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ORIGINAL ARTICLE

Hong Kong Dent J 2012;9:11-20

Periodontal and microbiological status of patients undergoing orthodontic therapy

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ABSTRACT

Objective. To determine the benzoyl-DL-arginine-naphthylamide test scores of periodontopathic bacteria, including that of red complex bacteria *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythia*, as well as the organism morphotypes, probing depths, and plaque scores in patients undergoing orthodontic treatment. **Methods.** In a prospective study, plaque samples were collected from 13 patients at baseline, four monthly visits after treatment, and at 30 days after removal of the appliance. A benzoyl-DL-arginine-naphthylamide test was performed to identify the periodontal pathogens. Dark field microscopy was used to recognize the morphotypes. The O'Leary Plaque Index and probing depths were assessed at each test interval to determine the hygiene and periodontal status of the patients. Data were analyzed using analysis of variance and Tukey's Honestly Significant Difference test. **Results.** Significant increases in plaque score, probing depths, and benzoyl-DL-arginine-naphthylamide scores were found at each interval after placement of orthodontic appliances. However, the levels returned to baseline after removal of the appliances. Dark field microscopy confirmed increases in small spirochetes (7.5%), large spirochetes (2.5%), non-motile rods (9%), fusiforms (5%), and filaments (1%) with orthodontic treatment. **Conclusions.** Patients undergoing orthodontic therapy have an increase in plaque accumulation, probing depth, and microbial activity that may be associated with periodontal destruction. Thirty days after removal of the orthodontic appliance, the plaque score, probing depth, and benzoyl-DL-arginine-naphthylamide test score returned to almost baseline level.

Key words: Bacterial adhesion; Orthodontic brackets; *Porphyromonas gingivalis; Treponema denticola*

Introduction

Fixed orthodontic appliances introduce mechanical plaque (biofilm) traps and impair plaque removal, proper oral hygiene, and gingival health [1]. This promotes specific alteration in the oral environment, including decreased pH, increased plaque accumulation [2], and elevation of microbial counts in the saliva and the biofilm [3,4]. Gingivitis may develop in patients who do not institute proper oral hygiene measures and can become quite profound in 21 days [5]. Patients often exhibit gingival hypertrophy, bleeding, increased plaque accumulation, and calculus formation during orthodontic treatment [6]. Oral hygiene measures are recommended because bands, brackets, ligature wires, and

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elastics encourage the accumulation of microbial flora and food residues [7,8]. In time, the plaque accumulation around orthodontic appliances may cause periodontal disease and caries [9]. A higher level of oral microorganisms increases not only the risk of caries and periodontal diseases [10], but also the chances of systemic complications [11-14], since certain orthodontic procedures can cause transient bacteremias [15-17].

Three periodontal pathogens that are known to inhabit the plaque and contribute to periodontal disease are *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* [18]. These bacteria are anaerobic periodontal pathogens capable of initiating periodontal destruction. In many studies, the presence of these bacteria has been correlated with the common forms of adult periodontitis [19-28]. Although most patients undergoing orthodontic therapy tend to be younger and are less likely to experience periodontal disease, the host resistance to bacteria is compromised in orthodontic patients due to the appliances on the teeth. Studies using DNA probes have proven a close relationship between the three aforementioned bacteria in the subgingival plaque of patients with periodontal disease [1,4,19-28]. These bacteria are termed the 'red complex' among five bacterial complexes that group bacteria together based on relationships and associations. These three bacteria have a symbiotic relationship in a highly ordered system or biofilm, which serves as protection, facilitates communication, and promotes adhesion to the oral environment [18].

The benzoyl-DL-arginine-naphthylamide (BANA) test (Knowell Periodontal Technologies, Toronto, Canada) has been used by many investigators to rapidly screen contributing putative red complex periodontal pathogens in dental patients [4,22-28]. Although some controversy exists, the BANA test is accurate and efficient in determining whether the red complex bacteria are present in a plaque sample [4,22-28]. Patients who demonstrate susceptibility to these bacteria can be identified and monitored for increases in the bacterial load with the BANA test. To complement data obtained from the BANA test, dark field microscopy was used to ascertain the morphotypes of periodontopathogens residing in the plaque. The purpose of this study was to determine the BANA-positive periodontopathogens and periodontal health in patients undergoing orthodontic therapy. We hypothesized that: (1) there is no statistically significant difference between the level and activity of periodontopathic bacteria (BANA test and microscopy analysis) before, during, or after orthodontic treatment; and (2) there is no statistically significant difference in any of the clinical indicators of periodontal health (probing depth and plaque score) before, during, and after orthodontic treatment.

