PREVALENCE OF CANDIDA ALBICANS IN CHRONIC PERIODONTITIS PATIENTS

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CERTIFICATE

This is to certify that this dissertation titled “PREVALENCE OF CANDIDA ALBICANS IN CHRONIC PERIODONTITIS PATIENTS” is a bonafide record of work done by Dr.PL.SASIKUMAR, under our guidance and to our satisfaction, during his postgraduate study period of 2013-2016.

This dissertation is submitted to THE TAMILNADU DR. MGR MEDICAL UNIVERSITY in partial fulfilment for the award of the degree of MASTER OF DENTAL SURGERY - PERIODONTICS, BRANCH II. It has not been submitted (partial or full) for the award of any other degree or diploma.

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**LIST OF ABBREVIATIONS**

AGNR : Anaerobic Gram-negative rods

C.albicans : Candida albicans

CFU : Colony forming units

CAL : Clinical attachment level

CEJ : Cemento enamel junction

DNA : Deoxyribonucleic acid

IOPA : Intra oral periapical radiograph

IgA and IgG : Immunoglobulin A and G

PBS : Phosphate buffered saline

PCR : Polymerase chain reaction

PII : Plaque index

PD : Probing depth

RAPD : Random amplified polymorphic DNA

SDA : Sabouraud’s dextrose agar.

TBC : Trypsin bile salts cysteine

TSBV : Tryptic soy serum bacitracin vancomycin

VMGA : Viability medium Goteborg agar
Chronic periodontitis, formerly known as adult periodontitis, is the most prevalent form of periodontitis. It is generally considered to be a slowly progressing disease. Chronic periodontitis is most frequently observed in adults, it can occur in children and adolescents in response to chronic plaque and calculus accumulation. Chronic periodontitis is defined as “an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss”.

Chronic periodontitis is associated with a widely diverse and complex subgingival micro biota encompassing both Gram-positive and Gram-negative bacteria, facultative and anaerobic organisms, viruses and yeasts. More than 500 bacterial strains have been recovered from the subgingival plaque. Plaque also comprises of fungal species like *Candida albicans* (*C.albicans*), *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, and *Candida dubliniensis*. Most of these strains are commensals and some are potential opportunistic pathogens.

The *Candida* species are one of the opportunistic pathogens that can cause disease in hosts, who were compromised by underlying local or systemic pathological processes. The first known description of candidal infection as oral thrush in patients with underlying diseases was given by Hippocrates in fourth century BC. Bennett isolated the fungus in 1844 from sputum of a patient suffering from tuberculosis.

*Candida* species are commensal yeasts and opportunistic pathogens that reside on the mucosal surfaces and can cause oropharyngeal infections. It occurs usually in the immuno compromised individuals, which includes endocrinial disorders, blood diseases, and with long term use of broad spectrum antibiotic therapy.
INTRODUCTION

The possible relevant factors for *Candida* species colonization are nutrition, bacterial interaction and the presence of specific antibodies like IgA, and IgG in saliva.\(^5\) In healthy oral carriers, *Candida* species typically resides on the tongue, palate, buccal mucosa and in the saliva.\(^6\)

*C. albicans* is the most prevalent yeast of oral micro biota. It constitutes 60 to 70% of total isolates of this genus, but other *Candida* species including, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida dubliniensis*, *Candida glabrata*, *Candida kefyr*, *Candida lusitaniae*, and *Candida viswanathii*, are also found.\(^5\)

Yeasts, especially *C. albicans*, are recovered not only from the oral mucosae, but also in other oral sites such as pulp chamber, carious lesions and periodontal pockets.\(^7\) At the subgingival sites, there was an increase in colonization with *Candida* species in chronic periodontitis and aggressive periodontitis than the subjects with healthy periodontium.\(^8\)

The *Candida* species have virulence factors that facilitate colonization and proliferation in the oral mucosa and possibly in periodontal pockets. These fungal organisms can coaggregate with bacteria in dental biofilm and adhere to epithelial cells. These interactions, which are associated with their capacity to invade gingival connective tissue, may be important in microbial colonization that contributes to progression of oral diseases.\(^9\)

With this background, the present study aims to find the prevalence of *C. albicans* in subgingival plaque samples of patients with chronic periodontitis in the Outpatient Department of Periodontics at Sri Ramakrishna Dental College and Hospital in Coimbatore.
AIMS & OBJECTIVES
AIM:

To study the prevalence of *C. albicans* in subgingival plaque samples of patients with chronic periodontitis who reported to outpatient Department of Periodontics, Sri Ramakrishna Dental College and Hospital, Coimbatore for a period of 3 months.

OBJECTIVES:

1. To evaluate the presence of *C. albicans* among patients with chronic periodontitis.
2. To correlate the presence of *C. albicans* in mild, moderate, and severe form in chronic periodontitis.
3. To assess the presence of *C. albicans* in smokers and non-smokers with chronic periodontitis.
REVIEW OF LITERATURE
Arendorf TM and Walker DM (1980) conducted a study to analyse the prevalence of oral carriers of *C. albicans* in healthy dentate adult population, and to examine the possible influence of age, sex, caries, periodontal status and smoking habits on the carrier rate. The study also compared the sensitivity of four standard methods for detecting Candidal carriers and to demonstrate the intra-oral distribution of *C. albicans* in 54 healthy subjects evenly divided between the sexes. The results showed that *C. albicans* in healthy subject was 44.4% as determined by imprint culture, 29.6% by salivary samples and 13% by impression cultures. Cigarette smokers had a significantly increased carrier rate compared with non-smokers. Females were more frequent carriers than males, as were subjects with lower salivary pH on the surface of the tongue, but these differences were not significant. The age, DMF index, Russell’s periodontal index, plaque index and intraoral temperature of subjects did not influence the carrier rate. The frequency of isolation of *C. albicans* and its mucosal density per unit area, as measured by imprint culture, was highest on the dorsum of the tongue, particularly the posterior half, and they concluded that *Candida* is not uniformly distributed throughout the mouth, but that the tongue is the primary oral reservoir for the fungus, from which the rest of the oral mucosa, plaque-coated surfaces of the teeth and the saliva may become secondarily colonized in a proportion of carriers.

Bastiaan RJ and Reade PC (1982) analysed the prevalence of *C. albicans* in the oral cavity and correlated it with their sex, age, oral mucous membrane keratoses and tobacco-smoking habits. The study included 127 subjects who were divided into Group I consist of 79 patients with no detectable
disease of the oral mucous membrane. Group I included 50 men (63%) and 29 women (37%). Their average age was 51 years, with a range of 21 years to 88 years. The Group I was subdivided into Group IA comprising of 66 subjects 84% who smoke tobacco regularly and Group IB of 13 patients 16% who did not smoke. Group II consisted of 48 healthy patients that included 29 males and 19 females with average age of 57 years with age range between 28 and 85 years with clinically obvious keratotic lesions of the oral mucosa that were considered to have an irritational component. The group was subdivided into Group II A consisting of 39 patients 81% who smoked tobacco regularly and Group II B 9 of patients 19% who were non smokers. Microbiologic testing done in Group II patients with sterile cotton applicators tips moistened with 0.15 ml NaCl swabs on the keratotic patches. In Group I, subjects swabbing was done on the non-keratotic areas and swab samples are incubated aerobically for 72 hours at 37°C. The results showed that *C.albicans* was positive for 20 of 79 patients 25% in Group I and Group IA 6 out of 16 patients 24% who smoke with mucosal disease and Group IB 31% 4 out of 13 in non smokers are positive for *C.albicans*. In Group II 15 out of 48 patients 31% *C.albicans* present. In Group II A 12 out of 39 patients are smokers and in Group II B 3 out of 9 non smokers were *C.albicans* positive. The study concluded that tobacco smoking did not influence the prevalence of Candida nor was the prevalence of this organism related to the presence of oral mucous membrane keratoses of an irritational nature. The prevalence of the fungus in the mouth was significantly greater in women than in men, as was its prevalence in the older age groups of women compared to young women.
Oliver DE and Shillitoe EJ (1984)\textsuperscript{11} conducted a study to analyse whether smoking affects the prevalence and intraoral distribution of \textit{C.albicans} 100 healthy subjects were studied which included 43 men and 57 women. Swab samples was collected from imprint culture method in the posterior tongue, anterior tongue, anterior palate, left buccal mucosa and right buccal mucosa. Each pad was dipped in Sabouraud’s broth and pressed against the mucosal surface for 60 seconds, 2 to 3 ml of whole unstimulated saliva was collected by spitting into a sterile vial. In laboratory processing, saliva (0.2 ml) was spread evenly onto 90 mm dishes of Mycobiotic agar and incubated at 37°C for 48 hours after which \textit{Candida} colonies were counted. The results showed that smokers and nonsmokers had same prevalence of \textit{C.albicans}, which was 35%. The mean concentration and isolation frequency of \textit{C.albicans} colony-forming units in saliva of smokers was 750 cfu / ml and carrier state of \textit{C.albicans} was 53%, was higher when compared with non smokers which was 333cfu /ml and 46% respectively. \textit{C.albicans} was recovered most often from the posterior dorsum of the tongue. The study concluded that the effect of cigarette smoking influence on the carriers of \textit{C.albicans} is only minimal.

Kaminishi et al. (1986)\textsuperscript{12} conducted an experimental study to analyse the characteristics of collagenolytic enzyme production by \textit{C.albicans}. \textit{C.albicans} ATCC 1002 was used and maintained on Sabouraud’s agar. The organism was cultured for 18 hours at 37°C in glucose-peptone broth, collected, washed three times with a saline solution, and suspended in distilled water. To obtain collagen degrading enzyme the cells were inoculated in a growth medium. In media containing collagen as the nitrogen source, the pathogenic yeast \textit{C.albicans} secreted a collagenolytic enzyme.
Purification of the enzyme from a culture filtrate was achieved by DEAE-Sephacel chromatography at pH 6.7. The results showed that the molecular weight was found to be 46,000 by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the isoelectric point was at pH 4.2. The optimum pH for enzyme activity, measured by the hydrolysis of Azocoll, was 3.5 to 4.0. Enzyme activity began to decline above pH 4.5 and most stable pH for the enzyme was 6.0. The enzyme underwent alkaline denaturation observed at pH higher than 6.0. The enzyme was heat labile, and a decrease in activity was found above 55°C. The enzyme activity was inhibited by the addition of urea, cysteine, and pepstatin. No other inhibitor among those tested had any effect. The study concluded that *C. albicans* produces an extracellular, collagenolytic enzyme. Further studies are required to determine the role of this enzyme in dentinal caries.

**Gonzalez S et al. (1987)** conducted a study to analyze the yeast that was invading gingival connective tissue in juvenile periodontitis. The study included 12 females in the age group of 17 to 25 years at the Clinica de periodoncia, Facultad de Odontologia, Universidad de Concepcion, in Chile. 2 punch biopsies of 3mm in diameter was taken from the oral epithelium, and underlying connective tissue apical to the periodontal pockets before and after treatment with Spiramycin (500mg for every 12 hours). A total of 60 juvenile periodontitis samples taken from 12 females (5 biopsies per patient) was studied. Biopsies were done 15 to 20 days after spiramycin therapy. In addition, 6 biopsies were done apical to the sulci from normal gingiva and eight biopsies apical to pocket from adult advanced periodontitis and used as controls. 7 biopsies of juvenile periodontitis were treated in vitro with spiramycin 400 mg/ml in a sterile saline
solution for 5 hours and was incubated at 37°C. Samples were processed by scanning electron microscope. In microbiological processing 10 samples were homogenized in sterile saline solution, spread onto the Sabouraud’s dextrose agar, and incubated at 37°C for 72 hours. The results showed that negative yeast present in 10 of the juvenile periodontitis patients. In control patients, neither yeast nor bacteria were found. Scanning electron microscope reveals 2 types of yeast cells oval shaped 6.3 micrometer cells with abundant blebs in the cell surface and 10.7 micrometer of round cells in connective tissue of 26 out of the 60 juvenile periodontitis samples. The study concluded that yeast cells are more abundant after Spiramycin treatment in juvenile periodontitis patients due to opportunistic growth but not in controls.

