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Impact of Orthodontic Fixed Appliances on Type and Quantity of Certain Periodontal Microbial Pathogens

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ABSTRACT

Objectives: The main objective of this study was to detect the correlation between several periodontal pathogens and periodontal inflammation parameters and fixed orthodontic appliances.

Materials and Methods: Periodontally healthy subjects 32 (14 M, 18 F) with a mean age of 17.5 years (range: 14-28 years) attending the orthodontic clinic were recruited. Clinical periodontal parameters; plaque index (PI), bleeding on probing (BOP) and probing pocket depth (PPD) were measured and plaque samples were collected before bonding orthodontic appliances (T0) and 3 months later (T1). Real-time polymerase chain reaction (PCR) analysis was conducted on the collected samples and tested for *Aggregatibacter actinomycetemcomitans* (Aa), *Tannerella forsythia* (Tf), and *Porphyromonas gingivalis* (Pg).

Results: Periodontal parameters increased significantly at T1. The correlation analysis demonstrated that PI and BOP were significantly correlated at T0 ($P<0.01$) and at T1 ($P<0.001$). However, PPD was significantly correlated with BOP only at T1 ($P<0.01$). Regarding the bacterial concentration results, Tf had the lowest mean concentration with 0.66 and 1.47 DNA copy/sample at baseline, and following 3 months, respectively. On the other hand, the highest count was for Pg with a mean of 3.02×10^4 and 106×10^6 DNA copy/sample at baseline, and following 3 months, respectively.

Conclusions: Patients with fixed orthodontic appliances showed highest concentrations of periodontal pathogens which might confirm that orthodontic appliances contribute in the periodontal inflammation. Accordingly, oral-hygiene measures should be emphasized for fixed orthodontic patients.

Keywords: Orthodontic treatment, Fixed appliances, Dental biofilm, Periodontal indices, Periodontal pathogens, PCR.

1. Introduction

Dental biofilm, a complex microbial community encased in an extracellular matrix, is the primary etiological factor for most oral diseases, including dental caries, gingivitis, and periodontitis (1). The oral cavity's dynamic environment and the presence of dental appliances can significantly influence the composition and pathogenicity of this biofilm. Environmental shifts can lead to an ecological imbalance, favoring the proliferation of specific pathogenic bacteria and subsequently increasing the risk of oral disease (2). Clinically, numerous plaque retention factors, such as

misaligned teeth, defective restorations, fixed or removable prosthetic appliances, and orthodontic hardware, are consistently associated with increased plaque accumulation and localized gingival inflammation (3).

Orthodontic treatment, while beneficial for dental alignment, frequently presents challenges to maintaining optimal periodontal health. The presence of fixed orthodontic appliances, including brackets and archwires, creates additional retentive areas that facilitate plaque accumulation, particularly in the gingival margin and interproximal regions. This increased plaque burden is a well-documented cause of gingival inflammation during orthodontic therapy (4). Clinical and experimental studies consistently demonstrate that bacterial biofilm is the most crucial etiological factor in periodontal diseases (4).

While previous research has extensively investigated the clinical periodontal parameters, such as plaque index (PI) and probing pocket depth (PPD), around orthodontic appliances (5, 6), there remains a paucity of information regarding the specific microbiological changes and the colonization patterns of gingival tissues during orthodontic treatment. Studies have indicated that adverse shifts in the oral microflora can occur shortly after the placement of orthodontic appliances (3, 4). Notably, periodontal pathogens like *Aggregatibacter actinomycetemcomitans* (Aa), *Tannerella forsythia* (Tf), and *Porphyromonas gingivalis* (Pg) are strongly implicated in the progression of periodontitis (7). The inflammation of gingival tissues during fixed orthodontic therapy is a well-established clinical observation (4,7).

Traditional microbiological techniques for detecting and characterizing oral pathogens, including culture-based methods, immunoassays, DNA probes, and Polymerase Chain Reaction (PCR), have been employed (8). However, conventional culturing methods are often limited by their sensitivity, specificity, and time-consuming nature. Furthermore, a significant portion of the oral microflora, estimated at 50%, is not amenable to laboratory culturing (9). Molecular biology methods, particularly PCR, offer superior sensitivity, specificity, and speed for bacterial identification, enabling the detection of both viable and nonviable microorganisms with less time and effort compared to conventional approaches (10,11).

