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Detection of Candida Albicans and its Adherence to Epithelial Cells in Periodontal Health and Disease – A Clinicomicrobiological Study

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Abstract:

Objectives

To detect *Candidaalbicans* in periodontal health and disease , to check the effect of SRP on *Candidaalbicans,* invitro to check the adherence of *Candidaalbicans* to epithelial cells.

Materials and methods

60 subjects between 25-55 years categorized into 2 groupsperidontally healthy and chronic periodontitis. The parameters were checked at baseline and after 15 days, plaque samples were collected to check the presence or absence of *Candidaalbicans*. Scaling was carried out in chronic periodontitis group. Plaque samples were collected after 15 days in subjects tested positive for *Candidaalbicans* at baseline and invitro adherence was checked in them.

Results

Candidaalbicans were present in 4 subjects and in vitro study showed adherence Candida albicans to epithelial cells.

Conclusion

Candidaalbicans is present in the chronic periodontitis subjects. There is adherence of *Candidaalbicans* to epithelial cells in chronic periodontitis patients. Futher the role of different species of *Candida* in periodontal disease and in perimplantits, at varying pocket depths, the use of antifungal therapy in unresponsive cases needs to be evaluated.

Keywords:*Candida albicans*; periodontalhealth; periodontaldisease; Periodontitis.

Introduction

Periodontal disease is a chronic inflammation associated with destruction of supportive connective tissue and alveolar bone loss and pocket formation .Chronic periodontitis (CP), atype of periodontal disease can be classified as localized and generalized forms.The disease is associated with a variety of organisms among which the *Porphyromonasgingivalis*, *Aggregatibacteractinomycetemcomitans* and *Tannerella forsythia* are considered the initial colonizers .Other than the organisms ,the host defense mechanism are also important in the pathogenesis and tissue destruction . Chronic Periodontitis commonly occurs in the adults. Various studies provide us with the evidence that periodontal disease has both a high prevalence and severity in the world [1-3] .

Periodontal disease being poly microbial in nature , the role of fungi in this disease has

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received little attention .The fungi colonizing different regions of the human body, including the oral cavity and has been both healthy and diseased individuals [4] . *Candida* is commonly present in the saliva and oral mucosa of around 40% of healthy people [5,6]
Even though candida is presentn healthy people, it is not clear why some patients are infected with *Candida* sp. whereas others are not. Nutrition, bacterial interaction and the presence of specific antibodies in saliva may be few relevant factors [7].*Candida* is considered as a opportunistic pathogen , because the various local and/or general predisposing factors and their alterations which allow them to invade different mucosal tissues [4] . The endocrinal disorders, blood diseases, immunodeficiencies, antibiotic therapy, use of orthodontic appliances and total prosthesis can be considered as the predisposing factors for *Candida* sp. Colonization [8] . The wide use of large spectrum antibiotics, corticosteroids, anti-tumor agents, contraceptives and due to the increase of immunocompromised patients also.imcrease the risk of development of the infections [3] .The virulence factors of the Candida app facilitate colonization and proliferation in the oral mucosa and, possibly, in periodontal pockets. The virulence factors allow the *Candida. albicans*to penentrate the epithelium, inhibiting polymorphonuclear cells and causing lysis of monocytes [1] .Studies conducted have demonstrated the presence of yeasts in periodontal pockets hasbeen described and *Candida albicans*is the speciesmost commonly isolated from the oral cavity [9] . Oral candidiasis is associated with significant morbidity[9] and occurs in.cases of severely immune compromisedpatients ,in whom it leads to the development of invasive diseases [10] .The key role in host defense is played by immune response and the anti-candidal activity oforal epithelium against localized *C.albicans*infection [11] .Oral epithelialcells are considered as the first line of host defenseagainst mucosal *C.albicans*infections. These cellsproduce the pro-inflammatory cytokines and chemokines I'm response to candidal invasion and cell injury [12] .Recent evidence suggest that human oral epithelial cells ,increase induction of IL-8 and IL-1a in cases of *C.albicans*infectionand it was demonstrated that IL-6,IL-6R,IL-6 ST, and NF-IL6genes were expressed at higher levels in HGE cellschallenged with *C.albicans*. According to a study conducted by Urzúa, et al. (2008) they demonstrated. that*C.albicans*can colonize subgingival sites of patients with aggressive and chronic periodontitis [9] .In.another study clinical and salivary parameters of periodontal inflammation were higher in type 2 diabetic patients with oral *C.albicans*colonization[11] . The adjuvant use of the broad spectrum antibiotics can be considered as one the reasons for the presence of *Candida* sp. and the development of opportunistic infections in subgingival sites [10] . The saliva, pH, adhesion, cell surface hydrophobicity, hyphae formation and the expression of specific enzymes are the specific factors affecting the distribution of oral *Candida*[12] .An extremely important virulence factor in fungi colonization and infection of the oral tissues is directly related to their adherence capacity. The stronger adherence to epithelial cells and to a higher pathogenicity can be attributed to a higher phosholipase activity [1]. Co -aggrgation with bacteria in dental biofilm is another feature of these fungal organisms which allows them to adhere to epithelial cells. The invasion of the gingival connective tissue , microbial colonization are made possible through these interaction, that contributes to progression of oral diseases [4,9] .A variety of enzymes are produced by *Candida* spp. , such as the collagenases and proteinases that degrade extracellular matrix proteins, and immunoglobulins [4] .The first step in colonization process is attachment of *Candida* spp. to oral epithelium followed by which local and systemic host defense mechanisms are activated to combat fungal proliferation and infection.
Among the *Candida app, Candida albicans* is the main species responsible for the majority of mycotic infections in the oral cavity [4]. The fungi Candidainconditions of oral health, are usually present on the palatine and vestibular buccalmucosa, on the floor of mouth , tongue and saliva[6]. Studies have reported that the proportion of yeasts in the periodontal pockets is similar to some bacterial periodontopathogens, suggesting a possible role for *Candida* spp. in the pathogenesis of the disease [7].

