ORIGINAL ARTICLE

Clinical and Microbiological Evaluation of Stabilised Periodontal Patients Undergoing Early Stage of Orthodontic Treatment

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ABSTRAK

Rawatan ortodontik boleh menjejaskan keseimbangan mikrobiota oral yang memainkan peranan utama dalam etiologi penyakit periodontium. Kajian klinikal prospektif ini bertujuan untuk menilai kesihatan periodontal dan profil mikrobiologi pesakit periodontal yang sihat (Kumpulan 1) dan yang telah stabil (Kumpulan 2) selama tiga bulan pertama semasa rawatan ortodontik. Aplian ortodontik atas dan bawah tetap dipasang. Kesihatan periodontium dinilai menggunakan skor plak (PS), pendarahan pada probing (BOP) dan kedalaman poket (PD). 29 tapak telah diambil untuk persampelan plak subgingival. Sampel plak diinokulasikan pada agar Trypticase Soya Darah (TSBA) dan agar Trypticase Soya Bacitracin Vancomycin (TSBV) untuk penilaian aerob, anaerob, bakteria berpigmen hitam (BPH) dan Aggregatibacter actinomycetemcomitans. Semua ukuran diambil sebelum pendakap gigi dipasang (T0), 1 minggu (T1), 1 bulan (T2) dan 3 bulan selepas dipasang pendakap gigi (T3). Secara umumnya, kesihatan periodontium dalam kedua-dua kumpulan hampir sama. Selepas 1 minggu, bilangan aerob adalah lebih tinggi dalam Kumpulan 1 (88%) manakala anaerob adalah lebih tinggi dalam Kumpulan 2 (45%). A. actinomycetemcomitans lebih tinggi dalam Kumpulan 1 pada T0 dan T1 tetapi jauh lebih tinggi dalam Kumpulan 2 di T3. BPH adalah minimal pada setiap masa dengan tiada perbezaan signifikan. Oleh itu, semasa 3 bulan pertama rawatan ortodontik dijalankan, terdapat perubahan ketara

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dalam bilangan aerob-anaerob pada kedua-dua pesakit periodontal yang sihat dan stabil. Bakteria patogenik akan meningkat semasa rawatan awal ortodontik.

Kata kunci: mikrob, ortodontik, stabil periodontium

ABSTRACT

Orthodontic treatment may affect the equilibrium of oral microbiota which plays a major role in aetiology of periodontal disease. This prospective clinical study aimed to assess the periodontal health and microbiological profile of healthy (Group 1) and stabilised periodontal (Group 2) patients throughout three months of orthodontic treatment. Upper and lower fixed orthodontic appliances were bonded. Periodontal health was assessed using plaque score (PS), bleeding on probing (BOP) and pocket depth (PD). 29 sites were taken for subgingival plaque sampling. Plaque samples were inoculated on Trypticase Soy Blood Agar (TSBA) and Trypticase Soy Bacitracin Vancomycin (TSBV) agar for assessment of aerobe, anaerobe, black pigmented bacteria (BPB) and *Aggregatibacter actinomycetemcomitans*. All the measurements were taken before bonding (T0), 1 week (T1), 1 month (T2) and 3 months post-bonding (T3). Generally, periodontal health in both groups were almost similar. After 1 week, the number of aerobes was significantly higher in Group 1 (88%) while the anaerobes were significantly higher in Group 2 (45%). *A. actinomycetemcomitans* was higher in Group 1 at T0 and T1 but was significantly higher in Group 2 at T3. BPB was minimal at all time with no significant difference. Thus, during the first 3-month of orthodontic treatment, there were significant changes in the number of aerobes-anaerobes in both healthy and stabilised periodontal patients. Pathogenic bacteria would increase during early treatment of orthodontics.

Keywords: microbe, orthodontic, stabilised periodontal

INTRODUCTION

Orthodontic appliances are not just for children anymore. Nowadays, the dentofacial aesthetic has become a focus on most adults and this leads to increase demand for adult-orthodontics. In adults, special attention must be given to the periodontal status because they are more likely to have already experienced the periodontal disease.

Chronic periodontitis is a dental disease that progresses slowly and without pain (Shaddox & Walker 2010). The clinical sign of chronic periodontitis, such as inflammation pocket formation, attachment loss, and bone loss are considered to be due to the direct, site-specific effect of subgingival dental plaque accumulation. As a result of this local

effect, deep pocketing and bone loss may occur only on one particular surface of a tooth.

