*Original Article*

# Orthodontic appliances did not increase risk of dental caries and periodontal disease under preventive protocol

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**ABSTRACT**

**Objectives:** To assess periodontal parameters and microbial species levels after orthodontic appliance placement in patients who received oral hygiene instructions and who were monitored and motivated throughout the study.

**Materials and Methods:** The Periodontal Index was recorded and saliva collection was performed before (T0) and 30 (T1), 60 (T2), and 90 (T3) days after orthodontic appliance placement in 15 patients (mean age 17.53 ± 8.0 years). Analysis was carried out using checkerboard *DNA-DNA hybridization*. Nonparametric statistical analysis was performed.

**Results:** The Periodontal Index did not change. The total amount of the purple and red complexes and *Candida* species showed a significant decrease from T2. The green, yellow, and orange complex showed a significant decrease at T3. The specific species analysis showed that *Prevotella nigrescens, Pseudomonas putida, Fusobacterium periodonticum, Pseudomonas aeruginosa, Peptostreptococcus anaerobius,* and *Tanerella forsythia* showed high incidence before bonding, and their levels decreased at T2 and T3. Only *Porphyromonas gingivalis* showed increased levels at T2 and displayed the highest level at T3. The *Streptococcus* group decreased their levels from T2 onward.

**Conclusions:** A dynamic change in microbial levels was identified. The decrease in the levels of complexes present was only possible due to the mechanical method of oral hygiene implemented in this sample. (*Angle Orthod.* 2019;89:25–32.)

**KEY WORDS:** Periodontal microbiology; Orthodontic brackets; Bacteria; Checkerboard DNA-DNA hybridization; Oral hygiene

## INTRODUCTION

Microbes are present in both healthy and diseased environments. Synergistic, mutualistic, and antagonistic interactions among the microorganisms contribute to the development of a polymicrobial biofilm.[1,2] Initial biofilm formation plays an important role in understanding the growth and proliferation of microorganisms.[3–6]

The literature describes that 60% of all orthodontic patients experience some alteration in biofilm accumulation after the bonding of orthodontic appliances. Authors emphasize a need for preventive measures to prevent biofilm-related complications during orthodontic treatment. Knowledge of the microbial dynamics in the early stages of orthodontic treatment can assist in the adoption of effective measures to prevent these changes.[7]

Studies investigating microbial contamination during orthodontic treatment have evaluated the species that are related to dental caries[8–11] or periodontal disease.[11,12] Few studies have evaluated the microbial

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ecology in the oral cavity after the bonding of orthodontic appliances.[13,14]

According to Socransky and Haffajee,[15] there are specific associations among bacterial species in the supragingival biofilm. Based on this relationship, they are grouped into different complexes. Purple and green complex species are present in early biofilm formation because of the distinct patterns of coaggregation. Yellow complex returns very quickly after cleaning surfaces of the teeth; this is an intermediate complex that improves the attachment points of adhesion. The orange and red complexes are directly involved with periodontal disease and are frequently associated with the soft tissue inflammation observed during orthodontic treatment.[16,17]

The aim of this clinical study was to assess periodontal parameters and to examine microbial communities in saliva in the early stages of orthodontic treatment in patients who received oral hygiene instructions, monitoring, and motivation throughout the study.

## MATERIALS AND METHODS

The Institutional Research Ethics Committee granted approval for the research project (Process 0062.0.138.000-10). Fifteen patients of both sexes (one male and 14 females) aged 11 to 41 years (mean age = 17.53 ± 8.0 years) with permanent dentition were screened.

The following inclusion criteria were considered in this study: no previous orthodontic treatment; no use of antibiotics, antimicrobial mouthwashes, or any systemic medication within 3 months prior to the study; no periodontal treatment within the previous 3 months; no smoking; no clinical signs of gingivitis; and no systemic disorder that could alter the periodontal conditions prior to bracket bonding.

The plaque index (PI) and gingival index (GI) were measured at three sites per tooth (mesiobuccal = MB, buccal = B, and distobuccal = DB),[18] and gingival bleeding index (GBI)[19] using a PCPUNC-BR15 probe (HuFriedy of Brazil, Rio de Janeiro, RJ, Brazil) was determined to the nearest millimeter. These indices were measured before bonding (T0) and 30 (T1), 60 (T2), and 90 (T3) days after bonding in the upper and lower arches.

