

Evaluation of insertion loss and diversity of periodontopathogens in patients with controlled periodontitis during orthodontic treatment

Avaliação da perda de inserção e diversidade de periodontopatógenos em pacientes com periodontite controlada durante tratamento ortodôntico

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Abstract

Introduction: Orthodontic treatment, while beneficial for correcting dental misalignments, can alter the oral environment in ways that may influence periodontal health. **Objectives:** This study aimed to assess clinical periodontal parameters and analyse microbial changes in the subgingival biofilm before (T0) and after six months (T2) of orthodontic treatment of patients with controlled periodontitis (GDP) compared to healthy individuals (GC). **Methodology:** The study included ten patients' GDP and GC. Clinical parameters were measured at two visits. Biofilm samples were collected and analysed for periodontal pathogens: *Porphyromonas gingivalis* (Pg), *Aggregatibacter actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn), *Treponema denticola* (Td), and *Tannerella forsythia* (Tf) using Real-Time PCR. **Results:** In the GDP group, probing depth significantly decreased between visits ($p < 0.05$). Tf was the most frequently detected pathogen in both visits, while other bacteria were detected in less than 30% of samples. At T2, Pg was undetected, and Aa was found in only one sample, while Fn and Td detections increased. In the GC group, fewer than 40% of samples showed Pg, Aa, and Fn at T1, with Tf and Td more common. At T2, Fn and Tf detection increased, while Aa and Td decreased. No significant differences were found between the groups or visits ($p < 0.05$). **Conclusion:** Orthodontic treatment appears to positively influence periodontal health in controlled periodontitis patients, as shown by the reduction in probing depth. The microbial shifts suggest that while some pathogens persist, others, like *P. gingivalis*, decrease with improved periodontal management.

Keywords: microorganisms; periodontitis; probing depth; biofilm.

Resumo

Introdução: o tratamento ortodôntico, embora benéfico para corrigir desalinhamentos dentários, pode alterar o ambiente oral influenciando a saúde periodontal. **Objetivos:** Avaliar os parâmetros clínicos periodontais e analisar as alterações microbianas no biofilme subgingival antes (T0) e após seis meses (T2) de tratamento ortodôntico, em pacientes com periodontite controlada (GDP) e comparar com indivíduos saudáveis (GC). **Metodologia:** o estudo incluiu dez pacientes com GDP e dez controles saudáveis (GC). Os parâmetros clínicos foram medidos em duas visitas. Amostras de biofilme foram coletadas e analisadas para patógenos periodontais: *Porphyromonas gingivalis* (Pg), *Aggregatibacter actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn), *Treponema denticola* (Td) e *Tannerella forsythia* (Tf) usando PCR em Tempo Real. **Resultados:** no grupo GDP, a profundidade de sondagem diminuiu significativamente entre as visitas ($p < 0,05$). Tf foi o patógeno mais frequentemente detectado em ambas as visitas, enquanto as outras bactérias foram detectadas em menos de 30% das amostras. No T2, Pg não foi detectado e Aa foi encontrado em apenas uma amostra, enquanto as detecções de Fn e Td aumentaram. No grupo GC, menos de 40% das amostras mostraram Pg, Aa e Fn no T1, com Tf e Td sendo mais comuns. No T2, a detecção de Fn e Tf aumentou, enquanto Aa e Td diminuíram. Nenhuma diferença significativa foi encontrada entre os grupos ou visitas ($p < 0,05$). **Conclusão:** o tratamento ortodôntico parece influenciar positivamente a saúde periodontal em pacientes com periodontite controlada, como mostrado pela redução na profundidade de sondagem. As mudanças microbianas sugerem que, enquanto alguns patógenos persistem, outros, como *P. gingivalis*, diminuem com o melhor manejo periodontal.

Palavras-chave: Microorganismo; periodontites; profundidade de sondagem; biofilme.