**Slots J et al. (1988)** analysed the subgingival occurrence of yeasts and species of *Enterobacteriaceae* and *Pseudomonas* in 500 adults consisting of 239 males and 261 females with severe refractory periodontitis. Subgingival microbial samples were collected with paper points and transported in VMGA III medium. The bacterial samples were placed on enriched *brucella* blood agar and incubated anaerobically on TSBV, TBC, and Sabouraud’s agar, which were incubated in 10% CO₂. Yeasts were speciated using the API 20C micro-method system and the germ tube test for *C.albicans* In the 500 periodontitis patients, yeasts were detected in 84 (16.8%), enteric rods or *pseudomonads* in 51 (10.2%), and both yeasts and enterics or *pseudomonads* in 6 (1.2%). *C.albicans* comprised 83.3% of the isolated yeasts. With these findings, the study concluded that yeasts or enteric
rods or *pseudomonads* occur in the subgingival flora of about one third of "refractory" adult periodontitis patients with predominance of *C.albicans*.

Slot J et al. (1990)\(^{15}\) conducted a study to analyse the age and sex occurrence of subgingival enteric rods, *Pseudomonas*, yeasts and *Staphylococci* in refractory periodontitis patients. In this study 3075 subjects constituted consecutive referrals for microbiological analysis. A total of 175 periodontal practices in 44 states in the United States participated in the study, about 50% of the patients were from Pennsylvania, Newyork, New Jersey and Maryland. They were from 12 to 93 years of age had not received periodontal or antibiotic treatment within the preceding 2 months and presented with at least 3 sites in separate teeth with probing pocket depth of at least 6mm. Subgingival plaque samples obtained from 3 deep periodontal pockets with paper points and after placement for 10 seconds, all 3 paper points were pooled in a vial containing 2ml anaerobically prepared and stored in VMGA III medium. The samples arrived to the microbiological laboratory within 16-40 hours of sampling. Selective and non-selective media and commercial identification kit system were used for microbial isolation and speciation. The results showed that females constituted about 60% of the study and almost 1/3 rd of the patients were in their forties. Females 47.3% showed a higher prevalence of the organism than males 43.9%. Older females 15.9% and males 15.3% revealed significantly higher prevalence of enteric rods and *Pseudomonad’s* than younger individuals 10.9% and older infected females yielded significantly higher viable counts than younger infected individuals. No sex or age relationships were found for yeast 14% of individuals infected with periodontal pockets in refractory periodontal patients. The study
concluded that subgingival yeasts were found 10% more in women than in man. The high subgingival levels of enteric rods and pseudomonads increase the risk for destructive periodontal disease.

**Rams TE and Slots J (1991)**\(^16\) conducted a study to find the *C.albicans* biotypes in patients with severe periodontitis. 55 isolates originating from 22 males and 19 females with severe periodontitis in the age range of 22-65 years were isolated in the study. None of the subjects demonstrated clinical signs of oral candidiasis. Primary yeast isolation done on Sabouraud’s dextrose agar in 10% CO\(_2\)-90% air at 35°C for 3 days. One isolate of each type of morphologically distinct yeast colonies was sub cultured from each individual. Total viable microbial count was determined on anaerobically incubated enriched brucella blood agar plates. The API micro method system and a germ tube test were used for yeast identification. The API 20 C kit has demonstrated good sensitivity, specificity and reproducibility for *C.albicans* biotyping. The results showed that *C.albicans* 11 biotypes constituted 81.8% of all yeast isolates. A single biotype accounted for 57.8% of the subgingival *C.albicans* strains. The study concluded that biotype distribution of *C.albicans* in human periodontal pockets appears to follow a selectivity pattern similar to that of other oral surfaces.

**Sedgley CM and Samaranayake LP (1994)**\(^17\) conducted a study to determine the oral prevalence of aerobic and facultative AGNR and yeasts in saline oral rinse samples obtained from 300 community-dwelling Hong Kong Chinese patients, which includes 190 females and 110 males attending the primary care unit at the Prince Philip dental hospital, University of Hong Kong. An average number of 20 samples were obtained at each sampling session, 3 sessions per week over a 5-week period.
total of 154 participants 100 females and 54 males were sampled during morning session (8.45-9.45am) and 146 (90 females and 56 males) during afternoon sessions (1.45-2.45am). The results showed that oral prevalence of AGNR as to be 41.7%, *Enterobacteriaceae* species comprised 73% of all AGNR isolates with an overall prevalence of 32%. There was no difference in prevalence between females (n = 190) and males (n = 110). The morning samples (n = 154) yielded a significantly higher prevalence of AGNR 54.5% and *Enterobacteriaceae* 42.2% than afternoon samples (n = 146) 28.1% and 21.2% respectively. Subjects over 50 years had a significantly higher prevalence of AGNR than those aged 30-49 years. The oral prevalence of yeasts was 24% with *C.albicans* forming 77% of all yeasts isolated. Subjects taking medication (n = 38) or wearing dentures (n = 38) had a significantly higher oral yeast prevalence of 36.8 % and 44.7% respectively. Yeast prevalence was significantly higher in subjects over 50 years than those aged 30-49 years and 15-29 years. Comparison with the previous studies suggested that the oral prevalence of AGNR in Chinese might be higher in Hong Kong than in other parts of the world.

**Dahlen G and Wikstrom M (1995)** conducted a study to analyse the frequency and percentage of *Enteric rods*, *Staphylococci* and *C.albicans* in 973 subgingival plaque samples collected from 535 patients between 30 to 59 of years of the age from both sexes in specialist clinic within Swedish public dental care system. The analysis was performed with culture technique using Sabouraud’s dextrose agar plate incubated at 25°C for 5 to7 days. The results showed that *Enteric rods*, *Staphylococci* and *C.albicans* were detected in 65.5% of the samples from 76.7% of the patients. In most samples *Enteric rods*, *Staphylococci* and or *C.albicans* constituted a small amount of the total microbial viable count. *Enteric rods* exceeded
10% of the total viable count in 30 samples. *Staphylococci* occurred in more than 10% in only three samples. In these three samples, Enterics constituted more than 10% of the total viable count. Fungi were recovered from 7.3% of the samples from 19.6% of the patients. *C.albicans* was not found to exceed 10% of the total viable count in any of the samples. The study concluded that low frequency of predominant non-oral microorganisms in periodontitis among Swedish adults and no statistically significant correlation between the presence of any of the target microorganisms and kind of periodontal treatment procedure received antibiotic administration or sample transport time.

Moudni B et al. (1995)\(^{18}\) conducted a study to investigate a novel cytoplasmic *C.albicans* amino peptidase activity identified by means of highly sensitive substrates with methylcoumarin groups. The purification and characterization of a 52 kDa metallopeptidase from cell free extracts of *C.albicans* are evaluated. *C.albicans* strain 2091 was obtained from The Pasteur Institute (Paris, France). 9 clinical Candida isolates were examined and the 3 *C.albicans* isolates was collected from the urine, vagina and the mouth, and single isolates of *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. guillermondi* and *C. pseudotropicalis*. All strains were grown for 48 hours on Sabouraud's dextrose agar at 37°C. A novel amino peptidase was purified by high performance liquid chromatography from a cytosoluble 100000 g extract of *C.albicans* based on its ability to cleave Larginine 7-amino-4-methylcoumarin. The results showed that the purification factor was 36 and the yield was 20 %. The native enzyme had a molecular weight t of 52 kDa by SDS-PAGE in the presence or absence of reducing conditions and exhibited an iso-electric point of 4.3. The amino peptidase showed optimum activity at pH 7.2, a Michaelis constant of
50um and $aV_{\text{max}}$ at 19 mM 7-amino-4-methylcoumarin derivatives released min/mg of protein for L-Arg-AMC. This enzyme was cleaved at low affinity L-leucine-7-amino-4-methylcoumarin by the spectrofluorimetric method. The enzyme was strongly inhibited by specific metallo-enzyme inhibitors-EDTA and o-phenanthroline. The study concluded that the existence of an amino peptidase in *C. albicans* and had similar activity in other *Candida* spp. The occurrence of a peptidase activity in all *Candida* strains tested having a pH optimum of 7.2, similar inhibitor sensitivity and similar substrate specificity provides evidence that this peptidase could be a major enzyme in all *Candida* strains.

**Hannula J et al. (1997)** conducted a study to analyse a total of 48 yeast positive oral samples that includes 29 Finnish subjects and 19 American subjects from 25 females and 23 males in mean age of 43 years were phenotyped and genotyped to determine the frequency of simultaneous oral carriage of multiple yeast taxa. An oral sample from either periodontal pockets, oral mucosa or saliva was obtained. 4 out of 48 samples were cultured on Sabouraud’s dextrose agar to confirm *C. albicans* and 4 to 22 colonies per subject from a total of 362 colonies 267 from Finland and 95 colonies from USA were subcultured on CHROMagar medium plates that were incubated at 37°C for 3 days, and additional yeast species are identified as *Candida krusei, Candida glabrata* or *Saccharomyces cerevisia*. The API 20C Aux kit distinguished nine different carbohydrate assimilation profiles among the *C. albicans* isolates. In PCR analysis, using a random primer OPA-03 and a repetitive primer (GACA) 4, detected 2 major genotypic groups among the *C. albicans* isolates, from the 44 subjects into one amplicon pattern showing isolates with a “typical” PCR-profile with the size of major amplicons range between and 600 and 1600 bp.
with random primer and *C.albicans* isolates from 4 subjects shows “atypical” PCR-profile with amplicons sizes between 500 and 1600 bp with random primer. The study concluded that no difference was found in distribution of oral yeast species and of *C.albicans* phenotypes and genotypes between Finnish and American subjects. The PCR method may offer a rapid and easy means of distinguishing oral *C.albicans*.

Kamma JJ et al. (1999)\(^2\) compared the microbial profile of smokers and non-smokers in a group of patients with early onset periodontitis. A total of 60 healthy individuals that includes 40 males and 20 females in the age groups of 22 to 35 years. 30 patients were smokers (30.9cigarettes/day) and 30 non-smokers. Subgingival plaque samples were collected from the deepest periodontal pockets of each quadrant. The samples were cultured anaerobically in 10% CO\(_2\) and air using Sabouraud’s and MacConkey agar plates at 35°C for 4 days. The results showed that smokers had a higher proportion of deep pockets of >5mm especially in the maxillary anterior 22.5 ± 7.5 and premolar regions 14.6 ± 4.1 and presented a significantly greater mean probing depth and attachment loss in diseased sites and significantly greater alveolar bone loss in 53.7 ± 8.3 maxilla and 49.6 ± 6.7 mandible when compared to non smokers 41.6 ± 6.2 maxilla and 38.9± 6.9 mandible respectively. The most frequently isolated species in smokers are *P.gingivalis* 90%, *B.forsythus* 83% and *F.nucleatum* 77%. In non-smokers most frequently, isolates are *P.intermedia* 77% and *F.nucleatum* 67%. Total colony forming units in smokers \(8.3\times10^{10}\) is higher than \(6.4 \times10^{10}\) in non smokers. Bacterial species includes *S. aureus, Campylobacter, E. coli, Bacteroides* species, and *C.albicans* were found in significantly higher numbers and more frequently in smokers than in non-smokers.
Rodier M et al. (1999)\textsuperscript{21} conducted a study to demonstrate the ability of 95-kDa *C.albicans* metallopeptidase to degrade ECM components such as types I and IV collagen, laminin and fibronectin. *C.albicans* strain 2091 was isolated and grown for 48 hours at 37˚C on Sabouraud’s dextrose agar. All procedures were performed by a high performance liquid chromatography system. The enzyme activity was confirmed using the fluorogenic substrate LArg 7-amido-4-methylcoumarin. The results showed that type I collagen occurred when this protein was incubated during 4 hours with the purified *C.albicans* metallopeptidase, with the appearance of a band of >200 kDa, highest molecular band of the degradation products and fibronectin were totally degraded by the enzyme whereas type IV collagen and laminin were only partially degraded. The study concluded that *C.albicans* metallopeptidase play a role in the degradation of the sub endothelial extracellular matrix components. This enzyme could facilitate the migration of the yeast in the tissues after crossing the endothelial layer, allowing the fungal invasion of target organs.

