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

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.

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 IgA5 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. 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. IgA seems to play an important role by causing fungal aggregation and preventing the adherence to mucosa or oral surfaces.

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.

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 H₂SO₄ 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 log₁₀).

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-

<table>
<thead>
<tr>
<th>Species</th>
<th>EG</th>
<th>%</th>
<th>CG</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>13</td>
<td>44.9</td>
<td>12</td>
<td>57.1</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>3</td>
<td>10.3</td>
<td>1</td>
<td>4.8</td>
</tr>
<tr>
<td>C. krusei</td>
<td>3</td>
<td>10.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>4</td>
<td>13.8</td>
<td>2</td>
<td>9.5</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>2</td>
<td>6.9</td>
<td>1</td>
<td>4.8</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4.8</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>2</td>
<td>6.9</td>
<td>2</td>
<td>9.5</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>2</td>
<td>6.9</td>
<td>2</td>
<td>9.5</td>
</tr>
<tr>
<td>Total of isolates</td>
<td>29</td>
<td>100</td>
<td>21</td>
<td>100</td>
</tr>
</tbody>
</table>
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 ($n = 14$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean OD ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG1 (orthodontic appliances, with yeasts)</td>
<td>0.036 ± 0.051</td>
</tr>
<tr>
<td>EG2 (orthodontic appliances, without yeasts)</td>
<td>0.012 ± 0.006</td>
</tr>
<tr>
<td>CG1 (control, with yeasts)</td>
<td>0.044 ± 0.063</td>
</tr>
<tr>
<td>CG2 (control, without yeasts)</td>
<td>0.038 ± 0.036</td>
</tr>
</tbody>
</table>

**Figure 1** - Number of *Candida* cells adhering to each epithelial cell, for groups EG1, EG2, CG1, and CG2.
Silva et al.\textsuperscript{15} observed that patients with clinical diagnoses of vaginal candidiasis exhibited lower levels of anti-\textit{Candida} IgA in the saliva.

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

**Conclusion**

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

**Acknowledgements**

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**References**