Journal of
PERIODONTAL RESEARCH

J Periodont Res 2013; 48: 428–432

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/jre.12022

Association of subgingival colonization of *Candida albicans* and other yeasts with severity of chronic periodontitis

A. Canabarro[1,2], C. Valle[1], M. R. Farias[1], F. B. Santos[3], M. Lazera[3], B. Wanke[3]

[1]Department of Periodontology, Universidade Veiga de Almeida, Rio de Janeiro, Brazil, [2]Department of Periodontology, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil and [3]Laboratory of Mycology, Instituto de Pesquisa Clinica Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Canabaro A, Valle C, Farias M R, Santos F B, Lazera M, Wanke B. Association of subgingival colonization of Candida albicans *and other yeasts with severity of chronic periodontitis. J Periodont Res 2013; 48: 428–432. © 2012 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd*

Background and Objective: The aim of this study is to analyze the relationship between the subgingival colonization by *Candida albicans* and other yeasts with the severity of chronic periodontitis (CP).

Material and Methods: After sample size calculation, 40 patients with CP and 20 healthy subjects (HS) were included in the study. Cases of slight-moderate (MCP, $n = 23$) and severe CP (SCP, $n = 17$) were defined according to the Centers for Disease Control/American Association of Periodontology classification. Subgingival samples were acquired using sterile paper-points from the sulcus or the deepest periodontal pocket of each healthy and subject with CP, respectively, and were cultured aerobically on three selective media. Yeast colonies that grew on the surface of plates were later identified by biochemical reactions. Statistical tests were used to analyze the association between subgingival yeast colonization (number of yeast-positive individuals and colony forming units (CFU) per subject) and periodontal disease status, considering statistical significance when $P < 0.05$.

Results: Although several yeast species were found (*C. parapisilosis*, *Rhodotorula* sp., *C. dubliniensis* and *C. tropicalis*), only *C. albicans* was present in all the patients with yeast-positive CP. Twelve patients (30%) with CP presented yeasts in the subgingival biofilm while only three patients (15%) in the HS group were positive for these microorganisms. No statistical difference was found between the CP and HS groups ($P = 0.084$). However, when the CP group was divided on the basis of severity, statistical differences were observed between the SCP and MCP groups (47% vs. 17%, $P = 0.043$), and between the SCP and HS groups (47% vs. 15%, $P = 0.033$). No statistical difference was observed between the MCP and HS groups (17% vs. 15%, $P = 0.832$). High densities of yeasts were found only in patients with MCP and SCP (mean and range 61.25 (0–100) CFU/plate and mean and range 51 (0–101) CFU/plate, respectively).

Conclusion: In this group of patients, subgingival colonization of some yeasts, especially *C. albicans*, was associated with the severity of CP.

Prof. Antonio Canabarro, Universidade Veiga de Almeida. R. Ibituruna, 108, casa 3, sala 201-Rio de Janeiro-RJ 20271-020, Brazil
Tel: +55 021 25748871
Fax: +55 021 25748871
e-mail: andradejr13@gmail.com

Key words: *Candida albicans*; chronic periodontitis; severity of periodontitis; subgingival yeasts

Accepted for publication September 11, 2012

Periodontitis promotes irreversible destruction of soft and hard periodontal tissues, resulting in the formation of periodontal pockets (1). The World Health Organization (WHO) reported that severe periodontitis affects approximately 10–15% of adults worldwide (2). In dentate US adults, the prevalence of moderate clinical attachment loss (CAL), i.e. $\geq$ 3 mm was 53.1% (3). Prevalence of chronic periodontitis (CP) in adolescents and young adults in south Brazil ranges between 18.2% and 72.0%, respectively (4). Severe loss of attachment, which is confined to a minority of the population, is usually evident in only a few sites (5) but increases with age (6). However, there is usually a loss of many teeth, if the disease is left untreated (7), especially in those with advanced (severe) periodontitis (5).

Despite the strong association of the so-called "Red Complex" periopathogens with periodontitis (8), especially in the severe form of periodontitis (9), periodontal pockets can harbor a great variety of microorganisms, including yeasts (10). The presence of enteric *Staphylococcus aureus* and *Candida* sp. should be expected, especially in patients with systemic disorders, i.e. diabetes mellitus, neutropenia, agranulocytosis and AIDS (11).

In the oral cavity, yeasts are not only found on mucosal surfaces and in saliva but also in periodontal pockets (10), suggesting that they may be involved in the pathogenesis of CP. However, the presence of *Candida albicans*, the most prevalent yeast in the mouth (12) in subgingival sites of "healthy" patients has been little described (13–15), and the presence of *C. albicans* in a subgingival biofilm has not been associated with the severity of periodontal disease until now (13,14).

