Candida spp. adherence to oral epithelial cells and levels of IgA in children with orthodontic appliances

Célia Regina Gonçalves e Silva[a]
Luciane Dias de Oliveira[b]
Mariella Vieira Pereira Leão[a]
Antonio Olavo Cardoso Jorge[b]

[a] Bioscience Basic Institute, Universidade de Taubaté - UNITAU, Taubaté, SP, Brazil.

[b] Department of Biosciences and Oral Diagnosis, Instituto de Ciência e Tecnologia, Universidade Estadual Paulista - UNESP, São José dos Campos, SP, Brazil.

Abstract: Adhesion and colonization of the oral cavity by *Candida albicans* is an initial step in candidosis. Orthodontic and other oral appliances seem to favor candidal presence. The aim of this work was to compare the presence of *Candida* species in saliva, their adherence to oral epithelial cells, and the levels of anti–*C. albicans* IgA in children with or without orthodontic appliances. This study included 30 children 5 to 12 years old (9.1 ± 1.7 years old) who were users of removable orthodontic devices for at least 6 months and 30 control children of similar ages (7.7 ± 1.5 years old). The presence of yeast species in the saliva was evaluated by microbiological methods. *Candida* species were identified using phenotypic methods. Anti–*C. albicans* IgA levels in saliva were analyzed by ELISA. The yeasts adhering to oral epithelial cells were assessed by exfoliative cytology. No statistically significant differences were observed for saliva yeast counts and anti–*C. albicans* IgA levels between the studied groups. Children with orthodontic devices exhibited more yeast cells adhering to oral epithelial cells and a higher percentage of non-*albicans* species relative to the control group. In conclusion, orthodontic appliances may favor the adherence of *Candida* to epithelial cells but do not influence the presence of these yeasts in saliva, and the levels of anti–*C. albicans* IgA do not correlate with yeast adherence or presence of *Candida* in the oral cavity.

Descriptors: *Candida*; Immunoglobulin A; Orthodontic Appliances.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
Luciane Dias de Oliveira
E-mail: *luciane@fosjc.unesp.br*

http://dx.doi.org/10.1590/S1806-83242013005000031
Epub Dec 02, 2013

Submitted: Feb 05, 2013
Accepted for publication: Sep 29, 2013
Last revision: Oct 12, 2013

Introduction

The pathogenicity of *Candida* spp. is due to enzyme production, tissue invasion, and their capacity to adhere to oral mucosa.[1] The adherence of yeast to oral epithelial cells is influenced by yeast-related factors such as the expression of adhesion proteins, the presence of germinative tubes, and the production of extracellular polymers and enzymes. Host-related factors such as sexual hormones, the presence of fibrin and fibrinogen,[2] and salivary compounds including mucine,[3] salivary proteins,[4] and secretory IgA[5] may also influence this process. The effects of these components on the adherence of *C. albicans* differ, as some of them increase the adhesion capacity whereas others show inhibitory activity.[6]

Biasoli *et al.*[7] observed a correlation between the capacity for yeast to adhere and its ability to colonize mucosal surfaces. *C. albicans* exhibits the highest values of adherence to oral epithelial cells relative to other

Candida species. Bosh *et al.*[8] verified that moderate stress may affect the process of microbial colonization and the adherence of *C. albicans* to epithelial cells by altering the secretory activity of salivary glands.

Because adherence is an important virulence factor in *Candida*, the inhibition of this process is an important strategy in the prevention of oral candidosis. *C. albicans* antigens, host proteins, antifungal agents, and antibodies have been used to inhibit *C. albicans* adherence to host cells.[5] IgA seems to play an important role by causing fungal aggregation and preventing the adherence to mucosa or oral surfaces.[9]

Adhesion and colonization of the oral cavity by *C. albicans* is an initial step in candidosis. The presence of orthodontic and other oral appliances may alter the oral ecological environment. Hence, these appliances may tip the balance to favor the existence of *Candida* species.[10]

To gain a better understanding about *Candida* carriage status and the specific response against this microorganism in orthodontic patients, we compared the presence of *Candida* species in saliva, their adherence to oral epithelial cells, and the levels of anti–*C. albicans* IgA in children with or without orthodontic appliances.

