PREVALENCE OF CANDIDA ALBICANS IN CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

BACKGROUND

Chronic periodontitis is defined as "an infectious disease resulting in inflammation within the supporting tissues of the teeth and progressive attachment loss and bone loss." Chronic periodontitis is associated with a widely diverse and complex subgingival microbiota 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, Candida glabrata, Candida tropicalis, Candida krusei, and Candida dubliniensis. Most of these strains are commensals and some are potential opportunistic pathogens.

The objective of the study is to examine the prevalence of *Candida albicans* (dimorphic fungus) in subgingival plaque samples of patients with chronic periodontitis.

MATERIALS AND METHODS

It's a cross-sectional study of 108 chronic periodontitis patients, their age ranged from 30 to 55 years. Clinical parameters like plaque index (PlI), probing depth (PD), and clinical attachment level (CAL) were measured. We also observed the smokers from the analysed patients. Suitable microbiological media was used to culture *C. albicans* from clinical plaque samples. Data analysed with suitable statistical methods.

RESULTS

Clinical parameters of PlI, PD, and CAL, disease severity and sex revealed no significant relation between *C. albicans* and periodontitis patients, while smoking of individuals significantly correlated with the presence of the *C. albicans*.

CONCLUSION

Although *C. albicans* infection occurred in chronic periodontitis patients, smokers were at higher risk for *C. albicans* infection than non-smokers.

KEYWORDS

C. albicans, Chronic Periodontitis, Smoking, Sex.

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BACKGROUND

Chronic periodontitis is defined as "an infectious disease resulting in inflammation within the supporting tissues of the teeth and progressive attachment loss and bone loss".[1] Chronic periodontitis is associated with a widely diverse and complex subgingival microbiota 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.[2] Plaque also comprises of fungal species like *Candida albicans, Candida glabrata*, *Candida tropicalis*, *Candida krusei*, and *Candida dubliniensis.* Most of these strains are commensals and some are potential opportunistic pathogens.

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Candida species are commensal yeasts and opportunistic pathogens that reside on the mucosal surfaces and can cause oropharyngeal infections. It occurs usually in the immunocompromised individuals with endocrinal disorders, blood diseases and with longterm use of broad spectrum antibiotic therapy.[3] The possible relevant factors for *Candida* species colonisation are nutrition, bacterial interaction and the presence of specific antibodies like IgA and IgG in saliva.[4] In healthy oral carriers, *Candida* species typically resides on the tongue, palate, buccal mucosa and in the saliva.[5]

C. albicans is the most prevalent yeast of oral microbiota. 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. [4]

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.[6] At the subgingival sites, there was an increase in colonisation with

Candida species in chronic periodontitis and aggressive periodontitis than the subjects with healthy periodontium.[7]

The *Candida* species have virulence factors that facilitate colonisation and proliferation in the oral mucosa and possibly in periodontal pockets. These fungal organisms can co-aggregate 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 colonisation that contributes to progression of oral diseases.[8]

The objective of the present study was to examine the prevalence of *Candida albicans* among chronic periodontitis patients and to assess the relationship between smoking and *C. albicans* infection.

MATERIALS AND METHODS

It's a prevalence study for a period of three months, the daily outpatients of Sri Ramakrishna Dental College & Hospital, Coimbatore, Tamil Nadu state, India having chronic periodontitis were included in this study. All patients were carefully analysed, and specific points related to study were noted. The study was approved by the ethical committee of the institution. The inclusion criteria include age group between 30 and 55 years. Patients who had not received any dental treatment for the past 6 months, without any systemic complications and patients with chronic periodontitis, and any systemic and immunocompromised diseases receiving any drugs for the past 6 months were excluded from this investigation.

On each clinical examination, the following clinical parameters were calculated by Plaque Index (PlI), Conventional probing depth (PD) and Clinical Attachment Loss (CAL). Subgingival plaque samples were collected from each patient with the help of a sterile Gracey curette either in buccal aspects of molar teeth or one more teeth in the deepest pockets**.** Collected samples were 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*. 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. Yeast colonies growing on each Sabouraud's Dextrose Agar were re-suspended and 10 µL of suspension solution was used to inoculate plates with CHROM agar medium. Inoculated plates were 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.

Samples were Gram stained, which indicate Gram-positive budding yeast cells with pseudohyphae, and Gram-negative pus cells and bacilli. Small portion of an isolated colony was suspended in a test tube containing 0.5 mL of human serum then incubated at 37ºC for 2 hours, then examined microscopically at 30-minute intervals up to 2 hours for the presence of germ tube confirmatory test for *C. albicans*.

Study Design

Cross-sectional Study.

Statistical Analysis

Chi-square test done by the Software SPSS Version 13.