Bacterial colonisation in periodontal patients was found to be different from healthy patients. Periodontal disease resulted from colonisation of subgingival bacteria. Examples of the pathogenic periodontal bacteria that resulted in chronic periodontitis are the *Bacteroides forsythus, Porphyromonas gingivalis* and *Treponema denticola* (Maddi & Scannapieco 2013). *P. gingivalis* is referred to as black pigmented bacteria because they produce black or darkly pigmented colonies (BPB). *Aggregatibacter actinomycetemcomitans* is one of the bacteria that plays a major role in the more aggressive type of periodontal diseases. *A. actinomycetemcomitans* has the potential to adhere tightly onto the tooth surface and invade the gingivae epithelium (Raja et al. 2014).

Bonding an orthodontic fixed appliance in patients has been shown to alter the patient's microbiota (Arab et al. 2016; Topaloglu-Ak et al. 2011; Liu et al. 2011; Shukla et al. 2017; Kim et al. 2012; Sandić et al. 2014; Shirozaki et al. 2020). Orthodontic appliances changed the *A. actinomycetemcomitans* population (Paolantonio et al. 1999; Kim et al. 2010; Kim et al. 2012), especially when using the conventional metal bracket as compared to self-ligating brackets (Pejda et al. 2013). However, there are contradicting results from other studies that shown orthodontic appliances did not change the intraoral microbiota (Al-Anezi 2014) or even improved as shown by Sandić et al. 2014.

Few patients with a history of periodontal disease may be indicated for orthodontic treatment which consequently may result in a change in their oral's microbiota. Currently, there is no study which assessed the microbiological profile of stabilised periodontal patients who underwent orthodontic treatment.

Thus, this study aimed to assess the periodontal health (plaque score, pocket depth and bleeding on probing) in healthy and stabilised periodontal patients throughout three months of orthodontic treatment. Furthermore, we compared quantitatively the general microbiological profile of aerobe, anaerobe, *A. actinomycetemcomitans* and black pigmented bacteria throughout the 3 months of orthodontic treatment in healthy and stabilised periodontal patients.

MATERIALS AND METHODS

This prospective clinical study recruited orthodontic patients aged 16-55 years, who were conveniently selected from the orthodontic clinic at the Faculty of Dentistry, Universiti Kebangsaan Malaysia. Written informed consent was sought prior to the start of the study. The study protocol was approved by the University Research Ethics Committee (UKM 1.5.3.5/244/SPP2).

The subjects were divided into two groups. The subjects with healthy periodontium which acted as a control group (Group 1), while subjects with a history of chronic periodontitis but has been stabilised acted as the tested group (Group 2). All subjects in both

groups must have minimum dentition of 20 teeth and were not taking any antibiotic at least for 3 months before and during the orthodontic treatment. The inclusion criteria for Group 1 were: i) no history of periodontal disease and periodontal therapy; ii) no bleeding on probing, any pocket formation 4 mm, or radiographic bone loss. The inclusion criteria for Group 2 were patients with a history of periodontal disease, but have been stabilised at least 3 months before the start of orthodontic treatment. The exclusion criteria were non-cooperative patients, smokers and pregnant or lactating women.

All patients received upper and lower fixed orthodontic appliances. Patients underwent full mouth supragingival and subgingival ultrasonic scaling at two weeks before bonding. The archwire sequences for the 3-month study were; 0.014-inch nickel-titanium (NiTi) (Truforce, Ortho Technology Inc., Florida USA) for 2 months, followed by 0.018 inch NiTi (Truforce), Ortho Technology Inc., Florida USA) for 1 month.

For the periodontal health assessment, the amount of plaque (PS), mean of pocket depth (PD) and the number of positive gingival bleeding sites (BOP) were recorded. The presence of plaque (PS) was scored using the Silness and Loe Index (Silness and Loe 1964). All teeth were checked in the mesial, distal, facial, and lingual surface and given a score of 0 to 3 depending on the amount of plaque. PS for each patient was calculated using the following formula; the number of plaque contains surfaces divided by

the total number of available tooth surfaces.