INTRODUCTION

Periodontal disease (PD) is a common chronic inflammatory condition that affects the supporting structures of the teeth, leading to tissue destruction and, if left untreated, potential tooth loss. It is primarily driven by a microbial imbalance within the subgingival environment, with pathogens such as *Treponema forsythia*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* being closely linked to the progression of the disease^{1,2}. Given the complexity of its pathogenesis, effective management of periodontal disease often requires a multifaceted approach, including mechanical debridement, antimicrobial therapy, and improvements in oral hygiene practices. In recent years, the potential influence of orthodontic treatment on periodontal health has gained increasing attention, especially for patients with a history of periodontitis.

Orthodontic treatments depend on tooth movement, facilitated by the periodontal ligament that connects the tooth roots to the alveolar bone. The forces exerted on a tooth create stresses on the periodontal ligament, resulting in bone formation in areas of tension and bone resorption in areas of compression, thus allowing tooth movement. A healthy periodontium is essential to preserve tooth-supporting tissues during this process.

While beneficial for correcting dental misalignments, orthodontic treatment can alter the oral environment in ways that may influence periodontal health. The introduction of orthodontic appliances creates new areas for bacterial biofilm accumulation, which can lead to microbial shifts and possibly exacerbate existing periodontal conditions³. Some studies have indicated that the presence of orthodontic appliances can lead to changes in the microbial composition of the oral cavity, increasing the prevalence of periodontal pathogens like *Fusobacterium nucleatum*⁴. Conversely, others suggest that periodontal health can be preserved or even improved in orthodontic patients with prior periodontitis, provided that proper care and regular maintenance are implemented⁵. Studies have shown that combined orthodontic-periodontal treatment can stabilise or improve clinical periodontal parameters such as probing depth, tooth mobility, plaque index, clinical attachment level, and sulcus bleeding index⁶.

The relationship between periodontal disease and orthodontic treatment remains complex, with a dynamic interplay between microbial shifts and clinical outcomes. Understanding how orthodontic interventions affect periodontal disease progression or resolution requires a closer examination of microbial populations in the oral cavity, particularly in patients with a history of periodontitis. Notably, bacteria such as *T. forsythia* have been shown to persist in both healthy and diseased sites, indicating their integral role in maintaining the subgingival microbiome^{7,8}. Furthermore, shifts in the microbiome following orthodontic treatment could reflect changes in the periodontal environment, potentially influencing the disease outcome^{9,10}.

Given the ongoing debate about recommending orthodontic appliances for patients with a history of or current periodontitis, it is crucial to evaluate these patients' clinical and microbial evolution before and during orthodontic treatment. Finally, detecting the presence of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponema denticola*, and *Tannerella forsythia* in biofilm samples from patients with controlled periodontitis (GDP) and non-diseased (GC) patients and comparing it with probing depth measurements at the time of appliance installation (T1) and after 6 months (T2) will provide valuable insights into the impact of orthodontic treatment on periodontal health.

METHODOLOGY

Study Design

This prospective, cross-sectional study was conducted from June 1, 2022, to August 31, 2023, at the University of Uberaba, Brazil. Ten patients with well-controlled periodontitis (8 women and 4 men; age range: 30–45 years; mean age: 36.4 years; standard deviation [SD]: 2.1) were included in the study. After completing periodontal treatment, their periodontist referred these patients for orthodontic treatment. Additionally, a control group of ten patients without a history of periodontal disease was included for comparison. The ethics committee approved the study protocol, and all participants provided written informed consent (CAAE 16594919.2.0000.5145).

Patients included in the study met the following criteria: stage III or IV periodontitis and grade C (defined by attachment loss of $\geq 30\%$ of teeth with more than 5 mm of probing depth and radiographs showing alveolar bone loss around at least half the length of the root); completion of systemic periodontal treatment within the past 3 months with stable periodontal conditions (no probing pocket depth [PPD] ≥ 4 mm, plaque index [PLI] $\leq 30\%$, gingival index [GI] $\leq 30\%$, and no occlusal trauma); mild tooth crowding or spacing; good oral hygiene and non-smokers; no crowns, implants, or fixed bridges; no diabetes or other systemic conditions; no pregnancy; and no use of antibiotics or hormones in the past 3 months.