Standardized hygiene instructions (modified Bass brushing technique) were given to all patients by the same investigator. The subjects were asked to brush three times daily, after meals, and were instructed not to use any hygiene products other than toothpaste and dental floss. Recall visits were scheduled at 30 days, at which time the instructions were reinforced.

The patients received edgewise metallic orthodontic brackets (0.022 × 0.028-inch slot) (Dental Morelli, Sorocaba, SP, Brazil) in the upper and lower arches. The brackets were bonded with orthodontic light-cured adhesive (Transbond XT, 3M Unitek, Monrovia, Calif).

### Saliva Collection

Nonstimulated saliva (1 mL) was collected in Falcon™ Conical Centrifuge tubes (Thermo Scientific™ Nunc™ - Waltham, MA USA) at T0, T1, T2, and T3. The saliva was collected in the morning. After collection, the Falcon tube was centrifuged for 30 seconds, and 30 μL of saliva was transferred to an Eppendorf tube (Eppendorf AG, Hamburg, Germany) with a content of 120 μL of buffer solution (10 mM Tris-HCL [Sigma-Aldrich Co., St Louis, Mo]), pH 7.6). Following this, 100 μL of NaOH (Labsynth Product Laboratories, Diaderma, SP, Brazil) was added. The samples were stored at −20°C until the *Checkerboard DNA-DNA hybridization* analysis was performed according to the method of Bergamo et al.[20]

### Checkerboard DNA-DNA Hybridization

The levels of five *Candida* species and 38 bacterial species were analyzed (Table 1). After thawing, the samples were boiled for 5 minutes. After cooling, 800 μL of 5 M ammonium acetate was added, and the contents were applied to the extended slot in the MiniSlot apparatus (Immunetics Inc, Boston, Mass) and then concentrated onto a 15 × 15-cm nylon membrane (Yond N, Amershan Biosciences, Buckinghamshire, UK), followed by baking for 2 hours at 80°C. Control samples defined amounts of genomic DNA corresponding to either $10^5$ or $10^6$.

The membranes were prehybridized (buffer hybridization; NaCl 0.5 M; blocking reagent 0.4% [w/v]). After prehybridization, the membranes were placed in a Miniblotter 45 (Immunetics). Defined amounts of fluorescein-labeled whole genomic probes were diluted in 150 mL of hybridization solution, applied in individual lanes of the Miniblotter, and the whole apparatus was placed in a sealed plastic bag containing sheets of wetted paper towel. Hybridization was performed overnight at 60°C with gentle agitation. The following day, the membranes were washed twice in a solution of 2 M urea, 0.1% sodium dodecyl sulfate, 50 mM $NaH_2PO_4$ (pH 7.0), 150 mM NaCl, 1 mM $MgCl_2$, and 0.2 blocking reagent at 65°C for 30 minutes and were also washed twice in a solution of 1 M Tris base, 2 M NaCl, and 1 M $MgCl_2$ for 15 minutes at room temperature.

The hybrids were detected by chemiluminescence using the Gene Images CDP-Star detection module (GE Healthcare, Buckingham, UK). Chemiluminescent signals were detected by exposing the membrane to ECL Hyperfilm MP (GE Healthcare) for 10 minutes. The image obtained on the hyperfilm was digitized and analyzed by the TotalLab™ Quant v13 software (TotalLab Ltd, Newcastle, UK).



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**Table 1.** Microbial Count ($\mu g \times 10^5$) in the Saliva, Before Bonding Brackets and 30, 60, and 90 Days After Bonding Brackets[a]