The amount of periodontal attachment loss was assessed by measuring the periodontal PD using William's periodontal probe. The measurements were taken on the mesial, middle, and distal of facial and lingual surfaces of the upper and lower teeth. This procedure was calibrated to exert 20 g of probing force. The score was calculated with the following formula: the total average PD (in millimetres) in a tooth divided by the number of teeth presence. After 15 seconds of PD measurement, any presence of gingival bleeding site was noted as 'bleeding on probing' score (BOP). The total number of positive bleeding sites were recorded for each patient.

These clinical readings were taken before placement of fixed appliance (T0), 1 week (T1), 1 month (T2), 3 months post-bonding (T3) by a single examiner. A total of 34 sites were taken for plaque sampling from each group. For Group 2, the subgingival plaque was collected from the periodontally involved gingival sulcus, identified 2 weeks before bonding procedure, and from match sites in Group 1 using sterile Gracey's curette. The selected site was isolated with sterile cotton rolls. Prior to sampling of the subgingival plaque, the supragingival plaque was carefully removed to avoid contamination. The gingival surface was air-dried and subgingival plaque samples were obtained using a sterile Gracey's curette, at the deepest part of the specified gingival sulcus. The collected plaque samples

were then transferred into a vial containing Thioglycollate; a reduced transport fluid (RTF). These samples were transported to the laboratory and processed within 4 hours. These subgingival plaque samplings were done before the placement of fixed appliance (T0), 1 week (T1), 1 month (T2), 3 months post-bonding (T3).

Serial 10-fold dilutions were prepared in RTF. Dilutions of 10^{-1} to 10^{-5} were plated in triplicate onto trypticase soy blood (TSBA) and trypticase soy bacitracin vancomycin (TSBV) agar. After 7 days of aerobic incubation at 37°C and anaerobic incubation in an anaerobic chamber, the plates containing 30-300 colony-forming units were selected for enumeration. From these plates, each of the patients' colony-forming units (CFU) of aerobe, anaerobe and black pigmented bacteria were determined from the TSBA whereas the CFU of *A. actinomycetemcomitans* was determined from the TSBV. The total numbers of CFU of microbial flora (total bacteria count) were calculated by adding CFU of aerobe and anaerobe together. The mean percentages of aerobe, anaerobe, black pigmented bacteria and *A. actinomycetemcomitans* of all patients were calculated.

Gram stain technique was done to a few selected colonies for identification confirmation. The bacterial species were identified based on their distinct colony morphology and staining of smears prepared. From the TSBA and TSBV, selected black pigmented bacteria and *A. actinomycetemcomitans* colony were

subcultured for pure cultures. The pure cultures were identified by Gram staining procedure to differentiate the bacterial species into either the Gram-positive (blue stain) or Gram-negative (red stain).

Interclass coefficient correlation (ICC) was used to assess the examiner reliability of measuring the probing depth. A good level of agreement was reached between the examiner and the calibrated examiner for the assessment of probing depth. The ICC was >0.60 for each probing site.

Data was analysed using the Statistical Package for Social Sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA), for normality, and summary descriptive statistics. Comparisons between the groups and throughout the timeline were analysed using Mann-Whitney U and Wilcoxon-rank test as the data were found to be not normally distributed. The level of significance was set at $p < 0.05$.

RESULTS

A total of 68 subgingival sites were sampled i.e. Group 1, n=34 and Group 2, n=34. Subjects were aged between 16 to 55 years old with a mean age of 23.75 $\pm$ 6.12 in Group 1 and 30.8 $\pm$ 14.24 in Group 2.

Clinical Examination

Generally, the amount of plaque in Group 2 was lesser than Group 1 throughout the study, significantly at T0 and T1 ($p < 0.05$) (Table 1). In Group 1, plaque score was 1.25 at baseline and increased very minimally after 1

Table 1: Clinical measurements i.e. plaque score, pocket depth (in mm) and bleeding on probing score in healthy (Group 1) and stabilised periodontal subjects (Group 2) from baseline (T0), 1 week (T1), 1 month (T2) and 3 months (T3) in median and interquartile range (IQR).