Methodology

The present study was submitted to and approved by the São José dos Campos/UNESP Dental School Ethics Committee (process number 078/2002 - PH/CEP).

The study included 30 children 5 to 12 years old (9.1 ± 1.7 years old) who were users of removable orthodontic appliances for at least 6 months (experimental group; EG) and 30 children of the same age (7.7 ± 1.5 years old) who were not users of any orthodontic appliances (control group; CG).

Sampling

Intra-oral examination was performed to evaluate the presence of lesions suggestive of candidosis. Oral mucosa was collected using a wooden spatula from the cheek and the lateral surface of the tongue of each patient.

Saliva samples (2 mL) were also collected in sterile tubes and transported on ice to the microbiology laboratory to be assayed within 3 hours. The saliva samples were diluted 1:10 and 1:100 with sterile saline and immediately assayed for *Candida* isolation and identification. A fraction of 1 mL of each sample was also transferred to sterile tubes containing 5.0 mM phenylmethylsulfonyl fluoride and 0.002% sodium azide, and stored at –20°C until antibody analysis.

Candida isolation and identification

The undiluted and diluted saliva samples (0.1 mL) were plated in duplicate in Sabouraud dextrose agar (Difco, Detroit, USA) supplemented with 0.1 mg/mL chloramphenicol (vixmicin, União Química, São Paulo, Brazil). After incubation at 37°C for 48 h, the colonies were counted and the number of colony-forming units per milliliter (CFU/mL) was calculated. Saliva samples were also plated on CHROMagar (CHROMagar Microbiology, Paris, France) for the presumptive identification of *Candida* species. The different-colored colonies were confirmed by microscopy to be distinct, transferred to tubes containing Sabouraud dextrose agar and maintained at 4°C until identification. Phenotypic identification of the isolates was based on the germ tube test, hypha/pseudohypha and chlamydoconidium formation, and carbohydrate fermentation and assimilation OR germinative tubes, hyphae/pseudohyphae and chlamyidoconidia formation, fermentation, and assimilation of carbohydrates.

Anti-*Candida* IgA analysis in saliva

Saliva samples containing 5.0 mM phenylmethylsulfonyl fluoride and 0.002% sodium azide were stored at –20°C until antibody analysis by ELISA. *C. albicans* ATCC 10231 grown in Sabouraud broth for 48 h at 37°C was used as the source of antigen. *C. albicans* yeast cells killed with thimerosal (0.2 g/L) were harvested by centrifugation and washed three times with 125 mM Tris-HCl (pH 6.8). The antigens were extracted from cells by boiling in 125 mM Tris-HCl (pH 6.8), 20 mM 2-mercaptoethanol, and 6 M urea for 5 minutes. The boiled product was then harvested at 10,000 × *g* for 30 minutes at 4°C, and the

supernatant was dialyzed three times against 3 L distilled water and then lyophilized.

Flat-bottomed 96-well plates (number 3590, Costar, Cambridge, USA) were sensitized with 50 µL of crude antigen solution (100 µg/mL) dissolved in 0.1 M carbonate buffer (pH 9.6) and then incubated for 2 h at 37°C and overnight at 4°C. The wells were blocked with 0.5% gelatin (G) in phosphate-buffered saline (PBS) for 1 h. Then, the plates were washed five times with 0.5% Tween 20 PBS (T-PBS) and incubated with 50 µL of saliva sample diluted 1:8 in T-PBS plus 0.5% gelatin for 2 h at 37°C. After an additional wash step with T-PBS, the wells were filled with peroxidase-labeled goat anti-human immunoglobulin G, A, or M, and incubated for 1 h at 37°C. Finally, 100 µL/well of *o*-phenylenediamine in 0.1 M citrate buffer (pH 5.5) was added at room temperature. The reaction was stopped with 2.5 M H_2SO_4 and the absorbance was measured at 490 nm (3550 - Bio-Rad Laboratories, Hercules, USA). The results were expressed as values of optical density (OD) obtained from the mean of two readings.