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The objectives of this study were to:
1. To detect *Candida albicans* in periodontal health.
2. To detect *Candida albicans* in chronic periodontitis.
3. Check the effect of SRP on Candida albicans.
4. To detect the in vitro adherence of *Candidaalbicans*to epithelial cells isolated from periodontally healthy subjects and chronic periodontitis patients.

Material and Methods
A total of sixty subjects visiting the outpatient department, Department of Periodontics and Oral Implantology, S.D.M College of Dental Sciences and Hospital, Dharwad were recruited in this study. An ethical clearance was obtained from the institutional ethical committee and an informed written consent was obtained from all the subjects before their participation in the study.

Study groups
The detailed case history of these selected patients was recorded. The criteria for patient selection are as follows:
Patient Selection criteria
Inclusion criteria:
1. Systemically healthy individuals.
2. Non-smoker.
3. Chronic periodontitis subjects ,if they have periodontal probing depth and /or clinical attachment loss of ≥5 mm.

Exclusion criteria:
1. Subjects who have taken antibiotic, antimycotic and anti-inflammatory drugs in the past 6 months prior to the study.
2. History of periodontal treatment in past 3 months.
3. Use of orthodontic appliance, use of partial and/or fixed prosthesis.
4. Pregnant ladies and lactating women.

Armamentarium For Clinical Usage
1. Pair of examination gloves
2. Mouth mirror
3. UNC -15 periodontal probes
4. A pair of tweezers
5. Ultrasonic scalers
6. A set of standard gracey curettes.
7. 2ml of Phosphate Buffered Saline (PBS)

Armamentarium For Laboratory Usage
1. Sabouraud dextrose agar (SDA) medium
2. Loops
3. Test tubes
4. Bunsen burner
5. Glass slide and cover slip
6. Microscope
7. Incubator
8. Trypan blue dye
9. Gentian violet.
10. Gram's Iodine.
11. Ethyl alcohol.