Methods

Recruitment of patients

Fifteen orthodontic patients, with a mean (standard deviation [SD]) age of 14 (2) years, were recruited from the Department of Orthodontics at the West Virginia University School of Dentistry in the USA. The selection criteria included presence of full adult dentition, comprehensive orthodontic treatment with fixed appliances (metal brackets) in the upper and lower arches (archwire sequence included 0.012-inch nickel titanium [NiTi], 0.018-inch NiTi, 0.018-inch stainless steel [SS], and 0.018 x 0.025-inch SS wires), no pre-existing periodontal disease, no antibiotic treatment in the previous 14 days, availability for data collection during the first 4 months of orthodontic treatment (namely 'visit 2' to 'visit 5', respectively), and one recall visit 30 days after completion of orthodontic treatment (namely 'visit 6'). Institutional review board approval was obtained from the West Virginia University and informed written consent was obtained from all patients. Two patients were disqualified due to missed appointments. Standard oral hygiene instructions and oral hygiene aids were given at the beginning of orthodontic treatment.

Collection of plaque sample for microbiological analysis

Prior to placement of the orthodontic appliances and pumicing, a plaque score was recorded, probing depths were measured, and five plaque samples were taken for laboratory analysis. Four Stim-U-Dent interdental cleaners (Johnson and Johnson, New Brunswick [NJ], USA) were used for collection of the plaque samples. For consistency, plaque samples were collected from each quadrant from the interproximal area between the first molars and second premolars by one operator. The samples were taken with

the tip of a Stim-U-Dent, placed slightly subgingivally, with a scraping motion. Samples were transferred to the BANA Reagent Strips (BANAMet LLC, Ann Arbor [MI], USA). The BANA reagent (Perioscan; Sirona Dental Systems, Inc., Long Island City [NY], USA) and strips were plastic cards onto which reagent matrices were affixed. The Stim-U-Dent interdental cleaners with the plaque were scraped onto the inferior reagent matrix with linear scribes. This was done to provide a maximum length of plaque to evaluate the BANA reaction. The inferior reagent matrix, to which subgingival plaque samples were added, was impregnated with buffered N-BANA. The superior reagent matrix was impregnated with stabilized Evans black dye (chromogenic diazo reagent). The reaction was activated when the inferior strip was folded over the superior strip, which had been moistened with distilled water, thus enabling any naphthylamide liberated from the inferior reagent matrix (BANA impregnated strip) to diffuse into the superior strip. Naphthylamide could then react with the Evans black dye, yielding a permanent blue-black color spot on a pale reddish-brown background. An incubation period of 15 minutes at 55°C was used to increase the sensitivity of the Perioscan card. The reagent card was blotted lightly with distilled water from a Q-tip, folded over, and subsequently taped and placed in the BANA incubator slits (Knowell Periodontal Technologies, Toronto, Canada) for 15 minutes. The BANA incubator knob was positioned in the number 3 position or 'confirmation' red setting to ensure 15 minutes of incubation.

After incubation, the reagent cards were stored in sealed light-protected envelopes and analyzed at the end of the regular orthodontic appointment by the investigators. The results were examined 24 hours later for intra-rater reliability. The Perioscan reagent test criteria were read as follows: (1) no color observable on the background signifies a titer of periodontal pathogens of insignificant numbers (<10 000 colony-forming units [CFU]) or a negative recording; (2) a small, faint blue on the background signifies a titer of detectable numbers of periodontal pathogens of some clinical significance (10 000-99 999 CFU) or a weak positive recording; and (3) a distinct and darker blue color on the background signifies a substantial and clinically relevant titer (≥100 000 CFU) of pathogens present in the plaque sample or a positive recording. Data of the above three categories were recorded as negative, weak positive, or positive, respectively.

For dark field microscopy, a plaque sample was taken from the lower right canine/premolar area with a Stim-U-Dent and placed in a stored liquid dental transport medium. A Stim-U-Dent was used to stir the solution and the vial was closed and sent to the Research Laboratory of Microbial Pathology, Department of Pathology of the University, under the direction of one of the investigators. The dark field microscopic analysis consisted of a quantification and qualification of the nine periodontal pathogenic morphotypes. The nine categories of qualification were as follows: small spirochetes, intermediate spirochetes, large spirochetes, motile rods, coccoid forms, non-motile rods, fusiforms, filaments, and yeast. A magnification of 10 x (ocular) and 40 x (nosepiece) gave the 400 x magnification factor of microscopy. The field was divided into quarters, with one quarter used to count 100 microorganisms. The investigator qualified and quantified the organisms based on their shape. Proportions were calculated for each of the organisms relative to the 100 total counts. These measurements served as the baseline (namely 'visit 1') against which the BANA scores were compared. These steps were instituted at each of the next 5 visits, for a total of 6 recordings.