Subgingival plaque sampling is crucial for microbiological assessment of periodontal diseases, allowing for the identification and quantification of disease-associated bacterial populations. However, various sampling techniques, such as curettes, dental floss, and absorbent paper points, can influence the results due to variations in bacterial recovery and composition (12). The complexity of sampling multiple sites and pooling specimens further introduces variability, potentially affecting diagnostic interpretations. The process itself is time-consuming, requiring meticulous site selection, isolation from saliva, thorough drying, and precise instrument insertion (13). As an alternative, mouth rinse samples offer a non-invasive, cost-effective, and rapid method for microbiological diagnosis. Mouth rinses collect oral pathogens from various sites, including supra- and sub-gingival plaque, as well as oral mucosal surfaces, making them suitable for screening large populations and reducing patient discomfort (14).

Given these considerations, this research aimed to detect and quantify the periodontal pathogens Aa, Tf and Pg in mouth rinse samples from orthodontic patients. The study sought to compare microbiological profiles before and after the bonding of fixed orthodontic appliances using real-time PCR techniques and to determine whether a correlation exists between changes in these microbiological profiles and key periodontal parameters; namely, plaque index, bleeding on probing, and probing pocket depth.

2. Materials and Methods

Forty patients who attended the orthodontic clinic at Jordan University of Science and Technology (JUST) were approached. Inclusion criteria include patients who are medically fit, non-smokers, did not receive antibiotic treatment nor used antimicrobial mouth washes for 3 months prior to participation and throughout the study period. Intra-orally, inclusion criteria were extended to include participants with healthy periodontium, with no probing pocket depth more than 3mm and no loss of periodontal attachment throughout the dentition. Ethical approval was obtained from JUST Ethical Committee. An informed consent was signed by the participant or the accompanying guardian. Accordingly, 32 (18 adolescents and 14 adults) patients (14 males and 18 females) have been included in this study with an age range of 14-28 years and an age average of 17.5 years.

All subjects were treated with fixed orthodontic

appliances in both dental arches with direct bonded appliances (straight wire appliances). Fixed orthodontic appliances were cemented to teeth surfaces with glass ionomer bond (GC Fugi I®, GC Corporation, Tokyo, Japan), and consisted of bands, nickel-titanium arch wires (3M Unitek®, 3M company, CA, USA), stainless steel brackets of 0.022" slot, Roth prescription (3M Unitek®, 3M company, CA, USA), elastomers (elastic) and/or metal ligatures (3M Unitek®, 3M company, CA, USA). The types of malocclusion and extraction patterns were not considered as selection criteria. Only routine oral hygiene instructions for orthodontic patients were given to all patients. Neither professional oral prophylaxis nor additional instructions of oral hygiene were given during the study.

Baseline data, including clinical periodontal parameters and saliva samples, was obtained just before bonding of the fixed orthodontic appliances, and 3 months later. Periodontal indices recorded included plaque index (PI), probing pocket depth (PPD) (5), and bleeding on probing (BOP) (16). All clinical examinations were performed by the same examiner.

Mouth-rinsing samples were collected immediately after PI recording and before the assessment of BOP and PPD. Plaque samples were collected by rinsing with 10 ml sterile saline (0.9 w/v Sodium Chloride) for 30 seconds and expectorating in sterilized tubes (VACUETTE®, Austria). Samples were stored at -70°C for preparation for real-time polymerase chain reaction (RT-PCR) analysis. DNA in each plaque sample was extracted using an enzyme lysis buffer; purified by DNeasy (QIAGEN, QIAGEN Ltd, UK) and run on Quantitative Real Time PCR (7000 Sequence Detection System, Applied Biosystems, UK) using species-specific primers (17,18). Samples were thawed at 37°C in a water bath, centrifuged for 8 minutes (Centrifuge 5402, eppendorf, Germany). Regular PCR protocol was followed in which the QuantiTect SYBR Green PCR Kit was used for the detection of amplified DNA.

Each plaque sample was tested for the Pg, Aa, Tf, and a universal mix of all these bacteria. The sequences of the primers used have been utilized in previous reports (17,18).

Statistical analysis was performed using SPSS-16 (SPSS®, Chicago, USA) software. Paired test (student's t-test) was used for comparison of differences in PI and PPD before and 3 months after bonding of the fixed orthodontic appliances. Chi-square test was applied to identify differences between the two time sets for BOP.