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12. Safranin.
13. Neubauer chamber
14. Millipore filters.
15. Centrifuge

Methodology

In periodontally healthy and chronic periodontitis subjects supragingival plaque was removed with cotton pellets and the area was isolated using cotton rolls.Subgingival plaque samples were collected by using curette from the gingival sulcus in case of periodontally healthy subjects and deepest periodontal pocket in case of chronic periodontitis subjects .

All chronic periodontits patients underwent scaling and root planing and were recalled after 15 days and the plaque samples were obtained from those patients who had tested positive for *Candida albicans* , to check for the effect of scaling and root planing on *Candida albicans*. The chronic periodontitis patients who tested negative for *Candida albicans* were also recalledand the clinical parameters were recorded.

Subjects of control group who tested positive for *Candida albicans* at baseline were recalled on 15^{th} day the subgingival plaque sample were collected from the gingival sulcus and analysed again for the presence of *Candida albicans*.

The following parameters were recorded both at baseline in both the groups and follow up on 15^{th}day :

1. Gingival index (GI)[13].
2. Plaque index (PI) [13].
3. Probing pocket depth (PPD)[14] – PPD was recorded using UNC -15 probe at baseline and 15 days postoperative.
4. Relative attachment level (RAL)[14] - A stent extending from a fixed reference point to the base of periodontal pocket was recorded in the patients belonging to group2.

Processing of samples for identification of *Candida albicans*:

Each sample was centrifuged and resuspended in PBS and inoculated into Sabouraud dextrose agar plate and incubated at 37^0 C and room temp for 48 hrs the colony was checked after 48 hrs for *Candida albicans*.

A smear was prepared from each sample on a glass slide, dried in air and heat. The smear on a glass slide was covered with few drops of one of the primary stains. Gentian violet is a mixture of methyl violet and crystal violet. The primary stain renders all the bacteria uniformly violet. After a minute of exposure to the staining solution, the slide was washed in water. The smear was treated with few drop of Gram's Iodine and allowed to act for a minute. This results in formation of a dye-iodine complex in the cytoplasm. Gram's iodine serves as a mordant. The slide wass again washed in water and then decolorized in absolute ethyl alcohol . A mixture of acetone-ethyl alcohol (1:1) can also be used for decolorization. The process of decolorization was fairly quick and should not exceed 30 seconds for thin smears. Acetone was a potent decolorizer and when used alone can decolorize the smear in 2-3 seconds. A mixture of ethanol and acetone acts more slowly than pure acetone. Decolorization was the most crucial part of Gram staining and errors can occur here. Prolonged decolorization can lead to over-decolorized smear and a very short decolorization period may lead to under-decolorized smear. After the smear was decolorized, it was washed in water without any delay. The smear was finally treated with few drops of counterstain safranin. After drying the slide it was observed under the microscope for presence of *Candida albicans* which appears as oval shaped violet colored structures.

Obtaining the epithelial cells and In-vitro adherence of *Candida albicans*:

The epithelial cells were obtained from the subjects by scraping the epithelial cells from gingival sulcus in periodontally healthy and periodontal pocket in Chronic periodontitis

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subjects using a curette. The epithelial cells were centrifuged at 3000rpm for 5 minutes, supernatant was discarded and to the test tube 5ml of saline was added and the mixture was vortexed. This method of centrifugation was repeated twice. After the centrifugation the epithelial cells was counted in Neubauer chamber and a suspension containing 105 cells/ml was obtained with the aid of Neubauer chamber. *Candida albicans*, after identification from subgingival plaque samples, was subcultured on SDA ,emulsified in saline and adjusted to macfarland 0.5.After this the *Candida Albicans* was centrifuged at 3000 rpm for 5minutes ,supernatant was discarded ,5ml of saline is added to remaining mixture in test tube vortexed. The centrifugation was repeated twice. Again the *Candida albicans* was adjusted to macfarland 0.5 ml. Next, the suspension of C.*albicans 0.5ml* and epithelial cells 0.5ml was mixed and incubated at 37^0C for 1 hr. C.*albicans*cells that did not adhere to epithelial cells was eliminated using a 12mm isopore membrane. The filter was stained with 50 mm of methylene blue and number of yeasts adheredto 25 epithelial cells was counted.