Plaque score and probing depth

After the plaque samples were taken for the BANA test, probing depths of selected teeth and tabulated plaque scores were measured by one operator. Probing depths were measured with a Michigan periodontal probe (Hu-Friedy International, Chicago [IL], USA) and taken on the mesial, middle, and distal sides of the facial and lingual surfaces of the upper and lower first molars and second premolars (first premolars were used when second premolars were not present). Thus, 12 surfaces were probed in each of the four quadrants. The plaque score was then assessed using the O'Leary Plaque Index [29] whereby all teeth were disclosed with plaque-disclosing solution and each tooth had a mesial, distal, facial, and lingual surface scored for plaque. The plaque score was calculated by dividing the number of plaque surfaces by the total number of tooth surfaces.

At each subsequent appointment, plaque samples were taken for BANA test and dark field microscopic analysis, and the plaque scores and probing depths measured exactly as described above.

Data analysis

Plaque scores were measured and recorded as percentages. Probing depths were measured and recorded as millimeters (mm). The BANA test results measured as negative, weak positive, or positive were converted to 0, 1, and 2, respectively for data analysis. Plaque scores, probing depths, and BANA scores were consolidated and averaged for all patients at each visit to determine general trends between patients and time intervals. Means, SDs, and relative frequencies were tabulated. Analysis of variance (ANOVA) was used to analyze data, with a P value of ≤ 0.05 regarded as statistical significance. A Tukey's Honestly Significant Difference (HSD) test was used to examine differences between patient visits or months.

Results

The mean plaque scores at baseline and each subsequent visit are shown in Figure 1a. The ANOVA test revealed significant differences between patient visits (P<0.001). The Tukey's HSD comparison test revealed significantly higher mean (±SD) plaque scores for the subsequent four visits (visits 2-5) during orthodontic treatment (47.92±13.05%; 52.23±14.15%; 51.85±13.96%; 55.15±19.91%) compared with baseline (38.15±14.84%) [P<0.05]. No significant differences were found between the plaque score obtained 30 days after removal of the orthodontic appliance (44.50±13.71%) and baseline. The individual O'Leary Plaque Index scores for each visit for all 13 patients are shown in Table 1. The ANOVA test revealed significant differences among patients (P<0.001), indicating variable differences between plaque scores both within and between patients.

The mean probing depths for all patients at baseline and each subsequent visit are shown in Figure 1b. The ANOVA test revealed significant differences between the 6 visits at P<0.001. The Tukey's HSD test revealed significantly higher mean (±SD) probing depths for visit 3 (2.71±0.65 mm; P<0.05), visit 4 (2.86±0.63 mm; P<0.05), and visit 5 (2.99±0.68 mm; P<0.05) than that for the baseline (2.45±0.57 mm). No significant differences were found between visit 2 (2.52±0.58 mm) and 30 days after removal of the appliances (2.52±0.55 mm). The probing depth averages for patients ranged from 2.1 mm to 3.3 mm. The individual probing depths for all 13 patients at each visit are shown in Table 2. The ANOVA