RESULTS

A total of 108 patients were analysed, which include 69 males and 39 females. The age group of the patients ranged between 30 and 55 years, the average was 42.9 (Table 1). The CAL measured from the CEJ to the base of the periodontal pocket using Williams periodontal probe, analysed as mild, moderate and severe chronic periodontitis based on the CAL. Among the study subjects, males had CAL with 4.5 ± 1.1 mm whereas females had 4.4 ± 1.1 mm. Among 69 males analysed, 5 patients had mild, 26 patients had moderate and 38 patients had severe chronic periodontitis. In females, 2 patients with mild, 18 patients with moderate and 19 patients with severe chronic periodontitis were observed (Table 2, Figure 1). Comparing the severity of diseases among the sexes showed no statistical significant differences. (Table 3, Figure 2)

In the present study, when correlating the sexes with severity of chronic periodontitis and *C. albicans,* none of the male and female 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* whereas severe chronic periodontitis with 9 males (23%) and 4 females (21%) were positive for *C. albicans.* The results showed no significant differences between sexes and severity of disease to the presence of *C. albicans* (Table 4, Figure 3).

The comparison of smokers and nonsmokers to the exposure of *C. albicans* infection- none of the subjects with mild chronic periodontitis tested positive for *C. albicans*. Among the smokers, 3 subjects (33.3%) with moderate chronic periodontitis and 6 subjects (66%) with severe chronic periodontitis tested positive for *C. albicans*. Among nonsmokers, 4 subjects (36.3%) with moderate chronic periodontitis and 7 subjects (63%) with severe chronic periodontitis tested positive for *C. albicans*. The result revealed that there is no statistically significant difference (p =0.888) between smoking and *C. albicans* positive individuals with severity of chronic periodontitis (Table 5, Figure 4).

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

Table 1. Age Characteristics of the Sample

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 2. Clinical Attachment Level with Severity of Chronic Periodontitis

Chronic Perio-dontitis	Sex				Total		p Value
	Male		Female				
	Count	%	Count	%	Count	%	
Mild	5	7.20%	2	5.10%	7	6.50%	0.670
Mode rate	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**	

Table 3. Severity of Chronic Periodontitis- Gender wise

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

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%	

Table 4. Severity of Chronic Periodontitis and Candida albicans Positive Cases- Gender wise

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

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

Table 5. Smoking Habits of Candida albicans Positive Cases with Chronic Periodontitis

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

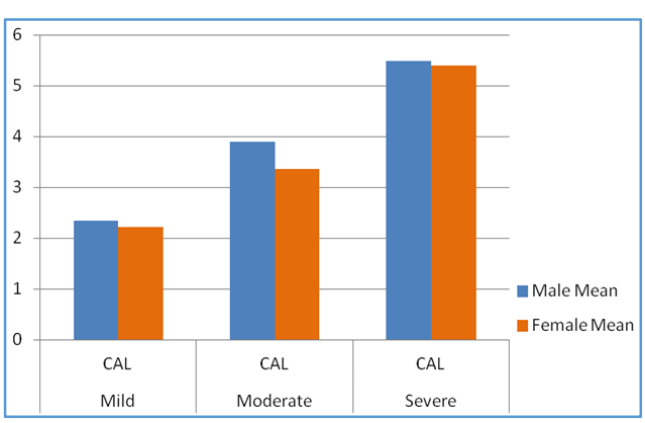


Figure 1. Clinical Attachment Level with Severity of Chronic Periodontitis

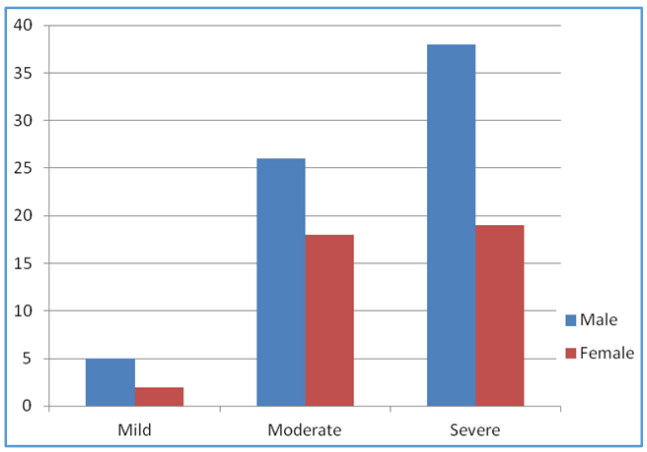
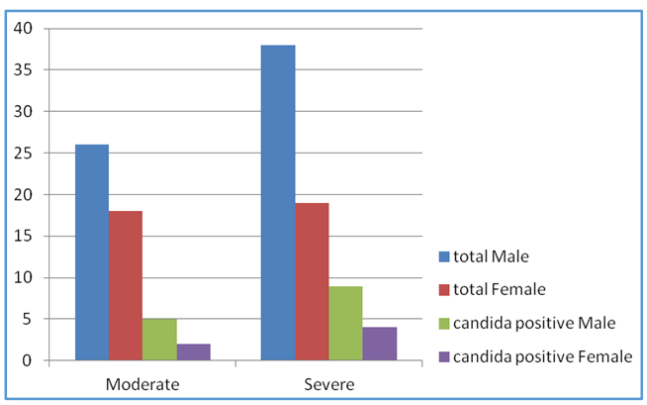