STATISTICAL ANALYSIS

A total of 60 subjects with 30 subjects in each group were included.
Data was statistically analysed by using the following procedures:
1) Mann Whitney-U- test.
2) Wilcoxon matched pair test.
Statistical significance was set at 0.001.

Results

This clinico-microbiological study was conducted in Department of Periodontics and Oral Implantology S.D.M College of Dental Sciences and Hospital, Dharwadto detect*Candida albicans* and its adherence to epithelial cells in periodontal health and disease. A total of 60 individuals in the age group of 25 to 55 years participated in the study. The participants were categorized into the following two groups:
Group1 (control): Periodontally healthysubjects (n=*30).*
Group2 (test): Chronic periodontitis subjects (n=30).

Study demographics

Of the total 60 subjects who participated in the study 23 were males and 37 were females. Group 1 that is control group had 26.7 % of males and 73.3 % of females .Group 2 that is test group had equal distribution with 50% of males and 50% females. All the subjects fell within the age range of 25 to 55 years with the average age in group 1 (control) being 29.10 years and in group 2 (test group) 41.20 years .There were five parameters assessed at baseline in both the groups including GI ,PI ,PPD ,RAL and *Candida albicans.* GI, PI, PPD ,RAL and *Candida albicans* were assessed again after 15 days following non-surgical periodontal therapy in group 2(test).The group 1 subjects were followed up with reassessment of parameters at end of 15 days without any treatment to observe changes if any.
The results of individual parameters obtained are as presented below:

Plaque index (PI)[13]

Inter- group comparison of PI scores was performed using Mann-Whitney U test at (table 3 ,graph 3). A statistically significant difference was found between the 2 groups at baseline with higher scores with the respect to test as compared to control(table 1 ,graph 1).

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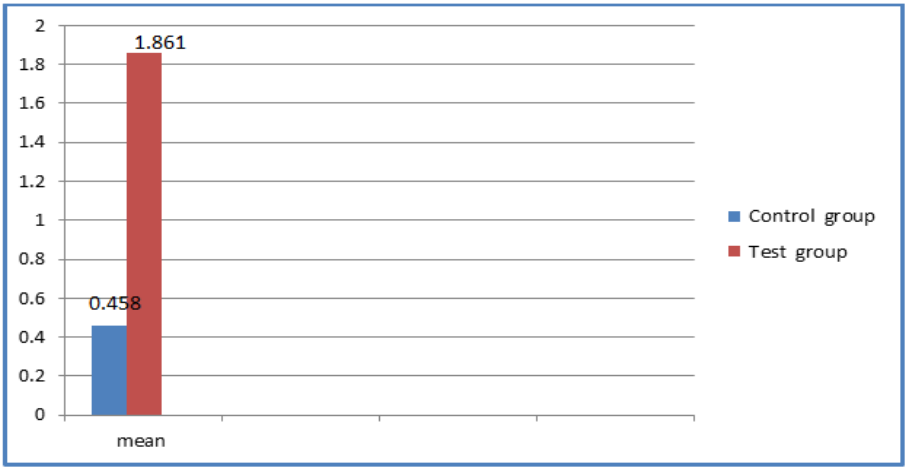
Table 1: Comparison of Plaque index at baseline using Mann-Whitney U test between control and test group.

	Group	N	Mean	Std. Deviation	Mean difference	P-value
Plaque index (PLI)(B)	Control	30	0.458	0.231	1.402	<0.001*
	Test	30	1.861	0.383		

Note: * denotes statistically significant

Graph 1: Comparison of Plaque index at baseline using Mann-Whitney U test between test and control groups



Intra-group comparison was performed using Wilcoxon matched pair t test group (Table 2,graph 2) according to which test group showed a significant decrease in the post treatment values as compared to the baseline values. Intra-group comparison in control group showed no significant difference in the follow up values.