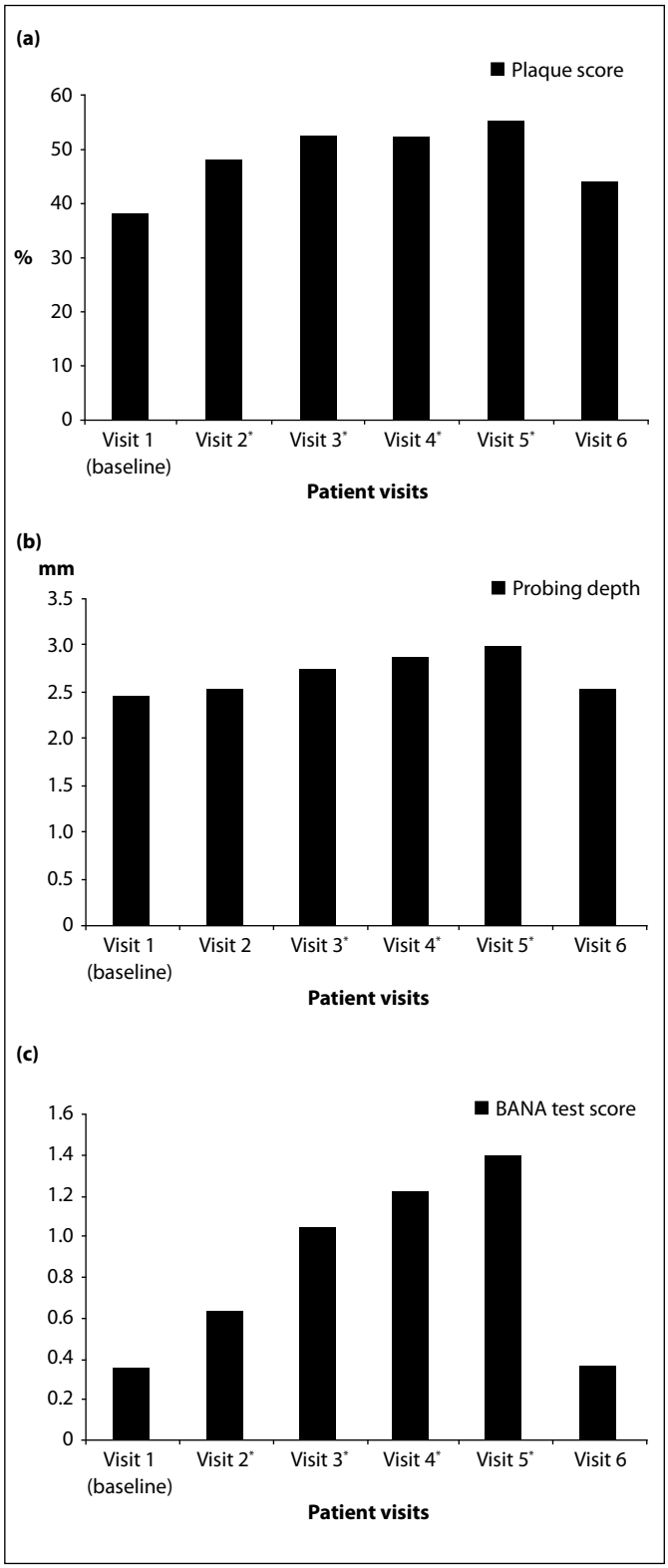

Figure 1 Mean **(a)** plaque score, **(b)** probing depth, and **(c)** benzoyl-DL-arginine-naphthylamide (BANA) test score of the patients at each visit

* Significantly different from baseline (P<0.05)

Table 1	Mean plaque score for each patient at each visit				
Patient No.	Plaque score (%)				
	Visit 1 (baseline)	Visit 2	Visit 3	Visit 4	Visit 5
1	38	40	39	48	50
2	23	43	62	45	43
3	33	30	34	34	33
4	50	51	68	79	72
5	58	74	72	53	65
6	24	36	31	38	42
7	27	41	56	37	60
8	63	61	52	66	58
9	27	49	53	51	54
10	51	57	63	62	79
11	64	55	62	68	69
12	48	55	54	59	56
13	18	29	30	34	33

Table 2	Mean probing depth for each patient at each visit				
Patient No.	Probing depth (mm)				
	Visit 1 (baseline)	Visit 2	Visit 3	Visit 4	Visit 5
1	2.60	2.65	2.70	2.90	2.80
2	2.40	2.35	2.68	2.75	3.00
3	2.45	2.40	2.45	2.70	2.85
4	2.40	2.68	2.85	3.00	3.20
5	2.50	2.40	2.43	2.75	2.80
6	2.25	2.42	2.80	3.00	3.25
7	2.56	2.68	2.70	2.90	2.90
8	2.25	2.50	2.60	2.80	2.95
9	2.42	2.60	2.75	2.89	2.95
10	2.42	2.52	2.58	2.90	3.24
11	2.19	2.60	2.90	2.89	3.25
12	2.60	2.70	2.73	2.74	2.75
13	2.70	2.73	2.74	2.80	2.90

test did not reveal significant differences among patients (P=0.08).

Benzoyl-DL-arginine-naphthylamide test

The mean BANA test scores for all patients at baseline and each subsequent visit are shown in Figure 1c. The ANOVA test revealed significant differences among the six time intervals (P<0.001). The Tukey's HSD test revealed significantly higher mean (±SD) BANA test scores during orthodontic treatment for visit 2 (0.63±0.49), visit 3 (1.04±0.47), visit 4 (1.22±0.47), and visit 5 (1.39±0.53) compared with baseline (0.35±0.50) and 30 days after removal of the appliances (0.36±0.49) [P<0.05]. The individual BANA test scores for each visit are shown in Table 3. The ANOVA test revealed significant differences among patients (P<0.001), indicating variability among patients.