| Microorganism (ATCC No.) | T0 M(Q1-Q3) | T1 M(Q1-Q3) | T2 M(Q1-Q3) | T3 M(Q1-Q3) | *P* Friedman |
|---|---|---|---|---|---|
| Purple complex | | | | | |
| *Veillonella parvula* (10790) | 5.17 (4.34–6.14) | 5.16 (4.81–5.54 | 3.45 (2.94–3.94) | 3.70 (3.09–4.05) | .00001* |
| *Neisseria mucosa* (25996) | 3.01 (0.00–3.29) | 4.18 (3.954.43) | 0.00 (0.00–0.00) | 0.00 (0.000.00) | .00001* |
| Green complex | | | | | |
| *Capnocytophaga gingivalis* (33624) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .112 |
| *Eikenella corrodens* (23834) | 3.55 (3.37–3.82) | 3.96 (3.86–4.02) | 2.69 (0.00–2.75) | 0.00 (0.00–0.00) | .00001* |
| *Aggregatibacter a a (*29523) | 3.01 (0.00–3.39) | 2.90 (0.00–3.03) | 0.00 (0.00–3.37) | 0.00 (0.00–2.73) | .066 |
| *Aggregatibacter a b (*29522) | 2.96 (0.00–3.27) | 2.81 (0.00–2.94) | 3.04 (0.00–3.52) | 0.00 (0.00–0.00) | .021* |
| Yellow complex | | | | | |
| *Streptococcus mitis* (49456) | 3.84 (3.77–4.07) | 3.88 (3.41–3.99) | 4.51 (4.13–6.37) | 3.57 (0.00–4.52) | .013* |
| *Streptococcus gordonii* (10558) | 3.82 (3.55–4.32) | 3.99 (3.74–4.61) | 0.00 (0.00–3.73) | 3.31 (0.00–3.80) | .00001* |
| *Streptococcus constelatus* (27823) | 3.22 (0.00–3.57) | 0.00 (0.00–3.43) | 3.41 (2.82–3.99) | 0.00 (0.00–3.33) | .014* |
| *Streptococcus oralis* (35037) | 5.23 (4.35–6.61) | 5.21 (4.60–5.80) | 4.04 (0.00–5.38) | 0.00 (0.00–3.99) | .00001* |
| *Streptococcus sanguinis* (10556) | 5.73 (4.61–7.10) | 5.07 (4.62–6.01) | 2.76 (0.00–3.70) | 0.00 (0.00–3.34) | .00001* |
| Orange complex | | | | | |
| *Campylobacter rectus* (33238) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .733 |
| *Fusobacterium nucleatum* (25586) | 3.91 (3.54–4.08) | 4.02 (3.86–4.27) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .00001* |
| *Fusobacterium periodonticum* (33693) | 4.24 (3.98–4.75) | 3.99 (3.88–4.18) | 0.00 (0.00–2.72) | 0.00 (0.00–0.00) | .00001* |
| *Prevotella intermedia* (25611) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 5.47 (3.73–7.41) | 3.74 (3.07–5.34) | .00001* |
| *Prevotella melaninogenica* (25845) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 5.20 (3.59–5.97) | 0.00 (0.00–3.45) | .00001* |
| *Prevotella nigrescens* (25261) | 5.05 (4.29–6.12) | 5.17 (4.54–5.70) | 0.00 (0.00–0.00) | 0.00 (0.00–2.89) | .00001* |
| *Parvimonas micra* (33270) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–2.89) | 0.00 (0.00–0.00) | .003* |
| Red complex | | | | | |
| *Treponema denticola* (35405) | 4.02 (3.77–4.34) | 3.83 (3.70–3.97) | 0.00 (0.00–2.79) | 0.00 (0.00–0.00) | .00001* |
| *Tanerella forsythia* (43037) | 5.38 (4.44–6.00) | 4.29 (4.73–5.15) | 2.97 (0.00–3.56) | 0.00 (0.00–0.00) | .00001* |
| *Porphyromonas gingivalis* (33277) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 3.38 (0.00–4.22) | 3.68 (2.81–4.86) | .00001* |