Table 2: Comparison of Plaque index between at baseline and on 15th day after SRP using Wilcoxon Signed rank test in Test group

	Mean	N	Std. Deviation	Mean difference	P-value
Plaque index (PLI)(B)	1.861	30	0.383	0.717	<0.001*
Plaque index (PLI)(15)	1.144	30	0.186		

Note: * denotes statistically significant

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Gingival index (GI)[13]

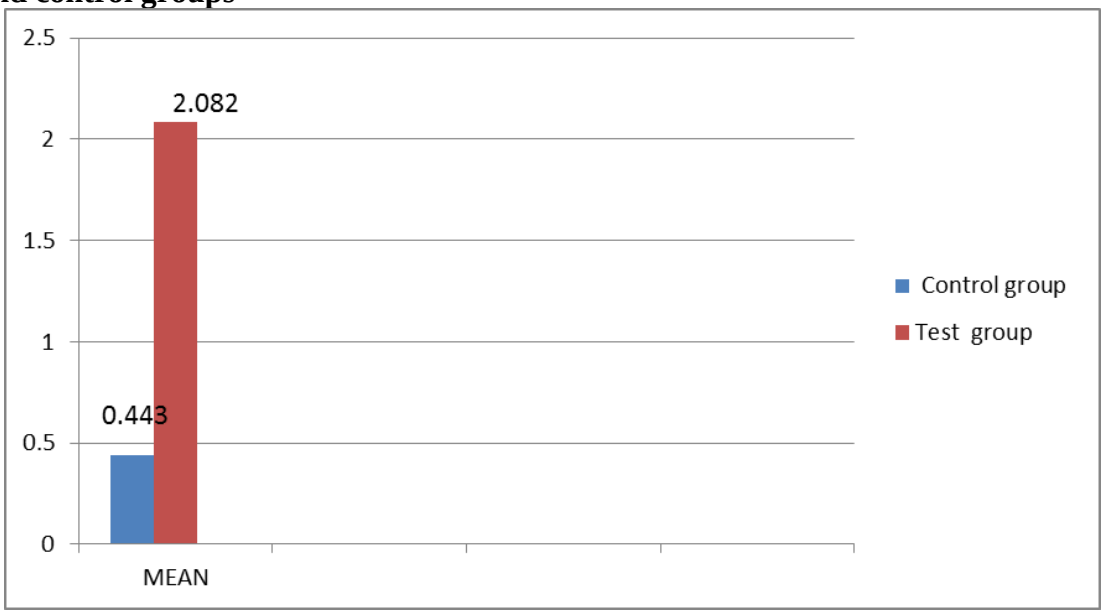
Inter-group comparison of GI scores was performed using Mann –Whitney U test both at baseline (table 3,graph 3). A statistically significant difference at baseline was obtained when test group was compared with control group(table 4,graph 4).

Table 3: Comparison of Gingival index atbaseline using Mann-Whitney U test between control and test group

	Group	N	Mean	Std. Deviation	Mean difference	P-value
Gingival index (GI)(B)	Control	30	0.443	0.339	1.639	<0.001*
	Test	30	2.082	0.481		

Note: * denotes statistically significant

Graph 3: Comparison of Gingival index at baseline using Mann-Whitney U Test between test and control groups



Intra- group comparisons was performed using Wilcoxon signed rank test(table 4,),where in test group showed a significant decrease in post treatment values as compared to the baseline values.Intra-group comparison in control group showed no significant difference in the follow up values(table 10,graph 10)

Table 4: Comparison of Gingival index at baseline and on 15th day after SRP using Wilcoxon Signed rank test in Test group

	Mean	N	Std.Deviation	Mean difference	P-value
Gingival index(GI)(B)	2.082	30	0.481	0.868	<0.001*
Gingival index(GI)(15)	1.214	30	0.275		

Note: * denotes statistically significant

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Probing pocket depth (PPD)[14]

Intra-group comparison of PPD scores was performed using Wilcoxon signed rank test which showed a significant decrease in the post treatment values in test group (Table 5).