Table 3	Mean benzoyl-DL-arginine-naphthylamide (BANA) test score for each patient at each visit				
Patient No.	BANA score				
	Visit 1 (baseline)	Visit 2	Visit 3	Visit 4	Visit 5
1	0.50	0	1.00	1.00	1.50
2	0.50	1.00	1.25	1.25	1.25
3	1.00	1.00	1.00	1.00	1.50
4	0.75	0.75	2.00	1.50	1.50
5	1.00	0.50	1.00	1.00	1.00
6	0	0	1.00	1.00	1.00
7	0	1.00	1.00	1.00	1.00
8	0.50	1.00	1.00	1.25	1.50
9	0	0	0.50	1.25	1.50
10	0.50	1.00	1.00	1.50	1.25
11	0.50	1.00	1.25	1.25	1.75
12	0	0.25	0.50	0.75	1.50
13	0.75	1.00	1.00	1.25	1.50

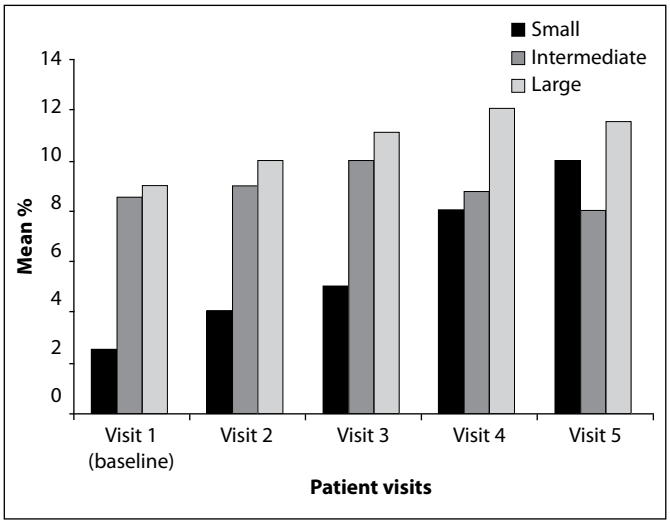

Figure 2 The proportions of small, intermediate, and large spirochetes in the plaque organisms over time

Dark field microscopy

Dark field microscopy was performed on plaque samples, and nine morphotypes were evaluated. As shown in Figure 2, the count for small spirochetes showed a steady increase in the numbers and relative percentages during orthodontic

treatment from 2.5% at the baseline to 10% for visit 5, representing a 7.5% increase in total organisms. The count for intermediate spirochetes showed an increase from 8.5% at baseline to 10% in visit 3 and declined in visits 4 and 5 down to 7.0%. The count for large spirochetes showed a 2.5% increase from 9.0% at the baseline to 11.5% in visit 5.

The count for motile rods showed a steady decrease from 20% at baseline to 11% in visit 5. The count for coccoid forms showed a large decrease from 28% at baseline to 12% in visit 5. The count for non-motile rods showed a steady 9% increase from 21% at baseline to 30% in visit 5 (Fig 3).

The count for fusiforms showed a slight but steady increase from 5% at baseline to 10% in visit 5. The count for filaments showed only a small increase of 1% from 5% at baseline to 6% in visit 5. The count for yeasts increased from 1% at baseline to 3% in visit 3, and decreased to 2% in visit 5 (Fig 4).

Discussion

This study examined the periodontal and microbiological status of patients undergoing orthodontic therapy. Although the main aim of the study was to evaluate the microbiological status, plaque scores and probing depths were also evaluated to assess the periodontal health of the patients. Several studies have investigated the effects of orthodontic treatment on periodontal health [30-35]. These studies concentrated mostly on the effects during treatment and the observation period was usually of short duration. Most of the investigators concluded that the overall gingival changes produced by appliances are transient with no permanent damage to the periodontal tissues. The long-term retrospective studies also concluded that no significant damage could be attributed to orthodontic treatment [35,36].

The current study found that the plaque scores increased over time in patients undergoing orthodontic therapy. The increase in plaque scores on teeth in this study is in agreement with several other studies [30,33,34,37]. However, other researchers have found that patients undergoing orthodontic therapy had either similar levels of plaque or a decrease in plaque levels from baseline [38,39]. The current study found that the greatest increase in plaque score occurred immediately after placement of orthodontic appliances, with an overall increase of 17%. Individual variations in plaque scores were also observed. Patient 3 exhibited the same plaque score at baseline and visit 5. When proper oral hygiene measures are instituted in patients undergoing orthodontic therapy, optimal hygiene and plaque control can be achieved [39,40].