Reynaud AH et al. (2001)\textsuperscript{22} assessed the prevalence of yeasts in periodontal pockets and possible associations with the clinical conditions of the sampled sites and other microorganisms. Group I consists of microbiological samples from periodontal pockets of 128 subjects 79 women and 49 men in the mean age of 16 to 85 years. In Group II includes 126 periodontal patients with untreated pockets of 4mm or more and presence of at least 4 teeth in the Institute of Clinical Dentistry University of Oslo, Norway are included in the study. Microbiological identification was performed after culturing on the blood and Sabouraud’s agar plates, and “checkerboard” DNA-DNA hybridization. The results showed in group I the prevalence of subjects with yeasts in the pockets was 15.6% in 20 out of 128 subjects and women harbor yeast
positive 20.3% in 16 out of 79 subjects which is greater than in men 8.2% in 4 out of 49 subjects. In Group II prevalence of yeast positive 17.5% in 22 out of 126 subjects. Yeasts positive individuals, which were found in both sampled pockets, are 27% in 6 subjects. Pocket depth of >7mm 19.7% in 12 out of 61 subjects and ≤7mm 15.6% in 10 out of 64 subjects. In 5 out of 6, individuals with yeasts in both pockets tested had at least one pocket with more than 9 cfu of \textit{C.albicans}. No correlation was found between age and the presence of yeasts. The study concluded that yeasts could be present in periodontal pockets in 1 out of 6 periodontal patients independent of gender and age.

**Hannula J et al. (2001)**\textsuperscript{23} conducted a study to determine the Clonal diversity of subgingival yeasts strains in relation to geographical location and coexistence of selected periodontal pathogenic bacteria. A total of 300 \textit{C.albicans} isolates from 60 \textit{C.albicans} positive dental patients obtained from microbiology laboratories at the Institute of Dentistry, University of Helsinki, Finland, School of Dentistry, University of Southern California Los Angeles, United States and the Faculty of dentistry University of Gazi, Ankara, Turkey were studied.

Each contributed 5 \textit{C.albicans} isolated from Finland that includes 11 women and 9 men at the mean age of 54.7 years, and 11 women and 7 men in United States, and 12 women and 8 men in Turkey, at the mean age of 31.8 years. In Finland, Subgingival microbial samples were collected from inflamed periodontal 4 to 6 sites and 3 sites with paper points in both United states and Turkey. These samples were transferred to VMGA III transport medium and inoculated in tryptic soy-serum bacitracin-vancomycin(TSBV) plates within 48 hours. The yeast isolates recovered from TSBV agar plates were subcultured on CHROMagar medium after incubation.
at 37°C for 2 days. *C.albicans* isolates were serotyped using slide agglutination and genotyped using polymerase chain reaction (PCR) amplification and a random sequence primer. The results showed that in 300 subgingival *C.albicans* isolates, 225 (75%) belonged to serotype A and 58 (19%) to serotype B, and 17(6%) isolates are non-serotypeable. *C.albicans* serotype A occurred more frequently in subjects from Finland and Turkey than in subjects from the United States. In patients from Finland, the United states and Turkey *C.albicans* serotype A, serotype B and non serotypeable isolates occurred with a frequency of 80%,45%,and 100%,15%,43%,and 0% and 5%,12%,and 0% respectively. A total of 27 PCR-based *C.albicans* genotypes were identified, of the 25 *C.albicans* genotype identified in sub gingival sites,11 occurred in multiple subjects and each of the remaining 14 genotypes occur only in a single subjects. *C.albicans* genotypes were detected in 12, 7, 9 subjects from Finland, United States and Turkey respectively. *C.albicans* positive Finnish and United states subjects exhibited fewer than three periodontal pathogenic bacteria more frequently than *C.albicans* negative subjects did. One *C.albicans* genotype occurred with particularly high frequency in subjects from Turkey and another genotype in subjects from the United States. The study concluded that present results showed geographical differences in the subgingival distribution of *C.albicans* serotypes and genotypes and suggested geographic clustering of *C.albicans* clones.
Martins C et al. (2002) conducted a study to analyse the prevalence of *Candida* species and *Staphylococcus* species in the human oral cavity. 68 individuals in the age group of 25 to 55 years old were analysed from the dental clinics of the School of Dentistry Sao Jose dos Campos SP, Brazil. Oral rinses were collected in the 10ml sterile phosphate-buffered saline and then cultured on Sabouraud’s medium supplemented with chloramphenicol and Baird-Parker agar, were incubated at 37°C for 48 hours. *Staphylococci* isolates were identified biochemically through coagulase, Vogesproskauer, D-threalose fermentation and beta galactosidase tests. The results showed that *Candida* species were isolated from 42 individuals 61.76% and in 30 subjects 61.22% were *C.albicans*, that are more frequently isolated species and *Staphylococcus* species were isolated from 65 individuals 95% among these isolates 41 strains 63% were coagulase negative. Among the coagulase positive strains, nine were *S. aureus*, 11 *Staphylococcus hyicus* and 4 *Staphylococcus schleiferi* sub species coagulans. No correlation was observed between the counts (cfu) of the isolated *Candida* species and *Staphylococcus* species. This study concluded that *Candida* species and *Staphylococcus* species have been increasingly reported as potential opportunistic pathogens, involved in super infection cases.

Song X et al. (2003) examined the distribution of genera, species and biotypes of yeasts in various sites of oral cavities of patients with chronic periodontitis. Samples were taken from the periodontal pockets, buccal mucosa and palate of 70 chronic periodontitis patients that includes 35 males and 35 females in the age group of 14 to 84 and 45 healthy individuals that includes 19 males and 26 females in the age group of 16 to 74 years at the Institute of Clinical Dentistry, Dental Faculty, University of Oslo, Norway. Subgingival plaque samples were
collected by inserting paper points into 2 to 4 of the deepest periodontal pockets, palatal and buccal mucosal samples were taken by streaking the sites with sterile cotton tipped swabs. The samples were streaked at the chair side directly onto the sterile Sabouraud’s agar plates incubated at 37°C for 3 days. After culturing yeast colonies were selected according to the morphology and the biotypes was determined by using a commercial kit Id 32C system. The results showed that oral yeasts were detected in 34.3% of the chronic periodontitis patients and 42.2% of the healthy subjects. 2 genera (Candida and Saccharomyces), 4 species (C. albicans, Candida dubliniensis, Candida parapsilosis and S. cerevisiae) and 19 biotypes were identified from the chronic periodontitis patients, while one genus (Candida), two species (C. albicans and Candida dubliniensis) and 11 biotypes were obtained from the healthy subjects. C. albicans was the most prominent species in all the oral sites, most often the buccal mucosa being colonized in 31.4% of the periodontitis patients and in 35.6% of the healthy subjects. Among the other Candida species, Candida dubliniensis being mostly present in the periodontal pockets. Two biotypes 7146340015 and 7347340015 of C. albicans and one biotype 7142100015 of C. dubliniensis were predominant in the periodontitis patients, whereas three biotypes 7147340015, 7147340015 and 7347340015 of C. albicans dominated in the healthy subjects. They study concluded that chronic periodontitis did not increase the carrier rate of yeasts in the oral cavity, but the variation in the yeast colony morphology, species and biotype was higher in the chronic periodontitis patients than in the health subjects. Suggesting cell, tissue or site tropism in oral yeast colonization.
Xu J and Mitchell TG (2003) compared the oral yeast flora of healthy people in China and Eastern North America. The samples of Chinese subject were obtained from 239 healthy volunteers from 5 geographically distinct areas. The North American samples were obtained from 483 individuals in 3 geographic areas of Eastern North America 2 samples from United States and 1 sample from Canada. Chinese and North American populations were comparable in age range from 15–76 years and sex 378 men and 344 women. Sterile swabs were used to sample the upper and lower outer gingiva of each person. After sampling, the tip of each swab was immediately severed and submerged in a sterile cryogenic tube for storage and transport medium that contains sterile enrichment broth 2% and culturing was done. Subcultures were streaked onto CHROMagar medium, which selects for yeast growth and permits the direct identification of *C. albicans* (green color) and *Candida tropicalis* (blue color). Every yeast colony of a different color or morphology was transferred to nutrient medium and identified with use of the standard yeast identification system, API 20C. All strains identified as *C. albicans* were further tested for growth at 45°C on Sabouraud’s glucose agar, and PCR amplification was performed with *C. albicans* species-specific primers. The results showed that in Eastern North America, the overall rate of carriage was 39.5% range from 29.3%–62% and species diversity was low and the recovery of yeasts was similar for all 3 geographic areas and different racial groups. *C. albicans* was the predominant isolate, with an overall frequency of 90.6% range, 64%–100%. *C. albicans* accounted for only 9.4% range from 0%–17.4% of all yeasts isolated in China. The study concluded that *C. albicans*, which is the predominant commensal and etiologic...
species of *Candidiasis* in Europe and the Western Hemisphere, was relatively rare in China.

Jarvensivu A et al. (2004) conducted a study to analyse the frequency of *Candida* infection in the periodontal tissues of chronic periodontitis patients and the extent of *Candidal* penetration into gingival tissues. 25 chronic periodontitis patients that included 15 females and 10 males at the mean age group of 53.4 ± 11.0 years with moderate to severe generalized chronic periodontitis were included in the study. Samples were collected from the premolar and molar regions during flap surgery that consisted of sulcular epithelium, subgingival plaque, and underlying connective tissue. The processed specimens were immediately formalin fixed and subsequently paraffin embedded and the subgingival plaque samples were collected by curettage from deep periodontal pockets from 17 out of 25 patients were cultured, at the Department of Oral pathology and Oral Microbiology, Institute of Dentistry, University of Helsinki, Finland. Samples were examined by immunohistochemistry using *C.albicans*-specific antibodies. Tissue Sections were also stained with periodic acid-Schiff stains. The results showed that yeast could be found in 15% of patients with chronic periodontal diseases. Immuno reactivity for *Candida* was present in 4 out of the 25 chronic periodontitis specimens 16%. Only one yeast-positive specimen was found when PAS-staining 4% was used and two yeast-positive specimens were found with plaque culture 8%. This study concluded that the sensitivity of specific antibodies was superior to periodic acid-Schiff stain or plaque culture in detection of *Candida* in tissues. *C.albicans* plays a role in the immune evasion of
plaque in periodontal disease and in the provocation of destructive inflammation in the underlying tissues.