Table 5: Comparison of Probing pocket depth at baseline and on 15th day SRP using Wilcoxon Signed rank test in Test group

	Mean	N	Std.Deviation	Meandifference	P-value
Probing pocketdepth(PPD)(B)	6.06	30	0.672	0.562	<0.001*
Probing pocket depth(PPD)(15)	5.498	30	0.603		

Note:*denotes statistically significant

Relative attachment level [14]

Intra-group comparison of RAL scores was performed using Wilcoxon signed rank test which showed a decrease in the post treatment values in the test group but it was not statistically significant(Table 8, graph 8).

***Candida albicans* in health and periodontal disease**

Out of the 30 subjects evaluated 4 samples from chronic periodontitis group (test group) showed the presence of *Candida albicans* at baseline (table 11, graph 11). Out of the 30 subjects evaluated in the control group none of them showed the presence of *Candida albicans* (table 11, graph 11). In the chronic periodontitis group after scaling and root planing ,after 15 days the samples were collected again from those patients in whom the *Candida albicans* were present at baseline and were analysed. After the non-surgical periodontal therapy when the samples were analysed again in group 2(test group) ,there were no presence of *Candida albicans* in those samples.

In vitro adherence of Candida*albicans* to epithelial cells

The *Candida albicans*obtained from the 4 test group were designated as test group 1,test group 2,test group 3 and test group 4.The *Candida albicans* obtained from these 4 samples were sub-cultured on SDA medium and checked for in vitro adherence to epithelial cells obtained from periodontal pocket. Out of the 4 test group samples evaluated for the adherence of *Candida albicans* to epithelial cells ,test group 1 showed the adherence of 42 *Candida albicans* to 25 epithelial cells ,test group 2 showed the adherence of 44 *Candida albicans* to 25 epithelial cells ,test group 3 showed the adherence of 36 *Candida albicans* to 25 epithelial cells and test group 4 showed the adherence of 55 *Candida albicans* to 25 epithelial cells(Table 12).

Discussion

This study was undertaken to detect the presence of *Candida albicans*in periodontally healthy subjects and in subjects with chronic periodontitis and to evaluate the effect of scaling and root planing*on Candida albicans*. An in vitro study evaluated the adherence of the *Candida albicans* isolated from the periodontally healthy subjects and in subjects with chronic periodontitis.

A variety of oral, vaginal, lung and sometimes *systemic infections can be caused by the Candida*. Considered asthe opportunistic pathogens,*Candida* spp cause disease in hosts compromised by

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underlying local or systemic pathological processes [15, 16]. Colonization sites of the Fungal organisms are commonly in the tongue, palate, and buccal mucosa. In.periodontotispts colonization may also occur in subgingival plaque of adults [3]. The saliva, Ph, adhesion, cell.surface hydrophobicity and hyphae formation are the specific factors affecting the distribution of oral *Candida* [17]. The host innate and cell mediated immunity *are well tolerated by the Candida* sp. [18].

In both the healthy and ill individuals the presence of yeasts colonizing different regions of the human body, including the oral cavity has been associated [19,20]. In healthy conditions the *Candida* may be present on the palatine and vestibular buccal mucosa, on the mouth floor, tongue and saliva, but rarely in the subgingiva. The various local and /or general predisposing factors confer *Candida* the capacity to invade different mucosal tissues [21-26].

A majority of mycotic infections in oral cavity are due to the *Candida albicans* is the main species [21,26]. According to the available evidence around 17% of patients with periodontitis, Candida *Spp.*may be isolated from the subgingivalmicrobiota [27] . The proportion of yeasts in the periodontal pockets is similar to some bacterial periodontopathogens, suggesting a possible role for *Candida spp*. in the pathogenesis of the disease[26].The virulence factors expressed by *Candida* may have an role to the pathogenesis of periodontal disease, allowing them to penentrate the epithelium, inhibiting polymorphonuclear cells and causing lysis of monocytes [7]. An.important virulence factor is the adherence is which is required by yeasts to colonize and infect of the oral tissues . The stronger adherence to epithelial cells and to a higher pathogenicity is.related to a.higherphosholipase activity [15].

