ABSTRACT

Aims: To determine the distribution of subgingival occurrence of Candida species in chronic periodontitis and peri-implantitis.

Materials and methods: A total of 20 patients with chronic periodontitis and 20 patients with peri-implantitis were evaluated. Periodontal parameters that include mean periodontal or peri-implant pocket depth, mean clinical attachment level or relative attachment level, mean gingival index, and mean plaque index were evaluated. Pooled subgingival sample from the deepest pockets using sterile paper points and sterile curettes was obtained from each patient and immediately streaked on to Sabouraud’s dextrose agar. Species identification was done by colony color on Chrom agar medium and Dalmau plate culture technique on corn meal agar.

Results: In periodontitis, the prevalence of Candida species in periodontal pockets was 26.8%, while in peri-implantitis, it is 27.2%. Comparing the prevalence of various sub-species of Candida between the two groups, we did not find any significant statistical differences. Also, there were no significant statistical differences between the two groups in terms of mean plaque index, mean gingival index, and mean probing depth, and mean CAL (p>0.05).

Conclusion: Candida albicans was the most common Candida species isolated from both the groups. It is followed by Candida dubliniensis, Candida krusei, Candida tropicalis, Candida glabrata, and Candida parapsilosis. No significant statistical difference was detected in the Candida count between the two groups.

Keywords: Candida albicans, Candida krusei, Chronic periodontitis, Microbiological, Peri-implantitis.


Source of support: Nil

Conflict of interest: None

INTRODUCTION

Bacterial plaque has been established as the primary etiological factor in the initiation and progression of periodontal disease. The plaque carries a plethora of microorganisms, and human knowledge is limited to just a fraction of the pathological species. One of such lesser-known species is the Candida species, which are yeasts and found inside the oral cavity. Candida albicans is the most frequently isolated bacteria of the Candida species. There is clear evidence that C. albicans adheres to oral surfaces including acrylic dentures and mucosa. However, Candida species have also been isolated from subgingival sites. In that milieu, it may act directly, or in concert with subgingival bacterial pathogens, or as a cofactor by inducing the production of proinflammatory cytokines to increase the occurrence of periodontal attachment loss. Recently, Candida species has been isolated from subgingival sites of women with chronic periodontitis and who were on hormonal contraceptives.

Besides, several studies showed that occasionally Candida species, Staphylococcus species and Enterococcus species are a part of the peri-implant flora in the infected peri-implant sites. Leonhardt et al have demonstrated that Candida species was associated with 55% of failing implant sites. Thus, it becomes essential to study the distribution of various Candida species in both chronic periodontitis and peri-implantitis. Hence, the aim of this study is to determine the distribution of subgingival occurrence of Candida species in chronic periodontitis and peri-implantitis.

MATERIALS AND METHODS

A cross-sectional study was conducted for a period of 12 months. Patients attending MS Ramaiah Dental College and Hospital were included in this study. The sample size was divided into two groups: Group I – patients with chronic periodontitis: 15 patients, and group II – patients with peri-implantitis: 15 patients. To be eligible for the study, the patient had to present the following inclusion criteria: Group I – (1) chronic periodontitis according to Tonetti and Claffey (presence of proximal attachment loss of 5 mm or more in 30% or more of the teeth present), group II – patients with one implant site...
testing positive for plaque (bleeding on probing, probing depth ≥5 mm, and bone loss were termed as having an “inflammation at the implant site”). The exclusion criteria were as follows: (1) Subjects with metabolic or systemic disorders, such as diabetes, epilepsy, hypertension, or metabolic syndrome, (2) subjects who have had antibiotic therapy in the past 6 months, (3) subjects who are lactating. The study protocol was approved by the institution’s Ethics Committee.

Method of Collecting Data

A detailed questionnaire regarding the full medical history was collected from each patient before clinical examination. All patients were examined by the same examiner.

Evaluation of Clinical Parameters

Plaque and gingival inflammation were measured for each site using the indices proposed by Silness and Löe and Löe and Silness respectively. The data for these measurements were presented as the percentage of sites for each patient exhibiting plaque (i.e., plaque index ≥1 and ≥2) and gingival inflammation (gingival index ≥1 and ≥2). A comprehensive periodontal examination including probing depths (six sites per tooth) and number of teeth was evaluated. Clinical attachment levels were calculated using the cementoenamel junction or margin of the crown as the reference landmark (Fig. 1A). For peri-implantitis, the relative attachment level was calculated by measuring from the implant shoulder to the base of the pocket.

