Impact of Treatment with Full-fixed Orthodontic Appliances on the Periodontium and the Composition of the Subgingival Microbiota

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Abstract

Aims: The purpose of this study was to evaluate the impact of full-fixed orthodontic appliances on the periodontium in adult patients.

Methods: Seventeen periodontally and systemically healthy subjects were selected from the Periodontal Clinic of Guarulhos University, 7 males and 10 females (mean age: 38.3 ± 6.3 years). The patients undergoing orthodontic treatment were submitted a clinical examination, a cone beam computed tomography at baseline and after 12 months of treatment. Subgingival biofilm samples were analyzed by Checkerboard DNA-DNA hybridization. Statistical analysis was performed by a Wilcoxon test.

Results: The percentage of sites with visible plaque increased (p =0.003), but no significant reduction in marginal bone was observed. The mean periodontal pocket depth was reduced (p=0.001) and the clinical attachment level significantly improved (p =0.001). There was a significant reduction in the mean proportions of the Actinomyces sp and an increase in the orange complex species. The proportions of the red complex species remained unchanged.

Conclusion: In spite of increase in plaque accumulation no significant clinical or tomographic iatrogenic changes in periodontally healthy adults undergoing orthodontic full-fixed appliance treatment could be detected. The microbiological changes did not affect the periodontal parameters in monitored adult patients that received short period of orthodontic treatment.

Keywords. Orthodontics, periodontal disease, microbiology

Introduction

There is increasing interest within the the adult population for good oral health and aesthetics, reflected in an increasing demand for orthodontic treatment. (Trossello and Gianelly, 1979; Boyd and Baumrind, 1992; McKiernan *et al.*, 1992; Karkhanechi *et al.*, 2013; Papageorgiou *et al.*, 2019). The use of orthodontic appliances is however, well known for making it difficult to maintain an optimal level of oral hygiene. Furthermore, the presence of orthodontic appliances has been also associated with alterations in the oral microbiota (Löe and Morrison, 1986; Diamanti-Kipioti *et al.*, 1987; Naranjo *et al.*, 2006; Lo *et al.*, 2008; Papageorgiou *et al.*, 2019). Treatment with fixed appliances may thus produce changes in biofilm formation (Diamanti-Kipioti *et al.*, 1987; Boyd and Baumrind, 1992; Karkhanechi *et al.*, 2013), thereby leading to an increased risk of gingivitis (Boyd and Baumrind, 1992; Naranjo *et al.*, 2006; Lo *et al.*, 2008) and consequently periodontal damage (Lo *et al.*, 2008).

A large number of studies have shown that full-fixed orthodontic appliances might lead to negative changes in clinical and/or microbiological periodontal parameters, even in periodontally-healthy patients (Diamanti-Kipioti *et al.*, 1987; Petti *et al.*, 1997; Perinetti *et al.*, 2004; Naranjo *et al.*, 2006; van Gastel *et al.*, 2008; Thornberg *et al.*, 2009; Liu *et al.*, 2011; Karkhanechi *et al.*, 2013; Ghijselings *et al.*, 2014). Nonetheless, only one of these studies evaluated adult patients. Karkhanechi *et al.*, (2013) showed that adult patients using full-fixed orthodontic appliances

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tended to present more plaque accumulation and a greater increase in mean probing depth (PD) values and presence of periodontal pathogens (*Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia*) than those treated with removable aligners during a 12 months observation period.

To the best of our knowledge, no studies have correlated changes occurring in the microbial profile of adults using full-fixed orthodontic appliances with changes in the clinical status, marginal bone assessed by Cone Bean Computed Tomography (CBCT), and clinical and microbiological changes after orthodontic treatment.

The aim of this prospective non-randomized study was to assess the clinical and microbiological changes in the maxillary buccal alveolar bone thickness over a period of 12 months, as a result of the presence of full-fixed bonded orthodontic appliances, with regular periodontal monitoring of periodontal clinical and tomographic parameters, and to correlate these data with the levels and proportions of 40 oral bacterial species, in adult patients.