This study also found an increase in mean probing depth with orthodontic treatment. The differences were generally small, but significant differences were found between visits 3, 4, and 5 from the baseline. The probing depths had more of a uniform increase than the plaque scores after the placement of orthodontic appliances. The measurement used was for probing depth, not clinical attachment loss, which are not the same. Probing depth is the measurement from the base of the sulcus, or pocket, to the free gingival margin. Attachment loss is the measurement from the cemento-enamel junction (CEJ) to the base of the sulcus or pocket. Loss of attachment is determined either by the direct measurement from the CEJ to the base, or by subtracting the distance from the gingival margin to the CEJ from the total probing depth. Thus, probing depth does not necessarily signify a loss of clinical attachment. Occasional studies have shown a statistically significant increase in the mean loss of clinical attachment in post-treated patients compared with untreated controls [5,41]. The probing depth increases found in this study were small, but significant. It would not be realistic to call the increase in probing depth as clinical attachment loss for several reasons. A common clinical sequela of orthodontic therapy is gingival hyperplasia. This is a result of the inflammatory process and plaque accumulation, and will cause the probing depth to increase, especially in the papillary areas. Another possible reason for increased probing depth in patients undergoing orthodontic therapy is the increased risk for penetration of the periodontal probe through the junctional epithelium in inflamed gingiva [42].

This study found an increase in BANA scores with orthodontic treatment. The scores at visits 2 to 5 were significantly greater than that at the baseline, showing the largest increase at 4 months after the orthodontic appliances were placed. The BANA test (Perioscan) has been shown to be a reliable indicator for the presence of the three putative periodontal pathogens (red complex): *P gingivalis, T denticola,* and *T forsythia* [4,19-28]. These bacteria have been correlated with the common forms of adult periodontitis. This

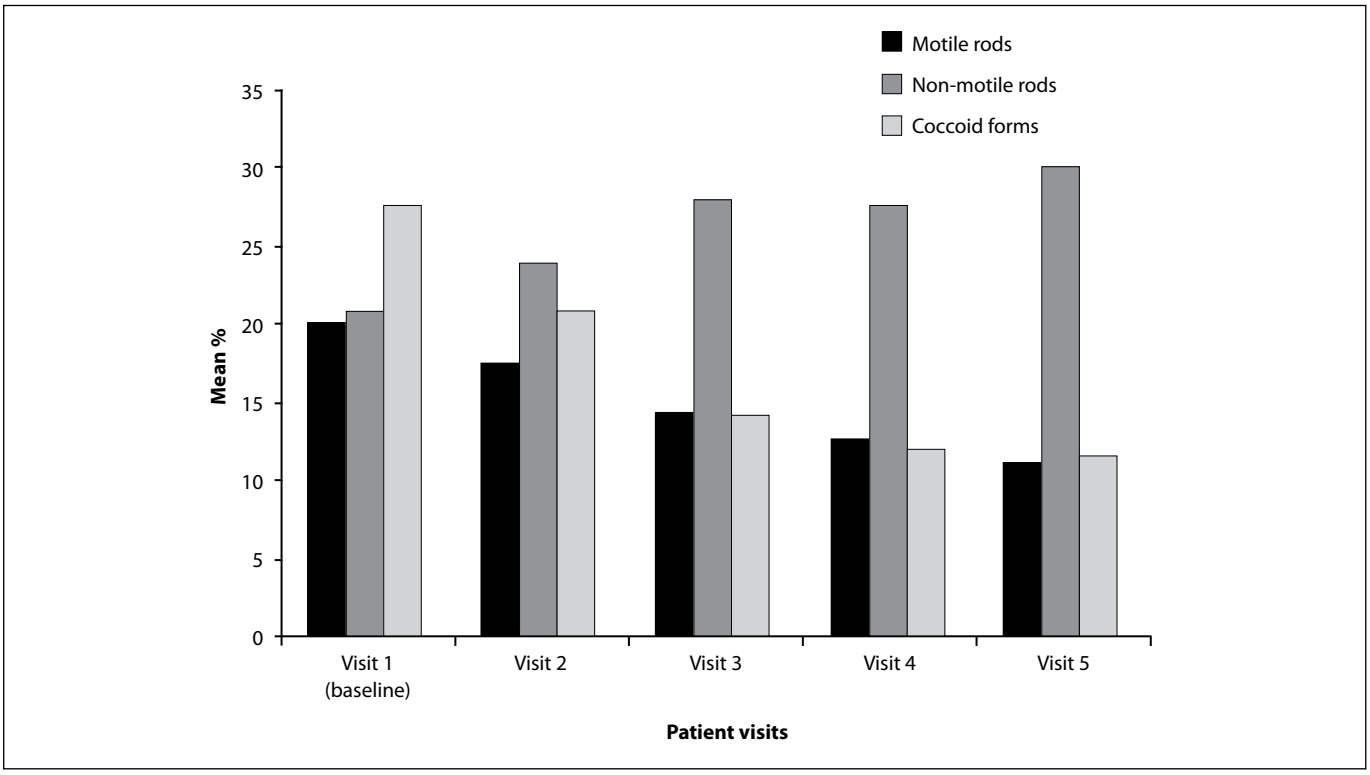

Figure 3 The proportions of motile rods, non-motile rods, and coccoid form in the plaque organisms over time

