In vitro adherence of *Candida albicans* isolated from patients with chronic periodontitis

Adriana Gadotti MACHADO[1], Edson Yukio KOMIYAMA[2], Silvana Soléo Ferreira dos SANTOS[3], Antonio Olavo Cardoso JORGE[4], Fernanda Lourenção BRIGHENTI[5], Cristiane Yumi KOGA-ITO[6]

1- DDS, Graduate student, Department of Biosciences and Oral Diagnosis, São José dos Campos Dental School, State University of São Paulo - UNESP, São José dos Campos, Brazil.
2- DDS, MSc, Graduate student, Department of Biosciences and Oral Diagnosis, São José dos Campos Dental School, State University of São Paulo - UNESP, São José dos Campos, SP, Brazil.
3- DDS, MSc, PhD, Associate Professor, Department of Dentistry, University of Taubaté (UNITAU), Taubaté, SP, Brazil.
4- DDS, MSc, PhD, Full Professor, Department of Biosciences and Oral Diagnosis, São José dos Campos Dental School, State University of São Paulo - UNESP, São José dos Campos, SP, Brazil.
5- DDS, MSc, PhD, Postdoctoral Research Associate, Department of Biosciences and Oral Diagnosis, São José dos Campos Dental School, State University of São Paulo – UNESP, São José dos Campos, SP, Brazil.
6- DDS, MSc, PhD, Associate Professor, Department of Biosciences and Oral Diagnosis, São José dos Campos Dental School, State University of São Paulo - UNESP, São José dos Campos, SP, Brazil.

Corresponding address: Cristiane Yumi Koga-Ito - Faculdade de Odontologia de São José dos Campos - UNESP - Departamento de Biociências e Diagnóstico Bucal - Av: Eng. José Francisco Longo, 777 - 12245-000 - São José dos Campos, SP - Brasil - Phone: +55-12-3947-9033 - Fax: +55-12-3947-9010 - e-mail: cristiane@fosjc.unesp.br

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ABSTRACT

Adherence is considered an extremely important virulence factor in yeast. Objective: The aim of this study was to analyze the adherence to epithelial cells of C. albicans isolated from patients with chronic periodontitis in comparison to healthy patients. Material and methods: Candida albicans cells isolated from individuals with chronic periodontitis (n=25) and healthy controls (n=25) were included in this study. Suspensions of C. albicans (10^6 cells/mL) and epithelial cells (10^5 cells/mL) were mixed and incubated at 37°C for 1 h. The number of yeasts adhered to 25 epithelial cells was counted. Results: The number of C. albicans cells adhered to epithelial cells was statistically higher in the chronic periodontitis group than in the control group (Student's t-test, p=0.000). Conclusion: The results of the present study suggest a higher Candida adherence of samples isolated from patients with chronic periodontitis.

Key words: Candida albicans. Cell adhesion. Periodontitis. Virulence factors.

INTRODUCTION

The presence of Candida spp. on oral cavity of healthy patients varies from 35 to 60%[4]. C. albicans is the most prevalent yeast of oral microbiota. It constitutes 60 to 70% of total isolates of this genus, followed by C. tropicalis and C. glabrata[27].

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 have been suggested are relevant factors[27].

Among predisposing factors for *Candida* sp. colonization there are endocrinal disorders, blood diseases, immunodeficiencies, antibiotic therapy, use of orthodontic appliances and total prothesis[29]. The increase of *Candida* sp. infections is related to the wide use of large spectrum antibiotics, corticosteroids, anti-tumor agents, contraceptives and due to the increase of immunocompromised patients[3].

Besides oral mucosa, recent studies have shown the presence of *C. albicans* in other oral sites such as root canal, including persistent infection[22], caries lesions[18] and periodontal pockets[10,28].

C. albicans express virulence factors that may have an important role to the pathogenesis of periodontal disease, such as the ability of penetrating the epithelium, inhibiting polymorphonuclear cells and causing lysis of monocytes[1]. Urzúa, et al.[28]

(2008) showed that *C. albicans* can colonize subgingival sites of patients with aggressive and chronic periodontitis. Javed, et al.[10] (2009) showed that clinical and salivary parameters of periodontal inflammation were higher in type 2 diabetic patients with oral *C. albicans* colonization. Gonzalez, et al.[7] (1987) evaluated the presence of yeasts of juvenile periodontitis punch biopsies and found an increase of yeasts frequency after antibiotics treatment and the presence of budding. The presence of *Candida* sp. and the development of opportunistic infections in subgingival sites are attributed to the use of broad spectrum antibiotics as an adjuvant[23].

Chronic periodontitis is one of the most common periodontal destructive diseases in adults. It is characterized by progressive loss of bone and soft tissue that support the teeth[9,21]. In cases that are refractory to conventional treatment, the presence of opportunistic microorganisms can be observed[13,25]. *Candida* spp. has been previously isolated from periodontal abscess[8], advanced periodontitis[25], AIDS patients[16] and patients with chronic periodontitis treated with antibiotics[8].