Material and Methods

Subjects

In total, 17 systemically healthy subjects to be treated with full-fixed orthodontic bonded appliances were selected from a group of patients referred to the Periodontal Clinic at Guarulhos University. Medical and dental anamnesis were obtained, a full-mouth periodontal examination was performed and then the patients were invited to participate in the study according to the following criteria:

Inclusion criteria: 30 years of age or older; with at least 20 teeth; periodontally healthy (no sites with increased PD, and clinical attachment level (CAL) measurements exceeding 3 mm (Armitage, 1999); no previous orthodontic or periodontal treatment and negative dental space discrepancy of no more that 8 mm.

Exclusion criteria: pregnancy or lactation; smoking; presence of chronic or systemic diseases; anti-inflammatory or antibiotic therapy in the previous 6 months and presence of fixed prosthetic rehabilitations.

The study protocol was explained to each patient, and a signed Term of Free and Informed Consent was obtained. The Guarulhos University Ethics Committee in Clinical Research approved the study protocol.

Periodontal preparation and monitoring

At baseline and then every three months, all patients received full-mouth oral prophylaxis (OP) (supragingival scaling and polishing) and monthly oral hygiene instructions (OHI). Patients were regularly asked not to use any other oral care products, such as mouthwashes during the period of the study.

Clinical evaluation

When treatment began, one trained and calibrated examiner performed the clinical examination including the registration of the variables visible plaque (VP), gingival bleeding (GB), bleeding on probing (BoP), suppuration. PD and CAL (mm) measurements were recorded to the nearest millimeter using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA). All parameters were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual) in all teeth excluding third molars. The variables VP, GB, BoP and suppuration were measured as the presence or absence of biofilm on the tooth surface or gingival bleeding, respectively. The identical examination was repeated after 12 months of treatment.

The methodology used for calibration was recommended by Araujo *et al.*, (2003), in which the standard error of the measure (s.e.m) was evaluated for continuous periodontal clinical parameters (PD and CAL). The intra-examiner s.e.m value indicated an acceptable reproducibility within the periodontal clinical research parameters. The intra-examiner standard error was calculated and the variability observed was 0.21 mm for PD and 0.26 mm for CAL. The agreement for categorical variables (VP and BoP) was 94 % and 92 %, respectively (Kappa-light Test).

Cone beam computed tomography evaluation

All images used in this investigation were obtained using i-CAT Imaging System (Imaging Sciences International, Hatfield, PA, USA), resolution 0.2 mm Voxel, 22 cm field of view (FOV) and 22 seconds for image acquisition. The files were acquired in Digital Imaging and Communications in Medicine (DICOM) format and the images were processed and reconstructed using Mimics 11 software (Materialise, Leuven, Belgium). Maxillary and mandibular volumetric rendering was performed and bucco-lingual central slices of the following teeth: 11, 21, 31, 41, 15, 25, 35, 45, 16, 26, 36 and 46 were generated according to Cattaneo *et al.* (2011). Mimics was used by a different calibrated examiner from the one who performed the clinical examination; and one line was drawn to the axial slice passing through the incisal point (IP) crossing to the center of the root - apical point (AP) for each individual tooth/root (Figure 1). This line described can be drawn irrespective of the angulation/rotation of the tooth relative to the alveolar process or the presence of crowding. On these images the maxillary buccal alveolar bone thickness values (MBABT) were assessed at 3, 6 and 9 mm from the cementum-enamel junction (CEJ).