All patients underwent fixed appliance treatment for both the maxillary and mandibular arches starting at the fourth-week visit. Self-ligating metal brackets (Morelli, Brazil) were used for the orthodontic treatment. During the first 6 months of treatment, oral hygiene instructions, including modified bass brushing and flossing with an interdental brush, were provided at each visit. Periodontal treatments and antibacterial mouthwashes were prohibited during the study.

Clinical periodontal examinations were conducted at six sites per tooth (excluding the third molars) using a North Carolina periodontal probe (PCP-UNC 15, Hu-Friedy Manufacturing Inc., Chicago, USA). The following clinical parameters were recorded: bleeding on probing

(BOP), presence of calculus (PC), probing depth (PD), and clinical attachment level (CAL). The highest scores for PD, CAL, BOP, and PC were recorded for each tooth. A single, trained, calibrated dentist (RDN) conducted all periodontal and oral examinations. Cohen's kappa coefficients for the intra-examiner agreement were 0.89 for PD and 0.93 for CAL.

Patients provided biofilm samples at appliance placement (T0) and 6 months after appliance placement (T2). Clinical examinations were performed at each visit, and biofilm sampling occurred before the clinical examination. The samples were stored at -80°C.

Sample Collection

Sub- and supragingival biofilm samples were collected from the mesial surfaces of teeth 31. Sampling was performed prior to the fixed appliance (T1) installation and repeated at the second visit (T2). Using pre-contoured sterilised wires, the biofilm was collected and immersed in Eppendorf tubes containing 250 µL of buffer solution (1X PBS, pH 7.0). The samples were then sent to the Biopathology Laboratory of UNIUBE for storage at -70°C. After laboratory analysis, the samples were autoclaved for decontamination before being discarded.

Detection of Bacteria in the Samples

DNA extractions were performed from the biofilm samples, followed by Real-Time PCR assays with specific probes for bacterial detection. Chromosomal DNA was extracted using the DNA MINI KIT (PURELINK), following the manufacturer's protocol. The quantity and purity of the extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). DNA purity was considered adequate, with an A260nm/A280nm ratio between 1.8 and 2.0. DNA samples were diluted in ultrapure water to a final concentration of 15 ng/µL for PCR analysis.

Real-time PCR was performed using specific primers for *Treponema denticola*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. Oligonucleotide primers (Exxtend Biotecnologia Ltda) used in the PCR amplification were as follows:

- *Treponema denticola*: F- AGAGCAAGCTCTCCCT-TACCGT and R- TAAGGGCGGCTTGAAATAATGA
- *Tannerella forsythia*: F- CGGGCGTGCATCTTGTCG-TCTAC and R-CTTAACCGGCCCTCTTTGAA
- *Aggregatibacter actinomycetemcomitans*: F- GGCGAGCCTGTATTTGATGTGCG and R- GTGCC-CGGTGCTGCGTCTTTG
- *Fusobacterium nucleatum*: F- ACCTAAGGGAGAA-ACAGAACCA and R- CCTGCCTTAATTCATCTCCAT
- *Porphyromonas gingivalis*: F- TGCAACTTGCCT-TACAGAGGG and R-ACTCGTATCGCCCGTTATTC

The PCR reactions were performed using SYBR Green (Quantinova, Qiagen) for fluorescence detection. The thermocycler protocol included DNA denaturation at 95°C for 1 minute, annealing at 62°C for 20 seconds, and extension at 68°C for 5 seconds, with a total of 40 cycles. A melting curve was generated at the end of the PCR reaction to confirm amplification specificity.