Song X et al. (2005)²⁸ conducted a study to compare the genetic relatedness of oral yeasts from different oral sites between two different groups that included 70 patients (35 males and 35 females) with marginal periodontitis with the age group of 14 to 84 years and 45 individuals that includes 19 males and 26 females from health subjects with group of 16 to 74 years, in the Department of Oral Biology and Periodontology, Faculty of Dentistry University of Oslo, Norway. The samples were collected from periodontal pockets with depth of ≥ 5mm from marginal periodontitis and gingival sulci of the healthy subjects by inserting sterile paper points into 2 to 4 of deepest pockets, palatal and buccal mucosa samples were taken by streaking the sites with sterile cotton tipped swabs. The samples were streaked directly onto Sabouraud’s dextrose agar plates and incubated at 37°C for 3 days. Random amplified polymorphic DNA fingerprinting and the Dendron computer-assisted program for gel analyses was applied for estimation of genetic relatedness of yeasts. The results showed that the dentrograms of the marginal periodontitis group had similarity coefficient ranged from 0.49 to 1 and three genetic clusters comprising 73 genotypes whereas the similarity coefficient of the oral health group ranged from 0.62 to 1. Three genetic clusters and 55 genotypes. In the pooled dentrograms, 57% of the yeast isolates and the type strain of C.albicans fell in a major cluster V. This study concluded that genetically heterogeneous yeasts were found in the oral cavities of marginal periodontitis patients and oral health subjects. Similar genetic clustering patterns were obtained from the yeasts of the two groups, with cluster V being most
predominant. The study also concluded that yeasts of the marginal periodontitis group were more genetically diverse than yeasts of the oral health group, and some yeasts of the marginal periodontitis group exhibited unique genetic patterns. There was no clear association between yeast genetic clusters and oral sites in the two participant groups.

**Urzua B et.al. (2008)** conducted a study to analyse and compare the composition of yeast present in the mucosa and subgingival sites of healthy individuals and patients with aggressive and chronic periodontitis. In Group I, 28 health subjects that includes 18 females and 10 males with an average age of 27.9 ± 6.7 years were included. Group II, consisted of 20 subjects with aggressive periodontitis that includes 13 women and 7 men with an average age of 28.7 ± 6.9 years with ≥ 4mm loss of attachment on more than 2 first molars and or incisors and 3 or more affected cuspids, premolars or second molar. In Group III, 26 individuals with chronic periodontitis that includes 18 women and 8 men with an average age of 40.8 ± 10 years with loss of attachment of ≥ 4mm on at least 30% of residual teeth were included. The samples of oral mucosa were collected using small cotton rolls and pooled from the internal cheek and from the dorsal side of the tongue, and subgingival plaque samples were obtained by introducing a paper cone in the gingival sulcus for 10sec and transferred to 2ml PBS buffer saline and transported to the laboratory. The samples was inoculated onto the Sabouraud’s agar plate and incubated at 37°C for 48 hours. The results showed that patients with chronic periodontitis had higher yeast carrier’s status of 69.2% and CFU ranges from 0-2140 compared to healthy individuals which was 35.7% and 0-62 respectively. The study concluded that only *C.albicans* and *C.dubliniensis* were capable of colonizing the
periodontal pockets in patients with chronic periodontitis. While only *C. albicans* was identified in subgingival sites of healthy individuals and patients with aggressive periodontitis.

**Barros LM et al. (2008)** conducted a study to analyse the genetic diversity and production of exoenzymes of *C. albicans* and *C. dubliniensis* isolated from the oral cavity of systemically healthy patients with periodontitis. 53 patients that includes 20 males and 33 females and 11 smokers are included in the study. Samples were collected from three oral cavity sites that grouped into periodontal pockets sites as site A with a probing depth of more than 3mm, bleeding and or suppuration, 6 gingival sulci sites as site B with a probing depth of 3mm or less and from the surface of the oral mucosa as site C, and plated, after isolation, suspect strains of *C. albicans* and *C. dubliniensis* were identified by PCR. The genetic diversity of the isolates was evaluated by RAPD and the activities of the secreted aspartyl proteinases and phospholipases were evaluated by the agar plate method. 21 patients showed positive results for *Candida* species. *C. albicans* was the most frequently found species, while *C. dubliniensis* was isolated from the periodontal pocket of only one patient. 16 genotypes were detected among the *C. albicans* isolates, and one among the *C. dubliniensis* isolates. All isolates produced high levels of Saps and most of them produced high levels of phospholipases. No relationship was found between the genotypes and the pattern of enzymatic production. There was no association between specific genotypes and their site of isolation. The study concluded that genetically homogeneous strains of *C. albicans* are present in the oral cavity of patients with periodontitis and that these strains are capable of producing high levels of exoenzymes.
Ito CY et al. (2008) conducted a study to establish a comparison between the phenotypic profile of oral Candida isolates from chronic periodontitis patients and control individuals, aiming to correlate specific phenotype features to the occurrence of periodontal disease. C.albicans isolates from chronic periodontitis (n=35) and control individuals (n=48) were included in this study. These strains were previously isolated and belonged to the strain collection of the Microbiology Laboratory at the Dental School of São José dos Campos, São Paulo State University. Isolates from chronic periodontitis were obtained from 88 individuals aged 25 and 62 years mean age range of 41.33 ± 5.54 years, with at least two 5-mm deep periodontal sites and diagnosed clinically as chronic periodontitis patients. Control group isolates were obtained from 68 individuals aged 25 to 55 years mean age of 34.45 ± 7.93 years diagnosed as periodontally healthy patients. The isolates were plated on the Sabouraud’s dextrose agar and incubated at 25ºC for 48 hours. Then, yeast suspensions were prepared in sterile distilled water adjusted to the turbidity of tube of McFarland scale. Using sterile swabs, the suspensions were plated on the surface of malt extract agar. Plates were maintained at room temperature in a lightproof environment for 10 days. After this period, the macro morphological aspects of the fringes and surface of the colonies were evaluated.

The results of morphotyping were recorded using 4-digit codes. Biotyping methods were performed with a combination of tests of tolerance to (pH 1.4 and NaCl), resistance (5-fluorocytosine, safranine and boric acid), enzymatic activity (proteinases) and growth in presence of urea, sorbose and citrate. The isolates were plated on Sabouraud’s dextrose agar and incubated at 37º C for 24 hours. In controls individuals plates were incubated at 37°C for 3 to 4 days. Positive tests were
considered when the strains grew at pH 1.4, 5-fluorocytosine, sodium chloride, boric acid, urea, sorbose and sodium citrate, as well as for the strains that formed colonies with diameter greater than 2mm in the presence of safranine. All 16 different morphotypes were observed for C. albicans, the most frequently observed being 0000 and 0001. The results of biotype 0000 (complete absence of fringe) was the most frequently prevalent 77.14% among the isolates obtained from periodontitis patients compared to those from control individuals 50%, with statistical significance (p=0.007). Biotyping revealed 5 different biotypes with higher prevalence of the biotype 357 among the isolates from control and periodontitis groups. This study concluded that by biotyping of the isolates did not permit to differentiate a characteristic model related to periodontal disease, whilst the morphotypes 0000 was most frequently isolated from periodontitis patients.

Javed F et al. (2009)31 conducted a study to analyse the relationship between periodontal condition, oral yeast colonization and Type 2 diabetic subjects with emphasis on gender. Total of 58 Type 2 diabetic subjects 23 males and 35 females in the age range between 45 to 64 years with random blood glucose level ≥11.1mmol/L were investigated at the Department of Dental Medicine, Division of Periodontology, Karolinska Institute, Huddinge, Sweden. Periodontal conditions was determined by plaque index, bleeding on probing, probing pocket depth, oral yeasts, salivary immunoglobulin IgA, IgG and total protein concentration and number of teeth present are determined. Oral samples was collected from the dorsum of the tongue with the sterile cotton swab, and identification of species level was determined by yeast identification system API 32-C system. Unstimulated whole saliva was collected in a clean plastic funnel connected to a measuring cylinder, and samples
were transferred to Karolinska University Hospital, Huddinge, Sweden. The results showed that out of 58 participants, 29 subjects that include 17 males and 12 females with the mean age of 50.5 and 49.3 years were *C.albicans* colonization positive. *C.albicans* colonization negative in 29 subjects that includes 9 males and 23 females at the mean age of 50.6 and 51.1 years respectively. Oral *C.albicans* colonization was (P<0.01) significantly higher in type 2 diabetic males compared to females. The periodontal condition PI (p<0.00001), BOP (p<0.01) and PD (4to6mm) p<0.001), Gig (ug)/mg protein (p<0.001) and Salivary total protein concentrations (p<0.05) were higher in Type 2 diabetic females with *C.albicans* colonization compared to males in the same group. The study concluded that in Type 2 diabetic females with oral *C.albicans* colonization are higher than in males.

**Cuesta AI et al. (2010)** conducted a study to analyse the presence of *Staphylococcus aureus* and *Staphylococcus* species in biofilm of subgingival plaque and oral cavity of individuals with gingival-periodontal disease, to identify isolates and the relationship with *Candida* species. The study included 82 immunocompetent adult individuals with gingival-periodontal disease divided into Group I gingivitis (n=26) and Group II periodontitis (n=56) who attended the out patients unit at the Faculty of Dentistry, University of Buenos Aires, Argentina. In the aged ranged from 18-70 years and mean age was 43.3 ± 15.4 (54.9% women and 45.1% men).Subgingival biofilm samples were obtained using Gracey curettes 7/8, after supra gingival biofilm removal, and a sample from the oral cavity (buccal mucosa, tongue and cheek mucosa) using a sterile swab. The material collected from the subgingival biofilm and oral cavity was soaked in sterile PBS, and stored at 4°C until subsequent processing. Direct microscopic analysis was conducted on the samples by
Gram staining method. To isolate *Staphylococcus* species samples were grown on selective and differential mannitol salt agar culture media. For the isolation of yeast species, a portion of the sample was grown on differential chromogenic solid medium incubated at 37°C for one week. Of all the patients 42.7%, exhibited *Staphylococcus* species in the periodontal pocket and 69.5% in the oral cavity while 25.6% exhibited *Candida* species in the periodontal pocket and 42.7% in the oral cavity, 13.4%, and 36.6% had both microorganisms in the periodontal pocket and the oral cavity respectively. The study concluded that the prevalence of *Staphylococcus aureus* was 13.4% in the periodontal pocket and 15.8% in the oral cavity. *C.albicans* was the most prevalent yeast in the periodontal pocket 76.2% and in the oral cavity 63.0%.

*Machado AG et al. (2010)* conducted a study to compare the number of *C.albicans* cells adhered to epithelial cells in the chronic periodontitis. *C.albicans* cells isolated from 25 individuals with chronic periodontitis and 25 healthy controls were studied. Subgingival dental biofilm samples were collected by inserting 3 sterile paper points into periodontal pocket for 30 seconds. The samples were plated on Sabouraud’s dextrose agar and incubated at 37°C for 24 hours, the yeasts were Gram stained in order to test the purity of the suspension, and the cells were centrifuged and washed 3 times in 15 ml of saline phosphate buffer. A suspension containing $10^6$ cells/ml was obtained in a Neubauer chamber using the trypan blue exclusion method. The epithelial cells were obtained from healthy individuals by scraping a sterile wood spatula against the buccal mucosa. The cells were centrifuged and washed three times in PBS, the suspension containing $10^5$ cells/ml was obtained with aid of a Neubauer chamber and suspension of *C albicans* and epithelial cells
were mixed and incubated at 37°C for 1 hour. *C. albicans* were eliminated using a 12mm isopore membrane. The filter was stained with 50mm of methylene blue and the number of yeasts adhered to 25 epithelial cells was counted. The results of the present study suggest that a number of *Candida* cells adhered to epithelial cells was statistically higher (p=0.000) 15.28 ± 2.32 in chronic periodontitis group than in the control group 6.44 ± 1.20. The study concluded that higher *C. albicans* adherence of samples isolated from patients with chronic periodontitis, which may be correlated to a higher pathogenicity than in controls.