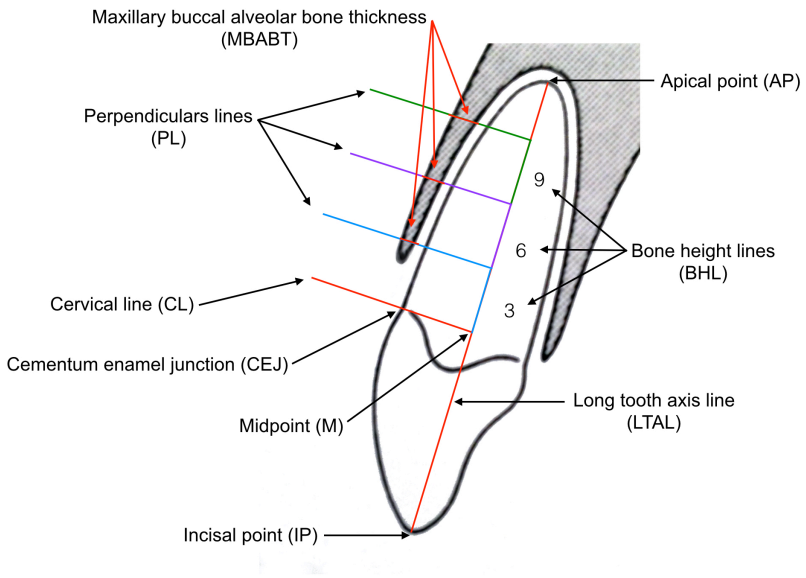

Figure 1. Illustration of maxillary buccal alveolar bone thickness measurement in maxillary central incisor with all reference points and lines represented. References lines used for measurements. Long tooth axis line (LTAL): Line starting from IP (Incisal point) or OP (Occlusal point) up to AP (Apical point) of tooth; Cervical line (CL): Line starting from LTAL up to the CEJ toward buccal surface of tooth; Bone height lines (BHL): in LTAL, 3 lines with different heights were determined from intersection of lines CL and LTAL, in 3, 6 and 9 mm measurements; Perpendicular lines (PL): another 3 lines were drawn perpendicular to LTAL, also parallel to CL, exceeding the maxillary alveolar bone limit of each tooth; segments of maxillary buccal alveolar bone thickness line (MBABT): in these three lines PL we can measure three segments of maxillary buccal alveolar bone thickness in different predetermined heights (3, 6, and 9 mm).

Microbiological monitoring

After recording the clinical parameters and removing supragingival plaque, nine sites of subgingival biofilm samples (PD<3 mm) were selected. The samples were taken with individual sterile mini Gracey curettes (#11-12- Golgran, SP, Brazil). For collection of the subgingival biofilm, the curette was positioned in the most apical portion of the site and a single scaling stroke was performed in the apical-coronal direction. The collected materials were immediately placed in separate Eppendorf tubes containing 0.15 ml of TE (10mM Tris-HCl, 1 mM EDTA, pH 7.6). One hundred microliters of 0.5 M NaOH was added to each tube, the samples were dispersed using a vortex mixer - before freezing at -200C – and stored until they were subjected to microbiological processing. The samples were evaluated for 76 bacterial species by checkerboard DNA-DNA hybridization (Socransky *et al.*, 1994) at the Laboratory of Microbiology of Guarulhos University.

Checkerboard DNA–DNA hybridization

The samples were boiled for 10 minutes, and neutralized using 0.8 ml of 5 M ammonium acetate. The DNA released was then placed into the extended slots of a Minislot 30 apparatus (Immunetics; Cambridge, MA, USA), concentrated on a 15x15 cm positively charged nylon membrane (Boehringer Mannheim; Indianapolis, IN, USA) and fixed to the membrane by baking it at 120°C for 20 min. The membrane was placed in a Miniblotter 45 (Immunetics; Cambridge, MA, USA) with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labelled whole genomic DNA probes for 40 bacterial species were hybridized in individual lanes of the Miniblotter 45. After hybridization, the membranes were washed at high stringency and the DNA probes were detected using the antibody to digoxigenin conjugated with alkaline phosphatase and chemiluminescence detection. The sensitivity of the assay was adjusted to permit the detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe. The reading was done 2 times by a single, trained, calibrated examiner (K test = 93%), on different days, to check the results. The intensity of each signal produced by a given probe in the biofilm sample was compared with the intensity of the signal produced by the same probe in the 2 control lines containing 10^5 and 10^6 bacteria.