Specimen Collection

Each subject provided a pooled subgingival sample from the deepest pockets using a minimum of four and a maximum of eight sterile paper points per patient. After supragingival scaling, paper points were inserted into the gingival sulcus/pockets for 60 seconds and placed thereafter in peptone water for transport. As per Portela et al., subgingival plaque samples which were obtained using sterile paper points were inoculated in Sabouraud’s dextrose agar (Fig. 1B). From the same subjects, as per Jabra-Rizk, subgingival samples were also collected from periodontal sites with a sterile curette (Fig. 1C). Subgingival samples were immediately streaked onto Sabouraud’s dextrose agar plates (Fig. 1D).

Specimen Identification and Speciation

Sabouraud’s agar plates were incubated at 35 to 37°C, for 48 to 72 h and checked daily for growth (Fig. 2A). Candida
species were identified by colony character and gram stain. Germ tube test was done to speciate C. albicans and non-C. albicans species. Species identification was done by colony color on Chrom agar medium (Fig. 2B) and Dalmau plate culture technique on corn meal agar.

Results were confirmed by carbohydrate fermentation and assimilation tests. Gram staining was done and all the germ tube positives were subjected to growth at 42°C to differentiate between C. albicans and Candida dubliniensis as C. dubliniensis does not grow at 42°C (Figs 3A and B).

Statistical Analysis

The means of all the sites were calculated for each individual, for all the outcome variables. The following methods of statistical analysis have been used in this study. The results for each parameter (numbers and percentages) for discrete data and averaged data (mean ± standard deviation) for each parameter were calculated.

Proportions were compared using chi-square test of significance. Student’s t test was used to determine whether there was a statistical difference between the groups in the parameters measured. Statistical analysis was performed per protocol, with the program Statistical Package for the Social Sciences (SPSS) for Windows (version 10.0). A level of significance of 5% was used in all the statistical tests.

RESULTS

Subjects were recruited from June 2012 to May 2013. The mean age group of patients in the peri-implantitis and chronic periodontitis groups was 30.12 and 36.71 years respectively. Among the chronic periodontitis group and the peri-implantitis group, the prevalence of Candida species in periodontal pockets was 26.8 and 27.2% respectively, while 73.2% in the chronic periodontitis group and 70.7% in the peri-implantitis group were culture negative (Graph 1).

Among the chronic periodontitis group, 14.6% of the subjects were culture positive for C. albicans, 2.4% for Candida tropicalis, 4.9% for Candida krusei, 4.9% for C. dubliniensis, 0% for Candida glabrata, 0% for Candida parapsilosis. Among the peri-implantitis group, 19.5%
of the subjects were culture positive for *C. albicans*, 4.9% for *C. tropicalis*, 2.4% for *C. parapsilosis*, 2.4% for *C. glabrata*, 0% for *C. krusei*, 0% for *C. dubliniensis* (Graph 2). The distribution of *Candida* subspecies in both the groups was compared and it was found to be statistically nonsignificant (*p* = 0.356). The distribution of the *Candida* species among the *Candida* positive subjects is shown in Graph 3.

**DISCUSSION**

In the oral cavity, yeasts commonly colonize the tongue, palate, and buccal mucosa. Yeasts, especially *C. albicans*, have been recovered from periodontal pockets in a large number of patients with chronic periodontitis, demonstrating
prevalence rates of 71 to 44.4%. In this study, we have shown a prevalence rate of 28.0%, which is in accordance with various studies published in the literature.

A recent study is in accordance with our study showing a prevalence rate of 30%. Twelve patients (30%) with chronic periodontitis presented yeasts in the subgingival biofilm, while only 3 patients (15%) in the healthy subjects group were positive for these microorganisms. Although several yeast species were found, such as C. parapsilosis, Rhodotorula sp., C. dubliniensis, and C. tropicalis, only C. albicans was present in all the patients with yeast-positive chronic periodontitis. Another study showed a prevalence rate of 34.3% from the periodontal pockets of marginal periodontitis patients and 42.2% of the healthy subjects. C. albicans, C. dubliniensis, and C. parapsilosis and 19 biotypes were identified from the marginal periodontitis patients.