Darwazeh MG et al. (2010)\(^4\) compared oral candidal colonization, both quantitatively and qualitatively, in groups of healthy dentate subjects with different levels of oral hygiene as determined by the plaque index and gingival index scores. A total of 149 healthy dentate subjects that included 75 males and 74 females with age range between 18 to 48 years attended the initial treatment unit in the Dental Teaching Center Faculty of Dentistry, Jordan University of Science, Jordan were included in the study. The concentrated oral rinse technique was used for oral candidal sampling. *Candida* species were cultured on Sabouraud’s dextrose agar plates and identified by germ-tube test and the automated Vitek system biochemical yeast card. The results showed that mean score of plaque index was statistically significant (p=0.005) 1.59 ± 0.46 higher among smokers compared with 1.32 ± 0.38 in non-smokers. However, the gingival index score was 1.28 ± 0.30 in smokers and 1.37± 0.35 in non smokers (p=0.19) not significant respectively. *Candida* species were isolated from 86 out of 149 57.7% subjects. The prevalence of candidal carriage increased significantly as a function of age but was comparable between males and females 58.7% and
The prevalence of oral candidal carriage was significantly higher in the subjects who were not using dental floss 81 out of 133 60.9% compared with those who were using dental floss 51 out of 16 31.2%. The prevalence of oral candidal carriage in 21 out of 35 60% are smokers and 65 out of 114 57% are non smokers respectively. The study concluded that Oral hygiene status, as determined by the plaque index and the gingival index scores per se, does not affect oral candidal colonization in healthy dentate subjects.

Nejad BS et al. (2011) conducted a study to isolate and determine the incidence rate of oral Candida species in periodontitis and gingivitis patients attended the educational clinics of Dentistry school, Ahvaz, Iran. 172 patients with periodontitis and gingivitis that includes 64 males and 108 females with an age range of 11 to 72 years were included in the study. Swabs samples were taken from salivary secretion, the palatal mucosa and dentine carious lesions were cultured directly on Sabouraud’s dextrose agar medium. Isolated yeasts were identified by CHROMagar medium, germ tube test and Clamidoconidia formation (corn meal agar plus Tween 80 medium). The results showed that 160 samples obtained from the oral cavities of 172 patients with periodontitis and gingivitis was positive for oral candidal infection 93%. Candidal species was positive in 110 females 64% and in 62 males 36%. The prevalence of 120 (75%) are C.albicans 20 (12.5%) are Candida glabrata, 10 (6.5%) are Candida tropicalis, 6(4.0%) are Candida dubliniensis and 3 (2.0%) were Candida krusei. Germ tube-test and chlamydospore formation were positive in the isolates that produced dark-green colonies and were considered as Candida dubliniensis and light-green colonies were identified as C.albicans.CHROMagar media is a satisfactory isolation
medium for oral and dental specimens. It is a satisfactory method for correct and rapid identification of common yeast species and easy recognition of mixed cultures in clinical samples.

McManus BA et al. (2012) conducted a study to analyse the prevalence and cell density of Candida species in periodontal pockets, healthy subgingival sites and in oral rinse samples of patients with untreated periodontitis, attending the Dublin Dental University Hospital, Ireland. 21 patients with untreated periodontitis consisting of 9 males and 12 females with the average age of 42.9 years age range from 27-61 years were included in the study. The control group included 50 healthy subjects consists of 21 males and 29 females with an age range from 26-73 years with mean age of 49.7 years were included in the study. The samples were collected from the periodontal pockets, gingival sites, and oral cavity. Subgingival Sampling techniques includes paper points, curette and oral rinse methods. Candida isolates were recovered on CHROMagar medium and representative isolates identified. C.albicans isolates were investigated by multilocus sequence typing to determine if specific clonal groups were associated with periodontitis. The results showed that Candida species were recovered from 10 out of 21 (46.7%) periodontitis patients and from 16 out of 50 (32%) healthy subjects. C.albicans predominated in both groups and was recovered from all Candida positive subjects. Candida positive periodontitis patients yielded Candida from periodontal pockets with average densities of 3,528 and 3,910 CFU/sample, from curette and paper points samples respectively, and 1,536 CFU/ml from oral rinses. Candida species were recovered from 16 out of 50 C.albicans positive healthy subjects yielded an average of (279 CFU/ml) from
oral rinses. Multilocus sequence typing analysis of 31 *C.albicans* isolates from periodontitis patients yielded 19 sequence types, 13 of which were novel. The majority 21 out of 31 of *C.albicans* isolates recovered from patients with periodontitis belonged to multilocus sequence typing clade 1, the most predominant. In contrast, 16 oral *C.albicans* isolates from healthy subjects belonged to 16 sequence types, with four from multilocus sequence typing clade 1. In 21 out of 31 patients with periodontitis clade 1 sequence types were recovered from *C.albicans* in 6 out of 9 subjects 71% greater than in healthy candida carriers sequence types identified in isolates from 4 out of 16 (25%). This study concluded that the distribution of sequence types between both groups was significantly different and indicates an enrichment of *C.albicans* isolates in periodontal pockets.

Canabaro A et al. (2013)\(^{37}\) conducted a study to analyse the relationship between the subgingival colonization by *C.albicans* and other yeasts with the severity of chronic periodontitis. Subjects are divided into 40 patients with chronic periodontitis and 20 healthy subjects attending the Periodontic and General dentistry Clinics, at the Veiga de Almeida University in Rio de Janerio, Brazil. Subgingival samples were collected using sterile paper points from the sulcus or the deepest periodontal pocket of each healthy and chronic periodontitis, respectively, and were cultured on three selective media includes Mycosel agar, Sabouraud’s Dextrose Agar and CHROMagar medium and streaked for isolation. To analyze the density of yeasts in subgingival sites, the number of colony forming units per subject was also determined. The plates were incubated at 37°C, in a non CO\(_2\) atmosphere, for 2–5 days in ultraviolet A and were checked daily for growth. When yeast colonies were
present, the colonies were recovered, counted and transferred to independent plates to obtain pure cultures, which were later identified by biochemical reactions (API 20C) system. The results showed that 23 patients aged 31–67 years made up the slight-moderate chronic periodontitis group and 17 patients aged 33–72 years the severe chronic periodontitis group. 20 healthy individuals aged 21–70 years were included in the control group. Out of 40 patients 12 (30%) are chronic periodontitis presented yeasts in the subgingival biofilm, while only 3 of 20 subjects 15% in the HS group were positive for these microorganisms. Patients with yeast-positive moderate chronic periodontitis out of 23 only 4 subjects 17% and the HS individuals presented only *C. albicans*. Out of 17 only 8 (47%), yeast-positive Severe chronic periodontitis yielded yeasts species like *C. parapsilosis*, *Rhodotorula* species, *C. dubliensis* and *C. tropicalis*, only *C. albicans* was present in all the patients with yeast-positive chronic periodontitis. No statistical difference was found between the chronic periodontitis and HS groups. Severe chronic periodontitis showed statistical significant difference of (P =0.043), (P = 0.033) than moderate chronic periodontitis groups and HS groups respectively. No statistical difference was observed between the moderate chronic periodontitis and HS groups. High densities of yeasts were found in patients with moderate chronic periodontitis and severe chronic periodontitis with mean and range of 61.25 (0–100) CFU/plate and mean and range of 51 (0–101) CFU/ plate respectively. HS group had a low density of yeast mean and range of 1 (0–1) CFU/plate. This study concluded that *C. albicans* and other yeast species are more likely to be present in periodontal pockets of patients with severe chronic periodontitis than in healthy individuals or patients with moderate
chronic periodontitis. Subgingival colonization of some yeast, especially *C. albicans*, was associated with the severity of chronic periodontitis

**Joshi PS et al. (2013)**\(^{38}\) conducted a study to analyze the clinical significance of isolation of *C. albicans* from subgingival plaque in patients with chronic periodontitis. 40 cases of chronic periodontitis and healthy individuals without periodontitis served as normal control attended the Department of Oral pathology and Microbiology, Vasantdada Patil Dental College and Hospital Kavalpur, Sangali Maharashtra were in the study. Periodontal status of the patient was assessed by using Russell’s Periodontal Index. Subgingival plaque sample was obtained aseptically from each patient with the help of Gracey curette. Subgingival plaque samples was smeared and stained with Gram’s stain. Part of the sample was inoculated on Sabouraud’s Dextrose Agar with chloramphenicol and incubated at 25-37°C for 2-3 days. Colony from SDA was inoculated in normal human serum and incubated at 37°C for 90 minutes to demonstrate germ tube formation. Mean Russell’s periodontal index score of 5.75 denoted that the periodontal disease was advanced. The results of the 40 smears showed that 3 were positive for *C. albicans* and 37 smears were negative for *C. albicans*. Colony smears on gram’s staining showed gram positive ovoid budding yeast cells with pseudohyphae. Positive Germ Tube test & demonstration of Chlamydosporre confirmed the yeast species as *C. albicans*. This study concluded that 7.5% isolation of *C. albicans* from subgingival plaque in patients with chronic periodontitis. *C. albicans* plays a role in the infrastructure of periodontal microbial plaque and in its adherence to the periodontal tissues.
Muzurovic S et al. (2013)\textsuperscript{39} conducted a study to analyse the presence of Candida species in the oral cavity and their relation with the smoking habit. A total of 140 healthy respondents 75 males and 65 females between the ages of 18 to 60 at the Dental clinic Health Centre, The Public Institution Medical centre of Sarajevo Canton, Bosnia and Herzegovina. Group I included patients aged from 18 to 30 years and Group II included patients aged between 31 to 60 years are included in the study. Isolation of Candida species samples were taken by sterile swab and stained by methylene blue and examined microscopically (40x) for detecting the presence of blastospores and pseudohyphae. Simultaneously each sample was cultivated on Sabouraud’s dextrose agar and Brilliance candida Agar. After 48 hours of incubation at 37°C, cultures were separated into positive growth of the yeast or negative cultures no yeast growth. On positive cultures, the number of yeast colonies was determined. The results were confirmed by yeast assimilation test API 20C AUX. In Group I, consists of 37 patients 52.8% and in Group II, included 40 patients 57.1% are smokers. There were significantly more male smokers, 49 (62.3%). Candida species were identified in 40 (29%) healthy respondents (carriers). The most frequently isolated species was C.\textit{albicans}. The study concluded that the presence of oral Candida in 82.5% subjects who are smokers compared to smokers without Candida were 44% subjects. Smoking has an influence on oral colonization with Candida species.

Joshi PR et al. (2014)\textsuperscript{40} conducted a study to analyse the oral manifestations in patients with Diabetes Mellitus and isolation of C.\textit{albicans} from subgingival plaque of patients with or without Diabetes Mellitus. A total of 80 cases selected randomly in the age group between 40-60 years attending the Out Patient
Department, in Department of Oral Pathology and Microbiology, Vasantdada Patil Dental College and Hospital, Kavalpur, Maharashtra. Group I consists of 40 cases of known Diabetes Mellitus with chronic periodontitis and (Group II) consists 40 control cases of patients with chronic periodontitis but without Diabetes Mellitus were included in the study. In Group I Glycosylated haemoglobin and Fasting blood glucose was estimated and in Group II, random blood glucose estimation was done. Subgingival plaques were collected from the labial surface of lower anterior teeth with the help of Gracey curette. Microbiological analysis of subgingival plaque samples was done by Gram’s staining and culture on Sabouraud’s Dextrose Agar with chloramphenicol and Corn meal agar at 25-37°C for 1-7 days. Germ tube test and demonstration of chlamydospores from culture was done for confirmation of *C.albicans* presence. The results of the study showed that in Group I more commonly in 27 males 67.50% than in 13 females 32.50% and the oral manifestations seen in subjects were gingivitis 100%, chronic generalized periodontitis 100% and Xerostomia 37.5%. Glycosylated hemoglobin levels showed that in Group I 19 subjects of 47.5% had good control, both good and fair control had 9 subjects 22.5% and 3 subjects had 7.50% of poor control. Group II were detected with increased random blood sugar above 210 mg/dl in 3 out of 40 cases. Group I revealed Gram positive *Candidal* pseudohyphae along with budding yeast cells in 36 subjects (90%) and in Group II 3 subjects (7.50%) which was statistically significant (p<0.001). The study concluded that Group I Diabetes mellitus with chronic periodontitis showed *C.albicans* growth was higher than non Diabetic with chronic periodontitis. Oral *Candidal* Carriage assessment from subgingival plaque can be
used as a quick and cost effective routine investigative procedure in management of chronic periodontitis patients with or without Diabetes Mellitus.