In spite of the role suggested for *Candida spp*. in periodontitis, it is currently unclear if yeasts participate in the etiology of this disease. The studies conducted till now regarding the yeast have evaluated the *Candida spp*but there is no documented study evaluating the effect of scaling and root planing on the *Candida spp*. Also to the best of our knowledge there is no study evaluating the adherence of the *Candida albicans* isolated from periodontally healthy subjects /periodontal pockets to the epithelial cells obtained from the gingival sulcus / periodontal pockets .

To assess the amount of plaque present plaque index as given bySilness and Loe (1964) [13]was used. At baseline the intergroup comparisons revealed a statistically significant difference when control group was compared with the test group. When the plaque scores were recorded again in the test sample after 15 days after SRP it was seen that there was a significant decrease in the plaque scores when the 15 day score was compared to the baseline scores in the test group, this is similar to the study performed by Isidor et al (1984)[23], Ludovico et al (1990)[24] who demonstrated that there was a significant decrease in plaque index after scaling and root planing .

Gingival index was selected as a method for assessing the severity and quantity of gingival inflammation among the subjects recruited in the study. When the test and control scores were compared with each other a statistically significant difference was found in scores of test and control group at baseline .When the baseline scores of gingival index of the test group was compared with 15 day scores a statistically significant reduction was found .The results of our study are in accordance to the the studies conducted by Isidor et al (1984)[23],Lobene et al (1986)[25], Ludovico et al (1990)[24]they demonstrated that there was a significant reduction in gingival index after scaling and root planing.

From the result of our study it can be interpreted that the significant reduction in the plaque scores and gingival index scores is due to the SRP which was done in the test group at baseline which lead to the decrease in amount of plaque and lead to decrease in the gingival inflammation

The results of our study showed that there was a reduction in probing depth in test group after SRP which is in accordance to the study conducted by Knowles et al (1979)[26] , Shibata Y (1989) [27] Mongardini et al(1999)[28] where there was a decrease in the probing

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depth after scaling and root planing. This reduction in the probing depth can be attributed to the SRP which was done at baseline leading to decrease in plaque accumulation and decrease in gingival inflammation thus reducing the probing [28,29]. Out of 60 plaque samples evaluated 4 test plaque samples were positive for *Candida albicans*. According to the results of our study, the prevalence of *Candidaalbicans* in periodontally healthy group(control group) was 0 % and in chronic periodontitis patients (test group) it was 13.3 % respectively. Studies conducted by Slots et al (1988 [29], Dahlen G -(1993)[30] have demonstrated that *Candida albicans* has been found in the subgingival sites of patients with chronic periodontitis [6] .Chronic periodontitis might have led to a localized compromise in the host immunity in these sites and increased colonization of *Candida albicans*.[45]

According to the studies conducted by Ye et al (2000)[31] and Jarvensivu et al (2004)[7] they have hypothesized in advanced periodontal lesions and the immune suppression caused by severe periodontal disease, the perturbation on epithelial structures seen all facilitate *Candida* subgingival colonization [31,7]. Changes in the subgingivalmicroflora can be produced by deep pockets predisposing a site for periodontal destruction. According to Hajishengallisa G in 2009[32] a deregulation in the local immune response can be created by periodontal pathogens, including *Porphyromonasgingivalis*, which inturn benefit cohabitin organisms colonizing the same subgingival niche .More damage to the underlying periodontal tissues are caused from the metabolites produced by the yeasts. The proteinases secreted by *C. albicans* are capable of degrading major extracellular matrices and basement membrane component. According to Rosa et al (2008)[33] under anearobic conditions some*C. albicans*strains,are recovered from periodontal pockets which enhance the production of some enzymes which suggest that the oxygen concentration in the atmosphere surrounding cells exerts a variable influence on the virulence attributes of *C. albicans*.

According to the results of our study in the chronic periodontitis group (test group) out of the 30 samples evaluated, *Candida albicans* were present in only 4 of the plaque samples . From the results of the our study it can be interpreted that the reason for the presence of *Candida albicans* only in the 4 patients out of the 30 patients evaluated in test group might be due to the presence of severe periodontitis in these 4 patients as compared to the other patients.