Thus, the number 0 was recorded when no signal was detected (0 cell); 1 was a less intense signal than that of the 10^5 cell control; 2 was that of 10^5 cells; 3 was that of between 10^5 and 10^6 cells; 4 was that of up to approximately 10^6 cells and 5 was that of over 10^6 cells. These records were converted to absolute counts by comparison with the standard lanes on the membrane and used to determine the levels of the different species investigated in the different samples evaluated.

Orthodontic treatment

Conventional straight-wire metal orthodontic brackets (0.022" x 0.028" slot) (Roth Max - Morelli Sorocaba, SP, Brazil) were bonded to both maxillary and mandibular teeth by using light-cured composite (Transbond XT; 3M Unitek, Monrovia, CA, USA). The leveling/aligning stage was performed by using NiTi (Thermo-Plus - Morelli, Sorocaba, SP, Brazil), CuNiTi (Thermo-Active Copper NiTi - Ormco Corp. Thermodynamic, Orange, CA, USA) and stainless-steel wires (Arco intraoral CrNi, Morelli, Sorocaba, SP, Brazil). Wire progression was performed at each appointment within four to five weeks, according to the following sequence: 0.014" NiTi, 0.016" NiTi, 0.018" NiTi, 0.019" x 0.025" CuNiTi, and 0.019" x 0.025" CrNi.

Statistical Analyses

The percentage of sites with VP, GB and BoP, and mean values for PD and CAL, number and the percentage of sites that gained or lost CAL (≥ 2 mm) between baseline and 12 months were computed for each patient and then averaged across patients. Similarly, changes in bone level height and bone thickness at 3 mm, 6 mm and 9 mm and mean counts ($x10^5$) and proportions (%) of individual bacterial species as well as of the microbial complexes (Socransky *et al.*, 1998; Armitage, 1999) were averaged within each patient and then averaged across patients.

The significance of differences between time points for the clinical, tomographic and microbiological parameters was sought by using the Wilcoxon Test. The analyses were made with the use of a statistical program developed by Socransky *et al.* (1998). The level of significance was set at 5 %. For the tomographic measurements, the error of the method was also calculated using the Dahlberg formula (Cattaneo *et al.*, 2011).

Results

The study was conducted between December of 2016 and August of 2018. Seventeen adults (seven males and ten females, mean age: 38.3 ± 6.3 years) were enrolled and completed the study.

The periodontal clinical parameters evaluated at baseline and at 12 months of orthodontic treatment are shown in Table 1. A statistically significant reduction in PD (2.8 ± 0.3 mm to 2.3 ± 0.3 mm, p = 0.001) and in CAL (2.9 ± 0.4 mm to 2.4 ± 0.3 mm; p = 0.001), but an increase in the percentage of sites with plaque (12.4 ± 17.3 % to 29.5 ± 13.5 %, p = 0.003) were observed. Table 2 presents the mean (± SD) number of sites gaining or losing 2 mm or more of CAL over the course of the study. The majority of sites did not lose or gain CAL from baseline to 12 months (86 %), and only 3 % showed a loss of CAL.

Relative to the microbiological data, Figure 2 shows that *Prevotella intermedia* was the only bacterial species that had increased counts in 12 months (p>0.05). Regarding the microbial complexes (Figure 3), at the baseline the samples had low proportions of red complex (7.8 ±

Table 1. Epidemiological characteristics and means of clinical periodontal parameters (± SD) at baseline and 12 months of orthodontic treatment.

Variable	Time	
Probing Depth (mm)	Baseline	2.8 ± 0.3 [a]
	12 months	2.3 ± 0.3 [b]
Clinical Attachment Level (mm)	Baseline	2.9 ± 0.4 [a]
	12 months	2.4 ± 0.3 b
Percentage of sites with:		
Visible Plaque	Baseline	12.4 ± 17.3 [a]
	12 months	29.5 ± 13.5 [b]
Gingival Bleeding	Baseline	2.4 ± 3.9 [a]
	12 months	2.5 ± 2.9 [a]
Bleeding on Probing	Baseline	9.4 ± 6.5 [a]
	12 months	8.2 ± 12.0 [a]
Suppuration	Baseline	0.0 ± 0.0 [a]
	12 months	0.0 ± 0.0 [a]

Distinct letters indicate differences between times (Wilcoxon's test).