	Group	T0	p-value	T1	p-value	T2	p-value	T3	p-value
Plaque score	Group 1	1.25 (0.45)	0.00*	1.40 (0.50)	0.02*	1.20 (0.4)	0.23	1.40 (0.5)	0.11
	Group 2	0 (1)		1 (1)		1 (1)		0 (1)	
Pocket depth (mm)	Group 1	1 (1)	1.00	1 (1)	1.00	1 (1)	1.00	1 (0.5)	0.51
	Group 2	1 (1)		1 (1)		1 (1)		1 (1)	
Bleeding on probing	Group 1	2 (1.5)	0.72	3 (2.5)	0.22	2 (2)	0.28	2 (3.5)	0.35
	Group 2	1 (1.5)		0 (1)		0 (0.5)		0 (2.5)	

*significant at p<0.05

week. The plaque score reduced at T2 but increased slightly at T3. The plaque score for Group 2 increased minimally after 1 week (T1) and 1 month (T2). However, the score reduced at 3 months similar to the baseline. Overall, the majority of subjects had good oral hygiene with very minimal plaque. All the changes within each group throughout this study were not statistically significant (p>0.05).

Overall, PD in Group 1 and Group 2 were similar throughout T0 to T3 (Table 1). All changes seen across groups and timeline were not statistically significant (p>0.05).

The number of bleeding on probing sites were presented as BOP score, as shown in Table 1. The BOP in Groups 1 varies within subjects as compared to Group 2 with an interquartile range of as minimum as 1.5 to as high as 3.5. A slight increment of BOP can be found in Group 1 after 1 week of wearing orthodontic appliances. However, the number of bleeding sites reduced afterwards. In Group 2, the number of bleeding sites were very minimal throughout the 3 months of this study. However, all the changes across groups and timeline were not statistically significant (p>0.05).

Microbiological Evaluation

In aerobic condition on TBSA, aerobic bacteria exhibited few different colony characteristics; circular, creamy white colonies, irregular glistening brown colonies or circular tiny white colonies with a colourless ring around them (Figure 1).

The TSBA in anaerobic condition showed similar colony characteristics as the aerobic bacteria (Figure 2). In addition to that, black pigmented round colonies with a diameter around 1mm were seen on this agar which was displayed by the BPB (Figure 3). *A. actinomycetemcomitans* colonies grew on TSBV with white glistening round shape colonies with diameters of 0.5-1 mm diameter (Figure 4).

The pure cultures from the black pigmented colonies on TBSA and the white colonies on TSBV were gram stained and investigated under the light microscope. The result showed

Figure 1: Aerobic bacteria's colonies on TSBA exhibited different colony characteristics; circular, creamy white colonies, irregular glistening brown colonies or circular tiny white colonies with a colourless ring

Figure 2: Anaerobic bacteria's colonies on TSBA exhibited similar characteristics as the aerobes

that the BPB were generally rounded or cocci shaped bacteria and were stained red (Gram-negative). *A. actinomycetemcomitans* showed a coccobacillus shaped and stained red (Gram-negative) under the light microscope.

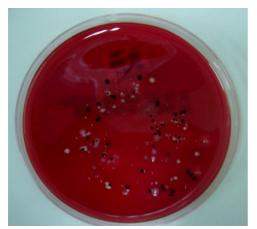Microbiological profile of the aerobes, anaerobes, BPB and *A. actinomycetemcomitans* were determined throughout baseline (T0), 1 week (T1), and 1 month (T2), 3 months post-bonding (T3). The result showed that in Group 1, from T0 to T1, the number of aerobe bacteria significantly increased from 80% to 88% (p=0.04) (Table 2). However, the counts significantly reduced from T2 to T3 with reduction of 43.3% (p=0.00). In contrast, the anaerobe counts slightly reduced from T0 to T1; but thereafter, insignificantly increased all

Figure 3: Black pigmented round colonies with a diameter around 1mm were seen on TBSA in anaerobic condition
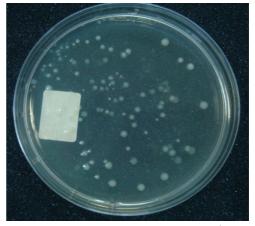

Figure 4 : *A. actinomycetemcomitans* colonies on TSBV characterised by the white glistening round shape colonies in 0.5-1mm diameter

Table 2: Amount of aerobe, anaerobe, Aggregatibacter actinomycetemcomitans (Aa) and black pigmented bacteria (BPB) in periodontal healthy (Group 1) and stabilised periodontal subjects (Group 2) at baseline (T0), 1 week (T1), 1 month (T2) and 3 months (T3) in mean percentage standard deviation.