For analysis of microbiological data, the average of 2 readings was used and the Wilcoxon test was used for testing the difference in bacterial concentrations of mouthwash samples at (T0) and (T1). P value less than 0.05 were considered significant. Correlations of the periodontal indices between (T0) and (T1) were tested using Pearson's correlation. Correlations of the bacterial species concentrations with each other and with the periodontal indices between (T0) and (T1) were tested using Spearman's correlation. The mean of the measurements from each tooth for each periodontal index was calculated.

3. Results

There were significant differences in all clinical periodontal parameters (PPD, PI and BOP) between the baseline and 3 months after the bonding of the fixed orthodontic appliances. A weak, but significant, correlation was found between PI and BOP (coefficient = 0.53). However, weak, but significant, correlations were found between BOP and PI (coefficient = 0.66) and with PPD (coefficient = 0.53).

Changes in BOP were significantly higher in males ($P<0.001$), while changes in PPD and PI were significantly higher in females ($P<0.01$). The values of all periodontal indices were higher at T1 for adolescents and adults; however, the differences were significant for PPD and PI scores in both, while BOP differences were significant in adolescents. On the other hand, changes in PPD were higher in the adult group than in the adolescent group, while changes in BOP and PI were higher in the adolescent group.

Females had higher (but insignificant) positive differences for microorganisms in case of Aa and Pg and equal positive differences for microorganisms with males in case of Tf. Each of Aa and Pg was detected in 34.4% of the patients at T0, while Tf was detected in 18.8%. At T1, the percentages of Aa and Pg reduced to 21.9% and 25%, respectively. The percentage of Tf increased to 31.3%.

The distribution of positive detection according to age shows that there was a decrease of detection of Aa in both age groups and an increase of detection of Tf in both age groups and an increase of detection of Pg in the younger age group and a decrease of detection of Pg in the older age group.

Tf had the lowest mean concentration of 0.66 DNA

copy/sample at baseline and after 3 months, where the mean concentration of this microorganism was 1.47 DNA copy/sample. Next came Aa with a mean concentration of 402.84 DNA copy/sample at T0, and 1970.41 DNA copy/sample at T1. The highest concentration was that of Pg at both time points, with a mean concentration of $3.02*10^4$ DNA copy/sample at T0, and a mean concentration of $1.06* 10^6$ DNA copy/sample at T1.

There were differences in the bacterial profiles between mouthwash samples collected at baseline (T0) and at end point (T1). The concentrations of Aa, Tf, and Pg were higher in mouthwash samples at (T1), however, the differences were not significant (Table 1).

Table 1: Changes between bacterial species concentrations

Bacteria	Mean ± SD (T0)	Mean ± SD (T1)	P-value
Aa	402.84±1079.32	1970.41±9983.75	0.3
Tf	0.66±1.72	1.47±4.53	0.3
Pg	30188.94±133078.48	1063968±5327275.4	0.2

Concentrations of Aa and Pg in male patients were higher at T0 than at T1, while Tf concentration was higher at T1. The only significant difference was in the case of Pg. The concentrations of all bacterial species were higher in female patients at T1 than those at T0, however, the differences were not significant. Differences between the means of concentrations of Tf at T1 were higher in males, whereas differences in means of concentration for Aa and Pg were higher in females (Table 2).

Table 2: Changes between bacterial species concentrations according to gender

Bacteria	Gender	T0	T1	Changes
Aa	Male	17.36	5.29	- 12.07
	Female	702.67	3498.83	2796.16
Tf	Male	0.57	2.29	1.72
	Female	0.72	.83	0.11
Pg	Male	57929.64	280.64	-57649*
	Female	8612.83	1891280.38	1882667.55

*P <0.05.

The changes between the means of concentration of all bacterial species were less in the adult group than in the adolescent group. The means of concentration of all bacterial species were higher in adolescents at T1 than at T0, but the differences were not significant. For the adult group, the means of concentration of Aa and Pg were higher at T0, while the concentration of Tf was higher. The difference was significant only in the case of Pg (Table 3).

Table 3: Changes between bacterial species concentrations according to age

Bacteria	Age group	T0	T1	Changes
Aa	Adolescent	600.00	2993.33	2393.33
	Adult	26.45	17.55	- 8.9
Tf	Adolescent	0.48	1.52	1.04
	Adult	1.00	1.36	0.36
Pg	Adolescent	3612.95	1620824.93	1259529.94
	Adult	80924.91	877.48	- 80047.43

Among the 3 bacterial species, Aa concentration was only significantly correlated (P<0.05) with periodontal

indices at baseline with PPD (coefficient = 0.38). On the other hand, at the end point, there were no significant correlations between periodontal indices and the concentrations of the 3 bacterial species (Table 4).