Madhumieatha A et al. (2015) conducted a study to analyse the influence of hormonal contraceptive usage on the distribution of Candida species in females of childbearing age and associations between hormonal contraceptive use and various periodontal clinical parameters. 82 female patients in the 19-45 years age group for a period of 12 months attending the Out Patient Department in M.S Ramaiah Dental College and Hospital, Bangalore. Group I consists of Hormonal contraceptive users with chronic periodontitis and Group II includes female patients with chronic periodontitis who were not taking any Hormonal contraceptives. Periodontal parameters such as periodontal pocket depth, clinical attachment Loss, gingival index and plaque index were evaluated. Pooled subgingival samples from the deepest pockets in each quadrant using sterile paper points and sterile curette was obtained from each patient and immediately streaked onto Sabouraud’s dextrose agar. Species identification was done by colony colour on CHROMagar medium and Dalmau plate culture technique on corn meal agar. The results showed that the Hormonal contraceptive user group was 26.8 % and the non-user group 29.3% of Candida species in periodontal pockets. The study concluded that C.albicans was the most common species isolated from both groups, followed by C. dubliniensis, C. Krusei, Candida tropicalis, Candida glabrata and Candida parapsilosis. No statistically significant difference in the candida count or periodontal clinical parameters between the hormonal contraceptive users and non-users group was found.
Petrovic S et al. (2015) studied to detect *Candida* species on the tongue and in the subgingival sites in healthy and type 2 Diabetes Mellitus (T2D) patients with chronic periodontitis and to compare the accuracy of sampling methods. A total of 131 patients divided into 4 groups that included Group A consisted of 35 subjects that included 14 males and 21 females with Healthy controls, Group B consisted of 30 subjects 14 males and 19 females with non Diabetics and chronic periodontitis. Group C included 26 subjects 14 males and 12 females diagnosed as Diabetics with good metabolic control and chronic periodontitis. Group D consists of 40 subjects that included 26 males and 14 females diagnosed as Diabetics with poor control and chronic periodontitis subjects attending University of Belgrade, School of Dental Medicine, Department of Oral Medicine and Periodontology Belgrade, Serbia. Oral Cotton swabs were collected by swabbing from the dorsum of the tongue with the help of a dry sterile cotton stick. Swab cultures were immediately inoculated on Sabouraud’s dextrose agar. Subgingival plaque samples were obtained from each patient with help of sterile paper points and a sterile curette. Both Subgingival plaque samples were inoculated on Sabouraud’s dextrose broth and incubated at 37°C for 48 hours and the number of CFU was counted. Receiving Operator Curve analysis was used to compare sampling methods for subgingival plaque collection for isolation of *Candida* species. The results showed that the presence of *Candida* species on the tongue in Group A (22.9%), Group B (22.9%), Group C (23.1%) and Group D (47.1%) respectively. *Candida* species present on the tongue was significantly higher in Group D (p=0.046) compared to non-diabetic groups. *Candida* species were present in the subgingival samples revealed that Group A (25.7%), Group B (26.7%), Group C (15.4%) and Group D (45%) showed higher presence of
yeasts than non-diabetic groups. Subgingival plaque sampling by means of a sterile curette showed sensitivity 0.576 and specificity was 0.919, and statistical difference (p=0.000) compared to paper points. This study concluded that *Candida* species is more prevalent on the tongue and the subgingival plaque samples in Diabetes Mellitus Group with poor control than in the healthy control group, regardless of periodontal status. Subgingival plaque may represent a reservoir of commensals.

**Deepa A et al. (2015)**\(^4^3\) conducted a study to analyse the antifungal resistance pattern, virulence attributes and spectrum of *Candida* species in oral cavities of patients with periodontal diseases and healthy individuals. A total number of 52 patients with periodontal disease that includes 5 gingivitis subjects, 22 chronic gingivitis subjects, 25 chronic periodontitis subjects and 100 healthy subjects attending the Department of Dental Surgery, Safdarjang Hospital, New Delhi are included in the study. 2 swabs were collected from the test group by gently rubbing gingival and supra gingival area of the affected teeth using sterile cotton swabs. First swab was inoculated on Sabouraud’s Dextrose Agar, incubated at 37˚C for up to 7 days, and observed daily for growth, and the second swab was used to prepare a smear for Gram’s staining. In control groups, swabs were collected from each subject by depressing the tongue and gently rubbing the surface of gum, tooth, tonsils and tongue, and processed like the test group respectively. Antifungal susceptibility testing was performed on all the isolates using antifungal agents including Fluconazole, Ketoconazole, Voriconazole and Amphotericin B by E-test strip method. The results showed that out of 52 patients screened 11 yielded 21% different *Candida* species, with *C.albicans* 83% being the commonest and non *C.albicans* species accounting for 17 %. Among 100 healthy controls, 23 were colonized by
REVIEW OF LITERATURE

various *Candida* species, with *C. albicans* as the predominant species. The study concluded that there is no significant difference in the distribution of *Candida* species among healthy subjects and patients with periodontal diseases. Antifungal resistance patterns and expression of some of the important virulence attributes also revealed no differences between the isolates from patients and control populations.

*Keten HS et al. (2015)* conducted a study to analyse the prevalence of *Candida* carriage and *Candida* species among cigarette and Maras powder users. Total of 180 volunteering men in 20 cafe houses in the city of Kahramanmaras, Turkey that includes 60 individuals smoking 3 cigarettes per day for at least 1 year constituted the cigarette group, 60 individuals using Maras powder at least 3 times a day for at least 3 years constituted the Maras powder group and 60 people declaring no use of tobacco products constituted the control group were included in the study. The mean age of the participants was $40.49 \pm 12.89$ years. Culture specimens were obtained from bilateral buccal mucosa and dorsum of the tongue with a sterile cotton-tipped swab. The specimens were inoculated into Sabouraud Dextrose Agar. The results showed that frequency of *Candida* carriers was found in 35 58% of the cigarette groups, 34 56.7% of the Maras powder groups, and 36.7% of the control groups. The most frequently isolated species was *C. albicans* at a rate of 30% in the cigarette users groups, 28.3% in the Maras powder users group and 18.3% in the controls. The prevalence of *Candida tropicalis* carriage was found to be at a rate of 20% in cigarette users groups and 21.7% in the Maras powder users group compared to 11.7% in the nonusers. The study concluded that the prevalence of oral *Candida* carriage was significantly higher in smokers and Maras powder users than control groups.
Shirazi J et al. (2015) evaluated the prevalence frequency of *C.albicans* in oropharyngeal infections and risk factors prevailing *C.albicans* infection in three major cities of the two densely populated provinces southern Punjab and Khyber Pakhtoon khawah in Pakistan. 503 swab samples were collected from dorsum of the tongue, vestibular sulcus and peak of the palatal vault. Sabouraud’s dextrose agar media containing chloramphenicol 10% was used for culturing the swab samples. Culture plates were incubated at 37°C for 24-48 hours. Plates having no yeast growth were incubated further for 72 hours. Presence of pseudohyphae and budding cells were observed to confirm the *C.albicans* presence. The results showed that *C.albicans* positive samples were 211 and rests were *C.albicans* negative, prevalence rate of *C.albicans* was observed as 41.94 % with three major risk factors antibiotic abuse, hygienic condition of oral cavity and alcohol consumption. The study concluded that high prevalence rate of *C.albicans* increases with antibiotic abuse.
Figure: 1

Armamentarium for clinical examination
Figure: 2

Preoperative photographs of chronic periodontitis
Figure: 3

Full mouth intra oral periapical radiographs
Figure: 4

Sub gingival plaque sample collected using Gracey curette
Figure: 5

Sample transferred to Eppendorf tube
Figure: 6

Growth of Candida albicans on Sabouraud’s dextrose agar plates after incubation
Figure: 7

Candida species identification on CHROMagar medium
Figure: 8

Microscopic identification of Candida species by Gram staining method
Figure: 9

Germ tube test positive confirmatory for Candida albicans
MATERIALS & METHODS
STUDY DESIGN AND PATIENT SELECTION

This study was conducted in the Department of Periodontics, Sri Ramakrishna Dental College & Hospital, Coimbatore for a period of 3 months. All patients were informed about the particulars of the study and informed consent was obtained from each subject. The study was approved by the ethical committee of the institution. This study included 108 subjects with chronic periodontitis.

INCLUSION CRITERIA:

- Age group between 30 to 55 yrs
- Patients who had not received any dental treatment for the past 6 months
- Patients without any systemic complications
- Patients with chronic periodontitis.

EXCLUSION CRITERIA:

- Patients with any systemic and immuno compromised diseases.
- Patients who received any drugs for the past 6 months.

ARMAMENTARIUM:

DIAGNOSTIC INSTRUMENTS:

Mouth mirror, Explorer, Tweezer, Williams’s periodontal probe, Gracey curettes, Cotton rolls.
CLINICAL EXAMINATION:

On each examination, the following clinical parameters were calculated:

Plaque Index (PlI):

The Full mouth plaque score was recorded by using Plaque Index system given by (Silness and Loe) 1964.46

| Scores | Criteria |
|--------|----------|
| 0      | No plaque |
| 1      | A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface. |
| 2      | Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin, which can be seen with the naked eye. |
| 3      | Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin |

Calculation: \[
\frac{\text{Sum of score of each teeth}}{\text{Total number of teeth examined}}
\]

Inference:

Excellent: 0
Good: 0.1 – 0.9
Fair: 1.0 – 1.9
Poor: 2.0-3.0
MATERIALS AND METHODS

Conventional probing depth (PD):

The probing pocket depth was assessed on each tooth from the gingival margin to the base of the sulcus using William’s periodontal probe at 6 specific surfaces per tooth.\(^4\)

1. Distobuccal surface
2. Midbuccal surface
3. Mesiobuccal surface
4. Distolingual surface
5. Midlingual surface
6. Mesiolingual surface

Clinical Attachment Loss (CAL):

The CAL was recorded from the Cemento enamel junction (CEJ) to the base of the pocket in all the 6 sites using Williams’s periodontal probe as mentioned for probing depth.\(^4\)

1. Gingival margin located on the anatomic crown- subtracting the depth of Pocket from the gingival margin to CEJ
2. Gingival margin coincides with CEJ the loss of attachment equals to the pocket depth
3. Gingival margin located apical to CEJ the distance between the CEJ and gingival margin should be added to the pocket depth.
MATERIALS AND METHODS

Based on the amount of clinical attachment loss, subjects were subdivided into Armitage GC (1999).  

Mild periodontitis: Periodontal destruction is generally considered slight when no more than 1 to 2 mm of clinical attachment loss has occurred.

Moderate periodontitis: Periodontal destruction is generally considered moderate when 3 to 4 mm of clinical attachment loss has occurred.