	T0			T1			T2			T3		
	Group 1	Group 2	p-value	Group 1	Group 2	p-value	Group 1	Group 2	p-value	Group 1	Group 2	p-value
Aerobe	80.06 ± 28.82	52.40 ± 40.54	0.01*	88.11 ± 18.88	40.37 ± 53.70	0.00*	84.66 ± 23.42	39.35 ± 35.00	0.00*	41.40 ± 45.98	33.30 ± 38.40	0.78
An-aerobe	19.94 ± 29.82	32.90 ± 36.72	0.51	11.88 ± 18.88	44.92 ± 41.33	0.04*	15.33 ± 23.42	37.12 ± 34.22	0.12	32.13 ± 43.15	52.00 ± 41.92	0.18
Aa	3.44 ± 9.05	1.80 ± 5.82	0.00*	6.56 ± 14.21	2.00 ± 3.41	0.05	1.27 ± 2.08	8.17 ± 13.17	0.77	1.85 ± 3.69	12.79 ± 22.78	0.00*
BPB	0.39 ± 1.37	0.09 ± 0.05	0.15	0.24 ± 0.80	0.79 ± 2.86	0.69	0.84 ± 2.51	0.87 ± 0.00	0.80	0.89 ± 0.48	1.00 ± 5.86	0.33

*significant at p<0.05

along till T3 (p>0.05).

The aerobe bacterial count in Group 2 showed a decreasing trend with the highest number at T0 (52%). For the anaerobe bacterial count, it increased insignificantly at T1 (p=0.34), slight reduction at T2 (p=0.78), then spiked up at T3 to 52% (p=0.22). However, all changes were not statistically significant (p>0.05).

In comparison for each bacteria between Group 1 and Group 2 (Table 2), aerobe bacteria in Group 1 was higher than Group 2 with a statistically significant difference at T0, T1 and T2 (p<0.05). Anaerobe bacteria in Group 1 showed significantly less count than Group 2 at T1 (p=0.04).

A. actinomycetemcomitans counts in Group 1 rose after T0 and was at a peak at T1 at 6.56% (p=0.46). After one month, *A. actinomycetemcomitans* counts reduced to 1.27% but then increased slightly at T3. All the changes between the timeline were not statistically significant (p>0.05). When compared to the baseline, *A. actinomycetemcomitans* in Group 1 significantly decreased (p<0.01) after the 3 months of orthodontic treatment with reduction of 1.59%.

A. actinomycetemcomitans in Group 2 increased from T0 to T3 with a statistically significant difference between before treatment (T0) and after 3 months of orthodontic treatment (T3) (p<0.05). *A. actinomycetemcomitans* showed its highest count at T3 (12.79%).

When comparing between the two groups, *A. actinomycetemcomitans* showed a statistically significant difference at T0 and T3 (Table 2).

In Group 1, the number of BPB at baseline was very minimal (0.4%). The BPB counts slightly reduced from T0 to T1. The counts then increased by 0.6% from T1 to T2 and a minimal increment at T3. Overall, the BPB in Group 1 were minimal with no significant differences detected (p>0.05) throughout the 3 months. In Group 2, BPB was only 0.09% at T0. BPB showed a consistently increasing trend from T1 to T3 with no significant changes (p>0.05). When compared between the two groups, no differences were detected (p>0.05) as shown in Table 2.

DISCUSSION

All subjects demonstrated healthy periodontium prior to the bonding of orthodontic appliances with minimum values for all clinical perimeters i.e. the PS, BOP and PD. Positive effects of oral hygiene instruction given to the patient two weeks before the placement of the fixed appliance may have a role in the good oral hygiene maintenance of the subjects. However, after 1 week of wearing orthodontic appliances, PS and BOP were slightly increased especially in patients without any previous history of periodontal disease. A previous study found a similar increase of the two clinical parameters after 1 month of orthodontic treatment (Guo et al. 2016). The slight increase in the PS and BOP during the first week of appliance placement may be due to the new adaptation of the appliance in the mouth causing discomfort or pain. The pain and discomfort from the appliance have been shown to be exacerbated by tooth brushing (Rakhshan & Rakhshan 2015), which may consequently affect their oral care.