Exfoliative cytology

After oral mucosa specimen collection, the slides were immediately fixed in a 1:1 solution of ether and alcohol and stained using the Papanicolaou method. The slides were analyzed under microscopy by a single researcher for the presence of yeast, which were counted in five randomly selected fields. Sixteen graded reticles were evaluated in every field, for a total of 80 reticles (200×). The number of cells with and without *Candida* and the quantity of hyphae and/or blastoconidia was counted.

Statistical analysis

Data obtained from yeast plating (CFU/mL), IgA analysis, and exfoliative cytology were subjected to ANOVA (5%) and Tukey's test.

Results

No significant differences (p = 0.843) between the CFU/mL of yeast species in saliva were observed between the EG and the CG. The EG exhibited 1.159 ± 1.33 CFU/mL whereas the CG exhibited 1.228 ± 1.37 CFU/mL (values are expressed in $\log_{10}$).

Table 1- *Candida* species isolated from the oral cavities of children in the EG (n = 29) and the CG (n = 21).

Species	EG		CG	
	n	%	n	%
C. albicans	13	44.9	12	57.1
C. lusitaniae	3	10.3	1	4.8
C. krusei	3	10.3	0	0
C. tropicalis	4	13.8	2	9.5
C. parapsilosis	2	6.9	1	4.8
C. guilliermondii	0	0	1	4.8
C. glabrata	2	6.9	2	9.5
Candida spp.	2	6.9	2	9.5
Total of isolates	29	100	21	100

A higher incidence of non-*albicans Candida* was observed among children in the EG (55.2%) compared with those in the CG (42.9%). The species of *Candida* observed in the studied groups are presented in Table 1.

Anti-*Candida* IgA in the saliva samples was analyzed by ELISA. OD values obtained for the groups were analyzed by ANOVA, and no significant differences were observed between the groups ($p = 0.16$). The mean OD values obtained were 0.024 ± 0.03 for the EG and 0.041 ± 0.05 for the CG.

To compare the IgA levels of the patients positive or negative for yeasts in the saliva, the groups were subdivided into four groups:

a. EG1, patients who had orthodontic appliances and yeasts in their saliva (n = 15);
b. EG2, patients who had orthodontic appliances but no yeasts in their saliva (n = 15);
c. CG1, control patients with yeasts in their saliva (n = 16); and
d. CG2, control patients with no yeasts in their saliva (n = 14).

No statistically significant differences were observed when the IgA counts in these groups were compared ($p > 0.05$; Table 2).

The exfoliative cytology results between the EG and CG children were significantly different ($p < 0.05$). Group EG1 was similar to EG2 and dif-

ferent from CG1 and CG2 ($p < 0.05$; Figure 1).

Discussion

This study showed no differences in the presence of *Candida* in the saliva of children in the EG and those in the CG. However, children in the EG showed more yeasts adhering to their epithelial cells in the exfoliative cytology examination. Hagg *et al.*[11] also observed that candidal carriage did not significantly increase due to the insertion of fixed orthodontic appliances using oral rinse or pooled plaque techniques, but an increase was detected using the imprint culture technique. According to these authors, the imprint technique is sensitive to the localization of yeast growth and the oral rinse technique is used to evaluate oral yeast carriage.[11] Similarly, examination of exfoliative cytology is better than evaluation of saliva to demonstrate adhering microorganisms. Our finding that the EG had higher counts of adhering *Candida* suggests that the use of orthodontic devices may cause stress or microtrauma in the oral mucosa that can affect the process of microbial colonization and the adherence of yeasts to epithelial cells.[8]