A recent study evaluated the prevalence of Candida species in subgingival sites of women using oral contraceptives. The prevalence of Candida colonization was 95.1% in the oral contraceptive (OC) group and 78.4% in the control group. The reason for the high percentage of Candida colonization which the authors quoted was unknown but it may be linked to gender and dietary factors. This high prevalence rate is contrary to our study.

Several yeast species have been isolated from periodontal pockets in patients with periodontitis. C. albicans was the species most frequently isolated but other species including C. dubliniensis, C. glabrata, C. parapsilosis, C. tropicalis, C. lipolytica, and C. guilliermondii have been isolated. Other species, such as Saccharomyces cerevisiae, Trichosporon mucoides, and Rhodotorula spp. have also been identified.

Only one study has demonstrated the presence of C. krusei isolated from periodontal pockets previously in the literature. To our knowledge, this is the second study demonstrating C. krusei from periodontal pockets.

Candida dubliniensis is a recently discovered yeast species principally associated with carriage and disease in the oral cavities of human immunodeficiency virus-infected individuals. To date, the majority of isolates of this species have been identified in Europe and North America. The authors have isolated this species in the sample population of healthy individuals. Isolation of C. dubliniensis from healthy individuals has been demonstrated in many studies.

The association between Candida and chronic periodontitis is controversial. Although different Candida species have been isolated from periodontal pockets of patients with periodontitis and hyphae have been found to invade the periodontal connective tissue, the absolute proof implicating Candida in the pathogenesis of periodontitis is still lacking. A recent study demonstrated that in most cases Candida isolates were accompanied by well-known periodontopathogens, such as P. intermedia, P. gingivalis, and A. actinomycetemcomitans. In those cases, it seems likely that the bacteria coisolated with Candida were the putative agents responsible for the development of periodontitis. No, such association regarding the severity of chronic periodontitis and the association of any Candida species was noted in our study.

The microbiota of peri-implantitis has been demonstrated in many studies to resemble the microbiota of chronic periodontitis. Our study also demonstrates a similar scenario with regard to the distribution of Candida species. Various analytical methods have found that the microbiota associated with peri-implant disease is mixed, somewhat variable, and, in most cases, dominated by diverse Gram-negative anaerobic bacteria, as is the case with chronic periodontal disease. A recent study has demonstrated the presence of Candida species in peri-implantitis sites with a prevalence rate of ~18.5% among the healthy individuals. But, it is unclear if the amount of oral Candida colonization affects the microflora of the peri-implant sulcus. Leonhardt et al detected Candida species only sporadically in the supragingival plaque of peri-implant lesions, preferentially in partial edentulous patients and none in healthy subjects. Alcoforado et al and Listgarten and Lai described the finding of C. albicans in the peri-implant sulcus of failing implants. It is unclear if the numbers of Candida species increase in the peri-implant sulcus after antibiotic therapy of peri-implant infections. But it is to be expected that a change of the microflora of the sulcus happens, from which Candida species benefits. Further long-term studies are needed to confirm the cause-effect relationship of Candida and peri-implantitis.

CONCLUSION

We found that C. albicans was the most common Candida species isolated from both groups, followed by C. dubliniensis, C. krusei, C. tropicalis, C. glabrata, and C. parapsilosis. No statistically significant difference in the Candida count between the groups was found.

The limitation of the study is that we have considered the sample collection at cross-sectional level and hence further studies should be conducted with a larger sample size and longitudinal study design to conclude definitively. While it is premature to make definitive statements regarding a protective cause-and-effect relationship between Candida and periodontal conditions due to the cross-sectional nature of the data, these findings suggest an important reexamination of the connection between various microbiological factors and periodontal diseases. Further studies are needed to clarify the role of Candida in periodontal disease and to determine the extent to which
oral health behaviors affect the initiation or progression of periodontal disease. Nevertheless, the dentist should always keep in mind that besides the well-known putative periodontal pathogens, there are an array of microorganisms that are not yet known which might have a possible role in chronic periodontitis and peri-implantitis. Probing into this issue with a wider perspective will offer a better approach toward the diagnosis and treatment planning of chronic periodontitis and peri-implantitis.

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REFERENCES