Out of the 30 patients evaluated in the test group 4 samples showed the presence of *Candida albicans* at baseline.In these patients the plaque samples were obtained after 15 days of SRP and tested for the presence of *Candida albicans*. The test group samples in whom there was a presence of *Candida albicans* at baseline, when tested again after 15 days of SRP in all the 4 Samples there was absence of *Candida albicans*. This indicates that SRP resulted in the elimination of *Candida albicans* from these sites.

Adherence is considered the first stage of the infection process for *Candida* spp. It is an essential step for the expression of the pathogenic potential and contributes for the persistence of the microorganism in the host, as the ability to adhere avoids the microorganisms of being eliminated.To persist in the oral environment, microorganisms should attach to teeth or mucosa.The continuous flow of gingival crevicular fluid can remove the microorganisms with a las of adherence.

Jarvensivu et al. (2004)[7]from their results concluded that the structure and adherence of periodontal biofilms present on chronic periodontitis was due to *C. Albicans.* They are observed at border of the sulcular epithelium and in connective tissue there was a presence of *Candida* [7]. Thepredominance of hyphae in the samples supports the visual finding of candidal tissue penetration and attachment. These authors also suggested that *C. albicans*may play a role in the infrastructure of periodontal microbiota as well as on adherence of periodontal tissues.

An in-vitro study was conducted after the detection of *Candida albicans* in the test group to evaluate the adherence capacity of the *Candida albicans* to the epithelial cells obtained from the periodontal pockets in test group in subjects whose plaque samples were positive

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for *Candida albicans*. In case of the control group none of the samples tested positive for *Candida albicans.*After the detection of the *Candida albicans* in the test group samples ,they were subcultured in the Sarbarouds dextrose agar medium(SDA) and incubated for 48 hours. After thisthey were checked for the adherence to epithelial cells. The *Candida albicans*obtained from the 4 test group were designated as test group 1,test group 2,test group 3 and test group 4.The *Candida albicans* obtained from these 4 samples were subcultured on SDA medium and checked for in vitro adherence to epithelial cells obtained from periodontal pocket. Out of the 4 test group samples evaluated for the adherence of *Candida albicans* to epithelial cells ,test group 1 showed the adherence of 42 *Candida albicans* to 25 epithelial cells ,test group 2 showed the adherence of 44 *Candida albicans* to 25 epithelial cells ,test group 3 showed the adherence of 36 *Candida albicans* to 25 epithelial cells and test group 4 showed the adherence of 55 *Candida albicans* to 25 epithelial cells. The results of our study are in accordance to the study conducted by Williams R(1999) and Machado et al (2010) who demonstrated that there is more adherence of *Candida albicans* to epithelial cells obtained from diseased sites as compared to the adherence of *Candida albicans* to epithelial cells obtained from healthy sites[10].

Summary and Conclusion

60 subjects visting the Department of Periodontics and Oral Implantology SDMCDSH, Dharwad were recrutited in this study.The 60 subjects were divided into 2 groups of 30 subjects each. Group1 was considered ashealthy subjects and group 2 consisted of chronic periodontitis patients. The objectives of this study were to detect *Candida albicans*in periodontal healthy subjects and in chronic periodontitis patients and to check the effect of SRP on *Candida albicans*. We also conducted a in-vitro study to evaluatethe adherence of *Candida albicans*to epithelial cells isolated from periodontally healthy subjects and chronic periodontitis patients.

The results of the study demonstrated that *Candida albicans* was absent in the control group and was detected in 13.3% of the test group patients. *Candida albicans* wereeliminated in chronic periodontitis patients after 15 days of SRP. *Candida albicans* obtained from test group patients showed adherenceto theepithelial cells.

From the result of the study we would like to conclude that :

1) *Candida albicans*were absentin periodontally healthy subjects.

2) *Candida albicans were* present in 13.3% of chronic periodontitis patients.

3) Scaling and root planingled to theelimination of Candida*albicans* in chronicperiodontits patients.

4) *Candida albicans* showedadherence to the 25epithelial cells obtained fromchronic periodontits subjects.

Hence within the limitations of this study we would like to conclude that *Candida albicans* might play a role in periodontal disease pathology.

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