C. albicans was the species most frequently isolated from the oral cavities of patients in both groups, followed by *C. tropicalis*. Among the many factors that contribute to the higher prevalence of *C. albicans* in the oral cavity are its excellent ability to adhere and the presence of many cell receptors, which confer versatility and resistance to removal by the fluids that bathe these surfaces.[8]

In this study, patients in the EG had a higher incidence of non-*albicans* species in saliva as compared with patients in the CG. Several studies have reported that predisposing factors such as mouth breathing[12] and HIV-infection[13] may increase the variability of *Candida* species in the oral cavity.

No correlation was observed between the level of anti–*C. albicans* IgA in saliva and the presence of *Candida* or its adherence to epithelial cells. The quantity of IgA was low in children of both the EG and the CG. Salivary IgA generally increases with age because the secretory immunological mechanism develops simultaneously with the humoral immune system.[14]

Koga-Ito *et al.*[12] did not observe differences in the levels of anti–*C. albicans* IgA in the saliva of treated and untreated children with mouth-breathing syndrome and control children. However, Gonçalves e

Table 2 - OD values obtained for EG1 (n = 15), EG2 (n = 15), CG1 (n = 16), and CG2 (n = 14).

Groups	Mean OD ± SD
EG1 (orthodontic appliances, with yeasts)	0.036 ± 0.051
EG2 (orthodontic appliances, without yeasts)	0.012 ± 0.006
CG1 (control, with yeasts)	0.044 ± 0.063
CG2 (control, without yeasts)	0.038 ± 0.036

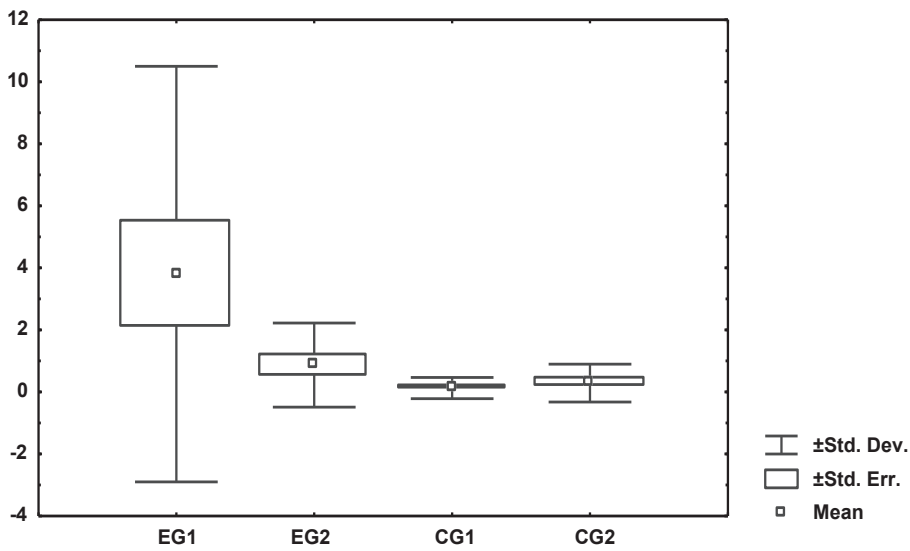

Figure 1 - Number of *Candida* cells adhering to each epithelial cell, for groups EG1, EG2, CG1, and CG2.

Silva *et al.*[15] observed that patients with clinical diagnoses of vaginal candidiasis exhibited lower levels of anti-*Candida* IgA in the saliva.

According to San Millán *et al.*,[9] in patients with dental prosthesis or removable orthodontic devices, salivary IgA reduces the adherence of *C. albicans* to polystyrene. Although the present study did not assess the adhesion of *Candida* to the surface of the orthodontic device nor the role of IgA in this phenomenon, our results indicate that anti–*C. albicans* IgA is not the most important factor to determine *Candida* carrier status.