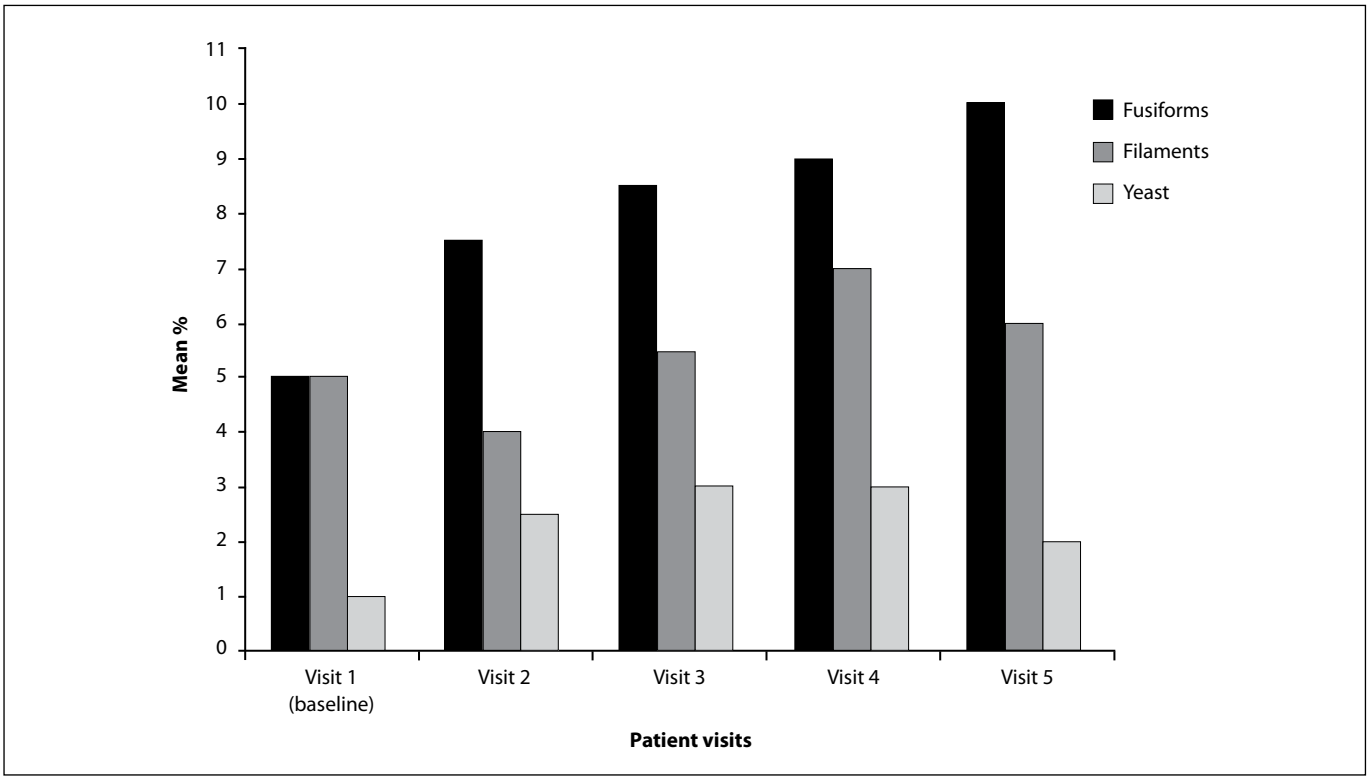

Figure 4 The proportions of fusiforms, filaments, and yeast in the plaque organisms over time

study involved two adults and 11 children or adolescents, and that these bacteria were found in the plaque samples of the latter, indicating that these three periodontal pathogens are found not only in adults, but also in children [4,43-45]. The BANA test indicated the presence of the three periodontal pathogens in all patients, with variations in levels detected. The results showed statistically significant differences in BANA scores over time in both adult and adolescent patients undergoing orthodontic therapy.