Table 4: Correlation coefficients between periodontal indices and bacterial species concentrations at T0 and T1

Bacteria	PPD		PI		BOP	
Time	T0	T1	T0	T1	T0	T1
Aa	0.38*	0.20	0.19	0.01	0.26	0.004
Tf	0.20	0.15	0.15	0.03	0.26	0.02
Pg	0.10	0.01	0.15	-0.20	0.10	-0.22

*P <0.05.

4. Discussion

The primary objective of this study was to detect and quantify the presence of specific periodontal pathogens; namely, Aa, Tf, and Pg, in mouth rinse samples from orthodontic patients. Measurements were taken at two time points; prior to the bonding of fixed orthodontic appliances and 3 months post-bonding.

Real-time PCR was selected as the detection method due to its high sensitivity, being capable of identifying even a single bacterial cell, and its ability to provide accurate quantification of bacterial load, offering a significant advantage over traditional culture techniques and nested PCR (8,14). Mouth rinse sampling was employed in this study for its simplicity, non-invasiveness, and proven efficacy in collecting periodontal pathogens (13,14).

Several periodontal indices are reported in the literature (3-6,19). For periodontal health assessment, the PI and PPD were chosen (15,16). These indices are well-suited for longitudinal studies, provide numerical data, and are widely accepted in the literature (20).

A statistically significant increase was observed in all recorded periodontal parameters (PI, PPD, and BOP) at the 3-month endpoint compared to baseline. The increase in plaque index scores aligns with previous research indicating plaque retention associated with orthodontic appliances. This is likely attributable to challenges in maintaining optimal oral hygiene due to the presence of fixed appliances, despite oral hygiene instructions (3).

The observed increase in probing pocket depth also resonates with several studies reporting increased probing depth during orthodontic treatment (21). However, some studies, such as Huser et al. (3), reported only a tendency toward increased PPD around teeth with orthodontic bands. Such discrepancies in the literature may arise from variations in orthodontic appliance types, individual oral flora, oral hygiene practices, measurement methodologies, and differences in patient age and sample size.

The significant increase in BOP at the end point was consistent with numerous studies linking fixed orthodontic appliances to increased BOP (4,5,6,22). A significant correlation was found between PI and BOP at baseline, which strengthened further at the endpoint. The PPD also significantly correlated with BOP at the endpoint (T1), but not at baseline (T0). These findings collectively suggest that orthodontic treatment can contribute to periodontal damage (4).

At the endpoint, all periodontal index values were higher in both males and females compared to baseline. Specifically, PPD and PI scores showed significant increases in both genders. In contrast, BOP differences were significant only in males. When comparing changes in mean periodontal parameters between genders, males exhibited higher changes in BOP, while females showed greater changes in PPD and PI.

Similarly, all periodontal index values were higher at the end point for both adolescents and adults. Significant differences were observed for PPD and PI scores in both age groups, whereas BOP differences were significant only in adolescents. Changes in PPD were more pronounced in adults, while changes in BOP and PI were greater in adolescents. These findings are partly consistent with a prior study that reported minimal periodontal inflammation in adolescents undergoing fixed orthodontic treatment when adequate plaque control was maintained (23). The observed reduction in PI with increasing age post-orthodontic treatment might be attributed to heightened oral hygiene

awareness in older patients or stabilized hormonal levels (5). Further investigation is warranted to explore these factors.

Comparisons of baseline and endpoint bacterial profiles were based on mean bacterial concentrations of Aa, Tf, and Pg in mouthwash samples. These microorganisms were selected due to their extensive investigation as key periodontal pathogens in the literature (24,25).

While being not statistically significant, our results indicated a trend of increased mean bacterial concentrations in mouthwash samples at the endpoint compared to baseline. This trend is noteworthy, as it suggests a potential increase in periodontal pathogen load in orthodontic patients, which could predispose susceptible individuals to periodontal disease. This observation aligns with previous reports that placement of orthodontic bands promotes subgingival plaque growth and influences subgingival microorganism composition in the absence of optimal oral hygiene (21). Furthermore, some longitudinal studies have documented microbiological shifts during orthodontic treatment, including an increase in total cultivable flora and a shift toward a more anaerobic environment, with increased percentages of black-pigmented gram-negative anaerobes (3). The rise in bacterial concentrations is also consistent with the findings of Paolantonio et al, who, in a longitudinal study, reported that orthodontic appliances promote supragingival plaque accumulation and hinder proper oral hygiene, consequently altering subgingival plaque ecology to favor the colonization and overgrowth of putative periodontal pathogens (22).