Conclusion

Orthodontic appliances may favor the adherence of *Candida* species to epithelial cells but did not influence the presence of these yeasts in saliva. The levels of anti–*C. albicans* IgA in saliva did not correlate with the presence of *Candida* or its adherence to epithelial cells.

Acknowledgements

We thank Bárbara Maegi and Ana Paula Barbosa for their help in the collection and analysis of the samples.

References

1. Bramono K, Yamazaki M, Tsuboi R, Ogawa H. Comparison of proteinase, lipase and alpha-glucosidase activities from the clinical isolates of Candida species. Jpn J Infect Dis. 2006 Apr;59(2):73-6.
2. Olsen I. Oral adhesion of yeasts. Acta Odontol Scand. 1990 Feb;48(1):45-53.
3. Edgerton M, Scannapieco FA, Reddy MS, Levine MJ. Human submandibular-sublingual saliva promotes adhesion of Candida albicans to polymethylmethacrylate. Infect Immun. 1993 Jun; 61(6): 2644-52.
4. Ueta E, Tanida T, Doi S, Osaki T. Regulation of Candida albicans growth and adhesion by saliva. J Lab Clin Med. 2000 Jul;136(1):66-73.
5. Holmes AR, Bandara BM, Cannon RD. Saliva promotes Candida albicans adherence to human epithelial cells. J Dent Res. 2002 Jan;81(1):28-32.
6. Hibino K, Samaranayake LP, Hägg U, Wong RW, Lee W. The role of salivary factors in persistent oral carriage of Candida in humans. Arch Oral Biol. 2009 Jul;54(7):678-83.
7. Biasoli MS, Tosello ME, Magaro HM. Adherence of Candida strains isolated from the human gastrointestinal tract. Mycoses. 2002 Dec;45(11-12):465-9.
8. Bosch JA, Turkenburg M, Nazmi K, Veerman EC, de Geus EJ, Nieuw Amerongen AV. Stress as a determinant of saliva-mediated adherence and coadherence of oral and nonoral microorganisms. Psychosom Med. 2003 Jul-Aug;65(4):604-12.
9. San Millán R, Elguezabal N, Regúlez P, Moragues MD, Quindós G, Pontón J. Effect of salivary secretory IgA on the adhesión of Candida albicans to polystyrene. Microbiology. 2000 Sep;146(Pt 9):2105-12.
10. Hibino K, Wong RW, Hägg U, Samaranayake LP. The effects of orthodontic appliances on Candida in the human mouth. Int J Paediatr Dent. 2009 Sep;19(5):301-8.
11. Hägg U, Kaveewatcharanont P, Samaranayake YH, Samaranayake LP. The effect of fixed orthodontic appliances on the oral carriage of Candida species and Enterobacteriaceae. Eur J Orthod. 2004 Dec;26(6):623-9.
12. Koga-Ito CY, Unterkircher CS, Watanabe H, Martins CA, Vidotto V, Jorge AO. Caries risk tests and salivary levels of immunoglobulins to Streptococcus mutans and Candida albicans in monthbreathing patients. Caries Res. 2003 Jan-Feb;37(1):38-43.
13. Brito GN, Inocêncio AC, Querido SM, Jorge AO, Koga-Ito CY. In vitro antifungal susceptibility of Candida spp. oral isolates from HIV-positive patients and control individuals. Braz Oral Res. 2011 Jan-Feb;25(1):28-33.
14. Brokor-Bratic M. Clinical significance of analysis of immunoglobulin A levels in saliva. Med Pregl. 2000 Mar-Apr;53(3-4):164-8.
15. Gonçalves e Silva CR, Melo KE, Leão MV, Ruis R, Jorge AO. Relationship between Candida in vaginal and oral mucosae and salivary IgA. Rev Bras Ginecol Obstet. 2008 Jun; 30(6): 300-5.