The results of dark field microscopy give a detailed picture of the microbial populations in the orthodontic plaque samples. The sampling in this study consisted of nine morphotypes of common oral microbes. The life cycle of bacterial plaque or biofilm has been established by several authors [33,38]. The early colonizers of the biofilm are mostly Gram-positive cocci and rods. These bacteria colonize on clean tooth surfaces with adhesins and they help to form the framework for the secondary colonizers, which colonize by congregation. The secondary bacteria may be Gram-negative, and have a preponderance of rods, fusiforms, cocci, filaments, and spirochetes. These bacteria include *P gingivalis, T forsythia*, and *T denticola*, and it is these organisms that cause concern. This study found statistically significant increase from baseline in the following morphotypes: small spirochetes, large spirochetes, non-motile rods, fusiforms, and filaments. The largest increase in population was seen in the small spirochetes, non-motile rods, and fusiforms. Huser *et al*. [33] found a significant increase in the percentage of spirochetes, motile rods, filaments, and fusiforms, and a concomitant decrease in cocci, the results of which are mostly consistent with ours, except that an increase in motile rods was not found. In the present study, a decrease in all coccoid forms was found. The results of this study are also comparable with previous studies on the progression of gingivitis and its associated microbiology [33,38]. The correlation between microscopy and BANA is not entirely coherent. *Treponema denticola* is a spirochete, and microscopy found an increase in both small and large spirochetes. However, *T forsythia* and *P gingivalis* are Gram-negative rods, and this study did not find an increase in motile rods. Nevertheless, the increases found in other morphotypes were of secondary colonizers that are associated with gingivitis. The use of checkerboard DNA-DNA hybridization allows a large number of DNA samples placed against a large number of DNA probes at the same time, thus enabling simultaneous determination of the presence of many bacterial species [46]. A study using this technique found metallic brackets in use for 1 month were multi-colonized by several bacterial species, including cariogenic microorganisms and periodontal pathogens [47].

This study also found a return to almost baseline level of plaque score, probing depth, and BANA score 30 days after removal of the orthodontic appliances. Clinically, the degree of gingival inflammation usually improves after removal of the appliances [5]. Some reports on the activity of the periodontopathic bacteria, after removal of orthodontic appliances, showed that the presence of these bacteria during treatment does not signify active periodontal disease. However, the presence of these bacteria may predispose the patient to periodontal disease. In addition, when host-immune resistance or equilibrium (locally and systemically) is altered or challenged, these bacteria may initiate periodontal disease. The biofilm may become unstable, allowing the red complex bacteria to play an active role in periodontal destruction. The high BANA score averages may be explained by the longer incubation time used (15 minutes instead of 5 minutes). This study used 15 minutes for incubation because it is believed that the 5-minute incubation period may not result in detectable levels of bacteria. Obviously, a detectable titer was found and the 15-minute incubation time may have been excessive. However, the increase in BANA scores is an important aspect of this study, because the operator consistently found higher bacterial titers over time.

Plaque is the primary etiological agent in almost all periodontal and gingival conditions. Thus, plaque control must be emphasized as the most important factor in preserving periodontal health in patients undergoing orthodontic therapy. Orthodontic appliances present a challenge to the proper removal of plaque from the tooth and gingival surfaces. The plaque may exist in equilibrium with the patient in the oral environment. However, this equilibrium may become unstable over time and with alterations in the external environment. Organisms commonly present in an early plaque are Gram-positive rods and cocci. Over time, these organisms are replaced by more Gram-negative and anaerobic organisms, which may initiate a periodontal reaction. The biofilm may become unbalanced and pathological as a result of changes in the environment from orthodontic appliances. The orthodontic appliances cause mechanical plaque traps where plaques may evolve

into a pathological state, because adequate oral hygiene measures are more difficult to achieve during orthodontic therapy. Thus, patient motivation and oral hygiene education are essential elements to a successful orthodontic outcome. It is not uncommon for patients undergoing orthodontic therapy to experience plaque challenges that may compromise their treatment results.

Conclusions

Plaque score and probing depths increased with successive orthodontic visits after placement of orthodontic appliances. There was a concurrent increase in the BANA-positive periodontopathogens *P gingivalis, T denticola*, and *T forsythia* with orthodontic treatment. Dark field microscopy confirmed an increase in the populations of small spirochetes, large spirochetes, non-motile rods, filaments and fusiforms, as well as a decrease in the populations of all coccoid forms and motile rods during orthodontic therapy. Thirty days after removal of the orthodontic appliance, the plaque score, probing depths and BANA score returned to almost baseline levels.

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