Thus, considering the pathogenic potential of *C. albicans*, the aim of this study is to analyze the relationship between subgingival colonization of *C. albicans* and other yeast species with the severity of CP. The hypothesis of this study is that the presence of severe CP (SCP) increases the odds of having subgingival yeasts, especially *C. albicans*.

Material and methods

Patients

The research design was a case–control study. Patients with CP (cases) and healthy subjects (HS) were selected from those referred to the periodontic and general dentistry clinics, respectively, at Veiga de Almeida University (UVA) in Rio de Janeiro, Brazil, after sample size calculation.

All consecutive patients who attended the periodontic clinic were recruited according to the following inclusion criteria: presence of CAL: slight = 1 or 2 mm CAL, moderate = 3 or 4 mm CAL, and severe = $\geq$ 5 mm CAL, Centers for Disease Control/American Association of Periodontology classification (16). CAL must be present in at least two proximal sites of two non-adjacent teeth (17). All teeth were examined using a standard periodontal probe (North Carolina periodontal probe, Hu-Friedy, Chicago, IL, USA) by a calibrated examiner. The calibration was performed in eight patients who were not included in the study. The data for full-mouth examination included CAL, probing depth and bleeding on probing. All measurements were taken twice (repeated by the examiner within 2 h to assess intra-examiner differences), in six sites per tooth on all teeth, except third molars. The intraclass correlation coefficients for the intra-examiner analysis were 0.82, 0.84 and 0.97 for CAL, probing depth and bleeding on probing, respectively.

The inclusion criterion for the HS group was the absence of proximal CAL.

The exclusion criteria for both groups were: any periodontal treatment in the past 12 mo; presence of aggressive periodontitis; pregnancy and lactation; denture wearers; and medical condition, which could affect the periodontal tissue and presence of yeasts, such as HIV and diabetes, chronic pulmonary disease treated by inhaled corticoids, non-steroidal anti-inflammatory drugs or antibiotic therapy in the past 6 mo.

The study was approved by the Ethics Committee on Research Involving Humans of Fundação Oswaldo Cruz, Rio de Janeiro, Brazil (protocol number 0020.0.325.009–08), and the subjects were enrolled into the study in compliance with the Helsinki Agreement.

Sample collection

After careful removal of supragingival plaque and saliva with sterile gauze and relative isolation with cotton rolls, the teeth were dried and subgingival biofilm samples were acquired using three size 45 sterile paper-points (Dentsply, Petropolis, Brazil) from the deepest periodontal pocket with bleeding or from the healthy sulcus (no bleeding upon probing) of each case or control subject, respectively, and removed 20 s after the last paper-point had been inserted.

Yeast culture

To determine the number of yeast-positive patients, subgingival samples were cultured immediately after the paper-point collection using a technique of bearing and semi-quantitative culture adapted from a previous study (18). Each paper-point was immediately rolled on sterile plates (adapted from 19) containing Mycosel agar (Difco Laboratories, Detroit, MI, USA), Sabouraud agar (Difco) or Candida CHROMagar (CHROMagar, Paris, France), and streaked for isolation. To analyze the density of yeasts in subgingival sites, the number of colony forming units (CFU) per subject was also determined (14). The plates were incubated at 37°C, in a non-CO_2 atmosphere, for 2–5 d 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 Aux system; bioMérieux Vitek, Inc., Hazelwood, MO, USA.) at the Mycology Laboratory of the Instituto

de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz.

Statistical analysis

The expected frequency of cases with exposure (i.e. positive for yeasts) was 50%, obtained by the evaluation of subgingival samples of 10 patients with SCP. The prevalence estimated among healthy individuals was 10%, obtained by the evaluation of the sulcus of 10 healthy individuals. Therefore, the minimum sample size was estimated to be 35 cases and 18 controls, to have an 80% power of demonstrating a significant difference, considering a confidence level of 95%. However, to compensate the refusals, a 10% increase in the calculated sample was carried out.

The chi-squared test was used to analyze the association between yeast colonization and CP, and the Kruskal–Wallis and analysis of variance (ANOVA) tests were used to compare non-parametric and parametric data, respectively. Differences were considered statistically significant when $P < 0.05$. Two statistical programs were used: EpiInfo version 7 for the sample size calculation and Statistical Package for Social Sciences (SPSS) software (version 17.0, IBM, Chicago, IL, USA) for the data analysis.