Interestingly, PS and BOP scores were generally lower in the stabilised periodontal patients (Group 2) than in the healthy periodontal patients (Group 1). The lower clinical readings from Group 2 may be due to having been in the periodontal therapy regime for a long term and consistently received oral hygiene education as part of periodontal maintenance therapy as compared to Group 1 who were only given oral hygiene instruction once. Thus, giving oral hygiene measures throughout orthodontic therapy to all patients regardless of the periodontal condition prior to treatment would be beneficial in maintaining optimal oral hygiene and plaque control (Levin et al. 2012). The PDs in Group 2 were identical to Group 1 even though these patients had already experienced some periodontal problems prior to the start of orthodontic treatment. Although the periodontal health in the subjects may be jeopardised insignificantly at some point of the treatment as presented in the PS and BOP scores, no permanent destructive effect on periodontal tissue was seen in the 3 months of this study.

Saliva acts as a self-cleansing mechanism to flush away from food debris from the tooth surface (Tiwari 2011). However, due to the presence of orthodontic brackets, this natural cleansing process is hindered. Orthodontic treatment has been

associated with increased plaque retention (Boke et al. 2014; Mahindra et al. 2017). Therefore, as time progresses, more subgingival plaque accumulated. Subgingival dental plaque, that is predominantly anaerobic flora (Ximénez-Fyvie et al. 2000; Ziouani et al. 2015), has been shown to be associated with periodontal disease (Daniluk et al. 2006; Tanner 2015).

In this study, the number of aerobic bacteria in healthy patients was consistently more than the anaerobes throughout the time interval. However, in the stabilised periodontal group (Group 2), more anaerobes were detected after 1 week and a marked difference after 3 months as compared to the aerobes. The higher count of the anaerobic bacteria may be reflected by the deeper periodontal pocket presence in Group 2. It has been shown that the deeper the periodontal pocket, the less oxygen tension in its environment. The anaerobic bacteria grows well in a lesser oxygen tension environment. Therefore, more anaerobe populated in a deep periodontal pocket (Ximénez-Fyvie et al. 2000; Tanner 2015). Although, the difference was not significant statistically, the increment of anaerobic bacteria has been shown to leads to the initiation and progression of periodontal disease as shown in published literature (Ximénez-Fyvie et al. 2000; Popova et al. 2013; Tanner 2015).

With respect to time, the results from this study showed an initial increase in adherence of *A. actinomycetemcomitans* in the subgingival plaque with the greatest increase being from the baseline

to the first week in Group 1 and a decrease in the months after. This is in agreement with a previous study which showed similar behaviour of *A. actinomycetemcomitans* (Ristic et al. 2007). In contrast with Group 2, *A. actinomycetemcomitans* started to increase after 1 week followed by its constant increment during the three months and a striking change from baseline to the 3rd month. This pattern is in agreement Naranjo et al. (2006) who found an increase in *A. actinomycetemcomitans* counts 3 months after bracket placement in healthy periodontal patients, but not statistically significant. However, Kim et al. (2012) found *A. actinomycetemcomitans* was detected at a low levels and showed no significant difference during their study period. The statistically significant increasing number *A. actinomycetemcomitans* within Group 2 may be because the patient who had a history of periodontal disease might already had that pathogenic bacteria in their plaque ecosystem; thus having fixed appliance could easily cause an increase in the bacterial count.

In our study, the BPB increased from the first week to the 3rd month. A few studies have found an increase in *P. gingivalis*, a type of black pigmented bacteria, 3 months after bracket placement (Naranjo et al. 2006; Kim et al. 2012). Another type of BPB, *P. Intermedia* was also found to be increased within 3 months of fixed appliance placement (Ristic et al. 2007 & Kim et al. 2012).

This implies that even if the periodontal condition of a patient has

been stabilised, they are still at risk of developing periodontal diseases. The mere presence of pathogen alone is not sufficient to cause disease, but they need to be present above a determined threshold to cause periodontal diseases. Therefore, effective oral hygiene instruction (Liew et al. 2011) and strict periodontal health monitoring must be indicated, especially to this group of patients. Furthermore, all orthodontic patients must be compliant and follow all given instructions, independent of the appliance type.

CONCLUSION

This study revealed that even though patients exhibited good periodontal health clinically, the microbiological ecosystem may reflect differently. Thus, regular periodontal health monitoring and strict oral hygiene measures must be implemented in all patient undergoing orthodontic treatment especially patients with a history of periodontal disease. -

ACKNOWLEDGEMENT

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