewing. All the three cases were accidentally discovered to be diabetic after random blood sugar estimations. Also it was noted that these patients were on long term antibiotic therapy and were suspected cases of refractory periodontitis. Machado AG et al[11] in 2010 in their in-vitro study related to candida adherence, have stated that adherence is an extremely important virulence factor in yeasts. The fact that candida species especially C. albicans can be isolated from periodontal diseases indicates that they are able to colonize subgingival environment and that they have a role to play in the infrastructure of periodontal microbiota. Candida species is also relatively tolerant to innate and cell mediated immunity.[4, 12] Adherence is an essential step for persistence of the microorganism in the host, as the ability to adhere avoids microorganisms of being eliminated by saliva.[11] Candidal organisms are one of the commonest opportunistic pathogens. So when C. albicans gets isolated, it means that they are no longer a passive inhabitant. Rather one has to search for underlying predisposing factors.

The gingival pocket and gingival crevicular fluid are favorable environments for germination and hyphal growth of Candida and hyphae are important in the attachment and disease process.

**CONCLUSION**

There was 7.5% isolation of Candida albicans from subgingival plaque in patients with Chronic Periodontitis. Though the results are not statistically significant, we can state that Candida albicans would have a role in the infrastructure of periodontal microbial plaque and in its adherence to the periodontal tissues. Not many studies demonstrating presence of yeasts in Chronic Periodontitis have been done. Further studies to demonstrate clinical significance of this finding need to be done.

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