Method of Analysis

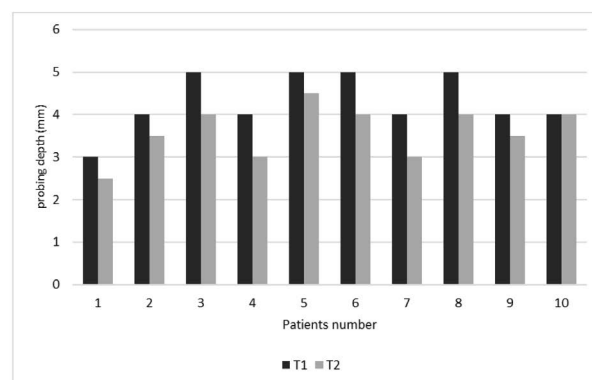
All statistical analyses were performed using BioSTAT software. One-way analysis of variance (ANOVA) and Bonferroni post-tests were used to compare probing depths at T1 and T2, as well as between groups. The frequency of bacterial detection and clinical data were compared between groups and visits using Fisher's exact test and Chi-square tests. A p-value < 0.05 was considered statistically significant.

RESULTS

Clinical data and subgingival plaque samples were collected from a total of 40 participants: 20 individuals diagnosed with periodontal disease (GPD) and 20 individuals with healthy teeth and gums (GC). In the GC group, all participants had probing depths within the healthy range (< 2mm), with no signs of bleeding, and no changes in probing depth were observed at T2 (p>0.05).

In the GPD group, the mean probing depth was 4.30 mm (SD: 0.66) at T1, and a statistically significant reduction was observed at T2, with a probing depth of 3.60 mm (SD: 0.60) (p=0.0313). Notably, no patients in the GPD group showed an increase in probing depth (Figure 1).

Figure 1- Probing depth (in mm) according to the patient number of the GDP group.



Source: self-authored

Regarding bacterial detection, *Treponema forsythia* was the most frequently detected bacterium in both groups, present in 50.0% of the samples at T1 and 56.7% at T2. Other bacteria were less commonly detected: *T. denticola* was found in 33.7% of samples at T1 and 30.0% at T2, *Fusobacterium nucleatum* in 23.4% of samples at T1 and 43.3% at T2, *Porphyromonas gingivalis* in 16.7% of samples

at T1 and 10.0% at T2, and *Aggregatibacter actinomycetemcomitans* in 16.7% of samples at T1 and 3.3% at T2.

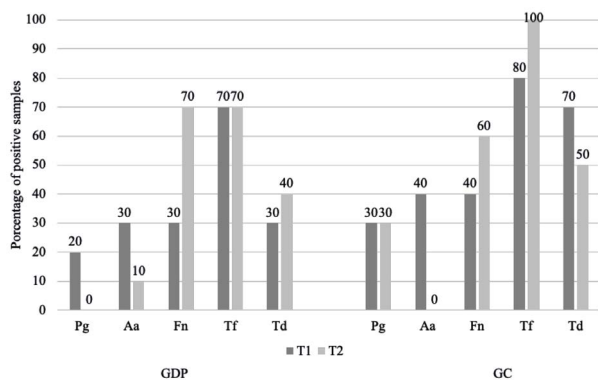
The frequency of bacterial detection in the biofilm samples of patients with periodontal disease (GDP) and healthy controls (GC) at T1 and T2 is presented in Table 1 and Figure 2. The results showed that *T. forsythia* was the most frequently detected bacterium in the GDP group at T1 and T2, while *P. gingivalis*, *A. actinomycetemcomitans*, *F. nucleatum*, and *T. denticola* were detected in less than 30% of the samples. At T2, *P. gingivalis* was no longer detected, and *A. actinomycetemcomitans* was detected in only one sample. However, the detection of *F. nucleatum* and *T. denticola* increased at T2.

Table 1 – Detection frequency of *P. gingivalis*, *A. actinomycetemcomitans*, *F. nucleatum*, *T. denticola*, and *T. forsythia* in biofilm samples collected from patients with controlled periodontitis (GDP) and healthy controls (GC).

	GDP N=10		GC N=10	
	T1 N	T2 N	T1 N	T2 N
Bacteria detection:				
<i>P. gingivalis</i>				
Yes	2	0	3	3
No	8	10	7	7
<i>A. actinomycetemcomitans</i>				
Yes	3	1	2	0
No	7	9	8	10
<i>F. nucleatum</i>				
Yes	3	7	4	6
No	7	3	6	4
<i>T. forsythia</i>				
Yes	7	7	8	10
No	3	3	2	0
<i>T. denticola</i>				
Yes	3	4	7	5
No	7	6	3	5

Source: self-authored

Figure 2 – Percentage of samples with detection of *P. gingivalis*, *A. actinomycetemcomitans*, *F. nucleatum*, *T. denticola*, and *T. forsythia* in biofilm samples collected from patients with controlled periodontitis (GDP) and healthy controls (GC).



