



Comparing the Average Number of Colonies of Candida Albicans in Periodontal Pockets of Patients with Chronic periodontitis before and after Periodontal Treatment phase I

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ABSTRACT

Chronic periodontitis is an inflammatory, infectious disease of tissues that support the teeth and caused by a particular microorganism or group of specific microorganisms. Periodontitis associated with other condition, such as candidiasis caused different and resistant clinical signs. The aims of this study is finding presence or absence of the fungus *Candida albicans* in patients with periodontal pockets in chronic periodontitis with various grades of slight, moderate and sever before and after periodontal treatment phase I. Patients with chronic periodontitis criteria into three groups of 21 people with slight moderate and sever groups. After sampling the deepest periodontal pockets of patients using paper point No.45. samples of paper points placed in 1 ml sterile saline, centrifuged and then were transferred to medium chrome agar. The green colonies of *Candida albicans* were found in the culture medium were counted for each patient then patients treated by mechanical periodontal treatment phase I, including health education, scaling and root planning. After 6 weeks, the patients re-sampling of deepest periodontal pockets were planted just like before and *Candida albicans* were counted again. The colony before treatment ($p < 0/001$) and after treatment ($p < 0/001$) was associated with disease severity; and disease severity in all three groups, the mean of colonies was significantly reduced after treatment ($p < 0/001$), so that the average number of colonies decreased in patients with sever disease and it was significantly higher than the mean and the median was more than slight ($p < 0/001$). It appears that fungus *Candida albicans* colonies influenced on the severity of the disease and periodontal treatment phase I can be effective in reducing the fungus and decrease was associated with disease severity.

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Introduction

The periodontium is composed of the gingiva, periodontal ligament, root cementum, and alveolar bone. In normal healthy gingivae, the free gingival margin and the tooth surface are in close proximity to each other, leaving very little space for microbial colonization (1). Periodontitis is an infection of the oral gingival tissue that is caused by a combination of microorganisms commonly found in dental plaque, such as streptococci, staphylococci, and many others.(2,3) As periodontitis manifests, the gingival margin becomes enlarged, causing the gingival tissue to detach from the tooth, resulting in the formation of periodontal pockets. As the disease progresses, the depth of these periodontal pockets (probing pocket depth) increases, and bleeding and/or suppuration upon the probing of periodontal tissues also occurs (4). The treatment of periodontitis consists of the mechanical cleaning of teeth and the debridement of the associated diseased tissue, followed by improved dental hygiene. Periodontitis associated with other conditions, such as Candidiasis caused different and resistant clinical signs. (5)Candidiasis is a primary or secondary infection by *Candida* species; including *Candida albicans* mostly. (6)*Candida* species are commensal yeasts and opportunistic pathogens that reside on mucosal surfaces and can cause

oropharyngeal infection albeit usually in immunodeficient individuals, those with severe underlying diseases, and upper denture wearers. (7) The transition of *Candida albicans* from a harmless commensal to a pathogenic organism appears to be dependent on minor changes in predisposing conditions which cause the expression of a variety of virulence factors. These factors include adherence, hyphal formation, thigmotropism, protease secretion, and phenotypic switching phenomenon. (8) *Candida* species have frequently been isolated from periodontal pockets; however, their role, if any, in the etiology of periodontitis remains to be elucidated. (9,10,11,2,12,3) Several previous studies investigated the prevalence and possible role of *Candida* species in periodontitis, all of which identified *C. albicans* as the *Candida* species most frequently isolated from periodontal pockets (13,14,15,16,17,12).The main goal of this study is finding presence or absence of the fungus *Candida albicans* in patients with periodontal pockets in chronic periodontitis with various grades of slight ,moderate and sever before and after periodontal treatment phase I.

Methods and Material

The research design was a clinical trial study. 63 patients with chronic periodontitis with ages between 25-55 were selected from those referred to the department of periodontics at

Islamic Azad University. All consecutive patients who attended the periodontics clinic were recruited according to the following inclusion criteria:

Presence of CAL: slight= 1 or 2 mm CAL, moderate=3 or 4 CAL, and sever = ≥ 5 mm CAL (American Association of periodontology classification) All teeth were examined, using a standard periodontal probe.

Twenty one patients with slight chronic periodontitis, twenty one patients with moderate chronic periodontitis and twenty one patients with severe chronic periodontitis were collected. The exclusion criteria for all groups were, any periodontal treatment in the past 12 month, presence of aggressive periodontitis, pregnancy and lactation, denture wears, and medical condition which could affect the periodontal tissue and presence of yeast, such as HIV and diabetes, chronic pulmonary disease treated by corticoids. NSAID drugs or antibiotic therapy in the past 6 month.