| Cariogenic | | | | | |
| *Lactobacilos casei* (393) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 3.71 (2.74–3.92) | 0.00 (0.00–3.78) | .00001* |
| *Streptococcus mutans* (25175) | 4.23 (3.77–4.57) | 3.82 (0.00–4.09) | 3.16 (0.00–4.16) | 3.67 (3.32–4.56) | .019* |
| *Streptococcus sobrinus* (27352) | 6.83 (4.87–9.85) | 5.58 (3.98–6.49) | 3.77 (2.84–4.47) | 0.00 (0.00–2.76) | .00001* |
| Other species | | | | | |
| *Bacteroides fragilis* (25285) | 0.00 (0.00–0.00 | 0.00 (0.00–0.00) | 3.24 (0.00–3.72) | 0.00 (0.00–0.00) | .00001* |
| *Escherichia coli* (10798) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .300 |
| *Enterococcus faecalis* (51299) | 0.00 (0.00–0.00) | 0.00 (0.00–3.05) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .352 |
| *Klebsiella pneumoniae* (700603) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–3.04) | 0.00 (0.00–3.72) | .001* |
| *Mycoplasma salivarium* (23064) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .543 |
| *Pseudomonas aeruginosa* (27853) | 4.16 (4.09–4.29) | 4.09 (3.97–4.18) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .00001* |
| *Peptostreptococcus anaerobius* (27337) | 5.51 (5.08–5.61) | 5.16 (4.90–5.37) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .00001* |
| *Pseudomonas putida* (12633) | 5.70 (4.84–6.33) | 5.25 (4.73–6.24) | 0.00 (0.00–3.51) | 0.00 (0.00–0.00) | .00001* |
| *Porphyromonas endodontalis* (35406) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 2.76 (0.00–3.02) | 0.00 (0.00–0.00) | .001* |
| *Staphylococcus aureus (*25923) | 0.00 (0.00–3.40) | 0.00 (0.00–3.01) | 0.00 (0.00–0.00) | 0.00 (0.00–3.40) | .093 |
| *Solobacterium moreei* (CCUG39336) | 5.17 (4.28–5.53 | 4.55 (4.34–5.24) | 5.40 (3.58–6.38) | 0.00 (0.00–3.68) | .00001* |
| *Streptococcus parasanguinis* (15911) | 6.33 (4.62–7.63) | 5.07 (4.60–5.66) | 3.76 (0.00–4.61) | 0.00 (0.00–0.00) | .00001* |
| *Staphylococcus pasteuri* (51129) | 4.49 (4.12–4.85) | 3.95 (3.47–4.36) | 0.00 (0.00–3.71) | 3.85 (2.21–4.81) | .005* |
| *Streptococcus salivarius* (25975) | 5.18 (4.57–6.64) | 4.97 (4.59–5.75) | 4.31 (3.26–5.92) | 3.04 (0.00–4.02) | .00001* |
| Candidas | | | | | |
| *Candida tropicalis* (13803) | 0.00 (0.00–3.44) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .055 |
| *Candida albicans* (10231) | 4.87 (3.90–5.31) | 4.64 (4.08–4.96) | 4.24 (3.45–5.00) | 0.00 (0.00–2.94) | .00001* |
| *Candida dubliniensis* (44508) | 4.45 (4.04–4.70) | 4.02 (3.95–4.09) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .00001* |
| *Candida glabrata* (66032) | 5.41 (4.70–6.05) | 6.08 (5.00–6.42) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .00001* |
| *Candida krusei* (2159) | 4.90 (4.4–6.64) | 5.79 (5.15–6.84) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | .00001* |