In our study, Tf exhibited the lowest mean concentration, followed by Aa, with Pg having the highest concentration. The low prevalence of Tf (11-15%) in periodontally healthy or minimally diseased individuals has been reported by several authors (24,26). Regarding Pg, which showed the highest concentration in our study, reports in the literature are conflicting. Di Murro et al detected Pg in 100% of sites in healthy individuals or patients with gingivitis (27), while other studies reported lower percentages or even its absence in healthy children, adolescents, and adults with minimal disease (24). These discrepancies may be attributed to variations in detection methods, sample size, and patient age. The disappearance of certain microorganisms in some patients at the endpoint might be due to unrecorded use of mouthwashes or antibiotics, or alternatively, the emergence of antagonistic species within the plaque that suppress existing bacteria through bacteriocins or environmental factors (28,29).

At baseline (T0), male patients showed a tendency toward higher concentrations of Aa and Pg compared to the endpoint (T1), though this difference was only significant for Pg. Conversely, Tf concentration was higher at the endpoint in males, but this difference was not significant. In female patients, concentrations of all bacterial species were higher at the endpoint than at baseline, although these differences were not statistically significant. Changes in Tf concentration were more pronounced in males, while changes in Aa and Pg concentrations were higher in females. Mullally et al reported similar concentrations of these organisms in males and females; however, their study utilized culture techniques, in contrast to real-time PCR employed in the current study, and involved an older patient demographic (18). Ethnic differences may also contribute to these discrepancies.

In adolescents, mean concentrations of all bacterial species were higher at the endpoint than at baseline, though these differences were not statistically significant. In adults, Aa concentration tended to be higher at baseline than at the endpoint, while Tf was higher at the endpoint. Notably, Pg concentration significantly decreased after 3 months of bonding in adults. Overall, changes in bacterial concentrations were smaller in adults than in adolescents, which aligns with previous studies (13,26). Prior research has reported the presence of Aa and Pg in all healthy subjects aged 19-38 years (13), while other studies have indicated that Aa decreases with age and Tf increases with age (24,26).

Discrepancies between our results and those of other studies may stem from methodological differences, study duration, oral hygiene practices, sample demographics, or ethnicity. At both baseline (T0) and endpoint (T1), concentrations of bacterial species were not significantly correlated with each other, with the exception of Aa which showed a significant correlation with Pg. Several studies have reported an increase in the numbers of Aa and Pg with increasing probing pocket depth (30, 31). However, Haffajee et al. reported a very strong relationship between Tf and Pg when PPD increased in both healthy and periodontitis patients (26). These variations in results can be explained by differences in methods and techniques, sample age and

size, oral hygiene habits, periodontal health status, ethnicity, and other demographic variables.

Our results indicated that the concentrations of bacterial species were not correlated with periodontal indices at baseline, except for Aa, which significantly correlated with PPD. At the end point, the concentrations of bacterial species were not correlated with any of the periodontal indices. These findings align with a previous cross-sectional study (22) which observed no significant difference in Aa plaque levels between patients with and without orthodontic appliances.

The limitations of this study include the small sample size and the short follow-up period. Nevertheless, its findings provide a valuable baseline for future research in our patient population, allowing for comparisons of the impact of different orthodontic appliances across various age groups and over extended follow-up durations.

5. Conclusions

Within the limitations of this study, it has been demonstrated that following bonding of fixed orthodontic appliances, periodontal parameters and specific periodontal pathogens increased significantly within 3 months following the bonding of fixed orthodontic treatment.

Higher concentration of bacteria in mouthwash samples was demonstrated in patients with fixed orthodontic appliances compared to the baseline; however, the concentrations of bacterial species were not correlated with periodontal indices at the end point. These findings might highlight the importance of periodontal evaluation both before and during fixed orthodontic treatment, as well as emphasizing the importance of oral hygiene measures for these patients.

Conflict of Interests

The authors declare that they have no conflict of interests.

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