Results

Sixty subjects participated in the study. Twenty-three patients aged 31–67 years made up the slight-moderate CP (MCP) group and 17 patients aged 33–72 years the SCP group. Twenty healthy individuals aged 21–70 years were included in the control group (HS). The statistical analysis revealed there were differences in the average age among the groups (one-way ANOVA test; $P = 0.014$). Patients with SCP were significantly older than patients with MCP (Duncan test; $P < 0.05$) and the HS subjects (Duncan test; $P < 0.05$). However, there were no differences in the average age between the MCP and HS groups (Duncan test; $P > 0.05$). No significant differences were found among the three groups for gender (chi-squared test; $P = 0.258$), smoking habits (chi-squared test; $P = 0.332$), number of teeth (one-way ANOVA test; $P = 0.126$), bleeding on probing (t test; $P = 0.612$) and extension of CP (chi-squared test; $P = 0.062$). The main characteristics of the subjects are shown in Table 1.

Twelve patients (12 of 40 = 30%) with CP presented yeasts in the subgingival biofilm while only three of 20 subjects (15%) in the HS group were positive for these microorganisms. Patients with yeast-positive MCP (four of 23, 17%) and the HS individuals presented only *C. albicans*. Other yeast species were also found but only in patients with yeast-positive SCP (eight of 17, 47%). These other yeasts included *C. parapisilosis* ($n = 2$), *Rhodotorula* sp. ($n = 3$), *C. dubliniensis* ($n = 1$) and *C. tropicalis* ($n = 1$), but always associated with *C. albicans*.

No statistical difference was found between patients with yeast-positive CP and healthy yeast-positive subjects (t-test; $P = 0.084$). However, when CP was divided on the basis of severity, statistical differences were observed between the SCP and MCP groups (chi-squared test; $P = 0.043$; odds ratio (OR) = 4.22; 95% confidence interval (CI) = 1.01–17.79), and between the SCP and HS groups (chi-squared test; $P = 0.033$, OR = 5.03; 95% CI = 1.06–23.82). No statistical difference was observed between the MCP and HS groups (chi-squared test; $P = 0.832$, OR = 1.19; 95% CI = 0.23–6.11) (Table 2).

High densities of yeasts were found in patients with MCP and SCP [mean and range 61.25 (0–100) CFU/plate and mean and range 51 (0–101) CFU/plate, respectively]. On the other hand, in the HS group a low density was observed [mean and range 1 (0–1) CFU/plate]. Considering the density of all yeast species found among the groups, statistical differences were observed between the MCP and HS groups ($P = 0.028$, Kruskal–Wallis test), and between the SCP and HS groups ($P = 0.013$ Kruskal–Wallis test). No statistical difference was found between the MCP and SCP groups ($P = 0.932$, Kruskal–Wallis test).

Discussion

In the present study, despite the limited number of patients examined, a statistical association between the subgingival colonization of yeast species, especially *C. albicans*, and the presence of deep periodontal pockets was determined in a group of Brazilian individuals. Hence, the presence of SCP increased the odds of having subgingival yeasts. In addition, a great variety of yeast species, such as *C. parapisilosis*, *Rhodotorula* sp., *C. dubliniensis* and *C. tropicalis*, always associated with *C. albicans*, was only identified in the subgingival sites of patients with yeast-positive SCP, suggesting that the advanced form of CP was associated to a more complex yeast community residing in the deep pockets.

Yeasts can be expected in periodontal pockets independent of gender and age (13). In the present study, gender and smoking were well distributed between the two groups (Table 1). The difference found between mean ages of the SCP and HS groups does not seem to be a crucial factor,

Table 1. Main characteristics of the subjects studied

Characteristics	SCP	MCP	HS
Age (years)[a]	53.20 ± 11.58	46.39 ± 9.22	40.59 ± 13.79
Gender: male/female (n)[b]	9/8	7/16	6/14
Smoking: yes/no (n)[b]	6/11	7/16	3/17
Number of teeth (n)[b]	21.30 ± 4.88	23.82 ± 5.55	25.88 ± 1.96
CAL (mm)[a]	4.01 ± 1.70	2.46 ± 0.65	–
BOP (%)[b]	23 ± 15.52	32.66 ± 26.31	–
Extension of CP (localized/generalized) (n)[b]	4/13	5/18	–

BOP, bleeding on probing; CP, chronic periodontitis; HS, healthy subjects; MCP, slight-moderate CP; SCP, severe CP.
[a]Statistically significant.
[b]Non-significant.

Table 2. Association between subgingival colonization of yeasts and the severity of CP

	[a]SCP	[b]MCP	HS	Total
Yeast-positive	8	4	3	15
Yeast-negative	9	19	17	45
Total	17	23	20	60

CP, chronic periodontitis; HS, healthy subjects; MCP, slight-moderate CP; SCP, severe CP.