Table 2. Mean (± SD) number and the percentage (%) of sites that gained clinical attachment (≥ 2 mm) or have lost (<2 mm) or remained stable (ranging from -1mm to 1mm) along the 12 months of orthodontic therapy.

Variable	Status	
Number of sites	Gain CA	15.9 ± 13.2
	Loss CA	4.7 ± 8.3
	Stable	135.5 ± 19.9
% of sites	Gain CA	11.0 ± 9.0
	Loss CA	3.0 ± 5.0
	Stable	86.0 ± 9.0

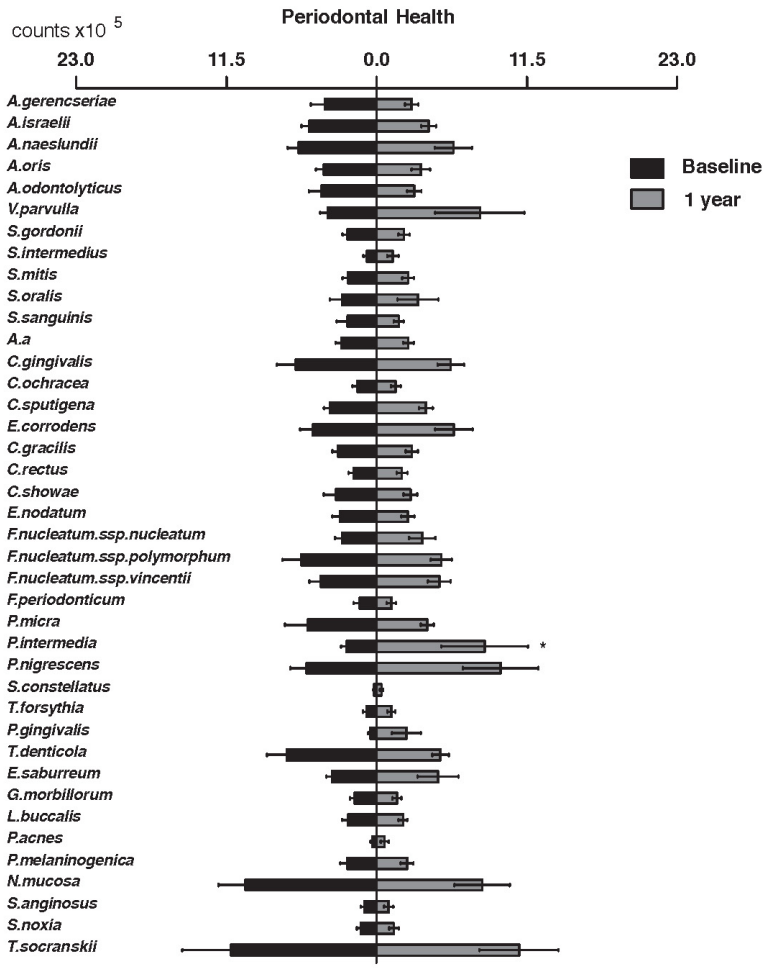

Figure 2. Mean counts (x 105) of 40 bacterial species evaluated in subgingival biofilm samples collected at baseline and at 12 months after orthodontic treatment. (*Wilcoxon test, p<0.05 - indicates differences between baseline and 12 months).