Specific factors affecting the distribution of oral *Candida* are saliva, pH, adhesion, cell surface hydrophobicity, hyphae formation and the expression of specific enzymes[29]. Moreover, *Candida* sp. is also relatively tolerant to innate and cell-mediated immunity[9].

Adherence is considered an extremely important virulence factor in yeasts because colonization and infection of the oral tissues is directly related to their adherence capacity[45]. A higher phospholipase activity is related to a stronger adherence to epithelial cells and to a higher pathogenicity[1].

Previous data showed that there are no differences on antifungal susceptibility of *Candida sp*. isolates from chronic periodontitis in comparison to the control group[13]. On the other hand, studying superinfecting microorganisms, Oliveira, Jorge and Santos[21] (2006) found that even 1,000 µg/mL minocycline was not sufficient to inhibit all periodontal tested isolates. Thus, it would be interesting to investigate deeply the virulence factors of periodontal isolates. The aim of this study was to analyze the adherence to epithelial cells of *C. albicans* isolated from patients with chronic periodontitis in comparison to healthy patients.

MATERIAL AND METHODS

This research project was approved by the Bioethics Committee of São José dos Campos Dental School/UNESP, Brazil (Protocol number 72/99-PH/CEP).

Oral isolates from chronic periodontitis were previously obtained from 88 individuals aged from 25 and 62 years (41.33±5.54), with at least two periodontal sites with 5 mm and diagnosed clinically as chronic periodontitis patient, as described by Koga-Ito, et al.[13] (2004). Control group isolates were obtained from 68 healthy individuals aged from 25 to 55 years (34.45±7.93). Subgingival dental biofilm samples were collected by inserting 3 sterile paper points into the periodontal pocket, for 30 s and processed according to Loberto, et al.[15] (2004).

Candida albicans isolated from chronic periodontitis (n=25) and control individuals (n=25) were included in this study. The *in vitro* adherence test of *C. albicans* to epithelial cells were performed according to Macura and Tondrya[16] (1989) and Wellmer and Bernhardt[30] (1997). The samples were plated on Sabouraud dextrose agar (Difco, Bencton Dickinson, Detroit, MI, USA) and incubated at 37°C for 24 h. Next, 3 colonies were transferred to 40 mL of Sabouraud broth (Difco).

After incubation at 37°C for additional 24 h, the yeasts were Gram stained in order to verify the purity of the suspension. Next, the cells were centrifuged (3,000 *g*; 15'.) and washed 3 times in 15 mL of saline phosphate buffer (PBS; pH 7.4). A suspension containing 10^6 cells/mL was obtained in a Neubauer chamber (Laboroptik, Friedrichsdorf, Hesse, Germany) using the Trypan blue exclusion method.

The epithelial cells were obtained from healthy individuals by scraping a sterile wood spatula against the buccal mucosa. The cells were centrifuged (3,000 g; 30 s) and washed three times in PBS. A suspension containing 105 cells/mL was obtained with the aid of a Neubauer chamber (Laboroptik). Next, the suspensions of *C. albicans* and epithelial cell were mixed and incubated at 37°C for 1 h. C. albicans cells that did not adhered to epithelial cells were eliminated using a 12 mm isopore membrane (Millipore, Millipore Indústria e Comércio Ltda., São Paulo, SP, Brazil). The filter was stained with 50 mm of methylene blue (Vetec Química Fina, Duque de Caxias, RJ, Brazil) and the number of yeasts adhered to 25 epithelial cells was counted.

The results were analyzed by Student's t-test (Minitab® 15.1.1.0. 2007, Minitab Inc, State College, PA, USA) comparing the number of candidal cells adhered to the epithelial cells in periodontitis and control groups. The significance level was set at 5%.

RESULTS

The number of *C. albicans* cells adhered to epithelial cells was significantly higher (p=0.000) in the chronic periodontitis group (15.28±2.32) than in the control group (6.44±1.20) (Figure 1).

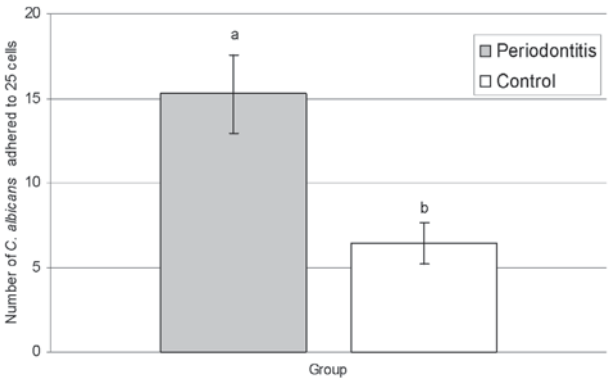

Figure 1- Number of C. albicans cells adhered to epithelial cells. Different letters show statistical significance (Student t test, p=0.000)

DISCUSSION

Increased periodontal colonization by yeasts has been found in patients with reduced immunity, such as women using oral contraceptives[2] and in HIV-positive patients with periodontal lesions[28]. *Candida* sp. has also been correlated to cases of severe and refractory periodontal infections, particularly in immunocompromised patients or individuals under antimicrobial therapy for long periods[8]. Despite *Candida* sp. isolation from patients with periodontitis and hyphal invasion in periodontal tissues[9], the role of *Candida* sp. in periodontal disease is still controversial.