[a] ATCC indicates American Type Culture Collection; T0, saliva sample before bonding; T1, saliva sample 30 days after bonding; T2, saliva sample 60 days after bonding; T3, saliva sample 90 days after bonding; M, median; Q1, first quartile; and Q3, third quartile.
* Statistically significant difference.

### Statistical Analyses

The Friedman's nonparametric test was employed. Multiple comparisons were achieved using the Dunn posttest. Differences were considered significant when $P < .05$. SPSS 21.0.0 statistical software (SPSS Inc, Chicago, Ill) was used for data analysis.

## RESULTS

No statistically significant difference was observed in PI, GI, and GBI scores throughout the study. Figure 1 shows the median, first, and third quartiles of these indexes for all evaluated periods.

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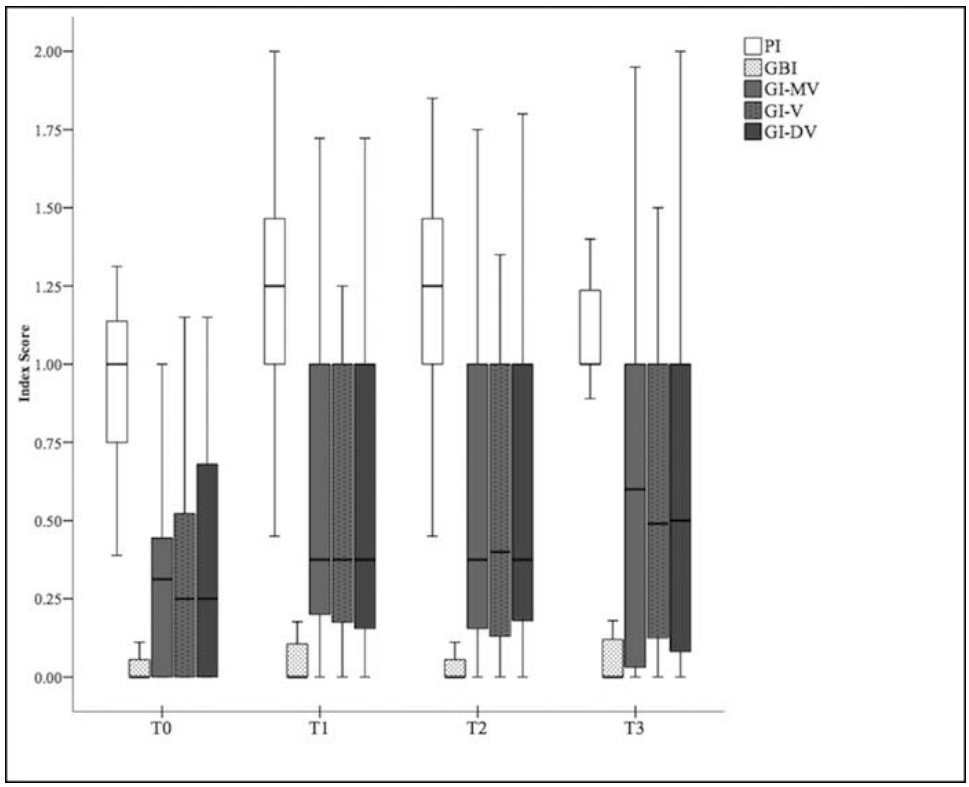


**Figure 1.** Periodontal Index distribution. T0 = Before bonding; T1 = 30 days after bonding; T2 = 60 days after bonding; T3 = 90 days after bonding of the orthodontic appliance. PI indicates plaque index; GBI, gingival bleeding index; and GI, gingival index.

All microbial complexes showed significant changes over the study period (Friedman test $P = .00001$), with a decrease in the levels of the microorganisms throughout the evaluated observational period (Figure 2). Purple and green complexes decreased levels over the observational period. A significant decrease of the purple started from T2, and the lowest level of the purple complex was also identified at T2. Green complex showed its lowest level at T3. The yellow and orange complex levels decreased significantly only at T3. The red complex and *Candida* spp decreased significantly at T2, and the lowest value was observed at T3 (Table 2).

Regarding individual analysis of the microorganisms, only eight species' levels did not change throughout the study (Table 1). There was no positive test observed for anaerobic species such as *Prevotella melaninogenica, Parvimonas micra,* and *Capnocytophaga gingivalis*. Some opportunistic species (*Bacteroides fragilis, Escherichia coli*) were not observed at T0. After bonding, significant changes were observed for *P melaninogenica* (T2-T0, T2-T1, and T2-T3; $P = .00001$), *P micra* (T2-T0 and T2-T1; $P = .034$), and *B fragilis* (T2-T0 and T2-T1; $P = .007$: T2-T3; $P = .048$), which had their highest levels identified at T2 and which decreased significantly at T3. Out of 15 species related to deep pockets and the status of advanced periodontitis, eight showed significant decreases in levels at T2 and T3: *A.a.b, Prevotella nigrescens, Pseudomonas putida, Fusobacterium periodonticum, Pseudomonas aeruginosa, Peptostreptococcus anaerobius, Treponema dentícola,* and *Tanerella forsythia* (Table 3). Only *P gingivalis* increased at T2, and its highest level occurred at T3 (T2-T0 [$P = .048$]; T2-T1 [$P = .020$]; T3-T0 [$P = .001$]; T3-T1 [$P = .00001$]).

Among nine species of the *Streptococcus* group analyzed, six species showed significantly decreased levels throughout the study: *S sobrinus, S mutans, S sanguinis, S salivarius, S parasanguinis,* and *S oralis* (Table 4). *Lactobacilos casei* increased significantly at T2 (T2-T0; $P = .002$: T2-T1; $P = .00001$). The levels decreased significantly at T3 (T3-T2; $P = .011$).

*Candida* species *C albicans, C dubliniensis, C glabrata,* and *C krusei* showed a significant alteration in their levels ($P = .00001$). However, the behavior of *C albicans* was different from that of the others: its levels decreased significantly only at T3, while the others showed a decrease starting from T2 (Table 1).