Severe periodontitis: Periodontal destruction is considered severe when 5mm or more of clinical attachment loss has occurred.

SAMPLE COLLECTION AND TRANSPORATION:

Sub gingival plaque samples are collected from each patient with the help of sterile Gracey curette either in buccal aspects of molar teeth or one more teeth in the deepest pockets. Collected samples was placed in a sterile container which consists of phosphate buffered saline in Eppendorf tube as a transport medium and was sent to the laboratory for the culturing of *C albicans*.

FUNGAL CULTURING:

Culture was performed in Sabouraud’s Dextrose Agar (SDA) incubated at 37°C for 2-3 days. *C albicans* growth on Sabouraud’s Dextrose Agar produces creamy, moist, yeast-like colonies in a streak like pattern.
MATERIALS AND METHODS

IDENTIFICATION OF CANDIDA SPECIES USING CHROMagar MEDIA:

Yeast colonies growing on each Sabouraud’s Dextrose Agar were resuspended and 10 μL of suspension solution was used to inoculate plates with CHROMagar medium. Inoculated plates was incubated at 37°C and read for up to 7 days. Plates were observed for fungal growth using morphology and colour to determine the presence of yeasts. C. albicans were identified by the production of green coloured colonies, respectively.

MICROSCOPIC ANALYSIS:

GRAM STAINING: Samples was gram stained shows gram positive budding yeast cells with pseudohyphae, and gram negative shows pus cells, and bacilli.

GERM TUBE TEST: Small portion of an isolated colony was suspended in a test tube containing 0.5 ml of human serum then incubated at 37°C for 2 hours then examined microscopically at 30 minutes intervals up to 2 hours for the presence of germ tube confirmatory test for C. albicans.
PREVALENCE OF CANDIDA ALBICANS IN CHRONIC PERIODONTITIS PATIENTS

PROFORMA
FORM I - SCREENING PROFORMA

NAME:
AGE: SEX:
POSTAL ADDRESS:

TELEPHONE NUMBER:
OCCUPATION:
CRITERIA OF INCLUSION:
  • Age group between 30 to 55 yrs,
  • Patients with chronic periodontitis.
  • Patients who had not received any dental treatment for the past 6 months.

CRITERIA OF EXCLUSION:
  • Patients with any systemic and immuno compromised diseases.
  • Patients who received any drugs for the past 6 months.
FORM II- HISTORY PROFORMA

CHIEF COMPLAINT WITH DURATION:

| PRESENT | ABSENT |
|---------|--------|
| 1. Bleeding gums |       |       |
| 2. Pain in gums   |       |       |
| 3. Swollen gums    |       |       |
| 4. Pus discharge from gums | |       |
| 5. Mobility       |       |       |
| 6. Hypersensitivity |     |       |
| 7. Any other complaints (Specify): | |       |

PERSONAL HISTORY:

1. Diet: Veg [ ] Non-Veg [ ] Mixed [ ]
2. Brushing habit:
3. Smoking: Yes [ ] No [ ]
4. Any other (specify):

FAMILY HISTORY:
FORM III- CLINICAL PARAMETER ASSESSMENT

DATE: __________

1. PLAQUE INDEX (SILNESS AND LOE 1964)

Calculation: \[
\text{Sum of score of each teeth} \quad \frac{\text{Total number of teeth examined}}{\text{}}
\]

Inference:

Excellent: 0
Good: 0.1 – 0.9
Fair: 1.0 – 1.9
Poor: 2.0-3.0
PERIODONTAL STATUS

a) PROBING DEPTH: (CONVENTIONAL PROBING METHOD)

CLINICAL ATTACHMENT LEVEL:
CONSENT FORM
CERTIFICATE BY INVESTIGATOR

I certify that I have disclosed all details about the study in the terms easily understood by the patient.

Date: ___________  Signature: ___________

Name: ___________
CONSENT BY SUBJECT

I have been informed to my satisfaction, by the attending physician, the purpose of the clinical trial and the nature of treatment and follow up including the laboratory investigation to be performed to monitor and safeguard my body functions.

Dated: _________  Signature or thumb impression
In this study, the prevalence of *C. albicans* in chronic periodontitis was statistically analysed using chi-square test, to evaluate the correlation between the severity of disease and to correlate the prevalence of *C. albicans* with smoking status and sex of the subjects. The statistical analysis was done using software SPSS version 13.

**Sample characteristics:**

The study was conducted in 108 subjects, which included 69 males and 39 females. The age group of the patients is between 30-55 years with the mean age of 42.9 ± 6.98 years. (Table 1)

Based on the amount of CAL, subjects were classified into mild, moderate and severe with chronic periodontitis Armitage GC (1999).48

In mild chronic periodontitis- 5 males and 2 females

In moderate chronic periodontitis- 26 males and 18 females

In severe chronic periodontitis- 38 males and 19 females

In the current study, there were 29 smokers and 79 non-smokers with *C. albicans* and chronic periodontitis.
1. Plaque index system:

The full mouth plaque index showed that 69 male subjects had a mean plaque score with standard deviation of $1.629 \pm 0.206$ and 39 female subjects had a mean plaque score with standard deviation of $1.45 \pm 0.349$. (Table 2, Figure 1)

2. Probing pocket depth level:

A total of 69 males in the study had a mean probing pocket depth with standard deviation of $3.766 \pm 0.916$ mm. Female patients who made the remaining 39 subjects had the mean probing depth with a standard deviation of $3.543 \pm 0.89$. (Table 3, Figure 2)

3. Clinical attachment level:

In the current study, the CAL measured from the CEJ to the base of the periodontal pocket using William’s periodontal probe, the subjects divided into mild, moderate, and severe chronic periodontitis based on the CAL. Among the study subjects, males had CAL with standard deviation of $4.470 \pm 1.067$ mm. Females had a mean CAL with standard deviation of $4.395 \pm 1.056$ mm. (Table 4, Figure 3)

4. Clinical attachment level with severity of chronic periodontitis:

Among 108 subjects, 69 males and 39 females were included in the study. Among males, 5 showed mild chronic periodontitis with mean value and standard deviation of $2.348 \pm 0.642$ mm, 26 showed moderate chronic periodontitis with mean value and standard deviation of $3.895 \pm 0.876$ mm and 38 showed severe chronic periodontitis with mean value and standard deviation of $5.486 \pm 0.422$ mm. Among
RESULTS

females, 2 had mild chronic periodontitis with mean value and standard deviation of 2.221±0.566 mm, 18 had moderate chronic periodontitis with mean value and standard deviation of 3.356±0.942 mm and 19 had severe chronic periodontitis with mean value and standard deviation of 5.433±0.141 mm respectively. (Table 5, Figure 4)

5. Sex and Candida albicans:

Among 69 males, 14 were positive for *C. albicans* (20.30%) and 55 were negative for *C.albicans* (79.7%). Among 39 females, 6 were positive for C.albicans (15.4%) and 33 were negative for *C albicans* (84.6%). The results showed that the presence of *C.albicans* among males and females was not significant with (p= 0.528.) (Table 6, Figure 5)

6. Sex and severity of chronic periodontitis:

In the present study comprising of 69 males and 39 females, the severity of chronic periodontitis was assessed and it was observed that 5 males (7.2%) and 2 females (5.1%) showed mild chronic periodontitis. Moderate chronic periodontitis was seen among 26 males (37.70%) and 18 females (46.2%). Severe chronic periodontitis was seen in 38 males (55.1%) and 19 females (48.7%). Comparing the severity of diseases among the sexes showed no statistical significant differences (p= of 0.670). (Table 7, Figure 6)
7. Sex with severity of chronic Periodontitis and Candida albicans positive:

In the current study when correlating the sexes with severity of chronic periodontitis and *C.albicans*, none of the males and females counterparts with mild chronic periodontitis tested positive for *C.albicans*. Among moderate groups of chronic periodontitis 5 males (19%) and 2 females (11%) were positive for *C.albicans*. Among severe chronic periodontitis, 9 males (23%) and four females (21%) tested positive for *C.albicans* The results showed no significant differences among sexes and severity of disease to the presence of *C.albicans*. (Table 8, Figure 7)

8. Smoking habit and Candida albicans:

In the current samples, 29 subjects were smokers and 79 subjects were non smokers. Among 29 smoking subjects 9 (31%), tested positive for *C.albicans*, 20 tested negative for *C.albicans*. Among non smokers 11 (13.3%) tested positive for *C.albicans*, (86.1%) tested negative for *C.albicans*. The study showed that 31% smokers tested positive for *C.albicans* compared to 13.3% of non smokers. The study demonstrated statistically significant (p= 0.042) correlations between smoking habits and *C.albicans*. (Table 9, Figure 8)

9. Smoking habit and Candida albicans with severity of chronic periodontitis:

Among the smokers and non smokers, none of the subjects with mild chronic periodontitis tested positive for *C.albicans* Among the smokers, 3 subjects (33.3%)
RESULTS

with moderate chronic periodontitis and 6 subjects (66%) severe chronic periodontitis tested positive for *C.albicans*. Among non smokers, 4 subjects (36.3%) with moderate chronic periodontitis and 7 subjects (63%) with severe chronic periodontitis tested positive for *C.albicans*. The result showed no statistically significant differences (*p* =0.888) when comparing smoking status and *C.albicans* positive individuals with severity of chronic periodontitis. (Table 10, Figure 9)
### Table 1: Age characteristics of the Sample

| Sex    | N  | Mean ± SD  |
|--------|----|------------|
| Male   | 69 | 43.3±7.3   |
| Female | 39 | 42.03±6.3  |
| Total  | 108| 42.9±6.98  |

### Table 2: Plaque index system

| Sex    | N  | Mean±SD   |
|--------|----|-----------|
| Male   | 69 | 1.629±0.206 |
| Female | 39 | 1.455±0.349 |
| Total  | 108| 1.558±0.783 |
### Table 3: Probing pocket depth

| Sex   | N   | Mean | Mean ± SD  |
|-------|-----|------|------------|
| Male  | 69  | 3.766| 3.766 ± 0.916 |
| Female| 39  | 3.543| 3.543 ± 0.892 |
| Total | 108 | 3.844| 3.844±0.911 |

### Table 4: Clinical attachment level

| Sex   | N   | Mean | Mean ± SD  |
|-------|-----|------|------------|
| Male  | 69  | 4.470| 4.470 ± 1.067 |
| Female| 39  | 4.395| 4.395 ± 1.056 |
| Total | 108 | 4.239| 4.239±1.063 |
### Table 5: Clinical attachment level with severity of chronic periodontitis

| Chronic periodontitis | N  | Male Mean ± SD          | Female Mean ± SD    |
|-----------------------|----|-------------------------|---------------------|
| Mild                  | 7  | 2.348 ± 0.642           | 2.221 ± 0.566       |
| Moderate              | 44 | 3.895 ± 0.876           | 3.356 ± 0.942       |
| Severe                | 57 | 5.486 ± 0.422           | 5.433 ± 0.141       |
| Total                 | 108| 4.470 ± 1.067           | 4.395 ± 1.056       |

### Table 6: Sex and Candida albicans

| Sex     | Candida albicans | Summation | p.value |
|---------|------------------|-----------|---------|
|         | Positive (+)     | Negative (-) |       |
| Male    | Count            | 14        | 55      | 69      |
|         | Percentage       | 20.30%    | 79.70%  | 100.00% |
| Female  | Count            | 6         | 33      | 39      |
|         | Percentage       | 15.40%    | 84.60%  | 100.00% |
| Total   | Count            | 20        | 88      | 108     |
|         | Percentage       | 18.50%    | 81.50%  | 100.00% |

\[ \chi^2 = 0.000 \ (p \text{ value} > 0.05 \text{ not significant}) \]