To the best of our knowledge, this study shows for the first time the adhesion degree to epithelial cells of *C. albicans* isolated from chronic periodontitis sites and gives more insight on the pathogenesis and implications of *Candida* sp. in periodontal disease. *C. albicans* was used in the present study because it comprises 83.3% of the yeasts isolated from subgingival plaque of refractory periodontitis patients[25].

The identification of virulence factors of microorganisms present in gingival sulcus should be evaluated when considering a microorganism as possibly involved in periodontal disease[26]. Several virulence factors have been attributed to *Candida* spp., such as dimorphism, phenotypic switching, interference on host's immune systems, production of hydrolases, ability to respond to environmental changes, and ability to adhere to and invade into the epithelium[29]. These factors are of possible relevance to periodontal disease.

Adherence is considered the first stage of the infection process for *Candida* sp. It is an essential step for the expression of the pathogenic potential and contributes for the persistence of the microorganism in the host[1], as the ability to adhere avoids microorganisms of being eliminated by saliva[12]. Adherence is a complex and multifactorial process that involves several types of adhesions on a morphogenically changing cell surface[20].

The very low prevalence of *C. albicans* in the gingival crevicular fluid found by Ergun, et al.[6] (2010) showed the importance of adherence ability for colonization of periodontal sites. To persist in the oral environment, microorganisms should attach to teeth or mucosa. Lack of adherence will lead the microorganisms to be removed by the continuous flow rate of the gingival crevicular fluid. Moreover, some periodontal conditions, such as nutrient limitation, may trigger phenotypic changes, like pleomorphism and tigmotropism, which represent an adaptive advantage for yeast colonization. *Candida* sp. colonization in periodontal environment is important not only for periodontal health but also for pulpal health. Pulpal colonization by *Candida* sp. in intact pulpal chambers demonstrated by Miranda, et al.[19] (2009) suggests that these yeasts are able to invade pulpal tissue from periodontal sulcus.

In vitro adherence of *C. albicans* to buccal cells[11,12], vaginal cells[12] and fibrin-platelet matrixes[17] has been shown. As germinated yeasts have been shown to have a great ability to adhere *in vitro*, germ tube formation is also implied in adherence development[11].

The fact that several *Candida* species, especially *C. albicans*, have been isolated from many types of periodontal diseases indicates that they are able to colonize subgingival environment[7]. Using scanning electron microscopy, Gonzalez, et al.[7] (1987) showed yeasts invading the gingival connective tissue of patients with juvenile periodontitis. Järvensivu, et al.[9] (2004) suggested that *C. albicans* may play a role in the structure and adherence of periodontal biofilm present on chronic periodontitis. These authors observed the presence of *Candida hyphae* at the border of the sulcular epithelium and in the underlying connective tissue. The predominance of hyphae in the samples supports the visual finding of candidal tissue penetration and attachment.

Brusca, et al.[2] (2010) found a significant association between *Candida* and periodontitis only for *C. parapsilosis*, suggesting that *C. albicans* is not related to periodontitis. However, Lima-Neto, et al.[14] (2009) showed a higher affinity of *C. albicans* for epithelial cells than *C. parapsilosis*, which is in accordance with Repentigny, et al.[24] (2000). Although only *C. albicans* were evaluated in the present study, the findings of the present study show that *C. albicans* may also be related to chronic periodontal disease, as its adherence was significantly higher.

Järvensivu, et al.[9] (2004) showed the presence of *C. albicans* and the extent of gingival penetration in patients with chronic periodontitis. These authors found that *C. albicans* may play a role in the infrastructure of periodontal microbiota as

well as on adherence of periodontal tissues, which corroborates with the results of the present study.

Although the role of *C. albicans* in periodontal diseases has not yet been established, this yeast is considered as important on disease persistence and progression. The results of the present study suggest a higher adherence of samples isolated from patients with chronic periodontitis, which may be correlated to a higher pathogenicity of these isolates. These findings are in agreement with those of Calderone and Braun[5] (1991), who found a positive correlation between *C. albicans* adherence to host surfaces and pathogenicity. Further studies are needed to determine the pathogenicity of yeasts isolated from periodontal disease, to better understand the putative role of yeasts as periodontopathogens, and to identify anatomic differences between yeasts isolates.

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