## DISCUSSION

Previous literature[1,2,21–23] emphasized the importance of knowing the relationship between microbial species and the complex oral cavity environment for the early



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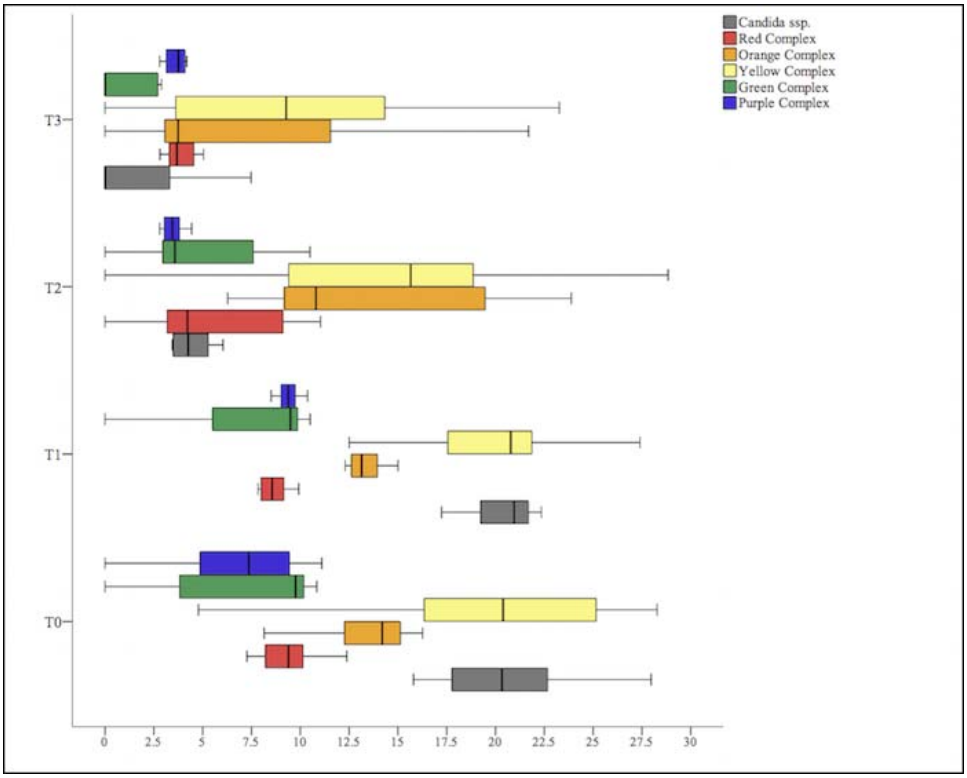


**Figure 2.** Bar chart. The total counts of different microbial complex levels ($\mu g \times 10^5$). T0 = Before bonding; T1 = 30 days after bonding; T2 = 60 days after bonding; and T3 = 90 days after bonding of the orthodontic appliance.

diagnosis of dental caries and periodontal disease. When the balance of the oral environment was broken, a dynamic fluctuation of microbial levels was identified.[24–26] Thus, the increase in levels of individual species should be viewed with caution in the orthodontic appliance environment.[27]

In this study, immediately after installation of the orthodontic appliance, there was an increase in the levels of purple and yellow complexes and *Candida* spp (T1). At T2, levels were mildly reduced, and at T3, the lowest levels were identified, indicating that homeostasis was recovered.

Some periodontal pathogenic species, such as *P melaninogenica* and *P intermedia,* were not identified at the baseline but showed an increase in levels at T2. Some anaerobic species have been shown[26–29] to be

**Table 2.** Post Hoc Dunn's Test[a]

| Complexes | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| Purple | 7.37 | 9.39 | 3.45* | 3.76* |
| Green | 9.76 | 9.50 | 3.58 | 0.00* |
| Yellow | 20.39 | 20.78 | 15.65 | 9.28* |
| Orange | 14.20 | 13.14 | 10.80 | 3.74* |
| Red | 9.40 | 8.56 | 4.22* | 3.68* |
| *Candida* spp | 20.33 | 20.95 | 4.26* | 0.00* |

Note: Median of Complexes Levels total count ($\mu g \times 10^5$)

[a] T0 indicates saliva sample before bonding; T1, saliva sample 30 days after bonding; T2, saliva sample 60 days after bonding; and T3, saliva sample 90 days after bonding.