\( \chi^2 \)-chi square test
RESULTS

Table 7: Sex and severity of chronic periodontitis

| Chronic periodontitis | Sex                      | Total          | p Value |
|-----------------------|--------------------------|----------------|---------|
|                       | Male                     | Female         |         |
|                       | Count | Percentage | Count | Percentage | Count | Percentage |
| Mild                  | 5     | 7.20%      | 2     | 5.10%      | 7     | 6.50%       | 0.670    |
| Moderate              | 26    | 37.70%     | 18    | 46.20%     | 44    | 40.70%      |         |
| Severe                | 38    | 55.10%     | 19    | 48.70%     | 57    | 52.80%      |         |
| Total                 | 69    | 100.00%    | 39    | 100.00%    | 108   | 100.00%     |         |

$\chi^2 = 0.000$ (p value >0.05 not significant) $\chi^2$ - chi square test

Table 8: Sex with severity of chronic periodontitis and Candida albicans positive

| Chronic periodontitis | TOTAL       | Candida Positive | Percentage | p Value |
|-----------------------|-------------|------------------|------------|---------|
|                       | Male | Female | Total | Male | Female | Total | Male | Female | Total |         |
| Mild                  | 5    | 2      | 7     | 0    | 0      | 0     | 0%   | 0%     | 0%     | 0.9185  |
| Moderate              | 26   | 18     | 44    | 5    | 2      | 7     | 19%  | 11%    | 14%    |         |
| Severe                | 38   | 19     | 57    | 9    | 4      | 13    | 23%  | 21%    | 22%    |         |
| Total                 | 69   | 39     | 108   | 14   | 6      | 20    | 20%  | 15%    | 18%    |         |

$\chi^2 = 0.000$ (p value >0.05 not significant) $\chi^2$ - chi square test
### Table 9: Smoking habit and Candida albicans

| Smoking habit | Candida | Summation | p Value |
|---------------|---------|-----------|---------|
|               | Positive (+) | Negative (-) |         |
| Smoker        | Count     | 9          | 20      | 29      | 0.042 |
|               | Percentage | 31.00%     | 69.00%  | 100.00% |
| Non Smoker    | Count     | 11         | 68      | 79      |
|               | Percentage | 13.90%     | 86.10%  | 100.00% |
| Total         | Count     | 20         | 88      | 108     |
|               | Percentage | 18.50%     | 81.50%  | 100.00% |

$\chi^2 = 0.000$ (p value $\leq 0.05$ significant) $\chi^2$ - chi square test

### Table 10: Smoking habit and Candida albicans positive with chronic periodontitis

| Smoking habit | Candida positive (+) | Chronic Periodontitis | p Value |
|---------------|----------------------|-----------------------|---------|
|               | Count | Mild | Moderate | Severe |         |
| Smoker        | 9     | 0    | 3    | 6      | 0.888 |
|               | Percentage | 0.00% | 33.30% | 66.70% |
| Non smoker    | 11    | 0    | 4    | 7      |
|               | Percentage | 0.00% | 36.36% | 63.63% |

$\chi^2 = 0.000$ (p value $> 0.05$ not significant) $\chi^2$ - chi square test
RESULTS

Graph 1: Plaque index system

Graph 2: Probing pocket depth level
RESULTS

Graph 3: Clinical attachment level

Graph: 4 Clinical attachment level with severity of chronic periodontitis
Graph 5: Sex and Candida albicans

Graph 6: Sex with severity of chronic periodontitis
RESULTS

Graph 7: Sex with severity of chronic periodontitis and Candida albicans positive

Graph 8: Smoking habit and Candida albicans

Graph 9: Smoking habit and Candida albicans positive with chronic periodontitis
DISCUSSION

The study was conducted to assess the prevalence of *C. albicans* in patients with chronic periodontitis and to correlate it with sex, severity of chronic periodontitis and smoking status of the subjects. The current study was done in the Department of Periodontics, Sri Ramakrishna Dental College and Hospital, Coimbatore for a period of 3 months.

*C. albicans* may aid the plaque microorganisms in evading the host defence mechanism as it is typically found on the outer layers of the plaque and has also been observed in deep periodontal tissues.\(^{27}\) *C. albicans* was identified in the subgingival sites of patients with severe chronic periodontitis, suggesting that the advanced form of chronic periodontitis was associated with a more complex yeast community residing in the deep pockets.\(^{37}\) Severe periodontal disease may be one of the causes for immunosuppression, which leads to colonization of this opportunistic pathogen.\(^{27}\)

Though Candida can appear in pseudohyphae or yeast forms, the pseudohyphae is the one found more common in tissues, whereas the yeast forms are found on epithelial surfaces. Hyphae have the ability to penetrate host tissue and are hence important in the disease process. The gingival pocket and gingival crevicular fluid provide a favorable environment for the germination and growth of these hyphae.\(^{27}\) *C. albicans* can secrete proteinases capable of degrading major extracellular matrices and basement membrane components that cause destructive inflammation of the underlying periodontal tissues.\(^{12, 18, 21}\)
Based on these observations, in the current study, subgingival plaque samples were collected from the deepest periodontal sites using sterile curettes from 108 subjects. This comprehensive sampling regimen was carried out in order to get an accurate representation of *C. albicans* prevalent in the periodontal pockets of patients with chronic periodontitis. The clinical parameters were assessed using plaque index, probing pocket depth and clinical attachment level. Sabouraud’s dextrose agar and the CHROMagar media were used to culture *C. albicans* from clinical samples in order to exhibit characteristic colony colors and for the detection of separate *Candida* species.

Based on the amount of clinical attachment loss, subjects were classified into mild, moderate and severe chronic periodontitis. In the current study, CAL for mild, moderate and severe chronic periodontitis for male subjects was $2.348 \pm 0.64$, $3.895 \pm 0.876$, and $5.486 \pm 0.422$ respectively and for female subjects $2.221 \pm 0.566$, $3.356 \pm 0.942$ and $5.433 \pm 0.141$ respectively.

In this study, the prevalence of *C. albicans* was found to be 18.5% of the 108 subjects. Among 20 subjects who were positive for *C. albicans*, 14 (20.3%) were males and 6 (15.4%) were females, which showed that the prevalence of *C. albicans* was not statistically significant ($p=0.528$) between males and females. This result was in accordance to the studies done by Slots et al. (1988) in USA, who analysed 500 subjects and showed 16.7% of the males and 16.9% of females were positive for *C. albicans*. This study was also in accordance with the study done by Reynaud et al. (2001) in Norway, who analysed 128 individuals and showed 17.5% of the total subjects and 20.3% and 8.2% of males and females respectively to be Candida positive. Canbarro et al. (2013) reported 17% prevalence of *C. albicans* in Brazilian
population that included 20.3% of the males and 8.2% of the females respectively. None of the studies observed significant difference between sexes and \textit{C.albicans}.

In this study, of the 7 subjects diagnosed with mild chronic periodontitis none were positive for \textit{C.albicans} was present in 7 out of 44 subjects (14%) with moderate chronic periodontitis and 13 out of 57 subjects (22%) with severe chronic periodontitis. This result was in contrast to the study done by \textit{Canbarro et al. (2013)}\textsuperscript{37} in Brazilian population where \textit{C.albicans} was present in 47% subjects with moderate chronic periodontitis though similar results were found with 17% of the subjects were \textit{C.albicans} positive in severe chronic periodontitis This difference could be attributed to the geographical and ethnic differences among the subjects in both the studies, and also the limited sample size in this study.

In the present study among the subjects who tested positive, \textit{C.albicans} was significantly higher in the 9 out of 29 smokers (31%) than in the 11 out of 79 non-smokers (13.0%) with (p=0.042), showing that tobacco smoking increases the prevalence of \textit{C.albicans}. This result was in accordance with the study done by \textit{Keten et al. (2015)}\textsuperscript{44} in Turkey who showed that 30% of the study samples were \textit{C.albicans} positive in smokers compared to 18.3% non smokers.

However, other studies have found that tobacco smoking did not have an influence on oral colonization with \textit{C.albicans}. \textit{Oliver & Shillitoe (1984)}\textsuperscript{11} in California USA showed \textit{C albicans} to be prevalent in 35% smokers and in 35% non smokers and \textit{Darwazeh et al. (2010)}\textsuperscript{34} isolated \textit{C albicans} from 84% of the smokers and 74% of the nonsmokers and they found no significant association between smoking habits and \textit{C.albicans}. 
Higher candidal count observed among the smokers may be attributed to the aromatic hydrocarbons in tobacco, which have been shown to act as nutrients to the yeast cells. Smoking may indirectly increase the level of salivary glucose, which enhances yeast growth. Also, smoking can depress the activity of oral leucocytes and other non-specific immune defences.\textsuperscript{34}

To the best of our knowledge, this is the first study to elaborate the prevalence of \textit{C.albicans}, in mild, moderate and severe chronic periodontitis among smokers and non smokers. Among the 9 smokers who tested positive for \textit{C.albicans}, 3 subjects had moderate chronic periodontitis (33.3\%) and 6 subjects had severe chronic periodontitis (66.7\%). Among the 11 non smokers, who tested positive for \textit{C.albicans}, 4 subjects had moderate chronic periodontitis (36.3\%) and 7 subjects had severe chronic periodontitis (63.6\%).

As a small number of samples were included in this study, the statistical validity of the associations found was limited. Hence, larger numbers of samples are required to confirm the clinical findings and further qualitative and quantitative analysis should be done to validate the results of this study.
SUMMARY AND CONCLUSION
SUMMARY AND CONCLUSION

This study evaluated the prevalence of *C. albicans* among patients with chronic periodontitis. The study included 108 subjects of which 69 were males and 39 were females. The study was conducted in the Department of Periodontics, Sri Ramakrishna Dental College and Hospital, Coimbatore, India over a period of 3 months.

The mean age of the study group was 42.9±6.98 years. The mean PI score was 1.558±0.783. The mean PD and CAL of the study subjects were 3.844 mm and 4.23 mm respectively.

In the current study, 20 subjects (18.5%) were tested positive for *C. albicans*. 7 subjects (14%) with moderate chronic periodontitis and 13 of subjects (22%) with severe chronic periodontitis were positive for *C. albicans* showing an increased prevalence of *C. albicans* in severe chronic periodontitis compared to milder forms of chronic periodontitis. Though *C. albicans* was increased in severe chronic periodontitis compared to moderate chronic periodontitis, the results are not statistically significant. The results also showed no statistical significant between prevalence of *C albicans* and sex of the individual.

In the current study, 31% of smokers were positive for *C albicans* compared to 13% of non smokers who were positive showed for *C albicans*. The results showed statistically significant relation between *C.albicans* and smoking status of the individuals.

In the current study, among the 9 smokers who tested positive for *C.albicans*, 3 subjects had moderate chronic periodontitis (33.3%) and 6 subjects had severe chronic periodontitis (66.7%). Among the 11 non smokers, who tested positive for
C. albicans, 4 subjects had moderate chronic periodontitis (36.3%) and 7 subjects had severe chronic periodontitis (63.6%). The result showed no statistically significant differences when comparing smoking status and C. albicans positive individuals with severity of chronic periodontitis.

The following conclusions were drawn from the study,

- *C. albicans* were present in higher amounts in the periodontal pockets of patients with severe chronic periodontitis.
- Smokers had greater chances of being *C. albicans* positive than non-smokers.
- *C. albicans* did not have a predilection for particular sexes.

Limitations of the study include smaller sample sizes and pooling of subjects from the limited geographical area. Larger sample size along with wider geographical area is needed. Further microbiological and immunological investigations are needed to prove the relationship between *C. albicans* and chronic periodontitis.
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