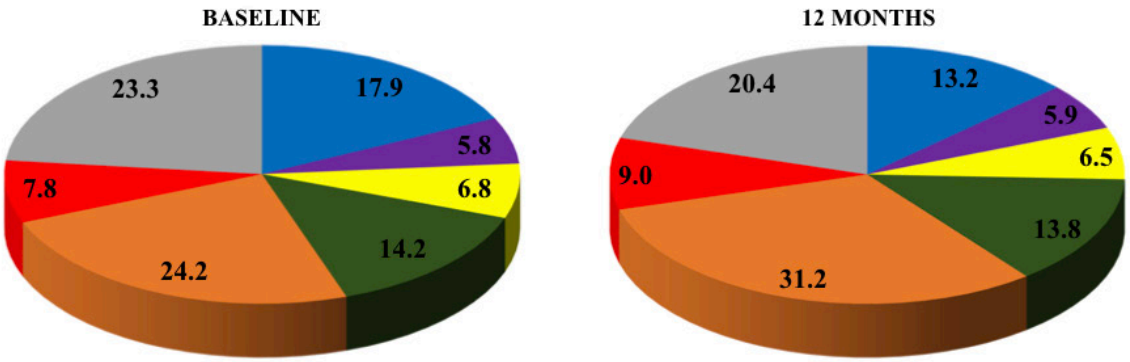

Figure 3. Mean proportions of microbial complexes (Socransky et al., 1998) of 40 bacterial species evaluated in subgingival biofilm samples collected at baseline and at 12 months after orthodontic treatment. (*Wilcoxon test, different letters indicate differences between baseline and 12 months, p<0.05).

4.3%) and high proportions of complexes associated with periodontal health, especially the *Actinomyces* species, the yellow and green complexes. After 12 months, there was a statistically significant reduction in the mean proportions of the *Actinomyces sp* (17.9% to 13.2%) and an increase in the mean proportions of orange complex species (24.2% to 31.2%). The proportions of the red complex species remained unchanged during the study period.

No statistically significant differences were detected for the tomographic variables since no reduction in maxillary buccal alveolar bone thickness was detected (Table 3).

Table 3. Mean (± SD) of the measurements obtained by tomography at baseline and at 12 months of orthodontic treatment.

Variable	Time	
Bone level height	Baseline	2.2 ± 0.3 [a]
	12 months	2.3 ± 0.5 [a]
Thickness		
3 mm	Baseline	1.2 ± 0.3 [a]
	12 months	1.3 ± 0.2 [a]
6 mm	Baseline	1.2 ± 0.2 [a]
	12 months	1.3 ± 0.2 [a]
9 mm	Baseline	1.5 ± 0.3 [a]
	12 months	1.6 ± 0.4 [a]

Equal letters indicate that there is no difference between the times within the group (Wilcoxon test).

Discussion

The data from the present study showed increased plaque accumulation, which had a negative impact in the composition of the subgingival biofilm. Nonetheless, our findings demonstrated that these effects were not sufficient to influence the clinical parameters in the individuals. In fact, mean PD and CA showed an improvement between baseline and 12 months and the maxillary buccal alveolar bone thickness did not change over time.

To date, Karkhanechi *et al.* (2013) have conducted the only study reporting the effects of full-fixed orthodontic appliances on the periodontium of adult patients. Similar to our results, the authors observed an increase in plaque accumulation in a group of patients treated with fixed appliances for one year. Studies evaluating adolescents and/or young adults have also shown that fixed appliances favor the accumulation of plaque (Huser *et al.*, 1990; Sukontapatipark *et al.*, 2001). Interestingly, some of these studies showed an increase in levels of plaque at 6 months, followed by a reduction at 12 months (Zachrisson and Zachrisson, 1972; Ristic *et al.*, 2008). This pattern has not been observed in adults, or in the present study, or in the study of Karkhanechi *et al.*, 2013. One possible explanation for this difference could be that the adolescents were more prompt in changing their oral hygiene habits than the adults (Zachrisson and Zachrisson, 1972; Sukontapatipark *et al.*, 2001).

Although there was an increase in plaque accumulation in the present study, it is worth noting that the percentage of teeth showing VP was moderate (29.5 %), and that the increase in plaque accumulation did not result in damage to the periodontium.

On the contrary the patients in our study exhibited a statistically significant reduction in mean PD and gain in CAL over the 12 months of treatment. These findings corroborate findings reported in a systematic review (Ristic *et al.*, 2008) that suggested an absence of reliable evidence on the detrimental effects of orthodontic treatment on periodontal health. Furthermore, Bollen (2008) found that there was only low-quality evidence for orthodontic therapy having detrimental effects on the periodontium. It is well known that bonded and banded appliances may vary considerably regarding their effects on the periodontium (Diamanti-Kipioti *et al.*, 1987; Boyd and Baumrind, 1992; van Gastel *et al.*, 2008).