* Statistically significance difference: Purple complex: T0-T2, $P = .002$; T0-T3, $P = .003$; T1-T2, $P = .00001$; T1-T3, $P = .00001$; Green complex: T0-T3, $P = .00001$; T1-T3, $P = .00001$; T2-T3, $P = .013$; Yellow complex: T0-T3, $P = .00001$; T1-T3, $P = .004$; Orange complex: T0-T3, $P = .00001$; T1-T3, $P = .00001$; T2-T3, $P = .001$; Red complex: T0-T2, $P = .001$; T0-T3, $P = .00001$; T1-T2, $P = .04$; T1-T3, $P = .002$; *Candida* spp.: T0-T2, $P = .011$; T0-T3, $P = .00001$; T1-T2, $P = .016$; T1-T3, $P = .00001$.

**Table 3.** Periodontal Pathogens in Which Levels Decreased[a]

| Microorganism | Decreased at T2 | Decreased at T3 |
|---|---|---|
| *A.a.b* | | T3-T0 ($P = .028$) |
| | | T3-T2 ($P = .016$) |
| *F periodonticum* | T2-T0 ($P = .00001$) | T3-T0 ($P = .00001$) |
| | T2-T1 ($P = .002$) | T3-T1 ($P = .001$) |
| *P nigrescens* | T2-T0 ($P = .00001$) | T3-T0 ($P = .00001$) |
| | T2-T1 ($P = .00001$) | T3-T1 ($P = .00001$) |
| *T denticola* | T2-T0 ($P = .00001$) | T3-T0 ($P = .00001$) |
| | T2-T1 ($P = .003$) | T3-T1 ($P = .00001$) |
| *T forsythia* | T2-T0 ($P = .00001$) | T3-T0 ($P = .00001$) |
| | T2-T1 ($P = .004$) | T3-T1 ($P = .00001$) |
| *P aeruginosa* | T2-T0 ($P = .00001$) | T3-T0 ($P = .00001$) |
| | T2-T1 ($P = .001$) | T3-T1 ($P = .00001$) |
| *P anaerobius* | T2-T0 ($P = .00001$) | T3-T0 ($P = .00001$) |
| | T2-T1 ($P = .001$) | T3-T1 ($P = .00001$) |
| *P putida* | T2-T0 ($P = .00001$) | T3-T0 ($P = .00001$) |
| | T2-T1 ($P = .00001$) | T3-T1 ($P = .00001$) |

[a] $P$ = post hoc Dunn's test. T0 indicates saliva sample before bonding; T1, saliva sample 30 days after bonding; T2, saliva sample 60 days after bonding; and T3, saliva sample 90 days after bonding.



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**Table 4.** Median Values ($\mu g \times 10^5$)—Streptococcus Group Post Hoc Dunn's Test[a]

| Microorganism | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| Yellow complex | | | | |
| *S mitis* | 3.84 | 3.88 | 4.51 | 3.57 |
| *S gordonii* | 3.82 | 3.99 | 0.00* | 3.31* |
| *S constelatus* | 3.22 | 0.00 | 3.40* | 0.00 |
| *S oralis* | 5.23 | 5.21 | 4.04 | 0.00* |
| *S sanguinis* | 5.73 | 5.07 | 2.76* | 0.00* |
| Cariogenic species | | | | |
| *S mutans* | 4.23* | 3.82 | 3.16 | 3.67 |
| *S sobrinus* | 6.83 | 5.58 | 3.77 | 0.00* |
| Others species | | | | |
| *S parasanguinis* | 6.33 | 5.07 | 3.76* | 0.00* |
| *S salivarius* | 5.18 | 4.97 | 4.31 | 3.04* |

[a] T0 indicates saliva sample before bonding; T1, saliva sample 30 days after bonding; T2, saliva sample 60 days after bonding; and T3, saliva sample 90 days after bonding.

* Statistically significant difference: . *mitis*: T0-T2, $P = .016$; T1-T2, $P = .006$; T2-T3, $P = .01$; *S gordonii*: T0-T2, $P = .016$; T0-T3, $P = .040$; T1-T2, $P = .001$; T1- T3, $P = .002$; *S constelatus*: T1-T2, $P = 0.020$; T2-T3, $P = .009$; *S oralis*: T0-T2, $P = .00001$; T0-T3, $P = .00001$; T1-T2, $P = .004$; T1-T3, $P = .001$; *S sanguinis*: T0-T2, $P = .00001$; T0-T3, $P = .00001$; T1-T2, $P = .004$; T1-T3, $P = .001$; *S mutnas*: T0-T1, $P = .19$; T0-T2, $P = .010$; *S sobrinus*: T0-T2, $P = .001$; T0-T3, $P = .00001$; T1-T2, $P = .048$; T1-T3, $P = .00001$; *S parasanguinis*: T0-T2, $P = .001$; T0-T3, $P = .00001$; T1-T3, $P = .00001$; T2-T3, $P = .048$; *S salivarius*: T0-T3, $P = .00001$; T1-T3, $P = .00001$; T2-T3, $P = .016$.