Source: self-authored

In the GC group, fewer than 40% of the samples at T0 showed the presence of *P. gingivalis*, *A. actinomycetemcomitans*, and *F. nucleatum*. At the same time, *T. forsythia* and *T. denticola* were detected in most samples. At T2, the GC samples exhibited an increase in the detection of *F. nucleatum* and *T. forsythia*, while *A. actinomycetemcomitans* and *T. denticola* showed a decrease in detection. No statistically significant differences were found between the groups or visits ($p < 0.05$, $q < 2.23$).

Figure 3 shows the individual detection of bacteria in each patient, categorised by group and visit. In the GDP group, *P. gingivalis* (Pg) was detected at T1 but not at T2. *A. actinomycetemcomitans* (Aa) was found in only one sample at both visits. The detection of *F. nucleatum* (Fn) increased from T1 to T2, rising from 3 to 7 samples, with the positive samples at T1 remaining positive at T2. For *T. forsythia* (Tf), there was variability in detection; of the eight samples that were positive at T1, five remained positive at T2. *T. denticola* (Td) was detected in two samples at both T1 and T2.

Figure 3– Schematic representation of patients (n=number) with detection of *P. gingivalis* (Pg), *A. actinomycetemcomitans* (Aa), *F. nucleatum* (Fn), *T. denticola* (Td), and *T. forsythia* (Tf) in biofilm samples collected from patients with controlled periodontitis (GC) and non-diseased (GP) at T1 and T2. The positive detection of the bacteria is represented by the areas marked in grey.

	n	Pg		Aa		Fn		Tf		Td	
		T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
GDP	1										
	2										
	3										
	4										
	5										
	6										
	7										
	8										
	9										
	10										
GC	11										
	12										
	13										
	14										
	15										
	16										
	17										
	18										
	19										
	20										

Source: self-authored

In the GC group, *P. gingivalis* was detected at T1 in one sample and remained present at T2 in the same sample. *A. actinomycetemcomitans* was not detected in any samples at either visit. The detection of *F. nucleatum* increased from T1 to T2, rising from 4 to 6 samples, with two positive samples at T1 remaining positive at T2. *T. forsythia* was the most frequently detected bacterium, found in 6 samples at both visits. *T. denticola* was detected in four samples at both T1 and T2.

DISCUSSION

The results of this study provide important insights into the microbiological and clinical changes observed in patients with periodontal disease (GPD) and healthy controls (GC) over time. The significant reduction in probing depth (PD) observed in the GPD group at T2 is noteworthy. A decrease in PD is commonly associated with improvements in periodontal health, which suggests that the clinical management in this group effectively controlled the periodontal condition³. Additionally, no increase in PD in the GPD group supports the idea that periodontal conditions can be stabilised or improved in certain circumstances, even in patients with pre-existing periodontal disease, provided that appropriate care is maintained⁵. This observation is consistent with other studies showing that periodontal treatment can significantly reduce PD⁴.

In terms of microbial profiles, the detection of *Treponema forsythia* as the most prevalent bacterium in both groups aligns with previous findings that identify *T. forsythia* as a key species in periodontal disease^{1,2}. It has been demonstrated that *T. forsythia* is a critical pathogen in the aetiology of periodontitis. It is often found in diseased and healthy periodontal sites, suggesting it might be part of the normal oral microbiome (7). This bacterium's high frequency in both the GPD and GC groups could indicate its persistence within the subgingival plaque regardless of periodontal health status. Similarly, *T. denticola* was also frequently detected in both groups, which supports its role as a significant member of the periodontal pathogen complex^{8,11}.