It is important to note that the increase in the orange complex is primarily associated with the increase of a single bacterial species - *P. intermedia*. In this same context, the levels of this bacterium are reflected by the accumulation of biofilm at 12 months. In addition, reduced proportions of *Actinomyces* species and elevated proportions of orange complex were observed at 12 months. In addition, it has been suggested that an increase in orange complex pathogens seemed to precede the colonization by species of the red complex pathogens (Socransky and Haffajee, 2002) and were associated with the presence of periodontitis. Indeed, a non-significant increase in the proportions of the red complex pathogens was observed (from 7.8 % to 9.0 %). Thornberg *et al.* (2009) have also observed an increase in levels of *P. intermedia* during fixed orthodontic treatment and in a recent systematic review Freitas *et al.* (2014) reported moderate evidence that fixed appliances influenced the quantity and quality of oral microbiota. In another study, Naranjo *et al.* (2006) found a significant increase in periodontal pathogens from the red complex after three months of bonded orthodontic treatment.

The negative changes observed in the subgingival microbial profile in the present study occurred despite the fact that patients received monthly prophylaxis and were highly motivated to maintain a good oral health. Therefore, these results should be considered an important warning in cases of long-term orthodontic treatments, since the transition from gingivitis to periodontitis would cause irreversible changes in periodontal tissues, and loss of periodontal attachment may show faster progression when associated with orthodontic tooth movement (Boyd and Baumrind, 1992; Diamanti-Kipioti *et al*,. 1987; Naranjo *et al.*, 2006; Lo BA *et al*,. 2008; Karkhanechi *et al*,. 2013).

The option to perform the tomographic examination was because it is considered the gold standard examination in Orthodontics. Although the CBCT should not be used routinely, in this research it was the only way to determine the maxillary buccal alveolar bone thickness and height with high resolution. One finding in this study was the full stability of the maxillary buccal alveolar bone

thickness, observed by CBCT. It has been suggested that orthodontic appliances might lead to various degrees of bone resorption; but this concept has been based on findings from adolescents and young adults and the majority of studies used bite-wing and/or periapical radiographies to evaluate bone level (Zachrisson and Alnaes, 1974; Edwards, 1976; Bondemark, 1998; Lund *et al*,. 2012; Ganji *et al*., 2019; Lee *et al*., 2019). To our knowledge, this is the first study to use CBCT to evaluate changes in maxillary buccal alveolar bone thickness after orthodontic treatment in adults (Fuhrmann, 1996; Scarfe *et al*., 2006; Cattaneo and Melsen, 2008; Lund *et al*., 2010). Conversely, Zachrisson and Alnaes (1974), by means of radiographic examination, demonstrated that adolescent orthodontic patients presented more alveolar bone loss than untreated patients. This disagreement with our findings could be due to poorer hygiene habits demonstrated by adolescents (Boyd and Baumrind, 1992) or to the strict plaque control we performed in this study.

The significance of this study relates to it being the first to evaluate both clinical and tomographic parameters, and changes occurring in the subgingival microbial profile (40 bacterial species). Another important aspect of this study was that all patients were treated by the same orthodontist and evaluated by the same calibrated examiner. All the patients were highly motivated and received OP and OHI every month. The limitations of the study design were the small number of patients included and the absence of control group for ethical reasons.

Conclusions

The results of this study demonstrated that the orthodontic treatment of periodontally healthy adult individuals by means of bonded brackets does not generally have a deleterious impact on the periodontium. However, in spite of oral hygiene instructions performed every month, an increase in plaque accumulation and a negative change in the composition of the subgingival microbiota were observed. Nevertheless, the microbiological changes did not affect the periodontal parameters and the results indicated that regularly monitored adult patients presented no great signs of periodontal problems resulting from this short period of orthodontic treatment with fixed appliances.

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