[a]Statistical difference between subgingival colonization of yeasts in patients with SCP and MCP and with the SCP and HS group.

[b]No statistical difference between mild/moderate CP and HS group.

especially considering that such difference was not observed between the MCP and SCP groups, and even so, the subgingival colonization of yeasts was statistically increased in patients with SCP.

Candida albicans has been found in the subgingival sites of patients with CP (10,11,15), independent of gender and age (13). These patients seem to have a greater percentage of yeast colonization than healthy individuals (14), although no differences were found in the subgingival colonization comparing the subtypes (chronic or aggressive) (14) or the severity (13) of periodontitis. In the present study, the prevalence of yeasts in CP and HP individuals was 17% and 15%, respectively. These results are similar to the prevalence of about 16% reported by other studies (10,13,15). However, 47% of patients with SCP were yeast-positive, suggesting that the degree of yeast subgingival colonization is related to the severity of CP. This is the first work in Brazil that describes this association. Interestingly, the distribution of yeast CFUs was very heterogeneous among CP groups. In 25% and 37% of MCP and SCP, respectively, CFU per plate was 100 or more. This agrees with previous studies that reported subjects with CP were more heavily colonized than others (14,19) without CP (14).

It can be argued that periodontal pockets can easily become contaminated by saliva, which may harbor *Candida* sp. To minimize such bias, in the present study the paper-point samples were inserted in periodontal pockets of teeth without saliva or biofilm on their crowns. As *C. albicans* is the fungal species most commonly associated with biofilm formation (20), the above-mentioned strategy seems to be sufficient to show that yeasts recovered from periodontal pockets were probably associated with subgingival biofilm. However, it is well known that molecular approaches are the only accurate way to verify whether the yeast found in subgingival sites came from saliva or not.

Although no study attempts to explain the mechanisms of periodontal disease on the enhancement of *Candida* sp. colonization, periodontitis, especially in its severe form, appears to be an important predisposing factor for yeast colonization. Possibly *C. albicans* has a role in the immune evasion of the plaque microorganisms and in its adherence to the periodontal tissues, because it has been typically found on the outer layers of the plaque and has been seen deep in periodontal tissues (15). It could be hypothesized that the profound perturbation on epithelial structures seen in advanced periodontal lesions (21) and the immunosuppression caused by severe periodontal disease (15) facilitate *Candida* subgingival colonization. In fact, deep pockets can produce a change in the balance of the subgingival microflora predisposing a site for periodontal destruction (22). Periodontal pathogens, including *Porphyromonas gingivalis*, can deregulate the local immune response and may benefit cohabiting organisms colonizing the same subgingival niche (23). When yeasts gain access to underlying periodontal tissues, more damage may result from the metabolites produced by them (24). For example, *C. albicans* can secrete proteinases capable of degrading major extracellular matrices and basement membrane components (25–27). A recent *in vitro* study shows that some *C. albicans* strains, recovered from periodontal pockets and growing under anaerobic conditions, enhance the production of some enzymes, suggesting that the oxygen concentration in the atmosphere surrounding cells exerts a variable influence on the virulence attributes of *C. albicans* (28).

To suggest new modalities of treatment and prevention of certain diseases at both individual and collective levels, it is important to establish the cause and pathogenic mechanisms involved in the disease. As there is usually a loss of many teeth, especially in those with SCP (5), more studies should be made addressing the role of *C. albicans* and other yeast species on the pathogenesis of this disease.

In conclusion, *C. albicans* and other yeast species are more likely to be present in periodontal pockets of patients with SCP than in healthy individuals or patients with MCP, which suggests that these microorganisms are not ubiquitous in subgingival sites and may participate in the progression of CP.

Acknowledgements

This study was supported by grants from the "Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro" (FAPERJ), Rio de Janeiro, Brazil.

References

1. Buduneli N, Kinane DF. Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. *J Clin Periodontol* 2011;**38**: 85–105.
2. Petersen PE, Ogawa H. Strengthening the prevention of periodontal disease: the WHO approach. *J Periodontol* 2005;**76**: 2187–2193.
3. Albandar JM, Brunelle JA, Kingman A. Destructive periodontal disease in adults 30 years of age and older in the United States, 1988–1994. *J Periodontol* 1999;**70**: 13–29.
4. Susin C, Haas AN, Valle PM, Oppermann RV, Albandar JM. Prevalence and risk indicators for chronic periodontitis in adolescents and young adults in south Brazil. *J Clin Periodontol* 2011;**38**:326–333.
5. Locker D, Slade GD, Murray H. Epidemiology of periodontal disease among older adults: a review. *Periodontol 2000* 1998;**16**:16–33.
6. Brown LJ, Brunelle JA, Kingman A. (1996) Periodontal status in the United