Sample Collection

After careful removal of supra of supra gingival plaque and saliva with sterile gauze and cotton rolls, the teeth were dried and subgingival biofilm samples were acquired using paper point No.45 From the deepest periodontal pockets of each case and removed after 30 s. Then each samples of paper point placed in 1 mL sterile saline, Centrifuged (1000 rpm/5min) and then transferred to medium chrom agar by sampler (50 ML). The plates were incubated at 37 C° for 24-72 h. The green colonies of Candida Albicans were found in the culture medium and to analyze the average number of colonies of Candida albicans in subgingival sites; the number of colony forming unite (CFU) per subject was also determined. Then patients treated by mechanical periodontal treatment phase I, including health education, scaling and root planning. After 6 weeks the patients re-sampling of periodontal pockets were planted just like before and Candida were counted again on plates.

Statistical analysis

The chi-square test was used to analyze the association between disease severity and gender and variance test was used to analyze the association between disease severity and age, and t-paired test was used to find association between subgingival colonization of yeast and the severity of chronic periodontitis before and after periodontal treatment phase I. ANOVA tests were used to compare non-mechanical parametric and parametric data. Differences were considered statistically when $p < 0.001$. SPSS 18 statical program was used for the data analysis.

Results

Sixty three subjects participated in the study, the statistical analysis revealed that there were no differences in the average age among the groups (Variance test $p = 0.12$). Also no significant differences were found among the groups for gender before and after treatment (chi-square test $p = 0.18$). When chronic penodontitis was divided on the basis of severity, statistical differences were observed.

Statistical differences were among the slight chronic periodontitis and moderate chronic periodontitis and sever chronic periodontitis before ($p < 0.001$) and after ($p < 0.001$) treatment. Before treatment the association between subgingival colonization of Candida albicans and the severity of chronic periodontitis was observed.

(Table 1). After treatment the differences among the groups was significantly observed and the average number of colonies of Candida albicans were decreased (t-test $p < 0.001$). (table 1)

Table 1: The average number of colonies of Candida albicans in periodontal pockets before and after periodontal treatment phase I

P-value*	After treatment		Before treatment		Severity of Chronic periodontitis
	Variance	Average	Variance	Average	
0<0/001	2	6/67	2/6	10/57	Slight
0<0/001	3/1	20/48	8/1	35/67	Moderate
0<0/001	7/9	33/95	10/5	67/14	Sever
	0<0/001		0<0/001		P-value**

* T-paired test

** One – way ANOVA test

Discussion

In the present study a statistical association between the subgingival colonization of Candida albicans in the periodontal pockets and the severity of chronic periodontitis before and after mechanical periodontal treatment phase I, was determined. Yeast can be expected in periodontal pockets in dependent of gender and age (11). In the present study, gender and age were well distributed among the three groups. There were no differences among the groups for age and gender before and after treatment. Candida albicans has been found in the subgingival sites of patients with chronic periodontitis (18,19,10), these patients seem to have a greater percentage of yeast colonization than healthy individuals (12). Urzua et al. (12) reported previously that patients with chronic periodontitis had a significantly higher level of colonization with Candida at subgingival sites than periodontally healthy individuals.

Candida albicans is the fungal species most commonly associated with biofilm formation. Pizzo et al. (20) suggested that heterogeneity within subgingival Candida albicans isolates results not only from the spreading of Candida microorganisms from saliva or biofilm, but also from new strain adapting to subgingival pockets and developing different virulence properties. In another study Barros et al. (21) identified a genetically homogenous population of Candida albicans strains in the oral cavities of patients with periodontitis by using random amplified polymorphic DNA (RAPD).

It appears that the fungus Candida albicans influenced on the severity of the disease and mechanical periodontal treatment phase I can be effective in reducing the fungus and this decrease was associated with disease severity. Possibly Candida albicans has a role in the immune evasion of the plaque microorganisms and its adherence to the periodontal tissues, because it has been typically found on the outer layers of plaque and has been seen deep in periodontal tissues.

Another study by ArdiLa et al. (22) revealed that presence of Candida albicans in subgingival plaque in patients with periodontitis, cause clinical sign and symptoms. When yeast gain access to underlying periodontal tissues, more damage may result from the metabolites produced by them. On the other hand deep pockets can produce a change in the balance of the subgingival microflora predisposing a site for periodontal destruction.

Conclusion

Candida albicans is more likely to be present in periodontal pockets of patients with chronic periodontitis than in healthy individuals and it seem the strong association between subgingival colonization of Candida albicans and the severity of chronic periodontitis.

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