unable to live for long periods in aerobic sites. This is in agreement with the current data, which showed a decrease in the levels of several pathogenic species (*A.a.b, P nigrescens, P putida, F periodonticum, P aeruginosa, P anaerobius, T dentícola,* and *T forsythia*) over the study period. *P gingivalis* increased levels at T2 and T3. The levels may have altered according to the presence of oxygen and nutrients. Some species are more sensitive to changes in these conditions, which could explain the different behaviors of these periodontal pathologic species.

In the *Streptococcus* group, a significant decrease occurred to most species at T3. This agrees with the findings of previous literature, which described that these species were present in the initial stage of biofilm development. They were the primary colonizers that then co-aggregated with other bacteria, thus leading to the development of a mature biofilm.[23,30,31]

*S mutans* and *S sobrinus* exhibited higher levels before bonding. *S mutans* levels decreased significantly at T2 and showed a mild increase at T3, while *S sobrinus* levels decreased significantly only at T3. This species has high virulence because of high adhesion capability, acidogenicity, and acid-uric properties. High levels of both species in a patient indicated more susceptibility to caries incidence than for patients who only had the presence of one species.[31,32] Therefore, in this study, this high risk for the incidence of caries was observed before bonding. However, the oral hygiene program implemented in the study was successful in reducing the levels, and control was obtained.

*S sanguinis* is considered a beneficial bacterium with regard to dental caries because it is an antagonist to *S mutans*. Epidemiological studies[31,33] of dental caries demonstrated that early colonization and high levels of *S sanguinis* in a patient's oral cavity correlated with significantly delayed colonization by *S mutans*. The current results were in agreement with those studies, since the highest levels of *S sanguinis* were observed at T0, decreased significantly at T2, and reached their lowest levels at T3, when the *S mutans* levels had a mild increase. This result highlighted the antagonism between species and emphasized the predisposition of orthodontic patients to dental caries even after instruction, motivation, and supervision of oral hygiene throughout the study period.

The *L casei* cariogenic species correlated with deep cavities and increased significantly at T2, but decreased significantly at T3, indicating a return to baseline levels. This species is correlated with carious dentin,[32] and the sample did not show cavity activity.

*Candida* spp are frequently correlated with a decrease of pH, increase of orthodontic appliance deterioration due to the release of metallic ions, and secondary infections.[34,35] In this study, the general levels of *Candida* spp decreased after T2. However, *C albicans,* the most frequently identified fungus, which is responsible for 75% of opportunist systemic infections and has an indirect role in gingivitis, periodontitis, and dental caries,[36] showed decreased levels only at T3. In turn, *L casei, P gingivalis,* and *P intermedia,* species that are favored with the presence of *C albicans,* showed an increase starting from T2. These data may suggest that *C albicans* proliferation could trigger imbalance in the oral environment.

The sample enrolled was composed of healthy patients, with GI, PI, and GBI indices that indicated health, and the patients' oral hygiene was supervised and monitored monthly. Despite the absence of significant changes in the clinical indices used in the present study, there was a difference in the microbiological parameters between the initial timepoint T0, T1, and T2, showing the important role of adequate mechanical hygiene in these patients. In this study, the Hawthorne Effect might have been expected to play some role in motivating patients to perform better oral hygiene. At the first observational period (30 days), the Hawthorne Effect should have had its greatest influence, because this was a novel situation, but at that timepoint, an increase in the measured parameters was observed. Patient motivation and the reinforcement of oral hygiene instruction resulted in oral hygiene improvements, resulting in subsequent decreases in the parameters evaluated. The limitations of this study,



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including the small number of enrolled subjects and the large variability in the ages of the patients, must be considered. Future research considering the effects of age, longer observation periods, and additional monitoring may add important new information to the current findings.

## CONCLUSION

- A dynamic change in microbial levels was found. The decrease in the levels of complexes present was only possible because of the mechanical method of oral hygiene implemented in this sample.

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