Interestingly, *Porphyromonas gingivalis*, a well-known pathogen associated with periodontal disease, was detected in 16.7% of the GPD group at T1, but it was absent in all GPD samples at T2. This reduction in the detection of *P. gingivalis* suggests that the bacterial composition in the GPD group may have shifted as the periodontal health improved, potentially because of treatment or better oral hygiene¹⁰. This finding is consistent with other studies that have demonstrated fluctuating levels of *P. gingivalis* in periodontal disease and its ability to be suppressed with proper management^{12,13}. Additionally, the very low prevalence of *Aggregatibacter actinomycetemcomitans* in both the GPD and GC groups at both visits may suggest that it plays a less prominent role in the studied cohort compared to other periodontal pathogens. This is in line with studies indicating that *A. actinomycetemcomitans* may be less prevalent in chronic periodontitis compared to aggressive forms of periodontal disease^{11,14}.

The increased detection of *Fusobacterium nucleatum* in both the GPD and GC groups, particularly from T1 to T2, is in line with current literature indicating that *F. nucleatum* plays a key role in the progression of periodontal disease^{15,16}. *F. nucleatum* acts as a bridging organism, facilitating the co-adhesion of other periodontal pathogens and contributing to the formation of complex bacterial biofilms¹⁷. Its increased detection over time could reflect

changes in the oral microbiota in response to environmental shifts, such as orthodontic treatments or other interventions.

The variability in the detection of *T. forsythia* in the GPD group, where five of the eight positive samples at T1 remained positive at T2, suggests that *T. forsythia* may be more resilient or persist in certain periodontal sites even after clinical improvement. Similar findings have been reported by Liu et al.⁷ (2019), who observed the persistence of specific pathogens despite clinical resolution in periodontal treatment. Interestingly, *T. forsythia* was the most frequently detected bacterium in the GC group at both time points, with increased detection from T1 to T2. This observation suggests that *T. forsythia* may be an integral part of the microbiome, even in individuals with healthy gums. A similar trend was noted by Silva et al.¹⁸ (2020), who identified *T. forsythia* as a dominant species in healthy individuals, which may indicate that its presence alone is not sufficient for periodontal disease development. Still, rather its interactions with other pathogens may determine disease progression.

The increased detection of *F. nucleatum* in GC samples also warrants attention. The dynamics of the oral microbiota are complex, and environmental factors, such as the introduction of orthodontic appliances, may lead to microbial shifts that favour the growth of certain species. Although *F. nucleatum* is considered part of the normal oral flora, its increased presence may reflect subtle microbial changes that could eventually influence periodontal health¹⁹. The absence of statistically significant differences between the groups or visits ($p > 0.05$) could be attributed to the natural variability of the oral microbiome, as previously suggested by other studies³.

CONCLUSION

In conclusion, the findings of this study highlight important microbiological and clinical changes in periodontal health in both patients with periodontal disease (GPD) and healthy controls (GC) over time. The significant reduction in probing depth (PD) in the GPD group suggests successful management of periodontal disease, potentially due to effective treatment or improved oral hygiene practices. This observation supports the idea that periodontal health can be stabilised or even improved in patients with pre-existing diseases if appropriate care is maintained. The microbiological analysis revealed a consistent presence of key periodontal pathogens, such as *Treponema forsythia* and *Fusobacterium nucleatum*, across both groups, indicating their potential role in maintaining the subgingival microbiome, regardless of periodontal health status. Interestingly, the reduction of *Porphyromonas gingivalis* in the GPD group at T2 suggests a positive shift in the microbial environment following treatment, further supporting the importance of bacterial control in periodontal therapy. Additionally, the study observed subtle shifts in microbial populations, such as

the increased detection of *Fusobacterium nucleatum*, which may reflect environmental changes like orthodontic interventions. While no significant statistical differences were observed between the groups or visits, these findings underscore the complexity of the oral microbiome and the dynamic interplay between periodontal health and microbial composition. Further research is needed to understand better the long-term impact of various treatments on the oral microbiome and their implications for periodontal health.

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Submetido em 06/09/2025

Aceito em 27/03/2025