Figure 2. Severity of Chronic Periodontitis- Gender wise



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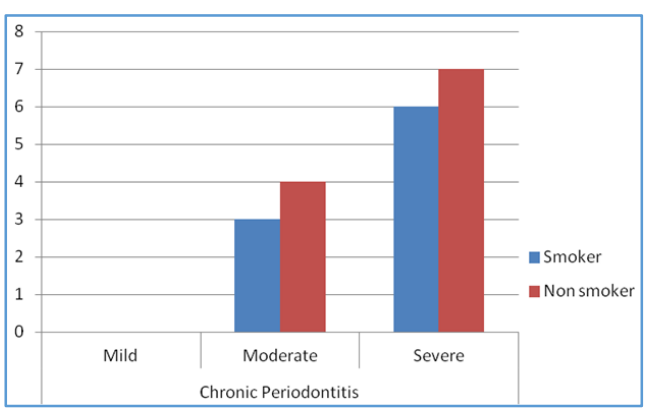

Figure 4. Smoking Habits of Candida albicans Positive Cases with Chronic Periodontitis

DISCUSSION

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.[9] *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.[10] Severe periodontal disease may be one of the causes for immunosuppression, which leads to colonisation of this opportunistic pathogen.[9] 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 favourable environment for the germination and growth of these hyphae.[9] *C. albicans* can secrete proteinases capable of degrading major extracellular matrices and basement membrane components that cause destructive inflammation of the underlying periodontal tissues.[11,12,13]

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 CHROM agar media were used to culture *C. albicans* from clinical samples in order to exhibit characteristic colony colours 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.[14] In the current study, CAL for mild, moderate and severe chronic periodontitis for male subjects was 2.348 ± 0.64, 3.895 ± 0.876, and 5.486 ± 0.422 respectively and for female subjects 2.221 ± 0.566, 3.356 ± 0.942 and 5.433 ± 0.141 respectively. In the present study, the prevalence of *C. albicans* was 18.5%. This was in accordance with studies done by Reynaud et al.[15] in Norway who demonstrated prevalence of 17.5% in 128 subjects that were studied and Canbarro et al[10] who reported a prevalence of 17% in the Brazilian population. Among 20 subjects who were positive for *C. albicans,* 14 (20.3%) were male and 6 (15.4%) were female, which showed that the prevalence of *C. albicans* did not show any statistically significant (p=0.528) difference between males and females. This result was in accordance with the studies done by Slots et al.[16]

In the present study, of the 7 subjects diagnosed with mild chronic periodontitis, none were positive for *C. albicans.* 14% of the subjects with moderate chronic periodontitis and 22% of the subjects with severe chronic periodontitis were *C. albicans* positive. This result was in contrast to the study done by Canbarro et al[10] in Brazilian population, where *C. albicans* was present in 47% subjects with moderate chronic periodontitis though similar results were found in 17% of the *C. albicans* positive patients with severe chronic periodontitis. These differences 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, *C. albicans* was significantly higher in smokers (31%) than in the non-smokers (13.0%) with p=0.042, showing that tobacco smoking increases the prevalence of *C. albicans*. This result was in accordance with the study done by Keten et al.[17] in Turkey which showed that 30% of the study samples were *C. albicans* positive in smokers compared to 18.3% in nonsmokers.

However, other studies have found that tobacco smoking did not have an influence on oral colonisation with *C. albicans.* Oliver & Shillitoe[18] demonstrated that *C. albicans* was prevalent in 35% of the smokers and in 35% of the nonsmokers. Darwazeh et al.[19] isolated *C. albicans* from 84% of the smokers and 74% of the nonsmokers and they found no significant association between smoking habits and *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 nonspecific immune defences.[19]

To the best of our knowledge, this is the first study to elaborate the prevalence of *C. albicans*, in mild, moderate and severe chronic periodontitis among smokers and nonsmokers. 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 nonsmokers, who tested positive for *C. albicans,* 4 subjects had moderate chronic periodontitis (36.3%) and 7 subjects had severe chronic periodontitis (63.6%).

As the sample size was small in this study, the statistical validity of the associations found was limited. Hence, large sample sizes are required to confirm the clinical findings, and further qualitative and quantitative analysis should be done to validate the results of this study.

CONCLUSION

C. albicans was present in higher amounts in the periodontal pockets of patients with severe chronic periodontitis. Smokers were at higher risk for *C. albicans* infection than non-smokers. *C. albicans* did not have a predilection for any particular sex.

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