States, 1988–1991: prevalence, extent, and demographic variation. *J Dent Res* 1996;**75**:672–683.
7. Harris RJ. Untreated periodontal disease: a follow-up on 30 cases. *J Periodontol* 2003;**74**:672–678.
8. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998;**25**:134–144.
9. Wara-aswapati N, Pitiphat W, Chanchaimongkon L, Taweechaisupapong S, Boch JA, Ishikawa I. Red bacterial complex is associated with the severity of chronic periodontitis in a Thai population. *Oral Dis* 2009;**15**:354–359.
10. Slots J, Rams TE, Listgarten MA. Yeasts, enteric rods and pseudomonades in the subgingival flora of severe adult periodontitis. *Oral Microbiol Immunol* 1988;**3**:47–52.
11. Dahlen G. Role of suspected periodontopathogens in microbiological monitoring of periodontitis. *Adv Dent Res* 1993;**7**: 163–174.
12. Arendorf TM, Walker DM. The prevalence and intra-oral distribution of *Candida albicans* in man. *Arch Oral Biol* 1980;**25**:1–10.
13. Reynaud AH, Nygaard-Ostby B, Boygard GK, Eribe ER, Olsen I, Gjermo P. Yeasts in periodontal pockets. *J Clin Periodontol* 2001;**28**:860–864.
14. Urzua B, Hermosilla G, Gamonal J *et al*. Yeast diversity in the oral microbiota of subjects with periodontitis: *Candida albicans* and Candida dubliniensis colonize the periodontal pockets. *Med Mycol* 2008;**46**:783–793.
15. Jarvensivu A, Hietanen J, Rautemaa R, Sorsa T, Richardson M. Candida yeasts in chronic periodontitis tissues and subgingival microbial biofilms *in vivo*. *Oral Dis* 2004;**10**:106–112.
16. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;**4**:1 –6.
17. Tonetti MS, Claffey N, European Workshop in Periodontology group C Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. *J Clin Periodontol* 2005;**32**:210–213.
18. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. *N Engl J Med* 1977;**296**:1305–1309.
19. Kleinegger CL, Lockhart SR, Vargas K, Soll DR. Frequency, intensity, species, and strains of oral *Candida* vary as a function of host age. *J Clin Microbiol* 1996;**34**:2246–2254.
20. Ramage G, Saville SP, Thomas DP, Lopez-Ribot JL. Candida biofilms: an update. *Eukaryot Cell* 2005;**4**:633–638.
21. Ye P, Chapple CC, Kumar RK, Hunter N. Expression patterns of Ecadherin, involucrin, and connexin gap junction proteins in the lining epithelia of inflamed gingiva. *J Pathol* 2000;**192**:58–66.
22. Edwardsson S, Bing M, Axtelius B, Lindberg B, Soderfeldt B, Attstrom R. The microbiota of periodontal pockets with different depths in therapy-resistant periodontitis. *J Clin Periodontol* 1999; **26**:143–152.
23. Hajishengallisa G. *Porphyromonas gingivalis*-host interactions: open war or intelligent guerilla tactics?. *Microbes Infect* 2009;**11**:637–645.
24. Jabra-Rizk MA, Ferreira SM, Sabet M, Falkler WA, Merz WG, Meiller TF. Recovery of Candida dubliniensis and other yeasts from human immunodeficiency virus-associated periodontal lesions. *J Clin Microbiol* 2001;**39**:4520–4522.
25. Kaminishi H, Hagihara Y, Hayashi S, Cho T. Isolation and characteristics of collagenolytic enzyme produced by *Candida albicans*. *Infect Immun* 1986;**53**:312–316.
26. El Moudni B, Rodier MH, Barrault C, Ghazali M, Jacquemin JL. Purification and characterisation of a metallopeptidase of *Candida albicans*. *J Med Microbiol* 1995;**43**:282–288.
27. Rodier MH, El Moudni B, Kauffmann-Lacroix C, Daniault G, Jacquemin JL. A *Candida albicans* metallopeptidase degrades constitutive proteins of extracellular matrix. *FEMS Microbiol Lett* 1999;**177**:205–210.
28. Rosa EA, Rached RN, Ignacio SA *et al*. Phenotypic evaluation of the effect of anaerobiosis on some virulence attributes of *Candida albicans*. *J Med Microbiol* 2008;**57**:1277–1281.